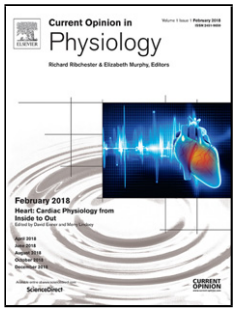

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Oxytocin and vasopressin signalling and myometrial contraction

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Abstract

Oxytocin and vasopressin are potent stimulants of uterine contractions. The importance of these hormones, particularly oxytocin, in pregnancy and labour, has been extensively studied and has paved the way for many important discoveries focused on the management of uterine contractions in labour. This includes development of therapies modelled on their structure or drug-delivery strategies targeting their receptors. This review will summarise our current knowledge of oxytocin and vasopressin signalling in myometrium and describe recent advances which have shed light on their roles in parturition, including novel roles for oxytocin as an inflammatory mediator and a regulator of gene transcription. How this information may impact the development and delivery of new oxytocin receptor-focussed therapies for preterm birth and dysfunctional labour will be discussed. Issues that warrant

further investigation and which are necessary for expanding the therapeutic potential of these important signalling molecules are also highlighted.

Key words: Oxytocin, Oxytocin receptor, Vasopressin, Myometrium, Parturition, GPCRs

1. Introduction

Oxytocin (OT) and arginine vasopressin (vasopressin, AVP) are two structurally similar neuropeptide hormones. They function via G protein-coupled receptor (GPCR)-mediated signal transduction and have both central and peripheral actions, with roles in many physiological and pathophysiological processes. In myometrium, the most noted roles for the OT-OT receptor (OTR) system include the initiation and stimulation of myometrial contractions during labour. Evidence for this comes from the significant OTR upregulation which is seen in both rats and humans during labour onset[1-3] (which increases myometrial sensitivity to OT) and that OT can be used to induce or augment labour, whilst OTR antagonists (OTR-As) can inhibit uterine contractions in late gestation and parturition[4].

Whilst the main physiological roles for arginine vasopressin (AVP) in the body are the control of osmolarity and blood pressure, AVP is also a strong uterotonic[5]. Hence, AVP's role in myometrium has also been studied. However, its role in parturition is less clear. Despite it being a potent inducer of contractions, circulating levels of AVP do not change with pregnancy or labour and there are only a few studies suggesting expression of AVP receptors is increased in late gestation and preterm labour[6,7].

This review will summarise our current knowledge of signalling by these neuropeptides, particularly oxytocin, in myometrium and highlight recent advances in our understanding of their role in parturition. Clinical considerations for their use as therapeutics to manage labour contractions will also be discussed well as important issues that warrant further investigation.

2. Classical signalling by the OT-OTR system in myometrium

The OTR is a Class 1 GPCR. As with all GPCRs, their activation leads to coupling of heterotrimeric G proteins, G_{α}, G_{β} and G_{γ}, to stimulate a number of signalling pathways and regulate diverse cellular processes. In heterologous cell systems, OTRs have been shown to recruit and activate G_q, G_i (G_{i1} G_{i2} and G_{i3}) and the Go (G_{oA}, G_{oB}) families of G_{α} proteins but

not G_s[8]. To date in myometrium, the OTR has been shown to couple to $G_{\alpha q/11}$, $G_{\alpha i}$ and potentially $G_{\alpha 12/13}$, however, there is a lack of knowledge around the exact role of these and other G_α subunits in human myometrium. Figure 1 details the currently known mechanisms leading to contraction in myometrium following oxytocin stimulation. OTRs couple to $G_{\alpha q/11}$ proteins to activate phospholipase Cβ (PLC-β) which controls the hydrolysis of phosphatidylinositol-4, 5-bisphosphate (PIP_2) into inositol-1, 4, 5-trisphosphate (IP_3) and diacylglyerol (DAG). These in turn control the mobilisation of Ca from the sarcoplasmic reticulum (SR) and the activation of protein kinase C (PKC) respectively. The increase in intracellular Ca ($[Ca]_i$) brings about contraction via stimulation of Ca-dependent calmodulin and activation of myosin light chain kinase (MLCK). MLCK phosphorylates Ser19 on the regulatory light chains of myosin enabling acto-myosin cross bridge cycling and myometrial contraction[4]. OT can also raise $[Ca]_i$ and hence contraction, through Ca entry. Candidate mechanisms include opening of L-type voltage-gated Ca channels[9], or store-operated Ca entry (SOCE)[10,11].

Calcium entry involving L-type Ca channels requires a change in membrane potential, but until recently, how OTR activation causes this depolarization was largely unknown. Santi's and England's groups elegantly showed that OT inhibits the sodium-activated, high-conductance, potassium leak channel, SLO2.1. This was via $G_{\alpha q}$-protein signalling and activation of PKC. Inhibition of SLO2.1 reduces outward K^+ current and leads to membrane depolarization[12]. Voltage-dependent calcium channels are then activated, resulting in calcium influx. Oxytocin's inhibition of SLO2.1 therefore provides a novel mechanism through which OT can induce Ca entry.

Oxytocin may also maintain $[Ca]_i$ by inhibiting Ca extrusion mechanisms such as the plasma membrane Ca^{2+} ATPase (PMCA)[13-15]. Mechanistic data on this is limiting but a recent study shows that exposure of human myometrial strips to OT, induces phosphorylation of PMCA[16].

Oxytocin signalling may also alter the interaction between myosin and actin independently of changes in $[Ca]_I$ via Ca sensitisation[17]. This occurs via activation of Rho proteins (likely via $G_{\alpha 12/13}$ proteins) which in turn leads to the activation of Rho kinase (ROCK) and subsequent phosphorylation and inhibition of MLCP[18]. Recently, the regulatory subunit of MLCP (PPP1R12B) was found to be phosphorylated during OT stimulation in human

myometrium[16]. Additionally, DAG –mediated production of PKC following OTR activation, can lead to inhibition of MLCP via the action of CPI-17[19].

Termination of OTR signalling is predominantly via G protein-independent, G protein-coupled kinase (GRK) mechanisms. Following OTR stimulation, GRK phosphorylates the C-terminal tail of OTR and β-arrestin proteins are recruited to the receptor which sterically hinders further G-protein coupling, facilitates OTR internalization and receptor desensitisation[20,21]. Post-internalisation and agonist removal however, OTRs have been shown to efficiently recycle to the cell surface, re-sensitise and restore the full receptor response within 4 hours [22]. In myometrium β-arrestin recruitment is dependent on GRK6 as GRK6 loss-of-function mice exhibit enhanced uterine contraction with an associated higher rate of stillbirth[23]. Thus, desensitisation of the receptor is important to prevent over-stimulation of the uterus in labour. β-arrestins can also act as scaffolding proteins for additional signalling pathways. In myometrium OT-induced β-arrestin recruitment has been linked to activation of MAPK and cell proliferation[24].

