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REVIEW

Diverse Roles of Oxytocin

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The oxytocin/oxytocin receptor (OT/OTR) system has been reported to influence a variety of physiological, behavioural, and emotional processes in humans. There are a wide range of studies focusing on their role in parturition, lactation and behaviour. More recent studies have investigated the novel effects of OT/OTR system including inflammation and miRNAs, as well as the effect of their genetic polymorphisms on the onset of labour and parturition. OT also appears to have a great influence in cardiomyocyte differentiation and heart homeostasis, and an age-specific regulation of muscle maintenance and regeneration. This review will summarize the previously established role of OT/OTR system as well as the studies on their novel roles, and highlight the potential therapeutic approaches.

Keywords: Oxytocin; Inflammation; miRNA; SNPs; Muscle regeneration

Abbreviations: OT, Oxytocin; PVN, paraventricular; PG, prostaglandins; MLCK, myosin light chain kinase; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; GEFs, guanine nucleotide exchange factors; ROK, Rho kinase ; CHO, Chinese hamster ovary; MMP, metrix metalloproteinase; PDGFB, platelet-derived growth factor beta polypeptide; UTR, untranslated region ; CX-43, connexin-43; PTB, preterm birth; VDBP, vitamin D-binding protein; SNPs, single nucleotide polymorphisms.

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Oxytocin

Oxytocin (OT) is classically considered to have a fundamental role in the mechanism of human labour. In 1906, it was first noted that the posterior pituitary extract had the ability to drive uterine contractions [1]. Subsequently, the active factor was identified to be oxytocin, a potent activator of contractions in the pregnant uterus (from the Greek *oxus*, meaning sharp and *tokos*, referring to childbirth). OT was first introduced into clinical practice as a uterotonin to stimulate labour within 1940s [2]. By 1953, the biochemical structure of OT was determined [3], and the gene was cloned 30 years later [4].

OT is a nonapeptide hormone (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2) with a disulfide bridge between the two cysteines. The presence of a disulfide bridge results in a six-amino acid cyclic part and a COOH-terminal α-amidated three-residue tail. This structure of OT is very similar to other nonapeptide neurohypophysial hormones. These can be classified into two families; vasopressin and OT families, depending on their amino acid at position 8, where vasopressin family contains a basic amino acid (Lys, Arg) and OT family contains a neutral amino acid. It has been reported that OT and vasopressin genes are on the same chromosome locus in opposite directions and therefore arose as a result of common ancestral gene duplication. However, the variance in these key amino acids can promote differences in the polarity of the peptides, enabling them to bind to their respective receptors [5].

It has been established that OT is synthesized principally in the magnocellular neurons of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei [6]. The produced peptide is then transported down the neuronal

