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NUTS AND BOLTS

An overview of the oxytocin-oxytocin receptor signaling network

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Abstract Oxytocin, a nine amino acid long neuropeptide hormone, is synthesized in the hypothalamus and stored and released from the neural lobe of the pituitary gland. Although commonly known for its central role in the regulation of parturition and lactation, oxytocin signaling also plays a key role in modulating social behavior, evoking contentment, initiating maternal behavior, inducing trust, generosity and bonding in humans and animals. Oxytocin signaling can prove to be of great importance in therapeutics and drug targeting because of its diverse range of actions. However, a well annotated map of oxytocin signaling pathway is currently lacking in the publicly available pathway resources. Therefore, we systematically curated the available signaling information of oxytocin from published literature and collated the data to develop a more complete map. We cataloged 66 molecules belonging to oxytocin signaling pathway, which included 9 protein-protein interactions, 39 post-translational modifications, 14 protein translocation events and 22 activation/inhibition events. Further, Oxytocin signaling network data is made freely available to academic fraternity by integrating this into NetPath (http://www.netpath.org/), a freely available human signaling pathway resource developed previously by our group.

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Abbreviations

OXT	Oxytocin
OXTR	Oxytocin receptor
CNS	Central nervous system
GPCR	G-Protein coupled receptor
PTMs	Post-translational modifications
PPIs	Protein-protein interactions
BioPAX	Biological pathway exchange
SBML	Systems biology markup language
PSI-MI	Proteomics standards initiative for molecular interaction

Introduction

The nine amino acid long cyclic peptide hormone oxytocin (OXT) is synthesized in the magnocellular neurosecretory cells of the supraoptic and paraventricular nuclei of the hypothalamus (Summar et al. 1990). It is expressed as an inactive precursor protein by the *OXT* gene located on chromosome number 20. The post-translational progressive hydrolysis of this inactive prohormone through the action of a series of enzymes results in the active form of OXT (Guillou et al. 1994). It is stored in the Herring bodies at the axon terminals of the posterior pituitary lobe before being released into circulation by exocytosis from the neurohypophysis nerve terminals (Jirikowski et al. 1990). OXT is also reportedly synthesized in several peripheral tissues such as uterus, placenta, amnion, corpus luteum, testis, thymus, kidney and pancreas (Gimpl and Fahrenholz 2001). OXT binds to oxytocin receptors (OXTR), which belongs to the G-protein coupled receptor (GPCR) superfamily (Arrowsmith and Wray 2014). The human *OXTR* gene is located on chromosome locus 3p25. The expression levels of oxytocin receptors in the myoepithelial cells surrounding the alveoli of mammary glands remain constantly elevated throughout the lactation period (Soloff et al. 1979).

OXT-OXTR signaling has been associated with various biological functions. However, its role in uterine contraction and labor induction was the most widely studied. OXT expression in the uterus is elevated during gestation and declines immediately after parturition. The cell specific difference in receptor expression levels aids oxytocin to switch targets between uteri during parturition, to mammary glands during the milk ejection reflex. OXT contributes to myometrial contractility and its receptor has been used as a target for tocolytic agents (Vrachnis et al. 2011). In mammals, OXT is released in a pulsatile manner by the neurohypophysis and is thought to have a key role in the peripartum period by stimulating smooth muscle contractility in the uterus and mammary gland (Smith et al. 2006). In uterus, OXT signaling causes increase in prostaglandins (PGs) after activation of OXTR, which are the mediators of the manifold actions of OXT. Several OXT agonists and antagonists are being used for therapeutic purposes, with differing effects, depending on the cellular context. The highly selective and orally active OXT antagonists are used for the prevention of preterm labor (Serradeil-Le Gal et al. 2004). Also, OXT is being used as an agonist to generate or enhance maternal labor, to prevent or treat postpartum uterine bleeding (Weeks 2015).

OXT-OXTR signaling also plays significant roles in heart and brain. It regulates hypothalamo-pituitary-adrenal axis, modulating behavioral response towards stress and social behavior (Neumann 2002). OXT plays an important role in potentiating a response to a range of pro-social behaviors and in evoking contentment, inducing trust, generosity, and bonding in humans and other animals (Grillon et al. 2013). Prostaglandins are mediators of OXT actions such as excitation in supraoptic nuclei of neurons released in response to OXTR agonists (Wang and Hatton 2006). Cardiac activity of OXT involves maintenance of blood pressure, increased angiogenesis and anti-inflammatory activity (Gutkowska and Jankowski 2012). Further, OXT has a significant role in bone development and is implicated in skeletal remodeling and osteoblast maturation (Majumder et al. 2013). In addition to this, it is involved in the pathophysiology of various disorders such as diabetes, osteoporosis and neuropsychiatric disorders (Elabd and Sabry 2015; Rozek et al. 2014). Considering the biomedical importance OXT signaling in the biological system with respect to various physiological and pathological actions, it is imperative to develop a well annotated and expanded signaling map for oxytocin. Towards this goal, we initiated this bioinformatics study to systematically bring together all the molecular reactions orchestrated by stimulation of OXTR by OXT.

