PHARMACODYNAMIC RESPONSES OF 5-HT$_2$ ANTAGONISTS AS AN AID TO DRUG DEVELOPMENT IN MAN.

A thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine

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TITLE: "PHARMACODYNAMIC RESPONSES OF 5-HT_2 ANTAGONISTS AS AN AID TO DRUG DEVELOPMENT IN MAN."

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SUMMARY.

The aims of this thesis were to describe the clinical pharmacology of two 5-hydroxytryptamine type 2 (5-HT_2) receptor antagonists being developed for cardiovascular (migraine and thromboembolic disorders) and central nervous system (schizophrenia, anxiety and depression) diseases:

ICI 169,369 (2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride) and ICI 170,809 (2-(2-dimethyl-2-methylpropylthio)-3-phenylquinoline hydrochloride)are structurally related compounds with differing potency, activity and duration of effect in animal models.

A series of experiments was planned to investigate their human pharmacodynamics. Established endpoints such as *ex vivo* platelet aggregation (reflecting peripheral activity) and changes in waking EEG (as an index of central effects) were employed together with pupillometry. The latter novel effect was found by serendipity, although not predicted by the known pharmacology of these compounds formed the basis of a HYPOTHESIS that:

> *"5-HT_2 antagonists would modify human pupillary responses, providing an ancillary non-invasive pharmacodynamic measurement which would reflect other surrogate endpoints. This would provide the means of demonstrating 5-HT_2 mediated pharmacodynamic activity in man, and permit the investigation of possible pharmacokinetic and dynamic relationships in clinical efficacy trials."*

ICI 169,369 was targeted for CNS activity due its relatively weak activity on platelet aggregation in animal models. Therefore effects of single oral doses (80 and 120mg) on the power spectrum of waking EEG, dark adapted pupil responses, sedation score & platelet aggregation were studied as a double blind, placebo controlled, randomised cross over within subject comparison, in six healthy male volunteers.

The effects of ICI 170,809 were studied, as single oral (3, 7, 15 and 30mg) and multiple (7mg twice daily for 21 days) doses, on the *ex vivo* platelet aggregatory response to 5-HT and the pupillary light constrictor response, as a double blind, placebo controlled, randomised partial cross over, within subject comparison, in 8 healthy male volunteers.

Measurements of blood concentration were made for both compounds to assess the correlation between pharmacokinetic and pharmacodynamic parameters.

CONCLUSIONS

Both 5-HT_2 antagonists studied induced a miosis consistent with the hypothesis, providing evidence for the involvement of 5-HT in the control of pupillary responses.This miosis compared favourably with surrogate endpoints such as EEG (with ICI 169,369) and platelet responses following single and limited multiple dosing (with ICI 170,809).However, tachyphylaxis after multiple dosing would probably limit the use of miosis as a surrogate endpoint.Significant correlations were obtained between circulating 5-HT2 antagonist concentration and the magnitude of pupillary miosis for both compounds, and with *ex vivo* platelet aggregation for ICI 170,809.Therefore,the combination of pharmacodynamic endpoints discussed in this thesis may be a useful indicator of 5-HT_2 antagonist activity in therapeutic trials.

ACKNOWLEDGEMENTS & DEDICATION.

The studies carried out in this thesis were carried out in the Clinical Pharmacology Unit at ICI Pharmaceuticals, whilst the author held a joint academic appointment as research associate in the Department of Pharmacology and Therapeutics at the University of Liverpool.

The author would like to thank Professor.A.M.Breckenridge,the Professor of Clinical Pharmacology, and Dr.J.Harry, former Head of Clinical Pharmacology at ICI, for their guidance and support during the tenure of this Clinical Pharmacology training programme.Thanks are also due to numerous people at both ICI and the University of Liverpool for their willingness and patience in offering me help and advice.In particular I am grateful to Mrs.D Wilkinson SRN,principal nurse at ICI who taught me many of the practical aspects of research;members of the research engineering workshops especially Mr.S.Hobson for his help with the computation of data;Dr.A Swaisland for measuring drug levels of the 5-HT_2 antagonists and for calculating the pharmacokinetic parameters; Dr.S.Haworth, Dr.A.Rushton, Dr.T.Blackburn and Dr.B.Cox for lively debate, intellectual stimulation and for allowing me to experience how research can be used in drug development.

Finally, I must add a note of dedication to my wife Catriona, who has supported and encouraged me to complete this thesis, and to my four children whom, I hope will forgive me for neglecting them during the prolonged gestation of this thesis.

QUOTATION:

Although 5-hydroxytryptamine (5-HT) research has intrigued scientists for more than a century, the modern scientific "birth" of 5-HT can be dated to Dr. Irvine Page's Science paper of 1948.

More recently, Page summarised his contributions to 5-HT research as follows:

"Serotonin, in short, has taken on a life of its own and we are no longer living together. I have no regrets, because a long life has taught me that the natural history of ones active participation in a discovery is about 5 to 10 years. Then the subject grows complicated. New very bright young faces appear with their better methods and they take over. If they are aware that anything preceded their work, they give no indication of it - which is natures way of preventing constipation of the mind."

Irvine H.Page,1985.

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CHAPTER ONE

AIMS OF THE THESIS

This section of the thesis describes the training, supervision and contribution of the candidate DSM, together with the background philosophy, hypothesis and clinical relevance of the research.

1.1 RESPONSIBILITIES AND TRAINING IN RESEARCH METHODS

As a research fellow in the clinical pharmacology unit (CPU) at ICI pharmaceuticals I was jointly responsible with Dr. Steven Haworth (medical advisor), for human volunteer studies with the post synaptic 5-HT_2 receptor antagonists which had just entered clinical development. I also received training in methodology, project planning, writing, submitting and executing protocols with a wide range of new chemical entities which were being administered to man for the first time. At this time, I also held a post in the Dept. of Pharmacology and Therapeutics (University of Liverpool) with regular clinical and teaching commitments at the medical school, and as an Honorary Senior Registrar at the Royal Liverpool Hospital. This expert supervision was provided enthusiastically by Dr. John Harry (Head of the CPU), and by Professor A. M. Breckenridge for the Clinical part of my attachment.

Unless otherwise stated, the candidate (DSM) played a major role in the planning and execution of the studies described in the thesis. Where pupillometry was carried out with other assessments, I was responsible for the overall conduct of these studies. This included carrying out medical assessments and experimental practical procedures (*e.g.* venous cannulation, EEG recording, administering

psychomotor tests etc), together with the presentation of protocols to the ethics committee and staying overnight in the CPU to provide medical cover for the studies. The major exceptions were that all the *in vitro* and *ex vivo* platelet estimations were carried out by Carol Jessup (now deceased), and the assay of whole blood and plasma samples were carried out by Dr. Alan Swaisland at ICI.

For the multiple dosing studies carried out at Hazleton Clinical Research (under the medical supervision of Dr.Alan Houston), I had a major role in designing the protocol and in training the scientific staff in the recording of resting pupil diameter using the "Polaroid" pupillometer. I also visited the centre on the first dosing occasion and regularly monitored the conduct of the study. The evaluation of the pupil photographs and data analysis were also carried out by myself. Other pharmacokinetic and dynamic data collected during the course of this study was collated and analysed by the ICI biometrics group and are reported together with the pupil data in chapter five.

All statistical evaluations were carried out by DSM in conjunction with the department of medical statistics (Dr. S. Ellis). In the majority of cases, I performed the initial analysis using a statistical package "Statsgraphics" which would allow for Multiple factor (allowing for period, subject, treatment and temporal factors) analysis of variance. At this stage, I would discuss the findings with the ICI statistician, and we would agree whether to include a correction for other factors (*e.g.* covariance with predose readings), decide if it was to appropriate to apply significance tests and calculate 95% confidence intervals. Where the statistical manipulations were beyond the capability of the "Statsgraphics" package and required more powerful techniques these were carried out using the statistical package "SAS" and are reported seperately in appendix three.

1.2. BACKGROUND TO THE RESEARCH PROGRAMME

ICI had a number of potential 5-HT_2 receptor antagonists in preclinical development. This was part of a strategy to examine the therapeutic potential of a novel class of drugs, which might modify the cardiovascular and central nervous system effects of 5-HT, considered to be deranged in diseases such as migraine and schizophrenia (for further details see introduction and Appendix I and II describing the pharmacology of 5-HT and animal pharmacology of the two development candidates)

One of the objectives of the phase one 5-HT_2 receptor antagonist programme was to describe the human pharmacology of these compounds. This included assessing the pharmacokinetics, metabolic disposition, safety and tolerability of these compounds in man. These, together with an attempt to establish surrogate pharmacodynamic endpoints in man, would help to describe pharmacodynamic/pharmacokinetic relationships and may be predictive of doses for use in clinical trials.

1.3 SERENDIPITY AND THE GENERATION OF A HYPOTHESIS

One of the new techniques which I began to investigate was the capability of a prototype hand held infra red pupillometer (Pupilscan), to measure resting pupil diameter and to detect dynamic response to a light induced pupillary constriction.

As part of a familiarisation exercise, I began by trying out this technique on colleagues and healthy subjects who were passing through the CPU, and could spare 5 minutes of their time. On one such occasion, a group of subjects had received oral doses of the 5-HT_2 antagonist ICI 169,369 or placebo as part of a pharmacokinetic study. These were captive and willing subjects who allowed me to measure their pupil diameter whilst waiting for a venepuncture.

During this post dosing period it was noted that a number of subjects had markedly constricted pupils, and that the pupillometer was capable of detecting this and making repeatable measurements. It was these subjects with the pupillary miosis, who after the study was concluded were found to have received active treatment. There was no other rational explanation, and the miosis could not be readily explained by the known pharmacology of ICI 169,369 or other 5-HT_2 antagonists.

Thus, a chance observation led me to investigate the underlying mechanism producing the miosis, to design experiments establishing the repeatability, pharmacology and specificity of this phenomenon.

Published evidence suggests that 5-HT_2 receptors may be involved in the control of pupillary responses. Radioligand binding and immunohistochemistry have localised 5-HT neurones in the human iris-ciliary body (Usitalo *et al*, 1984). L-trytophan injection (a 5-HT precursor) by close carotid arterial injection in man produced an ipsilateral mydriasis by increasing ciliary body 5-HT

(Mantegezzini; 1966). Similarly fenfluramine, (a 5-HT releasing agent) produced mydriasis, not abolished by pre-treatment with guanethidine or thymoxamine (Kramer *et al*, 1973). Mianserin and trazodone (Longmore *et al*, 1988) both with 5-HT_2 antagonist properties, produced a miosis which in the case of mianserin was unaffected by alpha 1 or muscarinic agonists (Shur *et al*, 1983). These findings suggest that 5-HT may be influencing pupillary responses in man, by mechanisms other than classical autonomic receptors.

Therefore, the aim of this thesis was to test the HYPOTHESIS that:

> *"Serotonin type II antagonists such as ICI 169,369 and 170,809 would modify human pupillary responses, providing an ancillary non-invasive pharmacodynamic measurement which would reflect other surrogate endpoints. This would provide a means of demonstrating 5-HT_2 mediated pharmacodynamic activity in man, and permit the investigation of possible pharmacokinetic and dynamic relationships in clinical efficacy trials."*

1.4 RESEARCH STRATEGY

Based on a knowledge of the animal pharmacology of the two 5-HT_2 antagonist development candidates (ICI 169,369 and 170,809) I set out to profile the clinical pharmacology of the compounds in man. A separate study set out to examine the relationship between drug induced sedation and pupillary miosis, as a possible explanation for the changes observed with both ICI 169,369 and 170,809.

1.4.1 The first compound: ICI 169,369

ICI 169,369 was shown to be a relatively weak inhibitor of 5-HT induced platelet aggregation in animals and this was subsequently confirmed in man by Prof. Heptinstall (Bevan and Heptinstall, 1985; see addendum to chapter 4). Furthermore, doses which could be administered to man were limited by potential problems with hepatotoxicity in animals. Therefore, higher doses could not be administered to increase antiplatelet activity.

Thus, the main focus of attention with ICI 169,369 was to demonstrate potential central nervous system (CNS) activity, by examining its effects on both waking (work described in this thesis) and sleep EEG (Cowen *et al* 1990). As a direct result of my chance observation, suggesting a pupillary miotic effect, measurements of pupil size and reaction to light were made together with visual analogue scale assessments to further examine CNS activity. A preliminary pharmacokinetic study was also included (using a prototype whole blood assay) to allow assessment of any pharmacokinetic/dynamic (PK/PD) relationship.

1.4.2 The second compound: ICI 170,809

ICI 170,809 was a more promising candidate, with a longer duration of action and greater potency for the platelet 5-HT receptor in animals. Therefore, the *ex vivo* antiplatelet activity of this compound was explored as a potential surrogate marker of 5-HT_2 antagonist activity. Again, other assessments were made which included pupillometry and visual analogue scales as an index of central activity, together with measurement of circulating plasma ICI 170,809 to allow description of any pharmacokinetic/dynamic relationship.

1.5 CLINICAL RELEVANCE

The ultimate aims of the research described in this thesis were to describe surrogate endpoints in healthy subjects, which reflected the pharmacodynamic activity of the 5-HT_2 antagonists, and to determine any relationship with circulating drug levels. Thus, in the treatment of CNS disorders (*e.g.* schizophrenia) and cardiovascular disease lacking a definitive therapeutic endpoint (such as in conditions other than hypertension), these dynamic measurements could be used to monitor the degree of 5-HT_2 antagonism.

1.5.1 **This might facilitate**:

a. Monitoring dose response relationships in drug concentration controlled clinical trials.

b. Choice of the appropriate dose in phase III clinical trials.

c. Individualisation of drug therapy by choosing and adjusting the dose according to clinical endpoints

d. The testing of a speculative hypothesis which suggested the involvement of 5-HT_2 receptors in a particular disease process. For example, the treatment of Raynaud's disease where the hypothesis would be that the vasospasm was related to 5-HT_2 receptor stimulation. A clinical trial could be devised, to assess the efficacy of a 5-HT_2 antagonist in preventing attacks whilst *ex vivo* agonist activity at 5-HT_2 receptors might be measured, along with pharmacokinetic parameters. Thus efficacy endpoints could be related to a pharmacokinetic/dynamic (PK/PD) model.

Promising PK/PD log-linear relationships were found for both inhibition of 5-HT *ex vivo* platelet aggregation and pupilllometry following single oral doses of ICI 170,809. Therefore, this PK/PD relationship was further explored after limited multiple dosing. Since, this might provide a rationale, enabling assessment of a pharmacodynamic endpoint reflecting 5-HT_2 antagonism in clinic studies, which in turn could be used to relate to the study of efficacy parameters during multiple dose therapy.

CHAPTER 2

INTRODUCTION

2.1 CLASSIFICATION AND NOMENCLATURE OF FUNCTIONAL RECEPTORS FOR 5-HYDROXYTRYPTAMINE.

Over a century ago, Ludwig (1868) first described the vasoconstrictor properties of defibrinated blood and serum. More than 50 years later this "adrenaline like" vasoactive material was first ascribed to the platelet (O'Connor, 1912). Eventually, the responsible agent 5-hydroxytryptamine (5-HT) was discovered by Page in 1954, and about the same time Rapport (1948) identified the chemical structure of this crystalline material, which was considered to be involved in the pathophysiology of hypertension. Page in his early attempts to isolate the factor responsible for the pathogenesis of hypertension, was more interested in 5-HT because of its "nuisance value in the search for the vasoactive substance angiotensin" (1958).

5-HT has often been similarly dismissed as irrelevant, or too complicated a problem to tackle. As recently as 1985, in a major review "Interactions of Platelets with the Vessel Wall", Oates *et al* pointed out that "during each minute of circulatory transit, 1000 billion platelets survey 1000 square metres of capillary surface, carpetted with 700 billion endothelial cells. Any break in the continuity of the vessel wall is met with an instant response from the platelets, which contact the zone of injury, spread and clump." Surprisingly however, despite dealing with other vasoactive substances (*e.g.*, prostanoids) concerned with the platelet- vessel wall interaction, the involvement 5-HT was not even considered worthy of discussion in the monograph.

A bewildering complexicity and multiplicity of vascular actions have been ascribed to 5-HT, whilst the selective antagonists to characterise them were lacking. This meant that little progress was made until the discovery of ketanserin, the first selective antagonist of a vascular 5-HT receptor (Leysen *et al*, 1981).

Documented actions of 5-HT on the vascular receptor were reviewed by Houston and Vanhoutte (1989). These effects included a direct vasoconstrictor effect by way of a specific 5-HT receptor, amplification of the vasoconstrictor actions of other neurohumoral mediators, actions at the post synaptic junctional alpha adrenergic receptor, or indirect sympathomimetic action by displacement of noradrenaline from adrenergic nerve terminals, and release of vasoactive mediators such as thromboxane A2. Vasodilatation also occurs, further complicating the matter, perhaps reflecting an influence on the release of endothelium relaxing factors (Furchgott and Zawadaski, 1980). Alternative possibilities included inhibition of adrenergic neurotransmission, activation of inhibitory nerves, vasodilator prostaglandin release and stimulation of beta adrenergic receptors (Hollenburg, 1988).

Given the multiplicity of actions, many of which were not blocked by 5-HT antagonists, it is not surprising that the role of 5-HT in normal circulatory physiology and in disease has remained obscure. A similar story can be told for the involvement of 5-HT in the pathophysiology of nervous diseases.

During the latter part of the 1950's Gaddum in Edinburgh and Rocha de Silva in Brazil continued to work on the neuromodulatory role of 5-HT, culminating in the now classical publication by Gaddum and Picarelli (1957). Two receptor subtypes were described in the isolated guinea pig ileum. "M" receptors (related to cholinergic depolarisation)and antagonised by morphine; and "D" receptors

(smooth muscle contraction) antagonised by phenoxybenzamine. The major problem with this 5-HT classification lay in the lack of specificity of either morphine or phenoxybenzamine. By the beginning of the 1960's 5-HT research had again declined significantly. However, twenty years later history had repeated itself, and during the 1980's interest in the 5-HT receptor classification and roles of 5-HT had been revived.

5-HT was first identified in the central nervous system (CNS) by Twarog and Page (1954). Subsequently Dahlstrom and Fuxe (1962) mapped 5-HT containing neurones in the CNS using fluorescent histochemistry. More sophisticated procedures have since allowed a detailed mapping of 5-HT neurones (Steinbusch, 1981) and 5-HT has now been clearly shown to meet the criteria for the role of a neurotransmitter in the brain. The actions of 5-HT in the CNS include controlling sleep, appetite, pain, neuroendocrine function, sexual behaviour, aggression, temperature regulation and various psychiatric states as well as mediating the hallucinogenic properties of certain pharmacological agents (*e.g.* LSD, Angel dust; Green 1989; Roberts, 1984; Blundell, 1984; Iversen, 1984).

The work of Peroutka and Snyder (1979), using a range of 5-HT agonists and antagonists with varying selectivity, combined with radioligand binding, led to the identification of two discrete sites in the central nervous system (CNS): a 5-HT_1 site with high affinity for the natural ligand 5-HT and for LSD (the potent CNS hallucinogen, lysergic acid diethylamide); and a 5-HT_2 site with a high affinity for spiperone (a relatively non-selective antipsychotic) and a low affinity for 5-HT. Subsequent work has shown the 5-HT_2 site to be the same as the "D" receptor, to be homogeneous and located both in the brain and in the periphery where it mediates some of the excitatory actions of 5-HT (Leysen, 1982). Potent agonists of this receptor include alpha-methyl-5-HT (Richardson, 1985) and selective antagonists such as ketanserin (Leysen, 1985). (See Table 2.1 and 2.2 for functional

responses mediated by 5-HT_2 receptors; after Bradley *et al* 1986).The classification and discovery of the 5-HT_3 receptor came later when workers began to examine the "M" receptor.

TABLE 2.1

SUMMARY OF PROPOSALS FOR CLASSIFICATION AND NOMENCLATURE OF FUNCTIONAL 5-HT RECEPTORS

PROPOSED RECEPTOR NOMENCLATURE	TYPICAL RESPONSES	SELECTIVE AGONISTS	SELECTIVE ANTAGONISTS	EQUIVALENT BINDING SITE
"5-HT_1-like"	Prejunctional inhibition of neuronal transmitter release, smooth muscle relaxation, contraction of some vascular smooth muscles and tachycardia in the cat.	5-Carboxamidotryptamine	Methsergide* Methiothepin**	5-HT_1?
5-HT_2	Gastrointestinal and vascular smooth muscle contraction, platelet aggregation, neuronal depolarisation.	—	Ketanserin Cyproheptadine Methysergide	5-HT_2
5-HT_3	Depolarisation of peripheral neurones	2-Methyl-5-hydroxytryptamine	Cocaine MDL 72222 ICS 205-930	None

*Weak antagonist (or partial agonist) at some "5-HT_1-like" receptors.
**Less potent antagonist than at 5-HT_2 receptors but inactive at 5-HT_3 receptors.

FUNCTIONAL RESPONSES WHICH APPEAR TO BE MEDIATED BY 5-HT$_2$ RECEPTORS

RESPONSES TO 5-HT	SPECIES/LOCATION	SPECIFIC ANTAGONISM BY KETANSERIN OR METHYSERGIDE	RESISTANCE TO ANTAGONISM BY 5-HT$_2$ RECEPTOR BLOCKING DRUGS	COMMENTS
In vitro				
Contraction of vascular smooth muscle	Rabbit aorta	Apperley, Humphrey and Levy, (1976); Feniuk *et al.* (1985a)	MDL 72222 inactive at 10μM (Feniuk, unpublished observation)	Comparison of dissociation constants at the 5-HT$_2$ binding site with equivalent values from pharmacological studies suggests that the binding site and pharmacological receptors are similar (see Humphrey, 1984).
	Rat tail artery	Bradley, Humphrey and Williams (1985); Van Nueten *et al.* (1981)	No data	
	Dog gastrosplenic v.	Van Nueten *et al.* (1981)	No data	
Contraction of extra-vascular smooth muscle	Guinea-pig trachea	Van Nueten, Leysen, Vanhoutte and Janssen (1982)	No data	
	Rat uterus	Ichida *et al.* (1983)	Fozard (1984b); Richardson *et al.* (1985)	
	Guinea-pig ileum (atropine treated)	Engel *et al.* (1985)	Engel *et al.* (1985)	Receptor in guinea-pig ileum is Gaddum and Picarelli's original "D"-receptor (see Engel *et al.* 1984,1985).
Platelet aggregation	Cat	De Clerck, Xhonneux, Leysen and Jannsen (1984)	No data	5-HT$_2$- binding sites have been identified on cat platelets.
	Rabbit and man	No data	Taparelli (unpublished observations)	
In vivo				Inhibition of 5-HT-induced [^{32}P]phosphatidic acid formation in human platelets also appears to be 5-HT$_2$ mediated (see Leysen *et al.* 1984).
Vasopressor responses	Pithed/anaesthetised rat	Van Nueten *et al.* (1981); Feniuk, Humphrey and Perren (1982); Fozard (1982) Saxena and Lawang (1985);	Kalkman *et al.* (1984)	
	Conscious rat	Dalton *et al.* (1985)	Dalton *et al.*(1985)	
Bronchoconstriction	Anaesthetised cat	Reiche and Frey (1983); Conner *et al.* (1986)	No data	
	Anaesthetised guinea-pig	Saxena and Lawang (1985)	Dhasmana *et al.* (unpublished)	
5-Hydroxytryptophan-induced head twitch	Conscious rat/mouse	Malick, Dosen and Barnett (1977)	Shearman (unpublished observations)	Selective 5-HT$_2$ antagonist, pirenperone, potent antagonist of 5-hydroxytryptophan-induced head twitch in mice (Green, O'Shaughnessy, Hammond, Schachter and Grahame-Smith, 1983). Effects of ketanserin not studied.
5-Hydroxytryptophan-induced wet dog shake	Conscious rat	Colpaert and Janssen (1983); Yap and Taylor (1983)	No data	
Oedema	Rat paw	Ortmann, Bischoff, Radeke Buech and Delini-Stula (1982)	Not tested	Although ketanserin not studied many "classical" 5-HT antagonists are potent inhibitors of 5-HT-induced rat paw oedema (Ortmann *et al.*, 1982).
Urinary bladder contraction (second phase)	Anaesthetised cat	Saxena, Heiligers, Mylecharane and Tio (1985a)	Saxena *et al.* (1985a)	Second prolonged phase of contraction (first transient phase is 5-HT$_2$ mediated)

TABLE 2.2

The 5-HT_1 radioligand binding site is not homogeneous. Agonist displacement studies have demonstrated marked heterogeneity with three or more sites being identified (Middlemiss and Fozard, 1983). Pedigo *et al* (1981) noticed atypical competition for the 5-HT binding site to rat cortex by spiperone, and suggested that the 5-HT_1 site could be subdivided into 5-HT_{1a} (high affinity for spiperone) and 5-HT_{1b} (low affinity for spiperone). Two further subtypes of the 5-HT_1 receptor were proposed these were designated 5-HT_{1c} (Pazos, 1987) and 5-HT_{1d} (Heuring, 1987). The main problem here is the lack of selective antagonists,and classification related to agonist potency. The net result is that 5-HT_1 receptors were identified by exclusion (*i.e.* they were not 5-HT_2 or 5-HT).

There are functional, second messenger correlates for each of these binding sites. The 5-HT_{1a}, 5-HT_{1b} and 5-HT_{1d} sites inhibit the adenylate cyclase system (Sanders-Bush, 1988), whilst the 5-HT_{1c} site stimulates phosphatidyl inositol turnover (Conn, 1986), as does the 5-HT_2 receptor (Kendall and Nahorski, 1985). Since there is now functional evidence to distinguish each of the four radioligand 5-HT binding sites, it is justifiable to accept each of these as 5-HT_1 receptor subtypes.

Other functional 5-HT_1 receptors (see Table 2.1) exist in the periphery (Bradley *et al*, 1986) which are similar to, but not the same as the binding sites described above. These share the common property, where 5-carboxyamidotryptamine is a potent agonist and methiothepin is an antagonist. However, there are marked differences between these receptors and no selective antagonists have yet been reported (Humphrey and Feniuk, 1990). They are important clinically, because sumatriptan (GR43175) the selective antimigraine drug, which can abort a migraine attack is thought to act via these " 5-HT_1 like receptors" (Saxena, 1990).Much controversy still surrounds the categorisation of these receptors and Perren *et al* (1989) maintain that they are neither 5-HT_{1a} or 5-HT_{1d} preferring to refer to them as "5-HT_1 like".

The receptor type recently designated '5-HT_3' by Bradley *et al* (1986) is probably synonymous with the "M" receptor Gaddum and Picarelli identified in guinea-pig ileum. The discovery of this receptor dates back to Fozard's work in Manchester (1984). Using the Langendorf, isolated spontaneously beating perfused rabbit heart, he identified a tachycardia response to 5-HT which was equivalent to the "M" receptor response in the ileum, since it could be antagonised by cocaine. The major discovery came when Fozard showed that metoclopramide a "D_1" dopamine antagonist also antagonised some actions of 5-HT on the heart, suggesting the involvement of a novel 5-HT receptor (Fozard and Mwaluko, 1976).

Although metoclopramide and cocaine have many diverse effects, they provided the impetus to several groups from Sandoz and Merrell Dow who went on to identify much more selective and potent antagonists, such as ICS 205,930 (Richardson, 1985) and MDL 72222 (Fozard, 1984). These compounds have in common an aromatic nucleus attached to a 'tropane' heterocyclic group (similar to cocaine) via a side chain containing a carboxyl group (Fig 2.1). Zacopride and granisetron (BRL 43694), compounds both in clinical trials for CNS and gastroenterological indications, also share this similarity with cocaine. The first selective 5-HT_3 antagonist to receive regulatory approval for a clinical indication (treatment of chemotherapy induced emesis), was ondansetron (Cunningham, 1987) which has a basic heterocyclic nitrogen that is not incorporated into the 'tropane' nucleus.

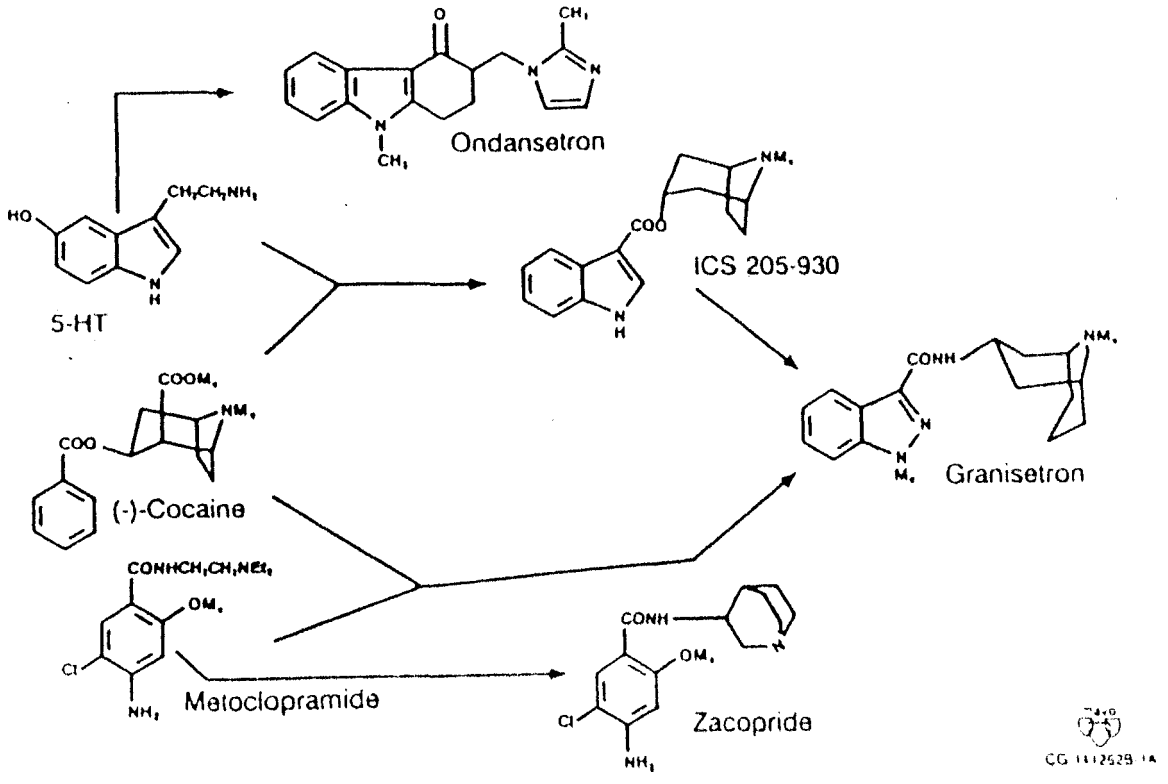


FIG 2.1

Illustrating the structural diversity of the 5-HT_3 antagonists. Structural similarities with both 5-HT and cocaine are exemplified (personal communication and courtesy of Dr. Talley, Mayo clinic).

Without these selective antagonists, the characterisation of 5-HT_3 receptors, and development of potential treatments for a wide range of CNS disorders would have been impossible. In animal experiments these compounds have four major influences on the critical "limbic-cortical" behavioural circuitry. They have the potential to reduce anxiety, improve cognition, reduce increased

psychomotor drive and to suppress disturbances of the reward system. Major advances in therapeutics may follow, with the possibility of an antipsychotic with both anxiolytic and cognitive enhancing properties (Barnes *et al* 1990).

Although the receptor type designated '5-HT_3' by Bradley *et al* (1986) corresponds most closely with the M receptor identified in guinea pig ileum by Gaddum and Picarelli, it has recently emerged that guinea pig ileum also contains a neuronal receptor which cannot be classified as 5-HT_3, and at which BRL 24924 is an agonist and high concentrations of ICS 205,930 exhibit antagonism with competitive kinetics (Craig and Clarke, 1989). A 5-HT receptor type exhibiting similar characteristics has also been identified in mouse embryo colliculi neurons and shown to be coupled with adenylate cyclase (Dumuis, 1988); this receptor has been provisionally designated 5-HT_4 (See Table 2.3 after Costall and Naylor, 1990) and has been identified in the gastrointestinal tract (N. Talley, Mayo Clinic, personal communication).

TABLE 2.3

SUMMARY CLASSIFICATIONS OF 5-HT RECEPTORS OR RECOGNITION SITES

	5-HT$_1$								
SUBTYPES	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1C}$	(a) 5-HT$_{1D}$	(b) 5-HT$_{1E}$	(c) 5-HT$_{1P}$	5-HT$_2$	5-HT$_3$	5-HT$_4$(d)
Location (example)	Enteric nerves	Sympathetic nerves Cortex	Choroid plexus Stomach fundus	Brain	Brain	Enteric neurons	Ileum Cortex	Vagus sympathetic nerves Enteric nerves brain	Hippocampus colliculi neurons
Function	Neuronal inhibition	Neuronal inhibition	Contraction	Neuronal Inhibition	?	Neuronal depolarization	Smooth-muscle contraction Neuronal depolarization	Neuronal depolarization	Cyclic AMP increase Neuronal depolarization?
Agonists	8-OHDPAT RU24969	RU24969	2(+)-α-Methyl 5-HT	Sumatriptan 5-carbox-amidotrypt-amine metergoline	?	5. and 6. hydroxy indalpine	2(+)-α-Methyl-5-HT	2-Methyl-5-HT Phenyl-biguanide	Metoclopramide zacopride renzapride
Antagonists	Spiperone	Cyano pindolol ←----Methysergide----→	Mesulergine Ritanserin	Methiothepin	?	5-HTP-DP	Ketanserin ritanserin mesulergine spiperone	ICS 205-930* MDL 72222 ondansetron	ICS 205-930**

See Bradley et al. for detailed classification and Refs. 41 and (a) 42, 43; (b) 44; (c) 45, 46; and (d) 31, 47, 48, 49, 50.(in Costall and Naylor, 1990)
GR43175 (Sumatriptam) =3-[2-(dimethylaminolethyl]-*N*-methyl-1H-indole-5methane sulphonamide; 8-OHDPAT =8-hydroxy-2(di-npropylamino) tetralin; RU24969 = 5-methoxy -3(1,2,3,6-tetrahydropyridin-4y0)1H-indole; 5HTP-DP = *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide; ICS 205-930 =(3-α-tropanyl) 1H-indole-3-carboxylic acid ester; MDL 72222 = 1α H,3α,5α H-tropan-3-yl-3,5-dichlorobenzoate.
* Nanomolar and ** micromolar concentrations

Functional correlates for the 5-HT_4 receptor have been identified. Both cisapride and renzapride have gastroprokinetic properties which are thought to be mediated via 5-HT_4 receptors (Naylor, 1990). Kaumann (1990) has demonstrated a 5-HT mediated tachycardia in human atrium which is not affected by blockade of 5-HT_1, 5-HT_2 or 5-HT_3 receptors and is antagonised by ICS 205,930. Furthermore, both cisapride and renzapride produce a dose related tachycardia in man and in the pig. This human atrial receptor produces increased atrial cyclic AMP, stimulates protein kinase A and is antagonised by ICS 205,930. He proposes that this receptor resembles 5-HT receptors of rodent brain and mammalian gut, and should be designated 5-HT_4. The clinical significance of these findings is uncertain, but should indicate caution in using these drugs in subjects with cardiovascular disease.

Recent DNA hybridisation and cloning techniques have facilitated identification and sequencing of 5-HT receptors at a molecular level (Hartig, 1989). The first groups to be cloned and sequenced were the 5-HT_{1a}, 5-HT_{1b} and 5-HT_2 receptors. These receptors share some sequence homology and belong to the G protein-coupled family of receptors. The 5-HT_{1c} receptor shares a closer homology with the 5-HT_2 receptor (51 percent identity) than the 5-HT_{1a} receptor (35 percent), suggesting it may in fact be better classified as a subtype of the 5-HT_2 receptor (Pritchett, 1988). This has important implications when describing the pharmacology of the 5-HT_2 receptor antagonists, ascribing their selectivity and explaining differences in therapeutic potential (see section on pharmacology of ICI 169,369).

The 5-HT_{1c} and 5-HT_2 receptor also have a common second messenger system (phosphatidyl inositol turnover), whereas the 5-HT_{1a}, 5-HT_{1b} and 5-HT_{1d} receptors regulate the intracellular concentration of cyclic AMP. Indeed, the 5-HT_{1a} receptor has been cloned by homology screening with a fragment of a beta adrenergic receptor gene (Fargin *et al*, 1988). The 5-HT_3 receptor is the

most recently purified receptor to date. This represents the only amine neurotransmitter-gated cation channel identified thus far (M^{c}Kernan, 1990). Thus, there is still much to be learned of the structure and function of 5-HT receptors and their interrelations with other receptors.

5-HT receptor pharmacology can also be described in terms of the electrophysiological actions of 5-HT, as expressed via membrane ion channels, the indole either opening or closing channels. This is particularly relevant when considering the neuronal effects of 5-HT and its involvement in the pathophysiology of CNS disorders. As reviewed recently by Wallis (1990), different 5-HT receptors are likely to be associated with different effects on ion channels.

2.2 ELECTROPHYSIOLOGY OF 5-HT

Firstly let us consider 5-HT effects on CHANNEL OPENING. These responses can be considered in two categories:

1A. Fast depolarization

5-HT_3 receptor activation involves the opening of a cationic channel permeable to Na and K ions, leading to a rapid phasic depolarisation (Wallis, 1989). This is recorded from cell bodies and axons, and is assumed to underlie the excitation of sensory endings and transmitter release via 5-HT_3 receptors, being responsible for the pain (acting on C fibre afferents) and flare response (releasing substance P via a local axon reflex) to intradermal 5-HT (Richardson, 1985).

1B. Hyperpolarization

5-HT_{1a} receptor activation in hippocampal pyramidal neurones is by opening of K channels, which are inwardly rectifying (Halliwell and Colino, 1990), and a similar mechanism may operate in lateral septal and myenteric neurones. Opening of K channels probably involves a pertussis toxin sensitive GTP-binding protein, which may directly couple the receptor to the K channel.

The other major effects of 5-HT can be described by CHANNEL CLOSURE. These can be divided into three categories:

2A. Slow depolorization

A further mechanism of excitation is the closure of the K channels, which typically shows a longer latency and slower response time course than channel-opening. K channels reflecting different 5-HT receptor types may be involved in the depolarisation (consider later when considering the 5-HT response in iris-ciliary muscle and relevance to pupillary constriction). For example, in the hippocampus depolarization currents are reduced by 5-HT acting via an unidentified receptor (Halliwell and Colino, 1990) whereas in the rat nucleus accumbens 5-HT reduces an inward conductance via a 5-HT_2 receptor (North, 1989). This is in keeping with observations concerning the potency of 5-HT_2 antagonists (ICI 169,369) in reversing the inhibitory effects of amphetamine on A9 and A10 dopaminergic neurones (a model to evaluate potential antipsychotic compounds; Goldstein, 1989). Similar depolarizations are seen in facial and lumbar motoneurones via receptors which fall broadly into the 5-HT_{1c} and 5-HT_2 category (Larkman, 1988; Elliot, 1990).

2B. Accommodation

In addition to changing membrane potential, 5-HT reduces the accomodation of the cell to produce tonic firing through suppression of a slow, Ca activated K activated conductance channel (Halliwell and Colino, 1990).

2C. Calcium Channels

Closure of Ca channels probably accounts for the action of 5-HT at nerve terminals which reduces transmitter release, either via an action at 5-HT_{1A} autoreceptors or of other neurotransmitters. These pre-synaptic or autosomal 5-HT effects are less well defined in terms of receptor selectivity. "5-HT_1 like " receptors are likely to be involved in these actions and have been demonstrated in dorsal raphe neurones (Kelly, 1989). The neuro-modulatory effects of 5-HT and its interaction with the autonomic nervous system are probably relevant here.

2.3 THERAPEUTIC TARGETS FOR PUTATIVE 5-HT2 ANTAGONISTS

The foregoing sections on 5-HT receptor classification emphasise the widespread distribution of 5-HT. This and its plethora of physiological effects would indicate the possible involvement of 5-HT in a variety of pathological states that should be susceptible to pharmacological manipulation. This thesis will specifically consider the location,function and physiological role of the 5-HT_2 receptor. It will also examine the rational basis for the therapeutic use and development of a selective 5-HT_2 antagonist. Thus, the principle aims of this research were to "measure the pharmacodynamic responses of 5-HT_2 antagonists as an aid to drug development in man".

2.4 THERAPEUTIC POTENTIAL IN CNS DISORDERS

The 5-HT_2 receptor is widely distributed in the brain, but with a particularly high density of receptor sites in cortical and limbic areas (Palacios *et al*, 1990). In animals, stimulation of these 5-HT receptors by direct or indirect agonists, leads to behavioural changes and stereotyped motor responses, which are antagonised by selective 5-$HT_{2/1c}$ antagonists such as ICI 169,369 (Blackburn, 1990).

Studies in depression demonstrate reduced cerebrospinal fluid levels of the 5-HT metabolite 5-HIAA (5-hydroxyindoleacetic acid) in some depressed individuals (Asberg, 1976). This is accompanied by an increase in both platelet and cortical 5-HT_2 binding sites in both depressed (Biegon, 1987) and suicide victims (Stanley and Mann, 1983). 5-HT_2 receptor antagonists have also been reported as displaying therapeutic activity in the treatment of depressive neurosis (Hoppenbrouers, 1986), schizophrenia (Ceulemans, 1985), anxiety (Bressa, 1987) and sleep disorders (Idzikowski, 1986, 1987; Speigel, 1980, Tortella, 1988, 1989).

In support of the involvement of 5-HT_2 receptors in other CNS disorders, is the finding of reduced 5-HT_2 receptor number in cortical membranes from patients suffering with senile dementia of the Alzheimer type (SDAT) (Crow *et al*, 1984). This together with the functional synergy of 5-HT_2 with 5-HT_3 receptors, in modulating hippocampal acetylcholine release from frontal cortex brain slices in the rat (Barnes, 1989), also supports their possible involvement in SDAT. Therefore, according to the cholinergic deficit hypothesis in SDAT (Crow *et al*, 1984; Costall *et al*, 1991), either 5-HT_2 or 5-HT_3 antagonists alone or in combination may provide a potential therapy for SDAT. Evidence with the 5-HT_3 receptor antagonist alosetron (GR68755), which partially reversed the acute memory deficit induced in healthy volunteers by the muscarinic antagonist scopolamine, would support this hypothesis (Preston, *et al*, 1991).

In keeping with the theory that mesolimbic dopamine turnover is increased in schizophrenia (for review see Gray *et al*, 1991), combined administration of the 5-HT_2 antagonists ICI 169,369 and the antipsychotic (dopamine, D_2 antagonist) haloperidol, has recently been shown to attenuate the development of dopaminergic supersensitivity (Blackburn *et al*, 1988). This is a property shared with atypical neuroleptics such as clozapine (Saller, 1990, Meltzer, 1989). Considered together with the relative A9/A10 dopaminergic selectivity of ICI 169,369 in the rat, this suggests that these agents might lead to a more selective treatment for schizophrenia. Mesolimbic dopamine might be differentially reduced, leaving nigrostriatal levels unaffected with reduced a propensity for extrapyramidal side effects (Goldstein, 1989)(Refer to Appendix A 1.2 for more details). Since 5-HT is involved in the modulation of dopamine release in the mesolimbic system (Costall et al 1991)this further supports the involvement of 5-HT as a possible presynaptic influence.

2.5 THERAPEUTIC POTENTIAL IN VASCULAR DISORDERS

Circulating 5-HT originates in the enterochromaffin cells (Erspamer 1954) of the gastrointestinal tract (GIT), being synthesised and secreted in response to digestive stimuli. Locally the monoamine facilitates the local reflexes that regulate gastrointestinal motility and secretion (Akkermans *et al* 1988; Bucheit *et al* 1985). Part of the 5-HT that is released overflows to the blood and is taken up by platelets (Stolz 1985). When the latter aggregate, the 5-HT is released from dense granules. The first targets for the 5-HT are the platelets themselves, activating 5-HT_2 receptors on the platelet membrane (Bevan and Heptinstall, 1985). This in turn accelerates the turnover of phosphoinositides, resulting in activation of protein kinase C and an augmented cytosolic calcium concentration (De Clerck and Van Nueten, 1982). The nett effect of this is to facilitate the aggregatory process (De Chaffoy de Courcelles, 1987).

5-HT is also involved in the initial reversible platelet shape change. Affolter *et al* (1984) and Erne and Pletscher (1985) showed that 5-HT, by activating 5-HT_2 receptors, caused a rapid increase in intracellular calcium which could be related to the shape change in platelets. Thus, by measuring platelet aggregation in whole blood using a platelet counter (Fox *et al* 1982), together with associated changes in light transmission for platelet rich plasma, differential effects of drugs on platelet shape change and aggregation can be observed (Bevan and Heptinstall, 1985).

5-HT is a weak direct activator of platelets, and primarily produces a transient shape change and aggregation of platelets, with little functional significance (Mullane *et al* 1982). However, as a consequence of this a 5-HT_2 mediated synergistic effect occurs faciliatating the platelet response to other agonists such as collagen, thromboxane A_2, and adenosine diphosphate (ADP) (Houston and

Vanhoutte 1989). This effect is antagonised by 5-HT_2 antagonists such as ketanserin (Van Nueten 1982), and ICI 170,809 and may be important in the genesis of coronary thrombosis (Cox *et al* 1991).

5-HT is also taken up and destroyed by the endothelial cells (Gillis 1985); these cells release endothelium-derived relaxing factor (EDRF) when exposed to the monoamine (Cohen 1989). The release of EDRF evoked by 5-HT is not blocked by 5-HT_2 antagonists (Luscher and Vanhoutte 1986), but involves a pertussis toxin sensitive G-protein. When 5-HT reaches vascular smooth muscle it usually causes contraction (Chester *et al* 1990); in most blood vessels this is prevented by 5-HT_2 antagonists (*e.g.* ICI 170,809; Cox *et al* 1991). The contractions evoked by 5-HT are often biphasic and are reduced considerably in the presence of a normal endothelium (Hollenburg 1989). Thus, contractions evoked by aggregating platelets, which release enough 5-HT to activate EDRF probably also act on 5-HT_2 receptors to produce smooth muscle contraction (Blauw 1988). Therefore, 5-HT_2 antagonists favour vasodilatation, not only by counteracting the amplifying effect that 5-HT exerts on further platelet aggregation, but also because, by blocking the direct 5-HT_2 mediated vascular contraction, they facilitate the production of EDRF (Vanhoutte, 1990).

The involvement of 5-HT_2 receptors in the pathophysiology of cardiovascular disease is however, contentious. In theory endothelial damage arising from atherosclerosis secondary to hypercholesterolaemia (Shimokawa, 1987), in hypertensives, diabetics and in normal ageing should predispose to 5-HT_2 receptor mediated vasoconstrictor responses. Clinically this might be expected to lead to an increased incidence in thrombotic arterial occlusion, presenting as myocardial and cerebral infarction or as peripheral vascular disease (Morishima *et al* 1991).

However, despite reports that ketanserin reduced morbidity and mortality in patients with critical artery stenosis to a greater extent than aspirin (Noble, 1990), a large prophylactic double blind trial in

subjects at risk from thrombo embolic disease failed to show a reduction in mortality. Unfortunately a subgroup with diuretic induced hypokalaemia had a marked prologation of QT_c on the electrocardiogram, which was associated with an increased incidence of sudden death (PACT study, 1989 and Cameron *et al* 1988).

Ketanserin is also under clinical evaluation in Raynauds phenomenon, and appears to be beneficial in some but not all cases of vasospasm, probably reflecting the multifactorial nature of the disease (Marasini, 1990).

2.6 HYPERTENSION AND ISCHAEMIC HEART DISEASE

Ketanserin is an effective treatment for mild/moderate hypertension in man (Breckenridge, 1988,), demonstrates ex vivo inhibition of 5-HT_2 mediated platelet aggregation during chronic therapy (Lijnen, 1987), and has a potential additional benefit in concomitant peripheral vascular disease (van Oene, 1988; Marasini, 1988). However the mechanism of action for the antihypertensive effect remains obscure. Ketanserin has some alpha-1 antagonist properties, yet it can lower blood pressure in subjects with peripheral autonomic neuropathy by mechanisms other than those involving classical autonomic receptors (Lijnen, 1987). The failure of both ICI 169,369 (Scott, 1988;Hedner et al 1987) and ritanserin (Stott *et al* 1987), both selective 5-HT_2 antagonists, to lower blood pressure in patients or healthy volunteers must cast doubt on the involvement of 5-HT_2 receptors in the pathophysiology of hypertension.

The involvement of 5-HT_2 antagonists in the aetiology of ischaemic heart disease (IHD) is still open to question. Coronary artery spasm displays very species dependent mechanisms. In normal healthy coronary arteries, non-human primates and man are less dependent on endothelium derived factors than the dog and phylogenetically more primitive species (Toda, 1990). This picture may change however when segments from normal and IHD subjects are compared (Chester *et al* 1990). Recent data would suggest that a correlation exists between the antiarrhythmic activity of a 5-HT_2 antagonist ICI 170,809 in a rat reperfusion arrhythmia model and its ability to inhibit ex vivo platelet aggregation to 5-HT (Coker, 1990). A possible synergism between 5-HT_2 receptor and thromboxane A_2 effects, and the consideration of a dual therapeutic approach certainly deserves further clinical evaluation (Hollenburg, 1988). The clinical utility of 5-HT_2 antagonists in coronary artery disease needs careful consideration.

2.7 MIGRAINE

Many of the drugs which have been used in the past to treat migraine are known to interact with 5-HT receptors. However, they were usually not specific for 5-HT and had troublesome side effects. An example par excellence would be ergotamine (Graham & Wolff,1938;Solomon, 1990) and its derivatives, which although undoubtably effective in both the prophylaxis and treatment of migraine, produces dependence by virtue of its withdrawal rebound attacks, together with with an unacceptable cardiovascular profile (hypertension and vasospasm). This is in addition to the psychomotor effects, nausea and gastrointestinal side effects of ergotamine. In spite of the lack of specificity these drugs do provide circumstantial evidence for the role of 5-HT in migraine; since this was the one property which they all shared (Fozard, 1989).

Migraine is a complex disease that involves both vasoconstriction and vasodilatation of some cerebral blood vessels, together with sensory disturbances attributable to cerebral ischaemia. (See Humphrey 1991 for recent review). Visual symptoms are the usual feature of an attack, but in severe cases, signs and symptoms resembling a transient ischaemic attack (TIA) are present, and may go on to produce permanent neurological sequaelae. Photophobia, severe unilateral headache, nausea and vomiting are also frequently reported. It is possible that 5-HT could play an important role in many if not all of these symptoms.

The turnover of 5-HT is increased following a migraine attack as measured by urinary 5-HIAA levels. This together with a parallel decrease in platelet 5-HT also supports the involvement of 5-HT in the aetiology of migraine (Sicuteri *et al*,1961;Curran *et al*,1965). Furthermore, drugs such as

fenfluramine which release 5-HT and the 5-HT_{1c} agonist methylchlorophenylpiperazine (MCPP) will produce a migraine type headache in susceptible subjects (Brewereton *et al*,1988;Fozard 1990).

Some migraineurs can have an attack triggered by dietary amine, and the possibility exists that these amines could either release 5-HT from blood platelets or act directly on 5-HT sensitive sites in the cerebral vasculature and the intramural nerves. A perivascular serotonergic innervation of central neuronal origin (from median and dorsal raphe nuclei) has been identified (Edvinsson, 1983 & 1984). The influence of 5-HT on the cerebrovascular circulation is further supported by observations that 5-HT concentrations within these vessels increase or decrease after the administration of drugs that modify the biosynthesis and degradation of 5-HT, or destroy nerve terminals by an uptake dependent mechanism (Reinhard, 1979). A perivascular nerve plexus had also been demonstrated in human cerebral and mesenteric blood vessels (Griffith, 1982). The possibility also exists that platelets may be responsible in part, for providing a vascular pool of 5-HT which is nonselectively taken up by sympathetic nerves, and is subsequently released along with noradrenaline, contributing to the intiation of the migraine attack (Verbeuren *et al* 1990).

Other migraineurs can have an attack triggered by stress or by sensory stimulation (often visual). In these cases one could hypothesise that the source of 5-HT is neuronal rather than platelet derived. This is a real possibility since the midbrain raphe cells have as one of their important functions the role of processing sensory information that is then passed on to other brain areas. They are therefore ideally placed to initiate a migraine attack. In support of this idea, is the finding that stimulation of trigeminal afferents can lead to increased release of inflammatory mediators and tissue extravasation in cerebral blood vessels, which can be modified by the 5-HT_1 like agonists sumatriptan (Buzzi, 1990). A migraine headache can also be triggered in healthy subjects by administration of a 5-HT_{1c}

agonist, suggesting that these receptors may be involved in the migraine trigger mechanism (Fozard, 1989).

Thus, 5-HT either blood borne, or released from serotonergic neurones can act on 5-HT receptors either on the cerebral blood vessels or sensory nerve endings. An action on the 5-HT_2/5-HT_{1c} receptors of cerebral blood vessels could lead to constriction followed by a reflex vasodilatation. Therefore intially cerebral blood flow decreases during the prodromal phase of migraine, whilst the headache phase is usually associated with an increase in blood flow (Drummond & Lance, 1984). Furthermore, the released 5-HT could act on sensory pain fibres, via 5-HT_3 or 5-HT_{1c} (Fozard,1991), so that noxious stimuli could be relayed along pain pathways via the trigeminal ganglia to the appropriate central nuclei in the thalamus. (See Fig 2.2 for summary of possible mechanisms; courtesy of Dr. B. Cox, ICI)

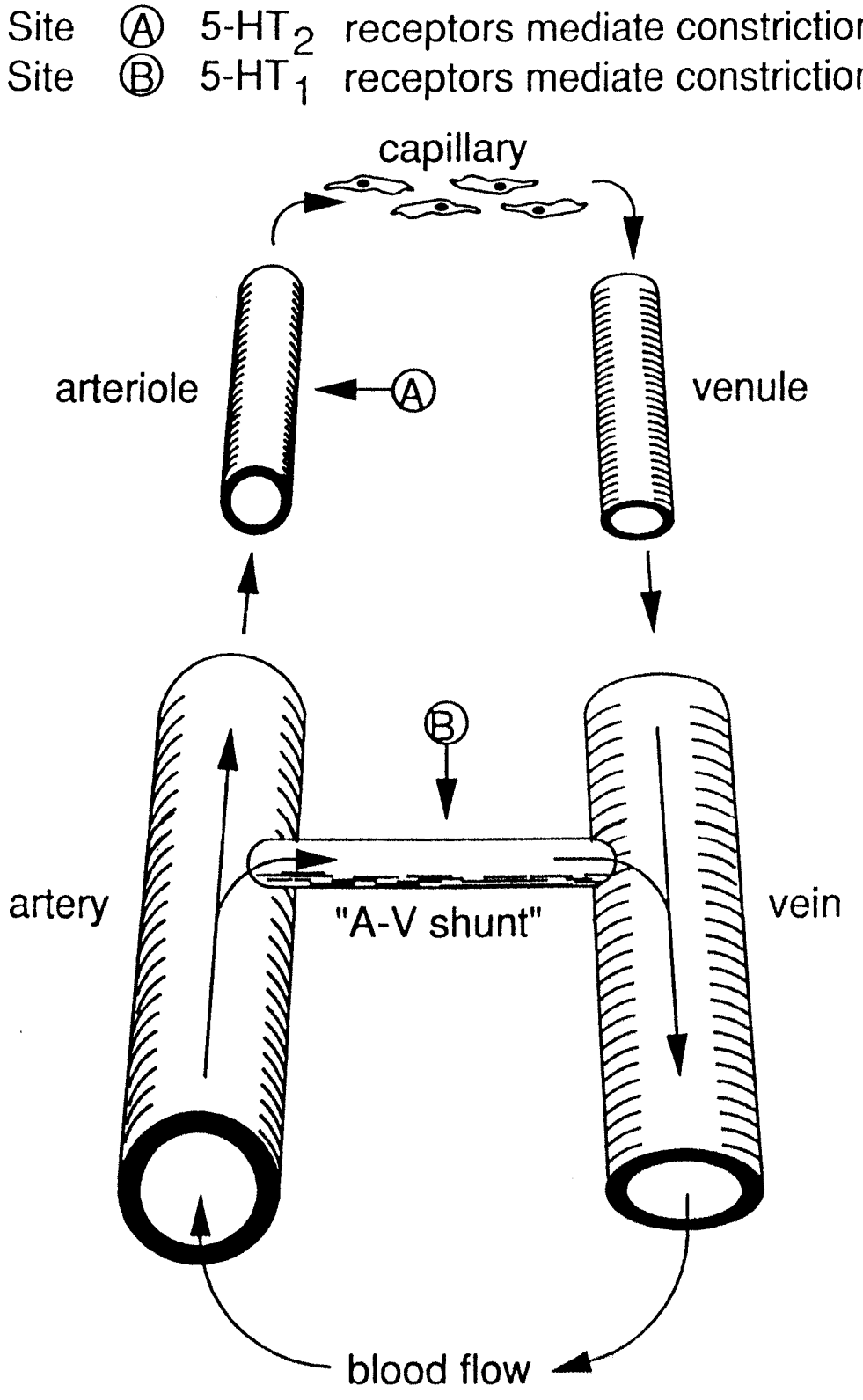


FIG 2.2

A diagram summarising possible serotonergic mechanisms producing the vascular changes in migraine. Site "A" represents the constrictor site responsible for initiation of the attack. Site "B" is situated on the putative arterio-venous (A-V) shunt, and is the site of action postulated for sumatriptan.

If these theoretical assumptions concerning the role of 5-HT in migraine prove to be correct, then the 5-HT_2/5-HT_{1c} antagonists should prevent the primary vasoconstriction, the 5-HT_3 antagonists should prevent the stimulation of painful C fibre afferents and "5-HT_1 like" agonists could reverse the secondary vasodilatation. As one might predict from its effects antagonising the agonist effects of 5-HT on human temporal artery (Jansen *et al* 1991) the 5-$HT_2/_{1c}$ antagonist ICI 169,369 does have some weak activity in the treatment of acute migraine, but the doses used were probably suboptimal and this class of drug should theoretically be more appropriately targetted at prophylaxis (Steiner, 1990; and see later section on pharmacodynamics of ICI 169,369).

Similarly, ICS 205,930 has some limited activity in acute migraine (Ferrari *et al*, 1991). However this drug is a relatively weak 5-HT_3 antagonist (it has almost equipotent 5-HT_4 antagonist properties; Costall and Naylor, 1990) and again may be effective only in the early prodromal phase.Granisetron, a more potent 5-HT_3 antagonist than ICS 205,930 is also only partially effective (Rowat *et al*, 1991), whereas sumatriptan the "5-HT_1 like" agonist does abort established migraine attacks (effective in >70% attacks). Sumatriptan is thought to normalise cerebral blood flow by selectively constricting certain cranial vascular beds (Perrin, 1989; Humphrey, 1990). Although the confirmatory evidence for this mechanism in man is indirect (Iversen *et al*, 1990), sumatriptan constricts porcine cranial arteriovenous anastamoses by an agonist action at the "5-HT_1 like" receptor, which could be a factor in the relief of symptoms during acute attacks (den Boer *et al*, 1991).

Very recently a controversy has arisen, following the unexpected finding that ergotamine and dihydroergotamine are the most potent agonists of

5-HT_{1c} receptors described thus far (Brown *et al*, 1991). This and doubts about the specificity of the MCPP headache (Spierings 1991) has led to the suggestion that 5-$HT1_c$ receptors are not involved in the initiation of a migraine attack. However, Fozard (1991) has strongly refuted this argument, claiming that the evidence still supports the concept that 1_d activation is responsible for terminating an attack.

Other clinical reports with sumatriptan (Dahlof *et al*, 1992) suggest that for a substantial number of subjects (up to 30%) treatment of the acute attack is followed by a recurrence which in some cases is worse than the original attack. The authors consider this to be related to the relatively short elimination half-life for sumatriptan (about 2 hours). Another possible explanation for this recurrence could be related to a lack of specificity for the 5-HT_{1c}/5-HT_{1d} receptor, so that as the plasma level falls then 1_c agonism begins to predominate leading to a recurrence of the original attack. Only carefully designed clinical trials will determine whether this hypothesis is correct.If this does prove to be the case,then prophylaxis with a 5-$HT_2/1_c$ antagonist such as ICI 170,809 could be used in combination to prevent recurrence. Alternatively, development of a more selective 5-$HT1_d$ agonist may be an improvement on sumatriptan.

2.8 THERAPEUTIC POTENTIAL IN CARCINOID SYNDROME

Carcinoid tumours arising from the enterochromaffin cells of the gut produce a number of different mediators such as 5-HT, kallikrein, tachykinins, substance P and other neuropeptides, prostaglandins and catecholamines. These are released into the systemic circulation producing distressing symptoms such as flushing, diarrhoea and sometimes wheezing and right heart failure. A conservative pharmacological approach to treatment includes 5-HT_2 antagonists. Cyproheptadine helps with the diarrhoea, ketanserin with flushing. Unfortunately because of the diverse nature of the mediators released no one agent is effective (Hodgson 1988).

In conclusion, the last decade has seen an almost exponential increase in publications concerning our understanding of the pathophysiological role of 5-HT, resulting from the discovery of a variety of selective agonists and antagonistsm (Personal communication; Dr. Steven Peroutka). However, it is somewhat disappointing that ketanserin the first of the 5-HT_2 antagonists has not been more successful. It has really only a limited therapeutic potential in hypertension and peripheral vascular disease (Breckenridge, 1988). The development of 5-HT_2 antagonists for CNS indications is even more problematic, where the therapeutic endpoints are more difficult to define.