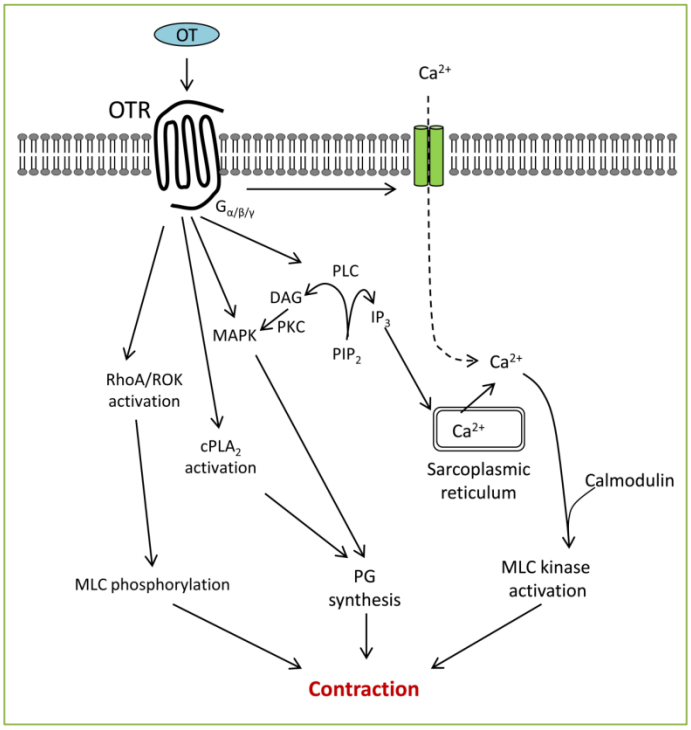

Figure 1. OT/OTR signaling pathways involved in myometrial contraction. OT binds to OTR and OTR coupled with G*α/β/γ* leads to activation of PLC, which hydrolyses PIP_2 to IP_3 and DAG. Activated PLC and IP_3 cause release of Ca^{2+} from the sarcoplasmic reticulum. Both OTR and DAG activate PKC to initiate MAPK cascade which in turn causes increased $cPLA_2$ activity and PG synthesis. OTR also triggers RhoA/ROK cascade and results in increase in MLC kinase activation. Intra-cytoplasmic Ca^{2+} increases further via membrane Ca^{2+} channels and these combined with calmodulin activates MLC kinase. The increase in PG synthesis and MLC activation induce myometrial contractions. OT: oxytocin, OTR: oxytocin receptor, PLC: phospholipase, PIP_2: phosphatidylinositol 4,5-bisphosphate, DAG: diacylglycerol, PKC: protein kinases type C, MLC: myosin light-chain, MAPK: mitogen-activated protein kinase, $cPLA_2$: cytosolic phospholipase A2, ROK: RhoA associated protein kinase.

projections (axons) of the posterior pituitary gland where it is released to modulate its function. Rossoni *et al.* have demonstrated that some of the OT from the hypothalamus is transported to the dendrites in order to regulate the firing patterns of the OT neuronal network [7]. Moreover, OT has been found to be synthesized locally in humans to contribute to the processes of labour [8]. Apart from mediating parturition, OT is also important for milk ejection during lactation [9], maternal behaviour, sexual receptivity and partnership bonding [10], thereby facilitating species propagation.

Oxytocin and Parturition

Oxytocin drives uterine contractions

In general, the OT/OTR system within the pregnant uterus plays a significant role in two distinct physiological functions; stimulation of uterine contractions and production of prostaglandins (PG). The effect of oxytocin on human uterine smooth muscle can be distinguished into at least three components including the increase in frequency of contractions, transient increase in the base tone, and long-lasting increase in the amplitude and duration of phasic contractions.

Stimulation of contractions by OT/OTR system in smooth muscle cells, such as uterine myometrial cells or mammary gland myoepithelial cells, is driven by an increase in intracellular Ca^{2+} that leads to a calmodulin-mediated activation of myosin light chain kinase (MLCK) [11]. Binding of OT to the OTR activates the heterotrimeric protein complex of a G-protein consisting of an α, β and γ subunits [12]. In the myometrium, binding of OT to its receptor leads to release of the $G_{\alpha q/11}$ and $G_{\beta\gamma}$ (from $G_{\alpha i}$) subunits, leading to activation of phospholipase C (PLC) isoforms β_1, β_3 and β_2, β_3, respectively [13]. This enzyme catalyzes hydrolysis of phosphatidylinositol-4, 5-bisphosphate (PIP_2) to inositol-1, 4, 5-trisphosphate (IP_3) and diacylglycerol (DAG). The increase in intracytoplasmic Ca^{2+} is further amplified by IP_3 which promotes release of Ca^{2+} from the sarcoplasmic reticulum, and a Ca^{2+} influx from the extracellular space through membrane Ca^{2+} channels [14]. Concurrently, the DAG generated by the actions of PPLCβ on PIP_2 stimulates protein kinase C (PKC), maximising the force generation during the phasic contraction (Figure 1). Further Ca^{2+}-promoted processes include gene transcription and protein synthesis.

