Chapter

# Molecular Aspects and Structure Activity Relationship of Oxytocin Agonists and Antagonist's Role in Health

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## Abstract

Oxytocin (OT) and Oxytocin receptor signaling mechanism had emerged as a pathway for treatment of metabolic disorders like obesity, and diabetes. Both agonists and antagonists activity of the oxytocin receptor has beneficial aspects. This chapter will outline the receptor agonists and antagonist's activity as a function of the features: hydrophobic regions, activity shapes, and positive and negative electrostatics. Also, their phenotype characteristics in various diseases like diabetes, obesity, cardiovascular and immune related diseases will be outlined. Finally, therapeutic development strategies for using various nanomaterials, and other biomaterials, as well as those in present use will be discussed.

**Keywords:** oxytocin, agonists and antagonists, drug delivery, nanomaterials, biomaterials, diabetes, obesity, regenerative medicine

## 1. Introduction

Oxytocin (OT) was the first hormone to have its composition determined and was synthesized in a biologically active manner [1]. OT is a major neuropeptide, and its roles involve control of neuroendocrine reflexes [2], the development of particular social and bonding habits [3], and the reproductive and maternal functions [4]. Cyclic nonapeptide OT and its structurally related peptides facilitate reproduction in many vertebrates.

In earlier years, OT was believed to be restricted to stimulate uterine contractions during childbirth [5] and milking (lactation) but the fact that OT is found in both men and women in the brain and plasma indicates there are other significant roles.

Most of the OT like peptides are nonapeptides and have a disulfide bridge between cysteine residues 1 and 6. The residue Lysine at position 3 and arginine or lysine at position 8 are responsible for biological activity of oxytocin peptide, that is, to activate oxytocin receptor [1]. Generally, the nonapeptides are classified as vasopressins and OT families, and are based on the presence of basic amino acid at position 8 and neutral amino acids. **Figure 1** shows the phylogenetic tree corresponding to oxytocin like peptides, retrieved from Uniprot webpage [6].

The oxytocin gene is located on chromosome 3p25 and comprises 3 introns and 4 exons and belongs to the G protein-coupled receptor (GPCR) superfamily. Oxytocin binds to receptors that activate intracellular signaling pathways, triggering the activation of proteins. **Figure 2** was developed by the authors to show in a schematic way, the oxytocin-oxytocin receptor (OT-OTR) signaling pathway in the body. Upon binding OT to OTR, Gq/phospholipase C (PLC)/inositol

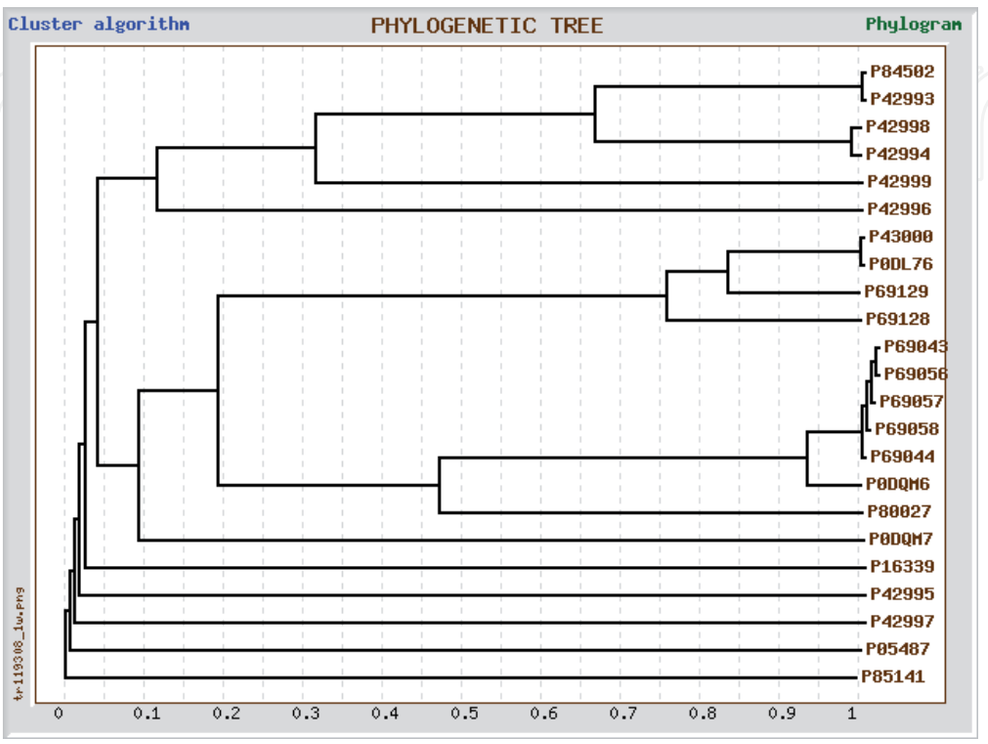


**Figure 1.**
*Phylogenetic tree of oxytocin like peptides. Oxytocin like peptides are denoted using their Uniprot ID (Uniprot webpage [6]).*