2.9 SUMMARY

Clearly, in choosing the appropriate dose for use in carefully controlled phase III clinical trials, the ability to monitor the therapeutic endpoint coupled with 5-HT_2 mediated pharmacodynamic effects, together with any related pharmacokinetic parameters would greatly facilitate this process. It was the aim of this thesis to address these issues with regard to the 5-HT_2 antagonists in healthy subjects. Further prospective analysis of clinical trials, undertaken with doses chosen from these phase I

studies, should then enable the design of a rational development programme for future 5-HT_2 antagonists.

CHAPTER THREE

3.1 METHODOLOGY

Human pupillary responses are under control of both divisions of the autonomic nervous system (Lowenstein and Loewenfeld, 1958). Contraction of the radial (dilator) muscle of the iris is brought about by sympathetic nerve impulses, whereas contraction of the sphincter (constrictor) muscle is mediated by parasympathetic impulses (Smith 1988). Resting pupil diameter reflects a balance between the two opposing forces. Assessment of these responses can reflect activity of the autonomic nervous system in man. The latency and amplitude of the miotic response to light is determined primarily by the parasympathetic reflex arc, which is the most robust and repeatable of the pupillary measurements (Smith *et al* 1988). Assessment of the sympathetic response is less precise, and is defined as the 75% recovery time of the pupillary response to light stimuli (Bakes *et al* 1990). This is determined partly by cessation of parasympathetic reflex activity, and partly by active redilatation brought about by the sympathetic innervation of the radial muscle (Smith, 1988)

Measurement of pupillary dynamics is also an established method of assessing the effects of drugs on autonomic function and has been successfully used to monitor the actions of antidepressant (Longmore *et al*; 1988) and antipsychotic (Szabadi *et al* 1980 and 1988) compounds in the past. Recently this approach has also been extended for more other novel drugs such as the alpha-2-adrenoceptor drugs clonidine, yohimbine (Morley *et al* 1991) efaroxan (Clifford *et al* , 1989), and for the mu opiate receptor antagonist fentanyl (Trew *et al* 1989). This has introduced the concept that receptors other than those dependent on the classical autonomic system may be operating. This is particularly pertinent for serotonergic drugs, where the effects of fenfluramine(Kramer and Turner, 1973) and flovoxamine (Wilson *et al* 1986) a 5-HT releasing agent and 5-HT reuptake inhibitor

respectively); and for mianserin and trazodone (antidepressants with activity at 5-HT_2 receptors) since their effects cannot be explained in terms of classical autonomic pharmacology (Shur *et al* 1983: Longmore *et al*, 1988).

The methods used have been either photographic (Smith and Dewhirst, 1986), crude subjective pupil rulers (Larson 1978) or complex, expensive non-portable infrared television pupillometers (Bakes *et al*, 1990). The latter allows assessment not only of static pupil diameter but also dynamic pupil response curves.

Recently, a compact portable hand held infrared pupillometer "Pupilscan" has become available, which has the potential to monitor changes in static and dynamic pupil response curves. It is less complex and expensive than the hitherto available television pupillometers. Such a system would be useful in the development of new drugs when testing novel concepts, and when trying to determine if they affect the pupillary reponses and hence the autonomic nervous system.

However, before "Pupilscan" can be used with confidence to determine the effects of new and unknown drugs on the pupil, validation of the system is required. This was accomplished by measuring the ocular effects of topically applied miotic and mydriatic pharmacological agents. This type of study also allowed the assessment of method sensitivity, together with some measure of variability both within subject, and between different observers.

The following strategy was employed:

Firstly a validation study was carried out with the polaroid photographic method of Smith and Dewhirst (1986). This was a pilot study using 6 volunteers receiving no drug treatment. It set out to establish that repeated measurements, using a flash photograph, at intervals of one hour would not affect subsequent measurements using portable "Pupilscan" III.

Secondly, a comparison was made between results obtained with portable "Pupilscan "III and polaroid photography, when used to measure the effects of miotic and mydriatic drugs.

Thirdly, the results obtained with the more sophisticated "Pupilscan" PC with pupil image display and artefact rejection were contrasted with the portable "Pupilscan" III.

3.2 STATISTICAL METHODS

These were applied consistently across the three experimental paradigms described.

Results are expressed as the mean with 95% confidence interval. Most of the statistical analyses were performed using Statsgraphics (see appendix 3.0), where standard analysis of variance methods and regression analysis were used. When an analysis of variance and variance ratio test based on the "F" distribution (Bishop, 1966) indicated significant treatment by time interactions, the nature of these differences were determined by using Student's t-test on the differences in the group means (Fischer and Yates, 1963). Some assessment of within and between observer error were made. The between observer standard deviation (SD) is the sum of all the differences in measurement between the two observers divided by the square root of the two mean values. This can be expressed as the percentage of the overall mean of the two observers to give the coefficient of variation.

3.2.1 Pupil Diameter measurements using the Polaroid method.

This was the method described in detail by Smith and Dewhirst (1976). Repeated measurements of pupil diameter were made directly from a polaroid flash photograph taken in complete darkness, following a 15 min period of dark adaptation. Six volunteers agreed to have flash photographs taken of their right eye at 30 minute intervals for 2 hours. The only deviation from the original method by Smith and Dewhirst, was that a small strip of metric graph paper (10mm x 5mm) was attached to the infra orbital ridge to allow verification of the magnification factor (approximately 2.5), when measuring the photograph with calipers and steel ruler. Fig 3A shows an example of a pupil photograph showing measurement points > and < spanning the diameter of the pupil.

FIG 3A.

A polaroid photograph of the right eye taken with the "Polaroid pupillometer".The margins of the iris (▶) and horizontal pupil diameter are demarcated (▷) to illustrate how measurements were taken. Magnification factor =2.5.

Results from this pilot study confirmed that repeat measurements were within 0.5mm and remained constant over a two hour period (see fig 3.1). Thus, the conclusion was that independent Polaroid flash photographs could be made at 30 min intervals without producing significant changes in pupil diameter.

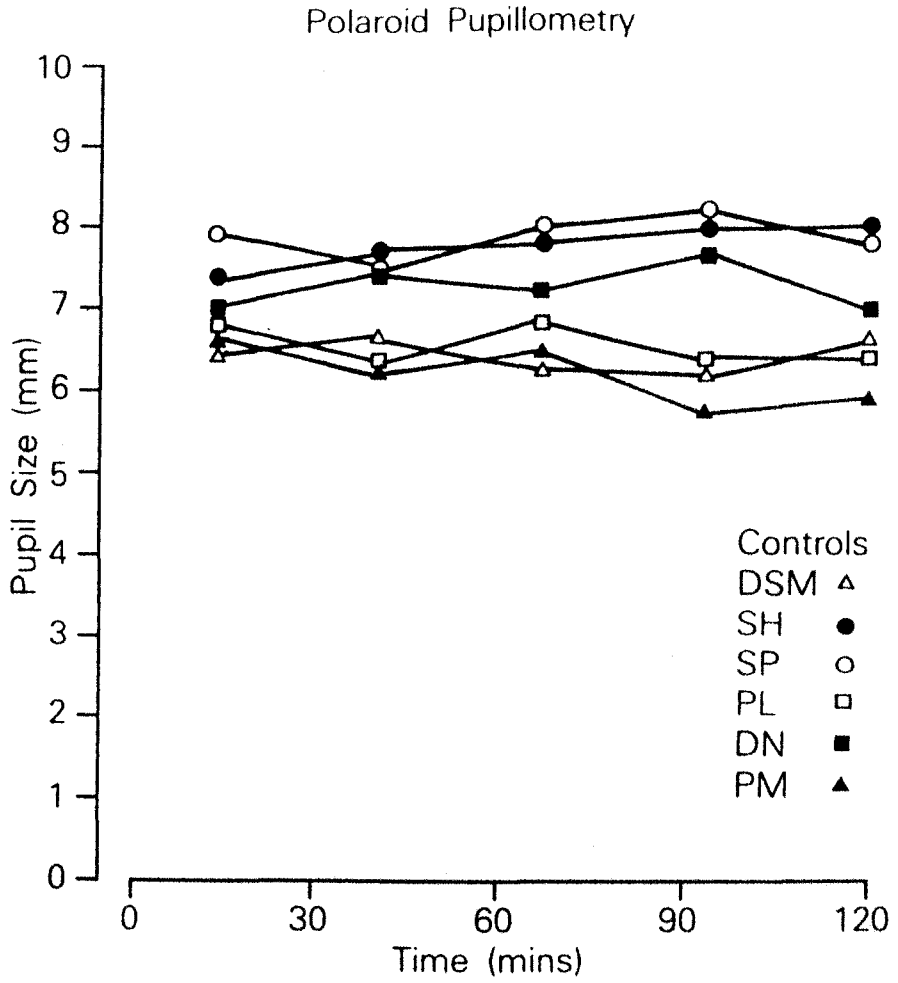


Fig 3.1

Measurements of horizontal dark-adapted pupil diameter using a "polaroid pupillometer" in six healthy subjects at regular intervals over a 2 hour period.

3.2.2. **The determination of drug induced pupillary changes using Pupilscan III (a portable infrared pupillometer) and the polaroid photographic method.**

Six healthy male volunteers with normal "corrected vision" and no previous ocular problems were randomised to receive one drop of either placebo (normal saline), a miotic agent (thymoxamine HCl 0.1%) or mydriatic agent (Tropicamide 0.5%) to the right eye, administered by a nurse unconnected with the study. Doses were administered double blind following a three way cross over design, separated by one week. All subjects gave informed consent to take part and the study was approved by the ICI research ethics committee.

3.2.3 **Assessment of pupillary responses using "Pupilscan III"**

Following a period of 15 minutes dark adaptation wearing dark goggles (BS.6795 GW), subjects were transferred to a darkened room and lay supine on a couch whilst staring at a cross on the ceiling. Prior to making any measurements the microprocessor unit was programmed for data acquisition using the keyboard input. Date, time, subject identification, impulse duration (0.5 seconds), stimulation intensity (565nm, 65 CD/sq metre) and measurent cycle duration (3 seconds) were selected for each subject. These stimulation parameter values were chosen empirically based on published literature values, and which in preliminary trials without drug instillation gave repeatable data.

The hand held optical unit (Fig 3B) was held a fixed distance from the eye utilising a padded cheek rest. A low intensity viewing light (6.5CD/sq metre) was activated using a trigger switch, cross wires were used to focus the centre of the unit on the pupil. Infrared illumination was adjusted

Dimensions	L 190 mm 7½"	W 50 mm 2"	H 32 mm 1¼"
Weight	285 gm./10 oz.		
Image Sensor	65K rectangular pixel array.		
Eye Illumination	(1) Yellow diode (1) 583 nm peak wavelength. Typical intensity 1 foot-candle.		
Image Sensor Illumination	(2) Infra-red emitting diodes (4) 880 nm peak wavelength. Intensity adjusted automatically by software in range 1.5 mw/cm^2 to 6.5 mw/cm^2.		
Stimulus Pulse	(3) High intensity green diodes (2) 565 nm peak wavelength. Intensity selectable in 3 steps in range 3 to 13 foot-candles. Pulse duration programmable in 0.1 second steps from 0 (no pulse) to 10 seconds. Reprogrammable in MEASURE mode.		
Alignment Aid	(4) Red diodes (2) on handle centerline indicate direction to move to center instrument on pupil.		

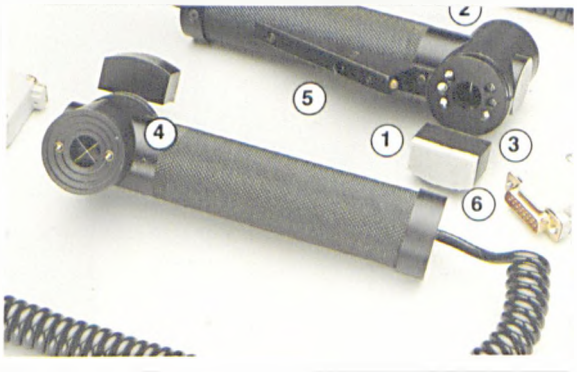


FIG 3B.

A photograph illustrating the placement of the "Pupilscan" optical unit on the cheek of a volunteer.The crosswires were used to centre on the illuminated pupil with the aid of the captured infra-red display on the television screen. Also shown are the technical specifications for the optical unit,together with a listing and display of the component parts.

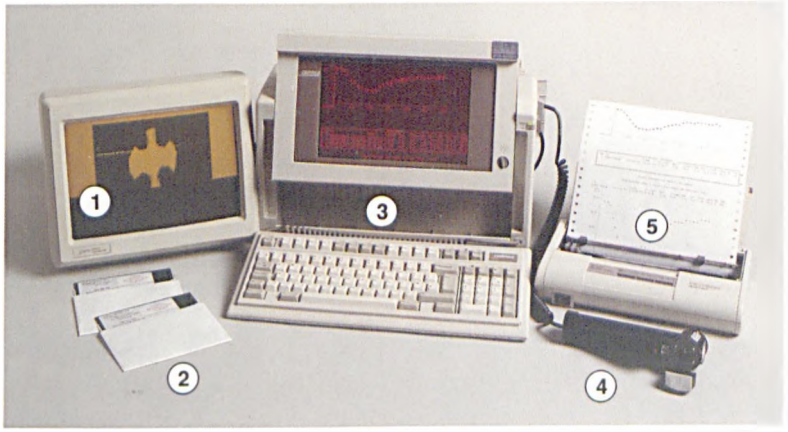
automatically. Subjects were instructed not to blink, following which the light stimulus was activated by releasing the trigger switch to commence the measurement cycle. The solid state image sensor within the optical unit scanned the vertical pupil diameter at 10 times per second producing a pixel count (19 pixels =1mm) versus time plot for analysis by the microprocessor.

At the end of the 3 second measurement cycle the printer and liquid crystal display gave a summary display of the response curve(see figs 3C and 3D). Resting pupil diameter (RPD), minimum pupil diameter (MPD) and recovered final pupil diameter (FPD) together with time to minimum diameter (seconds to MPD) were expressed (in mm). The pixel count every 10 milliseconds was printed alongside the summary values. Artifact rejection was employed: 1) if three consecutive pixel counts were missing; 2) if an error message flagged up on the screen; or 3) if the subject was noted to blink or initiate eye movement during the measurement cycle.

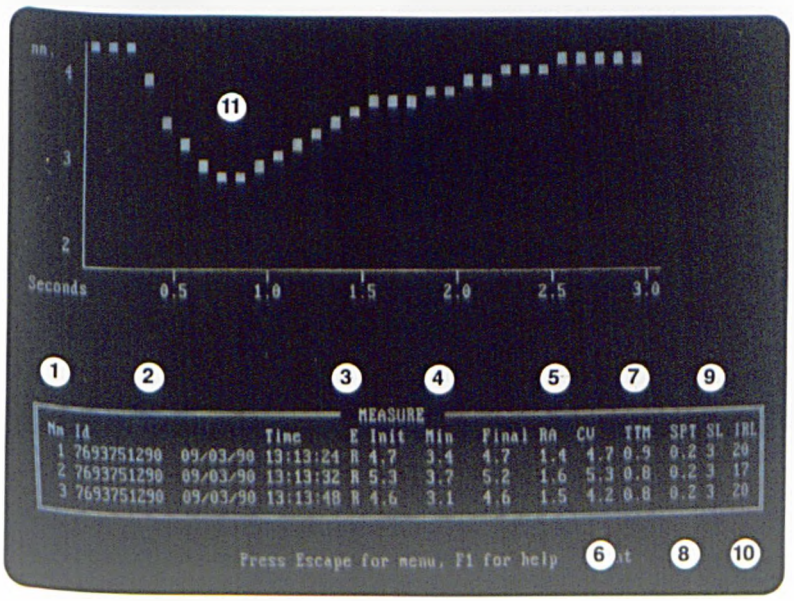
Five artifact free responses were recorded at 1 minute intervals. Assessments were made by two independent observers, DSM followed by DW, always in the same sequence and measurements were made before dosing and at 1, 2 and 3 hours after instillation of topical drug.

Polaroid photographs were taken immediately after the second observer had completed her measurement with Pupilscan III. [NB. Technical problems (shutter jamming) with the Polaroid camera precluded a full comparison between the two techniques to be carried out. Data was obtained for all six volunteers, whilst full data was available only on two out of three treatment periods].

COMPLETE SYSTEM



1. Optional auxiliary monitor
2. Program floppy disks (5¼" or 3½")
3. IBM PC or compatible computer
4. PUPILSCAN Optical Unit
5. Printer



MEASURE SCREEN

1. Measurement sequential serial number
2. Subject identification
3. Eye measured
4. Diameters (mm)
5. Reflex amplitude (mm)
6. Maximum constriction velocity (mm/sec)
7. Time to minimum diameter (sec)
8. Stimulus pulse duration (sec)
9. Stimulus intensity
10. Infra-red illumination level
11. Pupil response plot

FIG 3c.

A photograph illustrating the "Pupilscan PC" system, and its component parts, which capture data from the optical unit.Typical data from the measurement screen is also depicted.

3.3 RESULTS

3.3.1 Section A

Results obtained with Pupilscan III (portable pupillometer) and the "Polaroid" photographic method following topically applied mydriatic and miotic drugs.

This section is divided into two parts (I) **effects of drugs on pupillary responses** and (II) **variability and sensitivity of the technique.**

SECTION I.- Effects of drugs on pupillary responses.

3.3.2 Effects of thymoxamine and tropicamide compared with placebo.

A. **Resting pupil diameter** (RPD). Effects of a topical miotic and mydriatic on RPD (mm+/-95%CI) applied to the right eye are shown in Fig 3.2.

Predose estimates of Resting pupil diameter were not different between treatments and were not affected by placebo ($p>0.5$) at (predose) To, 1.2 and 3 hours after dosing with mean RPD values of 5.85, 5.50, 5.65 and 5.27mm respectively.

Thymoxamine significantly reduced RPD compared with placebo ($P<0.05$) at 1 and 2 hours after dosing (3.78 and 4.30mm) and compared with predose values at all time points ($p<0.001$). Tropicamide increased RPD compared with placebo ($p<0.001$) and predose

values at 1, 2 and 3 hours after dosing with RPD values of 8.59, 8.30 and 7.84mm respectively.

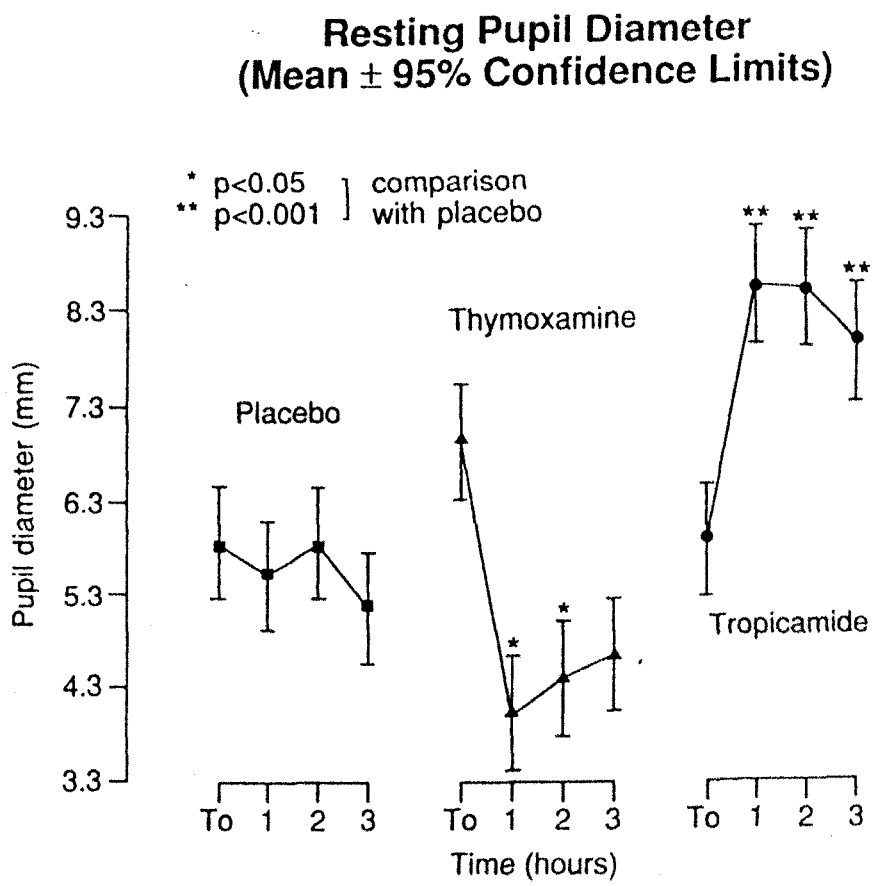


Fig 3.2

Effects of thymoxamine (▲) and tropicamide (●) compared with placebo (■) on resting pupil diameter following instillation into the right eye at predose (to) and at 1, 2 and 3 hours post dose.

B. **Light constricted pupil diameter** (MPD). Results are depicted in Fig 3.3.

A small but non-significant decrease in MPD was observed with both placebo (2.80 and 2.20mm) and thymoxamine (2.35 and 2.45mm) at 1 and 2 hours. This decrease returned to baseline values with placebo but was still present (2.35mm) at 3 hours after treatment with thymoxamine.

Tropicamide produced a marked increase in MPD to greater than 7mm, and was of similar magnitude for both RPD and FPD. This mydriasis persisted beyond 3 hours after dosing ($p<0.001$) compared with placebo.

Predose values for MPD were not significantly different from each other for any of the treatments ($p>0.5$).

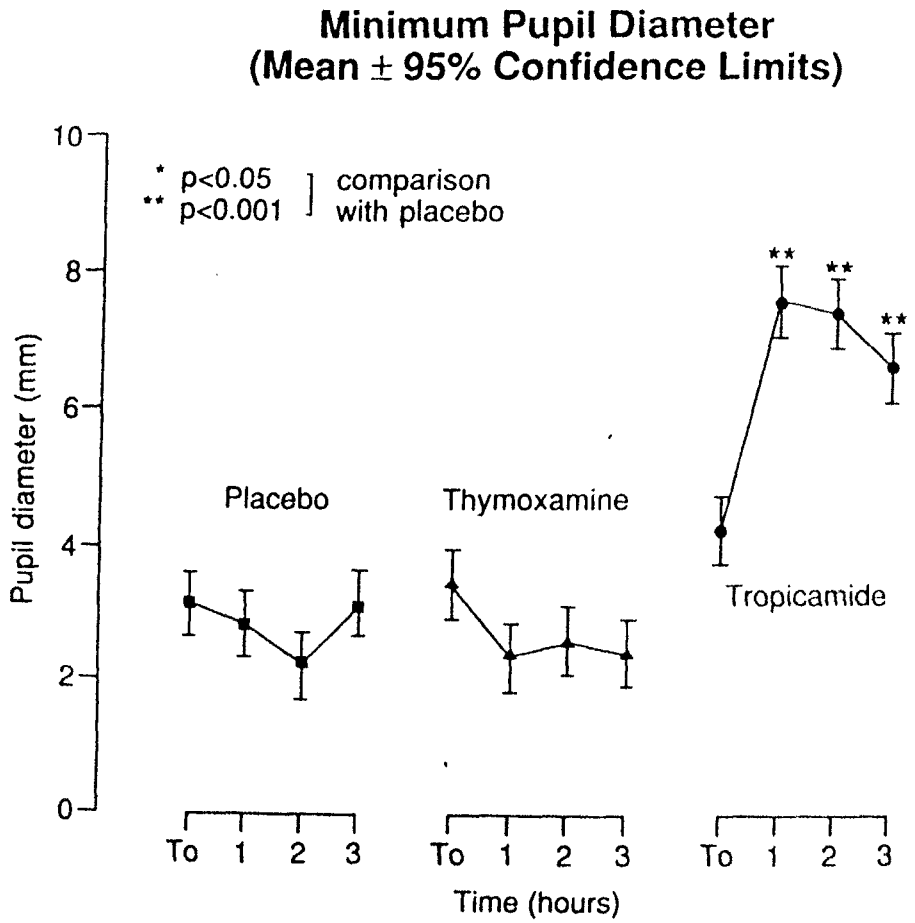


FIG 3.3

Effects of thymoxamine (▲), tropicamide (●) and placebo (■) instilled into the right eye on the minimum pupil diameter after constriction to a 0.5 second (365nm; 65cd/m-2) light stimulus at predose (to) and at 1, 2 and 3 hours after dosing.

C. **Final recovered pupil diameter** (FPD). Results are depicted for FPD in Fig 3.4.

The effects of thymoxamine and tropicamide were similar to those found on RPD. Thymoxamine significantly reduced FPD to 3.95 and 4.10mm compared with placebo values ($p<0.05$) at 1 and 2 hours of 4.90 and 5.45mm respectively and compared with all predose ($p<0.001$) values. Tropicamide increased FPD compared with placebo values of 4.90, 5.45 and 4.93mm ($p<0.001$) at 1, 2 and 3 hours after dosing to 8.40, 8.85 and 7.81mm respectively.

The changes in light induced reflex amplitude (MPD expressed as a percentage of the resting pupil diameter) for thymoxamine and tropicamide compared with placebo are shown in Fig 3.5. Reflex amplitude was not significantly affected by thymoxamine (an alpha adrenoceptor antagonist), reflecting the minor sympathetic component of pupillary constriction, whereas, tropicamide (a muscarinic receptor antagonist) produced a marked reduction in reflex amplitude (10% compared with between 40 and 50% for placebo at 1 hour after dosing). Recovery of reflex amplitude was incomplete even at 3 hours after tropicamide, in keeping with the persistent mydriasis observed.

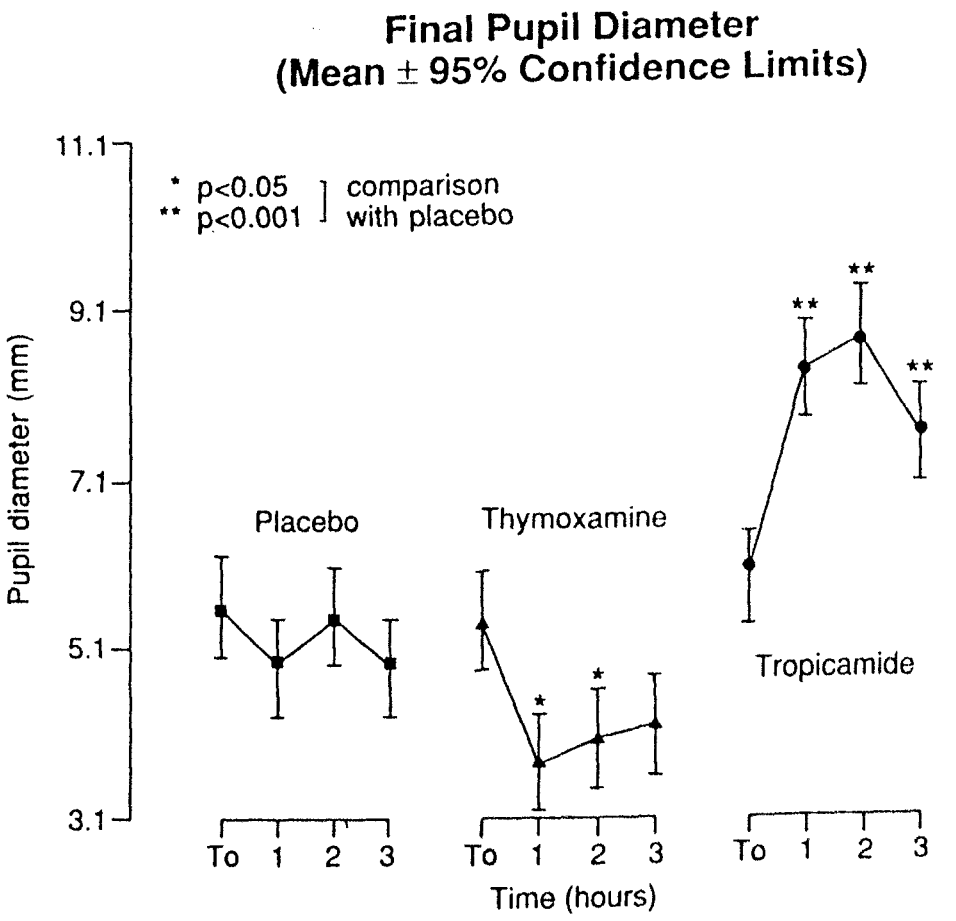


FIG 3.4

Effects of thymoxamine (▲), tropicamide (●) and placebo (■) instilled into the right eye on final recovered pupil diameter following a light stimulus.

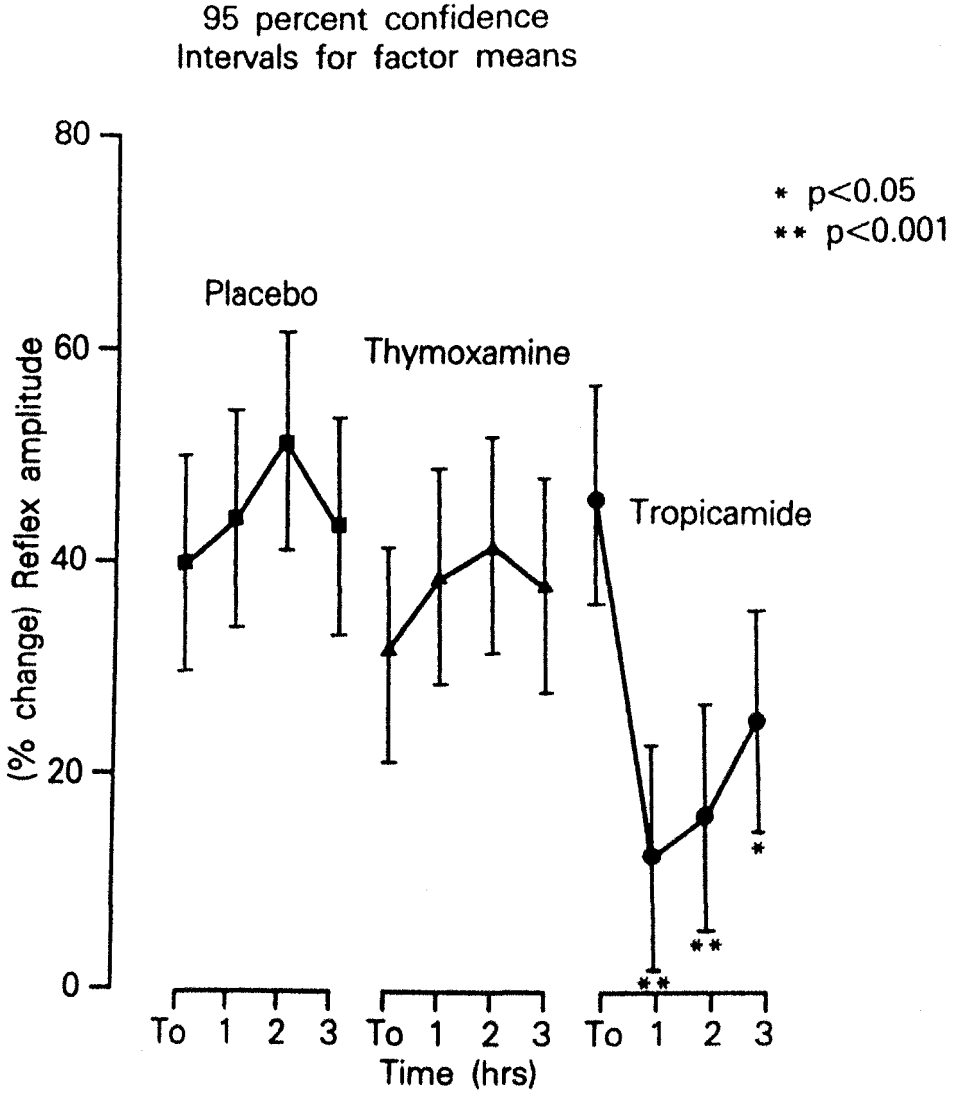


FIG 3.5.

Effects of thymoxamine (▲),tropicamide (●) and placebo (■) on changes in light induced reflex amplitude (mpd expressed as a percentage of resting pupil diameter) at predose (to) and at 1, 2 and 3 hours after dosing. values are calculated for changes in the right eye.

SECTION II- Variability and sensitivity of the technique

3.3.3 Within and between observer variability.

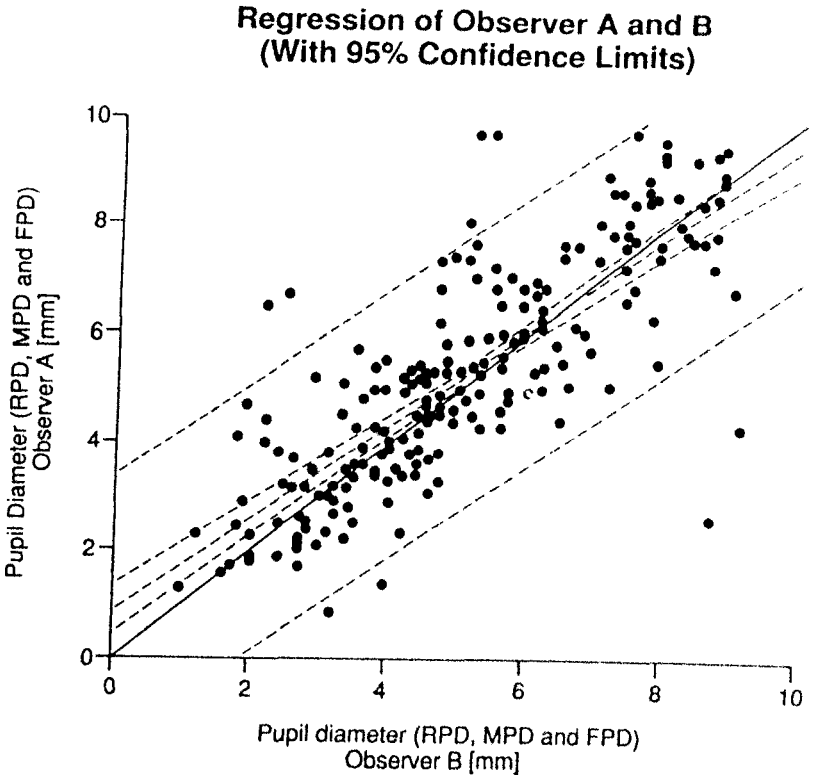
The mean coefficients of variation (CV%) for measurement of RPD, MPD and FPD within an observer (mean of observer A and observer B) across all time points calculated from the multifactor ANOVA were as follows:

	RPD	MPD	FPD
CV%	11.9	15.1	12.0

similarly variability between observers was calculated:-

	RPD	MPD	FPD
CV%	11.9	16.5	11.5

A regression (Fig 3.6) of all observer (N=216) estimates (RPD, MPD and FPD) for observer A versus observer B showed a significant ($p<0.001$) overall linear regression ($r=0.7997$; slope=0.863) and intercept of 0.89 although not significantly different from unity). Eight outlier points were identified for values outside the 95% confidence intervals.



Coefficient of variation (%) (multifactor anova)	RPD	MPD	FPD
Within observer	11.9	15.1	12.0
Between observer	11.9	16.5	11.5

Correlation between observers

(A) and (B) $r = 0.7996$ $p < 0.001$
$n = 216$

Intercept 0.8899 (SEM = 0.241)
Slope 0.863 (SEM = 0.044)

FIG 3.6

A regression plot showing the variability in pupil parameter estimates for two observers (A and B).All observer estimates are depicted (RPD, MPD and FPD) for both observers together with line of identity, regression line and 95% confidence intervals for both the fitted line and parameter estimates.A significant overall linear regression ($p<0.001$; $r=0.7996$; slope=0.863; intercept =0.89)

3.4 COMPARATIVE RESULTS OBTAINED WITH THE POLAROID PUPILLOMETER.

Results obtained with the Polaroid pupillometer gave comparable values for placebo, thymoxamine and tropicamide treated pupils. Both absolute and changes in mean pupil size following drug treatment were of the same magnitude (Fig 3.7). Resting pupil diameter (the only value which can be derived from the static Polaroid print) gave mean placebo values of 6.99, 6.79, 6.39, and 6.43mm at To, 1, 2, and 3 hours after dosing respectively. With thymoxamine the predose value To was 7.05mm (comparable to the placebo values)which fell to mean values of 5.38, 5.64 and 5.80mm at 1, 2 and 3 hours post dosing. For tropicamide again the predose To value (6.53mm) was within the range of placebo values and increased to mean values of 8.59, 8.30 and 7.84mm at 1, 2, and 3 hours after dosing.

Assessment of repeatability showed that measurements with the Polaroid pupillometer had a coefficient of variation of 6.9% for within subject measurements taken during the placebo period. Because of the loss of data with the polaroid camera during certain treatment periods, a formal comparison of the two methods was not possible. Some indication of comparability can be obtained from a simple regression plot (see Fig 3.8.1) and from the Altman and Bland (1983) Plot (see Fig 3.8.2) which shows a reasonable agreement (r=0.715, slope = 0.455) between RPD (derived from Pupilscan III) and the Polaroid RPD. The major difference between the two measurements is the employment of a low intensity viewing light by Pupilscan, whereas the polaroid photographs were taken in darkness. This probably accounts for the systematically larger RPD (between 1.0 and 2.0mm) obtained with the Polaroid prints, although the magnitude of drug induced changes was comparable.

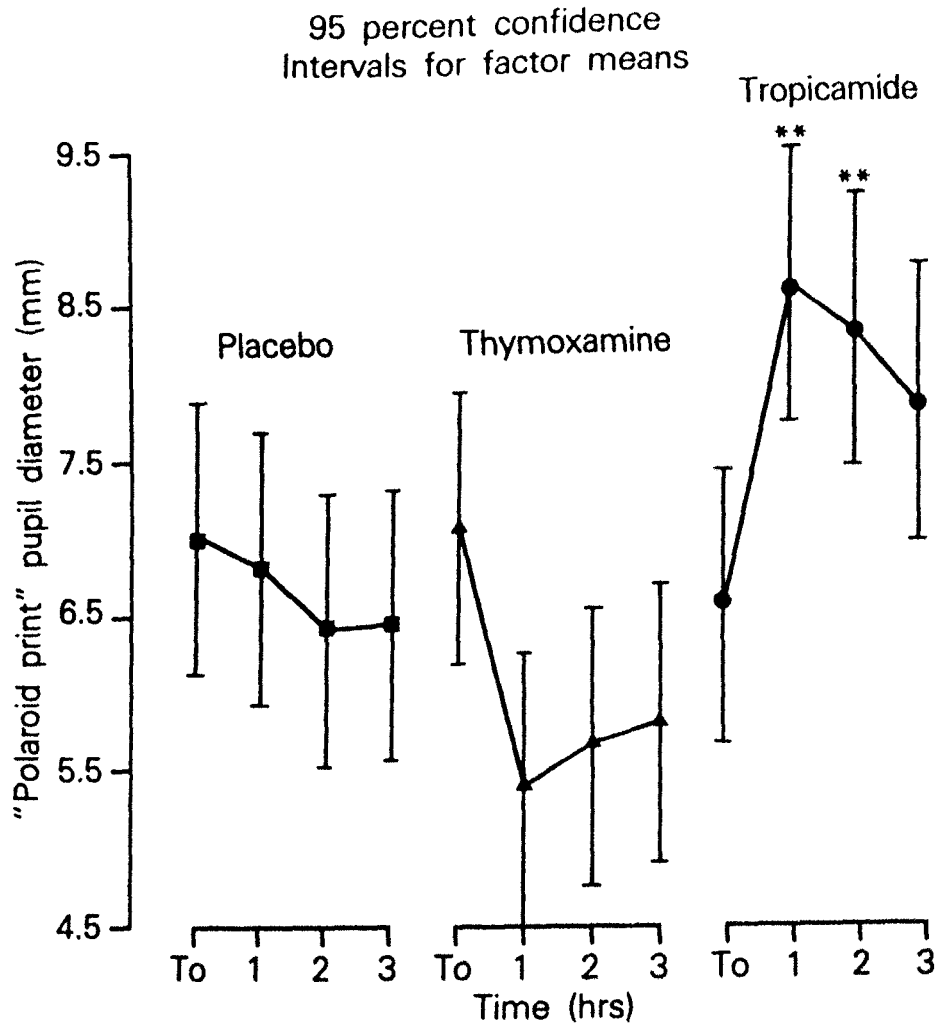


FIG 3.7

Effects of thymoxamine (▲), tropicamide (●) and placebo (■) on dark adapted resting pupil diameter as assessed by "Polaroid pupillometry" at predose (To) and at 1, 2 and 3h postdose.

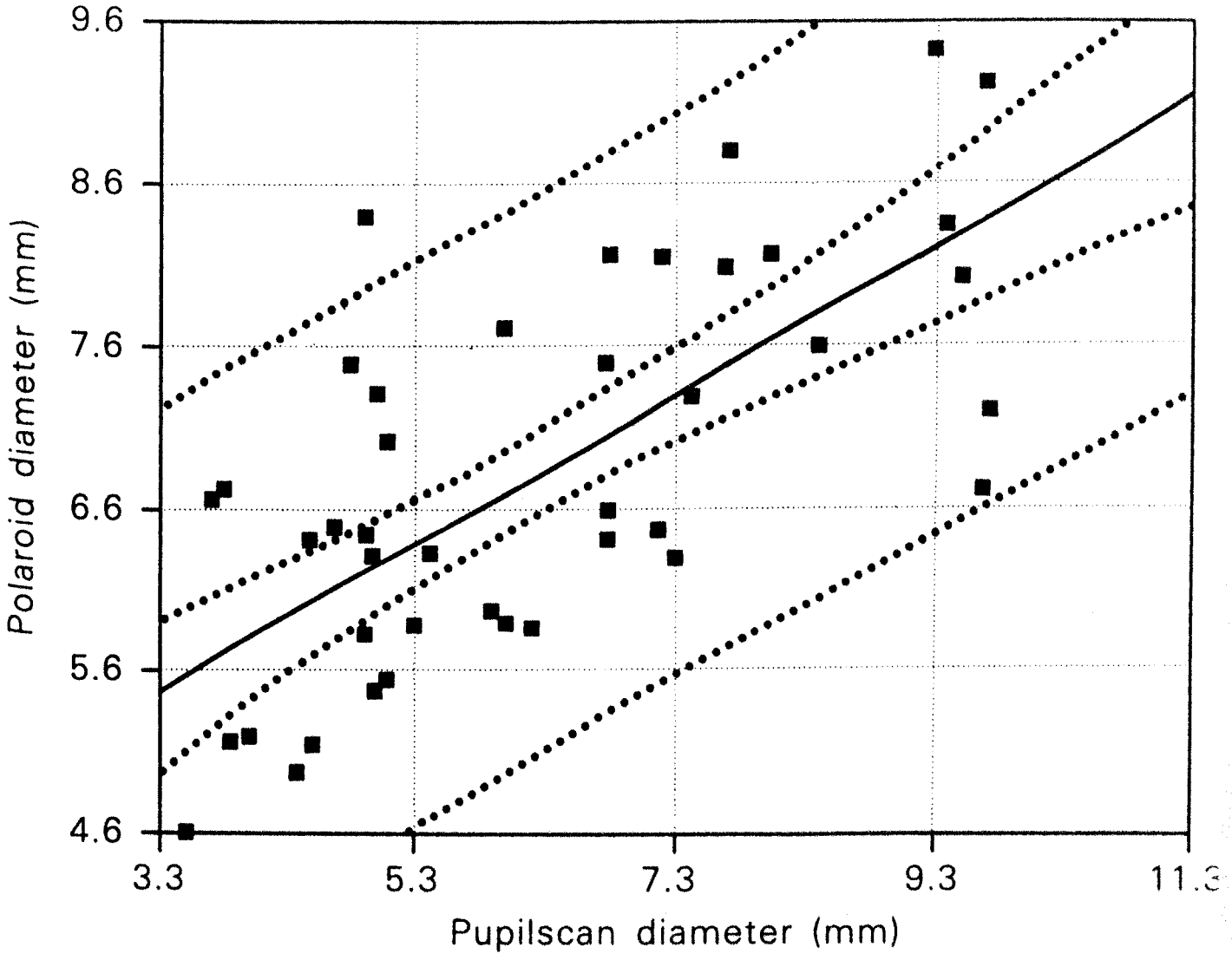


FIG 3.8.1

A regression plot showing the relationship between pupil diameter estimates obtained using the "Polaroid pupillometer" and Pupilscan.A linear model is depicted with the line of best fit ($r=0.715$, slope=0.4556, $p<0.0001$) together with the 95% confidence intervals for the fitted line and parameter estimates.

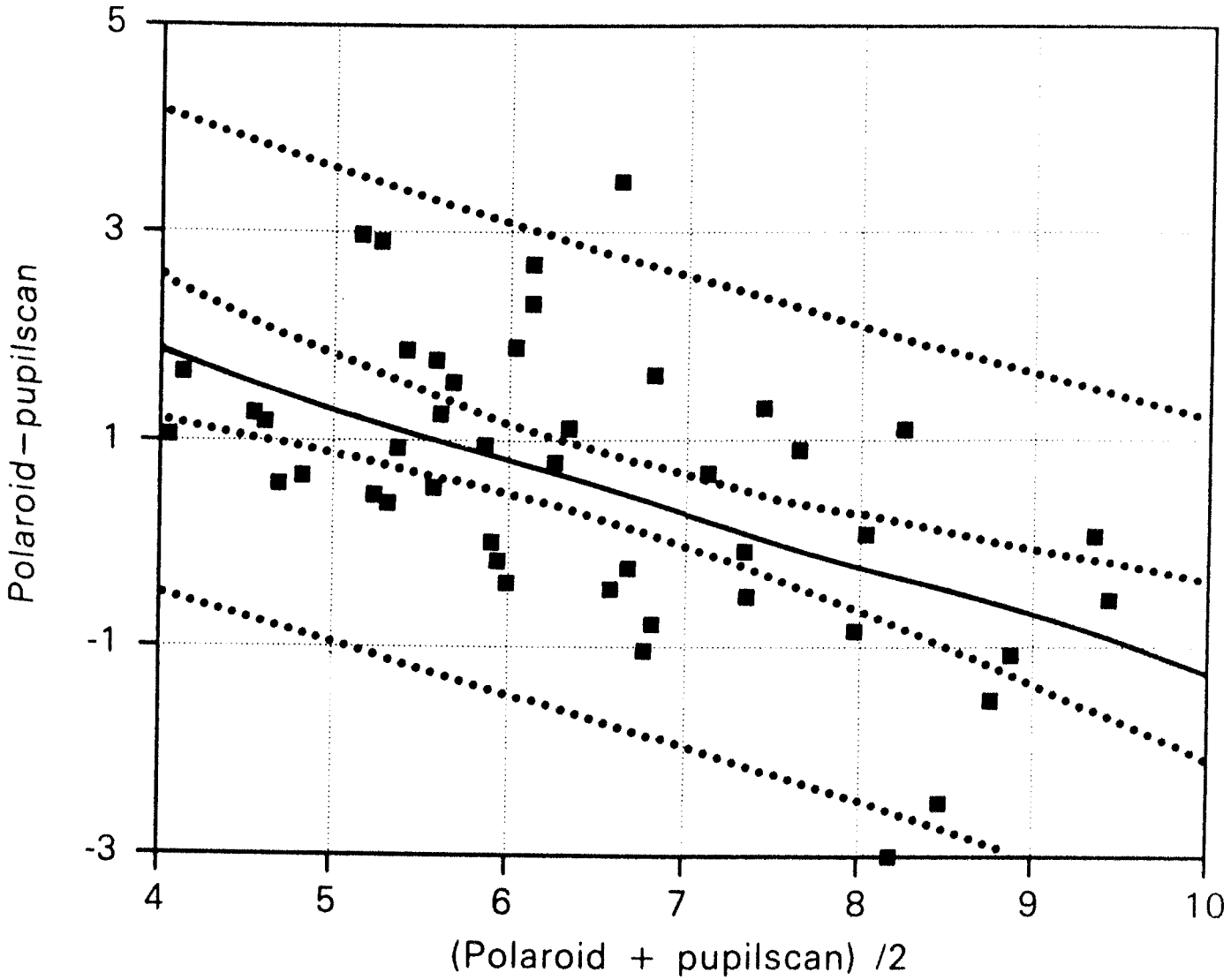


FIG 3.8.2

An Altman and Bland (1983) plot illustrating the within subject variability comparing "Polaroid pupillometry" with Pupilscan measurements. The difference between measurements is plotted against the mean of the two measurements. Visual inspection suggests that the errors are equally distributed across the range of measurements ($r=-0.555$, slope=-0.513, $p<0.0001$).

3.4.1 Data capture with Pupilscan - PC.

Pupil assessments by one observer, (A) were carried out in a separate group of eight volunteers under identical conditions to the studies with Pupilscan III, except that no topical drugs were instilled. Measurements were made at baseline (To) and at intervals of 1.0, 2.0, 4.0, 8.0 and 24 hours (Fig. 3.9). The Pupilscan optical unit was connected to a circuit board within an IBM PC and the real time image of the pupil displayed on a separate visual display unit (VDU).

A plot of pupil diameter (PD) versus time was displayed automatically at the end of each 3 second measurement cycle, following activation of the optical unit trigger. This data was coded with subject identification, date, time and trial number for storage in a Lotus 1-2-3 print file and later analysis. Artefact rejection was applied (1) automatically as a result of an error message, (2)when blink artefacts were noted on the PD versus time plot, or (3) if the subject was noted to blink or eye movement was noted by the observer. A hard copy of results obtained was also generated using the printer attached to the microcomputer.

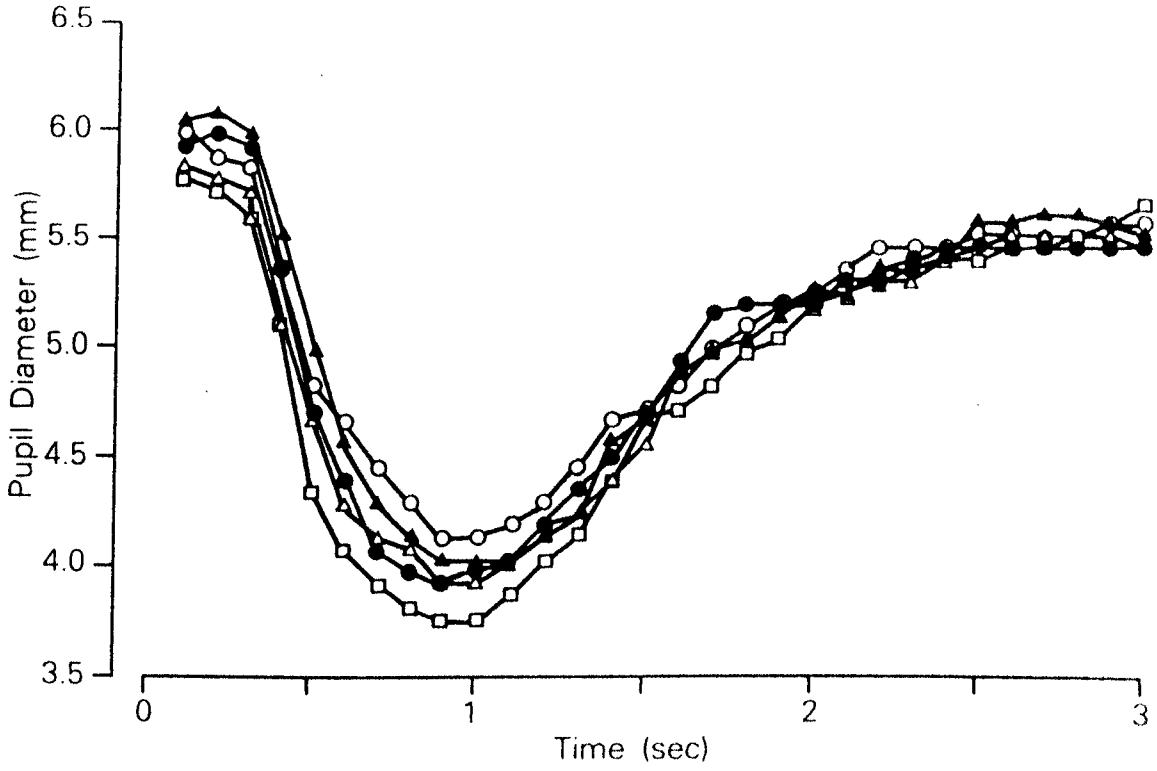


FIG 3.9.

Light response curves performed in an individual dark adapted male volunteer at various times (●1;▲2;○4;△8;□24hr) during a 24 hour period under placebo conditions.comparative mean data in a further five individuals under placebo conditions is described in chapter seven.

3.4.2 Data Analysis using the Pupilscan - PC

A Lotus 1-2-3 template file containing MACRO command (Fairville medical optics INC) was used to import data from the print file, which was saved during the pupil assessments. This allowed several pupil diameter versus time plots to be displayed on the same axis, with the facility for calculation of mean or median plots (Figs 3.9.1 and 3.9.2). Calculation of maximal constriction velocity and acceleration was also displayed using the algorithm forming part of the MACRO (Fig 3.9.5). Before accepting the derived parameters each velocity time plot was scrutinised using the VDU graphical display and only documented velocity measurements occurring in the first one second light constriction phase were accepted for analysis (Fig 3.9.5). Exploratory data analysis was used to construct a "pupil Hysteresis" curve for velocity changes with time. These displayed a characteristic shape but subsequent analysis failed to show any obvious drug effects (3.9.3).

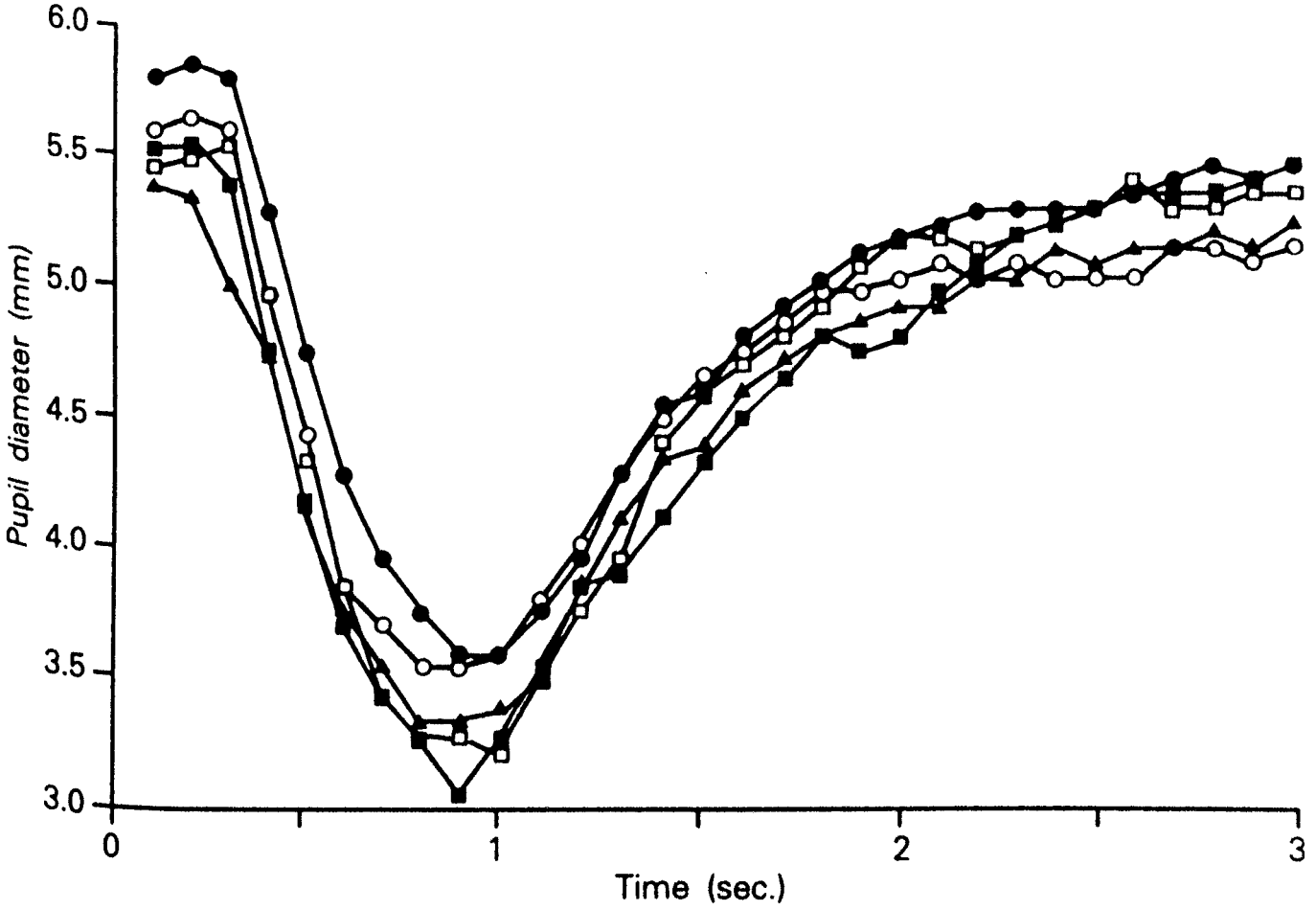


FIG 3.9.1

Shows typical light response curves obtained in a dark adapted pupil to a 0.5s duration stimulus (565nm, 65cd m-2) recorded over a 3s measurement cycle and data captured on to an IBM personal computer using "Pupilscan -pc". Recordings were made at 2 minute intervals (T_0○2;●4;■6;□8;▲ mins).

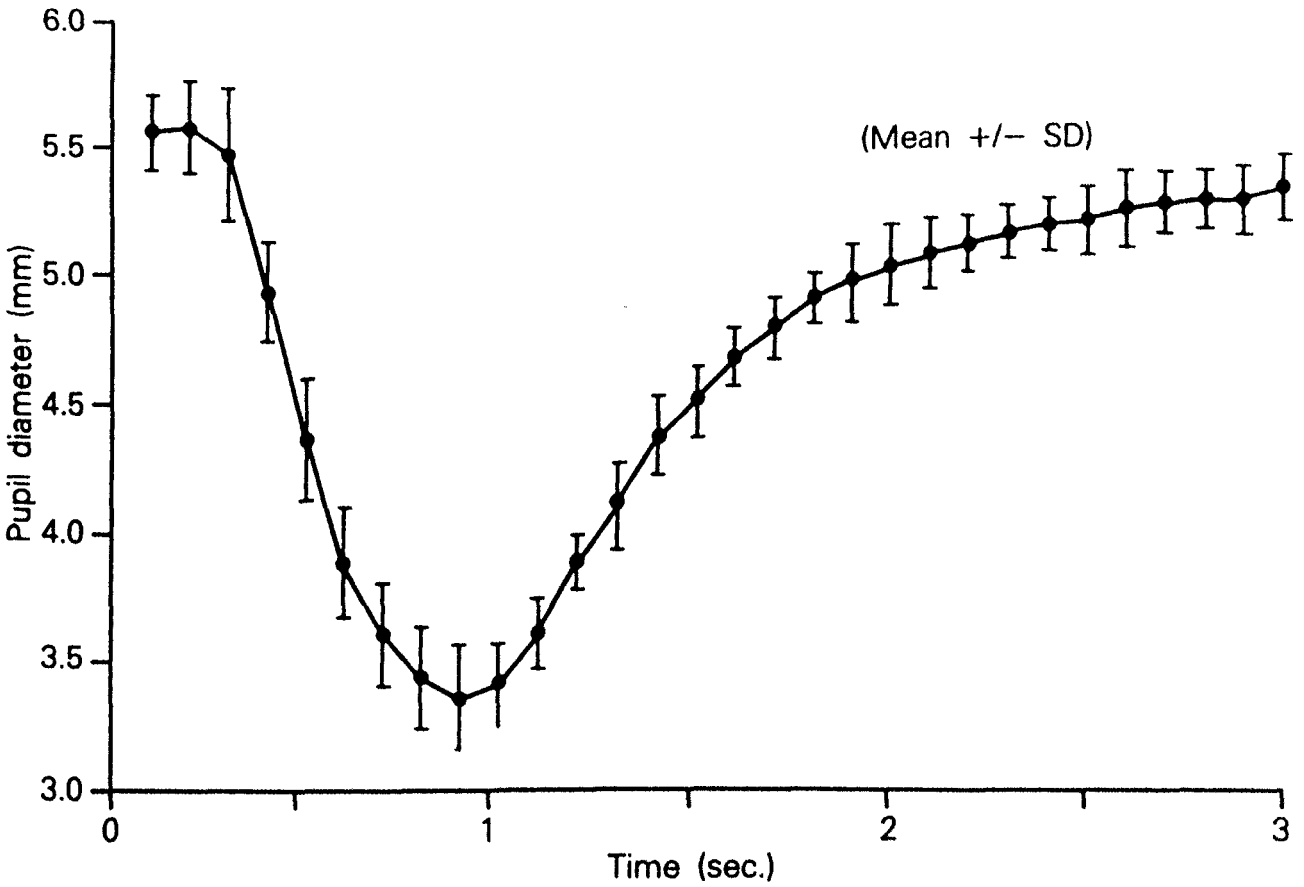


FIG 3.9.2

Shows data from fig 4.7 analysed using "Pupilscan software" and depicting the mean plots (n=5) with standard deviation.