In addition to regulating smooth muscle contractility by electromechanical coupling, pharmacomechanical coupling can also be accounted for the physiological mechanisms that regulate OT-induced contractions [15]. Activation of OTR in human myometrium can convert inactive RhoA-GDP to its active form, RhoA-GTP through guanine nucleotide exchange factors (GEFs). RhoA-GTP acts via Rho kinase (ROK) to phosphorylate the regulatory subunit of MLC, leading to contractions [16]. There has been records of RhoA and ROK upregulation in human myometrium during pregnancy [17] and ROK has been shown to mediate OT-induced myometrial contractions.

Oxytocin as an inflammatory mediator

Although labor onset is defined clinically by myometrial contractions, these are preceded by fetal membrane remodeling. The fetal membranes (composed of amnion and chorion) undergo carefully regulated biochemical changes that modulate functional pathways precipitating labor and birth. Labor is antedated by NF-κB activation and inflammatory stimulation in both the myometrium and the

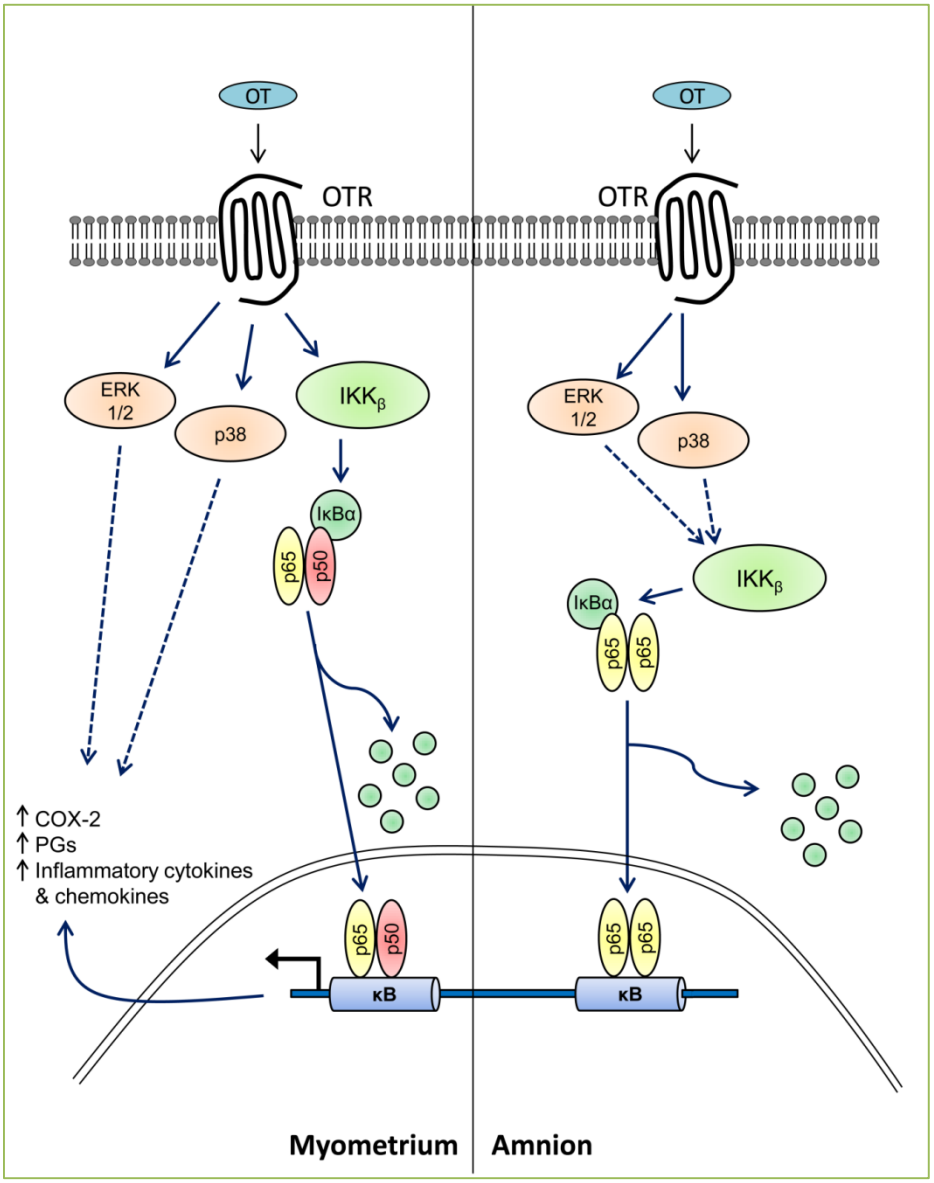

Figure 2. Models for OT/OTR signaling in the inflammatory activation in myometrium and amnion. In myometrium, OT binding to OTR drives the activation of MAPKs; ERK1/2 and p38 kinase, and canonical NF-κB signaling cascade involving p65/p50 heterodimers. MAPKs and the NF-κB dimers induce expression of pro-labour genes such as COX-2, prostaglandins, and proinflammatory cytokines and chemokines independently. In amnion, OT binding to OTR drives the sequential activation of MAPKs; ERK1/2 and p38 kinase, and the canonical NF-κB signaling cascade involving p65 homodimers. The NF-κB dimers translocate to the nucleus and binds to κB sites to induce expression of pro-labour genes.

amnion [18, 19], which triggers an inflammatory cascade involving elevation of inflammatory cytokines as well as PG, particularly PGE_2 [20]. It has been noted that OT stimulates an increase in cytoplasmic phospholipase A2 activity and induce COX-2 expression in Chinese hamster ovary (CHO) cells [21] as well as in cultured human myometrial cells and amnion cells [22]. These are two major enzymes required for PG synthesis. PG synthesis is one of the key steps involved in the onset of human labour [23]. There are various mechanisms of action PGs utilise to trigger labour. PGs can lead to cervical ripening and dilation, placental separation [24] and fetal membrane rupture by inducing metrix metalloproteinase (MMP) activity [25]. Another well described action of PGs is their role in promoting myometrial contractility. $PGF_{2\alpha}$ induced contractions are partly mediated by electromechanical coupling as well as pharmacomechanical coupling, resembling that of oxytocin induced contractions [26]. Release of $PGF_{2\alpha}$ by decidual OT/OTR system have been shown to enhance uterine contractions as well as promoting cervical ripening and

Table 1. Validated gene targets of oxytocin-regulated miRNAs.

miRNA	Change in expression with oxytocin	Validated gene targets	References
hsa-miR-146a-5p	Decrease	NF-κB activity IL-8, IL-6, MMP9 α-smooth muscle actin	[36-38]
hsa-miR-146b-3p	Increase	NF-κB regulation platelet-derived growth factor beta polypeptide (PDGFB)	[39]
hsa-miR-196a-3p	Decrease	No matches	
hsa-miR-876-5p	Decrease	No validated targets	

Gene targets of oxytocin-regulated miRNAs were identified using the miRTarBase platform.

lueolysis. Inhibition of PG synthesis decreases the upregulation of OT binding sites as well as the effect of OT on promoting myometrial contractions [27].

Collectively the increases in inflammatory stimulation play a role in driving myometrium to switch from a quiescent state to contractile state [19, 20]. However, the stimuli triggering inflammatory activation in normal human parturition are not fully understood. Recent study has shown that the role for OT in the onset of human labor exceeds stimulation of myometrial contractions and involves concurrent activation of inflammatory pathways. OT modulates key inflammatory pathways that promote the laboring phenotype revealing a novel role for OT in the onset of labor [28]. OT acts as an inflammatory mediator in human gestational tissues by inducing PGE_2 and inflammatory cytokine/chemokine release through a novel mechanism. In myometrium, OT activates the established canonical pathway involving p65/p50 heterodimers with no crosstalk between NF-κB and MAPKs (Figure 2). In amnion however, OT signalling is markedly different. NF-κB activation involves only p65 nuclear translocation and this is dependent on crosstalk with MAPKs. The subsequent translocation of activated NF-κB into the nucleus drives the expression of numerous pro-labor genes including COX-2, inflammatory cytokines and chemokines (Figure 2). Therefore, it is important to consider the dual role of OT in both stimulation of inflammation and onset of uterine contractions in the management of preterm and term labor.

