OTR, (OXYTOCIN RECEPTOR)

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Synonyms

OT receptor
OT-R
OXTR
OXT receptor

Historical Background

The biological activities of the pituitary gland have been recognised for over 120 years. In 1895, Oliver and Schäfer discovered that an element of pituitary extract could elevate blood pressure in mammals–the so-called '*pressor effect*'. A few years later, Howell identified that this activity resided from the posterior lobe of the pituitary. In 1906, Sir Henry Dale, while studying the vasopressor action of the extract, found that it also possessed a powerful stimulating action on the uterus of a pregnant cat . He termed the agent responsible, 'oxytocin' from the Greek words *ωχνξ, τοχοχξ* meaning 'quick birth'. Shortly afterwards the same extract was shown to cause milk secretion from mammary tissue. Around 50 years later, the American biochemist, Vincent du Vigneaud determined the chemical structure of oxytocin (OT). He characterised and sequenced a peptide of 9 amino acids which formed a 6 amino-acid cyclic ring structure via a disulphide bond between two cysteine residues and 3 residue tail. This was followed shortly by its synthesis. Oxytocin was in fact the first peptide hormone to be synthesised and for his work, du Vigneaud was awarded the Nobel Prize for Chemistry in 1955.

As with most endocrine systems however, the physiological actions of hormones are mediated through binding to specific receptors on target organs. Despite some of the biological activities of OT and its structure being known for some time, it wasn't until 20 years after its synthesis that the receptor responsible for eliciting these effects– the oxytocin receptor (OTR), was identified. In a 1973 study, Soloff and Swartz demonstrated using a pharmacological ligand binding assay, the specific and high affinity binding of radio-labelled oxytocin to the mammary gland of the rat. They proposed that the mammary gland possessed '*oxytocin binding substances which may be the hormone recognition sites of an oxytocin-receptor system*'. In addition they determined that the binding part of the receptor was a protein because it was susceptible to inhibition by trypsin. Similar studies in the uterus of the rat and sow and human soon followed.

It wasn't until 1992 however that the cDNA encoding the receptor for oxytocin was isolated and identified by Kimura. In their study they injected defolliculated oocytes from Xenopus with mRNA prepared from the muscle of the uterus (myometrium). Subsequent application of OT under voltage clamped conditions caused a large inward membrane current. The receptor mRNA responsible was determined to be between 4-5kb in size. Using a size-fractionated sub library of cDNA clones, followed by screening of progressively smaller sub libraries, they isolated a single cDNA clone which encoded the human OTR. When transcribed *in vitro* and the RNA injected into oocytes, this clone enabled them to be responsive to OT (Kimura et al. 1992). The molecular cloning of the OTR and determination of its amino acid structure paved the way for studies investigating the regulation of the gene's expression including the development of total and conditional OTR and OT gene knock out mice (Nishimori et al. 1996; Takayanagi et al. 2005; Lee et al. 2008).

The *OXTR* gene is present in single copy in the human genome and is mapped to chromosome 3 at locus 3p25-26.2 (Inoue et al. 1994) and spans approximately 17 kilobases (kb). In most mammals the gene consists of 3 exons however in the human and mouse a third intron interrupts the first exon. Therefore in

these species the *OXTR* gene consists of 4 exons and 3 introns (Gimpl and Fahrenholz 2001). Introns 1 and 2 are relatively short (639 and 166 bp). Intron 3 is the largest intron at approximately 12kb and separates the coding region found immediately after the sixth transmembrane spanning domain. Exons 1 and 2 correspond to the 5'- non-coding region, followed by exons 3 and 4 which contain the amino acids that encode the receptor: Exon 3 encodes from 142bp upstream of the translation start site (ATG initiation codon) and spans 922bp downstream which includes transmembrane domains 1-6 and beyond. Exon 4 contains the sequences encoding the seventh transmembrane domain and the C-terminus as well as the 3' non-coding region (**Figure 1**) (Inoue et al. 1994).

Figure 1. Organisation of the human oxytocin receptor gene. The OTR is located on chromosome 3 at position 3p25.3. It is comprised of 4 exons (boxes labelled 1-4). Only exons 3 and 4 encode the amino acid sequence for the receptor (areas highlighted in blue, flanked by the start (ATG) and stop codons (TGA). The regions encoding the 7 transmembrane-spanning domains are represented by dark blue boxes and are numbered by roman numerals I-VII (Adapted from Inoue et al., 1994).

Primer extension analysis identified that the transcription start sites are located 618 and 621 bp upstream of the initiation codon. A variety of nucleotide consensus sequences that are typically involved in transcriptional regulation in many genes were found in close proximity to this, including a TATA-like motif and a potential binding site for SP-1. A number of other transcription factor binding sites are also found in the 5' flanking region including those for AP-1, AP-2 c-Myb and four inverted GATA-1 motifs. The rat *OXTR* was also shown to contain a potential oestrogen response element (ERE) but a functional ERE has not been found in other species, including human (Kimura et al. 2003).

1- Receptor structure, ligand binding and signalling

The encoded receptor contains 389 amino acids in its polypeptide chain and belongs to the rhodopsin-type class I G protein-coupled receptor (GPCR) superfamily, typically characterized by their highly conserved 7 transmembrane (TM) alpha helical domains (**Figure 2**). Data from a number of species including pig, rat sheep, bovine, mouse and rhesus monkey have also been identified and show essentially the same basic structure. Two different sized messenger RNAs for the receptor were found: 3.6 kb in mammary gland and a larger 4.4 kb in ovary, endometrium, and myometrium.