3. Emerging roles for the OT-OTR system in parturition

Recent studies suggest that oxytocin also acts as an inflammatory mediator, playing a central role in the inflammatory cascade leading to labour onset [25](Figure 2). In cultured myometrial and amnion cells, Kim *et al.,* showed that OT activates mitogen-activated protein kinase (MAPK) and/or Nuclear factor kappa B (NFκB) pathways and this results in increased expression of prolabour genes such as cyclo-oxygenase 2 (COX-2), prostaglandins (PGs) and the pro-inflammatory cytokines and chemokines, IL-8, IL-6 and CCL5[26].

Oxytocin also causes PG (PGE_2 and $PGF_{2\alpha}$) production in other gestational tissues via upregulation of COX-2[27,28]. This local release of PGs, particularly $PGF_{2\alpha}$, will feedback to facilitate uterine contractions via similar mechanisms to OT (Figure 2). Prostaglandins also facilitate cervical ripening and dilation as well as fetal membrane rupture. Oxytocin can therefore be considered as both a stimulator of uterine contractions and an activator of inflammatory pathways in gestational tissues including myometrium. It is therefore important that this dual role is considered when developing new treatments targeting the OTR in labour.

Oxytocin may mediate some of these changes in gene expression through regulation of micro RNAs (miRNAs) within the myometrium. Micro RNAs have been shown to be post-transcriptional regulators of key gene expression pathways involved in parturition[29], including the OTR[30]. More recently, OT itself has been shown to affect the miRNA profile of human myometrium at term[31]. Gene targets for these miRNAs included NFκB, NFκB-regulated genes such as IL-8, IL-6 and MMP9 and α-smooth muscle actin. Down-regulation of these miRNAs would favour a pro-inflammatory and pro-contractile phenotype and hence, promote labour onset. This also further supports OT as an inflammatory mediator in myometrium. Further work is required to define gene targets of other miRNAs in myometrium, as well as clarify the biological significance of these changes in relation to parturition.

4. The AVP/AVPR system in myometrium

Similar to OT, AVP is a nonapeptide. Its sequence differs to OT by just two amino acids (Figure 3A). AVP also signals through GPCRs, $V_{1a}R$, $V_{1b}R$ and V_2R, (AVPRs) which display high homology with the OTR. In contrast to the OT/OTR system however, much less is known about AVP signalling in myometrium and its role in parturition. To date, only the $V_{a1}R$ and $V_{1b}R$ have been found in myometrium [5,32], although some recent data suggests V_2R is also expressed in human myometrium (S Arrowsmith et al., abstract in *Acta Physiol* 2016, 217 (Suppl. 708), 3–158). Because of the high homology with OTRs, there is significant crosstalk between the receptor families and their ligands (Figure 3B and C). AVPRs may be important in mediating the response to OT (and vice versa).

Differences in the affinity of OT and AVP for the different receptor families between species however, has hampered our understanding of the role of AVPRs in myometrium. For example in rodents, AVP-induced contractions are solely mediated by OTRs although this is not the same for human myometrium[33,34]. AVP's uterotonic effect is thought to involve IP_3-mediated store-Ca release with potential actions at both the $V_{1a}Rs$ and the OTR[7]. However, the model systems and the selectivity of the peptides and antagonists used to test this are likely to confound data. Deciphering the involvement and relative importance of AVP and AVPRs in human myometrium will require highly selective human receptor-subtype

agonists (e.g. see [35]) and antagonists as well as good models to test them[36]. New peptides displaying selectivity for the *human* $V_{1a}R$ and $V_{1b}R$ have been shown to augment human myometrial contractions indicating that the receptors are functional in myometrium (S Arrowsmith et al., abstract in *Acta Physiol* 2016, 217 (Suppl. 708), 3–158). Associations between OTRs and some AVPRs with potential important functional consequences have also been reported and are discussed later.

5. Clinical considerations

5.1. Targeting the OTR (or AVPRs) to relax or stimulate the myometrium

Due to its prominence in myometrial contraction, the OTR has long been a major target for therapies aimed at modulating uterine contractions in labour. OTR antagonists represent the only drugs used specifically for the management of preterm labour and atosiban is the only currently approved OTR-A used for this purpose. Interestingly, atosiban is primarily a $V_{1a}R$ antagonist and displays a lower affinity for the OTR[37]. Which receptor/s it antagonises to bring about inhibition of contraction has therefore been questioned and atosiban's activity at the $V_{1a}Rs$ in other tissues such as blood vessels may inadvertently cause unwanted side effects. In addition to inhibition of $G_{\alpha q}$ signalling in myometrium, atosiban has also been shown to *activate* $G_{\alpha i}$ signalling and inflammatory pathways in amnion[38] which is counterintuitive and undesirable for a tocolytic. Hence, understanding the contribution of signalling from the different G protein subunits which are coupled to OTR will be essential to aid the development of effective therapeutics.

Other more-selective OTR-As include barusiban, retosiban and nolasiban which have shown some promise in early *in vitro* studies[39-41]. However, despite a >300-fold selectivity for the OTR, barusiban was no more effective than placebo in stopping preterm labour in pregnant women[42]. Retosiban, a >1400-fold selective OTR inverse agonist, showed a favorable efficacy and safety profile in a phase 2 proof-of-concept study for the treatment of spontaneous preterm labour[43]. Interestingly, data from Smith's group suggest retosiban may prevent stretch-induced uterine contractions and that OTR may also act as a mechanosensor in which myometrial stretch increases contractions via agoinst-free activation of the OTR[44]. Retosiban may be suited to delaying or preventing preterm

labour in multiple pregnancy. Clinical trials to test this are needed. In addition to $G_{\alpha q}$ signalling, nolasiban, unlike atosiban, also inhibits $G_{\alpha i}$ signalling from the OTR and therefore may be beneficial in that it also inhibit OT's inflammatory action in gestational tissues[45].

Considerations for the use of oxytocins to exogenously manage dysfunctional labour and postpartum haemorrhage (PPH) include dosing and delivery strategies. Oxytocin's release from the pituitary is thought to be pulsatile which compensates for its relative short half-life (3-4 min)[46] and the rapid desensitisation of its receptor. However trials have shown that pulsatile infusion of oxytocin confers no clinical benefit for induction and is not recommended for augmentation[47].