Modulation of miRNAs and oxytocin

Since the discovery of their existence almost two decades ago, miRNAs (non-coding, single-stranded 19-25 nucleotide molecules) have been identified as a powerful post-transcriptional regulator of about a third of the human genome [29, 30]. Typically, these small RNA regulate gene expression by degradation of mRNA transcript or translational repression through binding to the 3` untranslated region (UTR) of the target gene. There has been increasing evidence highlighting the role for miRNAs in vascular smooth muscle as well as in female reproductive tissues, where they have been shown to be associated with differentiation, proliferation and embryo implantation [31-33]. More recently, miRNAs have been implicated in hormone responsiveness and the regulation of key gene expression pathways involved in parturition [34].

A recent study in human myometrium have described a novel pathway in which OT contributes to the onset of labour by regulating the expression of miRNAs, hsa-miR-146a-5p, hsa-miR-196a-3p, hsa-miR-876-5p, and hsa-miR-146b-3p (Table 1) [35]. Notably, hsa-miR-146a-5p was shown to be significantly reduced in myometrial samples of oxytocin-treated women and cultured myocytes. In numerous cancer cell studies, hsa-miR-146a-5p has been shown to be a suppressor of NF-κB activity ultimately reducing the expression of NF-κB-regulated genes including the key labor-associated genes IL-8, IL-6 and MMP9 [36, 37]. In addition, hsa-miR-146a-5p was found to down-regulate the expression of α-smooth muscle actin in human dermal fibroblasts [38], which is an essential component for the contractile machinery of uterine smooth muscle. The miRNA hsa-miR-146b-3p was increased in myometrial samples of OT-induced labors and is thought to be involved in the regulation of NF-κB activation [39]. It has also been implicated in the regulation of inflammatory cytokine, platelet-derived growth factor beta polypeptide (PDGFB), which was shown to be released by uterine myocytes in response to stretch [40]. OT-induced labor led to a significant decrease in hsa-miR-876-5p; however further research is required to understand the role of this miRNA in myometrial function as there is a lack of validated gene targets.

MiRNAs have not only been reported to be regulated by OT signaling to promote laboring phenotype, but also to increase the myometrial sensitivity to OT. Another study by Renthal *et al.* reported an increase in miR-200 family of

miRNAs in laboring murine and human myometrium at term. The family of miR-200 was found to be associated with labour by modulating the expression of the two critical contraction-associated proteins including connexin-43 (CX-43) and OTR [41], thus preparing the myometrium for contractions and enhancing the myometrial sensitivity to OT.

SNPs in oxytocin pathway and the risk for PTB

Predicting preterm birth (PTB) takes various factors into consideration including risk factors, fetal fibronectin, cervical length, bacterial vaginosis, and presence of other biomarkers such as cytokines, chemokines, albumin and vitamin D-binding protein (VDBP) [42, 43]. The women's history of previous PTB is one of the major risk factors and predictors for PTB [44]. It has been reported that 20% of women with previous PTB have successive PTB with the same partner, although changing paternity appears to reduce the risk by a third [45, 46]. Recent genome-wise linkage analyses in recurrent PTB and association studies have identified a cassette of candidate genes associated with PTB, and also provided evidence for maternal and fetal genetic variations predisposing to PTB [47-49].

To date, OTR gene polymorphisms have been investigated mainly in relation to psychiatric disorders and diseases [50]. There are dozens of single nucleotide polymorphisms (SNPs) identified within the OTR gene however, the association between these SNPs and the risk of PTB remains largely unknown. Kim *et al.* have identified three rare variants of OTR (rs202138705, rs200498154 and rs201783860) by resequencing and have reported that these coding variants appear to contribute to the increased risk of prematurity [51]. Characterization of the effects of these genetic variants *in vitro* showed a significant decrease in OT-specific binding compared to control cells, which highlights the importance of these residues in the function of the receptor. Previous study have confirmed that these variants are located in the second extracellular loops of OTR that determines agonist binding and selectivity [52]. Despite the potential for diagnostic or therapeutic relevance of OTR mutations in the risk of PTB, larger studies have not been carried out to confirm these findings.