The structure of the receptor, modelling of the ligand docking at the molecular level and subsequent activation, as well as the post-oxytocin signalling mechanisms have been well described. In general, when activivated, conserved sequence residues in GPCRs leads to signal transduction via the recruitment of G proteins. The switching from the inactive to active form of the OTR is assumed to follow that of other GPCR models with a change in the relative orientation of the transmembrane domains revealing a G protein binding site. Oxytocin is suggested to bind to the extracellular loops and the NH_2 terminal domain, as well as to residues within the transmembrane domains themselves (Gimpl and Fahrenholz 2001). Mutagenesis and molecular modelling has suggested that the binding site of OT is located in in a narrow cleft buried in the TM core of the receptor, formed by the ring like arrangement of the TM domains. The cyclic portion of the oxytocin peptide interacts with TM 3, 4, and 6 whilst the linear C-terminal tail region interacts with TM 2 and 3 and the first intracellular loop.

Biochemical studies with the human OTR and OT have identified four key residues that are important for ligand binding and agonist selectivity (see **Figure 2**): Arg^{34} positioned within an important 12 residue sequence within the N terminus, Phe^{103} positioned in the first extracellular loop and two aromatic residues, Tyr^{209} and Phe^{284} in TM 5 and 6 respectively, which are thought to bind to residues 2 and 3 of OT (Zingg and Laporte 2003). In terms of OTR signalling, site-directed mutagenesis has also identified

key residues in the OTR sequence that are responsible for conformational changes in the receptor, intracellular activation of G proteins and coupling to downstream effectors (see **Figure 2**). A point mutation in the amino acid aspartic acid at position 85 to alanine in TM2 in the human OTR, results in receptor inactivity. Changing a lysine to alanine residue at position 270 within the C-terminal part of the third intracellular loop of OTR decreases phosphatidyl turnover. In contrast an arginine to alanine transition at position 137 which forms part of a conserved glutamate (or aspartate)–arginine–tyrosine motif increases basal OTR activity.

The carboxy terminus contains two well conserved cysteine residues, thought to be sites of palmitoylation and therefore conferring anchorage of the receptor's cytoplasmic tail into the membrane lipid bilayer. Additionally the NH_2-terminal domain of the receptor also contains sites for possible glycosylation (**Figure 2**). These differences in glycosylation patterns may be responsible for differences in the molecular masses of the myometrial versus mammary OTRs reported. However, glycosylation does not appear to be important for the proper expression of the OTR or its function since receptor binding characteristics in deglycosylation mutants involving exchange of Asp for Asn in three positions remained unchanged (Gimpl and Fahrenholz 2001).

Figure 2. Schematic structure of the human oxytocin receptor. Membrane topology of the human OTR is shown where each amino acid is denoted by a blue circle and residues involved in ligand binding and signal transduction discussed in the text are highlighted. Residues in red indicate those identified as being involved in binding of oxytocin to the receptor whilst residues in purple denote those sites shown to confer altered receptor activity when mutated. Areas of proposed functional importance in the cytoplasmic C-terminal tail, including clusters of serine residues whish are thought to be involved in GRK or β-arrestin binding and receptor internalisation and desensitisation are highlighted in green. Putative N-glycosylation and palmitoylation sites are also

highlighted and a putative disulphide bond between the first and second extracellular loops is also shown. (adapted from Gimpl and Fahrenholz 2001)

Homology with other GPCRs

The OTR shares high sequence homology with the vasopressin receptors of which there are 3 subtypes: V1aR, V1bR and V2R. The highest homology between the vasopressin (AVP)/OT receptor types is found in the extracellular loops (~80%) and the transmembrane helices. This high sequence similarity especially in the extracellular domains can give rise to agonist cross-reactivity. In regards to AVP/OT agonist binding, the OTR is relatively unselective, having only a 10-fold higher affinity for OT over AVP. AVP can act as a partial agonist at OTR but requires 100-fold greater concentration to induce the same response as OT. In fact much of our understanding about the functional structure of OTRs including the determination of the important binding domains which confer different ligand affinities comes from chimeric 'gain of function mutants' in which different domains or residues of the OTR have been exchanged for the equivalent regions on the different AVPRs .

Oxytocin receptors are functionally coupled to the heterotrimeric G-proteins, comprised of three subunits G_{α}, G_{β} and G_{γ} which cycle between active and inactive signalling states in response to guanine nucleotides. Together they can stimulate a number of signalling pathways that regulate a diverse number of cellular processes. Oxytocin receptors have been shown to recruit and activate the G_q and G_i subfamily (G_{i1}, G_{i2} and G_{i3}) as well as the two members of the G_o family (G_{oA} and G_{oB}) of G proteins (Busnelli et al. 2012). There is less evidence however to suggest that OTR activation results in the recruitment or activation of $G_{\alpha s}$.

Classically, the activation of $G_{\alpha q/11}$ results in the activation of phospholipase C-β (PLC-β) which in turn, controls the hydrolysis of phosphatidylinositol 4,5-bispohosphate (PIP_2) into inositol-tris-phosphate (IP_3)

and diacylglycerol (DAG). Inositol-tris-phosphate controls the mobilisation of Ca^{2+} from intracellular stores such as the sarcoplasmic reticulum (SR) thereby raises intracellular Ca^{2+} and promoting cell contraction, whilst DAG leads to activation of protein kinase type C (PKC). OTR activation linked to G_q signalling also leads to the stimulation of phospholipase A2 production and an increase in cyclooxygenase 2 (COX-2) levels, both resulting in increased prostaglandin production. Other signaling pathways activated include the MAP-kinase (MAPK) cascade and induction of c-*fos* and c-*jun* which are linked to the proliferative effects of OT. Signalling through $G_{\alpha i/o}$ results in the inhibition of adenylate cyclase activity and a reduction in levels of cAMP. In addition, inhibition of cell growth is reported to be G_i-mediated.

In *in vitro* assays using HEK293 cell lines stably transfected with human OTR cDNA, the $G_{\alpha i}$ and $G_{\alpha o}$ isoforms were found to be activated by OT with an EC_{50} 10-fold greater than that required to activate $G_{\alpha q,}$ suggesting that the G_i and G_o-mediated pathways are activated at much higher concentrations of oxytocin than the G_q pathway (Busnelli et al. 2012). How this is translated into a physiological response *in vivo* however, will depend on the local concentration of OT as well as the relative expression of the individual G protein subunits. In addition, that OTRs can activate multiple G proteins can give rise to heterogeneity in the overall cellular response following their activation. The signalling pathways may therefore act synergistically or may have opposite effects on the same cell function.