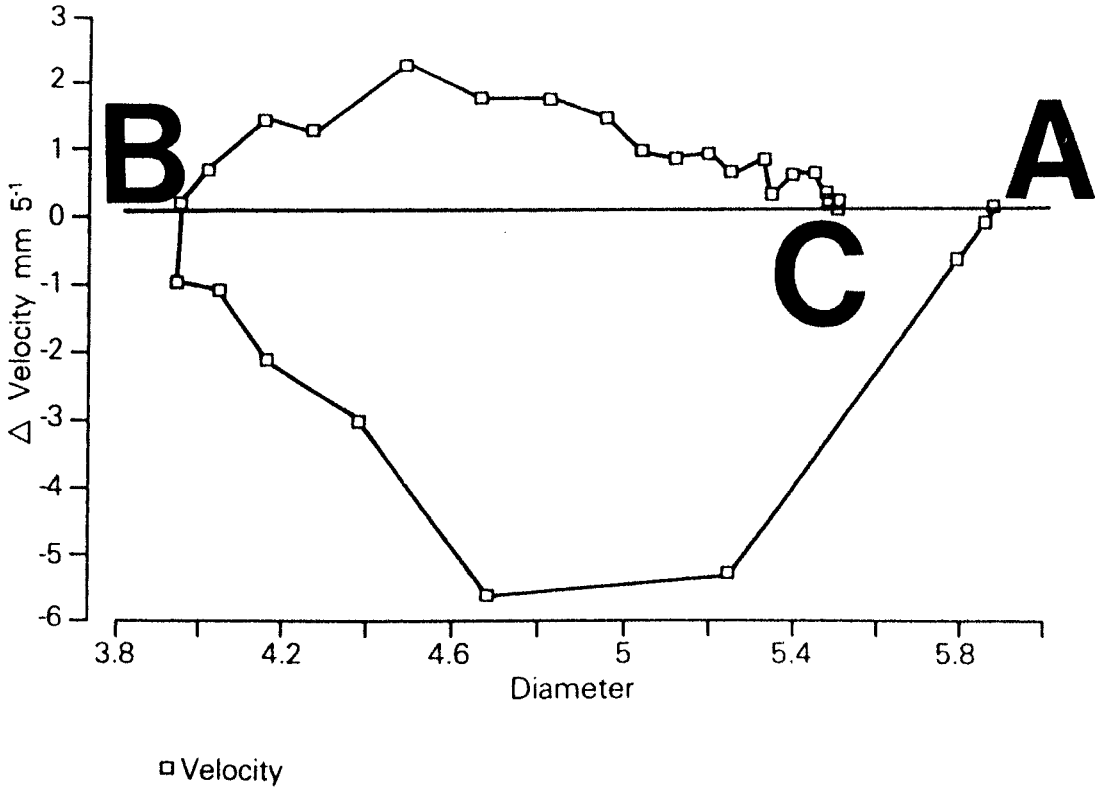


FIG 3.9.3

A hysteresis curve relating changes in the velocity of the pupillary light reflex over a 3 second observation period. A-B represents pupillary constriction and B-C redilatation.

3.4.3 Results obtained over a 24 hour period using Pupilscan -PC

Fig 3.9.1 depicts the baseline results, from a single volunteer with five typical light response curves to a 0.5 s duration stimulus (565nm, 65 cd m-2) recorded over a 3s measurement cycle. Fig 3.9.2 shows how the software can be used to generate mean plots and associated standard deviation.

Fig 3.9 shows the individual results obtained, for light response curves at various times over a 24hr period under placebo conditions in a subject as part of placebo controlled, double blind randomised study (see chapter 7 for mean data and more details).

Manual entry of data to the template ,although time consuming and tedious, is possible, and allows data from the portable Pupilscan III to be analysed. Fig 3.9.4 illustrates the median plots obtained for tropicamide, placebo and thymoxamine treatments. The shape of the plots concurs with the data obtained from Pupilscan III. If the percentage change in reflex amplitude is plotted for the various treatments (Fig 3.5), virtually no change in reflex amplitude compared with baseline values is noted for placebo and thymoxamine, whereas there was a considerable reduction in reflex amplitude for tropicamide consistent with a cycloplegic effect.

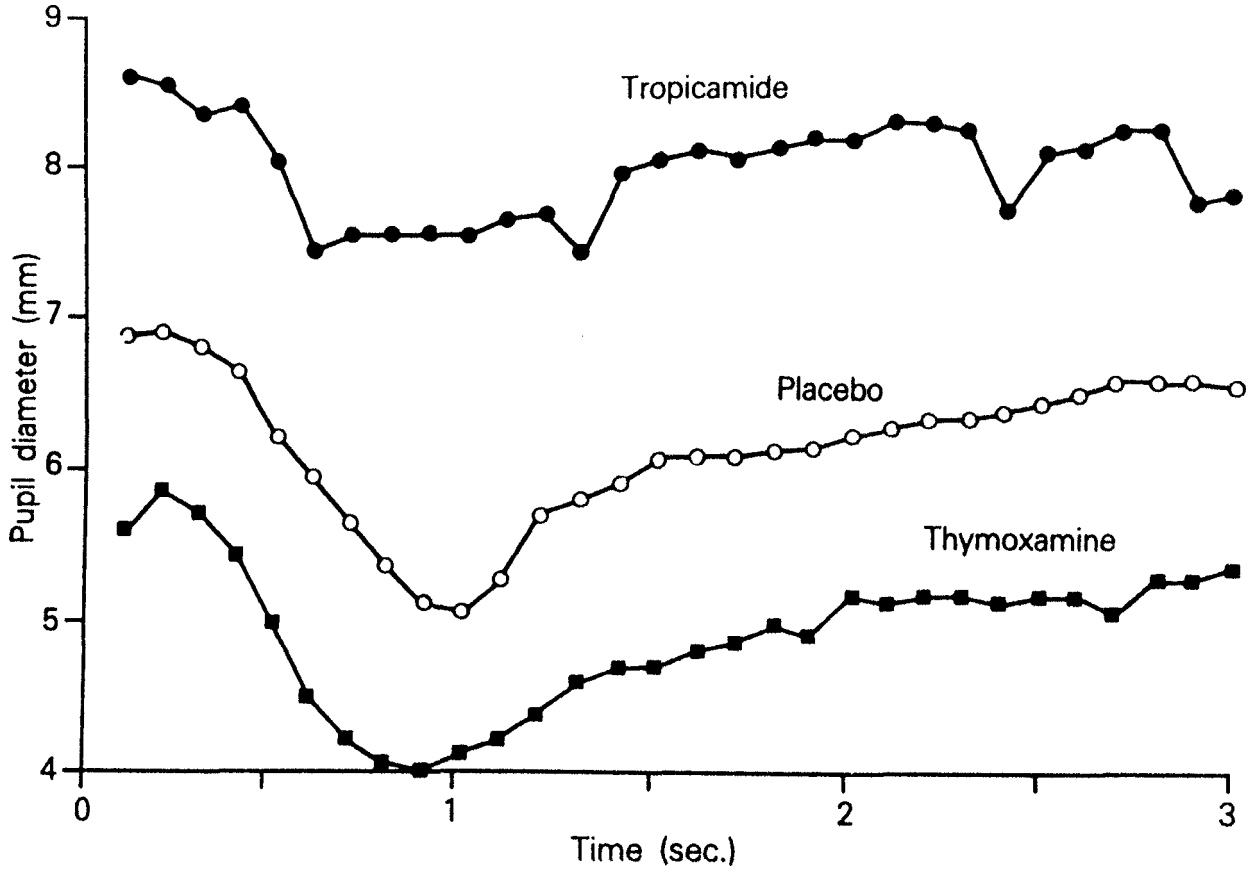


FIG 3.9.4

Median light response curves at 2 hours post dose showing the effects of thymoxamine (■), tropicamide (●) and placebo (○), which were topically applied into the dark adapted right eye in healthy volunteers.

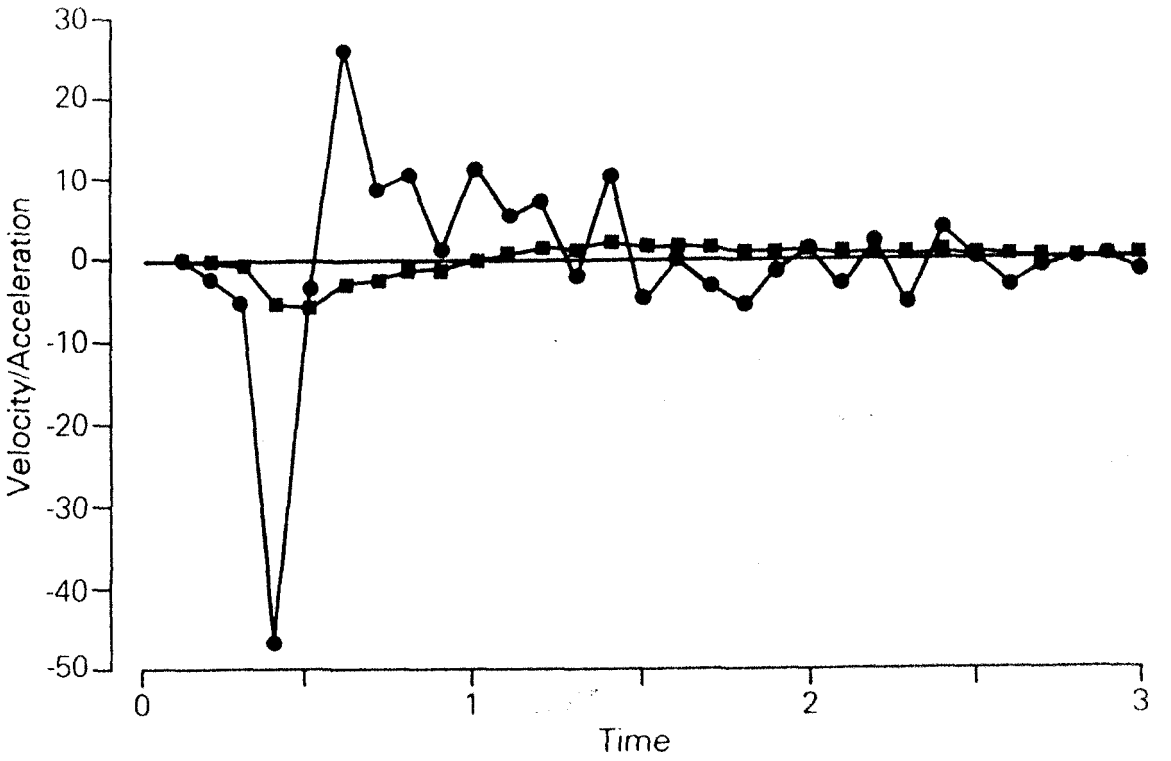


FIG 3.9.5

A plot of changing pupillary constriction velocity (■;mm/s) and acceleration (●;mm/s/s) elicited by the light response during the 3 second measurement cycle using Pupilscan.

3.5 DISCUSSION

The Pupilscan pupillometer has shown itself to be capable of measuring changes in dark adapted resting pupil diameter (RPD) induced by known mydriatic and miotic drugs, together with associated changes in light induced reflex amplitude. The range of resting diameter and the changes in reflex amplitude are consistent with those observed in the literature using infrared television pupillometry and similar light stimulation parameters (Longmore *et al*, 1988; Theofilopoulus *et al*, 1988).

The mydriatic changes induced by 0.5% tropicamide in this study,under dark adapted conditions, of 2mm, are less than the values obtained by Levine *et al* (1983). However, their values were obtained using an entoptic pupillometer with ambient illumination equivalent to direct opthalmoscopy. Which explains why they observed a pupillary miosis following placebo (due to light stimulation), and may have contributed to the large mydriatic effect which they observed. Despite these differences the absolute mydriatic diameters recorded in this study were similar to those of Levine (7-8mm). Pollack *et al* (1981) studied the dose response effect of tropicamide using a photographic method. They found that with tropicamide a stable mydriasis was achieved by 30 minutes, and that this began to diminish by 3 hours with a mydriatic diameter of 8mm, which is in accordance with the results from this study.

The miotic effects of thymoxamine gave a pupillary response of 2mm, which is in agreement with Lee *et al* (1983) using infra red pupillometry. They also noted marked variability in pupillary response to thymoxamine (mean difference from placebo of 1.3mm with a range of 3.0mm) which is comparable to the results from this study (mean difference of 2.28 mm with 95% CI of 3.02 to 1.54mm).

The reflex amplitude of the light constrictor response observed for both the miotic and mydriatic agents were in keeping with their expected pharmacological properties. Thymoxamine had no significant effect on reflex amplitude, since this was a predominantly parasympathetic response; whereas tropicamide markedly reduced it, amounting to a cycloplegic effect. Moreover, the duration of mydriasis and cycloplegia were comparable to those observed by other workers (Pollack *et al* 1981).

An attempt was made to assess both within and between observer differences for measurements of RPD, MPD and FPD. Both RPD and FPD showed comparable repeatability using the portable (Type III) machine, with a coefficient of variability (CV%) of around 12% for within observer and 13 to 15% for between observer estimates. These variability are higher than the 3.2% observed by Smith and Dewhirst (1986) with polaroid photography and IR television pupillometry, but better than those of Lee *et al* (1983) with a calculated CV% of greater than 60% using IR cinematography and intermediate in magnitude (CV% = 9.4%) with the photographic records.

The MPD due to light constriction (or reflex amplitude) demonstrated a higher degree of variability giving a within observer CV% of 19% and between observer estimate of 24.3%. These values compare favourably with those of Longmore *et al* (1988) who quoted a standard error of 11% for light induced constriction, giving a calculated between subject CV% of between 27 and 38%. Other workers, (Smith personal communication) have reported measurement repeatability for reflex amplitude to be much lower (7.0% within occasions and 8.2% between occasions). However, no published data exists defining the criteria used for exclusion of outliers or aberrant data at the different study centres.

No attempt was made to estimate recovery of the pupil reflex after light stimulation, other than measurement of FPD. Principally this was because the Pupilscan used was a hand held instrument and the limit for recording was set at 3 seconds after the light stimulus. Other workers have attempted to assess the redilatation phase using recovery to 50% and 75% of the baseline parameters measuring recovery to 5 seconds and beyond (Smith, 1988; Bates *et al*, 1990). It was considered beyond the technical capability of Pupilscan to measure full redilatation.

Differences in the magnitude of the errors in measurement repeatability using Pupilscan III compared with other methods are probably due to three major factors.

a). The effects of the low intensity viewing light, employed to allow visual fixation of the pupil and aligning the optical unit. This increased the amount of hippus, or random oscillation of the pupil, and hence the variability of the " unstimulated " resting pupil diameter.

b). The lack of sophistication in terms of artefact rejection. The presentation of summary parameters (RPD, MPD and FPD) along with a crude listing of pixel count, combined with "automatic" rejection was very limiting with the portable device. Unless arbitrary rejection was employed then a large number of outliers was encountered. This was partly negated by taking median values for subsequent statistical analysis.

The ability to generate a graphical representation of the pupillary response using the IBM PC version of Pupilscan greatly improved the ability to exclude, according to objective criteria, outliers and artefacts produced by blink or eye movements.

c). The use of the Pupilscan optical unit required a high degree of dexterity together with subject compliance. Again, using the IBM PC version and the ability to visualise and centre the image using the screen greatly improved measurement reproducibility (CV% was 5.7% and 9.1% for MPD).

This evaluation of the portable Pupilscan together with its comparison with PC-Pupilscan and polaroid photography has demonstrated their ability to detect changes in pupillary responses to topically applied drugs. Acceptable reproducibility has been established for the portable Pupilscan,which may allow its use in clinical settings such as drug dependency clinics to monitor opiate abuse, or in operating theatres to monitor the level of anaesthesia.

The further refinement of capturing data onto a microcomputer reduces its portability, but greatly enhances its ease of use, reproducibility and data handling, making it a very useful tool in the pharmaceutical industry for evaluating autonomic effects of new drugs.

CHAPTER FOUR

THE EFFECTS OF A 5-HT_2 ANTAGONIST (ICI 169,369) ON CHANGES IN WAKING EEG, PUPILLARY RESPONSES AND STATE OF AROUSAL IN HUMAN VOLUNTEERS

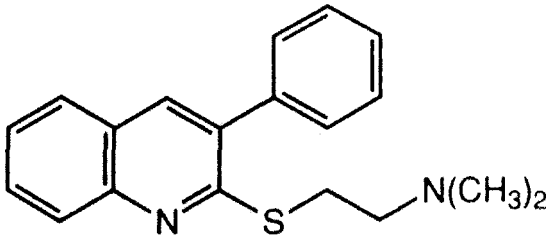
ICI 169,369 was the first development 5-HT_2 candidate, which was already under investigation when the candidate (DSM) began to investigate the pharmacodynamics of these compounds. Indeed, DSM had by chance already began to study the pupillary effects of ICI 169,369 during the course of unscheduled empirical pupillary measurements in a pharmacokinetic study. This le to the generation of a hypothesis concerning the possible involvement of 5-HT_2 receptors in the control of human pupillary responses (See chapter one; Aims of the thesis), and is the reason for their inclusion as an assessment in this study.

Evidence which suggests that 5-HT_2 receptors may be involved in the control of pupillary responses is restated here (for a more detailed rationale see Chapters one and seven). Radioligand binding and immunohistochemistry have localised 5-HT neurones in the human iris - ciliary body (Usitalo *et al*, 1984). L-trytophan injection (a 5-HT precursor) by close carotid arterial injection in man produced an ipsilateral mydriasis by increasing ciliary body 5-HT (Mantegezzini; 1966). Similarly fenfluramine, a 5-HT releasing agent, produced mydriasis, not abolished by pre-treatment with guanethidine or thymoxamine (Kramer *et al*, 1972). Mianserin and trazodone (Longmore *et al* 1988) both with 5-HT_2 antagonist properties, produced a miosis which in the case of mianserin was unaffected by alpha 1 or muscarinic agonists (Shur *et al*, 1983). These findings support the suggestion that 5-HT may influencE pupillary responses in man, by mechanisms other than classical autonomic receptors.

5-HT_2 receptors in man have been characterised to date with ketanserin, a selective 5-HT_2 antagonist with antihypertensive properties (Cameron *et al* 1987). Pharmacodynamic responses in healthy volunteers have been demonstrated at a vascular 5-HT_2 receptor using limb blood flow plesthysmography (Blauw *et al*, 1988) together with inhibition of platelet aggregation (Gotta *et al* 1987) and by measuring changes in venous compliance (Roddie *et al* 1955; Docherty 1986). CNS activity and the sedative properties of ketanserin were examined using the waking EEG (Reiman *et al*; 1986). Other selective 5-HT_2 antagonists such a ritanserin (Idzikowski *et al* 1987) seganserin (Dijk *et al*) and non-selective compounds such as trazodone, and mianserin with 5-HT_2 antagonist properties all increase slow wave sleep (SWS), on acute and chronic dosing (Spiegel, R; 1980).

ICI 169,369 (2-2-Dimethylamino ethyl thio) -3-phenyl quinoline (Fig 1) is a selective competitive antagonist of 5-HT at the 5-HT_2 receptor (Blackburn *et al* 1988). From the above findings in the literature and the chance observation made by DSM, it should be possible to demonstrate pharmacodynamic effects with ICI 169,369 in man by assessing its effects on waking EEG, pupillary responses and VAS mood rating scales. In this study, these were examined together with whole blood concentration measurements, to assess possible correlation with any biological effect.

An unscheduled pilot evaluation of ICI 169,369 as an antagonist of ex vivo 5-HT induced platelet aggregation was also carried out in this study by Dr. S. Heptinstall (University of Nottingham). This was independent of the principal aims of the study (CNS and pupillometry assesments) and is reported as an addendum to this chapter as a personal communication.



ICI 169,369

2–(2–Dimethylaminoethylthio)–3–phenylquinoline

Figure 1. Structural formula of ICI 169,369 hydrochloride

4.1. MATERIALS AND METHODS

Six healthy male volunteers were recruited (aged 18-37 years). Informed consent and local ethics committee approval were obtained. They were allowed to enter the study if no clinically relevant contraindications were found on medical history and examination, following blood tests for standard haematological and biochemical parameters and resting ECG. Volunteers were not allowed to enter the study if a baseline EEG was abnormal.

4.2 STUDY DESIGN (including dose and dosing regimen of ICI 169,369).

Six male volunteers were studied in a double blind, within subject trial, using a three period cross over design, each seperated by one week, to compare two single oral doses of ICI 169,369 (80 and 120mg) and matching placebo. Volunteers were randomly allocated to the 6 possible sequences of the 3 treatments subject to the constraint that each set of 3 subjects formed a latin square.

4.3 METHODS OF ASSESSMENT

4.3.1 Waking EEG Recording

A standard 10/20 seven electrode montage was applied using seven silver/silver chloride electrodes fixed in place with flexible collodion after dermal abrasion. Following this, subjects rested supine in a sound attenuated, temperature controlled room for 5 minutes with their eyes open, under observation via a closed circuit television system. EEG recording then commenced for 4 minutes with "eyes open" whilst performing a vigilance task (counting a random sequence of crosses on a VDU), followed by a further 4 minutes "eyes closed" during which dominant index finger tapping was carried out.

4.3.2 EEG Data Capture And Processing.

Four channels of EEG signal were continuously monitored via a Medlec 5000 preamp and polygraph. The signal was recorded onto magnetic tape for archiving, and simultaneously passed through a 1703 signal conditioning module (CED, Cambridge) amplifier, filter and anti-aliasing device which was programmed to set a band width of 30Hz for on-line analysis.

In conjunction with an IBM PC-AT micro computer based system the signals were subsequently digitised by a 1401 intelligent interface (CED, Cambridge) at a rate of 128Hz. Continuous monitoring of the simultaneous polygraph output facilitated recognition of oculographic and other artefacts and assessment of vigilance.

The last two minutes of EEG recorded during each sequence were divided into 20 separate 6 second epochs, for eyes open and eyes closed recording. All 20 under each condition (eyes open/closed) free of artefacts were further analysed. Each 6 second epoch was multiplied by a 10-80-10 cosine bell window (half cosine wave in the range 0-1, lasting 0.6 secs at each end of the epoch and unity for the central 4.8 secs) to minimise leakage. Each epoch was also detrended and the fast fourier transform (FFT) was then applied to give estimates of the power spectrum at 0.125Hz intervals. A further data reduction was then performed by averaging groups of 20 adjacent values producing 1Hz frequency bands, the DC term being ignored, and by then averaging the 20 epochs obtained under the same conditions (eyes open/closed). The first 30 of these 1Hz frequency bands were used to construct a power spectrum analysis.

4.3.3. Infra-Red Pupillometry Assessments

"Pupilscan", a portable infra-red (IR) pupillometer, was used to measure dark adapted resting pupil diameter (RPD) and light stimulated reflexes (Millson *et al*(a); 1988). A hand held optical unit with dual IR emitting diodes (940nM wavelength, power 2 x 10^{-5} to 2 x 10^{-3} MW cm-2) was aligned on the centre of the pupil with the aid of crosswires and a low intensity viewing light (585nm, 3.2 CD m-2). Reflected IR, and hence the "black hole" effect of the pupil, was detected by an IR solid state image sensor (65K, rectangular fixed array) scanning the pupil every 10 ms.

All assessments were made under "dark room conditions". Volunteers dark adapted for 15 mins wearing dark welding goggles (BS15422) prior to illumination of the right pupil with the viewing light (3.2cd/m2) for 30 secs, to allow central fixation of the optical unit using crosswires. Supine subjects were asked to fixate on a cross marked on the ceiling (2m distant) and directed not to blink for the 3 sec measurement period.A recording was made of the light induced pupillary constriction. 5

artefact free pupil light response curves were recorded at 30 sec intervals. An artefact was defined as a blink or eye movement noted by the observer, or appearing as a significant negative deflection from the normal pupil response profile.

4.3.4 Analysis Of Pupillary Light Reflexes

Vertical pupil diameter was measured automatically, at the start of the 3 sec measurement cycle (RPD), initiated by the trigger mechanism in the optical unit. This was followed by a stimulus impulse of 0.5sec duration (565nm, 65cd m-2) and a measurement of minimum pupil diameter (MPD), followed by a recovery to final pupil diameter (FPD). The change in vertical pupil diameter was captured by a portable microprocessor, and summary pupil data (RPD, MPD and FPD) displayed on a liquid crystal display, together with date, time and subject identification.

Individual light reflex curves were analysed using a pupil scan data handling template. Recordings,corresponding to 30 estimates of vertical pupil diameter during each three second measurement cycle, were analysed to derive the maximum velocity ($VEL_{max}C$) and maximum acceleration ($ACC_{max}C$) of pupillary constriction (*i.e.* the first and second differential of the constrictor response).

4.3.5 Determination of Pharmacokinetic Parameters For ICI 169,369.

A preliminary assessment of the whole blood ICI 169,369 was constructed for each dose. The maximum measured whole blood concentration (C_{max}) of ICI 169,369 was obtained by inspection. An estimate of the elimination half-life was obtained from the exponential elimination rate constant describing the terminal log-linear elimination phase. Only a fairly crude picture of the human

pharmacokinetics of this drug could be described, because of the limited sensitivity and difficulty in interpreting the results from a whole blood assay.

4.3.6 VISUAL ANALOGUE MOOD RATING SCALES

Subjects were given 16 dimensions on which to rate their subjective feelings (Bond and Lader, 1974). Rating was performed by marking a point on a computer generated 100mm line by moving the cursor along the visual display unit of the IBM PC-AT, meant to represent the full range of a particular dimension (e.g. alert - drowsy). The ratings were measured and transformed according to the procedure of Bond and Lader (1974).

4.4 WHOLE BLOOD ICI 169,369 ESTIMATION.

Heparinised whole blood (1ml) samples were analysed using a gas-liquid chromatographic procedure as described by Haworth *et al* (1987). Briefly, after solvent extraction into pH10 buffer containing 1.5% amyl alcohol in hexane the organic layer was acidified with 0.1N hydrochloric acid. After shaking and centrifugal separation the organic layer was then discarded. 0.1N sodium hydroxide and 1.5% amyl alcohol were added to the aqueous layer and after extraction into the organic layer this was transferred and blown to dryness at 40°C with oxygen-free nitrogen. The residue was redissolved in 1% (v/v) ethanol in octanol and injected into a Hewlett-Packard gas chromatograph fitted with a 5% methyl-phenyl silicone capillary column of 0.52 micron film thickness, 25 metres long, internal diameter 0.31mm. Detection was by means of a nitrogen detector. The lower limit of quantification was 2.7 ng/ml, with an intra-assay coefficient of variation of <10% over the range of whole blood concentration measurements (4.0 - 250 ng/ml).

4.5 STUDY PROTOCOL

Three subjects were studied each day. Volunteers fasted overnight and consumed no alcohol or caffeine for 24 hours prior to and after dosing. EEG measurements using 4 channels were made on each volunteer at 4 timepoints (before and at 2, 6 and 24 hours after dosing) with both eyes open and closed. Pupillometry and VAS assessments were made at 6 timepoints (before and at 3, 5, 8, 12 and 24 hours after dosing). The same measurements were made in each of the 3 treatment periods.

A 5ml sample of venous blood was taken, into an oxalate tube and stored at -20 C until assayed, before dosing on each study day and immediately preceding EEG and pupillometry assessments, the order of priority for measurements was venous blood sampling, EEG recording, pupillometry followed by VAS Bond-Lader scales.

4.6 STATISTICAL ANALYSIS

For the EEG data, four conventional frequency wave bands were defined as follows; delta (0.00-3.99Hz), theta (4.00-7.99Hz), alpha (8.00-12.99Hz) and beta (13.00-29.99Hz). Anterior channels 0 and 1 were summed and posterior channels 2 and 3 were summed. For each wave band, eyes open or closed, and for both pairs of channels, and analysis of variance model was performed on the log power at each post-dose timepoint having subtracted the corresponding pre-dose value.

Where statistically significant differences were found, least square means (LS mean) are presented together with the least significant difference (LSD), and the p value for the statistical significance of the difference between measurements with each dose of ICI 169,369 from placebo. Least square means are means that have been adjusted to allow for any imbalance in the design. The LSD is the least difference there must be between a treatment LS MEAN and a placebo LS MEAN for statistical significance at $p<0.05$ level.

Similarly for the pupil reaction data, the difference from pre-dose was analysed at each time point with the same analysis of variance techniques.

The Bond Lader VAS alert-drowsy scale was analysed by calculating the area under the curve over 15 timepoints and an analysis of variance performed. The 16 Bond Lader scales were analysed as three factors, "Alertness", "Contentness" and "Calmness". The LS MEAN over the contributing scales was calculated for each factor at each timepoint and differences from predose were analysed by an analysis of variance model.

Linear and log linear regression analysis was carried out and analysis of variance was applied to assess the linearity of the model together with the correlation coefficient, slope, and 95% confidence intervals for the fitted line.

4.7. RESULTS

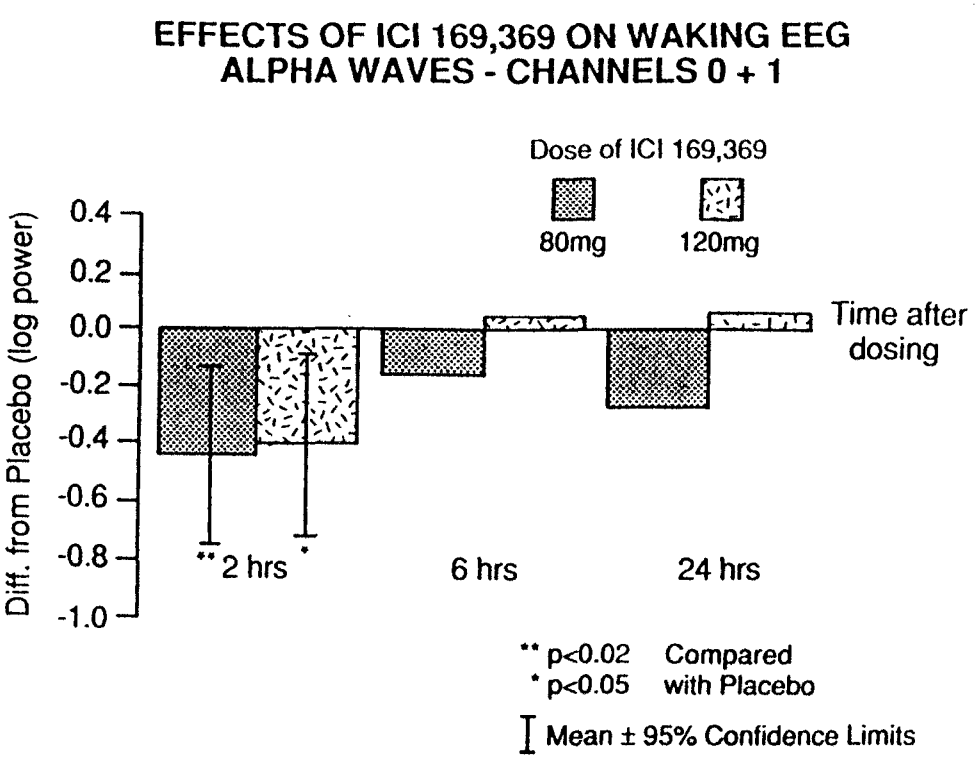
4.7.1. EEG POWER SPECTRUM ANALYSIS

ICI 169,369 has significant clear cut effects only on the "eyes closed" EEG at 2 hours after dosing (Fig 4.2), corresponding in time to the observed peak maximum whole blood concentration (Howarth *et al*; 1987). At 2 hours post dose theta wave activity was increased by 38% (range 20-86%; $p<0.01$) with 80 and 120mg doses of ICI 169,369 in both anterior and posterior channels. A corresponding 36% decrease in alpha wave activity occurred (range 10 - 54%; $p<0.02$) present in both channels, but was only significant for the anterior channel .

No consistent changes in delta or beta wave activity were noted. Both 80 and 120mg doses of ICI 169,369 appeared equipotent with respect to their activity on the waking EEG.

4.7.2 Pupil Responses

At 3 and 5 hours after dosing, resting pupil diameter (RPD) was reduced by approximately 30% ($p<0.01$) with both the 80 and 120mg doses of ICI 169,369 (Fig 4.3). MPD was reduced by 50% with the 120mg dose of ICI 169,369 at 5 hours after dosing. FPD was significantly reduced in a similar manner to MPD by both doses at 3 hours after dosing ($p<0.01$).



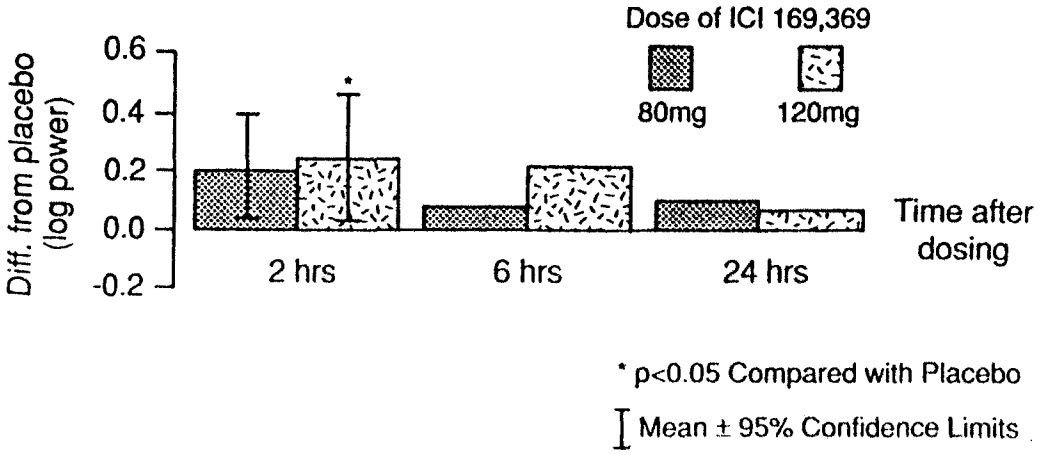
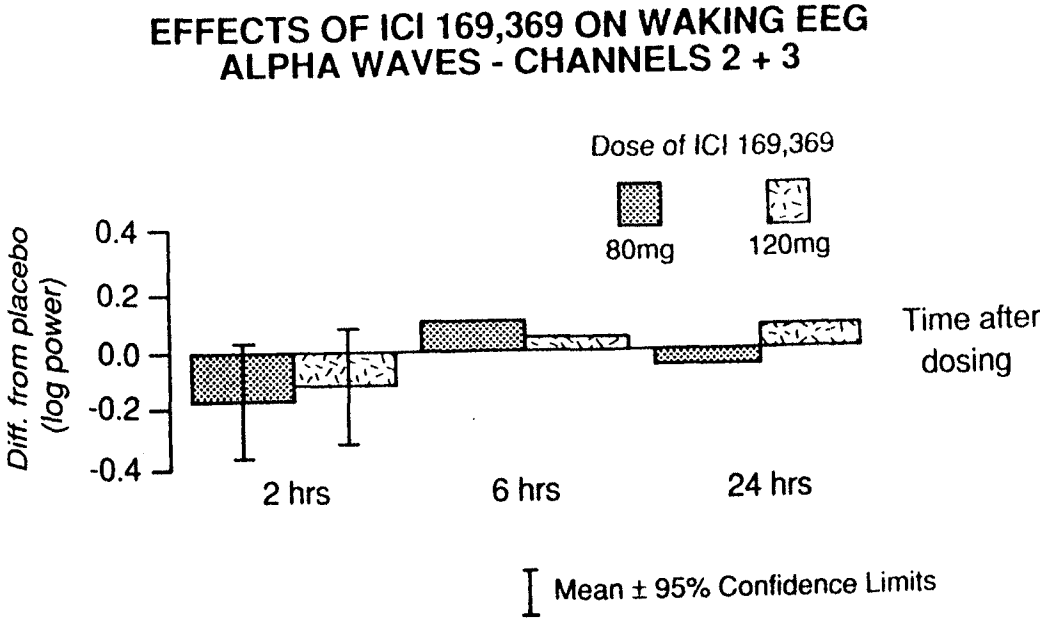


FIG 4.2

Effects of ICI 169,369 on EEG power spectrum analysis with eyes closed. Results depict the log 10 power difference between placebo and drug treatment at 2,6 and 24 hours after dosing. Alpha waves and theta waves are depicted for the anterior (0 + 1) and posterior (2 + 3) channels; with the 95% confidence intervals for a difference from placebo,with appropriate p values where significant.



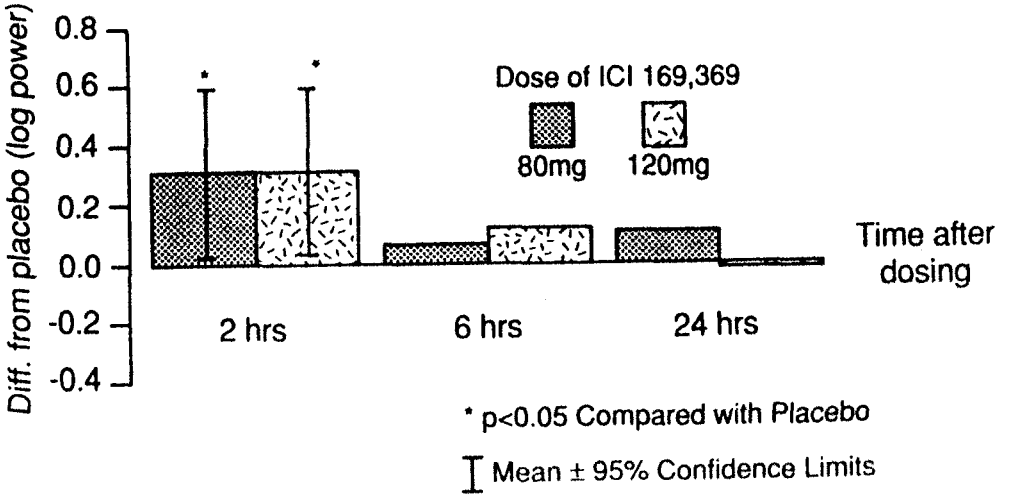


FIG 4.2

Effects of ICI 169,369 on EEG power spectrum analysis with eyes closed. Results depict the log 10 power difference between placebo and drug treatment at 2, 6 and 24 hours after dosing. Alpha waves and theta waves are depicted for the anterior (0 + 1) and posterior (2 + 3) channels; with the 95% confidence intervals for a difference from placebo, with appropriate p values where significant.

EFFECTS OF ICI 169,369 ON RESTING PUPIL DIAMETER

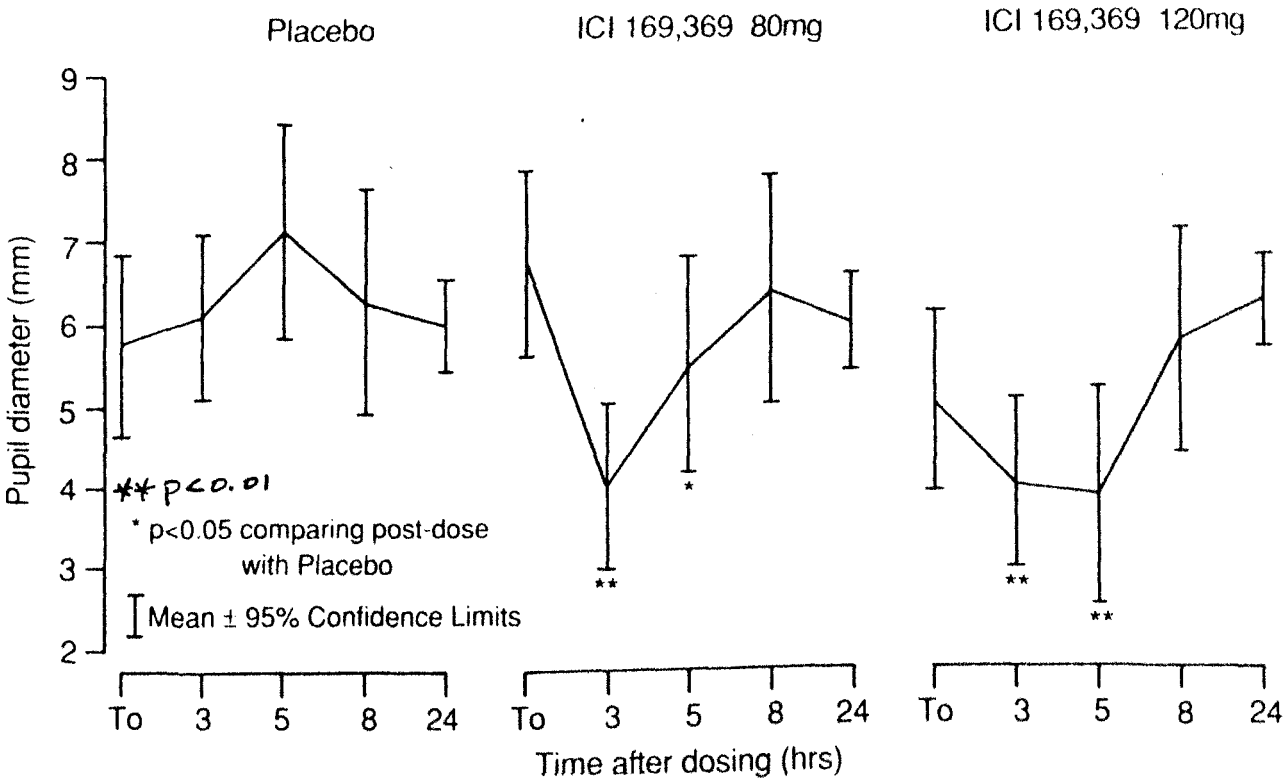


FIG 4.3.

Effects of ICI 169,369 on resting pupil diameter (RPD) for placebo, 80 and 120mg doses at To, 3, 5, 8 and 24 hours after dosing, with appropriate p values where treatments were significantly different from placebo.

4.7.3 Kinetics Of Pupillary Light Responses

Median light reflex curves were analysed for each of the 6 volunteers at 3 hours after dosing (corresponding to the maximally observed miosis) for Placebo, 80 and 120mg doses of ICI 169,369 (Fig 4.4). RPD was reduced by approximately 30% with both 80 and 120mg doses of ICI 169,369 compared with the placebo curve. The pupil response curves were diminished in amplitude after both doses of ICI 169,369, with reductions in the maximum velocity ($VEL_{max}C$) and maximum acceleration of constriction ($ACC_{max}C$), which were significantly different for the 120mg dose ($p<0.05$), (Table 4.1).

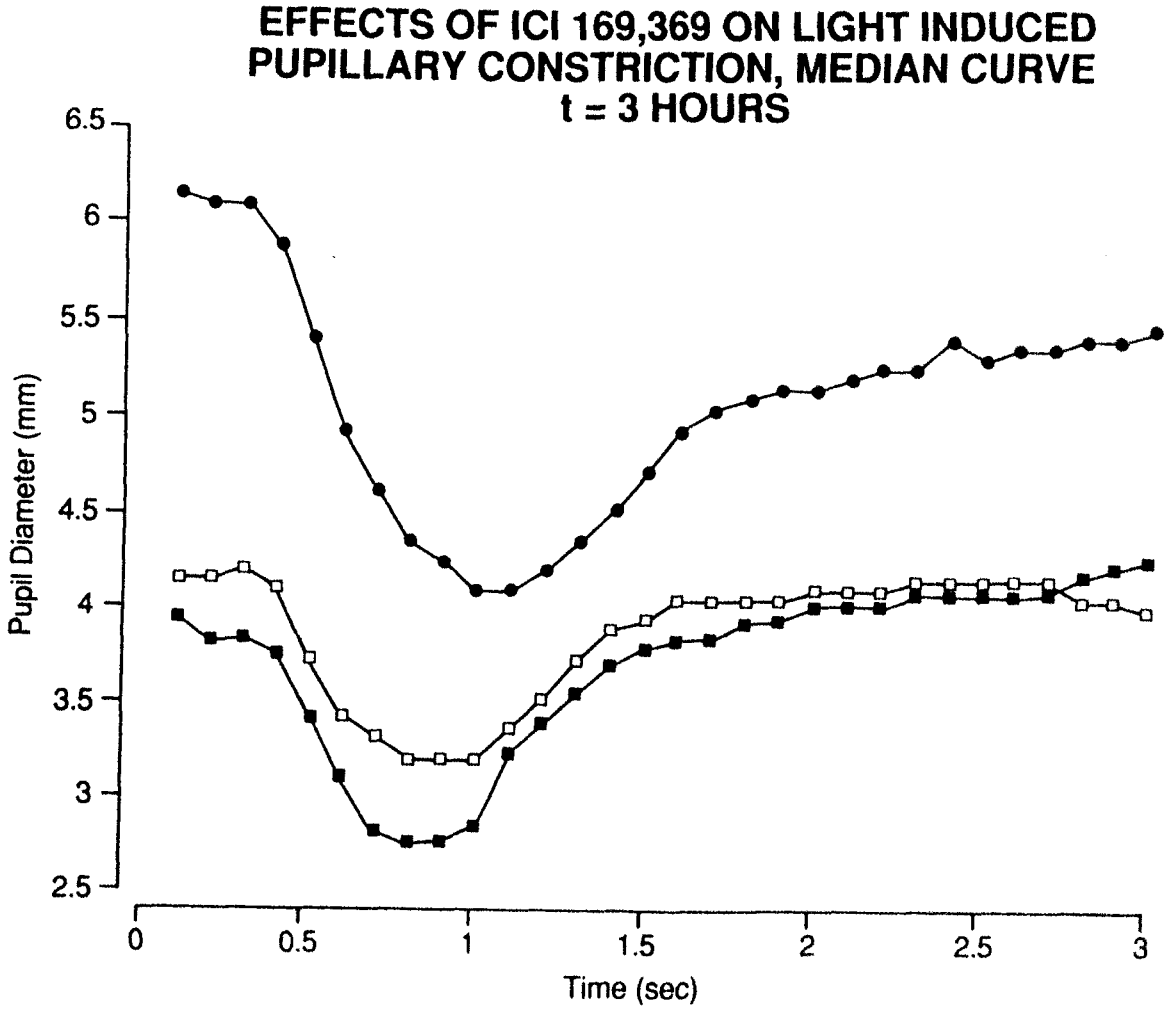


FIG 4.4.

Effects of ICI 169,369 on light induced pupillary constriction curves. Median curves are depicted for placebo (●), 80 (□) and 120mg (■) doses of ICI 169,369 at 3 hours after dosing. Pupil diameter is plotted against time (sec) for a three second measurement cycle.

EFFECTS OF ICI 160,369 ON THE KINETICS OF LIGHT INDUCED PUPILLARY CONSTRICTION AT t = 3 HOURS

Mean with (C.I. of difference between drug and placebo)

	Placebo (with sem)	80mg	120mg
Maximum constriction velocity (mm/s)	6.2 (1.02)	5.3 (± 1.8)	3.8 (± 0.7)*
Maximum constriction acceleration (mm/s/s)	40.7 (5.84)	31.0 (± 5.7)	29.7 (± 1.4)*

* $p<0.05$ comparing dose with placebo

TABLE 4.1

The effects of ICI 169,369, given as single oral doses (80 and 120mg) on the velocity (mm/s) and acceleration (mm/s/s) of the pupillary light response. Mean values are given with 95% confidence interval for the difference between placebo and drug effects.The results depicted are for three hours post dosing, corresponding to the time when circulating ICI 169,369 levels are at their highest.

4.7.4 Mood Rating VAS Scales

The alertness factor (Table 4.2) derived from the Bond Lader VAS was significantly higher in both the 80 and 120mg ICI 169,369 treatment group at 5 hour post dose compared to the placebo indicating a reduced level of alertness. No other consistent changes in the "Happiness" or "Relaxation" factors were recorded.

Examination of mean scores from the single "Alert-Drowsy" VAS scale, from the Bond-Lader before factorisation, confirms the statistical trend towards diminished arousal at between 3 and 5 hours after dosing with both 80 and 120mg doses of ICI 169,369 compared with placebo(Fig 4.5). Although the VAS had returned to baseline by 24h after dosing the Bond Lader alertness factor was still elevated, suggesting a possible residual effect. However, this was not significantly different from placebo ($p>0.5$).

EFFECTS OF ICI 169,369 ON "ALERTNESS FACTOR"

Mean with (C.I. of difference between drug and placebo)

Time (hr)	Placebo (with sem)	80mg	120mg
To	22.9 (4.2)	19.7 (± 9.1)	18.7 (± 9.1)
3	21.6 (6.1)	23.7 (± 5.1)	26.6 (± 5.1)
5	21.4 (4.2)	27.2 (± 3.0)**	25.0 (± 3.0)*
12	22.9 (5.4)	23.8 (± 3.4)	19.3 (± 3.4)
24	20.8 (6.0)	24.1 (± 5.9)	23.6 (± 5.9)

** $p<0.01$, *$p<0.05$ comparing dose with placebo

TABLE 4.2.

The effects of ICI 169,369 on the alertness factor derived from the "Bond Lader" visual analogue factor score. Mean values are given together with 95% confidence intervals for differences between placebo and active drug, for readings taken over a 24 h period.

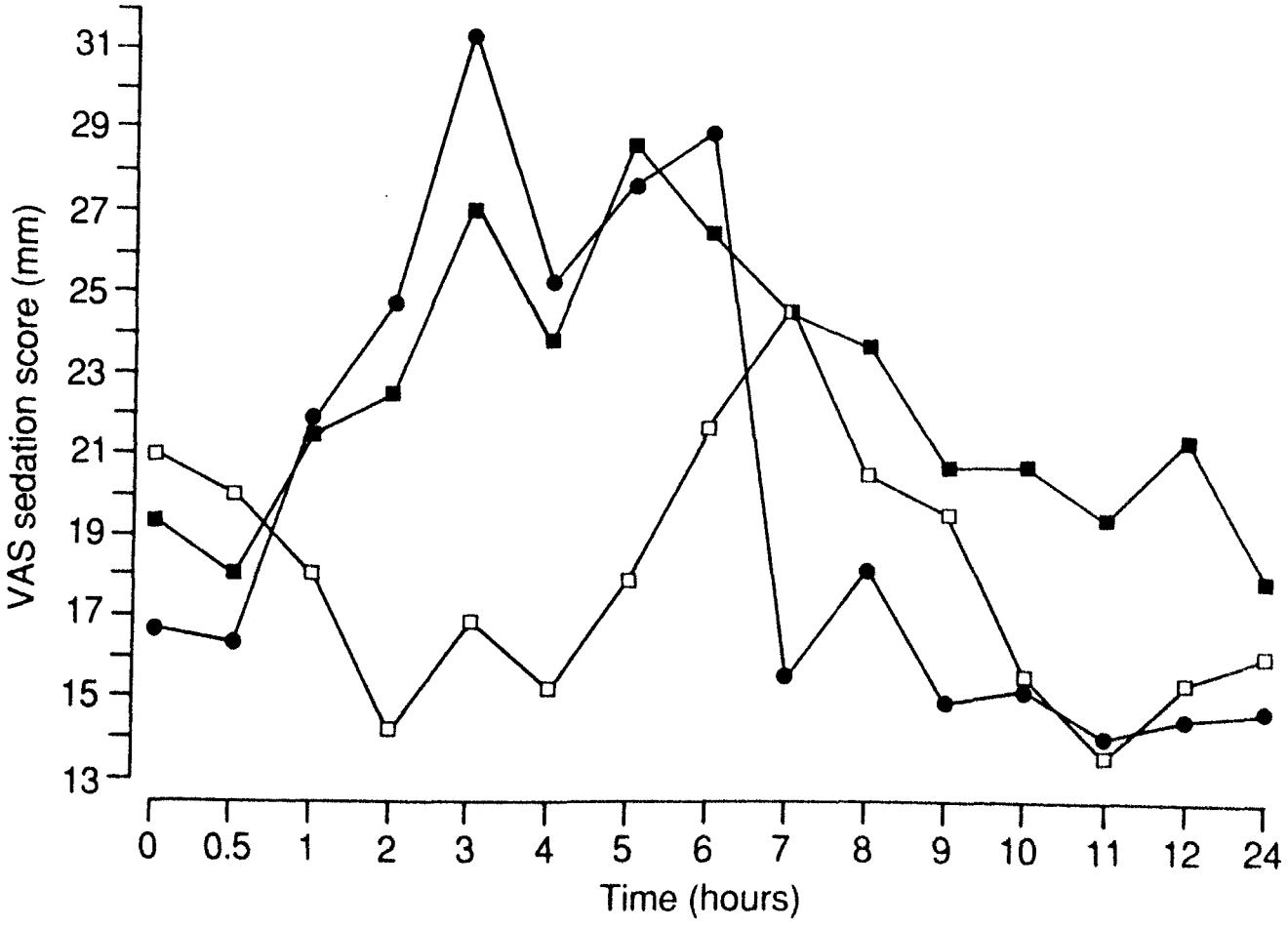


FIG 4.5.

Effects of ICI 169,369 on the Alert-drowsy visual analogue scale (100mm VAS), at various times after dosing for a 24 hour period. Mean VAS sedation score (mm) is plotted for placebo (□), 80 (■) and 120mg (●) doses of ICI 169,369.

4.7.5 Pharmacodynamic Correlation With Whole Blood Concentrations Of ICI 169,369.

Whole blood concentrations of ICI 169,369 (ng ml-1) taken prior to pupillary assessment of RPD, demonstrated a significant negative log linear correlation (Fig 4.6) with resting pupil diameter ($r=0.697$, slope=-0.99, $p<0.0001$).

4.7.6 Whole blood pharmacokinetic profiles for ICI 169,369 and pharmacokinetic parameters

The pharmacokinetic profiles described were obtained using a relatively unsophisticated whole blood assay and can give only a crude indication of the true pharmacokinetic profile of compound (Fig 4.7). Nevertheless, the parameters obtained show that peak maximum (Cmax) concentrations were achieved within 2 hours after dosing, and that the elimination halflife was between 8.0 and 9.0 hours for both doses

4.7.7 The relationship between constriction velocity, acceleration and changes in drug related pupil size.

Figure 4.8 demonstrates a linear relationship between maximum constriction velocity and initial resting pupil diameter (RPD) for subjects receiving ICI 169,369.There was no corresponding relationship between acceleration of constriction and RPD (Fig 4.9.). These results support the data obtained at 3 hours post dose, where dose related changes in constriction velocity were obtained with both doses of ICI 169,369 relative to placebo, whereas the changes in acceleration were not dose dependent and occurred only with the highest dose.

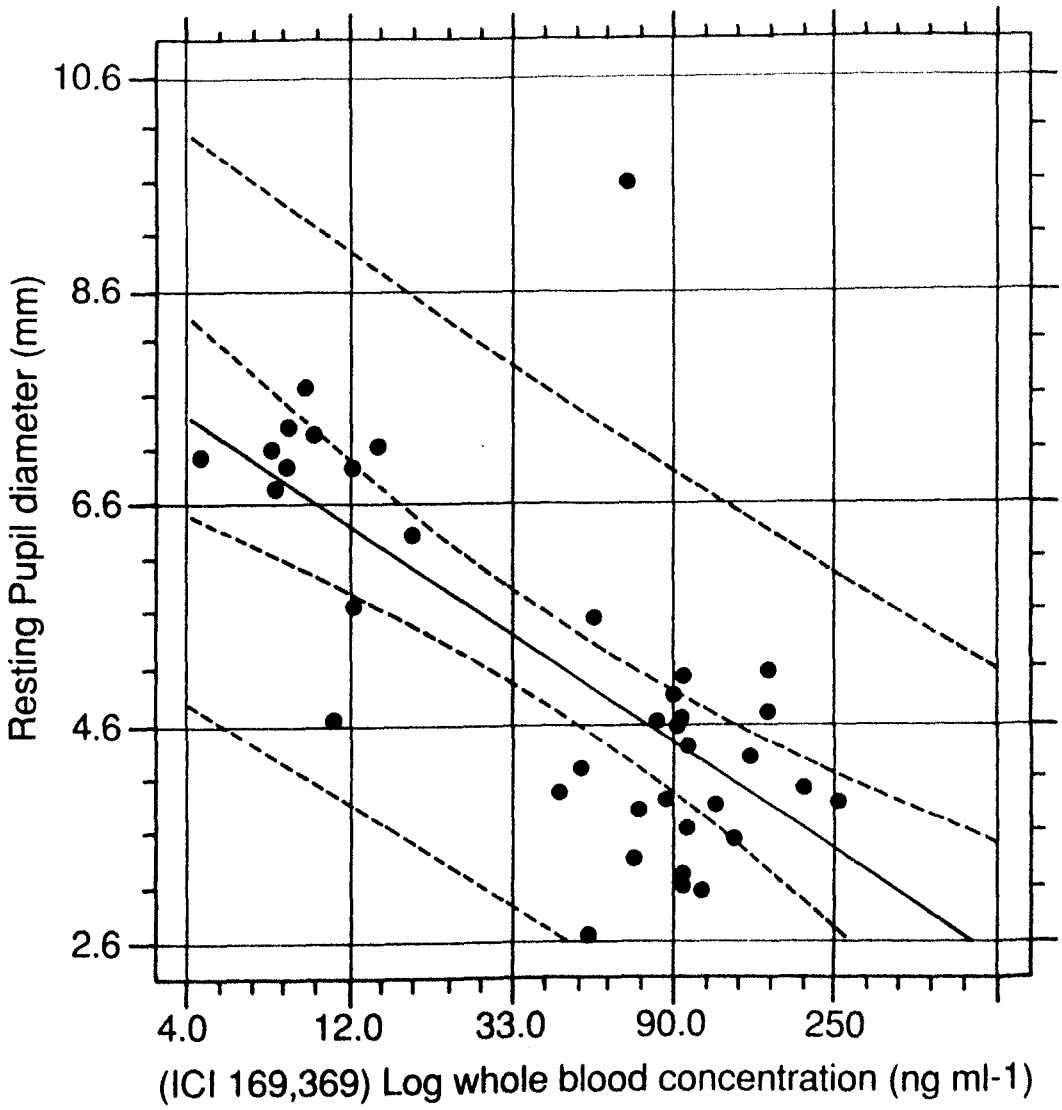


FIG 4.6.

Log linear regression analysis of resting pupil diameter (mm) versus log10 whole blood concentration (ng/ml) of ICI 169,369, following 80 and 120mg single oral doses. The line of best fit ($r=0.69$, slope $= 0.99$, $p<0.0001$) is depicted with 95% confidence intervals for the fitted line and value estimates.

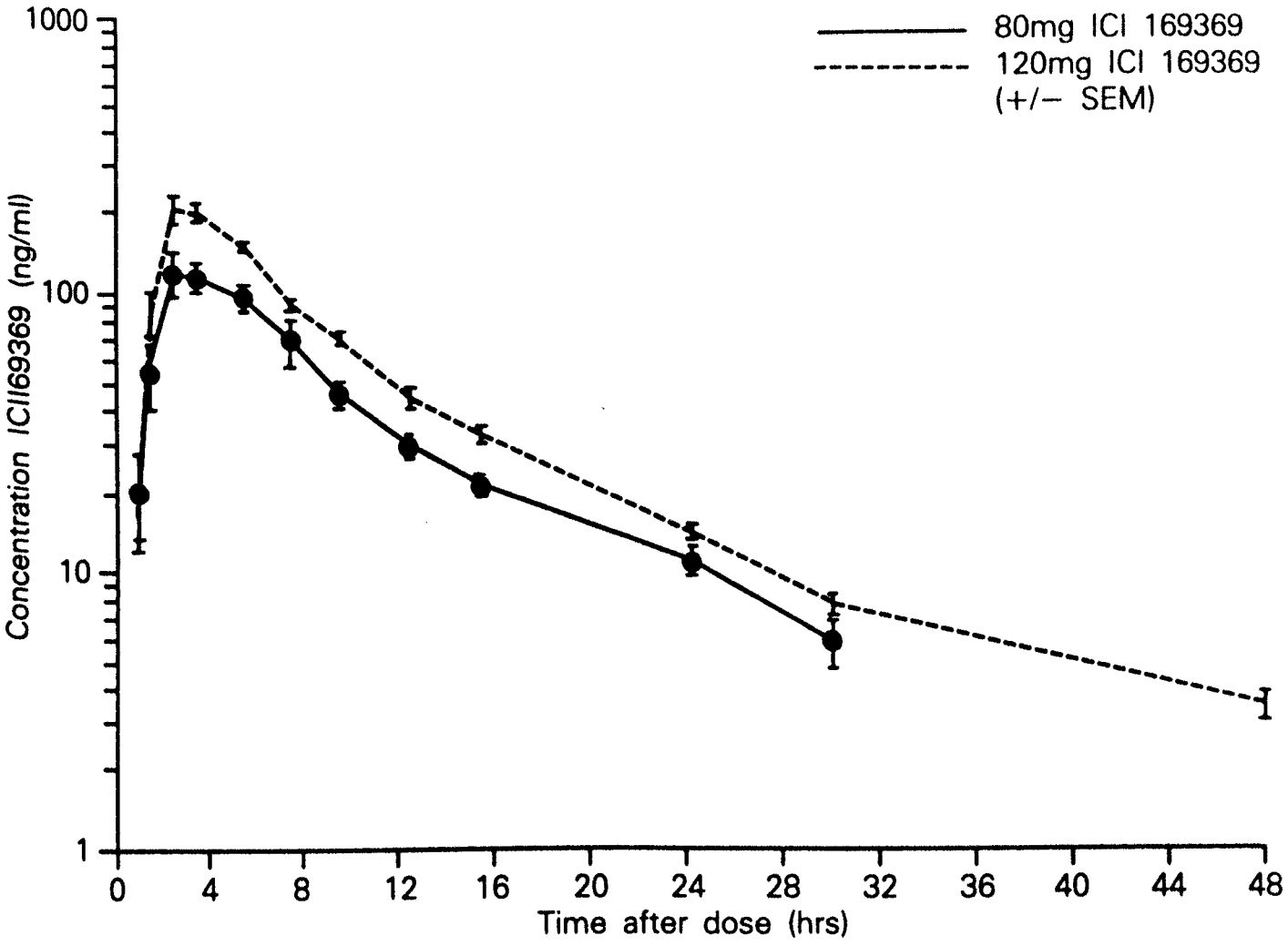


FIG 4.7

Pharmacokinetic analysis was performed by estimating whole blood concentrations at various times after dosing with 80 and 120mg of ICI 169,369. Peak maximum (C_{max}; median with range) concentrations achieved at 2 hours after dosing were 93.7ng/ml (53.2 - 162) and 135ng/ml (98.2 - 250) with an elimination half life of 8.6 and 9.4 hours respectively. Whole blood concentration profiles are depicted for 80 (● and 120mg (—) doses of ICI 169,369 over a 48 h period.