Currently, there has not been a report linking any individual OTR polymorphism to PTB, which led to the investigation of their combined effects. A haplotype analysis in Caucasian women by Kuessel *et al.* revealed a combination of OTR gene polymorphisms (rs2254298 A allele, rs2228485 C allele and rs237911 G allele) that is significantly associated with increased risk of PTB [53]. However, further studies investigating the role of OTR gene polymorphism and PTB and examination of the impact of the mutations on other downstream biological pathways are required to fully understand their significance as diagnostic or therapeutic targets.

Oxytocin and Lactation

Oxytocin plays a key role in mammals as a mediator of milk-ejecting activity of the lactating mammary gland. A complementary role in regulating milk production through control of prolactin secretion has long been considered. Previous gene ablation studies in mice with inactivated OT gene demonstrated that OT is required for milk ejection from the glands but not the production of milk [9, 54]. Treatment with OT antagonist, atosiban, inhibited milk ejection and this inhibition was reversed with the administration of OT [55, 56].

OT leads to milk ejection by stimulating contraction of myoepithelial cells that form a basket-like network around the alveoli and milk ducts in the mammary gland [57]. This is often in concert with suckling. The suckling of the infant promotes the release of OT from the posterior pituitary and it interacts with OTR of the myoepithelial cells to induce intracellular calcium release, leading to contraction . The myoepithelial contracion reduces the alveolar lumen resulting in the expulsion of milk from alveoli into the lactiferous ducts, and smooth muscle alpha-actin (ACTA2) have been shown to be involved in enhancing contractile force required [58]. The contrations occur 4-10 times per 10 minutes with each contraction lasting approximately 1 minute [59], which coincides with the pulses of OT in the blood stream [60].

High-affinity binding sites for OT were demonstrated in particulate fractions from mammary glands and confirmed that similar to myometrial cells, alveolar myoepithelial cells contain OTR. Studies in rodents suggest that there is a gradual gestational-dependent increase in the mammary gland OTR expression that reaches a maximum in the first week postpartum [61, 62] whilst uterine OTR undergo a rapid decrease [63, 64]. It is possible that circulating OT is able to switch its target organs during parturition and lactation via a tissue-specific regulation of OTR expression.

Oxytocin and Maternal Behavior

The first potential effect of OT on maternal behaviour was demonstrated in 1979, where it was shown to induce maternal behaviour in non-pregnant rats [65]. Unlike human and non-human primates, majority of mammalian species rarely exhibit spontaneous maternal behaviour. In sheep, ewes are only maternally responsive towards a lamb after the onset of parturition [66], and OT plays a significant role in inducing maternal responses post-partum. The brain OT

levels in sheep were elevated with vagino-cervical stimulation occurring at the time of birth, which can have immediate effect on inducing full maternal responses in non-pregnant, estrogen-primed animals [67]. Similar increase in OT was observed in human cerebrospinal fluid at the time of birth [68]. However, there is lack of evidence linking OT to mouse, rat or human maternal behaviour. The OT gene knockout mouse strain, C57BI6, is spontaneously maternal when presented with pups [69], and mere presence of pups evokes maternal responses in rats. The role of brain OT release in the control of maternal behaviour may be facilitatory.

There have been reports on the effects of OT on social and bonding behaviours. In sheep, OT has been shown to facilitate the formation of olfactory recognition associated with the maternal bond as OTR expression and OT immunoreactivity increases significantly in the olfactory bulb, which is a key site of formation of olfactory memory [70]. This was further confirmed as OT infusions given to hormone-primed animals led to selective bonding with their lambs in 2 to 4 hours [71]. OT infusions also facilitate social recognition in rats [72], and OT knockout male mice showed impaired social recognition skills [73]. A study in OTR knockout mice demonstrated displays of several aberrations in their social behaviours, including mother-offspring interaction [74]. It has been suggested that the diversity in the genetic regulation of OTR rather than the peptide itself may bring about natural variation in social behaviour [75]. Similar to bonding behaviours of sheep, human mothers also have the ability to selectively recognise the smell of their own infants within 30 minutes of birth, and in a recent study, OT has been reported to be associated with human parent-infant bonding [76]. Moreover, mothers with low plasma concentration of OT during pregnancy has been shown to have increased risk for postpartum depression [77].