Many GPCRs, including the OTR have also been shown to associate and form dimers which can be homo- or heterodimers in nature. OTR heterodimerisation reported includes *in vitro* dimerisation with the highly related V1aR and V1bRs and heterodimerisation with the dopamine D2 and adrenergic β2 receptors *in vivo*.

2- Expression, localisation and regulation

Oxytocin has both central and peripheral actions, with roles in many physiological and pathological processes including reproduction, for example; parturition and lactation, maternal behaviour, erectile dysfunction and ejaculation. Oxytocin can also modulate social behaviour via increasing empathy, trust and pair bonding. Not surprisingly therefore, the OTR has been found to be expressed in humans throughout the body; in reproductive structures including myometrium, endometrium, gestational tissues (amnion and decidua) ovary, testes and breast, as well as other organs including kidney, heart, adrenal gland and in neural regions of the brain, such as the frontal cortex, amygdala, hypothalamus and olfactory nucleus.

The localisation of receptors to the plasma membrane was determined by investigating the distribution of [^{3}H] oxytocin binding sites amongst various subcellular fractions of rat myometrium obtained by differential centrifugation. More specifically, OTRs have been found to be localised to the cholesterol-rich and caveolin-containing membrane domains of the plasma membranes known as caveolae which is Latin for 'little caves'(Gimpl and Fahrenholz 2001). These form small omega shaped cell surface invaginations. The localisation of the receptors to these domains may be conveyed by a cholesterol-binding motif within the extracellular domains of the receptor . In contrast to most other GPCRs, the OTR undergoes quite large and cell-specific up- and down-regulation of expression. A number of factors have been shown to regulate OTR expression.

Sex steroids: In uterus, brain and kidney, oestrogens are a major stimulant of OTR expression. However OTR mRNA levels in the mammary gland remain unaffected by oestrogen administration. Studies of the rat and human *OXTR* promoter identified a potential but non-classical oestrogen responsive element (ERE) but there are doubts over its functionality (Kimura et al. 2003). Oestrogen's effects may not follow the classical ER- ERalpha-ERE- target gene pathway. Instead, the mechanism through which

oestrogen regulates expression is likely to involve multiple factors acting indirectly or via other promoter elements with the OTR. Progesterone (P4) on the other hand is inhibitory towards OTR expression. Pregnant rats treated with P4 failed to show the same upregulation of OTR mRNA at parturition as controls. Ovariectomy of pregnant rats in mid gestation results in a significant increase in myometrial OTR mRNA levels compared to sham-operated controls and induces preterm labour. The elevation in OTR mRNA levels associated with ovariectomy-induced preterm labour was completely blocked by the administration of progesterone (Ou et al. 1998).

Cholesterol: Cholesterol is one of the most abundant lipids within the lipid bilayer and therefore its concentration can regulate the function and organization of many membrane proteins including GPCRs. The OTR is known to favour residing within cholesterol rich portions of the membrane (Gimpl and Fahrenholz 2001). It is within these cholesterol rich domains that they confer a higher affinity for agonist binding. OTRs are said to be sensitive to levels of membrane cholesterol and disruption of cholesterol content affects OTR signalling.

Stretch: Mechanical stretch e.g. of the myometrium (smooth muscle of the uterus) during pregnancy has also shown to be involved in the upregulation of OTR expression and it suggested that this may contribute to the higher rates of preterm birth in multiple pregnancy. In studies of unilaterally pregnant rats in which the non-gravid uterine horn was mechanically stretched, the non- gravid stretched horn shows equivalent OTR upregulation as the gravid horn at parturition (Ou et al. 1998). The non-gravid, non-stretched uterus however shows low expression suggesting that both endocrine signals and stretch are a contributing factor to OTR expression in pregnancy and labour.

Internalisation and desensitisation: A further dimension to the regulation of OTR signalling is via its capacity, like other GPCRs, to desensitise and internalise. In the setting of persistent agonist binding, desensitisation is initiated by phosphorylation of the receptor by G-protein-coupled receptor kinases

(GRKs). These phosphorylate GPCRs which increases their affinity for β- arrestins. β-arrestins contain motifs which allow them to function as scaffold proteins linking the receptor to components of the clathrin-dependent endocytic machinery and hence prompts receptor endocytosis. The OTR has been shown to recruit β-arrestin following OTR stimulation and has been shown to co-localise with β-arrestins in endocytic vesicles. Removal of the receptor from the membrane therefore uncouples it from further G protein signalling. The recruitment of β-arrestin to the OTR is dependent upon a highly conserved region within its carboxy terminal containing a series of serine clusters. Mutations within this region lead to unstable OTR-β-arrestin interactions and prevent agonist-mediated receptor internalisation (Smith et al. 2006). There is also evidence to suggest that OTR can undergo receptor desensitisation via a non-β-arrestin pathway involving PKC.

***Epigenetics and OXTR methylation*:** The *OXTR* gene contains a CpG island that stretches through exon 1 to exon 3 from about 20 to 2350 bp downstream of the transcription start site. Luciferase reporter gene assays showed that this CpG region has significant promoter activity (Kusui et al. 2001). A specific region of this CpG island (termed MT2) appears to be responsible for the majority of DNA methylation-induced silencing of OTR.

3- Physiological functions in labour and lactation

Oxytocin is most widely known for its ability to contract the uterus and for milk ejection during lactation. In the uterus, oxytocin stimulates and maintains uterine contractions by elevating intracellular Ca levels. Binding of oxytocin to OTRs primarily leads to the activation of $G_{\alpha q/11}$ resulting in the PLC mediated hydrolysis of PIP_2, increased IP_3 formation and hence increased intracellular Ca via release of Ca^{2+} from the SR (**Figure 3**). In smooth muscle cells such as myometrium, elevated Ca brings about contraction via stimulation of Ca^{2+}-dependent calmodulin which in turn, activates myosin light chain kinase (MLCK).

