Review

Role of Oxytocin/Oxytocin Receptor System in Regulation of Cell Growth and Neoplastic Processes

(oxytocin / oxytocin receptor / G proteins / phospholipase C / kinases / signalling pathways / phosphoinositides / carcinoma cells / cell proliferation)

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Abstract. Novel sites of oxytocin receptor expression have recently been detected in central nervous system, cardiomyocytes, endothelial cells, various carcinoma cells, etc. These and other discoveries have greatly expanded the classical biological roles of oxytocin, which are stimulation of uterine smooth muscle contraction at parturition and milk ejection during lactation. It is becoming clear that the great diversity of oxytocin actions in the brain and peripheral organs is paralleled by activation of a diversity of signalling pathways. On the other hand, until now only one single oxytocin receptor type has been detected. This receptor belongs to G protein-coupled receptors and in dependence on cell conditions it binds to different G proteins; this phenomenon is called receptor-G protein promiscuity. Thus, in the same cells oxytocin can activate multiple responses at the same time. Recently, the oxytocinergic system has also been implicated in the growth modulation of various neoplastic cells, where it may inhibit or stimulate cell proliferation in dependence on cell type and activated metabolic pathways. The discovery of novel oxytocin receptor-linked signalling cascades brings interesting knowledge opening new avenues for research in oncology and molecular pharmacology with perspectives of finding new therapeutic agents.

Introduction

Oxytocin (OT) was the first peptide hormone to have its structure determined and the first to be chemically synthesized in biologically active form. Its main physiological role is regulation of the contraction of uterine smooth muscle at parturition and ejection of milk from the lactating breast. The expression and presence of OT receptors (OTRs) have been revealed not only in the myometrium and mammary gland, but also in the endometrium, ovary, testis, epididymis, vas deferens, thymus, heart and kidney, as well as in various regions of the brain. Central actions of OT range from the modulation of the response on individual synapses to the establishment of complex social and bonding behaviours related to the reproduction and care of the offspring. The long-term effects of OT in the central nervous system, on processes of memory and learning, mother's behaviour, and stress were reviewed by Hynie and Klenerová (2008) and Klenerová and Hynie (2008).

It is interesting to note that *OT* genes belong to the oldest ones; they should be even older than 500–700 million years (Acher et al., 1995; Gimpl a Fahrenholz, 2001). Despite the great diversity of the proposed functions of the OTRs, only one type of this receptor has been identified (Kimura et al., 1992, 2003; Inoue et al., 1994). The *OTR* gene is a single-copy gene consisting of four exons and three introns, localized at chromosome 3 of the human genome (3p25-3p26.2). In spite of the fact that there is only one type of OTR, its stimulation can produce more cell functions, and moreover, sometimes in the opposite manner. For example, OT has the ability both to stimulate and inhibit cell proliferation. OTR thus transduces growth-proliferatory or -inhibitory signals.

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Abbreviations: 3p25-3p26.2 – small arm of chromosome 3, area 25-26.2, AC – adenylyl cyclase, AKT – protein kinase Akt (protein kinase B), cAMP – cyclic adenosine monophosphate, DAG – diacylglycerol, eNOS – endothelial nitric oxide synthase, ESCC – oesophageal squamous cell carcinoma, GPCR – G protein-coupled receptor, HFF – human fibroblastic cells, HUVEC – human umbilical vein endothelial cells, Ins(1,4,5)P_3 or IP_3 – inositol 1,4,5-trisphosphate, NO – nitric oxide, OT – oxytocin, OTR – oxytocin receptor, PH – pleckstrin homology, PIP_2 – phosphatidylinositol 4,5-bisphosphate, PIP_3 – phosphatidylinositol 3,4,5 triphosphate, PI3-K – phosphatidylinositol 3-kinase, PKA – protein kinase A, PKC – protein kinase C, PLC – phospholipase C, SCLC – small-cell lung carcinoma.