Materials and methods

Literature searches were carried out using PubMed to compile the reactions induced by oxytocin-oxytocin receptor signaling using key search terms such as ‘oxytocin’, ‘oxytocin receptor’, ‘OXT signaling’ and ‘OXTR’. Experimental studies showing oxytocin receptor stimulation by oxytocin or oxytocin analogues (agonists and antagonists) were further selected for curation. All the signaling reactions were archived under the categories such as protein-protein interactions (PPIs), post translational modifications (PTMs), gene regulation, protein activation/inhibition and translocation. The reactions were manually curated and catalogued using PathBuilder, a web-based pathway curation tool developed in-house (Kandasamy

et al. 2009). NetPath annotation pipeline was followed to develop OXT signaling, which has been described previously by other groups at our institute, to develop several signaling pathways including Leptin (Nanjappa et al. 2011);Thyroid stimulating hormone (Goel et al. 2011), corticotropin-releasing hormone (Subbannayya et al. 2013), brain-derived neurotrophic factor (Sandhya et al. 2013), Interleukin-17 (Sharma et al. 2015), and Interleukin-10 (Verma et al. 2015) signaling pathways.

Each molecular reaction has been hyperlinked to the research articles in the PubMed from which it was curated. Each reaction was annotated with information about the experimental conditions and cell types used and brief comments about the study. Sites and residues for post-translational modification have been mapped to a RefSeq sequence from the sequence information provided in the respective experiments. Pictorial representation of OXT pathway was generated using PathVisio (Version 3.2.2), freely available software for