Subsequent phosphorylation of the regulatory myosin light chains by MLCK brings about cross-bridge cycling and generation of force (Arrowsmith and Wray 2014). Relaxation is brought about by dephosphorylation of myosin via MLC phosphatase (MLCP) and restoring normal intracellular Ca levels.

There is also evidence to suggest that Ca^{2+} from extracellular sources also contributes to the oxytocin-induced rise in intracellular Ca^{2+}. Oxytocin-induced intracellular Ca^{2+} increase is greater in the presence of extracellular Ca^{2+} than that in its absence, suggesting that activation of OTR also affects Ca influx mechanisms. One mechanism is via voltage gated i.e. L-type channels or receptor operated channels. However, this Ca^{2+} influx was found to be insensitive to the L-type channel blocker nifedipine. It is therefore suggested that oxytocin augments Ca^{2+} entry via a process known as capacitative Ca^{2+} entry or store-operated Ca^{2+} entry (SOCE) – a process which is independent of voltage-operated Ca entry. Instead the lowering of SR Ca concentration triggers Ca entry. It is likely that oxytocin affects L-type channel activity indirectly such as via the opening of other cation channels (e.g. Ca^{2+}-activated Cl- channels) or SOCE, which would then lead to membrane depolarisation and subsequent opening of voltage-operated channels. The evidence to suggest that oxytocin can affect Ca extrusion mechanisms e.g. by inhibition of Ca^{2+}-ATPases and Ca efflux from cells, thus prolonging the elevation of Ca^{2+} is limited.

Oxytocin binding to its receptor can also lead to activation of Rho proteins (likely via $G_{\alpha 12/13}$ proteins) which can regulate the interaction between actin and myosin independently of Ca^{2+}. Rho activation leads to activation of Rho kinase and subsequent phosphorylation and inhibition of MLC phosphatase (MLCP). Inhibition of MLCP therefore removes the inhibitory break on contraction and allows for modulation of force production without the need for a change in intracellular Ca concentration. This process is known as Ca sensitisation (Somlyo et al. 1999). In rats, pre-treatment with a specific inhibitor of Rho-kinase decreased the level of oxytocin-induced MLC phosphorylation, suggesting that the RhoA/Rho kinase cascade is involved in the contraction response mediated by oxytocin. When examined in human myometrium however, the inhibition of RhoA-Rho kinase pathway produced only moderate effects.

Additionally, OTR activation leading PKC-mediated production of DAG can also contribute to sensitisation via inhibition of MLCP directly, or via a smooth muscle specific inhibitor of MLCP known as C-kinase-activated protein phosphatase-1 inhibitor 17 kDa (CPI-17) (see **Figure 3**).

Figure 3. Proposed signalling pathways activated by oxytocin binding to the OTR in myometrium and other uterine tissues including decidua and amnion (from Arrowsmith and Wray 2014).

Binding of oxytocin to its receptor activates $G_{\alpha q/11}$, which activates phospholipase C-β, which in turn hydrolyses phosphoinositide-bis-phoshate (PIP_2) into inositol-tris-phosphate (IP_3) and diacylglycerol (DAG). IP_3 causes release of Ca from the sarcoplasmic reticulum (SR) and DAG activates protein kinases type C (PKC). Activation of $G_{\alpha q/11}$ is also suggested to cause the opening of voltage-operated Ca^{2+} channels (VOCCs) and Ca^{2+} entry. This may be as a result of direct activation or indirect activation of channel opening e.g. via receptor operated channel (ROC) opening. Inhibition of the Ca exit from the cell by inhibition of Ca^{2+}-ATPase also promotes increased $[Ca^{2+}]_i$. The reduction in lumenal SR $[Ca^{2+}]$ is considered to trigger store-operated Ca^{2+} entry (SOCE). The elevation in $[Ca^{2+}]_i$ leads to formation of the Ca^{2+}-calmodulin complex which then activates myosin light-chain kinase (MLCK), resulting in acto-myosin cross-bridge cycling and myometrial contraction. In addition, DAG activation of PKC activates the mitogen-activated protein kinase (MAPK) cascade resulting in increased phospholipase A2 (PLA2) activity and prostaglandin E2 (PGE_2) production, which also contributes to contraction (mechanism not shown). DAG-activated PKC also signals for phosphorylation of C-kinase-activated protein phosphatase-1 inhibitor 17 kDa (CPI-17), whereas oxytocin binding to OTR also activates Rho-A which in turn activates RhoA-associated protein kinase (ROCK). Both phosphorylated CPI-17 and ROCK inhibit myosin light chain phosphatase (MLCP), leading to increased MLC phosphorylation and is the proposed mechanism of Ca^{2+} sensitisation in the myometrium. Oxytocin receptor signalling in other uterine tissues (e.g. decidua and amnion) also signals for production of prostaglandins and pro-inflammatory cytokines (not shown), which may mediate local paracrine

signalling with the myometrium. Red pathways indicate signalling pathways with direct influences on $[Ca]_i$, whereas purple and turquoise lines indicate Ca^{2+}-independent pathways to contraction, including Ca^{2+} sensitisation (purple lines) and the production of prostaglandins (turquoise pathways). Dotted lines indicate where mechanisms are not yet fully determined.

OTR signalling in cultured myometrial and amnion cells (cells of the fetal membranes) also activates NFκB and MAPK signalling and stimulates the production of pro- inflammatory cytokines such as IL-6 and IL-8, and prostaglandin production via up-regulation of cyclooxygenase type 2 (COX-2) (**Figure 3**). This inflammatory signalling and upregulation of prostaglandin synthesis is known to play roles in parturition including fetal membrane remodelling, cervical ripening and myometrial activation. Additionally OT is also implicated in the achievement of co-ordinated, synchronous myometrium contractions required for labour. This is via its role in promoting upregulation of connexin proteins, including connexin 43 which is a major protein involved in the formation of gap junctions providing communication between myometrial cells. Activation of MAPK signalling pathways in myometrium, which is thought to be mediated by $G_{\alpha q\text{-}11}$ and $\beta\gamma$ release, may also lead to MAPK mediated induced myometrial cell proliferation.