To overcome the limits of endogenous OT, a number of OT analogues have been developed [48,49]. The newest which has been tested in clinical trials is carbetocin. It was developed to be a long-lasting (80-100 min half-life), potent, selective agonist of the OTR[50]. It has shown a favourable side-effect profile compared to OT in trials for the prevention of PPH and has some desirable effects including some reduced blood loss[51] but more trial data is needed to confirm this. Its longer half-life however, makes it unsuitable for use in labour induction[52]. Interestingly, carbetocin was found to display 'functional selectivity' in that, unlike OT, it only activates the OTR/$G_{\alpha q}$ pathway. In addition, carbetocin promotes OTR internalisation via a novel and yet unidentified β-arrestin-independent pathway. It was also shown to negatively influence OTR recycling to the plasma membrane[53].

5.2. Drug-delivery systems

Using a drug delivery system which targets the uterus has the potential to minimise toxicity from unwanted side effects in maternal and/or fetal organs and increase drug efficacy. The OTR is one such attractive target. So far, liposomes conjugated to antibodies against the extracellular domain of OTR have been used to successfully deliver tocolytics or uterotonics to human myometrial tissues *in vitro* and modify contraction. *In vivo* studies in pregnant mice also demonstrated that the liposomes predominantly localised to uterine tissues[54]. Others have used the OTR-A, atosiban as their targeting element to deliver indomethacin to the uterus[55]. Whilst no significant change on rates of induced preterm birth in mice was observed, they were able deliver double the concentration of indomethacin whilst

importantly, decreasing fetal levels. These promising approaches open new horizons for drug development in obstetrics that could greatly impact the management of preterm birth and dysfunctional labour without multi-organ side effects.

5.3. Cross talk with other receptors

In addition to receptor cross-talk by the neuropeptides (discussed above), OTRs can also form homodimers (and oligomers) as well as heterodimers with other GPCRs[56]. Examples include V_{1a}R/OTR, V_2R/OTR, β_2-adrenergic receptor (β_2-AR)/OTR and PG receptor/OTR. The relevance of these dimers is not known, but it is easy to imagine how heterodimers involving GPCRs which both mediate pro-contractile effects in the myometrium (e.g. V_{1a}R, FP) could have functional roles in the onset of labour. Indeed, low dose $PGF_{2\alpha}$ was shown to improve myometrial responses to OT[57] whilst FP receptor antagonists can suppress OT-induced myometrial contraction[58]. Similarly, OTR antagonism has recently been shown to affect $PGF_{2\alpha}$-induced contractions[41]. OTR and β_2-AR however, have opposing roles in myometrium. Despite this, they have been shown to physically interact[59,60], and β_2-AR signalling can also affect OTR signalling in myometrium[61]. Further studies are required to determine the biological significance of these and other potential dimers involving OTRs. Importantly they must be examined in native human tissues to make their interactions and function physiologically relevant.

Additionally, understanding OTR dimerization and crosstalk with other receptors may also open up the opportunity for the development of more effective combination treatments. In *ex vivo* studies of human contractions, the potency of a number of tocolytics is significantly reduced in the presence of low dose oxytocin (0.5nM)[62,63]. Hence combination tocolytics involving OTR-As warrants further investigation.

5.4. Genetic variants in the OT/OTR system

Single nucleotide polymorphisms (SNPs) in the *OTR* gene have been reported in neurologic disorders but the relevance of *OTR* SNPs in parturition remains largely unknown. Studies suggest that genetic variants of the *OTR* may alter oxytocin dose requirement for labour

induction[64,65] and increase the risk for preterm birth[66,67]. Variants in *OTR* have been observed in both coding and non-coding regions of the receptor, including in domains involved in agonist binding (which results in reduced OT binding and IP_3 production), and truncation of the receptor[64,66]. SNPs in genes related to the process of OTR internalisation and desensitisation (e.g. GRK6), and the enzyme responsible for degradation and inactivation of OT, have also been identified[65,67]. Together these data suggest that there could also be a genetic factor in determining maternal sensitivity to OT which needs to be considered. But, before these genetic differences can be translated into therapeutic strategies, further studies to investigate the impact of these genetic variations on downstream signalling and their functional consequences are needed.

Interestingly mutations in OTR transmembrane residues also changes the binding of AVP at the OTR from partial to full agonist[68]. Hence, these residues are critical in determining OTR's response to any agonist (or antagonist) modelled on the structure of OT or AVP. They therefore have important consequences for the design of new therapies to modulate OTR function. Given AVP's uterotonic action, this also warrants further investigation.

6. Conclusions and future directions

The emerging roles for oxytocin in parturition are exciting, however they highlight the complexity of this system. As our understanding of GPCR signalling expands, so too will our understanding of neuropeptide signalling in the myometrium. We have been close to achieving safer, more effective tocolytics and uterotonics with preferred routes of administration for many years but we are not quite there yet. A lot still remains to be done and several important questions remain unanswered, particularly around receptor dimerization and whether receptor crosstalk affects function. Determining which G proteins are essential for signal transduction and hence, which we should 'turn on' or 'off' to regulate contractions will, in turn, determine whether functional selectivity of ligands is an important factor for therapy. Identification of receptor SNPs which are crucial for receptor function should help identify patient subpopulations who are likely to benefit from treatment as well as inform receptor targeting and drug-delivery strategies. Other factors

which have not been discussed here include how receptor expression is regulated. For this readers are directed to an earlier review[4].

As with all studies, the system in which we use to test our questions is important. The OT-OTR and AVP-AVPR systems are no exception. To obtain clinically and physiologically relevant data, we need studies in human myometrial tissues. Together with further advances in our understanding of neuropeptide signalling in myometrium, it is exciting to think that, we may soon be able to provide a personalized medicine approach for the management of labour and treatment of preterm birth.