4.7.7 The relationship between constriction velocity, acceleration and changes in drug related pupil size.

Figure 4.8 demonstrates a linear relationship between maximum constriction velocity and initial resting pupil diameter (RPD) for subjects receiving ICI 169,369.There was no corresponding relationship between acceleration of constriction and RPD (Fig 4.9.). These results support the data obtained at 3 hours post dose, where dose related changes in constriction velocity were obtained with both doses of ICI 169,369 relative to placebo, whereas the changes in acceleration were not dose dependent and occurred only with the highest dose.

REGRESSION OF MAXIMUM CONSTRICTION VELOCITY (mm/s) VERSUS RPD (mm)

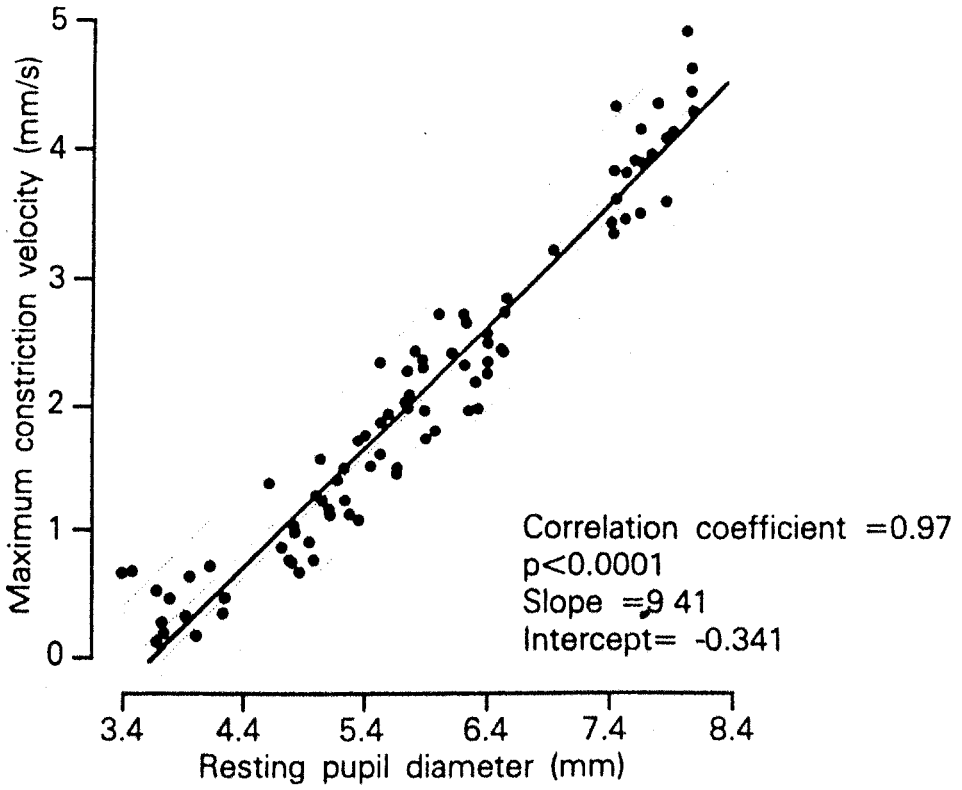


FIG 4.8.

The relationship between maxiumum constriction velocity (mm/s) for light induced pupillary constriction and initial resting pupil diameter for subjects receiving a range of oral doses of ICI 169,369 (80 and 120mg). The least squares regression line is given with 95% confidence intervals for the line of best fit and parameter values (r=0.97, slope=0.941, p<0.0001).

REGRESSION OF MAXIMUM CONSTRICTION ACCELERATION (mm/s/s) VERSUS RPD (mm)

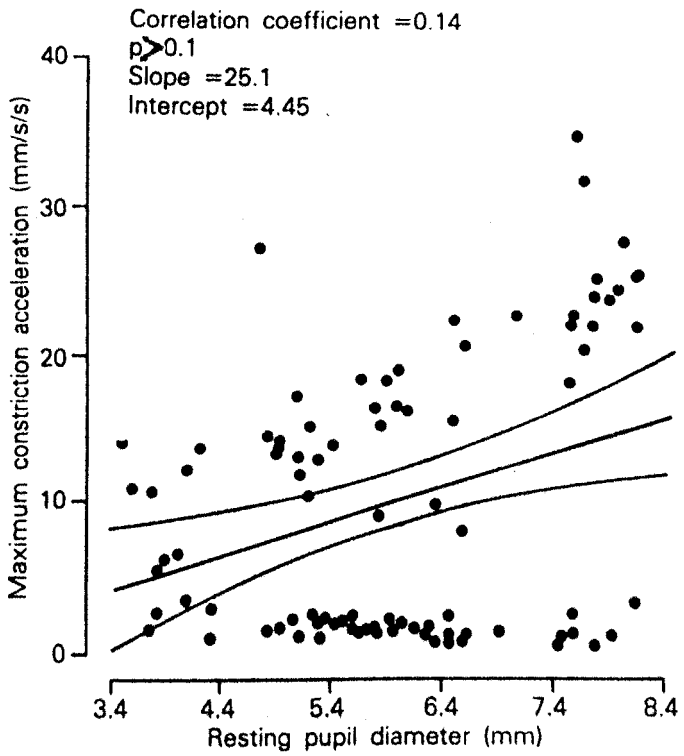


FIG 4.9

The relationship between maximum constriction acceleration (mm/s/s) and initial resting pupil diameter for subjects receiving ICI 169,369 single oral doses (80 and 120mg) is given with a least squares regression analysis,depicting 95% confidence intervals for the line of best fit (r=0.14, p>0.1).

4.8 DISCUSSION

This study has demonstrated that single oral doses of 80 and 120mg of ICI 169,369 produced a subjective reduction in arousal, as measured by the Bond Lader VAS, accompanied by objective changes in waking EEG (eyes closed), expressed as decreased alpha with a reciprocal increase in theta activity. Quantitative EEG methods are commonly used to identify the effects of drugs on the CNS (Itil, 1981), and are more sensitive than conventional psychometric tests.An increase of slow waves and decreased alpha wave activity was observed with mianserin (an anti-depressant with 5-HT_2 antagonist properties: Fink, 1984). These changes were dose related and considered to resemble the "thymoleptic EEG pattern" of the tricyclic antidepressants. Residual CNS "hangover" effects have been reported with the hypnotic zopiclone (Lader *et al* 1982), and with the "non-sedative" antihistamine triprolidine (Holland *et al* 1989). In contrast a lack of EEG effects or any interaction with lorazepam have been demonstrated for granisetron, a non-sedative 5-HT_3 antagonist with CNS activity in animal models of anxiolysis (Link *et al* 1991).

The changes in the waking EEG which occur during treatment with ICI 169,369 are comparable to those seen with the 5-HT_2 antagonist ketanserin (Reimann *et al*; 1986), where moderate sedation was accompanied by decreased alpha and increased theta activity. In sleep EEG studies ICI 169,369 produced similar changes to ritanserin in man (Cowen *et al* 1990; Idzikowski *et al* 1991), promoting slow wave sleep (SWS). Thus, it is thought that SWS is under 5-HT_2 inhibitory control, and that 5-HT_2 antagonists promote SWS.

Tortella *et al* (1989) failed to demonstrate an increase in SWS with either ICI 169,369, or ritanserin in the rat, although a specific REM suppressant effect was demonstrated. Despite evidence for waking EEG changes in both man and animals, paradoxically ritanserin produced no significant reduction in

arousal (Awouters *et al* 1983). Therefore, whilst a reduced state of arousal is often an accompaniment to waking EEG changes, this is not always the case.

The pupillary miosis observed with ICI 169,369 suggests that 5-HT_2 receptors may be directly involved in the control of human pupillary responses. A neurotransmitter role for 5-HT in the retina is now well established (Osbourne *et al*, 1986); however the involvement of 5-HT in the anterior chamber of the eye is more speculative. In the dog and rabbit intravenous injection of 5-HT lowered intraocular pressure (Chiang 1974; Schumacher and Classen, 1962), whilst local anterior chamber injection produced a mydriasis together with increased intraocular pressure and protein concentration (Palkama *et al*; 1984). In contrast local injection of 5-6 DHT (5-6 Dihydroxytryptamine, a 5-HT depleting agent) produced miosis with receptor supersensitivity to exogenous 5-HT induced mydriasis (Moro *et al*, 1987).

Recently Tobin and Osbourne (1988) concluded that 5-HT_2 receptors were involved in controlling rabbit pupillary reponses. 5-HT uptake was demonstrated in iris ciliary body, with a dose dependent 5-HT mediated increase in 3H-inositol phosphate turnover, selectively antagonised by ketanserin, methysergide and mianserin (5-HT_2 antagonists) but not by ICS 205,930 (5-HT_3 antagonist; Fozard 1984), prazosin (alpha 1 antagonist) or atropine (muscarinic antagonist). Furthermore, sympathetic denervation by superior cervical ganglionectomy had no effect on 5-HT mediated responses, suggesting a selective serotonergic innervation. Therefore animal studies would support the involvement of 5-HT_2 receptors in controlling pupillary responses.

Possible mechanisms underlying this miosis are :

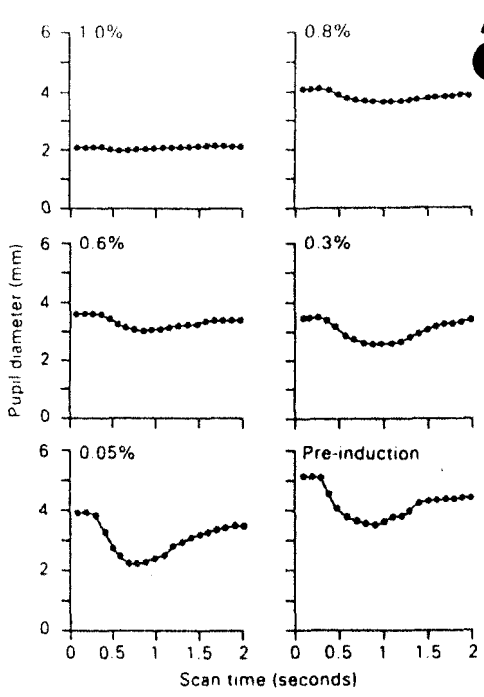
1) a direct post synaptic 5-HT_2 mediated dilatation, which, when antagonised induced miosis,
2) presynaptic 5-HT_2 inhibitory modulation of parasympathetic tone, which, when antagonised, increased parasympathetic tone and
3) a central effect of 5-HT_2 antagonism acting on efferents from the IIIrd nerve nucleus.

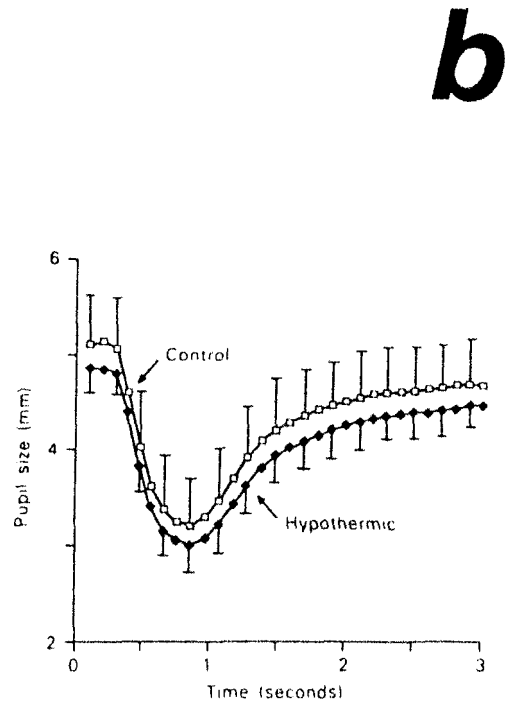
The reduced arousal and associated CNS effects observed with ICI 169,369 may have contributed to the pupillary miosis observed, as for example, subjects with narcolepsy demonstrate a pupillary miosis during attacks (Yoss, 1969). However, the link between sedation and miosis is tenuous, since general anaesthesia with thiopentone produced the opposite effect *i.e.* a mydriasis (Larson, 1981).

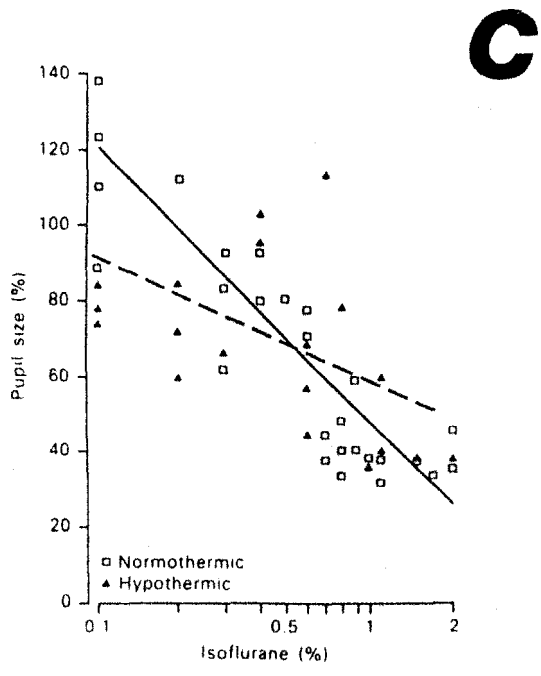
A more recent paper by Larson *et al* (1991) demonstrated a dose related pupillary miosis using "Pupilscan",with a similar pattern of results to those described in this study (see Fig 4.9.1). However, clearly there was a major difference in the state of arousal of the anaesthetised subjects in his study and the minor degree of sedation seen in this study.

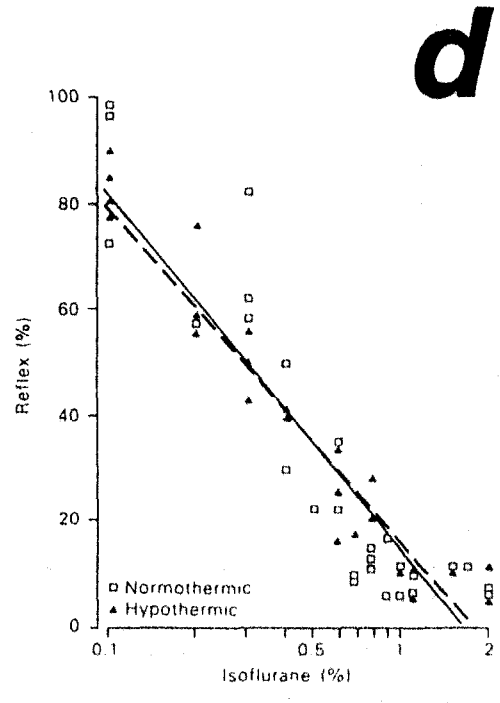
FIG 4.9.1

Figures showing results obtained by Larson *et al* (1991) using "Pupilscan " to investigate the effects of anaesthetic doses of isoflurane on the pupillary light response in healthy volunteers.Figs A and B show changes in the shape of the reflex with increasing endtidal isoflurane concentrations, together with the effects of experimental hypothermia. Figs B and C illustrate the linear relationships between changing pupil size and reflex amplitude(relative to pre-induction) and endtidal isoflurane concentration


a
1.0%
0.8%
0.6%
0.3%
0.05%
Pre-induction
Pupil diameter (mm)
Scan time (seconds)


b
Control
Hypothermic
Pupil size (mm)
Time (seconds)


c
Normothermic
Hypothermic
Pupil size (%)
Isoflurane (%)


d
Normothermic
Hypothermic
Reflex (%)
Isoflurane (%)

In support of the central 5-HT_2 effect, Goldstein *et al* (1989) demonstrated that ICI 169,369 reversed the inhibitory effects of amphetamine on A9 and A10 dopaminergic neurones, reflecting an effect on central dopaminergic activity and possible anti-psychotic activity. Beaumont *et al* (1987) demonstrated a miosis and diminished light reflexes in parkinsonian patients, with comparable miosis to those seen in this study. Therefore ICI 169,369 may have induced miosis in volunteers by reducing central dopaminergic actitivity to mimic pupillary changes observed in parkinsonian patients.

None of the volunteers involved in these studies complained of visual disturbances under normal lighting conditions, and the effects of ICI 169,369 on visual parameters is under evaluation. However, the miosis observed was correlated with whole blood concentrations of ICI 169,369 however, and may be a useful pharmacodynamic measurement in evaluating other putative 5-HT_2 antagonists in man. The observation relating initial pupilsize to constriction velocity is in accord with results discussed by Smith *et al* (1978), where he showed that reflex amplitude and constriction velocity were linearly related. The absolute values for constriction velocity (about 5 to 7mm/s) are also in agreement with his result and those of Beaumont *et al* (1987).

Therefore, this study has demonstrated pharmacodynamic changes in waking EEG, pupillary responses and state of arousal in human volunteers. The EEG changes and diminished state of arousal suggest a sedative effect, and on the basis of pharmaco-EEG analysis may be predictive of anti-depressant or anxiolytic activity (Fink; 1984). The miosis and changes in pupillary responses may indicate a central or a peripheral effect and may represent new evidence to support the involvement of 5-HT in the control of human pupillary responses.

The lack of efficacy of ICI 169,369 as an *ex vivo* inhibitor of 5-HT induced platelet aggregation in whole blood (described in the addendum), is in agreement with the potency of this compound as

described in animal models (see Blackburn *et al* 1988 and appendix I for more details). The use of higher doses than the 120mg dose used in this study was constrained by toxicological concerns that ICI 169,369 may interfere with porphorin metabolism in the canine liver. This was subsequently found not to be the case in man, and may have unjustifiably restricted the scope of human studies with this compound (personal communication Dr. B. Cox ICI)

APPENDIX TO CHAPTER FOUR

As part of the assessment of ICI 169,369 its effects were investigated on the *ex vivo* platelet aggregatory response to a range of concentrations of 5-HT (0.1 to 10.0uM). This work was carried out informally as a pilot study, and involved taking a 50ml blood sample at 2hours (roughly coinciding with time to C_{max}) after dosing with single doses (80 and 120mg) of ICI 169,369 or placebo.

The method chosen was that of Heptinstall *et al* (1986) which used the whole blood platelet aggregation response to 5-HT. The reason for this was that a previous study, using platelet rich plasma, had failed to demonstrate a convincing antagonism of the 5-HT_2 mediated aggregation response by ICI 169,369. It was considered appropriate to try the whole blood method since this was more sensitive to the intial aggregatory response to 5-HT.

The results presented (Figs 1a and 1b) are by kind permission of Dr. Stanley Heptinstall of the University of Nottingham, and the work was undertaken by him as part of the experiment to examine the effects of ICI 169,369 on the waking EEG and pupillometry. Platelet aggregation is expressed as a percentage of the maximum aggregatory response to 5-HT obtained in the abscence of drug.

(FIG 1b) EFFECTS OF SINGLE ORAL DOSES OF ICI 169-369 ON 5-HT INDUCED PLATELET AGGREGATION

(% OF MAXIMUM RESPONSE)

	5-HT CONCENTRATION (μM)				
(n=6) Doses	0.1	0.3	1.0	3.0	10.0
Placebo 80mg					
x	0.68	13.68	25.58	32.05	33.55
+-s.d.	5.38	5.59	4.24	5.92	5.75
s.e.m.	2.20	2.28	1.73	2.42	2.35
Active 80mg					
x	0.57	7.15	20.30	30.5	32.67
s.d.	2.33	4.97	9.02	8.67	12.12
s.e.m.	0.95	2.03	3.68	3.94	4.95
Placebo 120mg					
x	3.60	11.00	21.05	27.2	28.65
s.d.	4.03	8.41	11.19	9.77	10.98
s.e.m.	1.65	3.43	4.57	3.99	4.48
Active 120mg					
x	3.00	8.08	18.47	27.6	33.25
s.d.	3.69	5.16	12.01	11.2	10.92
s.e.m.	1.51	2.11	4.90	4.57	4.46

(FIG iii) [5-HT] μM % PLATELET AGGREGATION

	5-HT CONCENTRATION (μM)				
Patient No.	0.1	0.3	1.0	3.0	10.0
Placebo 80mg					
1	-1.9	14.6	25.8	32.9	33.9
3	-1.6	14.4	26.7	33.0	32.9
5	11.5	23.6	32.4	39.9	43.1
7	0.1	10.3	25.8	36.0	35.3
9	-1.2	12.0	23.3	26.6	30.3
11	-2.8	7.2	19.5	23.9	25.8
Active 80mg					
1	-3.8	4.6	12.6	23.5	18.5
3	1.3	15.5	32.0	40.9	49.4
5	0.5	5.9	20.9	29.3	31.4
7	0.9	5.9	26.6	38.9	41.8
9	3.2	9.9	22.3	32.1	35.0
11	1.3	1.1	7.4	18.5	19.9
Placebo 120mg					
2	3.0	5.6	27.2	35.3	37.7
4	-2.4	-1.7	1.8	11.7	10.3
6	8.0	8.7	21.3	26.6	27.3
8	0.7	7.3	15.8	20.4	22.8
10	7.6	23.2	33.7	37.3	39.3
12	4.7	12.9	26.5	32.0	34.5
Active 120mg					
2	1.8	5.7	15.6	26.4	30.2
4	-0.7	2.9	2.3	12.2	18.6
6	4.3	3.7	18.4	24.3	34.3
8	0.6	7.9	11.9	22.7	26.5
10	2.3	12.2	25.0	35.4	39.7
12	9.7	16.1	37.6	44.7	50.1

SUMMARY OF RESULTS and CONCLUSIONS (Platelet responses)

Table 1a show the individual platelet aggregation results for each subject at each *ex vivo* concentration of 5-HT under both placebo and active drug treatment conditions. The first thing to note is the large inter-individual variability in platelet reponse to exogenous 5-HT, and that only 3 out of six subjects (subjects1, 5 and 11) showed a general reduction in aggregation to 5-HT across the whole concentration range with the 80mg dose.A similar pattern is displayed for the 120mg dose. Therefore, although there is a small reduction in the mean platelet 5-HT aggregatory response (at the lower concentrations; Fig 1b) it is not surprising to find that this did not reach statistical significance.

The main conclusion to draw from this data is that the 5-HT_2 antagonist properties of ICI 169,369 are very weak, which is in accordance with predictions from animal studies (see chapter 2). Therefore, although it is not possible to exclude an effect of ICI 169,369 at the platelet 5-HT_2 receptor, it is likely to occur at doses (and hence plasma concentrations) which are in excess of those which produce both central (EEG changes) and pupillary effects (see data in preceeding chapter).

CHAPTER FIVE

THE EFFECTS OF A SELECTIVE 5-HT_2 ANTAGONIST (ICI 170,809) ON PLATELET AGGREGATION AND PUPILLARY RESPONSES IN HEALTHY VOLUNTEERS

5.1 INTRODUCTION

In this chapter, the first administration of ICI 170,809 to man is described, together with an attempt to gain some knowledge of the pharmacological profile, both pharmacodynamic and kinetic, using single oral doses of this 5-HT_2 antagonist. Doses of 3mg up to 30mg per average 70kg man (approximately 0.04 to 0.4mg/kg) were chosen, on the basis of being 1/5th of the minimally effective dose in the most sensitive *in vivo* animal model, (antagonism of the fenfluramine induced hyperthermia in the rat) in which a 0.2mg/kg intraperitoneal dose was active (Blackburn *et al* 1988).

Subsequent to the successful completion of the single dosing study with oral ICI 170,809 a limited multiple dosing study was carried out at Hazelton Research Laboratories under the medical supervision of Dr. Alan Houston.

Human volunteer studies reported in chapter four with ICI 169,369 ((2-(2-dimethylamino ethylthio)-3-phenyl quinoline), a 5-HT_2 antagonist which is structurally related to ICI 170,809, raised the possibility that 5-HT_2 receptors may be directly involved in the neuronal control of human pupillary responses (Millson *et al* 1991). This is supported by human data where fenfluramine, a 5-HT releasing agent both topically applied and systemically administered, caused a mydriasis (Kramer and Turner, 1973) and by animal data where 5-HT_2 receptors have been identified in rabbit iris (Tobin *et al* 1988).

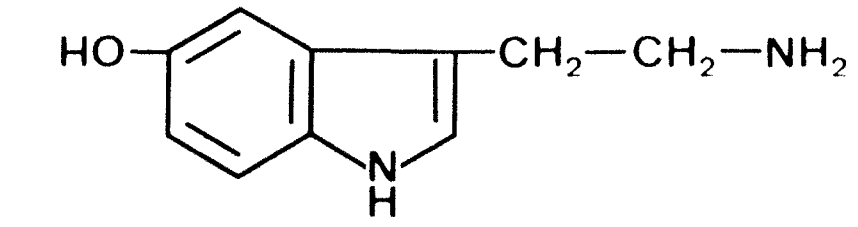
Although ICI 169,369 displayed only weak *ex vivo* activity at the platelet 5-HT_2 receptor (see addendum to chapter four), this was predictable from platelet studies for this antagonist at the circulating blood levels observed in animal studies (see Appendix I). However, because antagonism of platelet 5-HT_2 receptors had been sucessfully used to monitor pharmacodynamic effects with both ketanserin (Gotta *et al* 1987) and ritanserin (Leysen *et al* 1985), it was decided to proceed to similar studies with ICI 170,809. Furthermore, ICI 170,809 was a more potent platelet 5-HT_2 antagonist in animal models (Coker and Ellis 1990), and would therefore be predicted to show a similar pharmacodynamic profile in man.

ICI 170,809, whose structure is shown in Fig 5.1, is a potent selective 5-HT_2 receptor antagonist,which expresses its activity at vascular, neuronal and gastrointestinal 5-HT_2 receptors in both *in vitro* and *in vivo* animal models (See Blackburn *et al* 1988 and appendix II for more details)). It is necessary to demonstrate that ICI 170,809 has 5-HT_2 antagonist properties in man. Two pharmacodynamic responses will be explored together with measurements of plasma ICI 170,809 to investigate the ensuing pharmaco-dynamic/kinetic relationships.

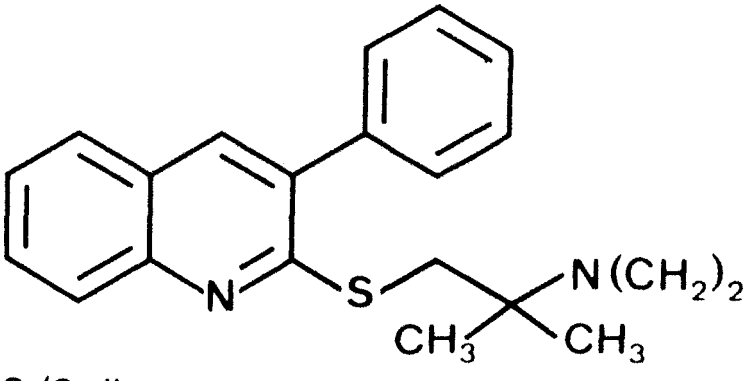
a). The *in vitro* antagonist activity of ICI 170,809 at functional 5-HT_2 receptors on human platelets (Bevan and Heptinstall, 1986; Gotta *et al* 1987), together with the duration of action of any effect, using 5-HT induced *ex vivo* platelet aggregation.

b). Changes in light induced pupillary reflexes and dark adapted resting pupil diameter resulting from dosing with ICI 170,809, together with the duration of action of any effect.

The ability to detect both neuronal effects and platelet disaggregatory properties in volunteers might provide a useful model in which to predict an active dose of 5-HT_2 antagonist at the human 5-HT_2 receptor. This would allow assessment of potential efficacy in diseases of both the cardiovascular and central nervous systems, and provide confirmatory evidence for the involvement of 5-HT_2 receptors in the control of human pupillary responses.



5 - Hydroxytryptamine (5-HT)



2-(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline hydrochloride (ICI 170,809).

FIG 5.1.

Illustrating the structure of ICI 170,809 and its relationship to 5-HT.

5.2 METHODS.

5.2.1 ASSESSMENTS

5.2.2 Platelet Aggregation Tests

Platelet rich plasma was prepared as a preliminary to examining the aggregatory effects of 5-HT. Subjects recruited into the study were bled from an antecubital vein into tubes containing tri-sodium citrate solution (3.2% w/v.one part to nine parts whole blood) and platelet rich plasma (PRP) was harvested after centrifugation (200g for 15 min.) on each of three separate occasions.

In vitro platelet aggregation was carried out using human PRP in a Payton aggregometer. Aliquots (250ul) of PRP were stirred (900rpm) and incubated ($37^{o}C$) for 60 seconds in an aggregometer before ICI 170,809 or vehicle were added. Platelets were incubated for a further 60 seconds and a single concentration of 5-HT was then added. This procedure was repeated using a range of 5-HT concentrations (5×10^{-9} to 5×10^{-5} M). The extent of 5-HT induced aggregation was measured and expressed as a percentage of the maximum obtained with vehicle controls (Fig 5.2).

Ex vivo platelet aggregation was performed on PRP from volunteers following oral dosing with either ICI 170,809 or placebo. Aliquots of PRP (250ul) were incubated for 120 seconds in an aggregometer (900rpm. $37^{o}C$) before concentrations of 5-HT were added. The extent of 5-HT induced aggregation, following oral dosing with ICI 170,809 or placebo, was expressed as a percentage of the mean individual maximum platelet aggregation response as previously described (Fig 5.2).

5.2.3 Pupil Responses

A "pupilscan" hand held infra-red pupillometer (Millson, 1988) was used to measure dark adapted resting pupil diameter (RPD) after 15 minutes dark adaptation by wearing dark goggles (BS 6975EW). A 0.5 second (565 NM; 36 CDM^{-2}) light stimulus induced pupillary reflex, minimally light constricted (MPD) and recovered (FPD) pupil diameter after a 3 s cycle were measured together with dynamic pupillary changes every 10 ms. A "pupil scan " data analysis template was used to analyse the maximum velocity (mm s-1) and acceleration (mm s-2) associated with the pupillary constriction.

5.2.4 Plasma Assay Of ICI 170,809

These assays and subsequent pharmacokinetic analysis were carried out by Dr. A. Swaisland (ICI Pharmaceuticals).

A 7ml sample of venous blood was taken into a tube containing oxalate as anticoagulant and, after thorough mixing was centrifuged at 2000 rpm for 10 minutes. The plasma was decanted off and stored at -20°C prior to assay.

Following thawing of stored plasma a 1ml aliquot was mixed with 1ml pH 10 buffer and agitated with 10ml 1.5% amyl alcohol in hexane for 15 minutes. Centrifugation was carried out to separate the phases, 9ml of the solvent layer was transferred to a tube containing 2ml 0.1N hydrochloric acid and after shaking and centrifugation the solvent layer was discarded. The aqueous layer was mixed with 2.5ml 0.1N sodium hydroxide to which 10ml 1.5% amyl alcohol was added. After mixing and centrifugation 9ml of the solvent layer was evaporated to dryness at 40°C. The residue was

redissolved in 0.2ml eluent (20% pH 4.0 triethylamine buffer (0.28%): 80% acetonitrile) and aliquots were analysed by high pressure liquid chromatography (HPLC) at a 1.0ml/min flow rate using a 10cm x 3mm (internal diameter) Hyperil ODS column with detection by ultraviolet absorbtion at 259nm.

A calibration series covering the expected sample concentration range was constructed for each set of analyses. The unknown samples were determined by a least square fit calculation from the standard curve. The limit of quantification was 2.0 ng/ml with an intra-assay coefficient of variation of <10% over the concentration range 2.0 -100 ng/ml.

5.2.5 Determination of pharmacokinetic parameters for ICI 170,809.

Data from plasma concentration time plots were subjected to a log linear plot using an iterative curve fitting programme. The completion of the absorbtion/distribution phase was determined by interpolation, which was followed by the terminal beta elimination, from which the elimination rate constant and half life were determined. Area under the concentration time curve (AUCinf) was determined by integration following extrapolation to infinity. Mean plasma concentration time plots (with standard error of the mean) are depicted in figure 5.7.

5.2.6 Statistical Methods

A multifactor analysis of varience was used to analyse pupillometry and platelet data using the following terms in the analysis: Volunteer, Treatment and Time after dosing. The main objective of this repeated measures analysis was to test whether significant differences were detectable in measurements of 5-HT induced platelet aggregation and pupil diameter parameters, between the placebo and groups treated with ICI 170,809 at various times after dosing. The Greenhouse Geisser

correction factor for repeated measures analysis was applied. If a statistically significant ($p<0.05$) difference was found between the profiles, then analysis of covariance was performed separately at each timepoint, using the pre-dose value as the covariate.

A least square regression analysis was used to examine the correlation between pharmacodynamic parameters, such as pupil diameter or percentage change in platelet responses to 5-HT, and pharmacokinetic measurements, *i.e.* plasma concentrations of ICI 170, 809. The 95% confidence intervals (CI) for the line of best fit with 95% CI for the parameter estimate are displayed graphically together with associated correlation coefficient, slope and intercept values (see Figs 5.4 and 5.8).

Differences in velocity and acceleration between placebo and ICI 170,809 treated volunteers at 3hr after dosing, were analysed using values derived from the median pupillary light constriction curves. Median curves were plotted for each volunteer, and were summated to give a mean 3hr (+/-sem) curve for each dose.

5.2.7 Volunteer Selection and Study Design

The study was a double blind, randomised, ascending dose, partial cross over design in which 8 male volunteers (aged 18-45 years, weighing between 63 and 91Kg) received three out of a possible four single oral doses (3, 7, 15 and 30mg) of ICI 170,809, or placebo on four dosing occasions 7 days apart.

This study was part of the phase I assessment of ICI 170,809, in which both dynamic and kinetic data were considered to be hypothesis generating. Therefore, no formal power calculations were performed. Empirical observations from previous studies with ICI 169,369 suggested that the subject

numbers employed were adequate to ensure that this study would be unlikely to fail to detect biologically significant changes in the parameters measured.

Before commencing the study, the protocol was approved by the ICI research ethics committee and written informed consent was obtained. Volunteers were only allowed to proceed into the study if no abnormalities were found after medical examination, laboratory blood and urinary safety screens.

TABLE 5.1

STUDY DESIGN AND COMPOSITION OF EACH DOSE

WEEK OF STUDY.	DOSE OF ICI 170,809	SUBJECT NUMBERS	NUMBER OF TABLETS ICI 179.809				Placebo
			1mg	5mg	15mg	30mg	-
1	3mg	6	3	-	-	-	-
	placebo	2	-	-	-	-	3
2	7mg	6	2	1	-	-	-
	placebo	2	-	-	-	-	3
3	15mg	6	-	-	1	-	2
	placebo	2	-	-	-	-	3
4	30mg	6	-	-	-	1	2
	placebo	2	-	-	-	-	3

5.2.8 Protocol Followed

ICI 170,809 was administered orally to volunteers who had fasted overnight. *Ex vivo* platelet sensitivity to 5-HT, measured as the extent of aggregation, was determined on three separate occasions before, and 2, 5, 8 and 24 hrs after oral administration of ICI 170,809 or placebo.

Infra-red pupillometry was performed at 3, 5, 8 and 24 hours after dosing, 6 consecutive artefact free recordings were made and mean values derived for resting (RPD), light constricted (MPD) and the final recovered (FPD) pupil diameter. Except for the 3mg dose (where sampling was limited to 24hours) venous blood sampling for estimation of ICI 170,809 plasma concentration was performed predose and at 1, 1.5, 2, 3, 5, 8, 12, 15, 24, 30 and 48 hours after dosing.

Because of a technical failure with "Pupilscan" on week two "Polaroid" pupillometry was substituted from week 2 of the study onwards.By week three the equipment had been repaired and for weeks three and four both methods were employed to measure resting pupil diameter.

5.3 RESULTS

5.3.1 Effects of ICI 170,809 on *in vitro* platelet aggregation

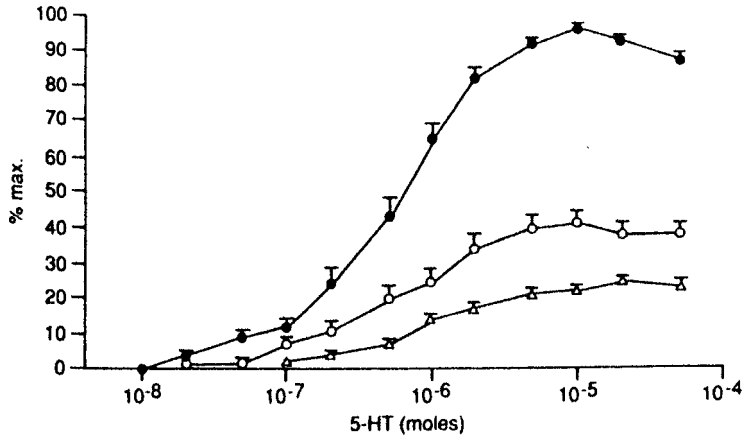
The percentage maximum aggregation achieved with 10^{-5}M 5-HT was reduced to less than 40% with both 10^{-7}M and 4 x 10^{-7}M concentrations of ICI 170,809, compared with the vehicle when added to PRP *in vitro* (See Fig 5.2). The dose response curve to 5-HT was significantly depressed at all concentrations of 5-HT with no evidence of a parallel shift ($p<0.05$ for 5-HT concentrations 10^{-8}M to 10^{-7}M and $p<0.001$ above 10^{-7}M).

5.3.2 Effects of ICI 170, 809 on *ex vivo* platelet aggregation

When dosed orally to human volunteers ICI 170,809 caused dose dependent inhibition of 5-HT induced platelet aggregation *ex vivo* (Fig 5.3). ICI 170,809 (3mg) did not significantly ($p>0.05$) modify aggregation responses. Two hours after administration of higher doses of the drug (7mg, 15mg, and 30mg) attenuation of 5-HT induced platelet aggregation was evident yielding mean (+/- s.e.) percentage inhibitions of 59.5 (+/- 4.) 6, 73.8 (+/- 4.2) and 82.4 (+/- 4.0) respectively, which were significantly different from placebo ($p <0.05$). This activity persisted for 5 hours with 7mg and 15mg doses (mean +/- s.e percentage inhibition) of 42.4 (+/-4.8) and 54.6 (+/-4.2) respectively. The minimal effective oral dose in human volunteers was 7mg (approximately 0.1 mg/kg). Platelet data from the 30mg dose of ICI 170,809 was not collected beyond two hours after dosing since this dose was considered to be in excess of the anticipated clinical doses and was included principally to provide pharmacokinetic data.

5.3.2.1 **Platelet aggregation as a function of ICI 170,809 concentration.**

5-HT_2 induced platelet aggregation (5 x 10^{-6}M) was significantly correlated ($r = 0.797$, $p<0.0001$, slope = -46.30, intercept = 106.43) with log concentrations (ng/ml) of ICI 170,809 (Fig.4).



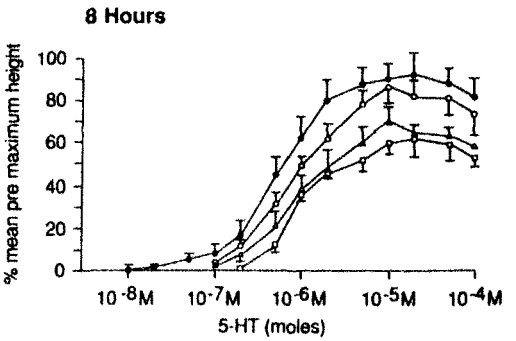
5-HT induced aggregation of human PRP in the presence of ICI 170,809 : 10^{-7}M (O—O, n=8) ; $4x10^{-7}$M (Δ—Δ, n=7) and vehicle (●—●,n=8).

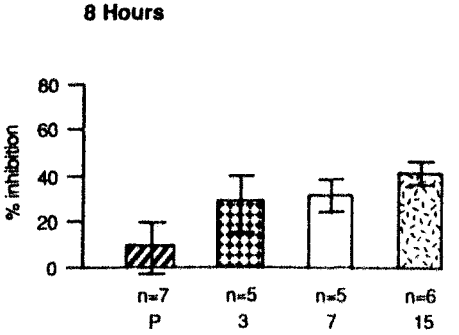
FIG 5.2.

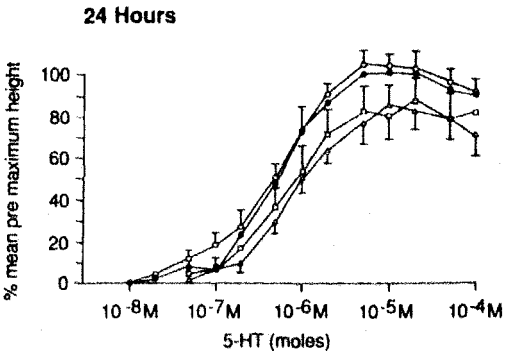
5-HT induced platelet aggregation of human platelet rich plasma in the prescence of ICI 170,809 (10^{-7}M (○ , n=8);4 x 10^{-7}M (△ ,n=7) and vehicle (● , n=8). The extent of the 5-HT induced aggregation was expressed as a percentage of the mean maximum aggregation response obtained with vehicle on three occasions prior to dosing.

FIG 5.3.

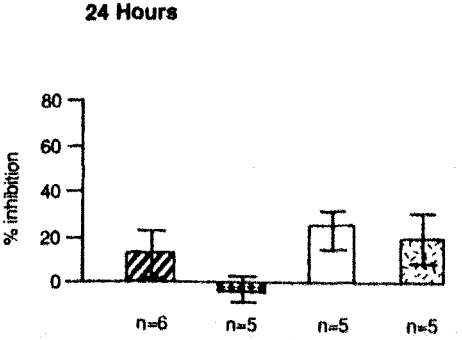
The effects of ICI 170,809 on *ex vivo* 5-HT induced human platelet aggregation at 2, 5, 8 and 24 hours after single oral dosing with drug or placebo.Data are expressed as inhibition of the mean maximum aggregation response to 5-HT in the presence of vehicle, on three occasions prior to dosing (see Fig 5.2). The histograms depict the maximum inhibition achieved for each treatment at 2, 5, 6 and 24 h with appropriate p values for values significantly different from placebo.





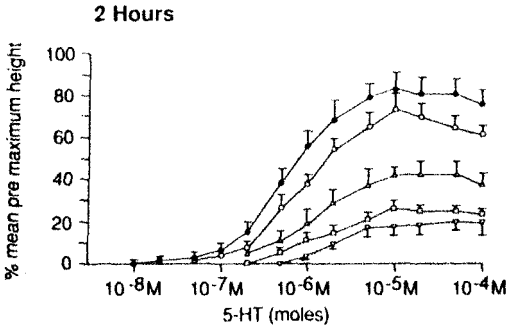


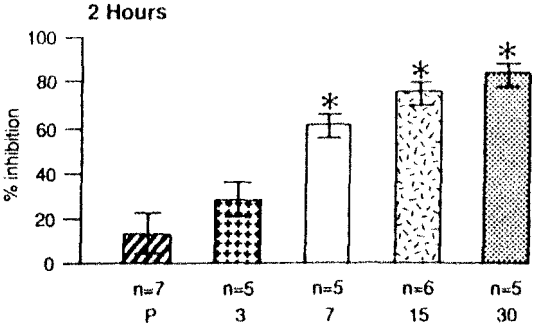
Effect of orally dosed ICI 170,809 : 3mg (o—o, n=5) ; 7mg (Δ—Δ, n=5) ; 15mg (□—□, n=6) ; 30mg (▽—▽, n=5) and placebo (●—●, n=7) on 5-HT induced platelet aggregation.

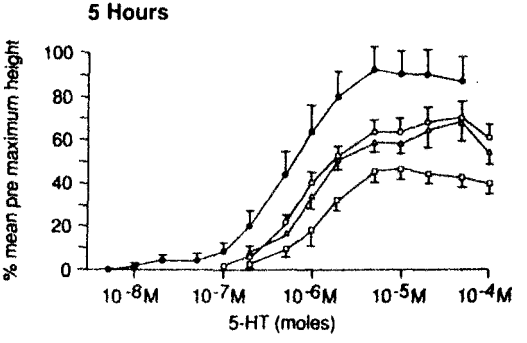


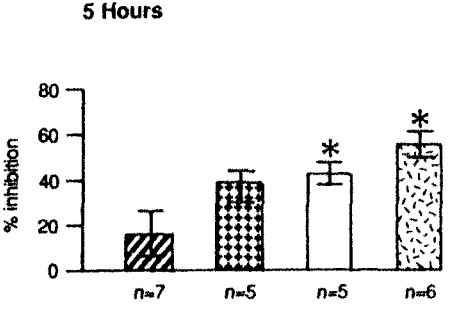
* p<0.02

Effect of ICI 170,809 : 3mg ( ) ; 7mg( ) ; 15mg ( ) and placebo ( ) on 5-HT induced platelet aggregation 8 & 24 hours after a single oral dose.


2 Hours
% mean pre maximum height
100
80
60
40
20
0
10 -8M
10-7M
10-6M
10-5M
10-4M
5-HT (moles)


2 Hours
% inhibition
100
80
60
40
20
0
*
*
*
n=7
n=5
n=5
n=6
n=5
P
3
7
15
30


5 Hours
% mean pre maximum height
100
80
60
40
20
0
10 -8M
10-7M
10-6M
10-5M
10-4M
5-HT (moles)


5 Hours
% inhibition
80
60
40
20
0
*
*
n=7
n=5
n=5
n=6

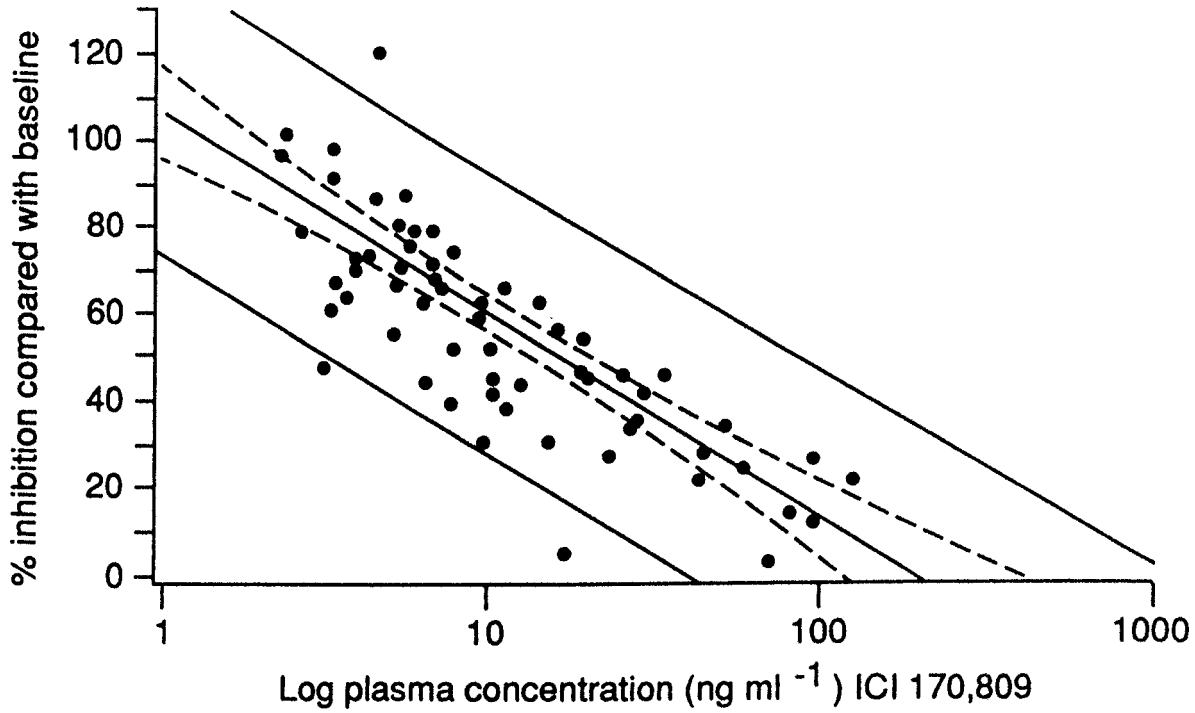


FIG 5.4.

The relationship between 5-HT induced platelet aggregation (5 x 10^{-6}M) expressed as percentage inhibition compared with baseline values,versus log plasma (ng/ml) ICI170,809 after oral dosing ($r=0.797$, $p<0.0001$, slope=-46.30, intercept=106.43). The least squares regression line is given with 95% confidence intervals for the line of best fit (-----) and parameter values.

5.3.3 Effects of single oral doses of ICI 170,809 on pupillary responses.

When dosed orally to human volunteers 15 and 30mg doses produced a dose dependant pupillary miosis (Fig 5.5) when measured by both "Pupilscan" and "Polaroid" pupillometry. No significant baseline changes were evident when pre-treatment groups were compared with placebo. Data from the 7mg dose was not available for "Pupilscan" due to a technical failure with the infrared sensing equipment on week 2 of the study.

A significant ($p < 0.01$) reduction in mean RPD and FPD occured at 3 and 5 hours after dosing, with reductions in vertical pupil diameter of up to 50% compared with placebo. MPD was also significantly reduced ($p<0.01$) by approximately 50% with 15 and 30mg doses of ICI 170,809 at 5 hours after dosing. Significant reductions in RPD, MPD and FPD were still present at 8 hours ($p<0.05$) with recovery to baseline values occurring 24 hours after dosing.

Mean pupil light response curves (Fig 5.6) derived from summation of median plots from each of 8 volunteers show a reduction in reflex amplitude together with a shift in RPD. Analysis of the kinetics of pupillary light constriction following oral ICI 170,809 demonstrated a reduction in both the velocity and acceleration of pupillary constriction in an dose dependent manner (Table 5.1).

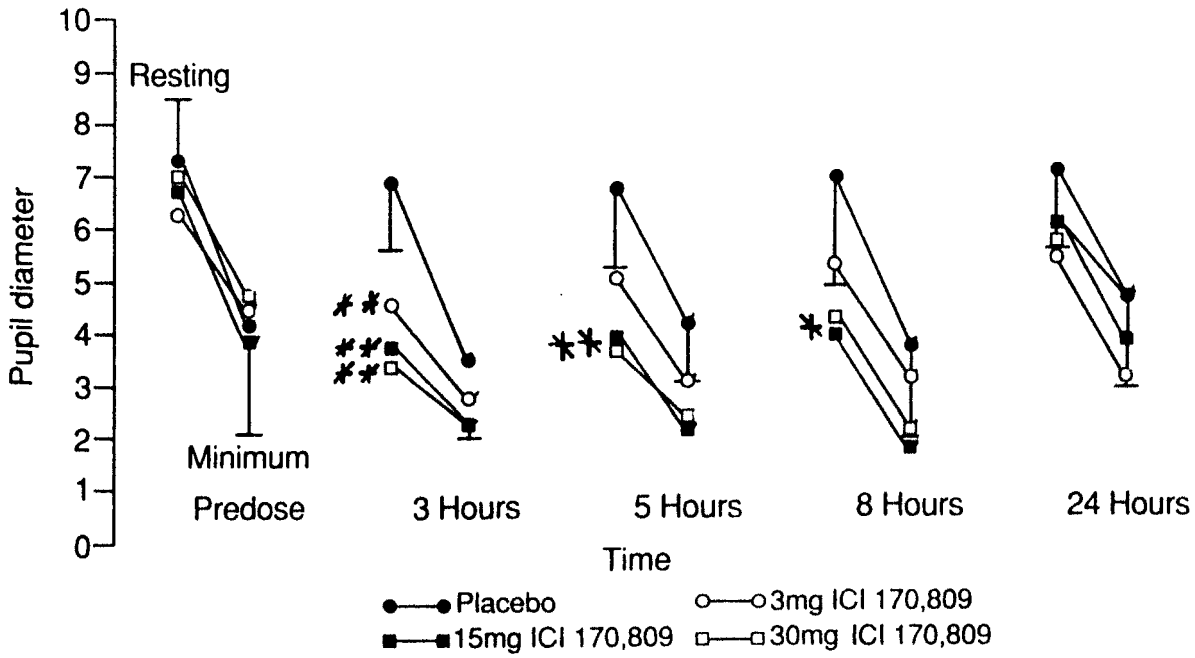


FIG 5.5.

Effects of ICI170,809 (○3,■15,□30mg and placebo●) on the mean (+/- 95% confidence intervals) for treatment differences from placebo,for resting pupil diameter (RPD),minimally constricted (MPD), Infra-red readings using "Pupilscan" were taken predose,and at 3, 5, 8 and 24 h after dosing. Significant differences from placebo are highlighted with appropriate p values. ($p < 0.01 = **$, $p < 0.05 = *$).

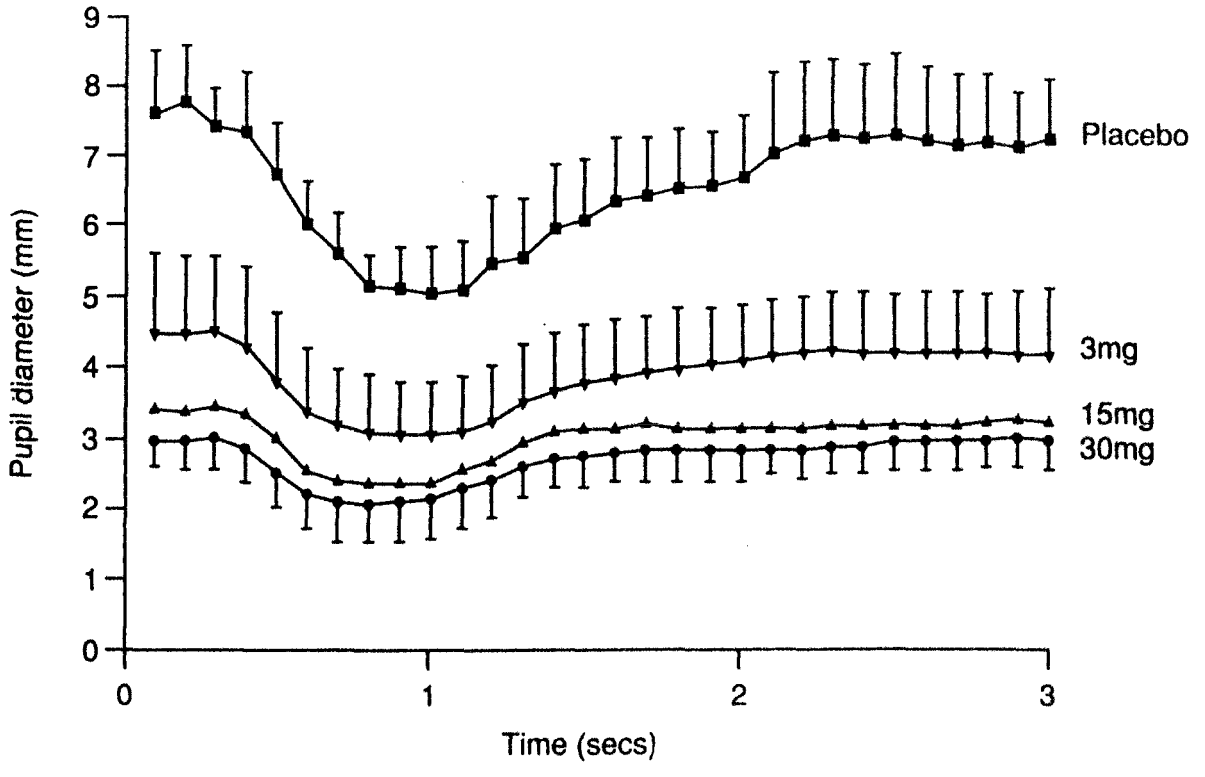


FIG 5.6.

The effects of ICI 170,809 (▼3, ▲15, ● 30mg, or placebo ■) on pupillary response curves.Each curve represents the change in mean dark adapted vertical pupil diameter (mm +/-,sem) from resting values at 3 h after dosing, (using data from the median curves in 8 subjects) following a light stimulus (0.52, 565nm, 36 cd M^{-2}) and with reflected infrared recordings every 10 ms.

Treatment	Maximum Constriction Velocity (mms^{-1}) and Acceleration (mms^{-2})					
	Mean	SE	CI	Mean	SE	CI
Placebo	7.87	1.2		64.9	13.9	
ICI 170,809 3mg	5.68	0.9	1.25	48.0	8.9	22.26
15mg	3.93	0.5	0.66**	47.0	10.4	22.62
30mg	4.00	0.4	0.58**	24.2	1.2	8.64**

CI = 95% CI for the differences from placebo mean. ** = $p<0.01$

TABLE 5.1.

Illustrating the maximum velocity and acceleration associated with the light induced pupillary constriction at 3 h after dosing with ICI 170,809 (3,15 and 30mg or placebo) expressed as the mean (+/- sem) and 95% confidence intervals for the difference between drug treatment and placebo with associated p values.

5.3.4 Effects of single oral doses of ICI 170,809 on alertness as assessed by VAS ratings.

Doses of ICI 170,809 from 3 to 15mg were well tolerated and no significant increase in sedation was documented relative to placebo.(see Fig 5.6.1).

5.3.5 Effects of single oral doses of ICI 170,809 on static "Polaroid" Resting pupil diameter.

Resting pupil diameter estimates derived from "Polaroid" prints showed the same pattern of results(see Fig 5.6.2) as the RPD measurements made by "Pupilscan". The data with the 7mg dose is not complete and represents results from only four out of the possible six subjects. Similarly the placebo data is incomplete representing seven out of a possible eight subjects. No data for the 7mg dose with "Pupilscan" was available because of instrument failure on the day of the study.

Therefore, the 7mg data was ignored because of the differences in predose RPD compared with the other groups.Only data with the 15 and 30mg doses was considered evaluable. The magnitude (2mm) and duration of the observed pupillary miosis was consistent, being greater than 8 hours in both cases.

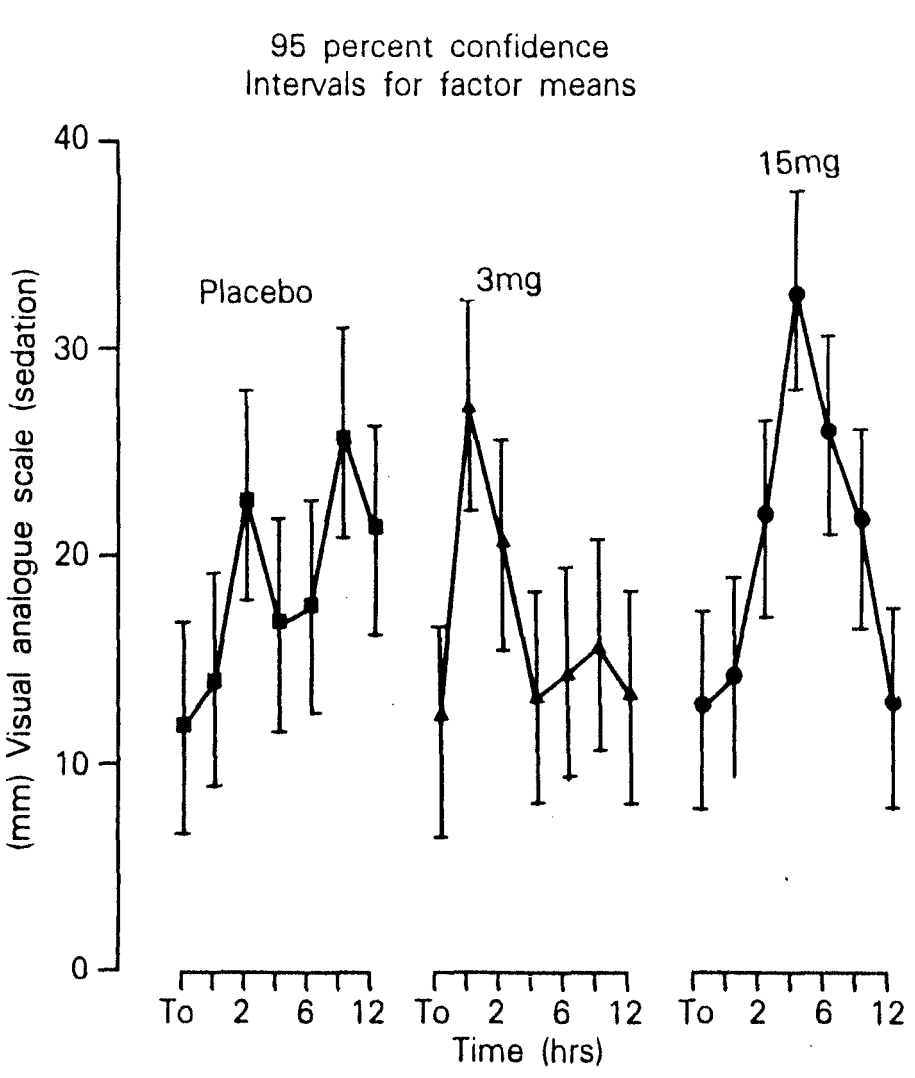


FIG 5.6.1.

The effects of single oral doses of ICI 170,809 on a visual analogue sedation score (mm). Mean results with placebo (■), 3.0 (▲) and 15mg (●) doses are given with 95% confidence intervals for predose (T_0) and at various intervals up to 12 h post dose.