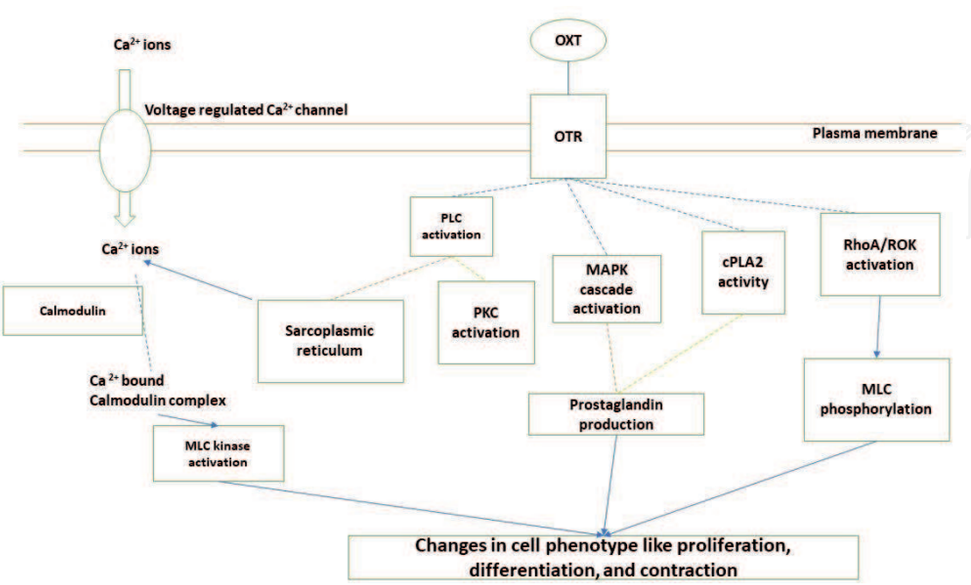


**Figure 2.**
*Molecular features of oxytocin receptor modulators calculated using forge tool. Oxytocin receptor linked signaling pathways resulting in cell phenotype. OT: Oxytocin, OTR: Oxytocin receptor, PLC: Phospholipase, PIP2: Phosphatidylinositol 4,5-bisphosphate, InsP3: Inositol 1,4,5- triphosphate, DAG: Diacylglycerol, PKC: Protein kinases type C, MLC: Myosin light-chain, MAPK: Mitogen-activated protein kinase, cPLA2: Cytosolic phospholipase A2, ROK: RhoA associated protein kinase.*

1,4,5-triphosphate (InsP3) pathway dominates the phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis and this results in InsP3 and diacylglycerol (DAG) (DAG). InsP3 releases $Ca^{2+}$ ions from the intracellular sarcoplasmic reticulum stores, but the DAG further activates protein kinases which phosphorylate other proteins. Mammary epithelial cells and milk ejection is caused by $Ca^{2+}$ ions. Activation of MAPK cascade is triggered by OTR and PKC resulting in increased expression of cPLA2 activity, which eventually results in development of prostaglandins. The profusion of prostaglandins development often causes muscle contractions. OTR activation elicits phosphorylation of calcineurin. All these signaling pathways result in many cellular effects such as cell formation, cell differentiation, and motility.

Transmembrane domains 3,4, and 6 of oxytocin receptor forms the docking site to oxytocin peptide cyclic part, and other c terminal domain of oxytocin interacts closely with domain 2 and domain 3 in the extracellular space of the oxytocin receptor. The key OTR residues R34, F103, D85, F284, and Y209, play a role in selectivity of oxytocin peptide. Based on the activity of oxytocin receptor, the oxytocin analogues were classified as agonists and antagonists.

## 2. OTR agonists and antagonists

Receptor binding and functional studies were generally used to identify oxytocin receptor agonists and antagonist activity. There are more than thousand compounds in literature as OTR agonists and antagonists, and this section was focused to demonstrate the applicability of molecular shape features as tool to develop agonists and antagonists. For this reason, peptide agonist and antagonist molecular shapes features are compared with oxytocin.

Substitution of hydoxyl group at position 1 and threonine at position 4 of original oxytocin peptide (peptide 1, **Figure 3**) revealed highest affinity to oxytocin receptor (OTR), with value of Ki 0.31 nM and served as oxytocin agonist and as a possible treatment of autism [7], and for the treatment of postpartum hemorrhage [8].