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Conflict of Interest

None

References

1. Fuchs AR, Fuchs F, Husslein P, Soloff MS, Fernstrom MJ: **Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor**. *Science* 1982, **215**:1396-1398.
2. Soloff MS, Alexandrova M, Fernstrom MJ: **Oxytocin receptors: triggers for parturition and lactation?** *Science* 1979, **204**:1313-1315.
3. Arthur P, Taggart MJ, Zielnik B, Wong S, Mitchell BF: **Relationship between gene expression and function of uterotonic systems in the rat during gestation, uterine activation and both term and preterm labour**. *J Physiol* 2008, **586**:6063-6076.
4. Arrowsmith S, Wray S: **Oxytocin: its mechanism of action and receptor signalling in the myometrium**. *J Neuroendocrinol* 2014, **26**:356-369.
5. Maggi M, Del Carlo P, Fantoni G, Giannini S, Torrisi C, Casparis D, Massi G, Serio M: **Human myometrium during pregnancy contains and responds to V1 vasopressin receptors as well as oxytocin receptors**. *J Clin Endocrinol Metab* 1990, **70**:1142-1154.
6. Fuchs AR, Behrens O, Maschek H, Kupsch E, Einspanier A: **Oxytocin and vasopressin receptors in human and uterine myomas during menstrual cycle and early pregnancy**. *Hum Reprod Update* 1998, **4**:594-604.
7. Bossmar T, Akerlund M, Fantoni G, Szamatowicz J, Melin P, Maggi M: **Receptors for and myometrial responses to oxytocin and vasopressin in preterm and term human pregnancy: effects of the oxytocin antagonist atosiban**. *Am J Obstet Gynecol* 1994, **171**:1634-1642.

8. Busnelli M, Sauliere A, Manning M, Bouvier M, Gales C, Chini B: **Functional selective oxytocin-derived agonists discriminate between individual G protein family subtypes**. *J Biol Chem* 2012, **287**:3617-3629.
9. Mironneau J: **Effects of oxytocin on ionic currents underlying rhythmic activity and contraction in uterine smooth muscle**. *Pflugers Arch* 1976, **363**:113-118.
10. Monga M, Campbell DF, Sanborn BM: **Oxytocin-stimulated capacitative calcium entry in human myometrial cells**. *Am J Obstet Gynecol* 1999, **181**:424-429.
11. Noble D, Borysova L, Wray S, Burdyga T: **Store-operated Ca2+ entry and depolarization explain the anomalous behaviour of myometrial SR: Effects of SERCA inhibition on electrical activity, Ca2+ and force**. *Cell Calcium* 2014, **56**:188-194.
12. Ferreira JJ, Butler A, Stewart R, Gonzalez-Cota AL, Lybaert P, Amazu C, Reinl EL, Wakle-Prabagaran M, Salkoff L, England SK, et al.: **Oxytocin can regulate myometrial smooth muscle excitability by inhibiting the Na(+) -activated K(+) channel, Slo2.1**. *J Physiol* 2019, **597**:137-149.
13. Popescu LM, Nutu O, Panoiu C: **Oxytocin contracts the human uterus at term by inhibiting the myometrial Ca2+-extrusion pump**. *Biosci Rep* 1985, **5**:21-28.
14. Soloff MS, Sweet P: **Oxytocin inhibition of (Ca2+ + Mg2+)-ATPase activity in rat myometrial plasma membranes**. *J Biol Chem* 1982, **257**:10687-10693.
15. Akerman KE, Wikstrom MK: **(Ca2+ + Mg2+)-stimulated ATPase activity of rabbit myometrium plasma membrane is blocked by oxytocin**. *FEBS Lett* 1979, **97**:283-287.
16. Hudson CA, Lopez Bernal A: **Phosphorylation of proteins during human myometrial contractions: A phosphoproteomic approach**. *Biochem Biophys Res Commun* 2017, **482**:1393-1399.
17. Somlyo AP, Wu X, Walker LA, Somlyo AV: **Pharmacomechanical coupling: the role of calcium, G-proteins, kinases and phosphatases**. *Rev Physiol Biochem Pharmacol* 1999, **134**:201-234.
18. Lartey J, Lopez Bernal A: **RHO protein regulation of contraction in the human uterus**. *Reproduction* 2009, **138**:407-424.
19. Ozaki H, Yasuda K, Kim YS, Egawa M, Kanzaki H, Nakazawa H, Hori M, Seto M, Karaki H: **Possible role of the protein kinase C/CPI-17 pathway in the augmented contraction of human myometrium after gestation**. *Br J Pharmacol* 2003, **140**:1303-1312.
20. Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ: **beta-Arrestin: a protein that regulates beta-adrenergic receptor function**. *Science* 1990, **248**:1547-1550.
21. Pitcher JA, Freedman NJ, Lefkowitz RJ: **G protein-coupled receptor kinases**. *Annu Rev Biochem* 1998, **67**:653-692.
22. Conti F, Sertic S, Reversi A, Chini B: **Intracellular trafficking of the human oxytocin receptor: evidence of receptor recycling via a Rab4/Rab5 "short cycle"**. *Am J Physiol Endocrinol Metab* 2009, **296**:E532-542.
23. Grotegut CA, Mao L, Pierce SL, Swamy GK, Heine RP, Murtha AP: **Enhanced Uterine Contractility and Stillbirth in Mice Lacking G Protein-Coupled Receptor Kinase 6 (GRK6): Implications for Oxytocin Receptor Desensitization**. *Mol Endocrinol* 2016, **30**:455-468.
24. Grotegut CA, Feng L, Mao L, Heine RP, Murtha AP, Rockman HA: **beta-Arrestin mediates oxytocin receptor signaling, which regulates uterine contractility and cellular migration**. *Am J Physiol Endocrinol Metab* 2011, **300**:E468-477.
25. Kim SH, Bennett PR, Terzidou V: **Advances in the role of oxytocin receptors in human parturition**. *Mol Cell Endocrinol* 2017, **449**:56-63.
26. Kim SH, MacIntyre DA, Firmino Da Silva M, Blanks AM, Lee YS, Thornton S, Bennett PR, Terzidou V: **Oxytocin activates NF-kappaB-mediated inflammatory pathways in human gestational tissues**. *Mol Cell Endocrinol* 2015, **403**:64-77.
27. Terzidou V, Blanks AM, Kim SH, Thornton S, Bennett PR: **Labor and inflammation increase the expression of oxytocin receptor in human amnion**. *Biol Reprod* 2011, **84**:546-552.