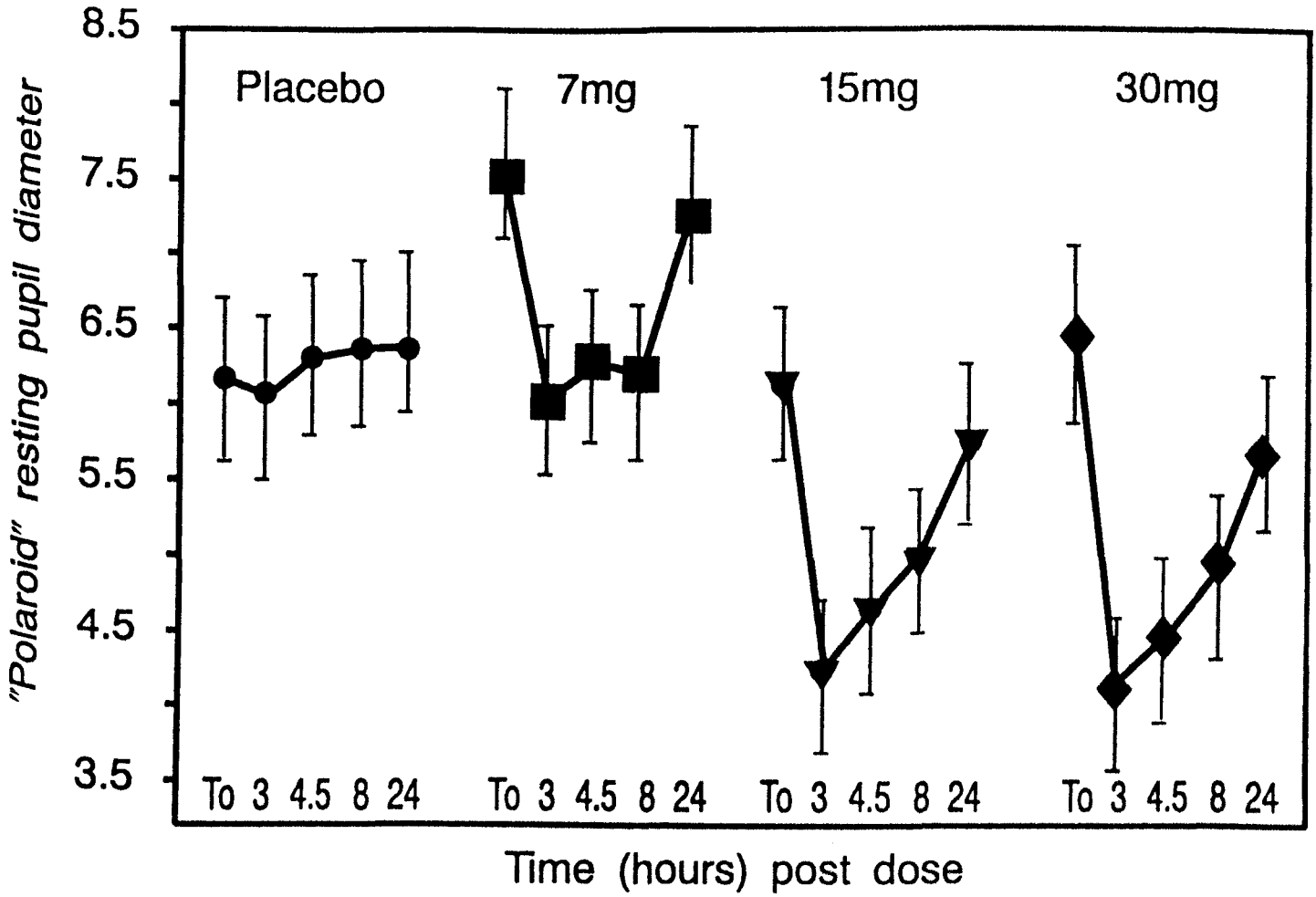


FIG 5.6.2

The effects of single oral doses of ICI 170,809 on dark adapted resting pupil diameter measured by "Polaroid pupillometry" for placebo (●), and drug, 7.0(■), 15.0 (▼) and 30mg (◆) doses at predose (T_0) and at 3.0, 4.5, 8.0 and 24 hours post dose. Mean values are given with 95% confidence intervals.

5.3.6 Pharmacokinetic analysis

Pharmacokinetic profiles for plasma ICI 170,809 with respect to time after dosing are given in Fig 5.7, together with the derived pharmacokinetic parameters in Table 3. Insufficient data was generated for the 3mg dose to calculate all parameters. The absorbtion phase was similar for all doses, with a median time to peak concentration of 2hrs (range 1-3hrs). The rise in peak plasma concentration (C_{max}) was approximately linear with increased dose, whereas the area under the concentration time curve displayed non-linearity above 7mg dose. The elimination phase was variable and became more prolonged with increasing dose. After the peak, the concentrations declined rapidly until about 15 hours post-dose, following which there was a slower decline or beta phase.

Pharmacokinetic data after a single daily (7mg) dose and after 21 days dosing (7mg twice daily) are shown in Table 5.2 and 5.3. The elimination half life is not significantly changed, but there is a suggestion of some accumulation reflected in the extended AUC (0-inf).

5.3.6.1 Duration of both pupillary and platelet effects following multiple dosing with ICI 170,809(7mg once daily for 3 days and twice daily for 19 days).

Compared with placebo (n=4) the eight subjects who received 7mg ICI 170,809 twice daily showed a pupillary miosis on day one at 3 and 12 hours after dosing compared with both placebo and predose readings. Similarly after eight days dosing a pupillary miosis was observed. The miosis was small (about 1mm) and was not detectable after 21 days dosing.

TABLE 5.2

PHARMACOKINETIC PARAMETERS FOR ICI 170,809 AFTER SINGLE ORAL DOSES

DOSE (mg)	C_{max} (ng/ml)	$T^1/_2$ (dist) (hours)	$T^1/_2$ (el) (hours)	AUC inf (ng.h/ml).
3.0	8.0 (1.5)	NC	NC	NC
7.0	15.9 (3.0)	2.6 (0.8)	18.0 (5.5)	197.0 (47)
15.0	37.7 (7.0)	2.6 (0.2)	40.0 (8.6)	1030.0 (388)
30.0	104.0 (2.6)	3.5 (0.2)	43.0 (7.8)	2259.0 (807)

values are expressed as the mean $^+/_-$ standard error of mean.

C_{max} = peak plasma concentration achieved.

$T^1/_2$ (dist) and $T^1/_2$ (el) are the distribution and elimination half lives respectively.

AUC inf is the area under the plasma concentration time curve extrapolated to infinity

TABLE 5.3

SUMMARY OF PHARMACOKINETIC DATA WITH SINGLE (7mg for three days) AND MULTIPLE ORAL DOSES (7mg B.D for 19 days) OF ICI170,80

(HAZLETON STUDY)

	DAY 1.	DAY 21.	
C_{max}	14.3 (3.0)	31.6 (12.2)	(ng/ml)
AUC (0-12)	101.0 (26.6)	273.7 (118.9)	(ng.h/ml)
AUC (0-inf)	246.4 (85.3)	1108.2 (620)	(ng.h/ml)
T_{max}	2.6 (0.9)	2.3 (0.6)	(h)
$T^1/_2$	33.4 (8.1)	37.9 (9.2).	(h)

Values are mean (standard deviation).

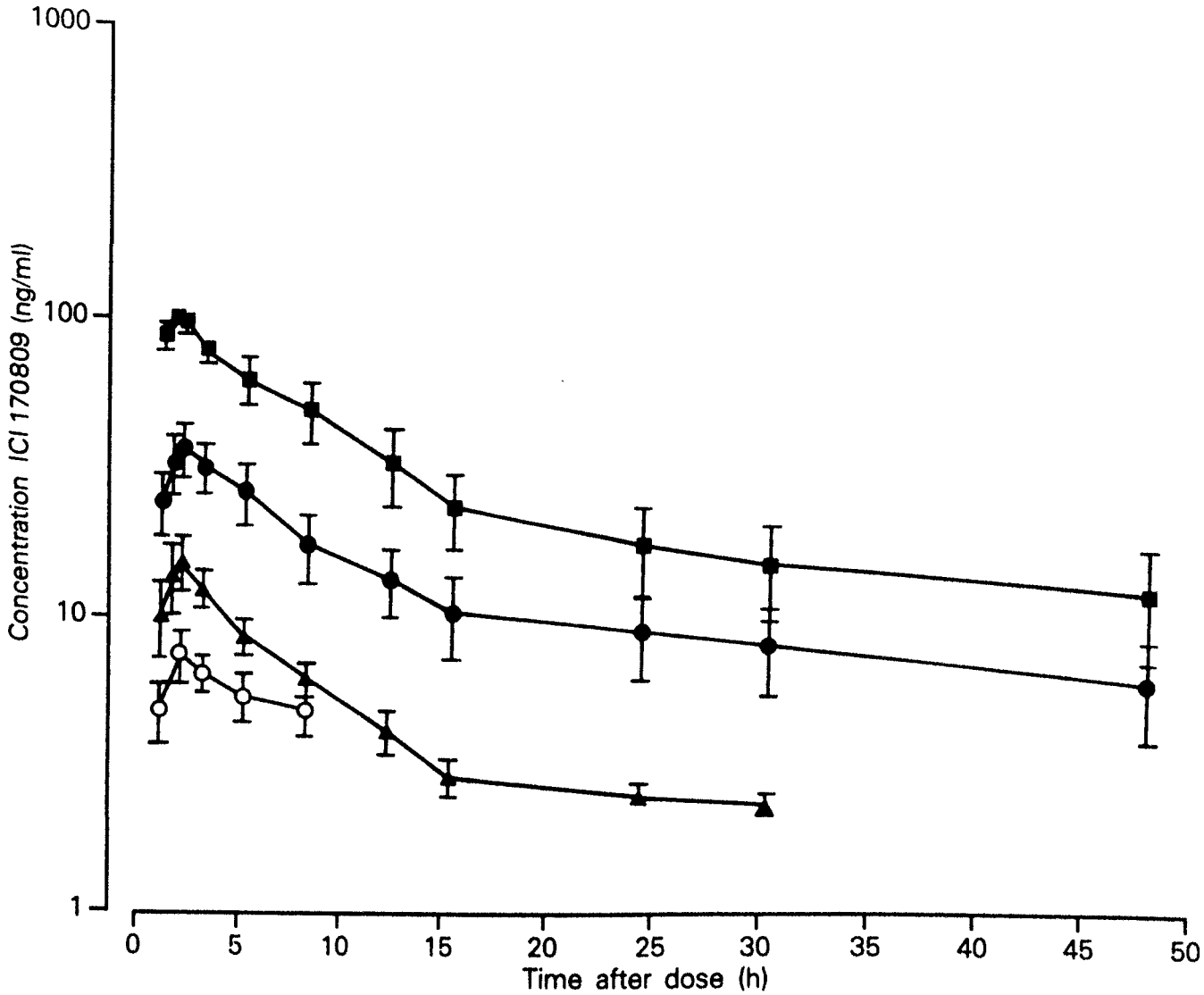


FIG 5.7.

Pharmacokinetic profiles over a 50 h period after treatment with single oral doses of ICI 170,809; 3 (○), 7 (▲) and 30mg (■). Values are shown as the mean and standard error of the mean (n=6).

Statistical analysis showed a significant overall regression on plasma concentration of ICI 170,809 and pupillary miosis relative to baseline on day 1 (p=0.049) but not on day 21 (p=0.568). When baseline values on days 1 and 8 were compared, no statistically different treatment-related differences were seen. The changes from baseline on days 1 and 8 showed significant differences between groups at 3 hours post-dose (p=0.002 and p=0.034 respectively), with volunteers on active drug showing a reduction in diameter, while the differences were not significant at 12 h post dose on all three days and at 3 h post-dose on day 21.(FIG. 5.9.6).

5-HT induced platelet aggregation was significantly reduced by ICI 170,809 on both days 1 and 21, with a highly significant regression for plasma concentration of ICI 170,809 (p=0.0004 and p=0.0002 respectively). Figs 5.9.0 to 5.9.2 show the right-ward shift compared to placebo on day 1, at 2, 8 and 24 hours after dosing, and on day 21, at 2, 8 and 24 hours after the last dose. There was evidence of pharmacodynamic activity for at least eight hours after dosing on both day 1 and day 21, which was no longer evident at 24 hours on day 1, but which was still apparent at 24h on day 21. This may reflect a certain degree of accumulation after multiple dosing at this dose and frequency of dosing.(FIGS 5.9.3 to 5.9.6).

5.3.7 Pupillary miosis as a function of plasma ICI 170,809 concentration.

Percentage changes in resting pupil diameter, compared with baseline values, were linearly related (Fig 5.8) to log plasma concentrations (ng/ml) in a similar manner to the platelet aggregatory response ($r = 0.55$, $p<0.0001$, slope =- 26.13, intercept = 98.89).

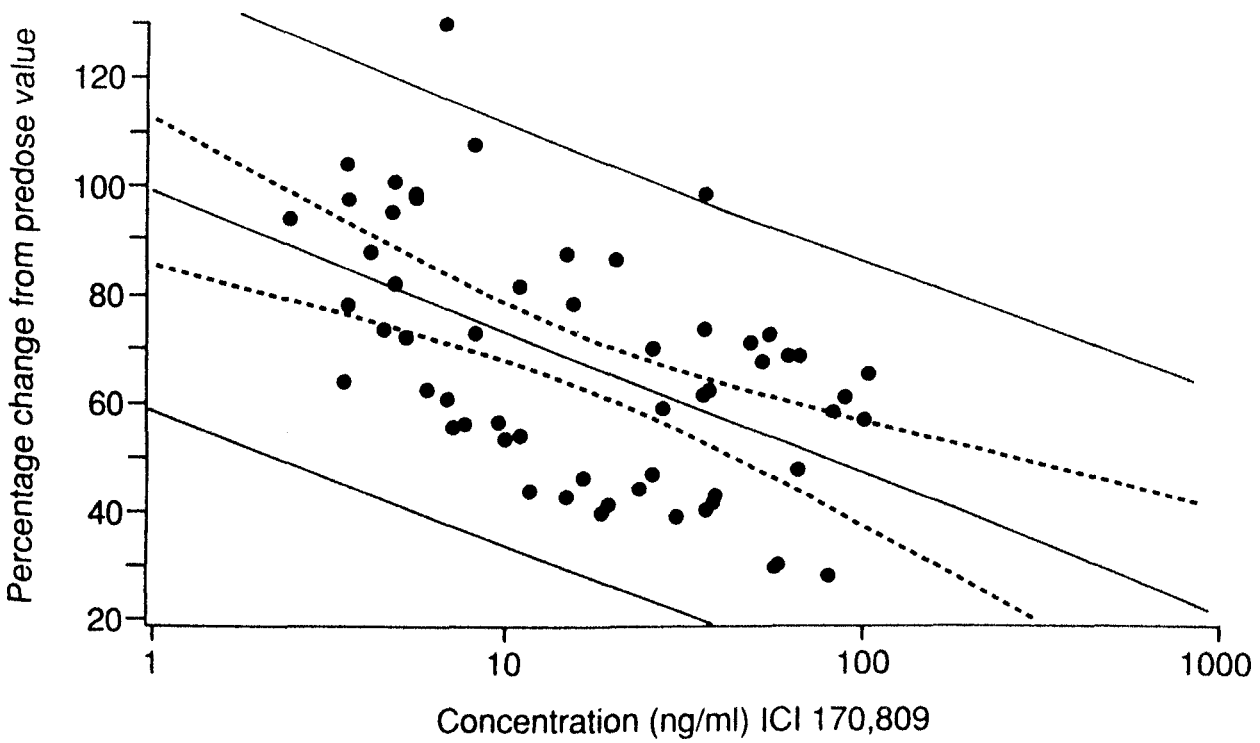
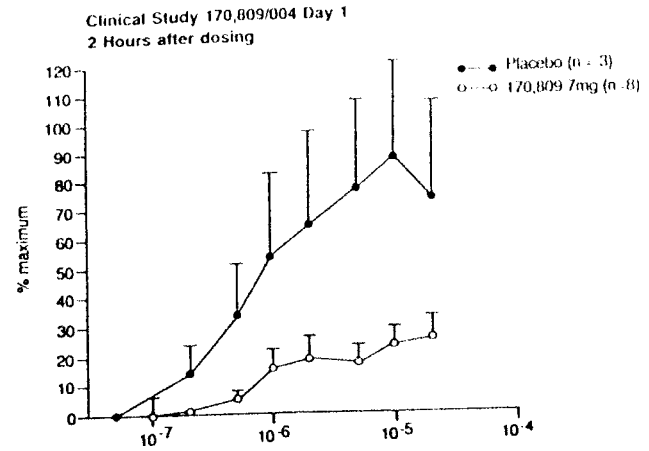
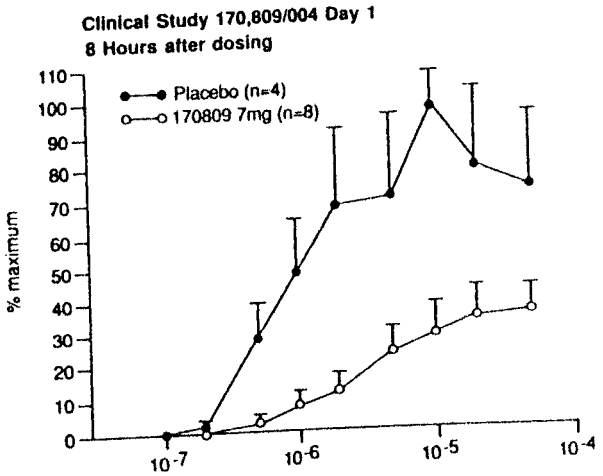
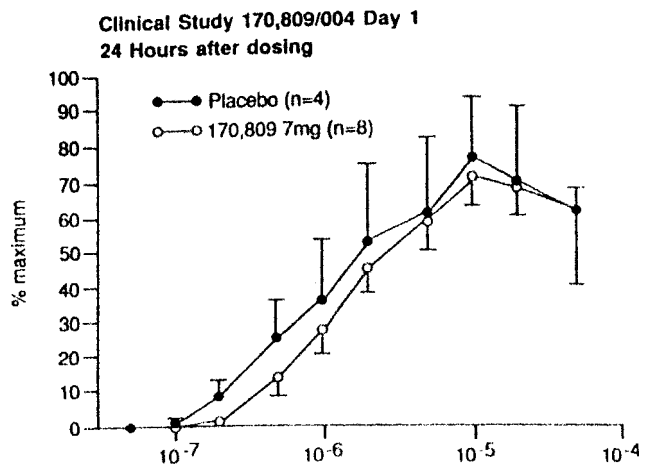


FIG 5.8.

The relationship between resting pupil diameter (RPD) expressed aas a percentage change from baseline values, versus log concentration (ng/ml) following single (3,15, & 30mg) oral doses of ICI 170,809. The least squares regression line is given ($r=0.55$, $p<0.0001$, slope=-26.13, intercept =98.89) with 95% confidence intervals for the fitted line (-----) and parameter estimates.

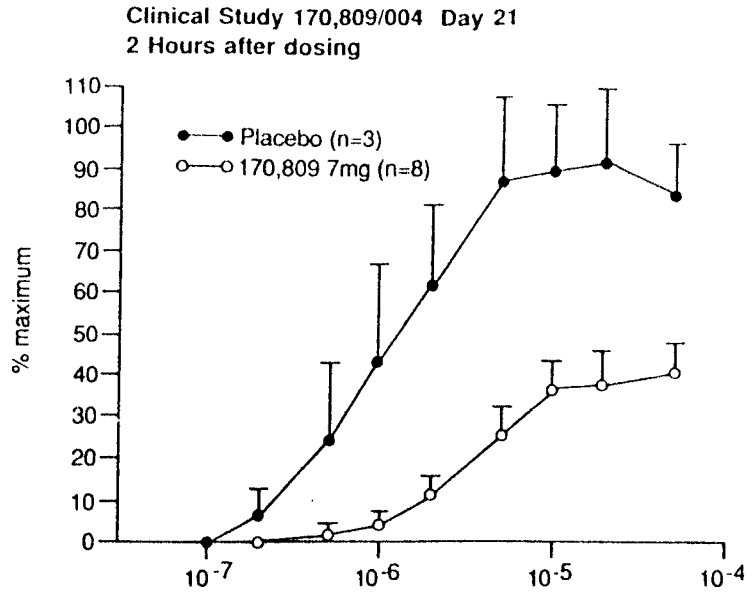






FIGS 5.9.0 TO 5.9.2.

The effects a of single (7mg) oral dose of ICI 170,809 in eight healthy male subjects on 5-HT induced platelet aggregation. This was assessed by a percentage change in platelet aggregation to a range of 5-HT concentrations relative to the mean maximum aggregation achieved on three occasions prior to dosing. Data from 3-4 subjects who received a matching oral placebo are included for comparison.



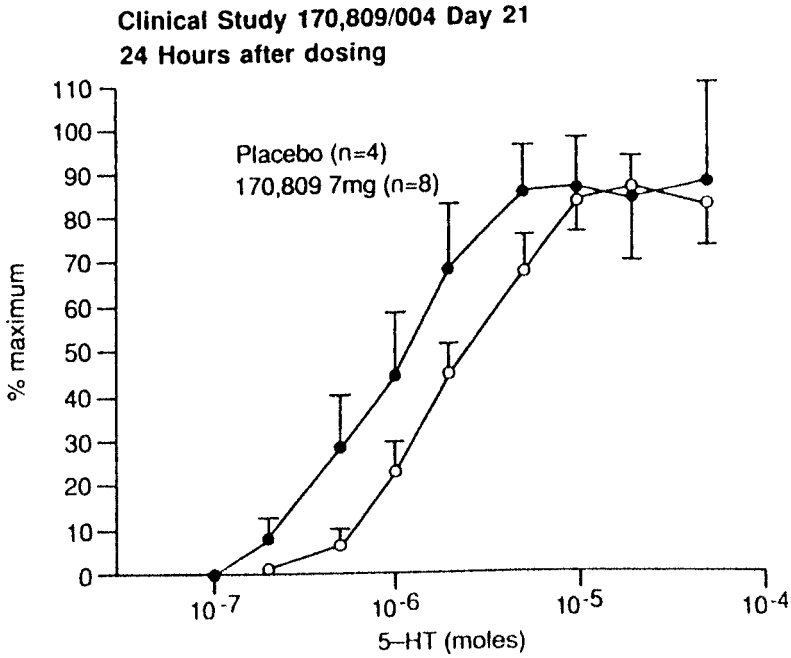
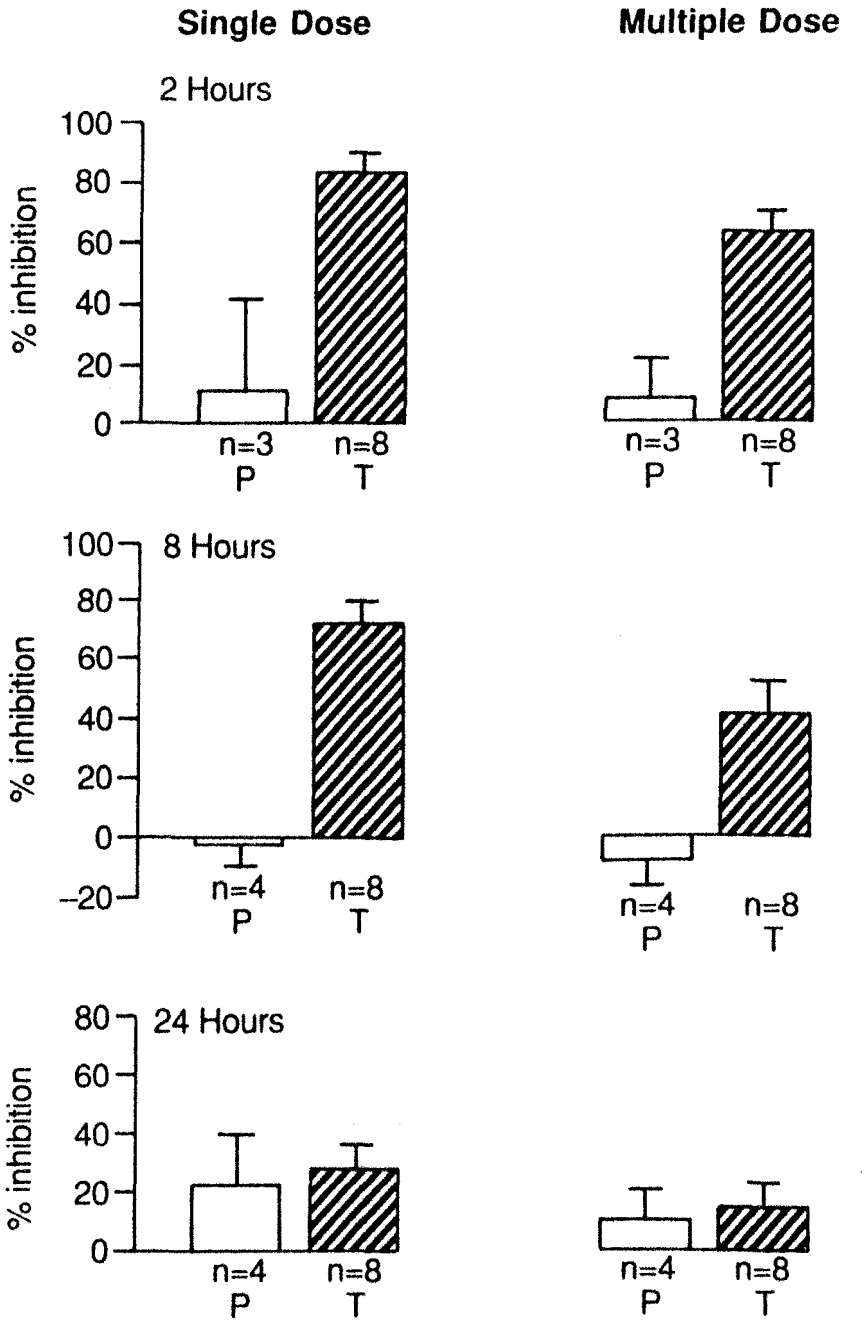


FIG 5.9.3 TO 5.9.5

The effects of multiple (7mg single dosing for three days;followed by 12 hourly for 21 days) oral dosing with ICI 170,809 in eight healthy male subjects on 5-HT induced platelet aggregation. This was as assessed by a percentage change in platelet aggregation to a range of 5-HT concentrations, relative to the mean maximum aggregation achieved on three separate occasions prior to dosing. Data from 3-4 subjects who received a matching placebo are included for comparison.

Fig. 5.9.5

Effect of ICI 170,809 on 5–HT Induced Human Platelet Aggregation Following a Single Oral Dose (7mg) and Multiple Dosing (7mg b.i.d x 14 days)



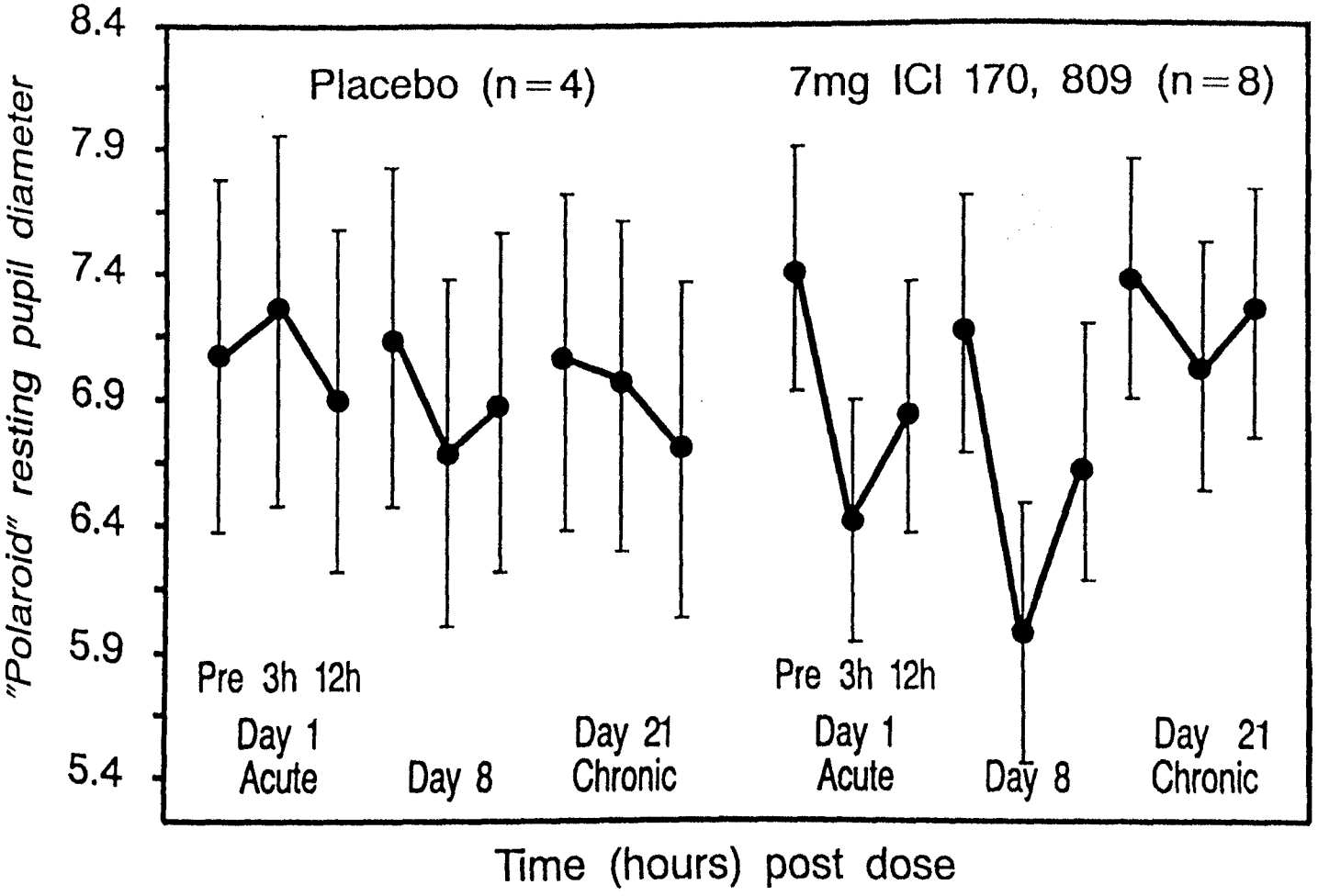


FIG 5.9.6

The effects of multiple doses of ICI 170,809 on dark adapted pupil diameter on day 1,8 and 21 of treatment.Pupil size was measured by "Polaroid photography" for placebo (n=4) and ICI170,809 treated for 3 days with single 7mg oral doses, and for the remaining 21 days with 7mg given twice daily. Pupil assessments were made prior to and at 3 and 12 hours after dosing with either ICI 170,809 or matching placebo.

5.3.8 Effects of multiple dosing on Bond-Lader visual analogue scores.

The three Bond-Lader factor scores were derived as described above and were administered at the same intervals as the pupil and platelet tests. None of the three scores showed a significant overall regression on plasma concentration of ICI 170,809. When values on days 1, 8 and 21 were compared with placebo, no statistically significant treatment-related differences were seen. There were no significant treatment-related effects in the changes from baseline.

Bond Lader scores for placebo treatment, on day 8 of dosing (n=4 subjects) were 36.8 $^{+}/_{-}$ 17.5 (alertness;mean $^{+}/_{-}$ standard deviation) 18.4 $^{+}/_{-}$ 7.8 (contentedness) and 6.2 $^{+}/_{-}$ 3.3 (calmness); the corresponding values were 42.0 $^{+}/_{-}$ 17.7, 16.7 $^{+}/_{-}$ 7.0 and 6.7 $^{+}/_{-}$ 2.4 on day 20 of dosing, with both measurements being made at 4h post-dose.Bond Lader scores following ICI170,809 treatment in a parallel group (n=8 subjects) were very similar with values on day 8 of 42.4 $^{+}/_{-}$ 2.3 (alertness), 22.7 $^{+}/_{-}$ 3.9 (contentedness) and 9.4 $^{+}/_{-}$ 0.5 (calmness) on day 8 . The corresponding values for day 20 after dosing with ICI170,809 were 36.2 $^{+}/_{-}$ 11.6, 20.1 $^{+}/_{-}$7.6 and 8.3 $^{+}/_{-}$ 2.5.

I am grateful to Dr.S.Haworth at ICI for allowing me to quote this Bond Lader data which was analysed by the biometrics group.

5.4 DISCUSSION

Single oral doses of ICI 170,809, a potent selective 5-HT_2 receptor antagonist (Blackburn *et al* 1988), produced dose related, plasma concentration dependent changes in both 5-HT_2 induced *ex vivo* platelet aggregation and pupillary responses in healthy volunteers. In both cases, a log linear dose response relationship was demonstrated The effective dose of ICI 170,809, expressed as the ID50 for the platelet inhibitory response (*i.e.* the dose of antagonist which inhibits the agonist response by 50%) was approximately 0.1mg/kg. This is in agreement with the effective range of doses active centrally (Cox *et al* 1988), and in the cardiovascular system at the antiplatelet and antiarrhythmic 5-HT_2 receptor using *in vivo* animal models (Coker and Ellis; 1990).

Antagonism of both the 5-HT induced *ex vivo* platelet aggregation, and pupillary miosis following the single 15 mg doses of ICI 170,809 were apparent for greater than 8h. The pharmacokinetic profile of ICI 170,809 following single oral doses displayed a variable and prolonged plasma elimination half-life, which increased from a mean of 18hrs with the 7mg dose to over 40hrs with the 15 and 30mg doses. There was a linear increase in both peak plasma concentration (C_{max}) and in the area under the concentration time curve (AUCinf) with increasing dose. The apparent increase in elimination half-life and AUCinf with increasing dose may be related to the better definition and resolution of the beta elimination phase. Therefore, proposed oral dosing with either 7mg or 15mg, as single or divided daily doses, would be appropriate for use in clinical trials

Bevan and Heptinstall (1986) have subdivided 5-HT_2 antagonists into two categories "competitive" and "non-competitive", based on their behaviour at the platelet 5-HT_2 receptor. The *in vitro* and *ex vivo* effects of ICI 170,809 in this study, the lack of a parallel shift and a depression of the maximum response with increasing dose of antagonist, would suggest non-competitive or high affinity

antagonism at the platelet 5-HT_2 receptor. An alternative possibility is that the 5-HT_2 receptor exists in one of two interchangeable conformations with both high and low affinity for ligands. This would produce a similar dose response curve to those observed in this study and would be in keeping with other observations for ICI 170,809 at the vascular 5-HT_2 receptor (Frenken and Kaumann;1989). From the clinical point of view, this apparently insurmountable antagonism with ICI 170,809 may be beneficial, when antagonising high local concentrations of 5-HT released from platelets, in disease states such as migraine (Davies and Steiner, 1990; Jansen *et al* 1991) or coronary artery spasm (Noble *et al* 1990).

In two recent papers, the potent *in vivo* activity of ICI 170,809 and other 5-HT_2 antagonists as inhibitors of coronary artery platelet thrombosis has been confirmed in the anaesthetised dog (Cox *et al* 1991 and Morishima *et al* 1991). This data, together with the observation that 5-HT_2 receptors are likely to be the principal mediator of contraction in human coronary arteries (Chester *et al* 1990), resulting from platelet aggregation (Hollenberg 1988), must make the clinical evaluation of this class of drugs in cardiac ischaemia a top priority.

Recently, antagonism of the 5-HT_2 platelet response by ketanserin has been used to monitor efficacy in patients with Raynaud's phenomenon (Marasini *et al* 1990). The authors recommended increased dosing frequency to improve clinical efficacy. This demonstrates the utility of being able to measure a pharmacodynamic endpoint, and the desirability of a prolonged duration of action for a drug designed to treat such cardiovascular disorders. ICI 170,809 has such a profile of activity.

The demonstration with ICI 170,809 of a pupillary miosis, which was dose and concentration dependent, provides evidence to support the hypothesis that 5-HT_2 receptors may be involved in controlling human pupillary responses (Millson *et al* 1991). Animal evidence to support this comes

from observations that intraocular injection of 5-6 dihyroxy 5-HT (a 5-HT depleting agent) produced a denervation supersensitivity, affecting both pupillary responses to 5-HT and production of aqueous humour (Moro *et al* 1987). This is supported by the biochemical findings demonstrating selective 5-HT uptake, post synaptic 5-HT binding sites, functional responses and second messenger generation linked to 5-HT_2 receptors (Tobin *et al* (1988), Palkama *et al* (1984), Uusitalo *et al*(1982 and 1984) and Osborne and Tobin ;1987). Thus compelling evidence exists for a serotonergic influence in the iris-pupillary body (Cutlifee and Osborne, 1987).

Data from the multiple dosing study at Hazleton confirmed the dynamic changes observed in the single dose study conducted "in house." This also demonstrated that both *ex vivo* platelet effects and pupillary responses are capable of detecting 5-HT_2 antagonist effects for up to 8 days after multiple dosing. However, by 21 days tolerance had developed to the miotic effect of ICI 170,809 although the *ex vivo* platelet reponse was still present. Data from the multiple dose study also confirmed the lack of subjectively rated sedation with ICI 170,809 and showed it to be well tolerated. The pharmacokinetic accumulation seen with ICI 170,809 is as predicted from the elimination kinetics of the compound and reflects the prolonged halflife.

Therefore, this study has demonstrated two pharmacodynamic responses which reflect pharmacokinetic changes in ICI 170,809 following single oral doses in healthy volunteers. Both *ex vivo* platelet and pupillary responses are thus techniques readily applicable to monitor the effects of 5-HT_2 antagonists in man.

CHAPTER SIX

THE EFFECTS OF THE SEDATIVE HYPNOTIC DRUG CHLORAL HYDRATE ON PUPILLARY RESPONSES AND MEASURES OF AROUSAL IN HEALTHY VOLUNTEERS

The aim of this pilot study was to establish whether chloral hydrate, a sedative-hypnotic drug would, when administered in doses known to induce moderate sedation, produce a pupillary miosis, such as that observed with the 5-HT_2 antagonists ICI 169,369 and ICI 170,809. Both the ICI 5-HT_2 antagonists produced a moderate degree of sedation on acute dosing, which in the case of ICI 169,369 was observed to coincide with waking EEG changes. However, as previously discussed sedation does not always lead to miosis. On the contrary, anaesthesia induced by barbiturates is accompanied by a mydriasis (Larson *et al* 1981).

Chloral hydrate was chosen (Kales *et al* 1970) for a its abscence of overt effects on the electroencephalogram (indeed it is used to sedate children undergoing EEG), because of its nonspecific sedative effect and its lack of known interaction with serotonergic systems. This is in contrast to the benzodiazepines (Nutt *et al* 1988) which have central EEG effects and might lead to pupillary changes through such a mechanism. Furthermore, chloral hydrate has a rapid predictable onset of action which is relatively short lived and suitable for administration to healthy volunteers (Breimer, 1977).

The primary assessment in this study was pupillary responses assessed by the Pupilscan-PC, with on-line data capture. The placebo limb from this study also provided useful repeatability data over a 24 hour period.

Psychometric measures included Bond-Lader visual analogue scales to assess arousal; choice reaction time for psychomotor depression and physiological tremor to assess sympathetic drive. Critical flicker fusion was also included since CFF is modified by both changes in pupillary measures and central processing (Smith and Misiak, 1976).

6.1 STUDY DESIGN

This was a double blind crossover, within subject double dummy comparison of a single oral dose of chloral hydrate (0.5g Noctec) or placebo on pupillary responses, VAS for sedation, choice reaction time, critical flicker fusion and physiological tremor.

No power calculations were conducted in this pilot study. Six volunteers were considered sufficient to demonstrate potential sedation induced miosis. Data from the previous studies with the 5-HT_2 antagonists suggested that this study should be capable of detecting a biologically significant (*ie*>50% reduction in resting pupil diameter) miosis, and a further definitive study could then be carried out.

6.2 VOLUNTEER SELECTION

Six healthy male volunteers between the ages of 18 and 45, gave informed consent to take part after the study had been approved by the ICI Research ethics committee.

6.3 METHODS OF ASSESSMENT

6.3.1 Pupillometry.

After 15 minutes dark adaptation pupil measurements were made as described in chapter three (methods). Data for subsequent analysis was captured onto an IBM-PC. Pupil size and reactivity was measured in a sequence of tests, which were completed prior to and at 1.25, 2.0, 4.0, 8.0 and 24 hour after each dose of trial medication.

6.3.2 Critical flicker fusion. (CFF)

CFF is defined as the fastest rate at which an intermittent light source appears to flicker as opposed to being steady.

Subjects were seated in temperature controlled, sound attenuated cubicles with a constant low light background. Communication was via a one-way mirror and intercom. Subjects placed their head on a restraint in order to view the variable intermittent light source at the end of a 47cm internally blackened cylinder (2cm diameter) to minimise reflected light.

After adapting to darkness for 5 minutes the subject viewed the light source (a red light emitting diode display) and was instructed to assess whether it appeared intermittent or continuous. A computer was used to control the light source sequence as follows:

A 50Hz preconditioning stimulus (30s) was followed by an intermittent light for 5s at 25Hz, rising by 0.5Hz increments every 5s to 50Hz, or until the volunteer indicated by pressing the keyboard that the light no longer appeared to flicker. This was considered to be the ascending CFF threshold and was captured on-line for later analysis.

Similarly, the descending CFF was measured following a 50Hz conditioning frequency (30s) followed by reducing steps every 5s, falling in 0.5Hz steps to 25Hz, or until the subject indicated that the light source appeared intermittent, which was recorded as the descending CFF threshold.

CFF was measured immediately prior to dosing and at 1.0, 1.5, 3.0, 6.0 and 24.0 hours post dose. All measurements were taken using the right eye in subjects with normal visual acuity. The left eye was

open during the assessment viewing a dark low-contrast background. Three threshold values were recorded and the median value used in subsequent analysis.

6.3.3 Hand Tremor Measurement

Hand tremor was monitored at intervals (predose, 0.75, 1.25, 2.0, 4.0, 8.0, and 24 hours postdose) throughout the study by means of an accelerometer attached to the index finger of the non-dominant hand. The technique and analysis used is described in detail by Reid et al (1987).

Briefly, this was measured using an accelerometer (Vibrio-Meter Corp,Boston USA) with a sensitivity in the vertical plane of 10.05mV g^{-1}. This was attached to the outstretched index finger of the dominant hand,with the arm supported at the elbow. Tremor was recorded after resting supine for 5 min and was captured for on line analysis over a 2 min period whilst the subject was occupied counting the number of crosses appearing on a visual display unit (VDU). The signal was subjected to a fast Fourier transformation using the CED package and an IBM PC-AT micro-computer. Tremor data was transformed into natural logarithms for statistical analysis as its distribution was non-normal. The transformed data was summmarised by calculating the "area under the curve" for the frequency range 0 to 30Hz. This utilised the same computer software package (CED Cambridge) which was used to analyse the waking EEG data (see chapter 4).

Overall treatment significance was assessed by an F test, and since this failed to show any difference ($p>0.05$) between placebo and chloral hydrate treatment no further significance tests were undertaken.

6.3.4 Bond -Lader Visual Analogue Mood Rating Scales.

These were administered in an identical manner to that described in detail in chapter four, at predose, 1.0, 3.0, and 6.0 hours after dosing.

6.3.5 Choice Reaction Time.

This was conducted using the timed response to an image on a VDU controlled by a microcomputer. A number of shapes were presented in random order to the subject who was asked to press the keyboard only when a given shape occurred (*i.e.* triangle). The time taken to react was recorded and a median of 5 recordings was taken. Measurements were made pre-dose, 1.0, 1.5, 3.0, 6.0 and 24 hours after dosing.

6.4 STUDY PROTOCOL

Two subjects were studied each day. Volunteers fasted overnight and consumed no alcohol or caffeine for 24h prior to and after dosing. When clusters of assessments occurred at the same timepoint the following priorities were observed

CRT, CFF, VAS Scales, Pupillometry And Tremor

6.4.1 Statistical analysis.

Standard analysis of variance techniques were applied. Overall treatment significance was assessed by an F test after which, if significant differences were found ($p<0.05$), pairs of treatments were compared using a two sided Student's t-test with the residual mean square estimating the standard error. The level of significance for the t-tests was $p<0.05$, calculated from the least squares estimates of the means,with the exception of the VAS scores where an absolute p value was quoted reflecting a statistical trend approaching significance.

6.5 RESULTS

Chloral hydrate produced a moderate degree of sedation, which just failed to reach statistical significance ($p=0.09$) compared to placebo at 1.5 and 3.0 hours after dosing (Fig 6.1). However, these differences were significantly different from baseline values for the chloral hydrate treated group ($p<0.05$). The magnitude of sedation was similar to that observed following single doses of both ICI 169,369 and ICI 170,809 (see chapters 4 and 5).

Resting pupil diameter was similar in both treatment groups (Fig 6.2) and did not differ significantly from baseline, or between treatment groups during the 24 hours of the study. Similarly the pupillary light reflex at 1.5 hour coincident with the time of maximum sedation appeared unaffected by chloral hydrate treatment relative to baseline or placebo (Fig 6.3)

There were no treatment related changes in critical flicker fusion (Fig 6.4) or physiological tremor (Fig 6.5). Choice reaction time showed some fluctuation with time (Fig 6.6) but no treatment placebo difference was evident.

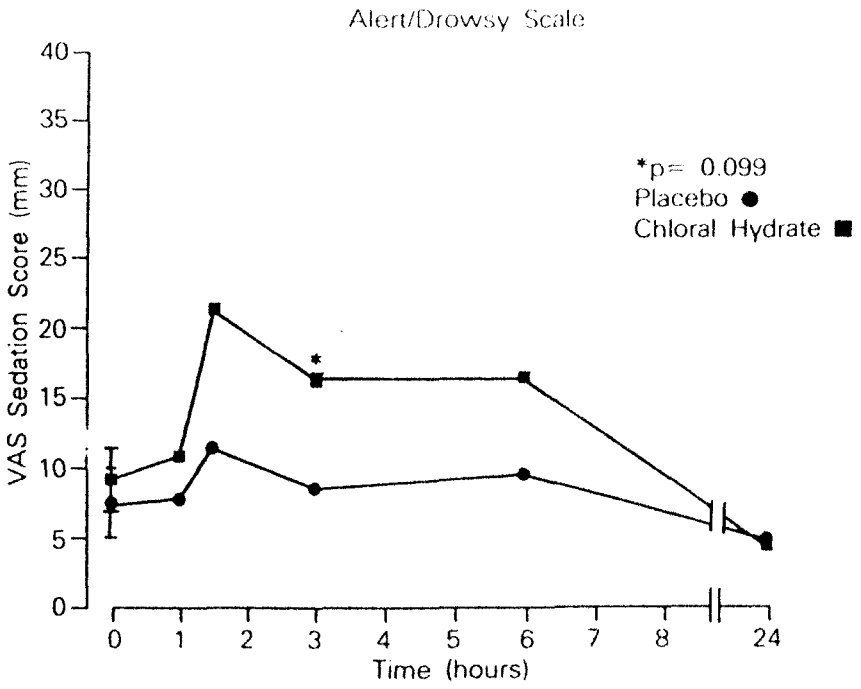


FIG 6.1

Effects of chloral hydrate (■) and placebo (●) on the state of arousal as assessed by visual analogue scale of sedation, predose (To) and at various times after dosing in six healthy subjects.

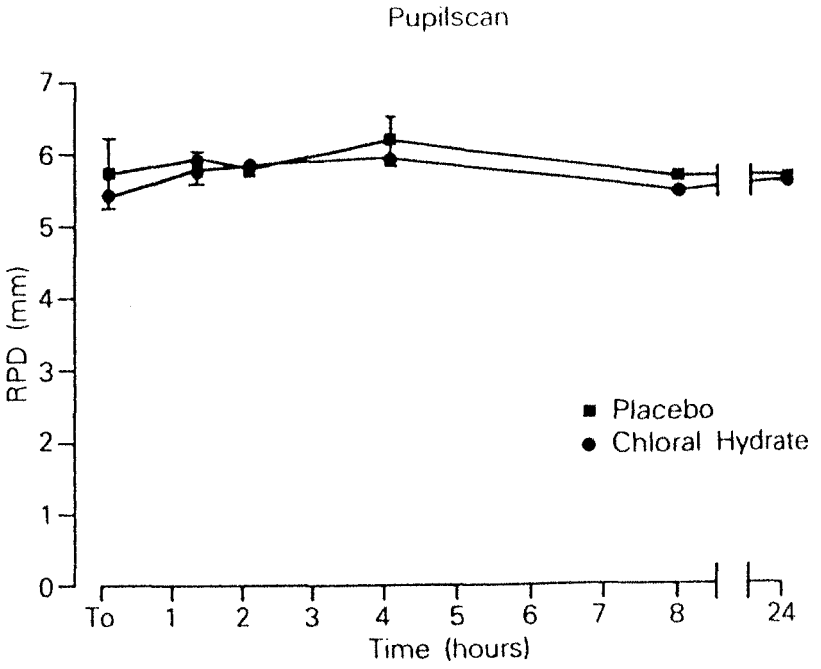


FIG 6.2

Effects of chloral hydrate (●) and placebo (■) on resting pupil diameter (RPD in mm;+/-standard deviation) at predose (To) and at various times after dosing.

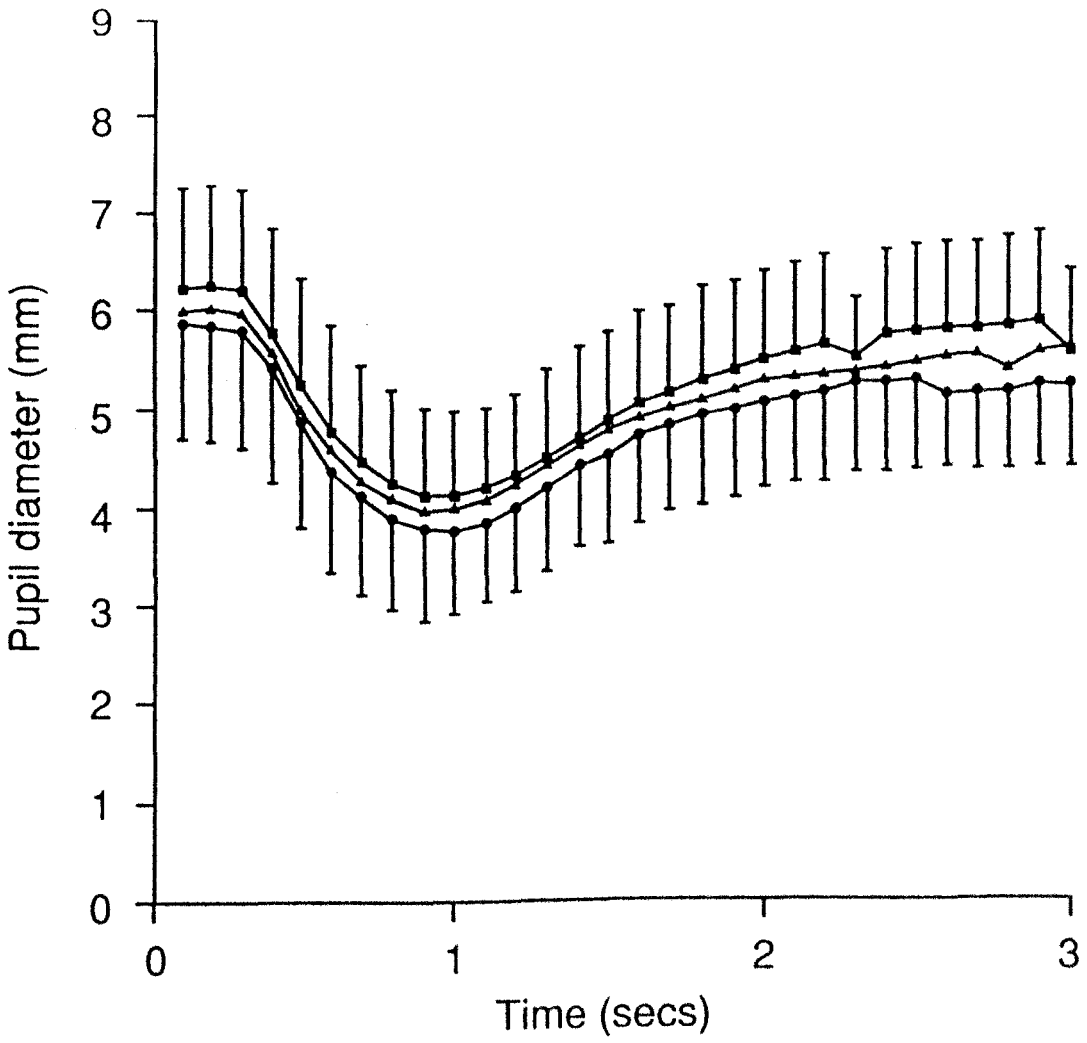


FIG 6.3

Effects of chloral hydrate (●) and placebo (■) on pupillary light response curves at 1.5h post-dose to a 0.5s duration stimulus (565nm, 65cdm^{-2}) recorded over a three second period in a dark adapted pupil, compared with predose (▲) recordings. Mean values are given with the standard deviation.

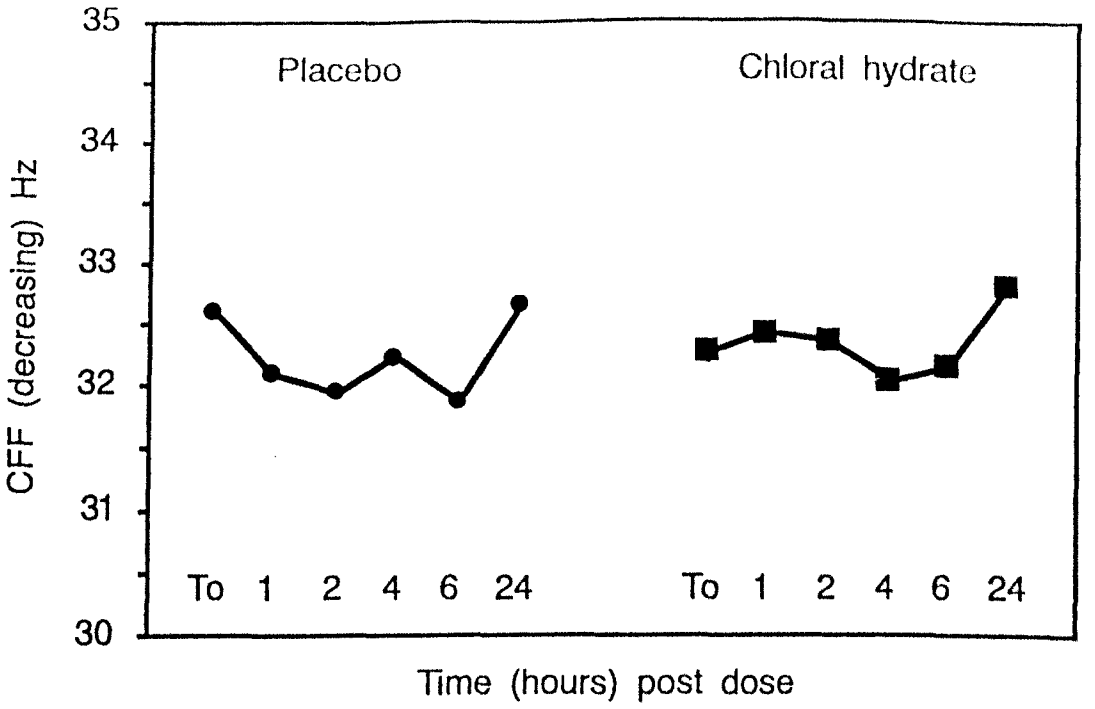


FIG 6.4

Effects of chloral hydrate (■) and placebo (●)on critical flicker fusion threshold (decreasing) at predose (To) and at various times up to 24 hours after dosing.(a similar pattern of results were obtained for the increasing CFF threshold).

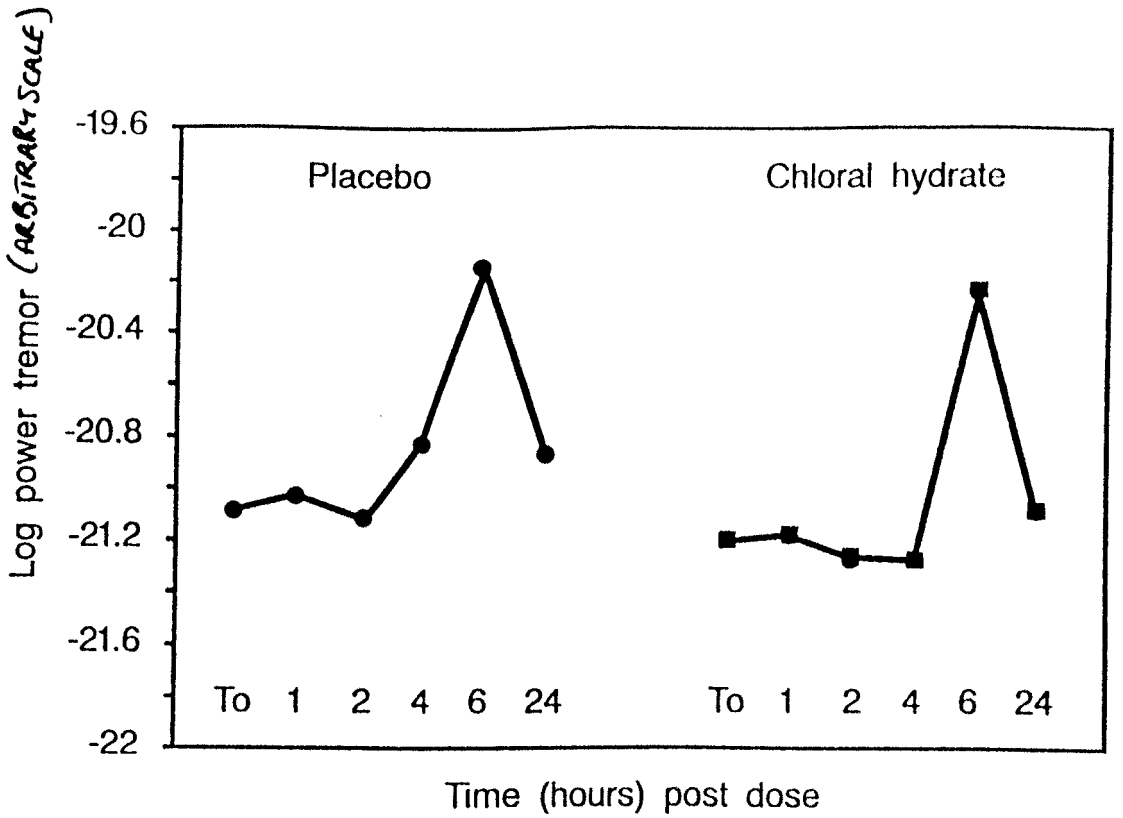


FIG 6.5

Effects of chloral hydrate (■) and placebo (●) on the log transformed power of finger tremor at predose (To) and at various times after dosing for a 24 h period.

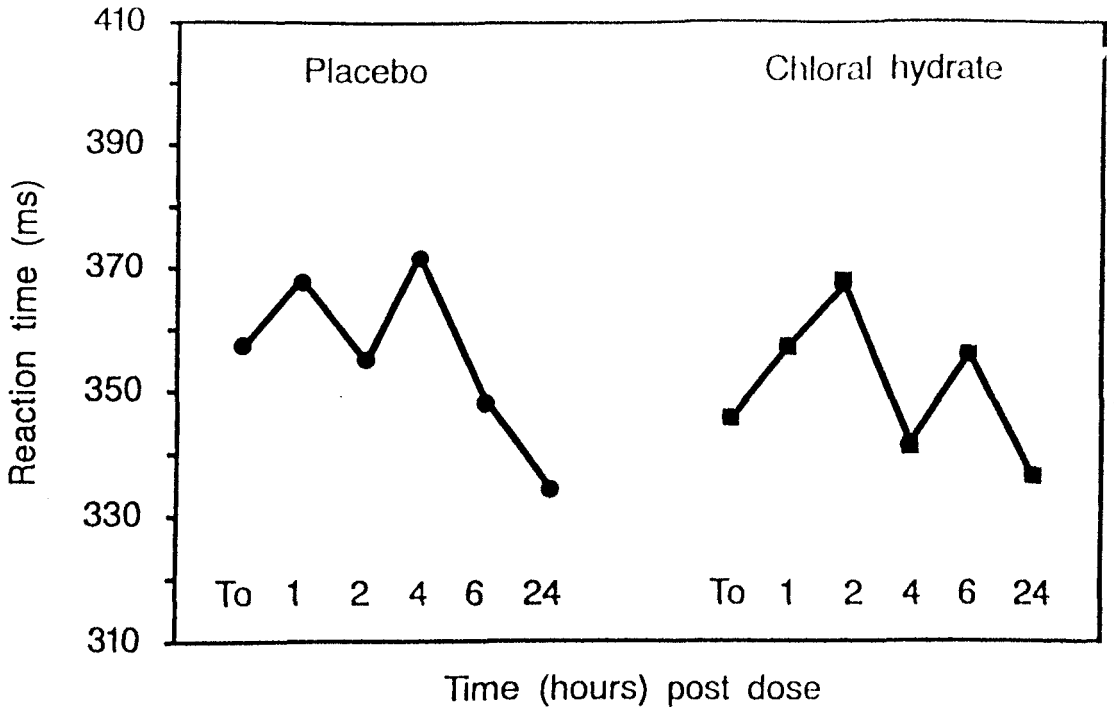


FIG 6.6

Effects of chloral hydrate (■) and placebo (●) on choice reaction time(ms) measured at predose(To) and at various times after dosing for up to 24 hours.

6.6 DISCUSSION

Chloral hydrate did not produce a significant sedative effect or any evidence for psychomotor retardation.No changes in either resting pupil size or pupillary responses were evident.The lack of a robust sedative effect may have reflected the small number of subjects used,since other workers have demonstrated sedation (Breimer,1977).Therefore,this study would neither confirm or refute the hypothesis that pupillary miosis is not necessarily accompanied by sedation.