Several findings indicate that OTR is regulated in a very complex manner. For example, it has been shown that OTR in the plasma membrane mediates inhibition of cell proliferation while OTR in caveolin-enriched microdomains (lipid rafts) mediates a mitogenic effect (Rimoldi et al., 2003; Herbert et al., 2007). The study of novel OTR-linked signalling cascades brings interesting knowledge opening new avenues for research in molecular pharmacology. The search for new OT analogues could bring a class of highly selective compounds with therapeutic relevance in obstetrics, oncology, psychiatry, and some other areas of medicine.

OT receptor

The encoded OTR is a 388-amino-acid polypeptide, which belongs to the family of G protein-coupled receptors (GPCRs) (Bockaert and Pin, 1999; Klenerova and Hynie, 2008). All GPCRs display seven heptahelical domains that are hydrophobic and span lipid bilayer. Extracellular domains and core of bundle of seven transmembrane segments act in signal discrimination and ligand binding. For OT the important binding domains are the extracellular N-terminus and the extracellular loops E2 and E3 (Gimpl and Fahrenholz, 2001; Gimpl et al., 2005; 2008). The E3 of OTR is able to contact the cyclic part of OT (Zingg and Laporte, 2003; Hasbi et al., 2004). Antagonists that are derived from the ground structure of OT bind to OTR at distinct sites partly non-overlapping with the agonist binding site. The affinity of the receptor for ligands is strongly dependent on the presence of divalent cations and cholesterol that both act as positive allosteric modulators. Notably, some evidence is provided that OTRs are also present in the form of dimeric or oligomeric complexes at the cell surface (Gimpl et al., 2008).

Intracellular domains of OTR function in signal transduction to heterotrimeric G proteins. G proteins are constructed of three types of subunits, an α-subunit uniquely capable of binding GTP and subsequently releasing β/γ-subunits. After degradation of GTP the β/γ-subunits are again attached to the α-subunit and their activating ability is finished. The nomenclature now popularly known as Gq, Gs, and Gi classes characterizes the interaction of the α-subunit with various effector molecules that control the concentrations of intracellular messengers (Morris and Malbon, 1999). Gs means that the α-subunit of heterotrimeric G protein interacts with adenylyl cyclase (AC) and stimulates production of cyclic adenosine monophosphate (cAMP), while Gi inhibits production of cAMP. Gq has been used for a class of G proteins which activate phospholipase C (PLC) (see below).

OTR is able to couple to different G proteins with subsequent stimulation of various signalling cascades (Fanelli et al., 1999; Chini and Fanelli, 2000; Rimoldi et al., 2003; Chini and Manning, 2007). In dependence on G-protein coupling, OTR can give rise to opposite effects on the same cellular function. The activation of OTR is connected with the coupling to the Gq protein in rat and human myometrium (Sanborn et al., 1995; Strakova and Soloff, 1997) and in human umbilical vein endothelial cells (HUVECs) (Cattaneo et al., 2008). Nevertheless, the study of Phaneuf et al. (1996) indicated that OT interacts with at least two types of G proteins in human myometrial cells: a member of the Gq class (pertussis toxin insensitive) and a member of the Gi class (pertussis toxin sensitive). For example, OTR coupling to Gq induces contraction in myometrial cells, while OTR activation of Gi delays preterm labour (Zhou et al., 2007).

Interesting findings concerning the coupling of OTR with various G proteins were reported in the brain. To investigate the potential role of Gq signalling in behaviour, Wettschureck et al. (2004) generated mice which lack the α-subunits of the two main members of the Gq/11 family. These authors found that forebrain Gq/11-deficient females did not display any maternal behaviour such as nest building, pup retrieving, crouching, or nursing. However, olfaction, motor behaviour, and mammary gland functions were normal in these mice. It seems therefore that Gq/11 signalling is indispensable to the neuronal circuit that connects the perception of pup-related stimuli to the initiation of maternal behaviour.