In late pregnancy and preterm labour, the expression of myometrial OTRs significantly increases shifting the myometrium towards increased sensitivity to OT. After parturition, OTR expression in myometrium rapidly declines, whereas mammary gland OTR expression remains raised throughout lactation. This tissue-specific regulation of OTR expression is suggested to be the one mechanism which enables circulating OT to switch its target organ and therefore its physiological function i.e. from to inducing and augmenting uterine contractions during parturition to milk ejection during lactation.

The milk-ejection reflex describes a process whereby oxytocin is released in response to neonatal sucking. During this process, pulsatile waves of OT are released from the posterior pituitary causing the myoepithelial cells surrounding mammary gland alveoli to contract, allowing the collected milk to be released into ducts and open into nipple pores. The signaling pathway leading to contraction of the myoepithelial cells is largely attributed to receptor coupling to $G_{\alpha q\text{-}11}$ and the activation of the PLC/Ca^{2+} signalling pathways. Unlike myometrium however, the increased Ca^{2+} in myoepithelial cells induced by OT is only transient and not sustained, which is indicative of Ca release from intracellular stores and not Ca influx mechanisms. The release of OT also occurs in most women before the tactile stimulus of suckling. A second release of OT follows in response to the suckling stimulus itself, highlighting other OTR signalling roles such as on maternal behaviour.

4- Physiological functions within the brain

The widespread distribution of OTRs within the brain has firmly established OT as a central neurotransmitter. However, whilst the peripheral role of oxytocin in labour and lactation is well known and the signalling pathways activated by OTR are well established, much remains to be understood regarding the behavioral and cellular mechanisms of OT's functions in the brain.

In rodents, receptors for OTR are abundantly expressed throughout the brain whilst in humans expression is more restricted. Receptor autoradiography studies and immunostaining for OTR found it to be highly expressed in in the basal nucleus of Meynert, diagonal band of Broca and lateral septal nucleus as well as the hypothalamus, anterior cingulate cortex, olfactory nucleus, and amygdala (Boccia et al. 2013). Many of these regions are associated with the central control of stress, anxiety and social behaviour including pair bonding, parental care, aggression and social memory. Therefore, in the brain, OT is suggested play a key role in the regulation of social cognition and behavior including roles in attachment, social exploration, and social recognition as well as having roles in the fear and stress response.

Following release from dendrites within the hypothalamic neurons, OT is suggested to reach other regions of the brain by diffusion. The precise signalling pathways elicited by OTR activation in these neuronal cells however is not fully understood. In immortalised neuronal cells OTR coupling to G_q or to $G_{i/o}$ resulted in opposite effects on cell excitability. G_q activation inhibits inwardly rectifying potassium channel conductance whilst $G_{i/Go}$ coupling was shown to promote inwardly rectifying currents (Gravati et al. 2010). The balance of signaling between the different G-proteins will therefore determine cell function and hence affect behavioural outcomes.

5- Pathophysiological implications

The pathophysiological functions of OT and OTR in reproduction has been demonstrated in mouse gene ablation studies (Nishimori et al. 1996; Young et al. 1996; Lee et al. 2008). Interestingly however, neither OT nor its receptor appear necessary for labour. Oxytocin-deficient females are fertile, display normal mating behaviour, become pregnant and deliver their offspring on time without complications and show normal maternal behaviour. However their pups die within 24 hours due to the mothers' inability to eject milk and nurse them. The mammary glands of the OT-deficient mice were shown to contain milk and re-administration of exogenous OT was able to restore milk ejection, confirming that OT is required for milk ejection but not its production. Similarly, normal parturition has been noted in the absence of OT in cases of women with clinical pituitary gland dysfunction.

OTR-null mice are also viable and show no obvious defects in fertility, sexual behaviour or parturition. However, they show defects in lactation due to lack of milk ejection and also reduced maternal nurturing. OTR-and OT-knockout females postpartum were shown to retrieve fewer pups when scattered compared to their wildtypes and those that do retrieve pups gather fewer pups. OTR knock-out female mice also groom themselves and their pups less than their wildtype counterparts. Additionally, OTR-null male pups

show increased aggressive behaviours compared to their wildtype littermates, further confirming that the OTR is also important in the regulation of development of social behaviour.

Labour dystocia, postpartum haemorrhage and preterm birth

Oxytocin is one of the most frequently used drugs in obstetrics, for promoting uterine contractions for labour induction and augmentation and to prevent postpartum haemorrhage. OTR antagonists have also been developed to inhibit preterm labour contractions and treating dysmenorrhea. The goal of labour augmentation is to enhance inadequate contractions to achieve vaginal delivery. Oxytocin is given intravenously and the rate of administration is tailored to the rate of contraction. Paradoxically, prolonged OT infusion results in decreased uterine contractility due to receptor desensitisation and internalisation. Radioligand studies in cultured cells have shown consistently that OT exposure results in decreased OT - OTR binding and leads to receptor internalisation. Clinical studies have shown a loss of uterine activity, measured by intrauterine pressure catheter recordings, with OT infusion. Desensitisation of the receptor can increase the risk of caesarean section delivery due to dysfunctional labour and also poses the risk of uterine atony leading to postpartum haemorrhage as forceful uterine contractions are also required to clamp the uterine blood vessels to stem bleeding after delivery. Therefore prolonged OT administration may be counterproductive to the augmentation of uterine contractions. In fact, OT is released from the pituitary in a pulsatile manner. The pulse frequency increases during labour reaching its maximum during the second stage. This pulsatile release may in fact be a mechanism to prevent agonist mediated desensitization. In contrast, uterine hyperstimulation (or hypertony) in which contractions become too strong or too frequent following OT administration can also occur which can be detrimental to the fetus.