In order to address this issue observations by Larson (1981) may be useful. He studied the mydriasis observed in subjects undergoing general anaesthesia. He speculated that this was due to inhibition of the pupilloconstrictor centre in the midbrain, where barbiturates are known to have an inhibitory influence (Lee and Wang 1975). The involvement of the sympathetic system would appear unlikely, since the dilatation following thiopentone anaesthesia in the cat is unaffected by ocular sympathectomy (Oono, 1965). Other workers have also shown this effect with chloroform, ethyl chloride, ethylene and nitrous oxide (Guedel 1937). Narcotic analgesics such as fentanyl and morphine apparently block the mydriasis often producing a nett miosis. This may reflect a direct action on the parasympathetic nucleus (Lee and Wang 1975) or an interaction at a pupillary level (Trew *et al* 1989).

Therefore, the only definitive way to establish the locus of 5-HT_2 antagonist induced pupillary constriction (*i.e.* central or peripheral) would be to examine the effects of topical application to the eye. Unfortunately, this experiment could not be carried out in man due to the high local irritancy of these compounds (Personal communication Dr. B. Cox), which might result in ocular toxicity.

CHAPTER SEVEN

7.1 FINAL DISCUSSION AND CONCLUSIONS

The aim of the work in this thesis was to test the hypothesis arrived at by chance observation namely that:

> *"5-HT_2 antagonists would modify human pupillary responses, providing an ancillary non-invasive pharmacodynamic measurement which would reflect other surrogate endpoints. This would provide the means of demonstrating 5-HT_2 mediated pharmacodynamic activity in man, and permit the investigation of possible pharmacokinetic and dynamic relationships in clinical efficacy trials."*

With ICI 169,369, the less potent of the two antagonists studied, a dose related, pupillary miosis was demonstrated which coincided with a change in waking EEG. The *ex vivo* changes in 5-HT induced platelet aggregation were small and non-significant. This probably reflects the lack of potency of ICI 169,369 (nmolar IC50 value = 17.9) at the 5-HT_2 receptor compared with both ICI 170,809 (1.8 nmolar) and ketanserin (3.2 nmolar), as determined by equilibrium binding to porcine choroid membranes (Major *et al* 1991). The relative potencies of both ICI compounds have recently been compared with RP62203, a novel 5-HT_2 antagonist being developed by Rhone Poulenc (see Fig 7.1) and with other 5-HT_2 antagonists, and are in agreement with the relative potencies observed in this study and with other published data (Doble *et al*, 1992; Koek *et al*, 1992)

Similarly ICI 169,369 was less potent than ICI 170,809 (about 8 to 10 fold) in that 120mg orally produced the same degree of miosis as a 15mg dose of ICI 170,809. This may also reflect the greater potency of ICI 170,809 for the 5-HT_{1c} receptor (0.9 nmolar) compared with ICI 169,369 (19.2 nmolar). Neither ICI 5-HT_2 antagonist can distinguish between the 5-HT_2 receptor and the putative

Table 1 Affinity of RP 62203 and other 5-HT_2 antagonists for 5-HT_2 receptors in rat frontal cortex

Antagonist	IC_{50} (nM)
RP 62203	0.12 ± 0.03
Ritanserin	0.24 ± 0.03
Spiperone	0.27 ± 0.10
Ketanserin	0.76 ± 0.14
ICI 170,809	0.77 ± 0.20
Pipamperone	1.13 ± 0.13
Methysergide	2.00 ± 0.32
Carpipramine	3.00 ± 0.20
Mianserin	3.67 ± 0.45
ICI 169,369	61.3 ± 17.1

The concentrations at which the compounds displaced 50% of the specific binding (IC_{50}) were obtained from seven-point displacement curves using [^{3}H]-ketanserin as the radiolabel. Data are expressed as the mean ± s.e.mean of three to four independent determinations of the IC_{50} values.

FIG 7.1

Data taken from Doble *et al* (1992) illustrating the rank potencies of ICI 169,369 & ICI170,809 for the 5-HT_2 receptor relative to other 5-HT_2/5-HT_{1C} receptor antagonists.

5-HT_{1C} receptor in choroid plexus. Ketanserin, on the other hand, does exhibit selectivity for the 5-HT_2 receptor with an IC50 in excess of 300 nmolar (Major *et al* 1991).

As discussed in chapters four and five the pupillary miosis produced by the two 5-HT_2 antagonists may be either centrally or peripherally mediated. The study with chloral hydrate (chapter six) and data from Larson (1971) with the anaesthetic agent thiopentone suggest that sedation is not invariably accompanied by a miosis. However, this does not entirely rule out a central mechanism, since ICI 169,369 showed associated changes in waking EEG and is reported to increase slow wave sleep in a similar manner to ritanserin (Cowen, 1990;Idzikowski *et al* 1991,1986 & 1987).

ICI 169,369 is also reported to lower blood pressure in the anesthetised rat by a central mechanism, and to activate vagal afferents by a non-5-HT_3 mechanism, producing a modified Bezold Jarisch reflex (Ramage, 1988). Paradoxically ICI 170,809 has no direct or indirect vagal effect on the isolated rabbit heart Langendorf preparation (Cox *et al* 1990). Also, ICI 169,369 had no hypotensive action in vasopressin supported pithed rats, suggesting that it was reducing central sympathetic central drive (Pires and Ramage, 1989). Neither ICI 169,369 (Scott *et al* 1988) or ritanserin posesses antihypertensive properties in man (Stott *et al*, 1987). Clearly from the pupillary miosis observed with topical thymoxamine (an alpha-1 adrenoceptor antagonist) this could not entirely account for the 5-HT_2 mediated miosis. However, data from Pires and Ramage (1989) indicates that ICI 169,369 also increased central vagal drive, since atropine partially attenuated the bradycardic response. Thus, the pupillary miosis could be due to a combination of an increased cholinergic drive to the pupil concomitant with a reduced sympathetic tone. This might explain the profound miosis experienced and the reduction in velocity of pupillary constriction. The arguments in favour of a peripheral mechanism are based on animal and human evidence. This suggests a functional 5-HT_2 receptor site in the iris linked to a second messenger, phosphatidyl choline turnover, with demonstrable receptor

supersensitivity following serotonin depletion, and a mydriasis to fenfluramine on topical application not mediated by classical autonomic mechanisms (see chapter five). Data with ketanserin (personal commmunication: Janssen pharmaceuticals) demonstrated a pupillary miosis in the dog following oral dosing, and in a limited human study a miosis with falls in intraocular pressure were recorded. Similarly, phase I volunteer studies with sertindole (a 5-$HT_{2/1c}$ antagonist with dopamine D2 properties) produced a dose related pupillary miosis (Lunbeck, personal communication). Therefore, whilst the miosis associated with the ICI and other 5-HT_2 antagonists is beyond dispute, the primary site of action is still open for debate. The use of topical fenfluramine applied to one eye and the differential effects of a 5-$HT_{2/1c}$ antagonist on the mydriasis might resolve this issue. Unfortunately, both ICI compounds are highly irritant and this would preclude their topical application.

An important question to debate is, what are the likely consequences of producing a miosis with the 5-HT_2 antagonists? The size of the pupil reflects the balance between constriction mediated by cholinergic parasympathetic nerves activating muscarinic receptors, and pupil dilatation mediated by adrenergic sympathetic nerves activating alpha adrenoceptors (Ishikawa *et al* 1977). A minor role is also played by inhibitory beta adrenoceptors, which relax ciliary muscle producing a reduction in accomodation, and are used in the treatment of glaucoma (Rushton *et al* 1979). The suspension of the sympathetic influence with drugs such as clonidine prolongs the time for the recovery reflex but produces little impairment of refraction, confirming that the major influence on visual function is cholinergic (Morley *et al* 1991).

7.1 Effects of 5-HT_2 antagonists on visual function

The impact of 5-HT_2 antagonism induced pupillary miosis on visual function was not addressed in this thesis. A separate study was carried out in conjunction with Professor Steven Smith (St Thomas's Medical school). This demonstrated that there was no significant impairment of visual acuity, contrast sensitivity, colour vision or constriction of the visual field following treatment with single doses (80 and 120mg) doses of ICI 169,369 in healthy volunteers compared with placebo. A significant finding in this study was that the degree of pupillary miosis reported was considerably less than that demonstrated with either ICI 169,369 or ICI 170,809; indeed it was only significant for the 120mg dose. The temporal relationship and duration of the miosis was the same in both studies. The major difference between the two techniques used was that Pupilscan (used at ICI) employed a low intensity viewing light which might activate the parasympathetic drive to the pupil, whereas Smith measured pupil diameter in complete darkness (personal communication).

The importance of ambient lighting conditions is illustrated by studying resting pupil diameter in anxious patients. Bond *et al* (1974) showed that under bright illumination the resting pupil diameter (RPD) of anxious patients was greater than normal controls, whereas Bakes *et al* (1990) found no difference between the two groups when measuring in the dark. In studies with ICI 170,809 both Pupilscan and polaroid photography (in total darkness) were employed in an attempt to address this question. The methods gave comparable results, with a percentage reduction in pupilsize relative to baseline of between *50%* and *35%* respectively, with the 15mg single oral dose. Thus, although methodological differences may have contributed to the discrepancy, it is more likely to have arisen from sampling errors associated with the small sample size used in the two studies.

The lack of any detrimental visual effects associated with this 5-HT_2 antagonist mediated miosis is probably because these drugs do not induce a spastic or paralytic miosis, which is further evidence of their novel mechanism.

Psychoactive drug induced miosis described to date has invoked two classical autonomic mechanisms.

7.1.1 Spastic miosis

Sedative drugs such as benzodiazepines produce such a response by increasing parasympathetic outflow, and can accentuate the reduction in critical clicker fusion frequency produced by sedation (Smith and Misiak, 1976). This spastic miosis may also enhance the paralytic miosis resulting from peripheral alpha-1 adrenoceptor blockade associated with amitryptyline (Szabadi and Bradshaw, 1988).

Opiate drugs produce a spastic miosis in man without sedation. This is believed to result from increased local cholinergic stimulation, probably mediated by Kappa receptors (Smith *et al* 1986), and associated with increased accomodation (Jaffe and Martin, 1980). Surprisingly, opiate addicts with pin point pupils rarely complain of impaired vision. Although definitive assessment of vision for this group under conditions of poor lighting (*e.g.* driving in dim lighting and changing lighting condtions) has not been undertaken.

7.1.2 Paralytic miosis

This occurs in two categories, the first with no involvement of accomodation, and is found with alpha-1 adrenoceptor antagonists such as prazosin and haloperidol. They cause paralytic miosis by an action at alpha-1 adrenoceptors in the iris, but leave the beta adrenoceptor dilator fibres in the ciliary body unaffected. No visual disturbance is associated with this class of drugs.

The second category is miosis with accomodation paralysis associated with phenothiazine neuroleptics (*e.g.* Chlorpromazine) and tricyclic antidepressants (*e.g.* amitryptyline) both of which produce a combined block of muscarinic cholinoceptors and alpha-adrenoceptors. In the iris the miosis resulting from alpha-1 blockade surmounts the mydriasis due to muscarinic antagonism, whilst in the ciliary body the paralytic effect of muscarinic blockade predominates unopposed (Ishikawa *et al* 1977). All these drugs produce blurring of near vision (Szabadi and Bradshaw, 1988).

The main conclusion to draw from this body of evidence is that systemically administered miotic drugs appear to be associated with visual disturbance, only if they also cause accomodation paralysis due to muscarinic receptor blockade.

Both ICI 169,369 and ICI 170,809 would appear to produce a spastic miosis, with no visual disturbance. Neither of the ICI 5-HT_2 antagonists discussed in this thesis show any demonstrable activity at autonomic receptors (either cholinergic or adrenergic) and are highly selective for the 5-$HT_2/_{1c}$ receptor type. Therefore, the work in this thesis taken together with other evidence, both animal and human, for the existence 5-HT_2 receptors in iris/ciliary body, would suggest that these receptors contribute to the control of human pupillary responses.

The second element in this thesis was that these pupillary responses could be used to monitor 5-HT_2 responses in man and could be used to model pharmacokinetic/dynamic relationships. For both ICI 169,369 and 170,809 the changes in pupillary responses reflect both circulating drug concentrations and are both a dose and concentration dependent. For ICI 169,369 the platelet responses to 5-HT were not sufficiently sensitive to be used as a measure of 5-HT_2 antagonism. However, for ICI 170,809 these responses were highly correlated with circulating plasma levels in a similar manner to the pupillary responses. Furthermore, there was no evidence of tolerance with ICI 170,809 to the antagonism of the 5-HT induced platelet aggregation, when the results of single and multiple dosing for 21 days were compared. This may reflect the renewal of platelet receptors as new platelets emerge every 10 days from the haemopoietic centres (Weiss 1975). Clinical studies with ketanserin have shown that this can be used to monitor therapy, and to provide a useful index of platelet 5-HT_2 antagonism, with which to modify and individualise dosing regimens (Marasini, 1990).

It was a disappointment, therefore, to find that despite an initial correlation between ICI 170,809 plasma levels and a reduction in resting pupil diameter, as measured by polaroid photography, that this correlation did not persist beyond 8 days dosing. This may reflect a tachyphylaxis to this neuronally mediated efffect. Whilst this was disconcerting this may well indicate the time taken to produce a therapeutic effect in the central nervous system. The clinical evidence to date with other 5-HT_2 antagonists such as ritanserin, suggests that efficacy in the treatment of anxiety and schizophrenia, does not occur after single doses and may reach a peak after a number of weeks (REF 1990).

Therefore, a dichotomy would appear to exist between (1) the therapeutic vascular effects of the 5-HT_2 antagonists which appear to be immediate and relatively shortlived after treatment withdrawal, taken together with the acute central nervous system (CNS) effects which occur after single doses (*i.e.*

EEG and pupillary effects) and (2) the CNS therapuetic efficacy in schizophrenia and anxiety. A possible explanation for this slow onset of therapeutic efficacy in the CNS is the involvement of an intermediary mechanism, which is slowly and cumulatively modified (either sensitised or densitised) by the 5-HT_2 antagonists. In schizophrenia a possible candidate for this would be the A9 dopaminergic neurones as suggested by Goldstein *et al* (1990), since these receive a 5-HT_2 mediated modulatory input. No clinical results with either ICI compound have yet been released by ICI which might refute or coroborate this hypothesis.

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APPENDIX I

ANIMAL PHARMACOLOGY OF THE 5-HT_2 ANTAGONIST ICI 169,369

As described in the introduction, 5-HT functions as a neurotransmitter in both the peripheral and central nervous system (CNS). The first of the ICI development candidates to enter phase I clinical evaluation in healthy volunteers was ICI 169,369 (2-(2-dimethylaminoehylthio-3-phenylquinilone hydrochloride; see Fig 1); a potent and relatively specific antagonist of 5-HT with an apparent selectivity for a subpopulation of 5-HT_2 receptors in the brain (Peroutka, 1979; Leysen, 1982). ICI 169,369 was also potent at the postsynaptic 5-HT receptors located on the dopamine neuroterminal, which has some similarities to the 5-HT_2 receptor, but was inactive on 5-HT autoreceptors within the CNS, indicating selectivity for postsynaptic 5-HT_2 sites. Receptor binding studies revealed at least a tenfold selectivity for the 5-HT_2 receptor. In functional tests, the compound had little or no agonist or antagonist activity at other adrenergic, cholinergic, dopaminergic or histaminergic receptor sites.

Although much of the animal pharmacology with ICI 169,369 has been published (Blackburn *et al*; 1988 and 1989) I am grateful to Drs. Barry Cox and Arthur Rushton at ICI, for permission to discuss in more detail the extensive pharmacological profile (as yet unpublished) of ICI 169,369; since this will provide essential background information to help explain the findings observed in man later in the thesis.

In *in vitro* studies, Blackburn *et al* (1988) demonstrated that ICI 169,369 had a higher affinity for 5-HT_2 binding sites in rat cortex than it has for 5-HT_1 sites (Ki 1.79×10^{-8} and 1.58×10^{-6}M, respectively). In a variety of isolated tissue preparations (rabbit aorta, pig coronary artery, rat caudal

artery) thought to represent functional responses at the 5-$HT_{2/1C}$ receptor ICI 169,369 was shown to be a competitive antagonist of 5-HT. In the rat caudal artery it had a similar affinity for the 5-HT_2 receptor to ketanserin (pA2 values of 8.18 and 8.42 respectively). Unlike ketanserin, which was inactive, ICI 169,369 was a non-surmountable antagonist at the rat stomach fundus 5-HT "D" receptor, recently reclassified as 5-HT_{1C}. It was inactive (>10^{-6}M) at the 5-HT_3 receptors found in the isolated perfused rabbit heart and myenteric plexus of guinea pig ileum. At receptors other than those for 5-HT, ICI 169,369 was inactive at concentrations of either 10^{-6} or 10^{-5}M.

Further pharmacological *in vivo* studies with ICI 169,369, again by Blackburn *et al* (1990), confirmed its potency and selectivity as an antagonist at 5-HT_2 and 5-HT_{1C} receptors. It caused a 50% inhibition of 5-HTP (5-hydroxytryptophan) induced head twitches in mice, and fenfluramine-induced hyperthermia in the rat at approximately 1mg/kg following parenteral administration. Results showed that ICI 169,369 had good oral bioavailability, since in the fenfluramine test the oral and parenteral ID50 values were similar. ICI 169,369 was also a selective antagonist of 5-HT-induced bronchoconstriction in the guinea pig and 5-HT induced pressor responses in the anaesthetised dog. In a series of other tests *in vivo* the compound was shown to be devoid of significant activity at alpha adrenoceptors (unlike ketanserin), dopamine (D_2), muscarinic (M_1) and histamine (H_1) receptors at 300 times its ID50 values used in the 5-HT tests. Thus ICI 169,369 was a selective, orally active 5-HT_2/5-HT_{1C} antagonist in animal models (Major *et al* 1991).

A1.1 EFFECTS ON PLATELET AGGREGATION

Contrary to expectations, (personal communication, T. Blackburn) ICI 169,369 was found to be a very weak antagonist at the platelet 5-HT_2 receptor. The inhibition of adenosine diphosphate (ADP) or collagen-induced platelet aggregation by ICI 169,369 was determined *in vitro* and *ex vivo*. In the *in vitro* experiments, ICI 169,369 showed weak inhibitory activity at 10^{-4} mol/L against collagen and ADP. In *ex vivo* experiments, groups of four rats were dosed orally with ICI 169,369 (20mg/kg) and blood samples taken at two hours postdose. Control rats were dosed with a saline vehicle only. A comparison between the ICI 169,369 dosed and control rats showed that ICI 169,369 had no effect on ADP induced platelet aggregation after oral administration.

Further *in vitro* platelet aggregation work, using either human platelet rich plasma or whole blood, measuring both the reduction in 5-HT induced potentiation of the platelet aggregatory response to ADP, and the transient aggregatory response and shape change to 5-HT (both 5-HT_2 mediated responses), showed ICI 169,369 to be a weak antagonist at the anticipated clinically relevent doses. Data from *ex vivo* whole blood platelet responses to 5-HT after oral dosing with ICI 169,369 (carried out in collaboration with Dr. S. Heptinstall) will be discussed later as part of a phase I study. These findings are consistent with those for other 5-HT_2 antagonists (*e.g.* ketanserin), where antagonist affinity at the platelet 5-HT_2 receptor is invariably lower than that for the smooth muscle receptor (Lijnen *et al* 1987).

A1.2 ANIMAL MODELS TO ASSESS ANTIPSYCHOTIC POTENTIAL

In electrophysiological tests predictive of antipsychotic drug activity, conventional antipsychotic drugs (*e.g.* D_2 antagonists such as haloperidol) will reverse the amphetamine induced suppression of firing rates of dopamine containing neurones in the rat substantia nigra (A9) and ventral tegmentum (A10), whereas drugs lacking antipsychotic properties will differentially reverse the effect of amphetamine on A9 cells but not A10. ICI 169,369 reversed the effects of amphetamine on both A9 and A10 cells of anaesthetized rats (Goldstein, 1990). The reversal was dose related over a range of 0.5 to 4.0 mg/kg i.v. The median effective dose was 1.5 mg/kg i.v. for A9 cells and 0.9 mg/kg i.v. for A10 cells. Comparable values for ritanserin, a selective 5-HT_2 antagonist with antipsychotic activity in man, were 0.2 and 0.5 mg/kg i.v. Thus, ICI 169,369 was selective in reducing amphetamine stimulated mesolimbic dopamine activity, which may be predictive of antipsychotic activity in man.

The effects of ICI 169,369 (10 mg/kg i.p.) on dopamine release and turnover were examined by measuring it effects on the concentrations of dopamine, and its two major metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in brain tissues. ICI 169,369 did not affect the concentrations of dopamine, DOPAC and HVA. Similarly, ICI 169,369 had no effect on the accumulation of 3-methoxytryptamine (3-MT) in rats pretereated with a monoamine oxidase inhibitor (pargyline). These results confirmed that ICI 169, 369 had no discernable effects on dopamine release or turnover under basal conditions.

In similar experiments, ICI 169,369 did not alter the concentrations of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in rat striatum, nor did it affect the accumulation of 5-HT following monoamine oxidase inhibition by pargyline. These results confirmed again that ICI 169,369 did not alter 5-HT turnover in normal animals.

In further experiments, rats were pretreated with the dopamine antagonist, haloperidol, or its vehicle 30 minutes before treatment with ICI 169,369 or placebo. By itself, haloperidol (0.1 and 0.25 mg/kg i.p.) elevated the concentrations of DOPAC and HVA in both the striatum and mesolimbic areas (olfactory tubercle). When ICI 169,369 (10mg/kg i.p.) was given to rats that were pretreated with haloperidol, it enhanced the haloperidol induced increases in DOPAC and HVA concentrations in brain tissues. These results suggested that 5-HT_2 receptor antagonism may enhance striatal dopamine release in the presence of dopamine receptor blockade, and thereby may attenuate the effects of dopamine receptor blockade in the striatum. Clinical studies with ritanserin have confirmed this hypothesis, where clinical efficacy as an antipsychotic is maintained, with an improvement in tardive dyskinesia when it is coadministered with haloperidol (Reynjens, 1986)

An ideal antipsychotic treatment should be more selective for the A10 mesolimbic versus the A9 nigrostriatal dopaminergic pathways, since this agent might be expected to possess less extrapyramidal side effects than conventional dopamine antagonists such as haloperidol. Other desirable features would be a modulatory influence on mesolimbic dopamine, only affecting transmission when hyperactivity was occuring. Thus the ideal agent would not affect dopamine or 5-HT turnover under basal conditions. Also, since combination therapy is often employed in antipsychotic therapy such an agent should enhance, rather than negate the effects of drugs such as

haloperidol. ICI 169,369 comes close to being the ideal hypothetical antipsychotic agent, but only randomised placebo controlled studies in schizophrenia will confirm its putative therapeutic benefits.

A1.3 ANIMAL ANXIETY MODELS

The potential anxiolytic activity of ICI 169,369 was tested in two models (unpublished data courtesy of Dr. B. Cox ICI) in which the standard anxiolytic chlordiazepoxide was active, and that are thought to be predictive of anxiolytic and/or anticonvulsant activity:

1). mouse shock induced aggression, and

2). mouse electroshock threshold. ICI 169,369 was tested in both of these models to determine if it significantly affected the potency of chlordiazepoxide in these tests.

In the shock induced aggression test pairs of mice were treated with various doses of chlordiazepoxide, both alone and with the addition of ICI 169,369 at 2.0 mg/kg i.p.; 30 minutes prior to chlordiazepoxide challenge. Dosed animals were then placed in a perspex chamber with an electrified grid floor. Application of a nociceptive current (1.5 mA to 2.7 mA) induced approximately 20 episodes per minute of fighting behavior in control animals. An ED50 value (the dose that reduced the episodes of fighting behaviour by 50%) for chlordiazepoxide alone and in combination with ICI 169,369 was determined.

Results from the shock induced aggression model, showed that ICI 169,369 reduced the ED50 for chlordiazepoxide from 6.8 mg/kg (5.1-9.3) to 1.5 mg/kg (0.6-3.6) which was significant ($p<0.05$ values are mean with 95% confidence intervals). ICI 169,369 had no effect on the threshold dose for chlordiazepoxide in the mouse electroshock threshold, which is more predictive of anticonvulsant activity. Thus in keeping with the clinical efficacy of the 5-HT_2 antagonists as putative anxiolytics, ICI 169,369 demonstrated an enhancement of the anxiolytic activity of chlordiazepoxide.

A1.4 ABSORBTION, DISTRIBUTION, METABOLISM AND EXCRETION STUDIES

These studies were carried out in the Safety of Medicines Department at ICI Pharmaceuticals.

Single dose pharmacokinetics were undertaken in the rat and dog. ICI 169,369 was given intravenously (5mg/kg) and orally (30, 50 and 150 mg/kg) to both male and female rats of the Alpk/AP (Wistar derived) strain. Whole blood estimations for ICI 169,369 and its N-desmethyl metabolite ICI 159,120 (a possible pharmacologically active metabolite) were performed by gas liquid chromatography (GLC) at designated times after administration. A similar procedure was followed for beagle dogs, but with smaller IV (2.5mg/kg) and oral (2.5, 5.0 and 10 mg/kg) doses, and blood for analysis taken by venepuncture in a randomised crossover study with a week between doses.

The mean elimination phase half-lives in the dog after oral doses of 2.5, 5.0 and 10mg/kg, respectively, were 1.7, 1.3 and 1.5 hours. These values were independent of dose, and were similar to that observed after intravenous administration. Bioavailability of ICI 169,369 after oral dosing was about 25% probably reflecting the extensive metabolism via a first pass effect. No circulating N-desmethyl metabolite was detected in either species.

Radiolabelled studies with oral 14C ICI 169,369 labelled in the quinoline ring demonstrated extensive metabolism in the rat, with most of the radiolabeled material consiting of unidentified metabolites. The latter did not pass readily into the brain, whereas ICI 169,369 and the N-desmethyl metabolite were selectively taken up with blood brain ratios of 8.2 and 10.1 respectively. In both blood and brain, the concentrations of ICI 169,369 were about four times greater than those of ICI 159,120.

Similarly in the dog extensive metabolism occured with mean blood concentrations of total radioactivity and unchanged drug peaking at 2 hours after dosing; total radioactivity was measurable up to 72 hours after dosing but declined to less than 80 ng/ml by 12 hours. Contrary to the rat ICI 169,369 and ICI 159,120 accounted for up to 86% of total radioactivity at 0.5 hours after dosing. After intravenous administration the blood concentrations of total radioactivity and parent compound peaked at 5 minutes; total radioactivity was measured up to 72 hours after dosing, but ICI 169,369 was undetectable after 5 hours. The N-desmethyl metabolite, ICI 159,120 was unquantifiable in these blood samples. Thus the N-desmethyl metabolite would appear to be produced as a result of first pass metabolism in both dog and rat.

Excretion studies in both rats and dogs with cannulated bile ducts demonstrated that between 60 to 70% of both oral and an intravenous dose were recovered in the bile. This confirms that ICI 169,369 is well absorbed and that biliary excretion is the major route of elimination.Urinary excretion accounts for only between 2 and 5% of total elimination in both species.

Chronic dosing with ICI 169,369 for six months with 150 mg/kg and 20 mg/kg, in rats and dogs respectively showed no evidence of accumulation of the drug.

Protein binding studies with ICI 169,369 to rat, dog, rabbit and human plasma at 37 C was determined by equilibrium dialysis following addition of 14C ICI 169,369. Unbound and total plasma concentrations of the coumpound at equilibrium were determined by scintillation counting.

The mean percentage binding for rat, dog, rabbit and human plasma, were 97.5%, 97.8%, 97.1% and 99.2% respectively. Clinical studies with other highly bound drugs which may be co-administered should be closely monitored, particularly if the therapeutic ratio of the other drug is low (e.g. Warfarin).

A1.5 HUMAN PHARMACOKINETIC DATA

The first phase I study (Haworth *et al* 1987) examined the tolerability of either single oral doses of 1, 3, 7, 15, 30 or 50 mg or multiple oral doses of 30mg BD with an initial assessment of pharmacokinetics. Meaningfully quantifiable whole blood concentrations were only detectable after the 7 and 15 mg doses, with mean peak concentrations of 8.0 and 17 ng/ml respectively. Mean peaks occured between 1 and 2 hours after dosing. Concentrations of the N-desmethyl metabolite ICI 159,120 were detected after the 15 mg dose, and were approximately one quarter the concentration of ICI 169,369, which was comparable to those seen in the dog. No quantifiable ICI 169,369 or ICI 159,120 were found in the urine collected over 24 hours implying that, as in animals, the main route of excretion was biliary.

In general over the dose range examined, peak whole blood concentrations and areas under the concentration time curve were nearly linear with dose. The apparent terminal half life in man for the higher two doses was between 3.5 and 5 hours. These pharmacokinetic parameters will be discussed in relationship to the pharmacodynamic endpoints in chapter 4.

APPENDIX II

A2 ANIMAL PHARMACOLOGY OF THE 5-HT_2 RECEPTOR ANTAGONIST ICI 170,809.

ICI 170,809 was the second development candidate with 5-HT_2 receptor antagonist properties to be developed at ICI.

ICI 170,809 (2-(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline hydrochloride is chemically related to ICI 169,369; with the introduction of a 2-methylpropylthio substituent in place of the aminoethylthio group (see Fig I and Blackburn *et al*, 1987 for comparative structures). These structural differences produced enhanced potency at the 5-HT_2 receptor, with improved brain penetration, a longer plasma elimination phase (and hence elimination half life) due to a different metabolic profile, together with unsurmountable blockade of the 5-HT_2 receptor; both producing a prolongation of pharmacodynamic activity compared with ICI 169,369.

To date the *in vitro*, *in vivo* and *ex vivo* pharmacology of ICI 170,809 has only been described briefly in published abstracts (Blackburn *et al* 1988; Cox *et al* 1988;Tortella *et al*1988) together with some comparative pharmacology with ritanserin and ICI 169,369 (Tortella *et al* 1989; Frenken and Kaumann, 1989). The pharmacology of ICI 170,809 will therefore be briefly reviewed, to compare and contrast its properties with ICI 169,369. The major pharmacological features are summarised in Table at the end of this section. I am grateful to Dr. Barry Cox (ICI pharmaceuticals) for access to, as yet unpublished data, concerning both ICI 169,369 and ICI 170,809, to supplement the data already published.

A2.1 5-HT RECEPTOR SPECIFICITY *IN VITRO*

The selectivity of ICI 170,809 for the CNS 5-HT_2/1c receptor was studied using radioligand binding studies similar to those reported by Peroutka and Snyder (1979). ICI 170,809 was shown to be selective for the 5-HT_2 binding site, being several orders of magnitude more potent in displacing tritiated spiroperidol or ketanserin (5-HT_2) than the endogenous ligand 5-HT (5-HT_1). The IC50 value (the concentration required for 50% inhibition of binding) for ICI 170,809 against tritiated spiroperidol binding in the frontal cortex (5-HT_2) was approximately ten thousand times greater than that for the 5-HT_1 binding site (*ie.*1.2 x 10^{-9}M and 1.4 x 10^{-5}M respectively). This is more selective than for ICI 169,369 where there was a 100 fold seperation (*i.e.* <1.79 x 10^{-8}M and 1.58 x 10^{-6}M respectively) according to data published by Blackburn *et al*(1988). The Ki value (Ki=IC50/(1 +S/KD)) for ICI 170,809 at the 5-HT_2 receptor was 6.6 x 10^{-10}M which is also 100 fold more potent than ICI 169,369 (1.79 x 10^{-8}M) and more or less equipotent with ketanserin and ritanserin (with Ki values of 9.9 x 10^{-10}M and 4.73 x 10^{-10}M respectively).

Furthermore ICI 170,809 was shown to be competitive in binding studies, as evidenced by a change in the apparent affinity (Kd's = 0.52 to 1.1nM) of tritiated ketanserin binding with no change in the number of receptor sites (Bmax). Binding studies to other neurotransmitter receptor sites revealed that ICI 170,809 had no apparent affinity for a wide range of ligand binding sites, including the benzodiazepine receptor (Blackburn *et al* 1988). ICI 170,809 did show weak affinity for the dopamine (D2) binding site (IC50 7.3 x 10^{-7}M), as measured by displacement of tritiated spiperone from rat striatal membranes, however this was an order of magnitude less than ritanserin (IC50 6.82 x 10^{-8}M).

Recent published data (Major *et al* 1991) with both ICI 169,369 and ICI 170,809 suggests that, whilst they have a high affinity for the 5-HT_2 receptor they cannot distinguish between the 5-HT and the putative 5-HT_{1c} receptors, present in membranes prepared from porcine choroid plexus. This clearly distinguishes these compounds from ketanserin, which does exhibit relative selectivity for the 5-HT_2 receptor.

Other CNS evaluation of ICI 170,809 involved neurotransmitter uptake studies, where no demonstrable inhibition of amine uptake was found. Further studies with neurotransmitter release showed that for potassium-evoked release of tritiated 5-HT from rat brain slices, employing a superfusion technique to determine the action of ICI 170,809 on presynaptic autoreceptors, the compound was a weak antagonist at 5-HT inhibitory autoreceptors (35% at 3 x 10^{-6}M). This suggests that *in vivo* potentiation of 5-HT release is unlikely, and coprescription with 5-HT reuptake inhibitors is unlikely to provoke the malignant 5-HT syndrome.

Neurochemical evaluation, using *ex vivo* techniques at parenteral doses ranging between 2.5 and 20mg/kg with ICI 170,809, did not alter the levels of the two major dopamine metabolites, 3, 4 dihydroxy-phenylacetic acid (DOPAC) or homovanillic acid (HVA) or the levels of 5-HT and 5-hydroxyindoleacetic acid (5HIAA)the major metabolite of 5-HT. Only a small significant effect (30%), $p<0.05$) was observed with ICI 170,809 at 20mg/kg on HVA levels with no apparent change in DOPAC levels.

A2.2 ANTAGONISM OF THE PERIPHERAL 5-HT RECEPTOR *IN VITRO*

5-HT agonist concentration effect curves were constructed in a variety of isolated tissue preparations, in the prescence and absence of ICI 170,809 (Blackburn *et al* 1988). ICI 170,809 was a potent selective surmountable antagonist of 5-HT on a variety of vascular preparations (rat caudal artery, rabbit aorta and pig coronary artery) which are believed to contain the 5-HT_2 receptor subtype. The threshold concentration for antagonism was approximately 10^{-9}M. Recently however, ICI 169,369 showed a progressive dose dependent right ward shift of a human temporal artery preparation over a concentration range of 10^{-7} to 10^{-5}M, whilst ICI 170,809 in these concentrations shifted the curve to the same degree with no dose dependency. The authors (Jansen *et al* 1991) attribute this to vasorelaxant properties, other possibilities are that ICI 170,809 was acting as an unsurmountable antagonist in human temporal and pial arteries. Similar differential effects for ICI 169,369 and ICI 170,809 were reported by Frenken and Kaumann (1989) using calf coronary artery, where the results were consistent with the existence of two interconvertible states of the 5-HT_2 receptor, to account for the apparent unsurmountable antagonism displayed by ICI 170,809.

Further studies of relevance to the cardiovascular effects of ICI 170,809 showed that it had similar potency to ketanserin at the platelet 5-HT_2 receptor (IC50's 3.8 x 10^{-8}M and 5.5 x 10^{-8}M respectively) which was 100 fold more potent than ICI 169,369. This antiplatelet activity for ICI 170,809 occured at doses which prevented reperfusion arrhythmias in the rat model of coronary ischaemia (Coker and Ellis, 1990).

In addition, ICI 170,809 also antagonised 5-HT on the D receptor subtype of the smooth muscle of the rat fundic strip and guinea-pig ileum, with a similar potency to that shown at the 5-HT_2 receptor. The

compound had no apparent selectivity for the 5-HT_3 receptor subtype in the latter preparation or in the Langendorff heart preparation (Clarkson *et al* 1990). Therefore, results from both isolated tissue and ligand binding studies indicated that ICI 170,809 was a potent selective 5-HT_2 antagonist devoid of significant effects at other neurotransmitter receptors (Blackburn *et al* 1988).

A2.3 *IN VIVO* PHARMACOLOGY OF ICI 170,809

This was investigated in a variety of species to determine the selectivity of the compound for the 5-HT receptor (Cox *et al* 1988). In two central tests indicative of activity at 5-HT_2 receptors, 5-HTP induced head twitches in the mouse and fenfluramine-induced hyperthermia in the rat (Blackburn *et al.*, 1983), ICI 170,809 produced a dose related inhibition with ID50 (inhibitory dose 50%) values of 0.3 (0.2-0.4) mg/kg i.p. and 0.8 (0.5-1.5) mg/kg p.o. respectively (numbers in parenthesis indicated 95% confidence intervals). ID50 values for a number of other classical 5-HT_2 antagonists were also determined in these models (*eg.*ketanserin 0.5(0.2-0.9)mg/kg i.p and 0.2 (0.1-0.3) mg/kg p.o., respectively and ritanserin 0.08(0.03-0.18)mg/kg i.p and 0.3 (0.02-5.8) mg/kg respectively). ICI 169,369 was about 4-5 fold less potent than the other antagonists (Blackburn *et al* 1986).

ICI 170,809 was also active against 5-HT induced pressor responses in the pithed rat (ID50 0.002mg/kg i.v.), and was inactive against noradrenaline induced pressor responses in the same model. The overall results from *in vivo* models show that the compound has marked selectivity for 5-HT receptors *in vivo*, with no apparent at other classical neurotransmitter receptors (Cox *et al* 1988). The selectivity of ICI 170,809 is born out by the differential affects of ICI 169,369 (a less selective 5-HT_2 antagonist) and ritanserin (a compound with appreciable dopamine D2 antagonist properties) on sleep regulation compared with ICI 170,809. While both these chemically novel 5-HT_2 antagonists from ICI clearly produced similar effects on REM latency and suppresion, their pharmacological profiles and effects on slow wave sleep differ from ritanserin (Tortella *et al* 1989).

A2.4 ELECTROPHYSIOLOGICAL STUDIES

ICI 170,809 reversed in a dose dependent manner the effects of amphetamine-induced suppression on the spontaneous activity of dopamine A9 and A10 cell bodies in rat brain. A test considered to be predictive of potential antipsychotic activity. ICI 170,809 was equipotent to ritanserin in this test, which is about 10 fold more potent than ICI 169,369 (Goldstein *et al* 1989).

A2.5 DURATION OF 5-HT ANTAGONISM

This was assessed by measuring the displacement of *ex vivo* tritiated ketanserin binding measured at various times after ICI 170,809 was administered to a group of rats (10mg/kg i.p). One hour after dosing, there was essentially complete occupancy of 5-HT_2 binding sites which were still substantially occupied at 5 hours. By 24hours occupancy was markedly reduced, and by 72 hours there was no displacement. In the rat fenfluramine hyperthermia model this was significantly inhibited by >50% for up to 8 and greater than 17 hours after administration of ICI 170,809 (1.0 and 10 mg/kg p.o. respectively). Similarly for the 5-HTP induced head twitches in the mouse, this was inhibited by >50% for 6 hours after administration.

Thus ICI 170,809 had a prolonged duration of action following a single dose. Furthermore, multiple dosing with this compound did not lead the development of tolerance or tachyphylaxis.

Ex vivo receptor binding studies following 26 days dosing with 10mg/kg i.p. ICI 170,809 produced a significant reduction in 5-HT_2 receptor number (Bmax) but no change in receptor affinity (Kd). This has also been reported with amitryptyline, where a functional desensitisation occured after chronic administration, accompanied by a reduction in number of receptors and in the 5-HT_2 mediated phosphoinositide hydrolysis response. This may represent an explanation for the delayed onset effect with an antidepressant (Sanders-Bush *et al.*, 1989), and is suggestive of potential efficacy for ICI 170,809 as an antidepressant.

A2.6 ABSORBTION, DISTRIBUTION, METABOLISM AND EXCRETION STUDIES

These animal studies were carried out in the Safety of Medicines Division at ICI under the supervision of Dr. A. Swaisland. A preliminary human pharmacokinetic evaluation, carried out as a collaborative project between DSM and Dr. Swaisland, is reported with the pupillary and platelet pharmacodynamics in chapter four.

Single oral and intravenous doses of [14C] -ICI 170,809 have been given to rats and dogs and plasma concentrations of radioactivity and drug measured(see Table 2.1).

TABLE 2.1 SHOWING A SUMMARY OF PHARMACOKINETIC PARAMETERS IN THE RAT & DOG.

SPECIES	DOSE	MAXIMAL PLASMA CONCENTRATION and Tmax (hours).	
	(mg/kg)	Radioactivity (ng equiv/g)	ICI 170,809*. (ng/g)
RAT	40	1285 +/- 732 (2.8 +/- 3.5)	351 +/- 270 (2.4 +/- 2.5)
DOG	10	1003 +/- 290 (1.5 - 4.0)	51 +/- 27 (0.5 - 1.5)
	1.0	85 +/- 12 (1 - 2)	NOT DETECTED

* = Mean peak +/- SD.

These results show that the peak concentrations are rapidly achieved but that there is considerable variability, both in time to peak and in the peak plasma concentration achieved. ICI 170,809 accounts for only a relatively small proportion of the circulating drug-related material, and it is probably the variable extent of metabolism which accounts for the wide range in plasma concentrations of the drug. Data in man, using 0.1mg/kg doses orally, gives plasma concentration values which are comparable to those in the dog after 10 mg/kg suggesting that the metabolic first pass effect is less in man (see Chapter 4).

The areas under the ICI 170,809 time curves after oral administration to rats (40mg/kg) and dogs (10mg/kg) were 1258 +/- 522 and 152 +/-65 mg.h/g. respectively. Comparison with the corresponding values obtained after intravenous administration showed that oral bioavailability was about 30% in the rat and ranged from 4 to 13% in the dog. No Intravenous formulation was developed for human use and therefore bioavailability comparisons were not possible.

A2.7 **PHARMACOKINETIC ELIMINATION PARAMETERS**

ICI 170,809 is rapidly cleared from both rat and dog plasma, mainly by metabolism. The terminal elimination half life (T1/2elim) in the rat after a single 40 mg/kg oral dose was 1.1 hours, and 1.6 hours after a single 1 mg/kg intravenous dose. After a single intravenous (i.v.) dose of 1mg/kg to dogs, the T1/2elim was 3.1 hours. These elimination rates would not be expected to lead to accumulation on chronic administration.

By contrast, radioactivity was eliminated much more slowly. In rats a mean apparent half-life of 14.2 +/-6.5 hours was found after i.v administration, and in dogs radioactivity was eliminated with apparent halflives ranging from 24 to 29 hours.

A2.8 MULTIPLE DOSE ADMINISTRATION

One and six month toxicity studies were monitored for plasma concentrations of ICI 170,809. Peak plasma concenrations were rapidly achieved (mostly within 3 hours of dosing in both species) but they showed a large inter-animal variation. Although mean concentrations increased with dose level, there was no linear correlation. Secondary peaking of plasma concentrations were also observed, at either 8 or 24 hours after dosing, suggesting biliary excretion, and may have been due to entero-hepatic circulation of the drug.

In the dog one month (20mg/kg) study, concentrations rose considerably during the course of the study, from a mean peak of 53 +/-39 ng/ml on day 1 to 350 +/-134 ng/ml on day 27. The corresponding AUC values at this dose level on the days sampled were 426 +/-184 and 1974 +/-765 ng.h/ml, respectively. This apparent accumulation was not accompanied by any increase in pre-dose values, and the increase in peak concentrations was attributed to either increased absorbtion, tissue saturation or a decrease in metabolism.

A2.9 PLASMA PROTEIN BINDING AND TISSUE DISTRIBUTION

In vitro plasma protein binding studies demonstrated that the drug is highly protein bound (>99%) over the concentration range 0.5 to 5 mcg/ml in rat, dog and human plasma.

Measurement of blood and brain concentrations of ICI 170,809 and its two major metabolites (the N-desmethyl and primary amine analogues) showed in the dog that the primary amine was not detected in blood after oral administration of 20mg/kg for 14 days. Two hours after the final dose on day fourteen, ICI 170,809 was present in both blood and brain in greater concentrations than the N-desmethyl metabolite, but the brain concentrations of both compounds were 11 to 26 times the corresponding blood concentrations. These results show that both parent drug and metabolite drug related material was preferentially distributed from blood to brain.

Excretion of ICI 170,809 was principally via the biliary route, after both oral and i.v dosing. This was confirmed in a study using bile duct cannulated rats:89% was recovered in bile from i.v dosed and 74% from orally dosed animals. Similar results were obtained in the dog, indicating that oral absorbtion was high in both species and that biliary excretion was the predominant route.

A3 CONCLUSIONS

ICI 170,809 is a potent selective 5-HT_2 antagonist which although structurally related to ICI 169,369 has a wider profile of pharmacodynamic activity, being more potent at both vascular and platelet 5-HT_2 receptors, whilst displaying comparable potency with ritanserin at the CNS 5-HT_2 receptor. Whilst there are also similarities (ie metabolic clearance and biliary elimination)in the biochemical pharmacology of the two compounds, important differences exist in the disposition of the compounds leading to a more prolonged duration of action for ICI 170,809.

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TABLE 06. LSMEANS FOR PUPIL REACTION - DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
INITIAL	TIME										
	3 HRS POSTDOSE	6	0.448	6	-3.283	1.793	0.001	6	-1.608	1.793	0.029
	5 HRS POSTDOSE	6	1.514	6	-1.842	1.588	0.001	6	-1.608	1.588	0.002
	8 HRS POSTDOSE	6	0.606	6	-0.433	1.336	0.110	6	0.483	1.336	0.838
	24 HRS POSTDOSE	6	0.378	6	-0.325	1.218	0.220	6	1.025	1.218	0.256
MINIMUM	3 HRS POSTDOSE	6	0.471	6	-2.000	3.366	0.129	6	-0.242	3.366	0.638
	5 HRS POSTDOSE	6	1.796	6	-0.583	2.801	0.086	6	-0.167	2.801	0.145
	8 HRS POSTDOSE	6	0.413	6	-0.183	2.221	0.553	6	0.917	2.221	0.615
	24 HRS POSTDOSE	6	0.497	6	-0.833	1.818	0.130	6	1.542	1.818	0.221

(CONTINUED)

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TABLE 06. LSMEANS FOR PUPIL REACTION - DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
FINISH	TIME										
	3 HRS POSTDOSE	6	0.349	6	-2.725	1.549	0.002	6	-1.025	1.549	0.075
	5 HRS POSTDOSE	6	1.391	6	-1.242	1.808	0.010	6	-1.159	1.808	0.012
	8 HRS POSTDOSE	6	0.516	6	-0.333	1.645	0.268	6	0.733	1.645	0.768
	24 HRS POSTDOSE	6	0.222	6	-0.750	1.594	0.197	6	1.242	1.594	0.178
TIME	3 HRS POSTDOSE	5	0.140	6	-0.125	0.243	0.036	6	0.017	0.243	0.270
	5 HRS POSTDOSE	5	0.455	6	-0.058	0.324	0.007	6	-0.000	0.324	0.013
	8 HRS POSTDOSE	5	0.118	6	-0.042	0.207	0.112	6	0.008	0.207	0.253
	24 HRS POSTDOSE	5	0.043	6	-0.042	0.271	0.485	6	0.083	0.271	0.733

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TABLE 02. SUMMARY OF PUPIL REACTION - INITIAL (MM)

		PREDOSE	+3 HRS	+5 HRS	+8 HRS	+24 HRS
80MG ICI 169369	MEAN	7.158	3.875	5.317	6.725	6.833
	MEDIAN	7.075	3.900	4.725	6.500	7.000
	MINIMUM	5.550	2.650	3.350	5.600	5.700
	MAXIMUM	9.750	5.000	9.600	9.500	7.300
	STD	1.514	1.003	2.230	1.425	0.590
	N	6	6	6	6	6
120MG ICI 169369	MEAN	5.625	4.017	4.017	6.108	6.650
	MEDIAN	5.650	3.950	3.875	6.025	7.025
	MINIMUM	4.850	3.050	3.550	4.600	4.650
	MAXIMUM	6.350	5.100	4.700	7.650	7.700
	STD	0.549	0.693	0.413	1.274	1.071
	N	6	6	6	6	6
PLACEBO	MEAN	5.969	6.417	7.483	6.575	6.347
	MEDIAN	5.800	6.175	8.050	6.575	6.442
	MINIMUM	4.400	4.450	3.300	5.000	5.550
	MAXIMUM	7.315	9.550	9.750	8.400	7.000
	STD	1.097	1.986	2.420	1.259	0.584
	N	6	6	6	6	6

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TABLE 03. SUMMARY OF PUPIL REACTION - MINIMUM (MM)

		PREDOSE	+3 HRS	+5 HRS	+8 HRS	+24 HRS
80MG ICI 169369	MEAN	4.425	2.425	3.842	4.242	3.592
	MEDIAN	4.050	2.650	2.625	4.025	3.800
	MINIMUM	2.150	1.200	2.400	1.550	1.350
	MAXIMUM	9.100	3.150	9.500	8.950	5.100
	STD	2.535	0.812	2.792	2.634	1.545
	N	6	6	6	6	6
120MG ICI 169369	MEAN	2.550	2.308	2.383	3.467	4.092
	MEDIAN	2.200	2.450	2.525	3.325	4.275
	MINIMUM	1.450	1.200	1.450	1.950	1.950
	MAXIMUM	3.850	2.850	3.000	4.900	5.750
	STD	1.029	0.615	0.616	0.988	1.297
	N	6	6	6	6	6
PLACEBO	MEAN	3.404	3.875	5.200	3.817	3.901
	MEDIAN	3.600	3.025	4.575	3.850	4.102
	MINIMUM	2.000	1.750	2.200	1.600	2.450
	MAXIMUM	4.300	9.050	8.800	6.200	4.650
	STD	0.839	2.686	2.949	1.569	0.800
	N	6	6	6	6	6

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TABLE 04. SUMMARY OF PUPIL REACTION - FINISH (MM)

		PREDOSE	+3 HRS	+5 HRS	+8 HRS	+24 HRS
80MG ICI 169369	MEAN	6.733	4.008	5.492	6.400	5.983
	MEDIAN	6.325	4.025	5.225	6.000	6.200
	MINIMUM	5.550	3.250	3.350	4.950	5.100
	MAXIMUM	9.300	4.850	9.550	9.650	6.650
	STD	1.387	0.656	2.186	1.667	0.600
	N	6	6	6	6	6
120MG ICI 169369	MEAN	5.017	3.992	3.857	5.750	6.258
	MEDIAN	5.125	3.900	3.725	5.900	6.575
	MINIMUM	3.850	3.050	3.450	4.300	4.300
	MAXIMUM	6.150	5.050	4.795	6.750	7.200
	STD	0.866	0.663	0.506	1.001	1.040
	N	6	6	6	6	6
PLACEBO	MEAN	5.759	6.108	7.150	6.275	5.981
	MEDIAN	5.500	5.750	7.050	6.125	5.892
	MINIMUM	3.700	4.100	4.050	4.950	5.350
	MAXIMUM	7.805	9.450	9.400	8.650	6.800
	STD	1.431	1.959	1.958	1.388	0.638
	N	6	6	6	6	6

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TABLE 05. SUMMARY OF PUPIL REACTION - TIME (SEC)

		PREDOSE	+3 HRS	+5 HRS	+8 HRS	+24 HRS
80MG ICI 169369	MEAN	0.942	0.817	0.883	0.900	0.900
	MEDIAN	0.925	0.800	0.875	0.875	0.925
	MINIMUM	0.800	0.750	0.800	0.750	0.800
	MAXIMUM	1.150	0.900	1.050	1.150	0.950
	STD	0.146	0.052	0.093	0.134	0.063
	N	6	6	6	6	6
120MG ICI 169369	MEAN	0.842	0.858	0.842	0.850	0.925
	MEDIAN	0.825	0.825	0.800	0.850	0.875
	MINIMUM	0.750	0.750	0.700	0.800	0.800
	MAXIMUM	1.000	1.150	1.200	0.950	1.250
	STD	0.097	0.150	0.188	0.055	0.164
	N	6	6	6	6	6
PLACEBO	MEAN	0.860	1.000	1.200	0.958	0.890
	MEDIAN	0.850	0.975	1.050	0.950	0.850
	MINIMUM	0.800	0.800	0.850	0.750	0.800
	MAXIMUM	0.950	1.250	1.950	1.250	1.050
	STD	0.065	0.158	0.416	0.172	0.096
	N	5	6	6	6	5

LISTING 01.

VOLUNTEER	TREATMENT	STAGE
001	80MG ICI 169369	03
	120MG ICI 169369	02
	PLACEBO	04
002	80MG ICI 169369	04
	120MG ICI 169369	03
	PLACEBO	02

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DETAILS OF PUPIL REACTION

TIME	INITIAL (MM)	MINIMUM (MM)	FINISH (MM)	TIME (SECS)
PREDOSE	5.900	2.300	5.550	0.80
+3 HRS	4.700	3.150	4.450	0.90
+5 HRS	4.850	2.550	5.600	0.85
+8 HRS	6.550	4.100	5.950	0.90
+24 HRS	7.250	2.550	6.650	0.95
PREDOSE	5.550	1.450	4.250	1.00
+3 HRS	4.000	2.600	4.000	0.85
+5 HRS	4.300	3.000	4.000	0.85
+8 HRS	7.300	4.100	6.750	0.85
+24 HRS	7.150	4.800	6.650	0.85
PREDOSE	7.100	4.300	6.550	0.95
+3 HRS	7.150	4.300	6.500	1.00
+5 HRS	7.850	4.150	6.600	1.00
+8 HRS	6.950	4.550	6.350	1.00
+24 HRS	7.000	4.650	6.800	0.90
PREDOSE	7.600	4.250	6.400	0.80
+3 HRS	4.600	3.050	4.450	0.75
+5 HRS	5.550	3.350	5.650	0.90
+8 HRS	6.450	3.950	6.350	0.85
+24 HRS	7.050	5.100	6.250	0.80
PREDOSE	4.850	1.800	3.850	0.75
+3 HRS	5.100	1.200	5.050	1.15
+5 HRS	4.700	1.450	4.795	1.20
+8 HRS	6.700	4.900	6.300	0.80
+24 HRS	7.700	5.750	7.200	0.90
PREDOSE	7.315	2.925	7.805	.
+3 HRS	9.550	9.050	9.450	1.10
+5 HRS	9.550	8.700	9.400	0.85
+8 HRS	8.400	6.200	8.650	0.85
+24 HRS	6.735	4.105	6.185	.

LISTING 01.

VOLUNTEER	TREATMENT	STAGE
003	80MG ICI 169369	02
	120MG ICI 169369	04
	PLACEBO	03
004	80MG ICI 169369	03
	120MG ICI 169369	04
	PLACEBO	02

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DETAILS OF PUPIL REACTION

TIME	INITIAL (MM)	MINIMUM (MM)	FINISH (MM)	TIME (SECS)
PREDOSE	9.750	9.100	9.300	1.15
+3 HRS	3.100	1.200	3.600	0.80
+5 HRS	9.600	9.500	9.550	1.05
+8 HRS	9.500	8.950	9.650	0.75
+24 HRS	6.750	4.700	6.350	0.85
PREDOSE	6.350	3.850	6.150	0.75
+3 HRS	4.400	2.850	4.300	0.75
+5 HRS	3.900	2.550	3.650	0.70
+8 HRS	5.050	3.400	4.950	0.80
+24 HRS	6.950	4.550	6.900	0.80
PREDOSE	6.000	4.000	5.800	0.80
+3 HRS	5.200	3.000	5.000	0.80
+5 HRS	9.750	8.800	9.000	1.95
+8 HRS	5.000	2.850	4.950	0.75
+24 HRS	5.850	3.600	5.600	0.85
PREDOSE	5.550	2.150	5.700	1.05
+3 HRS	3.200	2.250	3.250	0.85
+5 HRS	3.350	2.400	3.350	0.80
+8 HRS	5.700	2.050	5.450	0.85
+24 HRS	6.950	2.900	5.400	0.95
PREDOSE	5.750	2.100	4.850	0.80
+3 HRS	3.650	2.300	3.800	0.85
+5 HRS	3.550	2.500	3.450	0.70
+8 HRS	4.600	3.200	4.300	0.85
+24 HRS	6.350	4.000	6.000	0.90
PREDOSE	5.400	3.800	5.200	0.85
+3 HRS	4.450	1.750	4.100	0.95
+5 HRS	3.300	2.200	4.050	1.10
+8 HRS	5.500	1.600	4.950	1.00
+24 HRS	5.550	2.450	5.350	1.05

LISTING 01.

VOLUNTEER	TREATMENT	STAGE
005	80MG ICI 169369	02
	120MG ICI 169369	03
	PLACEBO	04
006	80MG ICI 169369	04
	120MG ICI 169369	02
	PLACEBO	03

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DETAILS OF PUPIL REACTION

TIME	INITIAL (MM)	MINIMUM (MM)	FINISH (MM)	TIME (SECS)
PREDOSE	6.650	3.850	6.250	1.00
+3 HRS	2.650	1.800	3.450	0.80
+5 HRS	3.950	2.550	3.950	0.80
+8 HRS	5.600	1.550	4.950	1.15
+24 HRS	5.700	1.350	5.100	0.95
PREDOSE	6.050	2.300	5.600	0.90
+3 HRS	3.050	2.100	3.050	0.80
+5 HRS	3.800	2.950	3.800	0.75
+8 HRS	7.650	3.250	6.700	0.95
+24 HRS	7.100	3.500	6.500	0.85
PREDOSE	5.600	3.400	5.200	0.90
+3 HRS	7.450	3.050	6.900	1.25
+5 HRS	6.200	2.350	6.350	0.90
+8 HRS	7.400	3.500	6.850	1.25
+24 HRS	6.800	4.500	6.600	0.85
PREDOSE	7.500	4.900	7.200	0.85
+3 HRS	5.000	3.100	4.850	0.80
+5 HRS	4.600	2.700	4.850	0.90
+8 HRS	6.550	4.850	6.050	0.90
+24 HRS	7.300	4.950	6.150	0.90
PREDOSE	5.200	3.800	5.400	0.85
+3 HRS	3.900	2.800	3.750	0.75
+5 HRS	3.850	1.850	3.450	0.85
+8 HRS	5.350	1.950	5.500	0.85
+24 HRS	4.650	1.950	4.300	1.25
PREDOSE	4.400	2.000	3.700	0.80
+3 HRS	4.700	2.100	4.700	0.90
+5 HRS	8.250	5.000	7.500	1.40
+8 HRS	6.200	4.200	5.900	0.90
+24 HRS	6.150	4.100	5.350	0.80

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LISTING 21. DETAILS OF SIDE EFFECTS

(1=MILD,2=MOD,3=SEV,8=SEVERITY N.R.)

			TIME				
			PREDOSE	+3 HRS	+6 HRS	+12 HRS	+24 HRS
TREATMENT	SIDE EFFECT:	VOLUNTEER					
80MG ICI 169369	DROWSINESS	001				1	
		002			2		
	DRY MOUTH	006	1				
	HEADACHES	002	1	1	2	2	
		003		2	1		
	HYPERSALIVATION	004	1				
		006	1				
	NAUSEA	002			1	1	
	POLYURIA	001		1		1	
	TIREDNESS	002	1	2	2	2	
	VISUAL DISTURBANCE	002			1		
120MG ICI 169369	COUGHING	003					1
	DROWSINESS	004		1			
		006		2	2		
	DRY MOUTH	006	1				

(CONTINUED)

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LISTING 21. DETAILS OF SIDE EFFECTS

(1=MILD,2=MOD,3=SEV,8=SEVERITY N.R.)

			TIME				
			PREDOSE	+3 HRS	+6 HRS	+12 HRS	+24 HRS
TREATMENT	SIDE EFFECT:	VOLUNTEER					
120MG ICI 169369	HEADACHES	006		1	1		
	HYPERSALIVATION	006	1				
	IMPAIRED CONCENTRATION	006			1		
	INCREASING DEAFNESS	004					1
	IRRITABILITY	005	1				
	MYALGIA	003	1				
	PAIN IN CHEST	003	1				1
	STIFFNESS IN JOINTS	004	1	1			1
	TIREDNESS	002			1		
		006	2	3	2		
	VISUAL DISTURBANCE	006		1			
PLACEBO	DROWSINESS	003	1				
	DRY MOUTH	004	1				
		006	1				

(CONTINUED)

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LISTING 21. DETAILS OF SIDE EFFECTS

(1=MILD,2=MOD,3=SEV,8=SEVERITY N.R.)