OTR and its transduction mechanisms

The coupling of OTR with Gq results in the activation of PLC, the effector of phosphoinositide signalling system (Strunecká and Patočka, 2007). Several reports support the conclusion that PLC is the major effector enzyme for transduction of OT signals for cellular functions (Sanborn et al., 1995; Strakova and Soloff, 1997; Cattaneo et al., 2008; Gimpl et al., 2008). It is generally accepted that phosphatidylinositol 4,5-bisphosphate (PIP_2) from the plasma membrane is hydrolysed by PLC and yields inositol 1,4,5-trisphosphate (Ins(1,4,5) P_3 or IP_3) and diacylglycerol (DAG) after receptor stimulation. Both products of this hydrolysis catalysed by PLC have a second-messenger role. IP_3 binds to receptors in membranes of endoplasmic reticulum, which results in the release of Ca^{2+} into the cytoplasm. DAG activates protein kinase C (PKC), which affects the activity of many enzymes via protein phosphorylation.

Since identification of PLC as the key enzyme of the phosphoinositide signalling pathway (Takenawa and Nagai, 1981; Streb et al., 1983), thirteen PLC isozymes have been identified in different mammalian tissues (Suh et al., 2008). They belong to six different subtypes and, moreover, every isoform of these subtypes has a few splicing variants. Little is known about the functions, relations, and hierarchy of PLC isozymes although it seems that their distribution in the body is tissue specific. All isoforms of PLC possess the so-called pleckstrin homology (PH) domain, which binds PIP_2, contain X and Y domains responsible for the catalytic activity, and C2 domains as well as EF hand motives, which are

involved in various biological actions (Suh et al., 2008). These regulatory domains serve to target the PLC isozyme to the vicinity of their activation or substrate through protein-lipid or protein-protein interactions.

PLC is an effector for OTR in several human tissues. In isolated myocytes from human uterus, where PLC is undoubtedly involved in physiological response to OT, only the isoform PLC-β was identified in older studies (Molnar and Hertelendy, 1990; Tasaka et al., 1991; Sanborn et al., 1995) and PLC-β (1-3) and PLC-γ (1 and 2) in following studies (Phaneuf et al., 1996). PLC-β isotypes are widely expressed in various tissues, especially in specific regions of the brain. The activation of PLC-β may proceed by OTR via pertussis toxin-insensitive Gq proteins or Gβγ-subunits.

Moreover, it seems that the PLC-δ isoform is very important, which is a basically soluble protein that is localized mainly in the cytoplasm and is translocated to the plasma membrane, where it functions to hydrolyse PIP_2 in response to receptor activation (Park et al., 1998). PLC-δ is composed of three isozymes, PLC-δ1, PLC-δ3, and PLC-δ4 (Irino et al., 2004). It has been shown that PLC-δ1 is tethered to the plasma membrane through interactions of its PH domain and PIP_2. This process may be triggered by a small increase in intracellular Ca^{2+} concentration (Suh et al., 2008).

It is interesting that the distribution of PLC-δ corresponds with tissues and organs where the distribution of OTR was described. PLC-δ1 is present in high abundance in the brain, heart, lung, skeletal muscle, and testis. Originally described PLC-δ2 seems to be a homologue of human/mouse PLC-δ4 (Irino et al., 2004). PLC-δ3 is detected abundantly in brain, skeletal muscle, heart, and reproductive organs. PLC-δ4 mRNA is expressed in various tissues with the highest levels detected selectively in the brain, skeletal muscle, testis, and kidney (Suh et al., 2008).

In addition to the function of PLC isoforms in signal transduction via membrane receptors, all PLC isoforms are also located in the nucleus, where they participate in the regulation of gene expression. PLC-β-mediated signalling occurs not only at the plasma membrane but also in the nucleus. PLC-β1 is the major PLC isozyme in the nucleus of various cells (Martelli et al., 2005). Several studies have demonstrated the existence of an autonomous intranuclear phosphoinositide cycle that involves activation of nuclear PLC-β1 and generation of DAG. Nuclear PLC-β1 has been linked with either cell proliferation or differentiation (Yagisawa et al., 2006; Faenza et al., 2008; Spadaro et al., 2008). The study of Fu et al. (2007) provided evidence that PLC-δ1 is a potent tumour-suppressor gene and plays an important suppressive role in oesophageal squamous cell carcinoma (ESCC) development and progression.