Preterm birth (<37 weeks gestation) accounts for the majority of neonatal morbidity and mortality. As premature uterine contractions are one of the most recognised signs and causes of spontaneous preterm labour, there has been much effort in developing anti-contraction medications (known as tocolytics) to

inhibit preterm labour contractions to prolong pregnancy. This includes the development of OTR antagonists. The OTR-antagonist, atosiban, is widely used clinically to reduce uterine activity in threatened preterm labour. Atosiban, is a peptide analogue of oxytocin which competes with oxytocin for binding at the OTR, thus preventing oxytocin-induced rises in intracellular calcium and promoting relaxation of the myometrium. Atosiban is however a biased ligand that antagonises $G_{\alpha q/11}$ signalling but acts as a $G_{\alpha i}$ agonist in a number of cell lines. Its coupling to $G_{\alpha i}$ signalling may in fact contribute to a pro-labour effect by activation of inflammatory pathways.

Additonally, owing to its similarity in structure to oxytocin and vasopressin, atosiban is also an antagonist at the vasopressin 1a receptor ($V1_aR$) and has higher efficacy at the $V1_aR$ over OTRs. The role of vasopressin receptors in human myometrium is less clear – the uterus is responsive to AVP and it expresses $V1_aR$. Interestly, a more selective OTR anatagonist barusiban was no better then placebo in inhibiting *in vivo* preterm labour contractions. There is much research interst in developing more effective OTR antagoinsits with greater selectivity and efficiacy for inhibiting preterm labour contractions.

Behavioural disorders

Research across species has shown that OT plays key roles modulating social perception, social cognition, and social behaviour, thereby promoting social approach, affiliation and promoting the maintenance of social relationships. Not surprisingly then, a dysfunction of OT or impairment of OTR signalling has been associated with a number of mental disorders including autism spectrum disorders and schizophrenia, as well as involvement in a collection of mood and anxiety disorders (Cochran et al. 2013).

Variations in the OTR gene may partly explain individual differences in OT-related social behavior. Two single nucleotide polymorphisms (SNPs) in the third intron have been suggested to be particularly promising candidates to explain differences in oxytocinergic functioning (Meyer-Lindenberg et al. 2011):

Genetic association studies have revealed reproducible and significant links of some OTR gene polymorphisms to specific social traits and behaviours**.** Rs225498 (G-A transition) has been linked to emotional deficits and females heterozygous for this polymorphism with a familial history of depression were found to have the highest levels of depression and anxiety. Whilst those with rs53576 (G-A transition) polymorphisms showed a deficit in social behaviours including empathy and mother's sensitivity towards her child's behaviour. However the effects of these SNPs are thought to be small and do not change the amino acid sequence of the receptor. Polymorphisms in the *OXT* and *OXTR* genes have also been associated with schizophrenia and autism. Downregulation of OTR mRNA and binding sites for OT have also been detected in a number of brain regions involved in social cognition in schizophrenic patients. *OXTR* methylation may be associated with autism, highcallous- unemotional traits, and differential activation of brain regions involved in social perception. Therefore both genetic and epigenetic factors, may have a large impact on defining social personalities and traits.

Exogenous OT administration has been reported to rescue social deficits in preclinical animal models characterised by autistic-like symptoms in which OTR expression was completely or partially absent. Some human studies have evaluated the therapeutic value of endogenously applied neuropeptides such as OT, typically administered via a nasal spray, in ameliorating social dysfunction in patients affected by many different psychiatric or neurodevelopmental disorders. But the evidence for consistency in their therapeutic potential is limiting. One factor being that only a small fraction of these peptides can pass through the blood brain barrier.

Summary

Activation of OTRs results in signalling via a number of pathways; the main pathway being the Gq/PLC/IP_3 pathway which results in increased intracellular Ca^{2+}. In myometrium this leads to

contraction and in mammary cells this contributes to milk ejection. Additionally OTR signaling in decidua and fetal membranes leads to increased prostaglandin synthesis and production of pro-inflammatory cytokines which can potentiate OT's actions and aid labour onset. A number of agonists and antagonists to the OTR have been developed for their therapeutic potential, including the antagonist atosiban which has tocolytic qualities and is used for inhibiting preterm labor contractions.

Oxytocin also plays a central role in social behavior and impairment of OTR signaling is indicated in a number or behavioral pathologies. Oxytocin analogues and OTR antagonists are therefore also useful research tools in furthering our understanding of OTR signaling and OT functions in systems such as the CNS. The therapeutic potential of OT analogs in the treatment of pathophysiological behaviors is still under examination but targeting the OTR brings hope for alleviating these social disorders.

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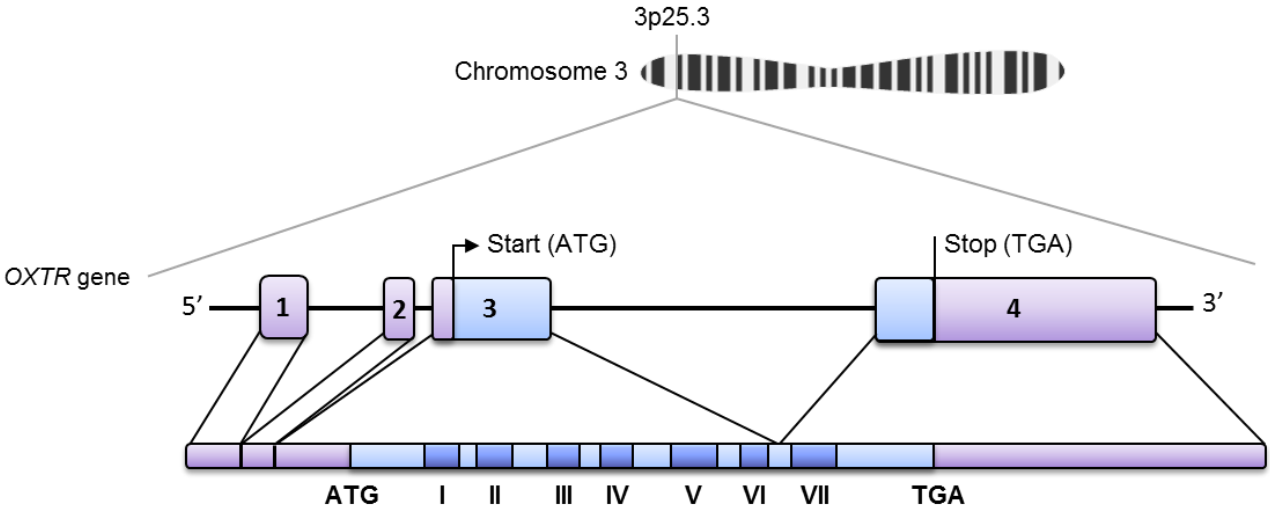