OT affects behaviour of both sexes especially in early life. Various studies on the effect of OT on social cognition and behaviour have been conducted with men, and these studies have shown that OT helps to promote trusting and protective social behaviour [76, 78], enhance facial emotion recognition [79], and memory for positive social information [80]. Moreover, disturbed OT signlaing due to functional polymorphism and epigenetic silencing causes increase in impulsive, aggressive temperament as well as violent, unemotional traits in males [81, 82].

Diminished OT is related to multiple pathlogical states including autism, osteoporosis and depression. Therefore, administration of OT improves social cognition in socially impaired individuals with autism and depression, alter dysfunctional cognitions in social phobia [75], and improve psychological well-being in the elderly. This introduces the therapeutic potential of manipulating OT/OTR system to enhance stress-alleviation and social support, however, this should be approached cauciously as there is increasing evidence that OT treatments can have asocial or negative conseuqneces such as increase in perception of threat [83] and enhancement of fear conditioning [84].

Oxytocin and Muscle Regeneration

In the 1980's, a novel function of the neurophyseal hormone, OT, has emerged. It was found to have a great influence on cardiomyocyte differentiation, cardiac healing and heart homeostasis [85, 86]. OT appears to play an extensive role in the cardiovascular system as there is an abundant functional OT system present in the early developing heart, and OT drives differentiation of embryonic stem cells into cardiomyocytes that respond to cardioactive drugs [87]. Plante *et al.* have reported that OT treatment can protect obese diabetic mice from cardiomyopathy via normalization of cardioprotective genes such as *Gata4*, *Anp* and *Bnp*, maintenance of cardiac structure, and in older mice, OT treatment prevented the development of cardiac hypertrophy and dysfunction [88]. Moreover, OT was found to aid the myocardium recovery after ischemic injury in rats [89].

More recently, several studies have emerged demonstrating the physiological role of OT on skeletal muscles. Skeletal muscles have been shown to express OTR [90]. De Jager *et al.*'s work in cattle skeletal muscles indicated that OT may contribute to muscle growth as treatment with anabolic steroids led to increase in the expression of OT [91]. The role of OT in the increase in muscle mass was also examined in relation to aging. Muscle aging due to deficiency in muscle regeneration after injury or muscle atrophy has been identified to be mainly due to inhibition of the muscle stem cells. Circulating OT levels decrease after ovariectomy, which mimics hormonal aging, led to a hypothesis that OT may be a key age-specific determinant of skeletal muscle maintenance and repair. Elabd *et al.* demonstrated a dramatic decrease in OT and OTR in muscle stem cells with age, increase in muscle stem cell proliferation by OT supplement via MAPK/ERK pathway, and impaired muscle regeneration and premature development of sarcopenia in OT knockout mice [92]. Collectively, these findings indicate that OT plays a significant role in the age-specific regulation of myogenesis. OT could provide an easier and safer treatment for prevention of sarcopenia as modulating other signaling pathways such as TGF-β/pSmad and Wnt could result in severe side effects such as oncogenic transformations and immune deregulation [93, 94].

Conclusions

A large body of evidence emerged emphasizing the role of OT/OTR system in a wide range of physiological and behavioral processes. The effects of OT on myometrial tissues have been widely investigated, but less is known about the function of OT/OTR in other gestational tissues. The role of OT/OTR system appears to exceed the stimulation of uterine contractions and involves modulation of miRNAs and activation of inflammatory responses to mediate fetal membrane remodeling and cervical ripening. The effect of OTR gene polymorphisms contributing to the increased risk of PTB introduces a possibility of a candidate in the prediction as well as management of PTB. The findings reported on the novel role of OT in muscle maintenance and regeneration also opens up new therapeutic approaches for diseases affecting striated muscles.

Conflicting interests

The authors have declared that no competing interests exist.

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