Oxytocinergic system in neoplastic cells

The oxytocinergic system (OT and OTR) is implicated in the growth modulation of various neoplastic cells, where it may stimulate or inhibit cell proliferation. OTRs are expressed or over-expressed in cells of several tumour types such as breast and endometrial carcinomas (Cassoni et al., 1994; Bussolati et al., 1995; Sapino et al., 1998; Cassoni et al., 2000; Suzuki et al., 2003; Reversi et al., 2005), carcinomas of the prostate (Whittington et al., 2007), neuroblastomas and glioblastomas (Cassoni et al., 1998), osteosarcomas (Novak et al., 2000; Petterson, 2008), choriocarcinomas (Cassoni et al., 2001b), Kaposi sarcomas (Cassoni et al., 2002), and small-cell lung carcinoma (SCLC) (Pegueux et al., 2004). OTRs were found in the large majority of ovarian carcinoma tissues (Morita et al., 2004). The *OTR* gene was demonstrated in 78 % of cases of endometrial carcinomas and its presence was confirmed by immunofluorescence and RT-PCR (Cassoni et al., 2000).

Moreover, all OTRs in carcinoma cells are structurally identical with OTRs in healthy cells and have the ability to bind OT (Copland et al., 1999). Kinsey et al. (2007) reported that human osteosarcoma (U2OS, MG63, OS15 and SaOS2), breast cancer (MCF7), and primary human fibroblastic cells (HFF) all exhibit the presence of OTRs not only on the cell membrane, but also in various nuclear compartments including the nucleolus, as detected by transfection and/or confocal immunofluorescence, respectively. Western blots indicate that OTRs in the nucleus and on the plasma membrane are likely to be the same biochemical and immunological entities. Constitutive internalization of OTRs was found only in osteosarcoma cells, while the findings of OTR in the nucleus in all other tested cells were dependent on ligand binding. Such atypical and unexpected nuclear localization of OTR suggests yet unknown biological functions of this receptor protein. Martelli et al. (2005) reported the finding of the PLC-β-PIP_2-DAG pathway in nuclear speckles (subnuclear structures) and found a possible involvement of this pathway in the progression of myelodysplastic syndrome to acute myeloid leukaemia.

Verbeeck et al. (1992) reported that the expression of *OT* genes is very rare in tumour cell lines. These authors investigated the presence of OT mRNAs in 13 tumour cell lines of neuroendocrine and in 23 tumour cell lines of non-neuroendocrine origin. They did not find the presence of OT mRNA in most of the investigated tumour cell lines with the exception of rat pituitary tumour cell line GH4C2 and rat pancreas tumour cell line RIN5. In these tumours very low OT mRNA signals were detected. Later, it has been established that some tumours also express and secrete OT, particularly in the SCLC (Péqueux et al., 2004).

OT inhibits proliferation of neoplastic cells of either epithelial (mammary and endometrial), nervous or bone origin *in vitro.* On the contrary, in neoplastic cells derived from trophoblast and endothelium, OT was found to promote cell proliferation (Cassoni et al., 2001a, 2004; Reversi et al., 2005). Morita et al. (2004) investigated the effects of OT on the growth of ovarian carcinoma cells. They found that OT inhibited proliferation

of ovarian carcinoma cells *in vitro*. In addition, their *in vivo* study showed that intraperitoneal administration of OT resulted in the reduction of intraperitoneal dissemination of ovarian carcinoma cells.