Figure 1

Organisation of the human oxytocin receptor gene. The OTR is located on chromosome 3 at position 3p25.3. It is comprised of 4 exons (boxes labelled 1-4). Only exons 3 and 4 encode the amino acid sequence for the receptor (areas highlighted in blue) flanked by the start (ATG) and stop codons (TGA). The regions encoding the 7 transmembrane-spanning domains are represented by dark blue boxes and are numbered I-VII. (adapted from Inoue et al., 1994)

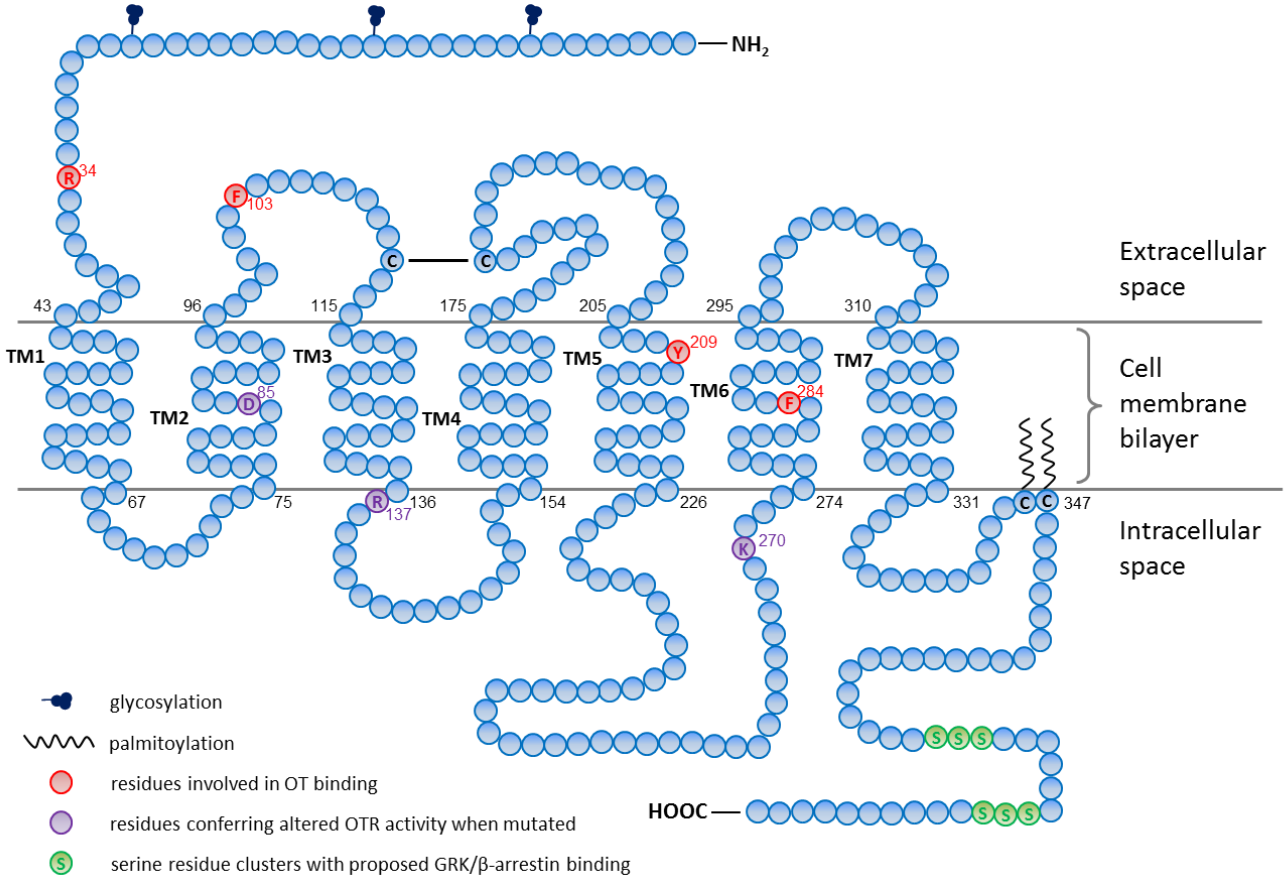

Figure 2

Schematic structure of the human oxytocin receptor. Membrane topology of the human OTR is shown where each amino acid is denoted by a blue circle and residues involved in ligand binding and signal transduction discussed in the text are highlighted. Residues in red indicate those identified as being involved in binding of oxytocin to the receptor whilst residues in purple denote those sites shown to confer altered receptor activity when mutated. Areas of proposed functional importance in the cytoplasmic C-terminal tail, including clusters of serine residues whish are thought to be involved in GRK or β-arrestin binding and receptor internalisation and desensitisation are highlighted in green. Putative N-glycosylation and palmitoylation sites are also highlighted and a putative disulphide bond between the first and second extracellular loops is also shown. (adapted from Gimpl and Fahrenholz 2001)