			TIME				
			PREDOSE	+3 HRS	+6 HRS	+12 HRS	+24 HRS
TREATMENT	SIDE EFFECT:	VOLUNTEER					
PLACEBO	FOUL TASTE	001			1		
	HEADACHES	002	1			1	
	HYPERSALIVATION	001			1		
	IMPAIRED CONCENTRATION	002	1				
	MYALGIA	004	1				
	TIREDNESS	002	1			1	
		003	1				
		006	2				

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TABLE 01. SUMMARY OF AGE, HEIGHT AND WEIGHT

		AGE (YEARS)	HEIGHT (CMS)	WEIGHT (KGS)
PATIENT NUMBER				
001		32.0	183.0	73.6
002		26.0	186.0	76.3
003		20.0	185.0	64.5
004		36.0	179.0	74.0
005		31.0	170.0	60.1
006		32.0	166.0	80.5
SUMMARY	MEAN	29.5	178.2	71.5
	STD	5.6	8.3	7.7
	N	6	6	6

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TABLE 23. LSMEANS FOR BOND-LADER VISUAL ANALOGUE SCALE SCORES

DIFFERENCES FROM PREDOSE

'ALERT' FACTOR

	PLACEBO		80MG ICI 169369				120MG ICI 169369			
	N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
TIME										
3HRS POSTDOSE	6	-1.352	6	4.389	7.832	0.129	6	7.926	7.832	0.026
5HRS POSTDOSE	6	-1.519	6	8.093	10.375	0.065	6	6.296	10.375	0.121
12HRS POSTDOSE	6	-0.037	6	4.685	8.600	0.241	6	0.611	8.600	0.866
24HRS POSTDOSE	6	-2.093	6	2.500	17.558	0.563	6	4.889	17.558	0.386

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TABLE 23. LSMEANS FOR BOND-LADER VISUAL ANALOGUE SCALE SCORES

DIFFERENCES FROM PREDOSE

'CONTENT' FACTOR

	PLACEBO		80MG ICI 169369				120MG ICI 169369			
	N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
TIME										
3HRS POSTDOSE	6	-0.067	6	2.667	4.782	0.224	6	1.633	4.782	0.436
5HRS POSTDOSE	6	-1.567	6	5.567	5.573	0.019	5	2.912	6.073	0.125
12HRS POSTDOSE	6	0.467	6	3.900	9.712	0.439	6	0.233	9.712	0.957
24HRS POSTDOSE	6	3.533	6	5.200	5.097	0.472	6	7.100	5.097	0.145

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TABLE 23. LSMEANS FOR BOND-LADER VISUAL ANALOGUE SCALE SCORES

DIFFERENCES FROM PREDOSE

'CALM' FACTOR

	PLACEBO		80MG ICI 169369				120MG ICI 169369			
	N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
TIME										
3HRS POSTDOSE	6	0.583	6	0.417	10.287	0.971	6	-0.333	10.287	0.842
5HRS POSTDOSE	6	4.417	6	3.833	11.673	0.909	5	-4.625	12.720	0.137
12HRS POSTDOSE	6	-0.583	6	6.000	11.474	0.222	6	-5.667	11.474	0.337
24HRS POSTDOSE	6	14.500	6	15.333	10.769	0.863	6	20.500	10.769	0.235

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TABLE 25. LSMEANS FOR LOG POWER FOR THETA WAVES (5-8 HERTZ) - CHANNELS O+1

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	0.09	6	0.31	0.23	0.069	6	0.34	0.23	0.037
	6 HRS POSTDOSE	6	0.11	6	0.19	0.39	0.654	6	0.33	0.39	0.239
	24 HRS POSTDOSE	6	-0.04	6	0.06	0.19	0.250	6	0.03	0.19	0.382
OPEN	2 HRS POSTDOSE	6	0.48	6	0.36	0.77	0.729	6	0.43	0.77	0.887
	6 HRS POSTDOSE	6	0.66	6	0.30	0.98	0.420	6	0.39	0.98	0.541
	24 HRS POSTDOSE	6	-0.23	6	0.23	1.28	0.432	6	0.06	1.28	0.618

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TABLE 26. LSMEANS FOR LOG POWER FOR ALPHA WAVES (9-13 HERTZ) - CHANNELS O+1

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	0.10	6	-0.34	0.33	0.017	6	-0.30	0.33	0.026
	6 HRS POSTDOSE	6	-0.37	6	-0.53	0.66	0.589	6	-0.33	0.66	0.888
	24 HRS POSTDOSE	6	0.01	6	-0.25	0.49	0.263	6	0.07	0.49	0.777
OPEN	2 HRS POSTDOSE	6	0.42	6	0.40	0.75	0.970	6	0.50	0.75	0.798
	6 HRS POSTDOSE	6	0.68	6	0.40	1.11	0.567	6	0.40	1.11	0.570
	24 HRS POSTDOSE	6	-0.36	6	0.16	1.45	0.430	6	0.13	1.45	0.457

TABLE 24. LSMEANS FOR LOG POWER FOR DELTA WAVES (1-4 HERTZ) - CHANNELS O+1

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	0.12	6	0.31	0.35	0.244	6	0.37	0.35	0.127
	6 HRS POSTDOSE	6	0.44	6	0.44	0.58	0.996	6	0.52	0.58	0.763
	24 HRS POSTDOSE	6	0.04	6	0.15	0.24	0.303	6	0.05	0.24	0.888
OPEN	2 HRS POSTDOSE	6	0.37	6	0.43	0.96	0.894	6	0.23	0.96	0.749
	6 HRS POSTDOSE	6	0.64	6	0.39	1.13	0.621	6	0.23	1.13	0.432
	24 HRS POSTDOSE	6	-0.21	6	0.30	1.53	0.459	6	0.10	1.53	0.654

TABLE 27. LSMEANS FOR LOG POWER FOR BETA WAVES (14-30 HERTZ) - CHANNELS O+1

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	0.11	6	0.06	0.29	0.693	6	0.13	0.29	0.860
	6 HRS POSTDOSE	6	0.10	6	-0.11	0.20	0.039	6	0.06	0.20	0.626
	24 HRS POSTDOSE	6	-0.17	6	-0.09	0.26	0.495	6	-0.11	0.26	0.555
OPEN	2 HRS POSTDOSE	6	0.47	6	0.29	0.64	0.542	6	0.45	0.64	0.941
	6 HRS POSTDOSE	6	0.69	6	0.32	0.87	0.347	6	0.38	0.87	0.430
	24 HRS POSTDOSE	6	-0.35	6	0.22	1.39	0.372	6	-0.04	1.39	0.619

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TABLE 28. LSMEANS FOR LOG POWER FOR DELTA WAVES (1-4 HERTZ) - CHANNELS 2+3

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	0.09	6	0.19	0.47	0.643	6	0.12	0.47	0.872
	6 HRS POSTDOSE	6	0.51	6	0.36	0.63	0.585	6	0.30	0.63	0.458
	24 HRS POSTDOSE	6	0.06	6	0.29	0.50	0.339	6	-0.04	0.50	0.651
OPEN	2 HRS POSTDOSE	6	0.28	6	0.22	1.03	0.895	6	0.40	1.03	0.790
	6 HRS POSTDOSE	6	0.69	6	0.36	0.87	0.405	6	0.23	0.87	0.258
	24 HRS POSTDOSE	6	-0.30	6	0.23	1.46	0.422	6	0.11	1.46	0.526

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TABLE 29. LSMEANS FOR LOG POWER FOR THETA WAVES (5-8 HERTZ) - CHANNELS 2+3

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	0.03	6	0.35	0.30	0.040	6	0.35	0.30	0.041
	6 HRS POSTDOSE	6	0.24	6	0.30	0.40	0.734	6	0.36	0.40	0.519
	24 HRS POSTDOSE	6	0.03	6	0.13	0.28	0.396	6	0.02	0.28	0.944
OPEN	2 HRS POSTDOSE	6	0.36	6	0.33	1.12	0.940	6	0.63	1.12	0.596
	6 HRS POSTDOSE	6	0.68	6	0.31	1.03	0.431	6	0.47	1.03	0.658
	24 HRS POSTDOSE	6	-0.21	6	0.22	1.42	0.511	6	-0.09	1.42	0.861

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TABLE 30. LSMEANS FOR LOG POWER FOR ALPHA WAVES (9-13 HERTZ) - CHANNELS 2+3

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	0.14	6	-0.03	0.23	0.141	6	0.02	0.23	0.280
	6 HRS POSTDOSE	6	-0.14	6	-0.05	0.34	0.533	6	-0.10	0.34	0.774
	24 HRS POSTDOSE	6	-0.13	6	-0.18	0.26	0.656	6	-0.05	0.26	0.532
OPEN	2 HRS POSTDOSE	6	0.40	6	0.40	1.05	0.999	6	0.56	1.05	0.729
	6 HRS POSTDOSE	6	0.52	6	0.29	0.97	0.600	6	0.34	0.97	0.686
	24 HRS POSTDOSE	6	-0.30	6	0.13	1.55	0.544	6	-0.10	1.55	0.770

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TABLE 31. LSMEANS FOR LOG POWER FOR BETA WAVES (14-30 HERTZ) - CHANNELS 2+3

ANALYSIS USING DIFFERENCES FROM PREDOSE

		PLACEBO		80MG ICI 169369				120MG ICI 169369			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
EYES	TIME										
CLOSED	2 HRS POSTDOSE	6	-0.04	6	0.11	0.19	0.101	6	0.22	0.19	0.013
	6 HRS POSTDOSE	6	0.10	6	0.15	0.30	0.737	6	0.12	0.30	0.928
	24 HRS POSTDOSE	6	-0.17	6	0.11	0.30	0.059	6	-0.13	0.30	0.750
OPEN	2 HRS POSTDOSE	6	0.32	6	0.13	0.95	0.648	6	0.35	0.95	0.953
	6 HRS POSTDOSE	6	0.54	6	0.08	0.95	0.295	6	0.28	0.95	0.534
	24 HRS POSTDOSE	6	-0.21	6	0.27	1.92	0.584	6	-0.51	1.92	0.722

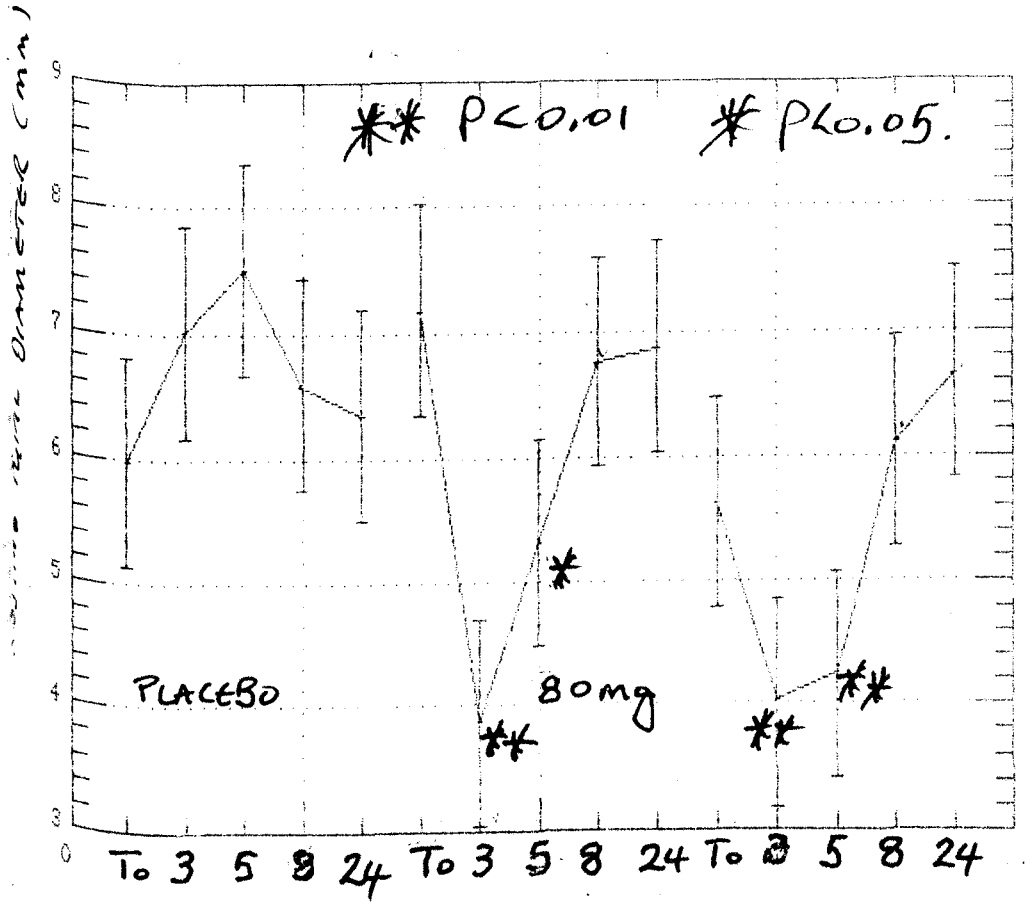
Analysis of Variance for D:ICI35IN.VAR1 = RESTING PULSE PARAMETER.

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	96.547233	11	8.777021	8.559	.0000
D:ICI35IN.VAR2 VOLUNTEER	35.786222	5	7.157244	6.980	.0001
D:ICI35IN.VAR4 TREATMENT	27.474056	2	13.737028	13.396	.0000
D:ICI35IN.VAR3 TIME	33.286956	4	8.321739	8.115	.0001
2-FACTOR INTERACTIONS	112.92513	38	2.9717140	2.898	.0006
D:ICI35IN.D:ICI35IN.	29.08828	10	2.9088278	2.837	.0092
D:ICI35IN.D:ICI35IN.	30.56011	20	1.5280056	1.490	.1392
D:ICI35IN.D:ICI35IN.	53.27674	8	6.6595931	6.494	.0000
RESIDUAL	41.018389	40	1.0254597		
TOTAL (CORR.)	250.49076	89			

Missing values have been excluded.

TIME POST DOSE (HOURS)

Effects of ICI 169,369 on Resting Pupil Diameter.



I = 95% Confidence Intervals.

TABLE 1. LSMEANS FOR PUPIL REACTION

		90MG ICI 169369					120MG ICI 169369				
		N	ESTIMATE	LOWER 95% C.L.	UPPER 95% C.L.	P	N	ESTIMATE	LOWER 95% C.L.	UPPER 95% C.L.	P
INITIAL	TIME										
	PREDOSE	61	1.189	-0.043	2.421	0.057	61	-0.344	-1.576	0.888	0.538
	13 HRS POSTDOSE	61	-2.542	-4.044	-1.040	**0.004**	61	-2.409	-3.962	-0.898	**0.006**
	15 HRS POSTDOSE	61	-2.167	-4.267	-0.067	**0.045**	61	-3.467	-5.567	-1.367	**0.005**
	18 HRS POSTDOSE	61	0.175	-1.746	2.096	0.839	61	-0.442	-2.362	1.479	0.610
	124 HRS POSTDOSE	61	0.486	-0.410	1.382	0.247	61	0.303	-0.594	1.199	0.459
MINIMUM	PREDOSE	61	1.021	-0.610	2.651	0.187	61	-0.854	-2.485	0.776	0.261
	13 HRS POSTDOSE	61	-1.456	-3.880	0.980	0.206	61	-1.567	-3.997	0.864	0.175
	15 HRS POSTDOSE	61	-1.358	-4.019	1.302	0.273	61	-2.817	-5.477	-0.156	0.040
	18 HRS POSTDOSE	61	0.425	-2.200	3.050	0.719	61	-0.350	-2.975	2.275	0.766

(CONTINUED)

TABLE 1. LSMEANS FOR PUPIL REACTION

		80MG IDI 169369				120MG IDI 169369			
		N	ESTIMATE	LOWER 95% C.L.	UPPER 95% C.L.	P	N	ESTIMATE	LOWER 95% C.L.	UPPER 95% C.L.	P
MINIMUM	TIME										
	24 HRS POSTDOSE	61	-0.309	-1.775	1.157	0.640	61	0.191	-1.275	1.657	0.772
FINISH	PREDOSE	61	0.974	-0.587	2.536	0.188	61	-0.743	-2.304	0.819	0.305
	3 HRS POSTDOSE	61	-2.100	-3.540	-0.660	0.010	61	-2.117	-3.557	-0.677	0.009
	5 HRS POSTDOSE	61	-1.658	-3.511	0.195	0.073	61	-3.292	-5.146	-1.439	0.003
	8 HRS POSTDOSE	61	0.125	-1.817	2.067	0.886	61	-0.525	-2.467	1.417	0.559
	24 HRS POSTDOSE	61	0.003	-0.769	0.774	0.994	61	0.278	-0.494	1.049	0.431
TIME	PREDOSE	61	0.084	-0.081	0.250	0.268	61	-0.016	-0.181	0.150	0.839
	3 HRS POSTDOSE	61	-0.183	-0.365	-0.002	0.048	61	-0.142	-0.323	0.040	0.109
	5 HRS POSTDOSE	61	-0.317	-0.624	-0.010	0.045	61	-0.358	-0.665	-0.051	0.027

(CONTINUED)

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TABLE 1. LSMEANS FOR PUPIL RECTION

		80MG ICI 169369				120MG ICI 169369			
		N	ESTIMATE	LOWER 95% C.L.	UPPER 95% C.L.	P	N	ESTIMATE	LOWER 95% C.L.	UPPER 95% C.L.	P
TIME	TIME										
	8 HRS POSTDOSE	61	-0.058	-0.128	0.011	0.099	61	-0.108	-0.179	-0.039	0.007
	24 HRS POSTDOSE	61	0.000	-0.172	0.172	1.000	61	0.025	-0.147	0.197	0.741

Regression Analysis - Linear model: Y = a+bX

PUBLICSCAN VALIDATION.

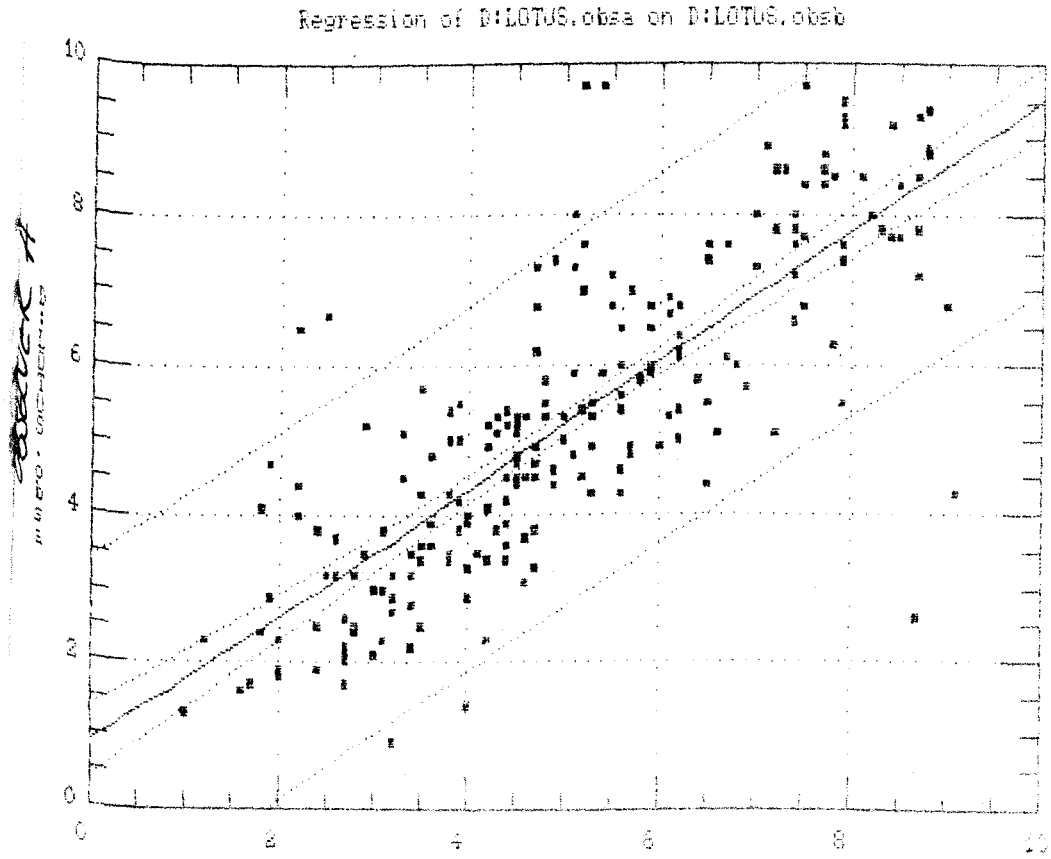
Dependent variable: D:LOTUS.obsa Independent variable: D:LOTUS.obsb

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	0.88997	0.240409	3.70191	2.72236E-4
Slope	0.862897	0.0442886	19.4835	0

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	594.41422	1	594.41422	379.60640	.00000
Error	335.09615	214	1.56587		
Total (Corr.)	929.51037	215			

Correlation Coefficient = 0.799692 R-squared = 63.95 percent
Std. Error of Est. = 1.25135



OBSERVER B.

ALTMAN + BLAND PLOT COMPARING OBSERVERS A+B.

ression Analysis - Linear model: Y = a+bX

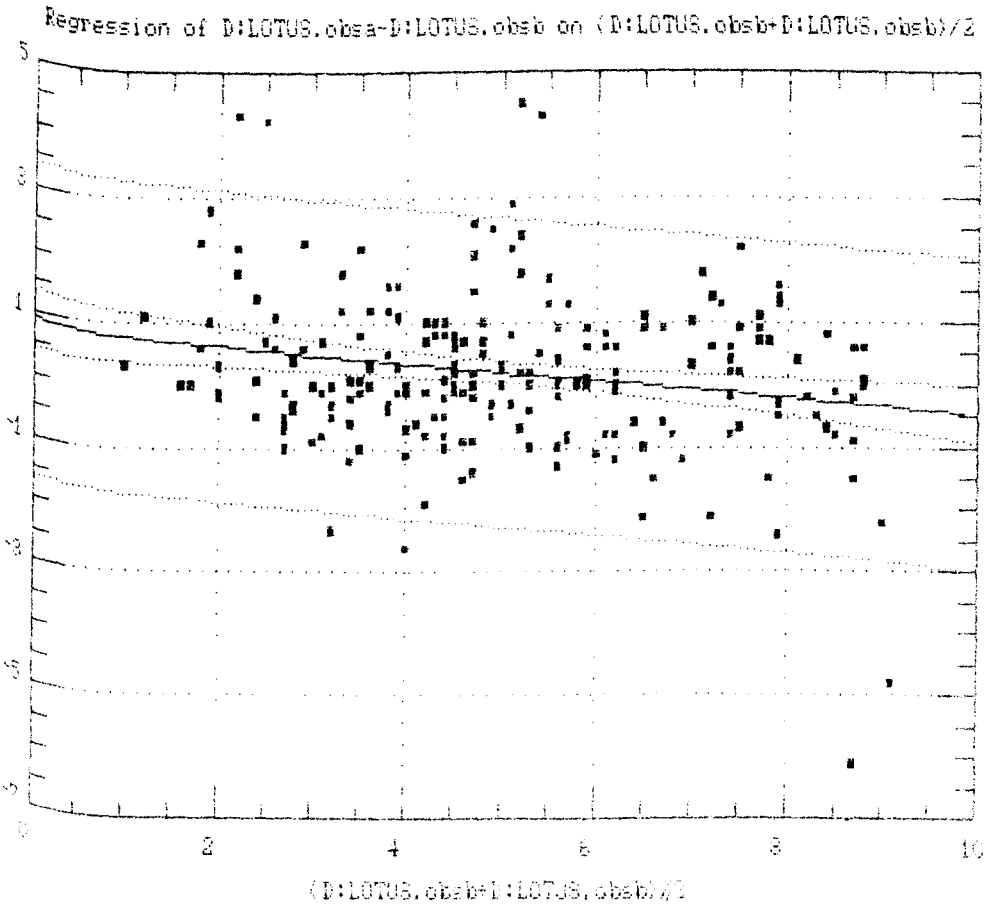
pendent variable: D:LOTUS.obsa-D:LOTUS Independent variable: (D:LOTUS.obsb+D:LO

ameter	Estimate	Standard Error	T Value	Prob. Level
ercept	0.88397	0.240409	3.70191	2.72236E-4
pe	-0.137103	0.0442886	-3.09567	2.22619E-3

Analysis of Variance

urce	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
del	15.006026	1	15.006026	9.583189	.00223
al	335.09615	214	1.56587		
al (Corr.)	350.10218	215			

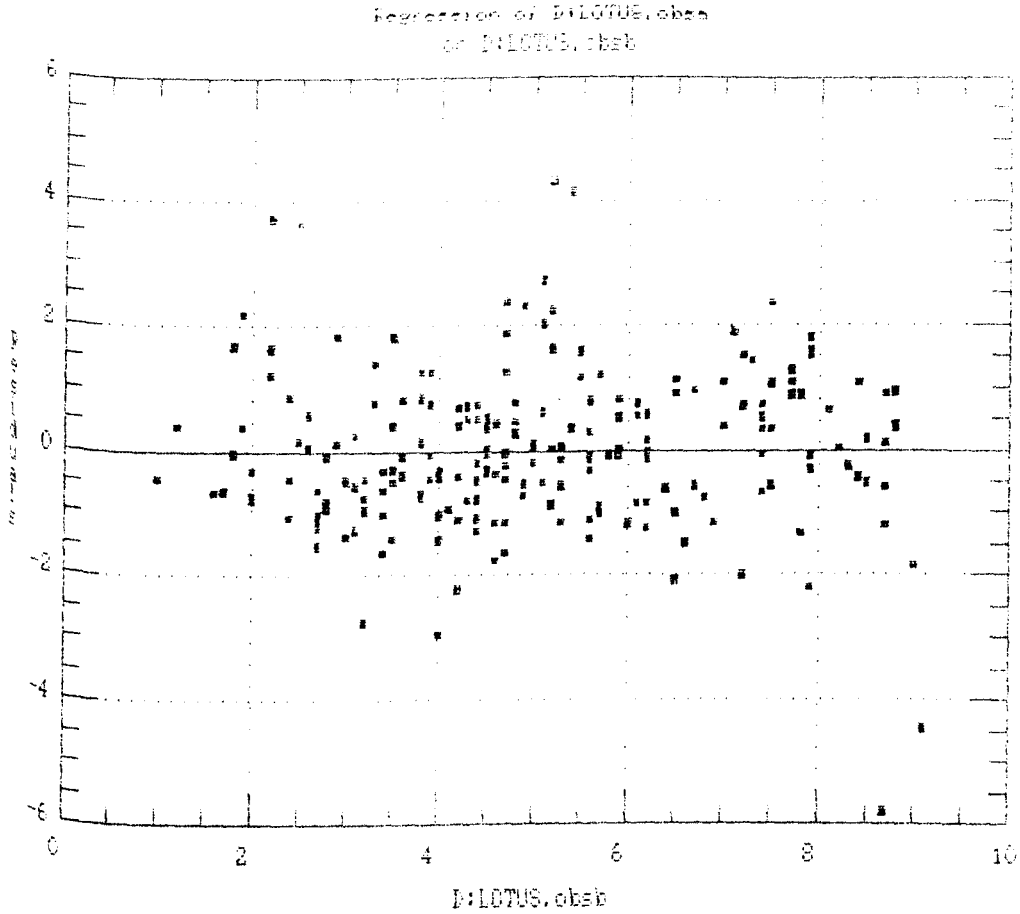
relation Coefficient = -0.207031 R-squared = 4.29 percent
d Error of Est. = 1.25135



OBSERVER A − OBSERVER B

VERSUS

$$\frac{\text{OBSERVER A + OBSERVER B}}{2}$$



OBSERVER A VERSUS OBSERVER B (REGRESSION OF RESIDUALS)

Statistical Analysis of Trial 170809/0001

Summary

Eight male volunteers were studied in a double blind randomised 4 way crossover design to compare placebo with 3 mg, 7 mg, 15 mg and 30 mg ICI 170809. One patient withdrew. For standing and supine systolic blood pressure and supine diastolic blood pressure there was a statistically significant ($p<0.05$) difference between the postdose means for the placebo group and the 3 mg ICI 170809 group. For standing heart rate there were several statistically significant differences between the ICI 170809 groups and placebo at some of the postdose timepoints. For some of the timepoints investigated, mean initial, minimum and finish pupil reaction in the ICI 170809 groups were statistically significantly lower compared to the placebo group. There were some statistically significant differences found between the means of the ICI 170809 groups and placebo for several haematology and biochemistry variables. However, the results should be interpreted with caution because of the number of significance tests performed.

Study Design

Eight male volunteers were studied in a double blind within patient trial to compare placebo with 3 mg, 7 mg, 15 mg and 30 mg ICI 170809. A randomised incomplete block design was used subject to the constraint that each patient received placebo and 3 doses of ICI 170809 in ascending order. The 8 volunteers were randomly allocated to the 16 possible sequences of the 5 treatments. The study consisted of 5 stages. The first stage was a prestudy screen. Stages 2, 3, 4 and 5 were the first, second, third and fourth treatment periods. In each of the treatment periods, the volunteers received either placebo or one of the doses of ICI 170809.

Volunteers and Data

One volunteer (003) withdrew from the trial. Details of heart rate and blood pressure are given in listings 01 to 05 and summarised in tables 01 to 06. Details of pupil reaction are given in listings 06 to 10 and summarised in tables 09 to 12. Details of drug levels in plasma and urine are given in listings 11 to 13 and plasma levels are summarised in tables 14 and 15. Details of symptom observations are given in listing 14 and additional comments relating to symptom observations are given in listing 15. Haematology and biochemistry measurements are given in listings 16 and 17 and summarised in tables 16 to 40. Values above the upper limit of the normal range are flagged 'U' and those below the lower limit are flagged 'L'. Details of urinalysis are given in listing 18. Details of side effects are given in listing 19 and summarised in table 42. Additional comments relating to the study are given in listing 20, and details of study completion are given in listing 21.

Details of the pre-study screen are given in appendices 1 to 24. Times of measurement for heart rate and blood pressure are given in appendix 25. Times of measurement for haematology and biochemistry are given in appendix 26. Times of measurement for drugs levels in plasma are given in appendices 27 and 28 and for drug levels in urine in appendix 29.

Statistical Methods

Heart rate and blood pressure (6 post dose timepoints) were analysed by repeated measures analysis of variance with the following general format:

Source	Degrees of Freedom
Volunteer	p-1
Treatment	4
Error 1	3p-4
Times	t-1
Times x volunteer	(t-1)(p-1)
Times x treatment	4(t-1)
Error 2	(t-1)(3p-4)
Total	4pt-1

The term stage cannot be fitted in the model to test for a period effect because it is confounded with treatment. The main objective of this repeated measures analysis is to test whether the post-dose time profiles of the ICI 170809 dose groups are parallel to that of the placebo group. This is defined by the times x treatment interaction and is tested by error 2. The Greenhouse Geisser correction factor was used. If a statistically significant ($p<0.05$) difference was found between the profiles then analysis of covariance was performed at each timepoint separately using the predose value as the covariate. Otherwise analysis of covariance was performed on the mean of the postdose values. Both analyses had the following general format:

Source	Degrees of Freedom
Volunteers	p-1
Treatment	4
Predose value	1
Error	3p-5
Total	4p-1

Pupil reaction data (4 post dose timepoints) were analysed in a similar manner as described above. There were no data measured at stage 3 except at predose and thus this data has been omitted from the analysis. Thus there were no 7 mg ICI 170809 data analysed.

Haematology and biochemistry were analysed by analysis of variance at each timepoint using the predose value as a covariate, with the same general format as given above.

MCH and MCHC were not analysed due to the limited range of values taken. Results are only presented for variables and timepoints where a statistically significant ($p<0.05$) difference was found between the treatments.

LSMEANS are presented for each treatment together with the LSD and p-value for the statistical significance of the difference of each dose of ICI 170809 from placebo. LSMEANS are means that have been adjusted to allow for any inbalance in the design. The LSD is the least difference there must be between a 170809 lsmean and the placebo LSMEAN for statistical significance at $p<0.05$ level.

Results

Heart Rate and Blood Pressure

No statistically significant differences ($p<0.05$) were found between the doses of ICI 170809 and placebo for supine heart rate or standing diastolic blood pressure. For both supine and standing systolic blood pressure the postdose mean the 3 mg ICI 170809 group was statistically significantly higher compared to the placebo group. For standing systolic blood pressure there was statistical evidence of a similar trend for the 7 mg ICI 170809 group compared to placebo ($p=0.056$). For supine diastolic blood pressure, the postdose mean for the 3 mg ICI 170809 group was statistically significantly higher compared to the placebo group.

Standing heart rate was analysed at each timepoint. At 8 hours postdose, the mean for the 3 mg ICI 170809 group was statistically significantly lower compared to the placebo group. At 24 hours postdose, the mean for the 15 mg ICI 170809 group was statistically significantly lower compared to the placebo group. At 2 and 3 hours postdose, the mean for the 30 mg ICI 170809 group was statistically significantly higher compared to the placebo group. There was evidence of a similar trend at 1 hour ($p=0.053$).

Pupil Reaction

At 3 hours postdose, mean initial pupil site for the 3 mg, 15 mg and 30 mg ICI 170809 groups was statistically significantly lower compared to placebo. The same was true at 5 hours postdose for the 15 mg and 30 mg ICI 170809 groups. There was statistical evidence that the same was true at 8 hours postdose for the 15 mg ICI 170809 group ($p=0.051$).

At 5 hours postdose, mean minimum pupil size for both the 15 mg and 30 mg ICI 170809 group was statistically significantly lower compared to the placebo group.

TABLE 1. LSMEANS FOR PUPIL REACTION

		PLACEBO		3MG ICI 170809				15MG 170809				30MG 170809			
		N	LSMEAN	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P	N	LSMEAN	LSD	P
INITIAL	TIME														
	PREDOSE	5	6.614	5	6.314	1.325	0.629	6	7.033	1.239	0.472	5	7.272	1.325	0.298
	3 HRS POSTDOSE	5	6.434	5	4.747	1.227	[illegible]	6	3.729	1.147	[illegible]	5	3.518	1.227	[illegible]
	5 HRS POSTDOSE	5	6.092	5	5.292	1.557	0.235	6	4.161	1.456	[illegible]	5	3.776	1.557	[illegible]
	8 HRS POSTDOSE	5	6.422	5	5.259	2.199	0.399	6	4.086	2.057	[illegible]	5	4.676	2.199	0.198
	24 HRS POSTDOSE	5	5.935	5	5.828	1.249	0.853	6	6.374	1.169	0.426	5	6.658	1.249	0.532
MINIMUM	PREDOSE	5	3.419	5	4.603	2.138	0.249	6	4.407	1.999	0.300	5	4.699	2.138	0.214
	3 HRS POSTDOSE	5	3.346	5	2.807	1.479	0.448	6	2.279	1.383	0.118	5	2.272	1.479	0.139
	5 HRS POSTDOSE	5	3.934	5	3.034	1.183	0.122	6	2.260	1.107	[illegible]	5	2.500	1.183	[illegible]
	8 HRS POSTDOSE	5	3.519	5	3.015	1.857	0.563	6	1.835	1.737	[illegible]	5	2.334	1.857	0.198
	24 HRS POSTDOSE	5	3.696	5	3.184	1.817	0.407	6	4.169	1.706	0.730	5	4.877	1.817	0.240

(CONTINUED)

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LISTING 06. DETAILS OF PUPIL REACTION - TREATMENT PLACEBO

VOLUNTEER	STAGE	TIME	INITIAL (MM)	MINIMUM (MM)	FINISH (MM)	TIME (SECS)
001	03	PREDOSE	8.30	5.80	6.40	0.70
		3HRS	.	.	.	.
		5HRS	.	.	.	.
		8HRS	.	.	.	.
		24HRS	.	.	.	.
002	05	PREDOSE	6.75	3.15	6.70	0.75
		3HRS	6.55	3.15	6.35	0.80
		5HRS	4.25	2.50	4.20	0.80
		8HRS	4.55	2.05	4.10	0.85
		24HRS	6.75	4.35	6.40	0.80
004	02	PREDOSE	6.45	2.95	6.10	1.00
		3HRS	7.25	4.05	6.15	0.95
		5HRS	6.35	3.50	5.90	0.90
		8HRS	6.55	2.95	6.70	1.10
		24HRS	6.10	3.50	5.65	0.85
005	02	PREDOSE	6.10	3.25	5.75	0.90
		3HRS	7.30	4.40	6.65	0.90
		5HRS	6.35	4.10	6.15	0.80
		8HRS	6.00	3.25	5.65	0.85
		24HRS	6.45	2.70	6.75	0.80

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LISTING 06. DETAILS OF PUPIL REACTION - TREATMENT PLACEBO

VOLUNTEER	STAGE	TIME	INITIAL (MM)	MINIMUM (MM)	FINISH (MM)	TIME (SECS)
006	04	PREDOSE	8.75	7.85	9.00	0.70
		3HRS	6.30	4.05	5.95	.
		5HRS	7.90	5.15	7.10	1.05
		8HRS	8.95	3.95	8.55	1.05
		24HRS	8.25	6.30	8.65	0.70
007	05	PREDOSE	8.15	3.45	6.95	0.90
		3HRS	6.70	1.75	6.30	0.75
		5HRS	8.55	5.70	8.00	0.85
		8HRS	9.05	6.30	7.80	1.05
		24HRS	7.45	6.25	7.35	0.85
008	03	PREDOSE	3.55	1.95	3.30	0.80
		3HRS	.	.	.	.
		5HRS	.	.	.	.
		8HRS	.	.	.	.
		24HRS	.	.	.	.

LISTING 07. DETAILS OF PUPIL REACTION

VOLUNTEER	STAGE	TIME	INITIAL (MM)
001	02	PREDOSE	5.20
		3HRS	3.15
		5HRS	3.80
		8HRS	2.90
		24HRS	4.25
002	02	PREDOSE	5.60
		3HRS	5.50
		5HRS	4.90
		8HRS	5.80
		24HRS	5.60
006	02	PREDOSE	7.30
		3HRS	5.35
		5HRS	7.10
		8HRS	6.85
		24HRS	7.05
007	02	PREDOSE	7.70
		3HRS	5.55
		5HRS	6.00
		8HRS	7.35
		24HRS	6.45

- TREATMENT 3MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
3.10	4.70	0.90
1.80	3.05	0.80
2.10	3.45	0.70
1.65	2.65	0.75
2.60	3.75	0.80
3.60	5.45	0.80
3.00	4.85	0.80
3.15	4.70	0.80
3.45	5.50	0.90
3.60	5.60	0.90
5.65	7.05	0.90
3.50	5.25	0.90
3.75	6.65	0.95
4.75	6.55	0.90
4.50	6.60	0.90
6.30	7.35	0.80
3.40	4.75	0.85
4.20	5.75	0.90
3.20	6.55	1.10
2.60	6.05	0.70

LISTING 07. DETAILS OF PUPIL REACTION

VOLUNTEER	STAGE	TIME	INITIAL (MM)
008	02	PREDOSE	5.60
		3HRS	3.15
		5HRS	3.10
		8HRS	3.50
		24HRS	3.60

- TREATMENT 3MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
3.60	5.45	0.80
1.95	2.85	0.70
2.00	2.85	0.70
2.45	3.60	0.70
2.15	3.40	0.75

LISTING 08. DETAILS OF PUPIL REACTION -

VOLUNTEER	STAGE	TIME	INITIAL (MM)
002	03	PREDOSE	8.30
		3HRS	.
		5HRS	.
		8HRS	.
		24HRS	.
003	03	PREDOSE	8.80
		3HRS	.
		5HRS	.
		8HRS	.
		24HRS	.
004	03	PREDOSE	6.00
		3HRS	.
		5HRS	.
		8HRS	.
		24HRS	.
005	03	PREDOSE	5.25
		3HRS	.
		5HRS	.
		8HRS	.
		24HRS	.

- TREATMENT 7MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
5.80	6.40	0.70
6.35	8.50	0.95
2.35	6.30	0.90
3.35	4.60	0.75

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LISTING 08. DETAILS OF PUPIL REACTION

VOLUNTEER	STAGE	TIME	INITIAL (MM)
006	03	PREDOSE	.
		3HRS	.
		5HRS	.
		8HRS	.
		24HRS	.
007	03	PREDOSE	7.90
		3HRS	.
		5HRS	.
		8HRS	.
		24HRS	.

- TREATMENT 7MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
.	.	.
.	.	.
.	.	.
.	.	.
.	.	.
6.00	6.30	0.75
.	.	.
.	.	.
.	.	.
.	.	.

LISTING 09. DETAILS OF PUPIL REACTION -

VOLUNTEER	STAGE	TIME	INITIAL (MM)
001	04	PREDOSE	6.85
		3HRS	2.75
		5HRS	2.65
		8HRS	3.20
		24HRS	2.90
002	04	PREDOSE	6.15
		3HRS	4.80
		5HRS	3.30
		8HRS	6.00
		24HRS	6.15
[illegible]	04	PREDOSE	8.45
		3HRS	5.90
		5HRS	7.40
		8HRS	4.50
		24HRS	8.50
005	04	PREDOSE	5.85
		3HRS	2.50
		5HRS	3.45
		8HRS	2.70
		24HRS	7.60

TREATMENT 15MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
4.00	6.40	0.90
1.95	2.70	0.70
1.70	2.70	0.85
2.40	3.25	0.70
1.85	3.05	0.80
1.70	5.85	0.85
3.10	4.80	0.80
2.30	3.25	0.75
2.25	5.50	1.10
2.90	5.90	0.85
7.15	8.20	0.70
2.25	4.30	0.85
2.35	7.45	0.80
1.00	3.40	0.85
5.60	7.70	0.95
2.70	5.25	0.80
1.25	2.45	0.70
1.80	4.20	0.80
1.05	2.50	0.80
4.00	6.55	0.80

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LISTING 09. DETAILS OF PUPIL REACTION -

VOLUNTEER	STAGE	TIME	INITIAL (MM)
007	04	PREDOSE	9.20
		3HRS	3.65
		5HRS	3.75
		8HRS	4.05
		24HRS	7.90
008	04	PREDOSE	3.95
		3HRS	2.85
		5HRS	2.65
		8HRS	2.90
		24HRS	3.40

TREATMENT 15MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
5.90	7.90	0.85
2.65	3.70	0.70
2.85	6.25	0.35
2.10	3.55	0.75
6.35	6.65	0.90
1.95	3.40	0.90
2.25	2.75	0.75
2.00	2.50	0.75
1.75	2.35	0.90
2.35	3.35	0.85

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LISTING 10. DETAILS OF PUPIL REACTION -

VOLUNTEER	STAGE	TIME	INITIAL (MM)
001	05	PREDOSE	5.50
		3HRS	3.20
		5HRS	2.60
		8HRS	3.75
		24HRS	3.45
004	05	PREDOSE	7.85
		3HRS	5.36
		5HRS	5.60
		8HRS	7.70
		24HRS	8.45
005	05	PREDOSE	7.65
		3HRS	2.15
		5HRS	2.30
		8HRS	3.20
		24HRS	6.20
006	05	PREDOSE	9.40
		3HRS	2.80
		5HRS	5.75
		8HRS	4.10
		24HRS	7.90

TREATMENT 30MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
2.40	5.15	0.80
2.30	3.10	0.70
1.90	2.75	0.80
2.40	4.30	0.80
2.35	3.45	0.80
7.05	7.35	0.70
4.00	5.63	.
4.15	5.10	0.75
4.10	6.70	0.75
7.35	8.30	0.80
4.25	5.65	1.10
1.00	2.25	0.70
1.40	2.35	0.75
1.75	3.15	0.70
4.15	5.50	0.70
8.35	9.10	0.70
1.55	2.75	0.80
2.25	3.90	0.80
1.55	4.05	0.80
7.05	7.55	1.05

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LISTING 10. DETAILS OF PUPIL REACTION -

VOLUNTEER	STAGE	TIME	INITIAL (MM)
008	05	PREDOSE	4.75
		3HRS	3.10
		5HRS	2.70
		8HRS	2.90
		24HRS	2.95

TREATMENT 30MG ICI 170809

MINIMUM (MM)	FINISH (MM)	TIME (SECS)
1.70	4.65	0.75
2.45	3.20	0.75
2.05	2.45	0.80
1.00	3.00	0.90
2.30	3.05	0.70

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LISTING 14. DETAILS OF SYMPTOM OBSERVATIONS

VOLUNTEER	TREATMENT	STAGE	TIME REL. TO DOSE	SYMPTOM	SEVERITY
001	PLACEBO	03	-0:10	SLEEP	.
001	15MG ICI 170809	04	04:10	SLEEP	MODERATE
		04	04:55	SLEEP	MODERATE
		04	06:45	SLEEP	SEVERE
		04	06:55	OTHER	MILD
001	30MG ICI 170809	05	00:21	SLEEP	.
		05	03:00	DROWSINESS	.
		05	05:08	DROWSINESS	.
		05	08:10	OTHER	.
002	PLACEBO	05	01:10	DROWSINESS	.
		05	06:55	DROWSINESS	.
		05	07:55	OTHER	.
002	3MG ICI 170809	02	02:45	DROWSINESS	.
		02	06:35	SLEEP	.
002	7MG ICI 170809	03	04:40	OTHER	.
		03	06:25	SLEEP	.
		03	07:40	OTHER	.
002	15MG ICI 170809	04	02:10	SLEEP	MILD
		04	04:40	SLEEP	MODERATE
		04	06:40	OTHER	.

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LISTING 14. DETAILS OF SYMPTOM OBSERVATIONS

VOLUNTEER	TREATMENT	STAGE	TIME REL. TO DOSE	SYMPTOM	SEVERITY
003	7MG ICI 170809	03	05:42	SLEEP	MILD
		03	07:55	OTHER	MODERATE
004	PLACEBO	02	01:40	DROWSINESS	.
004	7MG ICI 170809	03	06:25	DROWSINESS	.
004	15MG ICI 170809	04	00:05	YAWNING	MILD
		04	00:05	SLEEP	MODERATE
004	30MG ICI 170809	05	00:55	DROWSINESS	.
		05	01:01	SLEEP	.
		05	03:00	DROWSINESS	.
		05	03:00	OTHER	.
		05	07:45	SLEEP	MODERATE
006	3MG ICI 170809	02	00:01	SLEEP	.
006	7MG ICI 170809	03	.	SLEEP	MILD
		03	06:19	DROWSINESS	MILD
007	3MG ICI 170809	02	06:30	DROWSINESS	MILD
007	7MG ICI 170809	03	03:00	YAWNING	MODERATE
		03	05:35	SLEEP	SEVERE
		03	07:56	DROWSINESS	MILD

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LISTING 14. DETAILS OF SYMPTOM OBSERVATIONS

VOLUNTEER	TREATMENT	STAGE	TIME REL. TO DOSE	SYMPTOM	SEVERITY
007	15MG ICI 170809	04	01:10	DROWSINESS	MILD
		04	01:55	SLEEP	MODERATE
		04	05:00	DROWSINESS	MODERATE
		04	07:55	SLEEP	MODERATE
008	PLACEBO	03	02:45	SLEEP	MODERATE
008	3MG ICI 170809	02	-0:06	OTHER	.
		02	01:55	SLEEP	MILD
		02	05:45	DROWSINESS	MODERATE
008	15MG ICI 170809	04	01:55	SLEEP	MODERATE
		04	06:25	SLEEP	MODERATE
008	30MG ICI 170809	05	01:35	SLEEP	MODERATE
		05	02:00	OTHER	.
		05	05:20	SLEEP	MODERATE

Table B1 : 170809 CR0001. Concentrations of ICI 170,809 (ng/ml) in plasma of subjects after a single 3 mg dose of ICI 170,809.

Subject	Time after dose (hours)							
	Pre	0.5	1	2	3	5	8	24
1	ND	6.8	8.4	13.1	6.9	8.1	7.6	4.8
2	ND	ND	3.0	6.2	5.5	4.1	3.5	ND
6	ND	ND	3.9	5.8	4.5	3.6	2.4	ND
7	ND	ND	2.3	4.1	5.2	3.5	4.7	ND
8	ND	ND	5.9	7.1	9.5	7.2	6.0	3.5
Mean	NC	NC	4.7	7.3	6.3	5.3	4.8	NC
SE	-	-	1.1	1.5	0.9	1.0	0.9	-

SE - Standard error

NC - Not calculated

Peak concentrations underlined

ND - Not detectable; below limit of quantification, study limit = 2.0 ng/ml

	Time after dose (h)														
Subject	Pre	1	1.5	2	3	5	8	12	15	24	30	48	$t_{\frac{1}{2}}$(dist) (h)	$t_{\frac{1}{2}}$(el) (h)	AUC_{∞} (ng.h/ml)
2	ND	5.8	8.3	10.3	10.7	5.4	4.6	2.3	ND	ND	ND	ND	5.1	5.0	83
4	ND	17.9	22.0	20.8	14.3	9.7	7.1	5.3	3.7	2.8	2.7	2.3	2.0	19.7	257
5	ND	15.2	22.6	24.3	18.2	10.9	6.8	5.8	3.7	3.3	3.1	ND	1.7	26.8	316
6	ND	7.9	9.2	11.8	7.8	5.7	3.8	2.7	ND	ND	ND	ND	0.7	5.8	87
7	ND	2.6	5.9	8.2	10.6	10.6	8.2	4.6	3.0	2.5	2.1	ND	3.5	32.5	240
Mean	NC	9.9	13.6	15.1	12.3	8.5	6.1	4.1	2.9	2.5	2.4	NC	2.6	18.0	197
SE	-	2.9	3.6	3.2	1.8	1.2	0.8	0.7	0.38	0.25	0.22	-	0.77	5.5	47

SE - Standard error
NC - Not calculated
Peak concentrations underlined
ND - Not detectable, below limit of quantification, study limit 2.0 ng/ml

Table B2 : 170809 CR0001. Concentrations of ICI 170,809 (ng/ml) in plasma of subjects after a single 7 mg dose of ICI 170,809.

	Time after dose (h)														
Subject	Pre	1	1.5	2	3	5	8	12	15	24	30	48	$t_{\frac{1}{2}}$(dist) (h)	$t_{\frac{1}{2}}$(el)	AUC$_{\infty}$ (ng.h/ml)
1	ND	41.2	47.1	45.5	37.6	30.9	26.3	18.6	15.3	15.2	11.6	9.9	3.1	46.9	1484
2	ND	8.6	13.0	17.6	15.2	10.9	5.5	4.4	3.8	ND	ND	ND	1.8	10.7	175
4	ND	25.9	32.9	29.6	25.4	14.7	9.9	8.7	5.2	4.8	6.2	3.5	2.9	59.1	670
5	ND	25.8	34.5	47.3	39.5	28.0	16.9	13.2	7.8	6.7	5.1	3.9	3.1	34.3	693
7	ND	7.1	11.6	15.9	19.0	20.0	11.6	8.4	6.2	5.6	4.3	ND	2.7	23.7	411
8	ND	35.3	56.9	61.4	53.8	53.4	35.1	27.7	24.8	20.1	20.1	16.2	2.9	65.2	2745
Mean	NC	24.0	32.7	36.2	31.7	26.3	17.5	13.5	10.5	9.1	8.2	6.2	2.8	40.0	1030
SE	-	5.6	7.4	7.4	5.9	6.2	4.6	3.5	3.3	2.9	2.7	2.3	0.20	8.6	388

SE - Standard error
NC - Not calculated
Peak concentrations underlined
ND - Not detectable, below limit of quantification, study limit 2.0 ng/ml

Table B3 : 170809 CR0001. Concentrations of ICI 170,809 (ng/ml) in plasma of subjects after a single 15 mg dose of ICI 170,809.

	Time after dose (h)														
Subject	Pre	1	1.5	2	3	5	8	12	15	24	30	48	$t_{\frac{1}{2}}$(dist) (h)	$t_{\frac{1}{2}}$(el) (h)	AUC_{∞} (ng.h/ml)
1	4.4	81.6	114	98.9	84.0	67.5	61.1	43.2	33.8	24.9	23.0	17.8	3.6	37.9	2654
4	ND	93.2	85.3	84.4	65.8	48.0	35.9	17.5	12.7	8.1	7.7	6.1	3.2	53.5	1337
5	ND	56.7	88.4	98.4	80.4	58.5	39.1	23.6	18.1	10.8	6.8	5.9	3.5	38.4	1298
6	ND	85.6	81.9	74.0	57.2	36.5	24.3	15.7	9.6	6.6	5.9	ND	2.8	19.5	769
8	5.5	115	128	130	102	102	88.1	67.5	44.9	38.0	32.9	29.7	4.2	65.8	5238
Mean	NC	86.4	99.5	97.1	77.9	62.5	49.7	33.5	23.8	17.7	15.3	12.3	3.5	43.0	2259
SE	-	9.4	9.1	9.4	7.7	11.2	11.3	9.8	6.7	6.0	5.4	5.1	0.23	7.8	807

SE - Standard error
NC - Not calculated
Peak concentrations underlined
ND - Not detectable, below limit of quantification, study limit 2.0 ng/ml

Table B4 : 170809 CR0001. Concentrations of ICI 170,809 (ng/ml) in plasma of subjects after a single 30 mg dose of ICI 170,809.

DIAMETER

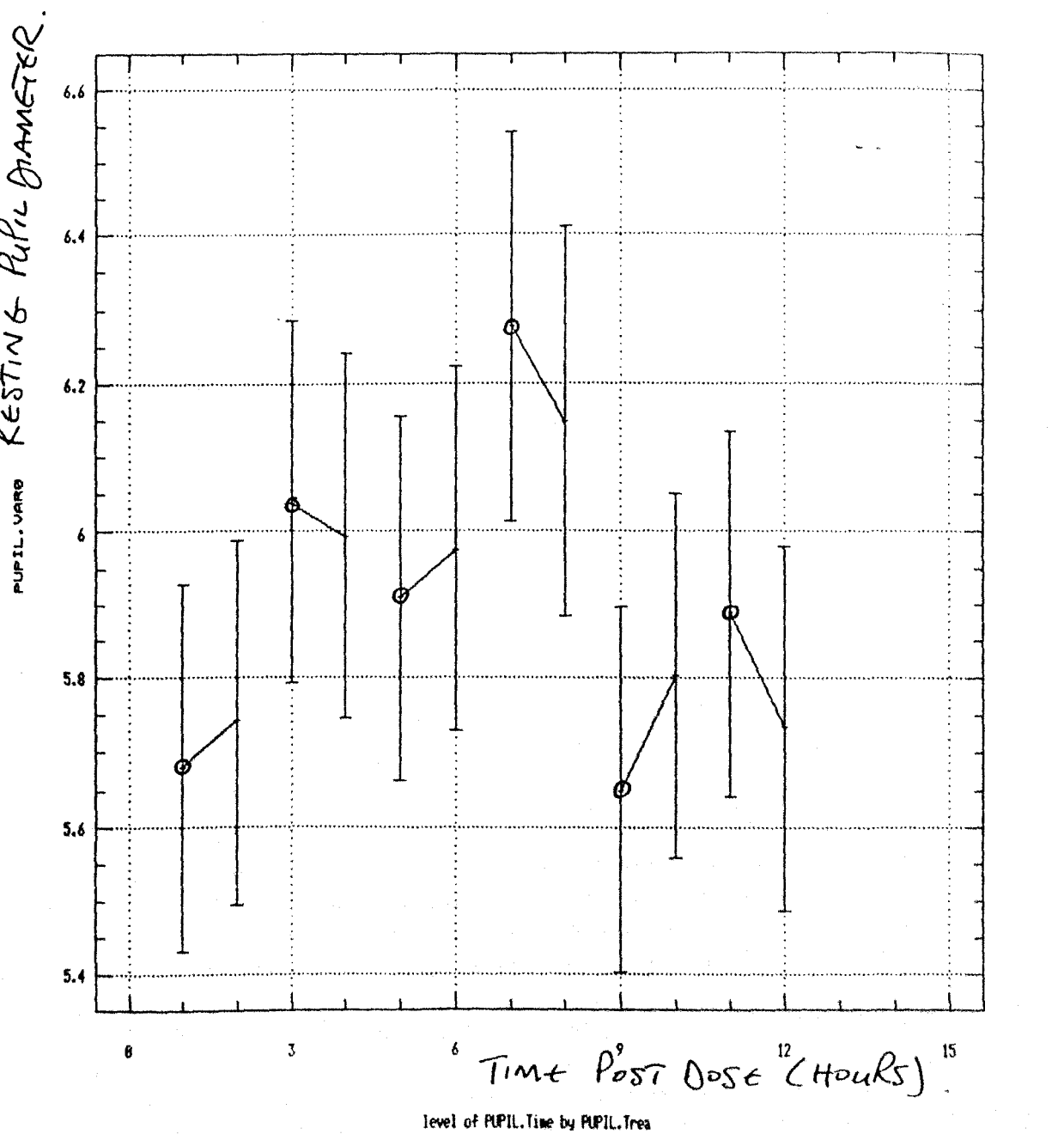
Analysis of Variance for PUPIL.VAR0

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. le
MAIN EFFECTS	143.77059	14	10.269328	83.549	.000
PUPIL.volunteer	140.24032	7	20.034331	162.996	.000
PUPIL.Time	1.41276	5	.282552	2.299	.053
PUPIL.stage	.59092	1	.590919	4.808	.031
PUPIL.Treatment	.00214	1	.002144	.017	.896
2-FACTOR INTERACTIONS	.2340300	5	.0468060	.381	.860
PUPIL.TimePUPIL.Trea	.2340300	5	.0468060	.381	.860
RESIDUAL	9.0955852	74	.1229133		
TOTAL (CORR.)	153.10021	93			

2 missing values have been excluded.

Table of means for PUPIL.VAR0

Level	Count	Average	Stnd. Error (internal)	Stnd. Error (pooled s)	95 Percent Confiden for mean	
PUPIL.volunteer						
1	11	3.8681818	.0713738	.1057068	3.6575088	4.0788
2	12	5.8200000	.1220345	.1012066	5.6182959	6.0217
3	11	4.7400000	.1423440	.1057068	4.5293270	4.9506
4	12	5.6716667	.1013233	.1012066	5.4699626	5.8733
5	12	5.1883333	.1367193	.1012066	4.9866293	5.3900
6	12	6.3491667	.0594349	.1012066	6.1474626	6.5508
7	12	7.6325000	.0886526	.1012066	7.4307959	7.8342
8	12	7.6400000	.0975534	.1012066	7.4382959	7.8417
PUPIL.Time (POST DOSE - HOURS)						
0	16	5.7112500	.2941795	.0876475	5.5365692	5.8859
1.25	16	6.0162500	.3228866	.0876475	5.8415692	6.1909
2	16	5.9425000	.3128851	.0876475	5.7678192	6.1171
4	14	6.2128571	.3345645	.0936991	6.0261155	6.3995
8	16	5.7268750	.3309969	.0876475	5.5521942	5.9015
24	16	5.8112500	.3783593	.0876475	5.6365692	5.9859
PUPIL.stage						
3	47	5.9855319	.1789092	.0511388	5.8836126	6.0874
4	47	5.8082979	.1961230	.0511388	5.7063786	5.9102
PUPIL.Treatment						
1 PLACEBO	47	5.8995745	.1800334	.0511388	5.7976552	6.0014
2 CHLORAL	47	5.8942553	.1959638	.0511388	5.7923360	5.9961
PUPIL.Time by PUPIL.Treatment						
0 1	8	5.6800000	.4247688	.1239523	5.4329640	5.9270
0 2	8	5.7425000	.4361018	.1239523	5.4954640	5.9895
1.25 1	8	6.0387500	.4686395	.1239523	5.7917140	6.2858
1.25 2	8	5.9937500	.4764899	.1239523	5.7467140	6.2408
2 1	8	5.9087500	.4635632	.1239523	5.6617140	6.1558
2 2	8	5.9762500	.4520427	.1239523	5.7292140	6.2233
4 1	7	6.2785714	.4536908	.1325105	6.0144788	6.5426
4 2	7	6.1471429	.5270390	.1325105	5.8830502	6.4113
8 1	8	5.6500000	.4757738	.1239523	5.4029640	5.8970
8 2	8	5.8037500	.4914154	.1239523	5.5567140	6.0508
24 1	8	5.8887500	.4681019	.1239523	5.6417140	6.1358
24 2	8	5.7337500	.6266491	.1239523	5.4867140	5.9808


0 PLACEBO.
CHLORAL HYDRATE.
TREATMENT
95 Percent Confidence
Intervals for Factor Means
RESTING PUPIL DIAMETER.
PUPIL.VARB
6.6
6.4
6.2
6
5.8
5.6
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0
3
6
9
12
15
TIME POST DOSE (HOURS)
level of PUPIL.Time by PUPIL.Trea

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The effects of a 5-HT_2 receptor antagonist (ICI 169,369) on changes in waking EEG, pupillary responses and state of arousal in human volunteers

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ICI 169,369 (2-(2-dimethylamino ethylthio)-3-phenyl quinoline) is a potent selective competitive antagonist of the 5-HT_2 receptor in animal models. Effects of ICI 169,369 as single oral doses (80 and 120 mg) separated by 1 week, on the power spectrum of waking EEG, dark adapted pupil responses and sedation score, were studied in a double-blind, placebo controlled, randomised cross over within subject comparison, in six healthy male volunteers.

Pupillary responses were measured using a portable infrared pupillometer following 15 min dark adaptation, assessing resting vertical pupil diameter (RPD), light constricted diameter (MPD) and recovered final diameter (FPD) at the end of a 3 s measurement cycle.

Both doses of ICI 169,369 produced a mean 36% (range 10–54%) decrease in log 10 power of the waking EEG alpha activity with eyes closed ($P < 0.02$), and mean 38% (range 2–86%) increase in theta activity at 2 h compared with placebo.

Both 80 and 120 mg doses of ICI 169,369 reduced RPD by approximately 30% from a predose value of 6.25 mm (±0.87; 95% CI) and from placebo values 6.41 mm (±1.06) and 7.48 mm (±1.49) at 3 and 5 h after dosing. MPD was reduced by 50% with the 120 mg dose at 5 h after dosing (placebo 5.2 mm; ICI 169,369 2.7 mm; $P < 0.05$). FPD was significantly reduced ($P < 0.01$) by both doses at 3 h after dosing.

The maximum acceleration (ACC_{max}, mm s^{-1} s^{-1}) and maximum velocity (VEL_{max}, mm s^{-1}) of the light induced pupillary responses were significantly reduced ($P < 0.05$) with the 120 mg dose of ICI 169,369 at 3 h after dosing (ACC_{max} = 29.7 ± 1.4 mm s^{-1} s^{-1}; VEL_{max} = 3.8 ±0.7 mm s^{-1}, mean ± 95% CI) compared with placebo (ACC_{max} = 40.7 mm s^{-1} s^{-1}; VEL_{max} = 6.2 mm s^{-1}).