OT and OTRs have been identified in the human prostate. Prostate hypertrophy results from abnormal growth of the gland. OT is implicated in the regulation of prostate growth; OT concentrations were increased in benign, and decreased in malignant prostate disease. While OT > 0.5 nmol/l had no effect on proliferation of prostatic epithelial cells when cells were cultured alone in the absence of androgens, low concentrations of OT (< 1 nmol/l) in the presence of testosterone stimulated proliferation of prostatic cells (Whittington et al., 2007). Changes in prostatic concentrations of OT that occur with aging and malignant disease may facilitate the cell proliferation. On the other hand, OT had an inhibitory effect on the proliferation of prostatic stromal cells. The effects of OT on proliferation and growth of various carcinoma cells are given in Table 1.

The diversity of OT effects on proliferation and growth of carcinoma cells can be caused by activation of different signal transduction pathways. It can be concluded that the inhibitory effects of OT are connected with the increase of cAMP and activation of protein kinase A (PKA). It is evident that cAMP and PKA have a key role in inhibition of the mitogenic response. On the contrary, the mitogenic effects are connected with the OTR coupling with Gq and activation of PLC; this results in the increased intracellular Ca^{2+}. In the case of OT-stimulated SCLC proliferation the coupling of OTR to Gq/11 and the increase of cytosolic Ca^{2+} is absolutely required (Pégueux et al., 2004). Simultaneously, Gi mediates the PLC-dependent inhibitory response of OT (Pégueux et al., 2004).

Additionally, the activation of particular signalling intermediates by OTR depends on its localization inside or outside caveolin-enriched domains of lipid rafts of the plasma membrane (Rimoldi et al., 2003). It has been shown that OT has a mitogenic effect when the OTRs are targeted to caveolin-enriched microdomains, and on the contrary that it inhibits cell proliferation when the vast majority of OTRs are excluded from these microdomains in plasma membranes (Emmer et al., 2003; Cassoni et al., 2004; Reversi et al., 2006).

Whittington et al. (2007) observed that the localization of OTR within the plasma membrane modulates OT's proliferative response in the prostate. Disruption of caveolae in the plasma membrane removed the inhibitory effect of OT on prostatic stromal cell proliferation but did not affect the stimulatory effect of OT on independent malignant PC-3 cells cultured in the presence of androgens. Herbert et al. (2007) found that both caveolin and OTR expression is drastically increased with age in both smooth muscle and epithelium of the prostate. These authors also found a significantly increased co-localization of the OTRs with caveolin in both the muscle and the epithelium and suggested that OT may have a proliferative effect on the prostate tissue through the caveolae-associated receptors. This process seems to be androgen receptor independent. Changes in prostatic concentrations of OT that occur with aging and malignant disease may therefore act to facilitate cell proliferation.

An important tool in studies of OTR transduction mechanisms is OT antagonist/agonist atosiban. This

Table 1. The effect of OT on proliferation of selected human carcinoma cells

Type of the human carcinoma cells	OT effect on cell proliferation and growth	Biochemical changes	Reference
Ovarian carcinomas	inhibition	not studied	Morita et al., 2004
Trophoblast cell lines	stimulation of cell proliferation	↑cytosolic Ca^{2+}	Cassoni et al., 2001b
Choriocarcinoma cell lines	stimulation of cell proliferation	↑cytosolic Ca^{2+}	Cassoni et al., 2001b
Endometrial adenocarcinoma	inhibition	↑cAMP/PKA	Cassoni et al., 2000; Suzuki et al., 2003
Breast cancer cells	inhibition	↑cAMP/PKA	Bussolati et al., 1995; Sapino et al., 1998; Cassoni et al., 2000; Reversi et al., 2005
Prostatic epithelial cells	activation in the presence of testosterone	not studied	Whittington et al., 2007
Prostatic stromal cells	inhibition	not studied	Whittington et al., 2007
Neuroblastomas and glioblastomas	inhibition	↑cAMP/PKA	Cassoni et al., 1998
Osteoblast-like cells (hOB)	stimulation of cell proliferation	decreased interleukin-6 (IL-6) production	Petterson, 2008
Osteosarcoma cells (SaOS-2)	stimulation of cell proliferation	not studied	Petterson, 2008
Kaposi's sarcoma	stimulation of cell proliferation; no effect on angiogenesis *in vivo*	↑cytosolic Ca^{2+}; increase of $CD4^+$ and $CD8^+$ lymphocytes	Cassoni et al., 2002
Small-cell lung cancer (SCLC)	stimulation of cell growth	↑cytosolic Ca^{2+}; no change in cAMP/PKA	Pégueux et al., 2004