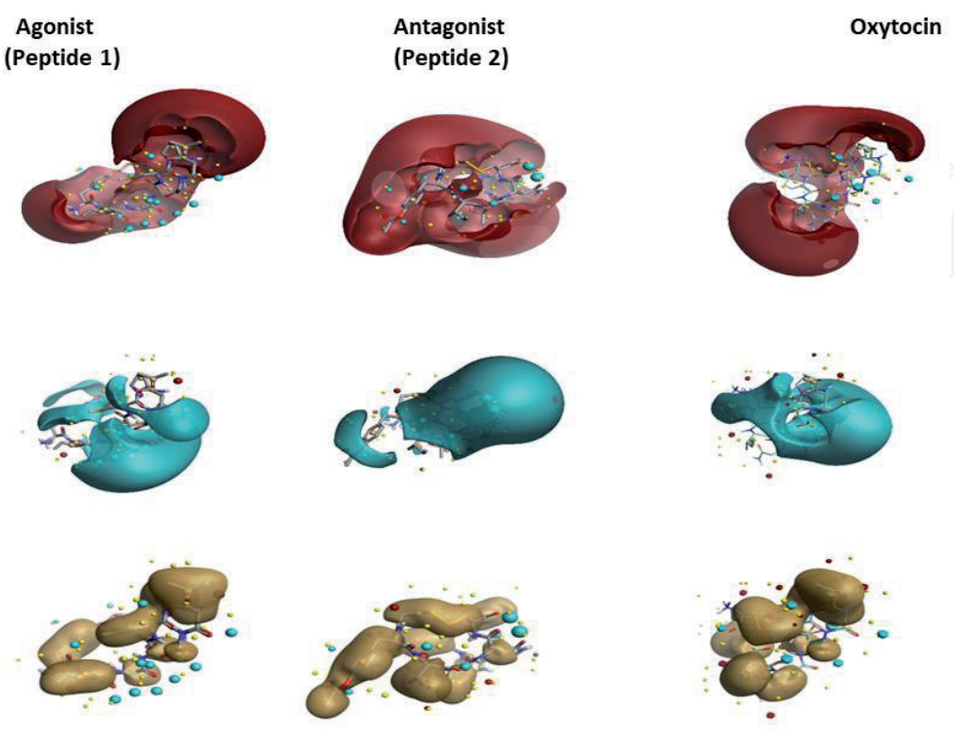


**Figure 3.**
*Molecular features of oxytocin receptor modulators calculated using forge tool.*

Peptide atosiban (peptide 2, **Figure 3**) revealed affinity to oxytocin receptor with a value of Ki 76 nM, and it is used in clinical as oxytocin antagonist [9]. Newly developed Cresset XED force field provided by software Forge [10] was utilized to understand the key features of oxytocin, agonist and antagonist of oxytocin receptor.

**Figure 3** was developed by the authors using Forge tool for oxytocin, agonist and antagonist of OTR, and features were visualized using Forge visualization software to show the comparison of features: red color (positive electrostatics), blue color (negative electrostatics), and gold color (hydrophobic shapes).

The molecular features shown in **Figure 3** are the result of activities and clearly reveal agonist and antagonist function on the oxytocin receptor. Visual examination either manually or using imaging algorithms of all the fields, reveals the presence of positive electrostatic feature in definite coordinates, and size and shape are responsible for agonist and antagonist activity of OTR. Increased electronegative regions favor agonist activity, and similarly, hydrophobic shape features are distinct for agonist and antagonist activity of OTR. The features mentioned in this study, indeed can be utilized by various investigators to discover, develop, and optimize agonist and antagonists of OTR. It is well possible to screen various libraries and chose the molecules of interest.

## 3. Phenotype characteristics in various diseases

Oxytocin receptor agonists and antagonists has potential benefits in health care, especially in treating diabetes, obesity, cardiovascular, and immune related disorders. It is well known that diabetes is considered one of the major causes of mortality and a metabolic disorder. Incidence of diabetes mellitus will increases over coming decades and is generally considered that affect many organs [11]. Many researchers has demonstrated beneficial effect of oxytocin, as endogenous factor, with significant changes in insulin sensitivity [12], lipidomic [13], and glucose metabolism [14]. Oxytocin stimulates glucose uptake [14, 15], and glucagon secretion [16] in pancreatic islets which is directly implicated in improvement and involvement of pathophysiology of diabetes.

Apart from diabetes, another major problem most of countries are concerned about is obesity, both in Mexico and worldwide [17, 18]. Oxytocin is well known for its effects in control of the energy balance [19]. It's well proven that oxytocin administration reduces body weight [20], food intake [21], and glucose tolerance was observed with administration of oxytocin [22]. In addition, oxytocin administration induces a marker of neuronal activation that is known as Fos [23], that is linked to control meal size, regulation of intake of food and indeed bodyweight.