28. Wilson T, Liggins GC, Whittaker DJ: **Oxytocin stimulates the release of arachidonic acid and prostaglandin F2 alpha from human decidual cells**. *Prostaglandins* 1988, **35**:771-780.
29. Renthal NE, Williams KC, Mendelson CR: **MicroRNAs--mediators of myometrial contractility during pregnancy and labour**. *Nat Rev Endocrinol* 2013, **9**:391-401.
30. Renthal NE, Chen CC, Williams KC, Gerard RD, Prange-Kiel J, Mendelson CR: **miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor**. *Proc Natl Acad Sci U S A* 2010, **107**:20828-20833.
31. Cook JR, MacIntyre DA, Samara E, Kim SH, Singh N, Johnson MR, Bennett PR, Terzidou V: **Exogenous oxytocin modulates human myometrial microRNAs**. *Am J Obstet Gynecol* 2015, **213**:65 e61-65 e69.
32. Lolait SJ, O'Carroll AM, Mahan LC, Felder CC, Button DC, Young WS, Mezey E, Brownstein MJ: **Extrapituitary expression of the rat V1b vasopressin receptor gene**. *Proc Natl Acad Sci U S A* 1995, **92**:6783-6787.
33. Kawamata M, Mitsui-Saito M, Kimura T, Takayanagi Y, Yanagisawa T, Nishimori K: **Vasopressin-induced contraction of uterus is mediated solely by the oxytocin receptor in mice, but not in humans**. *Eur J Pharmacol* 2003, **472**:229-234.
34. Chan WY, Wo NC, Manning M: **The role of oxytocin receptors and vasopressin V1a receptors in uterine contractions in rats: implications for tocolytic therapy with oxytocin antagonists**. *Am J Obstet Gynecol* 1996, **175**:1331-1335.
35. Muttenthaler M, Andersson A, Vetter I, Menon R, Busnelli M, Ragnarsson L, Bergmayr C, Arrowsmith S, Deuis JR, Chiu HS, et al.: **Subtle modifications to oxytocin produce ligands that retain potency and improved selectivity across species**. *Sci Signal* 2017, **10**.
36. Arrowsmith S, Keov P, Muttenthaler M, Gruber CW: **Contractility Measurements of Human Uterine Smooth Muscle to Aid Drug Development**. *J Vis Exp* 2018.
37. Akerlund M, Bossmar T, Brouard R, Kostrzewska A, Laudanski T, Lemancewicz A, Serradeil-Le Gal C, Steinwall M: **Receptor binding of oxytocin and vasopressin antagonists and inhibitory effects on isolated myometrium from preterm and term pregnant women**. *Br J Obstet Gynaecol* 1999, **106**:1047-1053.
38. Kim SH, MacIntyre DA, Hanyaloglu AC, Blanks AM, Thornton S, Bennett PR, Terzidou V: **The oxytocin receptor antagonist, Atosiban, activates pro-inflammatory pathways in human amnion via G(alphai) signalling**. *Mol Cell Endocrinol* 2016, **420**:11-23.
39. Reinheimer TM, Chellman GJ, Resendez JC, Meyer JK, Bee WH: **Barusiban, an effective long-term treatment of oxytocin-induced preterm labor in nonhuman primates**. *Biol Reprod* 2006, **75**:809-814.
40. Moraitis AA, Cordeaux Y, Charnock-Jones DS, Smith GC: **The Effect of an Oxytocin Receptor Antagonist (Retosiban, GSK221149A) on the Response of Human Myometrial Explants to Prolonged Mechanical Stretch**. *Endocrinology* 2015, **156**:3511-3516.
41. Kim SH, Riaposova L, Ahmed H, Pohl O, Chollet A, Gotteland JP, Hanyaloglu A, Bennett PR, Terzidou V: **Oxytocin Receptor Antagonists, Atosiban and Nolasiban, Inhibit Prostaglandin F2alpha-induced Contractions and Inflammatory Responses in Human Myometrium**. *Sci Rep* 2019, **9**:5792.
42. Thornton S, Goodwin TM, Greisen G, Hedegaard M, Arce JC: **The effect of barusiban, a selective oxytocin antagonist, in threatened preterm labor at late gestational age: a randomized, double-blind, placebo-controlled trial**. *Am J Obstet Gynecol* 2009, **200**:627 e621-610.
43. Thornton S, Miller H, Valenzuela G, Snidow J, Stier B, Fossler MJ, Montague TH, Powell M, Beach KJ: **Treatment of spontaneous preterm labour with retosiban: a phase 2 proof-of-concept study**. *Br J Clin Pharmacol* 2015, **80**:740-749.
44. Aye I, Moraitis AA, Stanislaus D, Charnock-Jones DS, Smith GCS: **Retosiban Prevents Stretch-Induced Human Myometrial Contractility and Delays Labor in Cynomolgus Monkeys**. *J Clin Endocrinol Metab* 2018, **103**:1056-1067.

