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Advances in the role of oxytocin receptors in human parturition

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ABSTRACT

Oxytocin (OT) is a neurohypophysial hormone which has been found to play a central role in the regulation of human parturition. The most established role of oxytocin/oxytocin receptor (OT/OTR) system in human parturition is the initiation of uterine contractions, however, recent evidence have demonstrated that it may have a more complex role including initiation of inflammation, regulation of miRNA expression, as well as mediation of other non-classical oxytocin actions via receptor crosstalk with other G protein-coupled receptors (GPCRs). In this review we highlight both established and newly emerging roles of OT/OTR system in human parturition and discuss the expanding potential for OTRs as pharmacological targets in the management of preterm labour.

1. Introduction

Oxytocin (OT) is a nonapeptide hormone, initially discovered by Sir Henry Dale in 1906 when it was noted that the extracts of human posterior pituitary gland had the ability to induce uterine contractions of a pregnant cat. It was named oxytocin from the Greek words *oxus* and *tokos* meaning sharp and childbirth, respectively (Du Vigneaud, 1956). Thus OT was soon introduced into clinical practice as a uterotonin to stimulate labour within 1940s (Theobold et al., 1948). In 1953, OT was sequenced and synthesised by Vincent du Vigneaud (Du Vigneaud et al., 1953), and the gene was cloned 30 years later (Land et al., 1983). In mammals, it has been established that OT is synthesized in the magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus (Scharrer, 1954). The produced peptide is then transported down the axons of the posterior pituitary gland where it is released to modulate its function. Moreover, OT has been found to be synthesised locally in humans to contribute to the processes of labour (Arrowsmith and Wray, 2014).

There is only one type of oxytocin receptor (OTR) identified to date, which belongs to the rhodopsin-type (class I) G protein-coupled receptor (GPCR) family. Similar to other GPCRs, activation of OTR drives conformational changes in the relative orientation of the transmembrane domains to enable G protein binding. On the basis of the amino acid sequence, the molecular mass of OTR can be calculated to be ~40–45 kDa. However, previous studies in guinea pig myometrial membranes identified OTR as a 68- to 80-kDa protein (Kojro et al., 1991), and photoaffinity labelling experiments in rat mammary gland and rabbit amnion cells found OTR to be 65 kDa (Hinko et al., 1992; Muller et al., 1989). Such discrepancy in the molecular mass of OTR in various tissues may be due to their differential glycosylation patterns, with each glycosylation core accounting for approximately 10 kDa. OTR has been found to have two or three putative N-glycosylation sites depending on the species. Despite the differential glycosylation patterns, OTR expression, ligand binding affinity, and its function are not affected as recombinant deglycosylation mutants of OTR showed unaltered functional properties (Kimura et al., 1997).

Oxytocin/Oxytocin receptor (OT/OTR) system can drive central as well as peripheral actions with a plethora of roles in both physiological and pathological processes, including reproduction/parturition, maternal behaviour, social behaviour, lactation, erectile dysfunction and ejaculation. Traditionally, OT/OTR has been

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considered to play a significant role in the myometrium during pregnancy and in labour. The most established effect of OT/OTR is the initiation of uterine contractions but it has been found to influence prostaglandin (PG) synthesis in other gestational tissues such as the decidua, amnion, and chorion.

Knowledge of G protein-coupled receptors (GPCRs) continues to evolve, and the diversity of their actions and the complexity of their signalling mechanisms are becoming increasingly evident. OT/OTR biology appears to be no exception. This review summarizes the knowledge surrounding the role of OT/OTR in parturition, with special emphasis on the recent advances, and highlights specific issues that warrant further investigation.

2. Previously established roles of OT/OTR system

There is conflicting evidence for the role OT in the initiation of parturition (Hirst et al., 1993a). The key prolabour responses induced by OT are indistinguishable to that of normal spontaneous labour and OT is one of the strongest uterotonins available to stimulate uterine contractions for labour induction or augmentation. Moreover, there is significant increase in uterine sensitivity to OT via upregulation of OTR in both rats and humans during the onset of labour (Fuchs et al., 1982; Soloff et al., 1979; Fang et al., 1996). Additionally studies in rats (Chan et al., 1991; Antonijevic et al., 1995; Fejgin et al., 1994), non-human primates (Hirst et al., 1991; Honnebier et al., 1989; Wilson et al., 1990) and in humans (Akerlund et al., 1987; Goodwin et al., 1994) reported that OT antagonists can inhibit late gestational uterine contractions and the process of parturition. The role of OT varies between different animal species. Although in rodents, OT is believed to play a significant role in the initiation and maintenance of parturition (Soloff et al., 1979; Russell and Leng, 1998), in humans, circulating OT does not seem to be essential for the initiation and maintenance of labour (Nishimori et al., 1996). Normal parturition has been observed in the absence of OT in mice (Nishimori et al., 1996) as well as in cases of clinical pituitary gland dysfunction (Phelan et al., 1978) and the concentrations of maternal or fetal OT does not increase with the onset of or during labour (Leake et al., 1981).