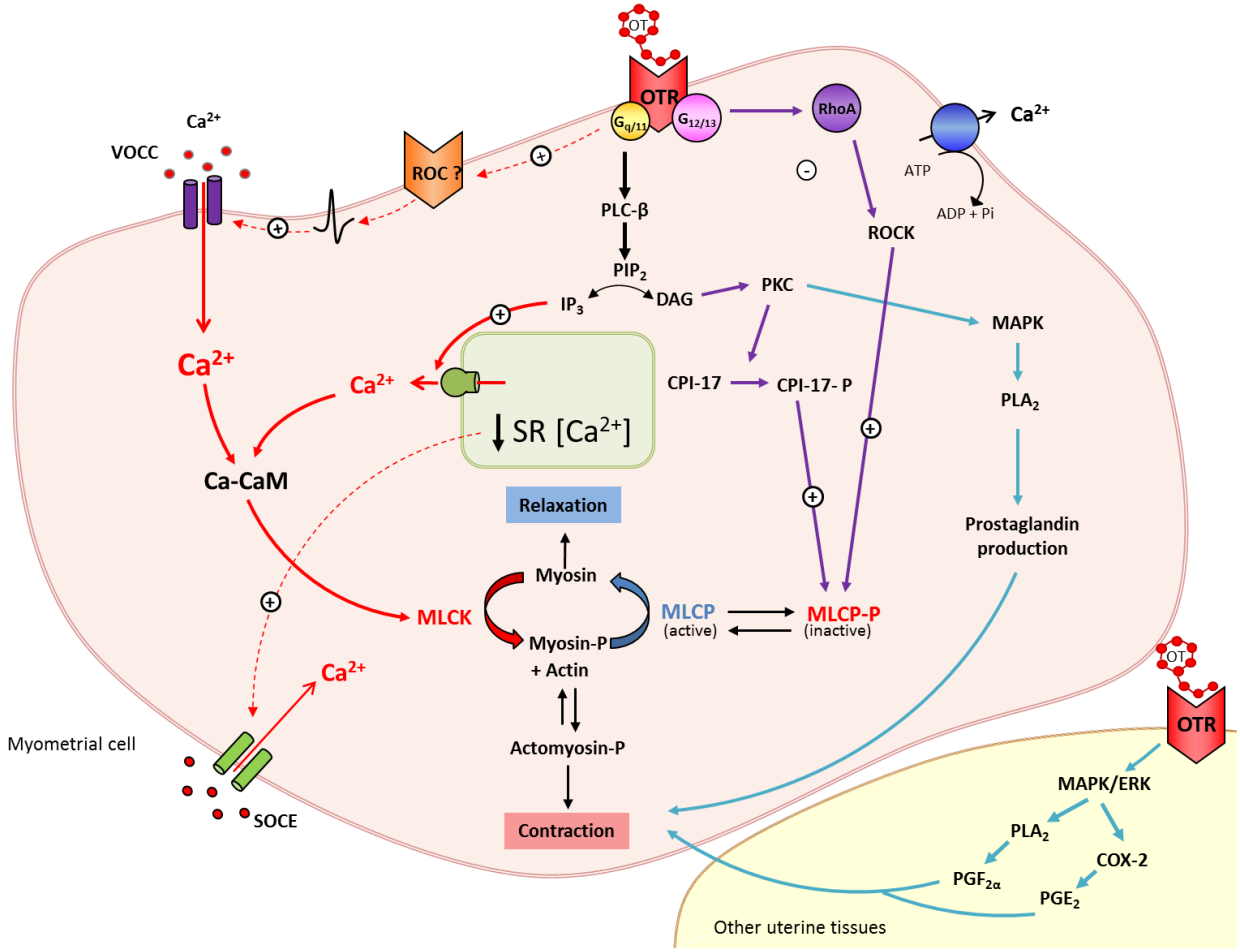

Figure 3

OTR (Oxytocin Receptor), Fig. 3 Proposed signalling pathways activated by oxytocin binding to the OTR in myometrium and other uterine tissues including decidua and amnion (from Arrowsmith and Wray 2014). Binding of oxytocin to its receptor activates Gaq/11, which activates phospholipase C-b, which in turn hydrolyses phosphoinositide-bis-phoshate (PIP2) into inositol-trisphosphate (IP3) and diacylglycerol (DAG). IP3 causes release of Ca from the sarcoplasmic reticulum (SR) and DAG activates protein kinases type C (PKC). Activation of Gaq/11 is also suggested to cause the opening of voltage operated Ca2+ channels (VOCCs) and Ca2+ entry. This may be as a result of direct activation or indirect activation of channel opening, e.g., via receptor-operated channel (ROC) opening. Inhibition of the Ca exit from the cell by inhibition of Ca2+_ATPase also promotes increased [Ca2+]i. The reduction in lumenal SR [Ca2+] is considered to trigger store-operated Ca2+ entry (SOCE). The elevation in [Ca2+]i leads to formation of the Ca2+-calmodulin complex which then activates myosin light-chain kinase (MLCK), resulting in acto-myosin cross-bridge cycling and myometrial contraction. In addition, DAG activation of PKC activates the mitogen-activated protein kinase (MAPK) cascade resulting in increased phospholipase A2 (PLA2) activity and prostaglandin E2 (PGE2) production, which also contributes to contraction (mechanism not shown). DAG-activated PKC also signals for phosphorylation of C-kinase-activated protein phosphatase-1 inhibitor 17 kDa (CPI-17), whereas oxytocin binding to OTR also activates Rho-A which in turn activates RhoAassociated protein kinase (ROCK). Both phosphorylated CPI-17 and ROCK inhibit myosin light chain phosphatase (MLCP), leading to increased MLC phosphorylation and is the proposed mechanism of Ca2+ sensitization in the myometrium. Oxytocin receptor signaling in other uterine tissues (e.g., decidua and amnion) also signals for production of prostaglandins and pro-inflammatory cytokines (not shown), which may mediate local

paracrine signaling with the myometrium. Red pathways indicate signalling pathways with direct influences on [Ca]i, whereas purple and turquoise lines indicate Ca2+-independent pathways to contraction, including Ca2+ sensitization (purple lines) and the production of prostaglandins (turquoise pathways). Dotted lines indicate where mechanisms are not yet fully determined