45. Kim SH, Pohl O, Chollet A, Gotteland JP, Fairhurst AD, Bennett PR, Terzidou V: **Differential Effects of Oxytocin Receptor Antagonists, Atosiban and Nolasiban, on Oxytocin Receptor-Mediated Signaling in Human Amnion and Myometrium**. *Mol Pharmacol* 2017, **91**:403-415.
46. Gazis D: **Plasma half-lives of vasopressin and oxytocin analogs after iv injection in rats**. *Proc Soc Exp Biol Med* 1978, **158**:663-665.
47. Tribe RM, Crawshaw SE, Seed P, Shennan AH, Baker PN: **Pulsatile versus continuous administration of oxytocin for induction and augmentation of labor: two randomized controlled trials**. *Am J Obstet Gynecol* 2012, **206**:230.e231-238.
48. Manning M, Misicka A, Olma A, Bankowski K, Stoev S, Chini B, Durroux T, Mouillac B, Corbani M, Guillon G: **Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics**. *J Neuroendocrinol* 2012, **24**:609-628.
49. Wisniewski K: **Design of Oxytocin Analogs**. *Methods Mol Biol* 2019, **2001**:235-271.
50. Cort N, Einarsson S, Schams D, Vilhardt H: **Blood concentrations of oxytocin equivalents after single injections of deamino-1-monocarba-[2-O-methyltyrosine]-oxytocin in lactating sows**. *Am J Vet Res* 1981, **42**:1804-1806.
51. Gallos ID, Papadopoulou A, Man R, Athanasopoulos N, Tobias A, Price MJ, Williams MJ, Diaz V, Pasquale J, Chamillard M, et al.: **Uterotonic agents for preventing postpartum haemorrhage: a network meta-analysis**. *Cochrane Database Syst Rev* 2018, **12**:CD011689.
52. Armbruster D, Burke T, Weeks A: **Heat-Stable Carbetocin to Prevent Postpartum Hemorrhage**. *N Engl J Med* 2018, **379**:2380-2381.
53. Passoni I, Leonzino M, Gigliucci V, Chini B, Busnelli M: **Carbetocin is a Functional Selective Gq Agonist That Does Not Promote Oxytocin Receptor Recycling After Inducing beta-Arrestin-Independent Internalisation**. *J Neuroendocrinol* 2016, **28**.
54. Paul JW, Hua S, Ilicic M, Tolosa JM, Butler T, Robertson S, Smith R: **Drug delivery to the human and mouse uterus using immunoliposomes targeted to the oxytocin receptor**. *Am J Obstet Gynecol* 2017, **216**:283 e281-283 e214.
55. Refuerzo JS, Leonard F, Bulayeva N, Gorenstein D, Chiossi G, Ontiveros A, Longo M, Godin B: **Uterus-targeted liposomes for preterm labor management: studies in pregnant mice**. *Sci Rep* 2016, **6**:34710.
56. Cottet M, Albizu L, Perkovska S, Jean-Alphonse F, Rahmeh R, Orcel H, Mejean C, Granier S, Mendre C, Mouillac B, et al.: **Past, present and future of vasopressin and oxytocin receptor oligomers, prototypical GPCR models to study dimerization processes**. *Curr Opin Pharmacol* 2010, **10**:59-66.
57. Baxi LV, Petrie RH, Caritis SN: **Induction of labor with low-dose prostaglandin F2 alpha and oxytocin**. *Am J Obstet Gynecol* 1980, **136**:28-31.
58. Friel AM, O'Reilly MW, Sexton DJ, Morrison JJ: **Specific PGF(2alpha) receptor (FP) antagonism and human uterine contractility in vitro**. *BJOG* 2005, **112**:1034-1042.
59. Wrzal PK, Devost D, Petrin D, Goupil E, Iorio-Morin C, Laporte SA, Zingg HH, Hebert TE: **Allosteric interactions between the oxytocin receptor and the beta2-adrenergic receptor in the modulation of ERK1/2 activation are mediated by heterodimerization**. *Cell Signal* 2012, **24**:342-350.
60. Wrzal PK, Goupil E, Laporte SA, Hebert TE, Zingg HH: **Functional interactions between the oxytocin receptor and the beta2-adrenergic receptor: implications for ERK1/2 activation in human myometrial cells**. *Cell Signal* 2012, **24**:333-341.
61. Engstrom T, Bratholm P, Vilhardt H, Christensen NJ: **Effect of oxytocin receptor and beta2-adrenoceptor blockade on myometrial oxytocin receptors in parturient rats**. *Biol Reprod* 1999, **60**:322-329.
62. Arrowsmith S, Neilson J, Bricker L, Wray S: **Differing In Vitro Potencies of Tocolytics and Progesterone in Myometrium From Singleton and Twin Pregnancies**. *Reprod Sci* 2016, **23**:98-111.

63. Arrowsmith S, Neilson J, Wray S: **The combination tocolytic effect of magnesium sulfate and an oxytocin receptor antagonist in myometrium from singleton and twin pregnancies**. *Am J Obstet Gynecol* 2016, **215**:789 e781-789 e789.
64. Reinl EL, Goodwin ZA, Raghuraman N, Lee GY, Jo EY, Gezahegn BM, Pillai MK, Cahill AG, de Guzman Strong C, England SK: **Novel oxytocin receptor variants in laboring women requiring high doses of oxytocin**. *Am J Obstet Gynecol* 2017, **217**:214 e211-214 e218.
65. Grotegut CA, Ngan E, Garrett ME, Miranda ML, Ashley-Koch AE, Swamy GK: **The association of single-nucleotide polymorphisms in the oxytocin receptor and G protein-coupled receptor kinase 6 (GRK6) genes with oxytocin dosing requirements and labor outcomes**. *Am J Obstet Gynecol* 2017, **217**:367 e361-367 e369.
66. Kim J, Stirling KJ, Cooper ME, Ascoli M, Momany AM, McDonald EL, Ryckman KK, Rhea L, Schaa KL, Cosentino V, et al.: **Sequence variants in oxytocin pathway genes and preterm birth: a candidate gene association study**. *BMC Med Genet* 2013, **14**:77.
67. Kuessel L, Grimm C, Knofler M, Haslinger P, Leipold H, Heinze G, Egarter C, Schmid M: **Common oxytocin receptor gene polymorphisms and the risk for preterm birth**. *Dis Markers* 2013, **34**:51-56.
68. Chini B, Mouillac B, Balestre MN, Trumpp-Kallmeyer S, Hoflack J, Hibert M, Andriolo M, Pupier S, Jard S, Barberis C: **Two aromatic residues regulate the response of the human oxytocin receptor to the partial agonist arginine vasopressin**. *FEBS Lett* 1996, **397**:201-206.

Annotated References

The following references have been selected as papers of special interest (*) or outstanding interest (**).

(12**) Ferreira JJ, Butler A, Stewart R, Gonzalez-Cota AL, Lybaert P, Amazu C, Reinl EL, Wakle-Prabagaran M, Salkoff L, England SK, et al.: Oxytocin can regulate myometrial smooth muscle excitability by inhibiting the Na(+) -activated K(+) channel, Slo2.1. *J Physiol* 2019, 597:137-149.

This is the first paper in a number of years to detail a mechanism of how oxytocin receptor activation can lead to depolarisation of the membrane. It also documents a novel K+ channel (SLO 2.1) in myometrium which is linked to enhanced contractility at term.

(16*) Hudson CA, Lopez Bernal A: Phosphorylation of proteins during human myometrial contractions: A phosphoproteomic approach. *Biochem Biophys Res Commun* 2017, 482:1393-1399

Using a global phosphproteomics approach, the authors identify proteins which are phosphorylated during spontaneous contractions and in response to oxytocin stimulation. These phosphorylation events may shed light on mechanisms responsible for altered contraction in response to oxytocin. Examples include (as discussed above) phosphorylation of PMCA and PPP1R12B, the regulatory subunit of MLCP.

(25**) Kim SH, MacIntyre DA, Firmino Da Silva M, Blanks AM, Lee YS, Thornton S, Bennett PR, Terzidou V: Oxytocin activates NF-kappaB-mediated inflammatory pathways in human gestational tissues. *Mol Cell Endocrinol* 2015, 403:64-77.

This paper provides insight into novel roles for oxytocin as an inflammatory mediator in facilitating labour onset.

(34*) Muttenthaler M, Andersson A, Vetter I, Menon R, Busnelli M, Ragnarsson L, Bergmayr C, Arrowsmith S, Deuis JR, Chiu HS, et al.: Subtle modifications to oxytocin produce ligands that retain potency and improved selectivity across species. *Sci Signal* 2017, 10.