Quantification of ICI 169,369 was performed on whole blood at various times after dosing with 80 and 120 mg. Resting pupil diameter after dosing was significantly correlated ($r = 0.697$, slope = 0.92; $P < 0.0001$) with log whole blood concentration.

Arousal assessed by Bond Lader visual analogue scales (VAS) demonstrated an increased sedation score at 5 h after dosing, with both 80 (VAS = 25 ± 3.0, $P < 0.05$) and 120 mg (VAS = 27.2 ± 3.0, $P < 0.01$) doses compared with placebo (VAS = 21.4).

Oral dosing with ICI 169,369 at both 80 and 120 mg doses produced changes in waking EEG, pupillary responses and sedation, suggesting that 5-HT_2 receptor antagonism may produce both central and peripheral effects in man.

Keywords 5-HT_2 antagonism ICI 169,369 human CNS-dynamics

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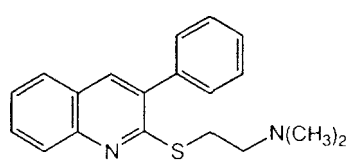
Introduction

5-HT_2 receptor binding sites have been demonstrated in frontal and limbic areas of human brain (Pazos *et al.*, 1987), linked to the turnover of phosphatidylinositol phosphate as a second messenger (Conn & Saunders-Bush, 1986). These 5-HT_2 receptors may be involved in the pathophysiology of CNS disorders such as anxiety, depression, schizophrenia and Parkinsonism (Cooper & Abbott, 1988). Outside the brain 5-HT_2 receptors mediate a variety of functional responses, involving smooth muscle contraction, platelet aggregation and neuronal depolarisation, with possible involvement in such diseases as hypertension, coronary artery spasm, Raynaud's disease and migraine (Cameron *et al.*, 1987; Davies & Steiner, 1990; De Clerck & Van Nueten, 1982; Hollenburg, 1988).

5-HT_2 receptors in man have been characterised to date with ketanserin, a selective 5-HT_2 receptor antagonist with antihypertensive properties (Cameron *et al.*, 1987). Pharmacodynamic responses in healthy volunteers have been demonstrated at a vascular 5-HT_2 receptor using limb blood flow plesthysmography (Blauw *et al.*, 1988) and on the waking EEG (Reiman *et al.*, 1986). Other selective 5-HT_2 receptor antagonists such as ritanserin (Idzikowski *et al.*, 1987) and trazodone, together with mianserin, an antidepressant with non-selective 5-HT_2 receptor antagonism, all increase slow wave sleep (SWS), on acute and chronic dosing (Spiegel, 1980).

Evidence suggests that 5-HT_2 receptors may also be involved in the control of pupillary responses. Radioligand binding and immunohistochemistry have localised 5-HT neurones in the human iris-ciliary body (Uusitalo *et al.*, 1984). L-tryptophan injection (a 5-HT precursor) by close carotid arterial injection in man produced an ipsilateral mydriasis by increasing ciliary body 5-HT (Mantegezzini, 1966). Similarly fenfluramine, a 5-HT releasing agent produced mydriasis, not abolished by pre-treatment with guanethidine or thymoxamine (Kramer & Turner, 1973). Mianserin and trazodone (Longmore *et al.*, 1988) both with 5-HT_2 receptor antagonist properties, produced a miosis which in the case of mianserin was unaffected by α_1-adrenoceptor or muscarinic agonists (Shur *et al.*, 1983). These findings suggest that 5-HT may be influencing pupillary responses in man, by mechanisms other than classical autonomic receptors.

ICI 169,369 (2-2-dimethylamino ethyl thio) -3-phenyl quinoline (Figure 1) is a selective competitive antagonist of 5-HT at the 5-HT_2 receptor (Blackburn *et al.*, 1988). From the above findings in the literature, it should be possible to demonstrate pharmacodynamic effects with ICI 169,369 in man by assessing its effects on waking EEG, pupillary responses and VAS mood rating scales. In this study, these were examined together with whole blood concentration measurements, to assess possible correlation with any biological effect.



ICI 169,369

2–(2–dimethylaminoethylthio)–3–phenylquinoline

Figure 1 Structural formula of ICI 169,369 hydrochloride.

Methods

Six healthy male volunteers were recruited (aged 18–37 years). Informed consent and local ethics committee approval were obtained. They were allowed to enter the study if no clinically relevant contraindications were found on medical history and examination, following blood tests for standard haematological and biochemical parameters and resting ECG. Volunteers were not allowed to enter the study if a baseline EEG was abnormal.

Study design (including dose and dosing regime of ICI 169,369)

Six male volunteers were studied in a double-blind, within subject trial, using a three period cross over design, each separated by 1 week, to compare two single oral doses of ICI 169,369 (80 and 120 mg) and matching placebo. Volunteers were randomly allocated to the six possible sequences of the three treatments subject to the constraint that each set of three subjects formed a latin square.

Study protocol

Three subjects were studied each day. Volunteers fasted overnight and consumed no alcohol or caffeine for 24 h prior to and after dosing. EEG measurements using four channels were made on each volunteer at four time-points (before and at 2, 6 and 24 h after dosing) with both eyes open and closed. Pupillometry and VAS assessments were made at six timepoints (before and at 3, 5, 8, 12 and 24 h after dosing). The same measurements were made in each of the three treatment periods.

The Bond Lader VAS alert-drowsy scale was analysed by calculating the area under the curve over 15 time-points and an analysis of variance performed. The 16 Bond Lader scales were analysed as three factors, 'alertness', 'contentness' and 'calmness'. The least square mean over the contributing scales was calculated for each factor at each timepoint and differences from predose were analysed by an analysis of variance model.

Linear and log linear regression analysis was carried out and analysis of variance was applied to assess the linearity of the model together with the correlation coefficient, slope, and 95% confidence intervals for the fitted line.

A 5 ml sample of venous blood was taken, into an oxalate tube and stored at −20° C until assayed, before dosing on each study day and immediately preceding EEG and pupillometry assessments, the order of priority for measurements was venous blood sampling, EEG

recording, pupillometry followed by VAS Bond-Lader scales.

Methods of assessment

Waking EEG recording A standard 10/20 seven electrode montage was applied using seven silver/silver chloride electrodes fixed in place with flexible collodion after dermal abrasion. Following this, subjects rested supine in a sound attenuated, temperature controlled room for 5 min with their eyes open, under observation via a closed circuit television system. EEG recording then commenced for 4 min with 'eyes open' whilst performing a vigilance task (counting a random sequence of crosses on a VDU), followed by a further 4 min 'eyes closed' during which dominant index finger tapping was carried out.

EEG data capture and processing Four channels of EEG signal were continuously monitored via a Medlec 5000 preamp and polygraph. The signal was recorded onto magnetic tape for archiving, and simultaneously passed through a 1703 signal conditioning module (CED, Cambridge) amplifier, filter and anti-aliasing device which was programmed to set a band width of 30 Hz for on-line analysis.

In conjunction with an IBM PC-AT micro computer based system the signals were subsequently digitised by a 1401 intelligent interface (CED, Cambridge) at a rate of 128 Hz. Continuous monitoring of the simultaneous polygraph output facilitated recognition of oculographic and other artefacts and assessment of vigilance.

The last 2 min of EEG recorded during each sequence were divided into 20 separate 6 s epochs, for eyes open and eyes closed recording. All 20 under each condition (eyes open/closed) free of artefacts were further analysed. Each 6 s epoch was multiplied by a 10–80–10 cosine bell window (half cosine wave in the range 0–1, lasting 0.6 s at each end of the epoch and unity for the central 4.8 s) to minimise leakage. Each epoch was also detrended and the fast fourier transform (FFT) was then applied to give estimates of the power spectrum at 0.125 Hz intervals. A further data reduction was then performed by averaging groups of 20 adjacent values producing 1 Hz frequency bands, the DC term being ignored, and by then averaging the 20 epochs obtained under the same conditions (eyes open/closed). The first 30 of these 1 Hz frequency bands were used to construct a power spectrum analysis.

Infra-red pupillometry assessments 'Pupilscan', a portable infra-red (IR) pupillometer, was used to measure dark adapted resting pupil diameter (RPD) and light stimulated reflexes (Millson *et al.*, 1988). A hand held optical unit with dual IR emitting diodes (940 nm wavelength, power 2×10^{-5} to 2×10^{-3} MW cm^{-2}) was aligned on the centre of the pupil with the aid of crosswires and a low intensity viewing light (585 nm, 3.2 cd m^{-2}). Reflected IR, and hence the 'black hole' effect of the pupil, was detected by an IR solid state image sensor (65K, rectangular fixed array) scanning the pupil every 10 ms.

All assessments were made under 'dark room conditions'. Volunteers dark adapted for 15 min wearing dark welding goggles (BS15422) prior to illumination of the right pupil with the viewing light (3.2 cd m^{-2}) for 30 s, to allow central fixation of the optical unit using crosswires. Supine subjects were asked to fixate on a cross marked on the ceiling (2 m distant), directed not to blink for the 3 s measurement period, and a recording made of the light induced pupillary constriction, five artefact free pupil light response curves were recorded at 30 s intervals. An artefact was defined as a blink or eye movement noted by the observer, or appearing as a significant negative deflection from the normal pupil response profile.

Analysis of pupillary light reflexes Vertical pupil diameter was measured automatically, at the start of the 3 s measurement cycle (RPD), initiated by the trigger mechanism in the optical unit. This was followed by a stimulus impulse of 0.5 s duration (565 nm, 65 cd m^{-2}) and a measurement of minimum pupil diameter (MPD), followed by a recovery to final pupil diameter (FPD). The change in vertical pupil diameter was captured by a portable microprocessor, and summary pupil data (RPD, MPD and FPD) displayed on a liquid crystal display, together with date, time and subject identification.

Individual light reflex curves were analysed using a pupil scan data handling template. Recordings (corresponding to 30 estimates of vertical pupil diameter during each 3 s measurement cycle) were analysed to derive the maximum velocity ($VEL_{max}C$) and maximum acceleration ($ACC_{max}C$) of pupillary constriction (i.e. the first and second differential of the constrictor response).

Visual analogue mood rating scales Subjects were given 16 dimensions on which to rate their subjective feelings by marking a point on a computer generated 100 mm line by moving the cursor along the visual display unit of the IBM PC-AT, meant to represent the full range of a particular dimension (e.g. alert-drowsy). The ratings were measured and transformed.

Whole blood ICI 169,369 estimation Heparinised whole blood (1 ml) samples were analysed using a gas-liquid chromatographic procedure. Briefly, after solvent extraction into pH 10 buffer containing 1.5% amyl alcohol in hexane the organic layer was acidified with 0.1 N hydrochloric acid. After shaking and centrifugal separation the organic layer was then discarded. Sodium hydroxide 0.1 N and 1.5% amyl alcohol were added to the aqueous layer and after extraction into the organic layer this was transferred and blown to dryness at 40° C with oxyen-free nitrogen. The residue was redissolved in 1% (v/v) ethanol in octanol and injected into a Hewlett-Packard gas chromatograph fitted with a 5% methyl-phenyl silicone capillary column of 0.52 μ film thickness, 25 m long, internal diameter 0.31 mm. Detection was by means of a nitrogen detector. The lower limit of quantification was 2.7 ng ml^{-1}, with an intra-assay coefficient of variation < 10% across the range of values studied (4.0–250 ng ml^{-1}).

Statistical analysis

For the EEG data, four conventional frequency wave bands were defined as follows; delta (0.00–3.99 Hz),

theta (4.00–7.99 Hz), alpha (8.00–12.99 Hz) and beta (13.00–29.99 Hz). Anterior channels a and b were summed and posterior channels c and d were summed. For each wave band, eyes open or closed, and for both pairs of channels, an analysis of variance model was performed on the log power at each post-dose timepoint having subtracted the corresponding pre-dose value.

Where statistically significant differences were found, least square means (LSmean) are presented together with the least significant difference (LSD), and the P value for the statistical significance of the difference of each dose of ICI 169,369 from placebo. Least square means are means that have been adjusted to allow for any imbalance in the design. The LSD is the least difference there must be between a treatment LSmean and a placebo LSmean for statistical significance at $P < 0.05$ level.

Similarly for the pupil reaction data, the difference from predose was analysed at each time point with the same analysis of variance techniques.

Results

EEG power spectrum analysis

ICI 169,369 has significant clear cut effects only on the 'eyes closed' EEG at 2 h after dosing (Figure 2) corresponding in time to the observed peak maximum whole blood concentration (Haworth *et al.*, 1989). At 2 h post dose theta wave activity was increased by 38% (range 20–86%; $P < 0.01$) with 80 and 120 mg doses of ICI 169,369 in both anterior and posterior channels. A corresponding 36% decrease in alpha wave activity occurred (range 10–54%; $P < 0.02$) present in both channels, but was only significant for the anterior channel.

No consistent changes in delta or beta wave activity were noted. Both 80 and 120 mg doses of ICI 169,369 appeared equipotent with respect to their activity on the waking EEG.

Pupil responses

At 3 and 5 h after dosing, resting pupil diameter (RPD) was reduced by approximately 30% ($P < 0.01$) with both the 80 and 120 mg doses of ICI 169,369 (Figure 3), MPD was reduced by 50% with the 120 mg dose of ICI 169,369 at 5 h after dosing. FPD was significantly reduced in a similar manner to MPD by both doses at 3 h after dosing ($P < 0.01$).

Kinetics of pupillary light responses

Median light reflex curves were analysed for each of the six volunteers at 3 h after dosing (corresponding to the maximally observed miosis) for placebo, 80 and 120 mg doses of ICI 169,369 (Figure 4). RPD was reduced by approximately 30% with both 80 and 120 mg doses of ICI 169,369 compared with the placebo curve. The pupil response curves were diminished in amplitude after both doses of ICI 169,369, with reductions in the maximum velocity ($VEL_{max}C$) and maximum acceleration of constriction ($ACC_{max}C$), which were significantly different for the 120 mg dose ($P < 0.05$) (Table 1).

Mood rating VAS scales

The alertness factor (Table 2) derived from the Bond Lader VAS was significantly higher in both the 80 and 120 mg ICI 169,369 treatment group at 5 h post dose compared with the placebo indicating a reduced level of alertness. No other consistent changes in the 'happiness' or 'relaxation' factors were recorded.

Examination of mean scores from the single 'alert–drowsy' VAS scale, from the Bond-Lader before factor-

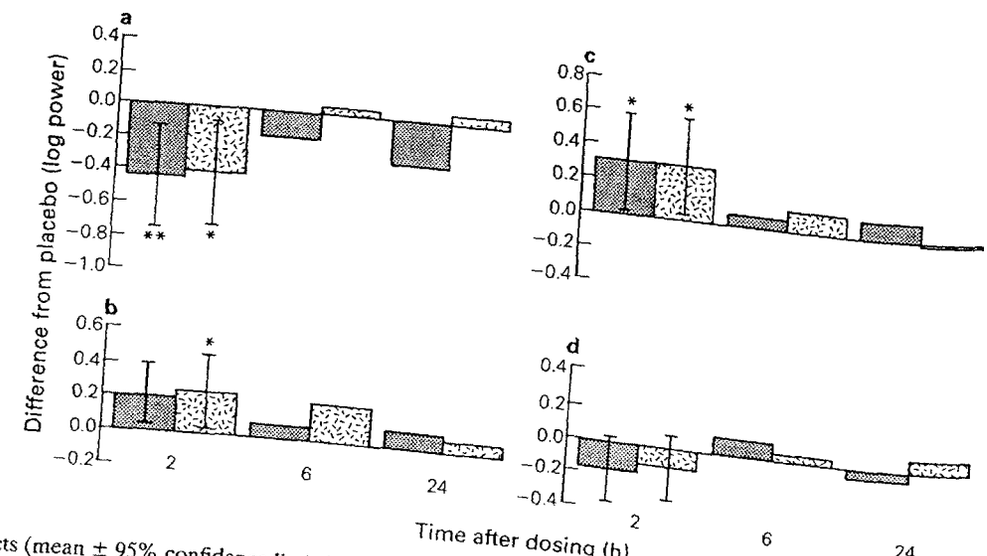


Figure 2 Effects (mean ± 95% confidence limits) of ICI 169,369 (▨ 80 mg, ▩ 120 mg) on EEG power spectrum analysis with eyes closed. Results depict the $\log_{10}$ power difference between placebo and drug treatment at 2, 6 and 24 h after dosing. Alpha waves and theta waves are depicted for the anterior (a + b) and posterior (c + d) channels; with the 95% confidence intervals for a difference from placebo, with appropriate P values where significant. $^*P < 0.05$, $^{**}P < 0.02$ compared with placebo.

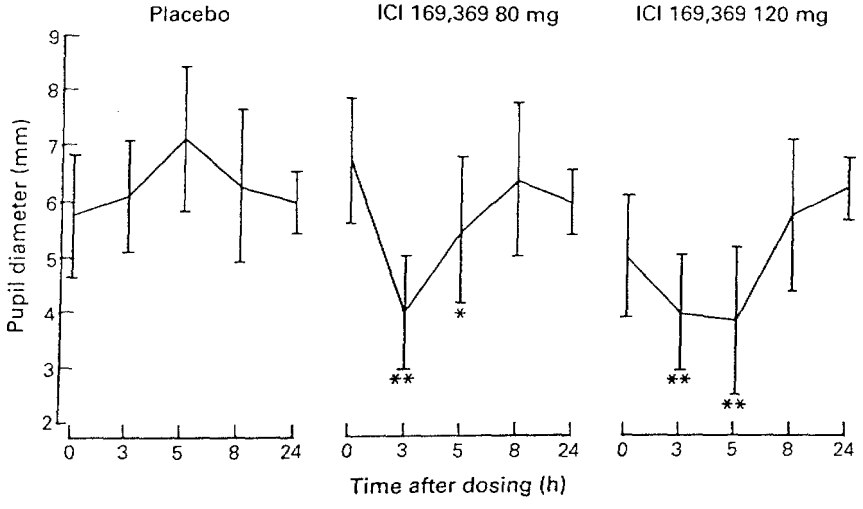


Figure 3 Effects (mean ± 95% confidence limits) of ICI 169,369 on resting pupil diameter (RPD) for placebo, 80 and 120 mg doses at 0, 3, 5, 8 and 24 h after dosing, with appropriate P values where treatments were significantly different from placebo. $^{**}P < 0.01$, $^{*}P < 0.05$ comparing post-dose with placebo.

Table 1 Effects of ICI 169,369 on the kinetics of light-induced pupillary constriction at $t = 3$ h. Mean (with C.I. of difference between drug and placebo)

	Placebo (with s.e. mean)	*ICI 169,369*	
		80 mg	*120 mg*
Maximum constriction velocity (mm s^{-1})	6.2 (1.02)	5.3 (± 1.8)	3.8 (± 0.7)*
Maximum constriction acceleration (mm s^{-1} s^{-1})	40.7 (5.84)	31.0 (± 5.7)	29.7 (± 1.4)*

$^{*}P < 0.05$ comparing dose with placebo.

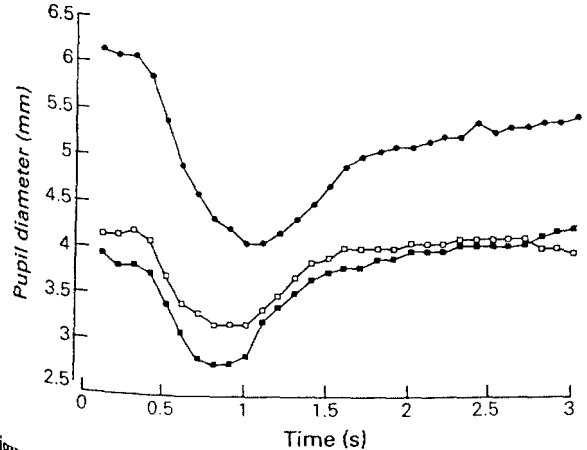


Figure 4 Effects of ICI 169,369 on light induced pupillary constriction curves. Median curves are depicted for placebo (●——●), 80 mg (□——□) and 120 mg (■——■) doses of ICI 169,369 at 3 h after dosing. Pupil diameter is plotted against time (s) for a 3 s measurement cycle.

sation, confirms the statistical trend towards diminished arousal at between 3 and 5 h after dosing with both 80 and 120 mg doses of ICI 169,369 compared with placebo. Although the VAS had returned to baseline by 24 h after dosing the Bond Lader alertness factor was still elevated, suggesting a possible residual effect. However, this was not significantly different from placebo ($P < 0.5$).

Table 2 Effects of ICI 169,369 on 'alertness factor'. Mean (with C.I. of difference between drug and placebo)

Time (h)	*Placebo (with s.e. mean)*	*ICI 169,369*	
		80 mg	*120 mg*
0	22.9 (4.2)	19.7 (± 9.1)	18.7 (± 9.1)
3	21.6 (6.1)	23.7 (± 5.1)	26.6 (± 5.1)
5	21.4 (4.2)	27.2 (± 3.0)**	25.0 (± 3.0)*
12	22.9 (5.4)	23.8 (± 3.4)	19.3 (± 3.4)
24	20.8 (6.0)	24.1 (± 5.9)	23.6 (± 5.9)

$^{**}P < 0.01$, $^{*}P < 0.05$ comparing dose with placebo.

Pharmacodynamic correlation with whole blood concentrations of ICI 169,369

Whole blood concentrations of ICI 169,369 (ng ml^{-1}) taken prior to pupillary assessment of RPD, demonstrated a significant negative log linear correlation (Figure 5) with resting pupil diameter ($r = 0.697$, slope $= -0.99$, $P < 0.0001$).

Discussion

This study has demonstrated that single oral doses of 80 and 120 mg of ICI 169,369 produced a subjective reduction in arousal, as measured by the Bond Lader VAS,

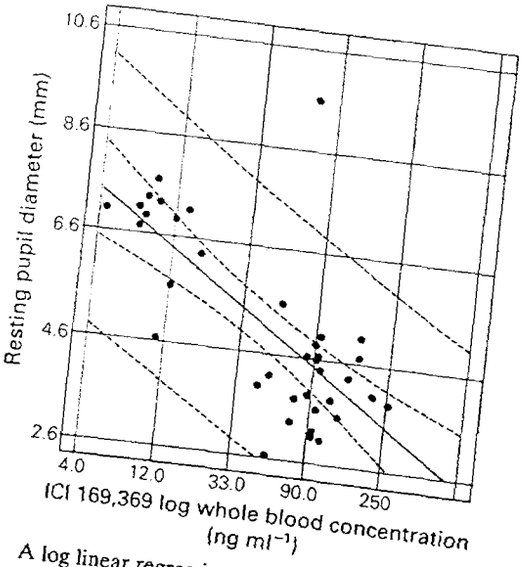


Figure 5 A log linear regression analysis of resting pupil diameter (mm) *vs* $\log_{10}$ whole blood concentration (ng ml^{-1}) of ICI 169,369, following 80 and 120 mg single oral doses. The line of best fit ($r = 0.69$, slope $= 0.99$, $P < 0.0001$) is depicted with 95% confidence intervals for the fitted line and value estimates.

accompanied by objective changes; in waking EEG (eyes closed), expressed as decreased alpha with a reciprocal increase in theta activity. Quantitative EEG methods are commonly used to identify the effects of drugs on the CNS (Itil, 1981), and are more sensitive than conventional psychometric tests. CNS effects have been reported with benzodiazepines (Lader & Denney, 1982), an antihistamine triprolidine (Holland *et al.*, 1989); and a lack of EEG effects or any interaction with lorazepam have been demonstrated for granisetron (Link *et al.*, 1991).

The changes in the waking EEG which occur during treatment with ICI 169,369 are comparable with those seen with the 5-HT$_2$ receptor antagonist ketanserin (Reimann *et al.*, 1986), where moderate sedation was accompanied by decreased alpha and increased theta activity. In sleep EEG studies ICI 169,369 produced similar changes to ritanserin in man (Cowen *et al.*, 1990; Idzikowski *et al.*, 1991), promoting slow wave sleep (SWS). Thus, SWS is thought to be under 5-HT$_2$ inhibitory control, and that 5-HT$_2$ receptor antagonists promote SWS.

Tortella *et al.* (1989) failed to demonstrate an increase in SWS with either ICI 169,369, or ritanserin in the rat, although a specific REM suppressant effect was demonstrated. Despite evidence for waking EEG changes in both man and animals, paradoxically ritanserin produced no significant reduction in arousal (Awouters *et al.*, 1987). Therefore, whilst a reduced state of arousal is often an accompaniment to waking EEG changes, this is by no means an *a priori* assumption.

The pupillary miosis observed with ICI 169,369 suggests that 5-HT$_2$ receptors may be directly involved in the control of human pupillary responses. A neurotransmitter role for 5-HT in the retina is now well established (Osborne *et al.*, 1986), however the involvement of 5-HT in the anterior chamber of the eye is more speculative. In the dog and rabbit intravenous injection of 5-HT lowered intraocular pressure (Chiang, 1974; Schumacher & Classen, 1962), and local anterior chamber injection, mydriasis together with increased intraocular pressure and protein concentration (Palkama *et al.*, 1984). In contrast local injection of 5-6 DHT (5-6 dihdroxytryptamine, a 5-HT depleting agent) produced miosis with receptor supersensitivity to exogenous 5-HT induced mydriasis (Moro *et al.*, 1981).

Recently Tobin *et al.* (1988) concluded that 5-HT$_2$ receptors were involved in controlling rabbit pupillary responses. 5-HT uptake was demonstrated in iris ciliary body, with a dose dependent 5-HT mediated increase in [^{3}H]-inositol phosphate turnover, selectively antagonised by ketanserin, methysergide and mianserin (5-HT$_2$ receptor antagonists) but not by ICS 205,930 (5-HT$_3$ receptor antagonist; Fozard, 1984), prazosin (α_1-adrenoceptor antagonist) or atropine (muscarinic antagonist). Furthermore, sympathetic denervation by superior cervical ganglionectomy, had no effect on 5-HT mediated responses suggesting a selective serotonergic innervation. Therefore animal studies would support the involvement of 5-HT$_2$ receptor involvement in controlling pupillary responses.

Possible mechanisms underlying this miosis are; 1) a direct post synaptic 5-HT$_2$ mediated dilatation, which when antagonised induced miosis, 2) presynaptic 5-HT$_2$ inhibitory modulation of parasympathetic tone, which when antagonised increased parasympathetic tone and 3) a central effect of 5-HT$_2$ receptor antagonism acting on efferents from the IIIrd nerve nucleus.

The reduced arousal and associated CNS effects observed with ICI 169,369 may have contributed to the pupillary miosis observed, as for example, subjects with narcolepsy demonstrate a pupillary miosis during attacks (Yoss *et al.*, 1969). However, the link with sedation and miosis is tenuous, since general anaesthesia with thiopentone produced the opposite effect, i.e. a mydriasis (Larson, 1981).

In support of the central 5-HT$_2$ effect, Goldstein *et al.* (1989) demonstrated that ICI 169,369 reversed the inhibitory effects of amphetamine on A9 and A10 dopaminergic neurones, reflecting an effect on central dopaminergic activity and possible anti-psychotic activity. Whereas, Beaumont *et al.* (1987) demonstrated a miosis and diminished light reflexes in parkinsonian patients, with comparable miosis to those seen in this study. Therefore ICI 169,369 may have induced miosis in volunteers by reducing central dopaminergic activity to mimic pupillary changes observed in parkinsonian patients.

None of the volunteers involved in these studies complained of visual disturbances under normal lighting conditions, and the effects of ICI 169,369 on visual parameters is under evaluation. The miosis observed was correlated with whole blood concentrations of ICI 169,369 however, and may be a useful pharmacodynamic measurement in evaluating other putative 5-HT$_2$ receptor antagonists in man.

Therefore, this study has demonstrated pharmacodynamic changes in waking EEG, pupillary responses and state of arousal in human volunteers. The EEG changes and diminished state of arousal suggest a sedative effect, and on the basis of pharmaco EEG analysis may be predictive of anti-depressant or anxiolytic activity

(Fink, 1984). The miosis and changes in pupillary responses may indicate a central or a peripheral effect and, may represent new evidence to support the involvement of 5-HT in the control of human pupillary responses.

The work reported in this study was carried out during the tenure of a Clinical Pharmacology training programme between the Department of Pharmacology and Therapeutics at the University of Liverpool and the Clinical Pharmacology Unit, at ICI Pharmaceuticals. The pupillary pharmacodynamic responses described were the subject of oral presentations at the April and July (1988) meetings of the British Pharmacological Society. I am grateful to Professor A. M. Breckenridge for his continuing encouragement and support.

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The effects of a selective 5-HT_2 receptor antagonist (ICI 170,809) on platelet aggregation and pupillary responses in healthy volunteers

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1 ICI 170,809 (2-(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline hydrochloride) is a potent 5-hydroxytryptamine (5-HT) type 2 postsynaptic receptor antagonist.

2 Effects of ICI 170,809 as single oral doses (3, 7, 15 and 30 mg) or placebo were studied on the duration of antagonism for the *ex vivo* platelet aggregatory response to 5-HT and to the pupillary light constrictor response in eight healthy male volunteers.

3 Pupillary dark adapted responses to a 0.5 s light stimulus were measured using a portable infrared pupillometer, for up to 24 h after dosing.

4 The *in vitro* platelet 5-HT aggregation response was reduced by ICI 170,809, with depression of the dose-response curve to 5-HT at all concentrations of 5-HT and with no evidence for a parallel shift.

5 The *ex vivo* platelet 5-HT response demonstrated a dose related significant ($P < 0.02$) decrease in aggregation reaching a maximum at 2 h after dosing with the effect persisting for at least 8 h after dosing with the 7 and 15 mg doses.

6 Resting pupil diameter (RPD), and light induced pupillary responses in the dark adapted pupil, showed a significant ($P < 0.01$) dose related reduction with significant ($P < 0.05$) effects still present with the 15 and 30 mg doses at 8 h after dosing.

7 We conclude that, changes in both *ex vivo* platelet aggregation to 5-HT and dark adapted pupil size, are significantly correlated ($P < 0.0001$) with log plasma concentrations (ng ml^{-1}) of ICI 170,809, enabling the assessment of 5-HT_2-receptor antagonism in man.

Keywords 5-HT_2-receptor antagonist ICI 170,809 platelet aggregation pupil response

Introduction

5-Hydroxytryptamine-2 (5-HT_2) receptors have been identified in both rat (Goldstein *et al.*, 1989; Peroutka *et al.*, 1981) and human central nervous system (Biegon *et al.*, 1987; Pazos, 1987; Reimann *et al.*, 1986). Subsequent work has demonstrated similarities of the 5-HT_2 site with the 'D' receptor of Gaddum & Picarelli (1957). The 5-HT_2 receptor is thought to be homogeneous, but to have structural homology with the 5-HT_{1c} site (Pritchett *et al.*, 1988), both of which are linked to a second messenger system stimulating phosphatidyl inositol turnover (Conn & Sanders-Bush, 1985). 5-HT_2 receptors are located also in humans on platelets (Bevan & Heptinstall, 1986; Gotta *et al.*, 1987) and in vascular smooth muscle (Blauw *et al.*, 1988; Houston & Vanhoutte, 1989) where they mediate some of the excitatory actions of 5-HT (De Clerk & Van Nueten, 1982).

Recently, human volunteer studies with ICI 169,369 (2-(2-dimethylamino ethylthio)-3-phenyl quinoline) a 5-HT_2-receptor antagonist which is structurally related to ICI 170,809 (Blackburn *et al.*, 1988, 1990), raised the possibility that 5-HT_2 receptors may be directly involved in the neuronal control of human pupillary responses (Millson *et al.*, 1991). This is supported by human data where fenfluramine (a 5-HT releasing agent), both topically applied and systemically administered, caused a mydriasis (Kramer & Turner, 1973); and animal data where 5-HT_2 receptors have been identified in rabbit iris (Tobin *et al.*, 1988).

ICI 170,809, whose structure is shown in Figure 1 is a potent selective 5-HT_2-receptor antagonist, which expresses its activity at vascular, neuronal and gastrointestinal 5-HT_2 receptors in both *in vitro* and *in vivo* animal models (Blackburn *et al.*, 1988). It is necessary

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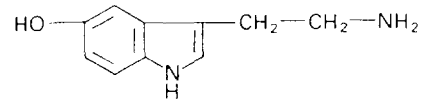
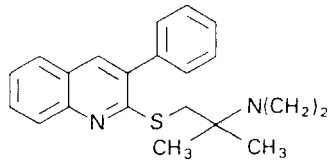


5-hydroxytryptamine (5-HT)



2-(2-dimethylamino-2-methylpropylthio)-3-phenylquinoline hydrochloride (ICI 170,809).

Figure 1 Structure of ICI 170,809 illustrating its relationship to 5-HT.

to demonstrate that ICI 170,809 has 5-HT_2-receptor antagonist properties in man. Two pharmacodynamic responses will be explored together with measurements of plasma ICI 170,809 to investigate the ensuing pharmacodynamic/kinetic relationships.

1) The *in vitro* antagonist activity of ICI 170,809 at functional 5-HT_2 receptors on human platelets, together with the duration of action of any effect, using 5-HT induced *ex vivo* platelet aggregation.
2) Changes in light induced pupillary reflexes and dark adapted resting pupil diameter resulting from dosing with ICI 170,809, together with the duration of action of any effect.

The ability to detect both neuronal and platelet disaggregatory effects in volunteers might provide a useful model in which to predict an active dose of 5-HT_2-receptor antagonist at the human 5-HT_2 receptor. This would allow assessment of potential efficacy in diseases of both the cardiovascular and central nervous system, and provide confirmatory evidence for the involvement of 5-HT_2 receptors in the control of human pupillary responses.

Methods

Volunteer selection and study design

The study was a double-blind, randomised, ascending dose, partial cross over design in which eight male volunteers (aged 18–45 years, weighing between 63 and 91 kg) received three out of a possible four single oral doses (3, 7, 15 and 30 mg) of ICI 170,809, or placebo on four dosing occasions 7 days apart.

Before commencing the study, the protocol was approved by an ethics committee and written informed consent was obtained. Volunteers were only allowed to proceed into the study if no abnormalities were found after medical examination, laboratory blood and urinary safety screens.

Protocol followed

ICI 170,809 was administered orally to volunteers who had fasted overnight. *Ex vivo* platelet sensitivity to 5-HT measured as the extent of aggregation, was determined on three separate occasions before, and 2, 5, 8 and 24 h after oral administration of ICI 170,809 or placebo.

Infra-red pupillometry was performed at 3, 5, 8 and 24 h after dosing, six consecutive artefact free recordings were made and mean values derived for resting (RPD), light constricted (MPD) and the final recovered (FPD) pupil diameter. Except for the 3 mg dose (where sampling was limited to 24 h) venous blood sampling for estimation of ICI 170,809 plasma concentration was performed predose and at 1, 1.5, 2, 3, 5, 8, 12, 15, 24, 30 and 48 h after dosing.

Assessments

Platelet aggregation tests Platelet rich plasma was prepared as a preliminary to examining the aggregatory effects of 5-HT. Subjects recruited into the study were bled from an antecubital vein into tubes containing tri-sodium citrate solution (3.2 w/v, one part to nine parts whole blood) and platelet rich plasma (PRP) was harvested after centrifugation (200 *g* for 15 min) on each of three separate occasions.

In vitro platelet aggregation was carried out using human PRP in a Payton aggregometer. Aliquots (250 μl) of PRP were stirred (900 rev min^{-1}) and incubated (37° C) for 60 s in an aggregometer before ICI 170,809 or vehicle were added. Platelets were incubated for a further 60 s and a single concentration of 5-HT was then added. This procedure was repeated using a range of 5-HT concentrations (5×10^{-9} to 5×10^{-5} M). The extent of 5-HT induced aggregation was measured and expressed as a percentage of the maximum obtained with vehicle controls (Figure 2).

Ex vivo platelet aggregation was performed on PRP from volunteers following oral osing with either ICI 170,809 or placebo. Aliquots of PRP (250 μl) were incubated for 120 s in an aggregometer (900 rev min^{-1}, 37° C) before concentrations of 5-HT were added. The extent of 5-HT induced aggregation, following oral

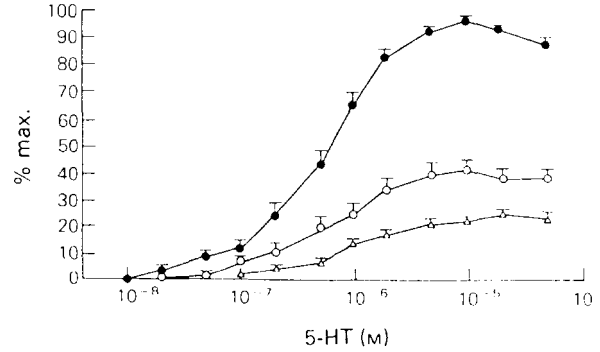


Figure 2 5-HT induced platelet aggregation of human PRP in the presence of ICI 170,809: 10^{-7} M (○, $n = 8$); 4×10^{-7} M (△, $n = 7$) and vehicle (●, $n = 8$). The extent of the 5-HT induced aggregation was expressed as a percentage of the mean maximum aggregation response obtained with vehicle, on three occasions prior to dosing.

Table 1 The maximum velocity and acceleration associated with the light induced pupillary constriction at 3 h after dosing with ICI 170,809 (3, 15, 30 mg or placebo), expressed as the mean with s.e. mean and 95% confidence intervals for the differences from placebo values and associated P values

	Maximum constriction					
	Velocity ($mm\ s^{-1}$)			*Acceleration* ($mm\ s^{-2}$)		
Treatment	*Mean*	*s.e. mean*	*CI*	*Mean*	*s.e. mean*	*CI*
Placebo	7.87	1.2		64.9	13.9	
ICI 170,809 3 mg	5.68	0.9	1.25	48.0	8.9	22.26
15 mg	3.93	0.5	0.66**	47.0	10.4	22.62
30 mg	4.00	0.4	0.58**	24.2	1.2	8.64**

CI = 95% CI for the differences from placebo mean. ** = $P < 0.01$.

dosing with ICI 170,809 or placebo, was expressed as a percentage of the mean individual maximum platelet aggregation response as previously described (Figure 2).

Pupil responses

A 'pupilscan' hand held infra-red pupillometer (Millson *et al.*, 1988) was used to measure dark adapted resting pupil diameter (RPD) after 15 min dark adaptation wearing dark goggles (BS 6975EW). A 0.5 s (565 nm; 36 cd m^{-2}) light stimulus induced pupillary reflex, minimally light constricted (MPD) and recovered (FPD) pupil diameter after a 3 s cycle were measured together with dynamic pupillary changes every 10 ms. A 'pupilscan' data analysis template was used to analyse the maximum velocity ($mm\ s^{-1}$) and acceleration ($mm\ s^{-2}$) associated with the pupillary constriction.

Plasma assay of ICI 170,809

A 7 ml sample of venous blood was taken into a tube containing oxalate as anticoagulant, and after thorough mixing centrifuged at 2000 rev min^{-1} for 10 min. The plasma was decanted off and stored at −20° C prior to assay.

After thawing stored plasma a 1 ml aliquot was mixed with 1 ml pH 10 buffer and agitated with 10 ml 1.5% amyl alcohol in hexane for 15 min. After centrifuging to separate the phases, 9 ml of the solvent layer was transferred to a tube containing 2 ml 0.1 N hydrochloric acid. After shaking and centrifugation the solvent layer was discarded. The aqueous layer was mixed with 2.5 ml 0.1 N sodium hydroxide to which 10 ml 1.5% amyl alcohol was added. After mixing and centrifugation 9 ml of the solvent layer was evaporated to dryness at 40° C. The residue was redissolved in 0.2 ml eluent (20% pH 4.0 triethylamine buffer (0.28%):80% acetonitrile) and aliquots analysed by high pressure liquid chromatography at a 1.0 ml min^{-1} flow rate using a 10 cm × 3 mm (internal diameter) Hyperil ODS column with detection by ultraviolet absorption at 259 nm.

A calibration series covering the expected samples concentration range was constructed for each set of analyses. The unknown samples were determined by a least square fit calculation from the standard curve. The limit of quantification was 2.0 ng ml^{-1} with an intraassay coefficient of variation of < 10% over the concentration range 2.0–100 ng ml^{-1}.

Determination of pharmacokinetic parameters for ICI 170,809

For each dose and plasma ICI 170,809 concentration time curve: the maximum measured concentration of ICI 170,809 in plasma (C_{max}), and the time corresponding to the maximum concentration in plasma (t_{max}) were obtained by inspection. The areas under the curve up to the last measured time point (AUCt) for ICI 170,809 concentrations were calculated using trapezoidal and log trapezoidal rules and extrapolated to infinite time (AUC∞) employing a terminal exponential rate constant (λ_z) estimated by log-linear regression of selected data points describing a terminal log-linear phase, wherever possible. The exponential elimination rate constant was used to extrapolate the area under the curve to infinite time (AUC∞) and to provide an estimate of the elimination half-life ($t_{½,z}$) where possible. Thus $t_{½,z} = 0.693/\lambda_z$. A similar approach was adopted to calculate a half-life for the absorption and distribution phase.

Statistical methods

A multifactor analysis of variance was used to analyse pupillometry and platelet data using the following terms in the analysis: volunteer, treatment and time after dosing. The main objective of this repeated measures analysis was to test whether significant differences were detectable in measurements of 5-HT induced platelet aggregation, and pupil diameter parameters between the placebo treated and ICI 170,809 treated groups, at various times after dosing. The Greenhouse Geisser correction factor for repeated measures analysis was applied. If a statistically significant ($P < 0.05$) difference was found between the profiles, then analysis of covariance was performed at each timepoint separately, using the pre-dose value as the covariate.

A least square regression analysis was used to examine the correlation between pharmacodynamic parameters (such as pupil diameter or % change in platelet responses to 5-HT) and pharmacokinetic measurements (i.e. plasma concentrations of ICI 170,809). The 95% confidence intervals (CI) for the line of best fit with 95% CI for the parameter estimate are displayed graphically together with associated correlation coefficient, slope and intercept values.

Differences in velocity and acceleration between placebo and ICI 170,809 treated volunteers at 3 h after

dosing, were analysed with values derived from the median pupillary light constriction curves. Median curves were plotted for each volunteer, which were summated to give a mean 3 h (± s.e. mean) curve for each dose.

Results

Effects of ICI 170,809 on the in vitro *platelet aggregation*

The percentage maximum aggregation achieved to (10^{-5} M) 5-HT was reduced to less than 40% with both 10^{-7} and 4×10^{-7} M concentrations of ICI 170,809, compared with the vehicle when added to PRP *in vitro* (Figure 2). The dose-response curve to 5-HT, was significantly depressed at all concentrations of 5-HT, with no evidence of a parallel shift.

Effects of ICI 170,809 on (ex vivo) *platelet aggregation*

When dosed orally to human volunteers ICI 170,809 caused dose dependent inhibition of 5-HT induced platelet aggregation *ex vivo* (Figure 3). ICI 170,809 (3 mg) did not significantly ($P > 0.05$) modify aggregation responses. Two hours after administration of higher doses of the drug (7, 15, 30 mg) attenuation of 5-HT induced platelet aggregation was evident yielding mean

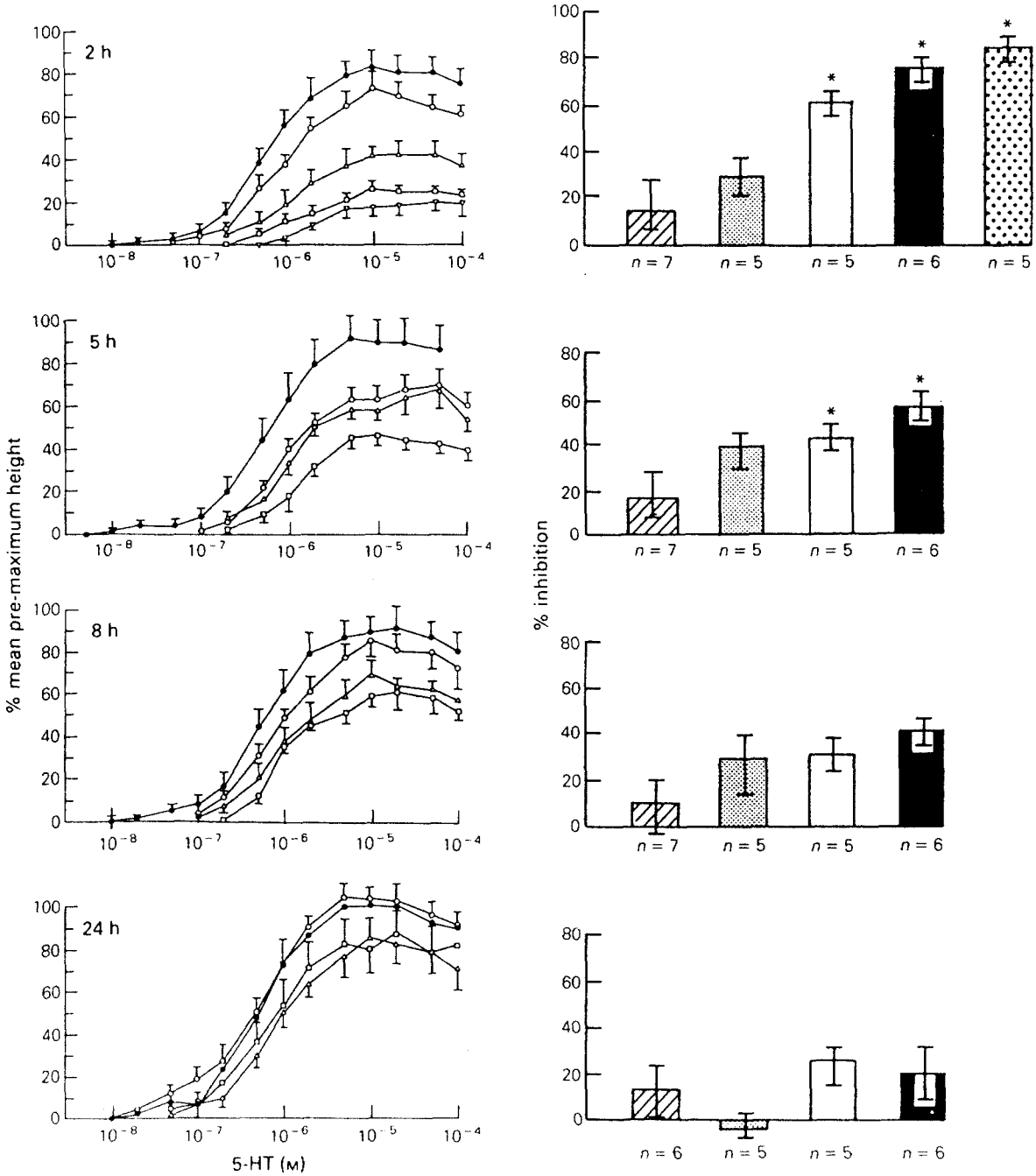


Figure 3 The effects of ICI 170,809 (3 mg, ○, $n = 5$; 7 mg, △, $n = 5$; 15 mg, □, $n = 6$; 30 mg, ▽, $n = 5$) and placebo (●, $n = 7$), on *ex vivo* 5-HT induced human platelet aggregation at 2, 5, 8 and 24 h after oral dosing, with data expressed as inhibition of the mean maximum aggregation response to 5-HT in the presence of vehicle, on three occasions prior to dosing. The histograms depict the maximum inhibition achieved for each treatment (ICI 170,809 3 mg ▨, 7 mg □, 15 mg ■, 30 mg ⊡, placebo ▨) at 2, 5, 8 and 24 h, with appropriate P values for values significantly different from placebo. $^*P < 0.02$.

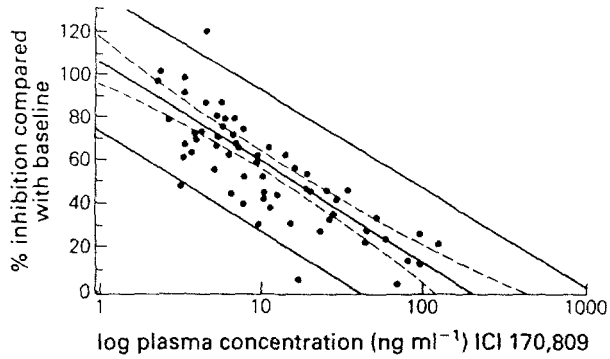


Figure 4 Relationship between 5-HT-induced platelet aggregation (5×10^{-6} M) expressed as % inhibition compared with baseline values, *vs* log plasma (ng ml^{-1}) ICI 170,809 after oral dosing ($r = 0.797$, $P < 0.0001$, slope = −46.30, intercept = 106.43). Least squares regression line is given with 95% confidence intervals for the line of best fit (– – –) and parameter values.

(± s.e. mean percentage inhibitions of 59.5 ± 4.6, 73.8 ± 4.2 and 82.4 ± 4.0 respectively, which were significantly different from placebo ($P < 0.05$). This activity persisted for 5 h with 7 and 15 mg doses (mean ± s.e. mean percentage inhibition of 42.4 ± 4.8 and 54.6 ± 4.2 respectively). The minimal effective oral dose in human volunteers was 7 mg. Data for the 30 mg dose of ICI 170,809 was not collected beyond 2 h after dosing since this dose was considered to be in excess of the anticipated clinical doses, and was included in this study principally to provide tolerability data.

5-HT_2 induced platelet aggregation (5×10^{-6} M) was significantly correlated ($r = 0.797$, $P < 0.0001$, slope = −46.30, intercept = 106.4) with log concentrations (ng ml^{-1}) of ICI 170,809 (Figure 4).

Effects of single oral doses of ICI 170,809 on pupillary responses

When dosed orally to human volunteers 3, 15 and 30 mg doses produced a dose dependent pupillary miosis (Figure 5). No significant baseline changes were evident when pre-treatment groups were compared with placebo. Data from the 7 mg dose was not available due to a technical failure with the infrared sensing equipment on week 2 of the study.

A significant ($P < 0.01$) reduction in mean RPD and

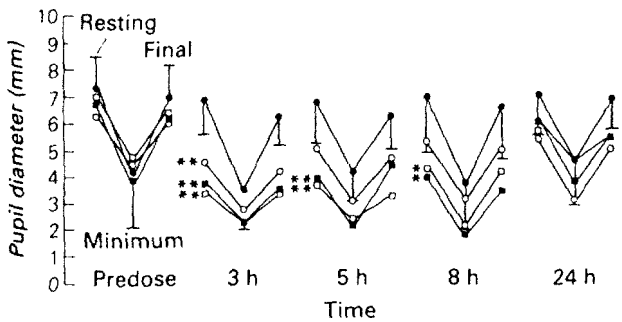


Figure 5 Effects of ICI 170,809 (3 mg ○, 15 mg ■, 30 mg □ and placebo ●) on mean (± 95% confidence intervals for treatment differences from placebo) for dark adapted resting pupil diameter (RPD), minimally constricted pupil diameter (MPD) and final resting pupil diameter (FPD), predose, 3, 5, 8 and 24 h after dosing. Significant differences from placebo are highlighted with appropriate P values. $^*P < 0.05$, $^{**}P < 0.01$.

Table 2 Pharmacokinetic parameters for ICI 170,809 after single oral doses

Dose (mg)	C_{max} *(ng ml^{-1})*	$t_{½,\lambda_1}$ *(h)*	$t_{½,z}$ *(h)*	*AUC∞ (ng ml^{-1} h)*
3.0	8.0 (1.5)	NC	NC	NC
7.0	15.9 (3.0)	2.6 (0.8)	18.0 (5.5)	197.0 (47)
15.0	37.7 (7.0)	2.6 (0.2)	40.0 (8.6)	1030.0 (388)
30.0	104.0 (2.6)	3.5 (0.2)	43.0 (7.8)	2259.0 (807)

Values are expressed as the mean ± s.e. mean.
C_{max} = peak plasma concentration achieved.
$t_{½,\lambda_1}$ and $t_{½,z}$ are the distribution and elimination half-lives respectively.
AUC∞ is the area under the plasma concentration-time curve extrapolated to infinity.

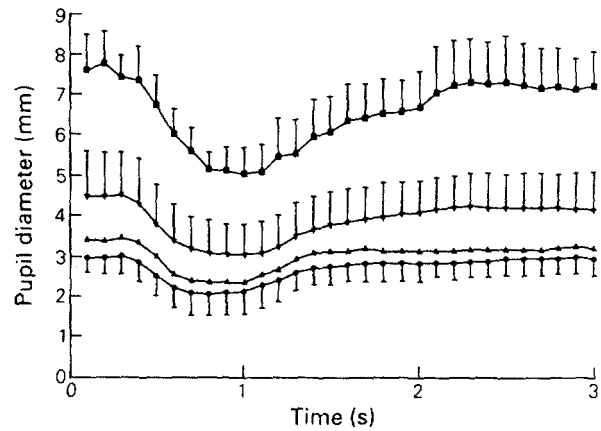


Figure 6 Effects of ICI 170,809 (3 mg ▼, 15 mg ▲, 30 mg ●, or placebo ■) on pupillary response curves. Each curve represents the change in mean dark adapted vertical pupil diameter (mm ± s.e. mean) from resting values at 3 h after dosing, following a light stimulus (0.5 s, 565 nm, 36 cd m^{-2}) and with reflected infrared recordings every 10 ms.

FPD occurred at 3 and 5 h after dosing, with reductions in vertical pupil diameter of up to 50% compared with placebo. MPD was also significantly reduced ($P < 0.01$) by approximately 50% with 15 and 30 mg doses of ICI 170,809 at 5 h after dosing. Significant reductions in RPD, MPD and FPD were still present at 8 h ($P < 0.05$) with recovery to baseline values occurring 24 h after dosing (Figure 5).

Mean pupil light response curves (Figure 6) derived from summation of median plots from each volunteer show a reduction in reflex amplitude together with a shift in RPD. Analysis of the kinetics of pupillary light constriction following oral ICI 170,809 demonstrated a reduction in both the velocity and acceleration of pupillary constriction in a dose dependent manner (Table 1).

Pupillary miosis as a function of plasma ICI 170,809 concentration

Percentage changes in resting pupil diameter (compared with baseline values) were linearly related (Figure 7) to log plasma concentrations (ng ml^{-1}) in a similar manner to the platelet aggregatory response ($r = 0.55$, $P < 0.0001$, slope = −26.13, intercept = 98.89).

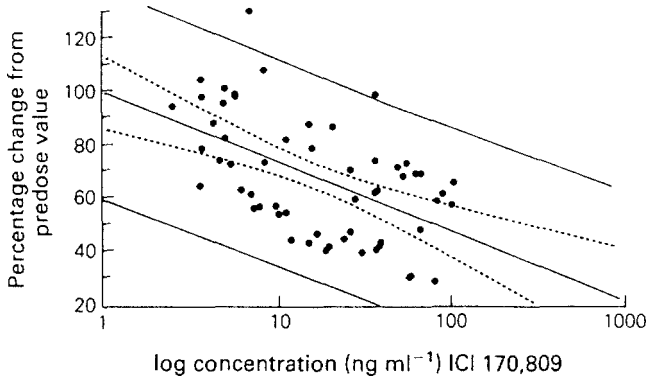


Figure 7 The relationship between resting pupil diameter (RPD) expressed as a percentage change from baseline values, *vs* log concentration of ICI 170,809 (ng ml^{-1}). Least squares regression ($r = 0.55$, $P < 0.0001$, slope = −26.13, intercept = 98.89) line is given with 95% confidence intervals for the fitted line (- - -) and parameter estimates.

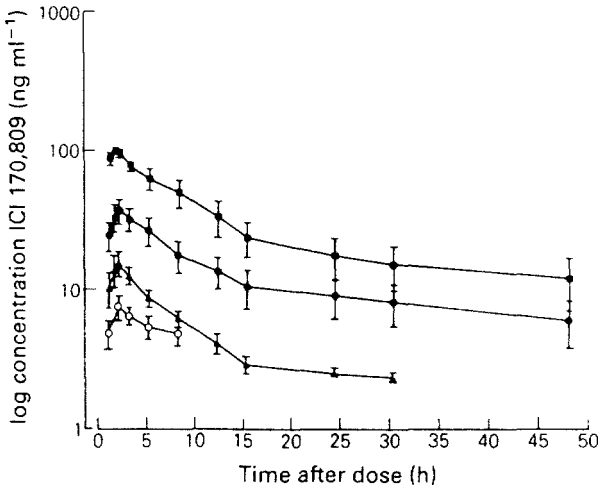


Figure 8 Pharmacokinetic profiles over a 50 h period of treatment with single oral doses of ICI 170,809 3 (○), 7 (▲), 15 (●) and 30 mg (■). Values are shown as mean and s.e. mean ($n = 6$).

Pharmacokinetic analysis

Pharmacokinetic profiles for plasma ICI 170,809 with respect to time after dosing are given in Figure 8, together with the derived pharmacokinetic parameters in Table 2. Insufficient data was generated for the 3 mg dose to calculate all parameters. The absorption phase was similar for all doses, with a median time to peak concentration of 2 h (range 1–3 h). The rise in peak plasma concentration (C_{max}) was approximately linear with increased dose, whereas the area under the concentration-time curve displayed non-linearity above 7 mg. The elimination phase was variable and became more prolonged with increasing dose. After the peak, the concentrations declined rapidly until about 15 h post-dose, following which there was a slower decline or beta phase.

Discussion

Single oral doses of ICI 170,809, a potent selective 5-HT_2 receptor antagonist (Blackburn *et al.*, 1988), produced dose related, plasma concentration dependent changes in both 5-HT_2 induced *ex vivo* platelet aggregation and pupillary responses in healthy volunteers. In both cases, a log linear dose-response relationship was demonstrated. The effective dose of ICI 170,809, expressed as the ID_{50} for both platelet and pupillary responses (inhibitory dose 50%) was approximately 0.1 mg kg^{-1}. This is in agreement with the effective range of doses active centrally (Cox *et al.*, 1988), and in the cardiovascular system at the antiplatelet and antiarrhythmic 5-HT_2 receptor using *in vivo* animal models (Coker & Ellis; 1990).

Antagonism of both the 5-HT induced *ex vivo* platelet aggregation, and pupillary miosis following the single 15 mg doses of ICI 170,809 were apparent for greater than 8 h. The pharmacokinetic profile of ICI 170,809 following single oral doses displayed a variable and prolonged plasma elimination half-life, which increased from a mean of 16 h with the 7 mg dose to over 40 h with the 15 and 30 mg doses. There was a linear increase in peak plasma concentration (C_{max}). The apparent increase in elimination half-life and AUC∞ with increasing dose, are probably related to the better definition and resolution of the beta elimination phase. Therefore, proposed oral dosing with either 7 or 15 mg as single, or divided daily doses, would be appropriate for use in clinical trials.

Bevan & Heptinstall (1986) have subdivided 5-HT_2-receptor antagonists into two categories 'competitive' and 'non-competitive', based on their behaviour at the platelet 5-HT_2 receptor. The *in vitro* and *ex vivo* effects of ICI 170,809 in this study would suggest non-competitive or high affinity antagonism at the platelet 5-HT_2 receptor. An alternative possibility, is that the 5-HT_2 receptor exists in one of two conformations with both high and low affinity for ligands, since this would produce a similar dose-response curve, as suggested for ICI 170,809 by Frenken & Kaumann (1989). From the clinical point of view, this apparently unsurmountable antagonism with ICI 170,809 may be beneficial, when antagonising high local concentrations of 5-HT released from platelets, in disease states such as migraine (Davies & Steiner, 1990; Jansen *et al.*, 1991) or coronary artery spasm (Noble *et al.*, 1990).

Recently, antagonism of the 5-HT_2 platelet response by ketanserin has been used to monitor efficacy in patients with Raynaud's phenomenon (Marasini *et al.*, 1990). They were able to recommend increased dosing frequency to improve clinical efficacy. This demonstrates the utility of being able to measure a pharmacodynamic endpoint, and the desirability of a prolonged duration of action for a drug designed to treat such cardiovascular disorders. ICI 170,809 has such a profile of activity.

The demonstration of a pupillary miosis with ICI 170,809, which was dose and concentration dependent, provides evidence to support the hypothesis that 5-HT_2 receptors may be involved in controlling human pupillary responses (Millson *et al.*, 1991). Animal evidence to support this comes from observations that intraocular injection of 5-6 dihydroxy 5-HT (a 5-HT depleting agent) produced a denervation supersensitivity, affecting both pupillary responses to 5-HT and production of aqueous humour (Moro *et al.*, 1987). This is supported by the biochemical findings of Tobin *et al.* (1988), Palkama

et al. (1984), Uusitalo *et al.* (1982, 1984) and Cutcliffe & Osborne (1987) demonstrating selective 5-HT uptake, post synaptic 5-HT binding sites, functional responses and second messenger generation linked to 5-HT$_2$ receptors. Thus compelling evidence exists for a serotonergic influence in the iris-pupillary body (Cutcliffe & Osborne, 1987).

Therefore, this study has demonstrated two pharmacodynamic responses which reflect pharmacokinetic changes in ICI 170,809 following single oral doses in healthy volunteers. Both *ex vivo* platelet and pupillary responses are thus techniques readily applicable to monitor the effects of 5-HT$_2$-receptor antagonists in man.

Addendum

The work reported in this study was carried out during the tenure of a Clinical Pharmacology training programme between the Department of Pharmacology and Therapeutics at the University of Liverpool and the Clinical Pharmacology Unit, at ICI Pharmaceuticals. The pupillary pharmacodynamic responses described were the subject of an oral presentation at the March (1988) meeting of the Serotonin club international symposium on serotonin held in Florence.

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