drug has biased agonist properties: it acts as an antagonist in the Gq pathway and as an agonist in the Gi pathway. Reversi et al. (2005) demonstrated that atosiban, by acting on the Gi-mediated pathway, inhibits cell growth of various cancer cell lines including prostate cancer cells expressing endogenous OTRs. Notably, atosiban leads to persistent ERK1/2 activation, i.e. the same signalling events leading to OTR-mediated cell growth inhibition via the Gi pathway (Klenerova and Hynie, 2008).

The role of the PIP_3/AKT pathway in OT proliferative effects

The serine/threonine protein kinase AKT/PKB is a major signal transducer of the phosphatidylinositol 3-kinase (PI3-K)/AKT pathway in all cells and tissues and plays a pivotal role in the maintenance of cell growth, proliferation and apoptosis. The product of PI3-K is phosphatidylinositol 3,4,5-triphosphate (PIP_3). The increase in PIP_3 recruits AKT to the plasma membrane, where it is activated by other kinases. Once phosphorylated, AKT loses the PIP_3 binding requirement and translocates to distinct cellular compartments, where it transduces the signal by phosphorylating numerous substrate proteins.

Cattaneo et al. (2008) demonstrated that OT stimulates phosphorylation of endothelial nitric oxide synthase (eNOS) via the PI3-K/AKT pathway during the process of angiogenesis using HUVECs. Angiogenesis is the initial event that allows tumour growth and survival. The activation of PI3-K and formation of nitric oxide are required for the pro-migratory effect of OT. Both the AKT and eNOS phosphorylation induced by OTR activation depended on PLC activity. This conclusion was supported by the observation that the HUVEC motility stimulated by OT was suppressed by more than 90 % by PLC inhibitor U73122. Based on the observed effects of OT, these authors identified the Gq coupling of OTR and PLC as the main effectors of OT effects on stimulation of motility and invasion in HUVECs.

Very interesting is the finding that PLC-δ has the ability of selective binding to PI3-K (Suh et al., 2008). Fu et al. (2007) found that PLC-δ1 could down-regulate phosphorylated AKT expression and suggested that the tumour-suppressive function of PLC-δ1 might be associated with the inhibition of the PI3-K/AKT signal transduction pathway.

Numerous studies have provided evidence of the importance of AKT signalling in cancer (Luo et al., 2003; Manning and Cantley, 2007). The interactions of AKT with oncogenic factors Ras and Raf further regulate the balance of cell proliferation and growth inhibition. Many components of the Ras-Raf pathway have been described as causal forces in cancer (Al Sheikh et al., 2008). Components of the PI3-K/AKT pathway become an attractive therapeutic target.

Conclusions and Perspectives

It is evident that OTR activates different signaling cascades, being functionally coupled to various G proteins, including at least Gq, Gi, and Gs. It may activate multiple responses in the same cell. OTRs are expressed in several cell types in brain and peripheral tissues (Klenerova and Hynie, 2008). Moreover, OTRs are expressed or over-expressed in cells of several tumour types. It is evident that the great diversity of proposed functions of OT is paralleled by a diversity of its signalling pathways, many of which have still remained unexplored. However, many observations indicate that the biological effects of OT result from the activation of complex intracellular networks and activation of different kinases using different signalling intermediates rather than one linear signalling pathway. Understanding the molecular mechanisms that underlie normal cell growth and biochemical changes in neoplastic pathology warrants further studies. Cooperative signalling by multiple G proteins may represent a novel concept implicated in the regulation of different cellular responses induced by the oxytocinergic system.

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