The authors detail how minor modifications to oxytocin's phamacophore can generate oxytocin analogues with different receptor selectivity profiles. The analogue described showed selectivity for the OTR and importantly, this selectivity was retained across both mouse and human species. It will be a useful peptide in determining the relative importance of OTRs compared to other GPCRs (e.g. V1aRs) in many systems including myometrium.

(40*) Kim SH, Riaposova L, Ahmed H, Pohl O, Chollet A, Gotteland JP, Hanyaloglu A, Bennett PR, Terzidou V: Oxytocin Receptor Antagonists, Atosiban and Nolasiban, Inhibit Prostaglandin F2alpha-induced Contractions and Inflammatory Responses in Human Myometrium. *Sci Rep* 2019, 9:5792

This paper shows that OTR-As can inhibit signalling by other GPCR hormones such as Prostaglandin F2α. It therefore provides evidence to suggest that there may be a functional cross talk between OTR and FP receptors in myometrium.

(43*) Aye I, Moraitis AA, Stanislaus D, Charnock-Jones DS, Smith GCS: Retosiban Prevents Stretch-Induced Human Myometrial Contractility and Delays Labor in Cynomolgus Monkeys. *J Clin Endocrinol Metab* 2018, 103:1056-1067

The authors describe the effects of a new OTR-A (inverse agonist), retosiban, on myometrial contractions under stretch. Interestingly, a new role for oxytocin receptor as mechanosensors is proposed.

(53**) Paul JW, Hua S, Ilicic M, Tolosa JM, Butler T, Robertson S, Smith R: Drug delivery to the human and mouse uterus using immunoliposomes targeted to the oxytocin receptor. *Am J Obstet Gynecol* 2017, 216:283 e281-283 e214

This paper describes a novel approach to delivering therapies to the uterus using OTR – targeted immunoliposomes as a platform for drug delivery.

Figure Legends

Figure 1. Oxytocin receptor signalling in the myometrium leading to contraction.

Binding of oxytocin to its receptor activates $G\alpha_{q/11}$ which activates PLC-β, which in turn hydrolyses PIP_2 to IP_3 and DAG. IP_3 causes release of Ca from the sarcoplasmic reticulum (SR) and DAG activates PKC. Activation of PKC leads to the inhibition of the SLO2.1 potassium leak channel, reducing K^+ efflux and depolarises the membrane. In turn, this opens voltage-operated (L-type) Ca channels (VOCCs) and Ca^{2+} enters the cell. Inhibition of the Ca-ATPase pump inhibits Ca exit from the cell, thus promoting elevated $[Ca]_i$. The reduction in lumenal SR [Ca] is thought to trigger store-operated Ca entry (SOCE) which is a process whereby depletion of luminal SR Ca stores (such as following agonist mediated SR Ca release or inhibition of SR/ER –ATPase 'SERCA') is coupled to Ca entry. That OT releases and thereby lowers SR Ca, is thought to trigger SOCE in myometrium. SOCE will lead to depolarisation and trigger the opening of L-type calcium channels and is therefore is also likely to contribute to Ca influx during OT stimulation. Stim and Orai and transient potential superfamily of protein (Trp) homologues which mediate SOCE in other cells, are expressed in myometrium and hence may be involved. The combined elevation in $[Ca]_i$ leads to formation of the Ca-calmodulin (Ca-CAM) complex which then activates MLCK, resulting in acto-myosin crossbridge cycling and myometrial contraction.

In addition, DAG-activated PKC also signals for phosphorylation of CPI-17 whilst oxytocin binding to OTR also activates Rho-A via ($G_{\alpha 12/13}$) which in turn activates ROCK. Both phosphorylated CPI-17 and ROCK inhibit MLCP leading to increased myosin phosphorylation and this is the proposed mechanism of Ca sensitisation in the myometrium.

OTR: oxytocin receptor, VOCC: voltage operated Ca channel, SLO2.1 sodium-activated, high-conductance, potassium leak channel, TRP: Transient receptor potential channel: PLC-β: phospholipase C-β, PIP_2: phosphatidylinositol 4,5-bisphosphate, IP_3: inositol 1,4,5-triphosphate, DAG: diacylglycerol, PKC: protein kinase type C, CPI-17: C-kinase-activated protein phosphatase-1 (PP1) inhibitor 17kDa, Ca-CAM: Ca-calmodulin complex, MLCK: myosin light-chain kinase, MLCP: myosin light chain phosphatase, ROCK: RhoA-associated protein kinase, SOCE: store-operated Ca entry.

Red pathways indicate signalling pathways with direct influences on $[Ca]_i$ whilst purple lines indicate Ca-independent pathways to contraction including Ca sensitisation. Dotted lines indicate where mechanisms are not yet fully elucidated.

Figure modified with updates from Arrowsmith and Wray 2014., *J Neuroendocrinol* 2014, **26**:356-369