A hypothesis proposed to explain the absence of OT increase in humans during labour was that it is not the peripheral OT but a local OT synthesis that initiates labour. In 1993, mRNA of OT was isolated in human uterine tissues in late gestation (Chibbar et al., 1993). A semi-quantification analysis revealed that OT mRNA is principally localised/or identified in decidua but also found in the chorion and amnion. Interestingly, significantly greater concentrations of OT mRNA were detected in the chorio-decidua after spontaneous onset of labour (at the expulsive stage of parturition) compared to before the onset of labour at term (Chibbar et al., 1993; Fuchs et al., 1991; Hirst et al., 1993b). Post labour chorio-decidua samples showed similar levels of OT mRNA to that of spontaneous labour, indicating the maximal level of gene expression. These findings demonstrate that localised synthesis of OT increases around the time of labour onset, and play a role in the physiology of human labour.

Moreover, the uterine sensitivity to OT markedly increases at the onset of labour. This is associated with an upregulation of OTR mRNA levels and a strong increase in the density of myometrial OTRs reaching a peak during early labour (Havelock et al., 2005). This was first described in rats (Soloff et al., 1979), and subsequently demonstrated in rabbits and humans (Fuchs et al., 1982; Jeng et al., 1995). In both species, OTRs are present in the endometrium as well as the myometrium, chorio-decidua and amnion (Fuchs et al., 1982; Chan, 1980). This increase in OTR is one of the most consistent findings in the study of parturition in several species, which introduces the possibility that changes in the expression or the function of the OTRs are important for the onset of labour. In addition, a more recent gene association study showed that maternal genetic variation such as single nucleotide polymorphisms (SNPs) in OTR coding region may be associated with gestational age-dependent susceptibility to preterm birth (Kim et al., 2013). This finding is in line with the hypothesis that OTR has a significant role in the process of labour onset.

The most established, and the longest known role of OT/OTR system in parturition is stimulation of uterine contractions. The uterus must contract and relax rhythmically to enable the propelling of the fetus into the vagina and delivery. Human myometrium comprises of smooth muscle cells arranged into bundles delineated by connective tissue and interspersed with microvasculature. Activation of OTR in the uterus triggers coupling of $G\alpha_{q/11}$ to phospholipase Cβ (PLCβ), which mediates the hydrolysis of phosphatidylinositol-4, 5-bisphosphate (PIP_2) to inositol-1, 4, 5-trisphosphate (IP_3) and diacylglycerol (DAG). Subsequently, these control the mobilisation of Ca^{2+} from the sarcoplasmic reticulum into the cytoplasm and the activation of protein kinase type C (PKC), respectively (Mitchell and Schmid, 2001) (Fig. 1). The increase in intracellular Ca^{2+} can lead to smooth muscle contractions via activation of Ca^{2+}-dependent calmodulin which triggers myosin light chain kinase (MLCK) activity. MLCK phosphorylates the regulatory myosin light chains and drives acto-myosin cross-bridge cycling and myometrial contractions (Kim et al., 2015a) (Fig. 1).

In addition to regulating smooth muscle contractility by electromechanical coupling, membrane potential independent, pharmacomechanical coupling can also be accounted for the physiological mechanisms that regulate contractions (Somlyo and Somlyo, 2000). Activation of OTR in human myometrium can convert inactive RhoA-GDP to its active form, RhoA-GTP through guanine nucleotide exchange factors (GEFs) (Shmygol et al., 2006). RhoA-GTP acts via Rho kinase (ROCK) to phosphorylate the regulatory subunit of MLC, leading to contractions. There have been records of RhoA and ROCK upregulation in human myometrium during pregnancy (Moore et al., 2000) and ROCK has been shown to mediate OT-induced myometrial contractions (Kupittayanant et al., 2001) (Fig. 1). Another alternative (and parallel) pathway to induce myometrial contraction may be through activation of the enzyme CPI-17 by protein kinase C (Ozaki et al., 2003) (Fig. 1).

3. Novel roles of OT/OTR system

3.1. Inflammation

Clinically, labour is defined by uterine contractions, however, there is growing evidence that this is preceded by carefully regulated biochemical changes in the gestational tissues including the fetal membranes (composed of amnion and chorion) (Skinner and Challis, 1985). Such changes involve NF-κB activation and inflammatory stimulation which triggers a cascade of pro-inflammatory signalling in both the myometrium and the amnion (Lim et al., 2012; Khanjani et al., 2011), and lead to elevation of inflammatory cytokines/chemokines as well as PGs, particularly $PGF_{2\alpha}$ and PGE_2 (Olson, 2003). Previous studies have shown that OT/OTR activation stimulate cytoplasmic phospholipase A_2 ($cPLA_2$) activity and induce COX-2 expression, the two major enzymes in PG synthetic pathway (Terzidou et al., 2011). PG synthesis is one of the key steps involved in the onset of human labour (Kelly, 1994). Increased PG synthesis occurs first in the region overlying the cervix where it is thought to mediate cervical ripening and dilation. Mechanical stretch of human amnion epithelial cells has been shown to lead to the activation of AP-1 and NF-κB systems and in turn, increase COX-2 and PG expression (Mohan et al., 2007). PGs have been shown to play a role in placental separation (Noort et al., 1989), fetal