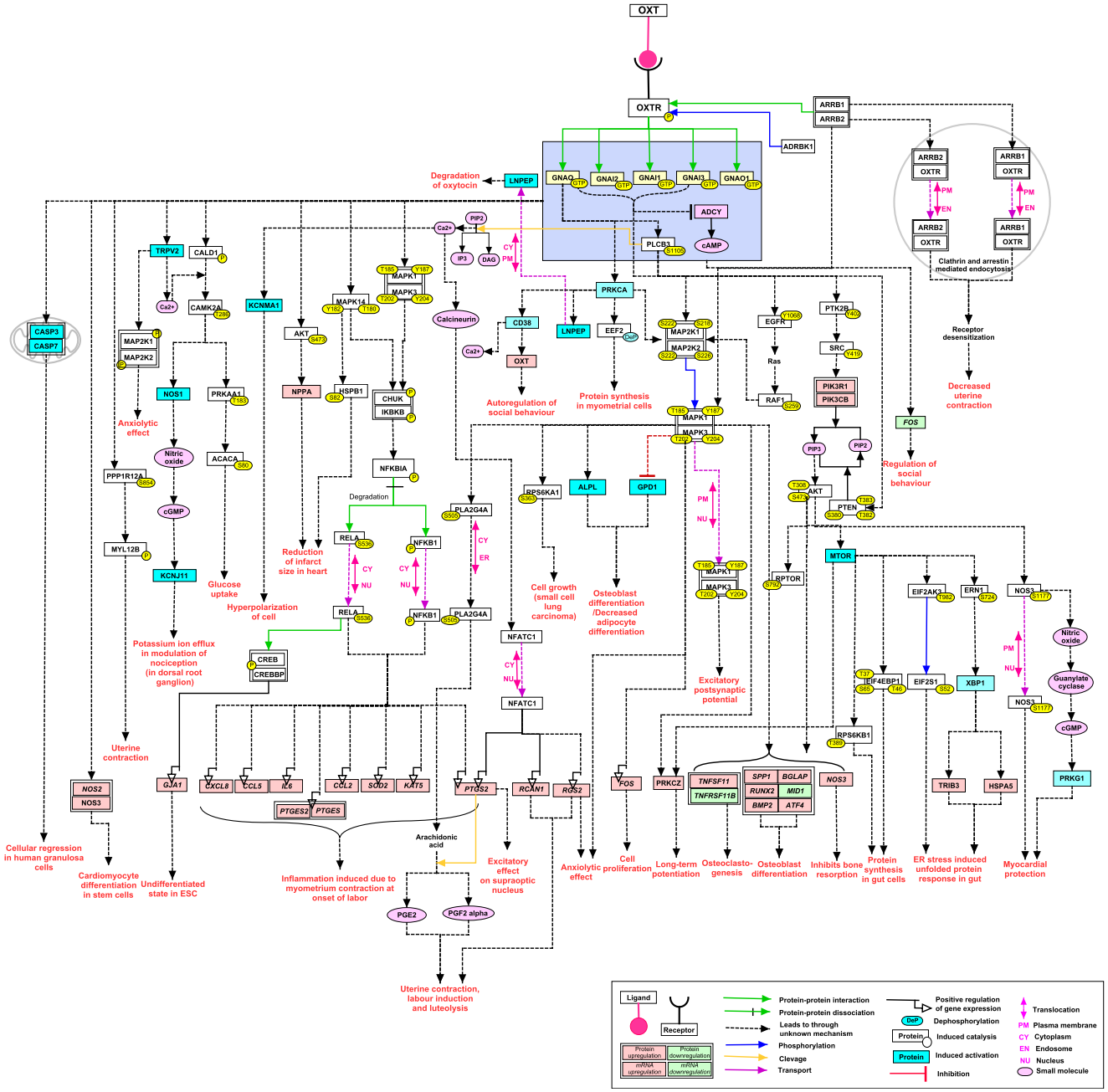

Fig. 1 A schematic representation of reactions induced by oxytocin. The pathway map depicts reactions induced by stimulatory action of OXT through OXTR. Reactions represented in map are protein-protein interaction, post-translational modification, activation/inhibition and gene/protein expression. Sites and residues for post translational modifications are also shown. Legend is provided for identification of different pathway reactions

drawing and visualization (Wang et al. 2012). The reactions were then exported to a web-based pathway resource called NetPath (Kandasamy et al. 2010). Pictorial representation of OXT pathway map depicts all the four broad categories of curation such as molecular association, catalysis, transport, and gene expression at transcription and/or translational level. To validate the authenticity of curated molecular events pertaining to oxytocin pathway, these reactions were also reviewed by a pathway authority, who is a subject expert in the field.

Results and discussion

An extensive PubMed search of research articles pertaining to OXT-OXTR signaling fetched in a total of 1803 articles. These articles were screened for information pertaining to the molecular events induced by oxytocin, resulting in 83 articles from which the pathway map was curated. We documented a total of 66 unique proteins involved in either of 9 PPIs, 39 PTMs, 14 translocation and 22 activation-inhibition reactions. We also catalogued regulation of genes induced by oxytocin in different mammalian systems and identified 62 and 57 genes regulated at transcriptional and translational levels, respectively (Supplementary Table 1).

The OXT pathway data has been made freely available to scientific community through the NetPath resource (http://www.netpath.org/pathways?path_id=NetPath_169). It includes description of the OXT signaling pathway and statistics of total number of molecules and molecular reactions present in signaling network. The molecules involved in the pathway have been linked to molecule page of NetPath and HPRD (Prasad et al. 2009), which provides concise description about the molecule. Data is available to scientific community in standard data exchange format such as Biological Pathway Exchange (BioPAX level 3) (Demir et al. 2010), Proteomics Standards Initiative for Molecular Interaction (PSI-MI version2.5) (Orchard and Kerrien 2009) and Systems Biology Markup Language (SBML version 2.1) (Hucka et al. 2003). The data has been made accessible in tab delimited and Microsoft Excel formats. Figure 1 depicts the OXT-OXTR signaling map.

Molecular events induced by OXT-OXTR signaling are involved in various processes such as uterine contraction, labor induction, cell proliferation, cardiomyogenesis, bone formation and neuromodulation. OXTR stimulation is primarily mediated through Gαi/Gαq/Gαo protein activation. OXT induces pro-inflammatory cytokine overexpression through mitogen-activated protein kinase (MAPK)/Nuclear factor kappa B (NFκB) pathway, which in turn is associated with onset of labor (Kim et al. 2015). Prostaglandins (PGE2 and PGF2 alpha) production through arachidonic acid cleavage is one of the effective mediators in oxytocin induced processes especially to those related to onset of labor (Terzidou et al. 2011; Jeng et al. 2000). OXT also triggers osteoclastogenesis and osteoblast differentiation through MAPK1/3 activation thus it may play a significant role as a therapeutic agent in osteoporosis (Tamma et al. 2009). It also plays significant role in functions associated with the CNS such as regulation of nociception, which is mediated by activation of potassium ion channel (KCJN11) (Gong et al. 2015) and anxiolytic effect mediated through expression of regulator of G-protein signaling 2 (RGS2) (Okimoto et al. 2012). In the heart, OXT elicits cardioprotective function by reducing infarct size and post-ischemic recovery, which is mediated through PI3K-AKT/NOS/natriuretic peptide A (NPPA) expression and p38 MAPK/heat shock 27 kDa protein 1 (HSPB1) phosphorylation (Ondrejcakova et al. 2012).

Conclusions

Availability of an expanded and well curated map of OXT signaling reactions in a centralized resource will accelerate the understanding of the role of various molecules in the context of normal physiological or pathological conditions induced by OXT. The signaling data has been made available in multiple community exchange formats to ensure easy integration of data with multiple public repositories and pathway analysis software such as gene set enrichment and Gene Ontology analyses.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

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