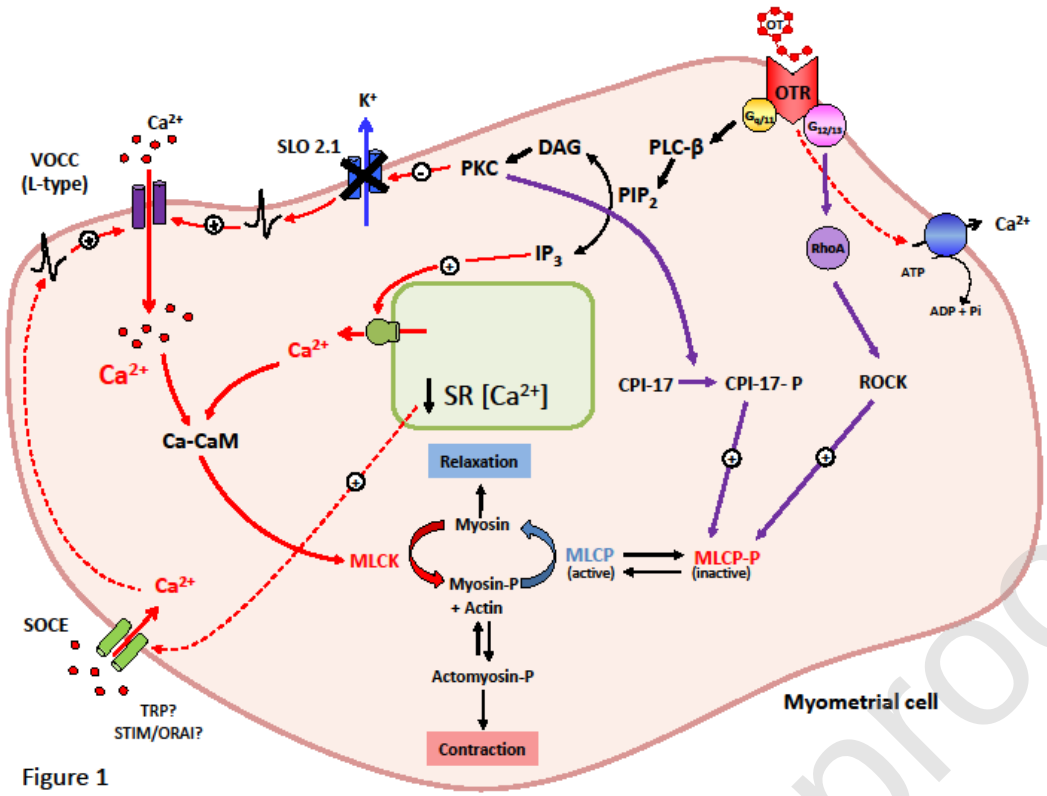


Figure 1

Figure 2. Oxytocin as an inflammatory signal in myometrium and other gestational tissues.

In addition to stimulating contractions, activation of the OTR leads to the concurrent activation of inflammatory pathways in myometrium and other gestational tissues such as amnion. In myometrium this is via activation of MAPKs, ERK1/2 and p38 kinase and NFκB p65/p50 subunits (shown as NFκB for simplicity). In amnion cells, OT signalling (via $G_{\alpha i}$ coupling) leads to ERK1/2 and p38 converging on NFκB p65 homodimers. NFκB dimers translocate to the nucleus where they induce expression of pro-labour genes including COX-2, PGs and inflammatory cytokines and chemokines.

Diacylglycerol (DAG)-induced activation of PKC and MAPK signalling also stimulates an increase in cytoplasmic phospholipase A2 (cPLA2) and COX-2 expression in human myometrial and amnion cells. In turn, this further increases PG synthesis including (PGE_2 and $PGF_{2\alpha}$). PGs can feedback to promote myometrial contractility as well as drive cervical ripening and dilatation.

OTR: oxytocin receptor, PLC-β: phospholipase C-β, PIP_2: phosphatidylinositol 4,5-bisphosphate, IP_3: inositol 1,4,5- triphosphate, DAG: diacylglycerol, PKC: protein kinase type C, cPLA2: cytoplasmic phospholipase A2, COX-2: cyclo-oxygenase 2, $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$, PGE_2: prostaglandin E_2, FP: $PGF_{2\alpha}$ receptor, NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells, MAPK: mitogen-activated protein kinase, ERK1/2: extracellular signal–regulated kinases, p38: p38 mitogen-activated protein kinase.

Red pathways indicate signalling pathways which will bring about contraction in myometrium whilst blue lines indicate pathways leading to activation of inflammation. Dotted lines indicate where sequences have been shortened for simplicity.

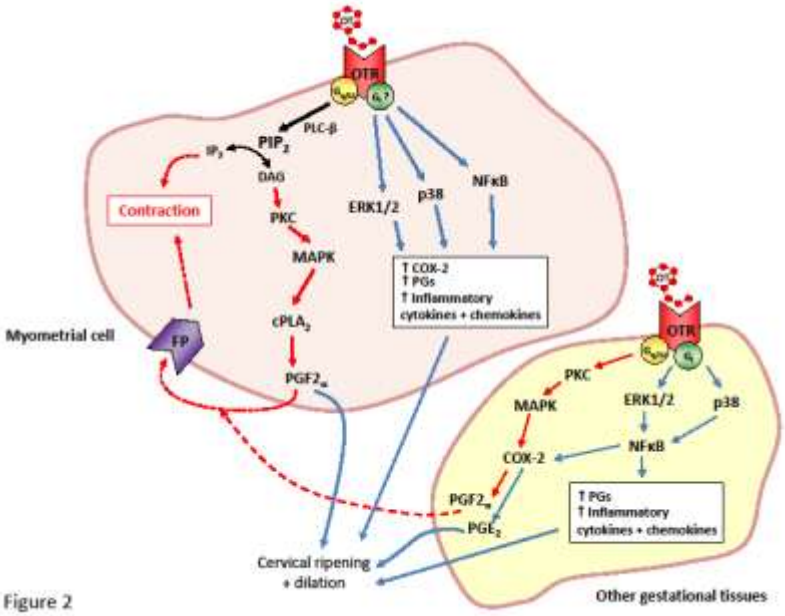


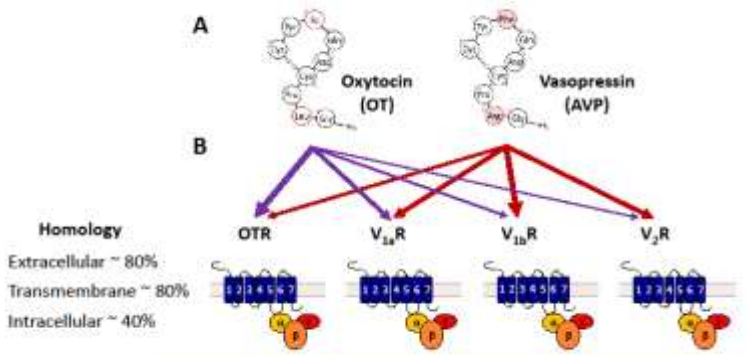
Figure 2

Figure 3: Significant cross-talk between OTRs and AVPRs

A) Oxytocin and Vasopressin peptides are structurally very similar. Both are nonapeptides (they contain 9 amino acids) with a disulphide bridge between two cysteines. This results in a 6 amino acid residue ring and a 3 amino acid residue tail. They differ by just 2 residues in positions 3 and 8.

B) The high structural similarity between the hormones and the high extracellular sequence homology between the 4 receptor subtypes (85% between OTR and $V_{1a}R$) allows for significant crosstalk between the receptors and their ligands (depicted by arrows).

C) The table indicates the selectivity of human oxytocin receptors (hOTR) and arginine-vasopressin (AVP) receptors, $hV_{1a}R$, $hV_{1b}R$ and $h_{V2}R$, to AVP and OT. Values denote affinity in K_i (nM). The lower the K_i, the greater affinity of that hormone for the receptor. *defined as selective as compared with the other receptors of that species by the selectivity criterion of having a two orders of magnitude lower K_i. Table modified from Manning et al., 2012 *J Neuroendocrinol* 2012, **24**:609-628.



C

	hOTR	$hV_{1a}R$	$hV_{1b}R$	hV_2R
OT	0.8*	120	>1000	3500
AVP	1.7	1.1	0.7	1.2

Figure 3