Another class of disease is cardiovascular disease, where 13% of deaths are due to this, specifically due to blood pressure, followed by tobacco, diabetes, and lack of exercise [24]. Cardiovascular diseases involve generally blood vessels or heart [25]. Oxytocin receptor decreases blood pressure [26] and lowers brain natriuretic peptide [27]. Oxytocin was also proven to facilitate the recovery of cardiovascular system (CVS) [28] from injuries [29] and decrease progression of atherosclerosis. Key pathways involved in cardiovascular protection upon treatment with oxytocin act by suppressing the increasing production of cytokines, apoptotic pathways are activated through the involvement of multiple signaling pathways [29].

Oxytocin is also involved in immune related health problem; oxytocin was proven to trigger thymocyte proliferation [30], inducing immune tolerance, which is generally observed in thymus which is a primary tissue responsible for [31]. The nonapeptide oxytocin interacts with neurohypophysial peptide receptors expressed by pre-T cells and induces phosphorylation of focal adhesion kinase [32]. Generally,

oxytocin production decreases with age, which is linked to thymic involution. Oxytocin also has antiviral activity and it has been reported its possible used to treat CoVID-19 through DPP-inhibition mechanism of action [33, 34].

## 4. Nano, micro and macro materials for delivery of agonists and antagonists

Up today, oxytocin receptor agonists and antagonists formulations including nano, micro and macro materials are not available in the market. Existing oxytocin receptor agonists and antagonists possess drawbacks like lack of efficacy, no improvement in neonatal outcomes, low stability, short half-life in-vivo, and low bioavailability.

Many researchers had started to utilize alternative approaches to address mentioned drawbacks, and among these, encapsulations for slow and steady oxytocin release are under investigation. Most of them are polymeric carriers, and liposome-based technologies.

Among the polymeric carriers for oxytocin, solvent displacement method was utilized to synthesize poloxamer hydrogel scaffold, to encapsulate oxytocin in hydrogel graft constituted of poly(d, l-lactide-co-glycolide) (PLGA), β-tricalcium phosphate, and hydroxyapatite (CP). This method yielded 89.5% encapsulation efficiency, slowly release encapsulated oxytocin, and finally yielded significant regeneration of bone loss in rat calvaria [35].

Another nanomaterial application was based on Bovine Serum Albumin (BSA) based nanocomposites. It is a twostep process to synthesize nanocomposites, nanoprecipitation followed by lyophilization. Transferrin (Tf) and Rabies Virus Glycoprotein (RVG) was conjugated to synthesized oxytocin-BSA nanoparticles for successful delivery of oxytocin across blood brain barrier (BBB). This composite was delivered intranasally, and an improvement in oxytocin bioavailability was observed. Delivery of oxytocin using nanocomposites led to greater pro-social effects in comparison to oxytocin alone within 3 days of intranasal administration. This delivery system definitely benefits social-deficit disorders and indeed enhance the brain delivery peptides [36].

Another important nanocarrier utilized in successful delivery oxytocin receptor agonists and antagonists is liposomal drug delivery systems. In specific, antibody-based liposomes were applied where PEGylated liposomes were conjugated with atosiban (ATO-Lipo, OTR antagonist) or anti-OTR monoclonal antibodies (OTR-Lipo) using the technology of dried lipid film hydration. This method increased intracellular internalization, and revealed that the cellular uptake is dependent on caveolin-mediated mechanisms, with no cellular toxicity observed. Antibody-conjugated liposomes gave way to improved treatment for obstetric complications [37].

## 5. Future perspectives

Since the therapeutic effects of agonists and antagonists are growing, more studies will be needed to protect them against biodegradation, to increase intestinal absorption particularly with use of technologies, such as enhancer absorption (cyclodextrines), surfactants, cell penetration peptide, chemistry modifications, nano, micro and macromaterials.

Besides improving drug delivery methods, additional cell-based experiments are required to understand the role of oxytocin such as the use of fluorescent tags

(HaloTag) to monitor new functions for agonists or antagonists, the use of imaging techniques to classify and develop imaging biomarkers is needed to explore the ability of oxytocin receptor agonists or antagonists across tissues, cell-host interactions. In addition, the molecular features described in this study can be used to discover new and novel oxytocin receptor agonists and antagonists.

## 6. Conclusion

Molecular shape features of oxytocin receptor agonist and antagonist activity as a function of positive and negative electrostatic, and hydrophobic shapes features were developed and detailed. Also, liposome and polymeric based strategies in improving most of the drawbacks associated with oxytocin delivery were discussed. More studies are needed towards the application of diverse nanomaterials to deliver neuropeptides and improve their efficacy. Some of the tools proposed in this chapter can guide the future research in improving the delivery strategies of oxytocin receptor agonists and antagonists.

It has to be remembered that, technologies need to be developed for each route of administration, maintain stability, and steady state release of encapsulated active ingredients.

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## Conflict of interest

The authors declare no conflict of interest.

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