Biological relevance of oxytocin and oxytocin receptors in cancer cells and primary tumors

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Summary

For a long time, the hypothalamic nonapeptide oxytocin (OT) is known to play a crucial role in many reproductive and behavioral functions. In recent years, a new biological effect of OT has been identified in neoplastic pathology. In this context, OT acts as a growth regulator, through the activation of specific G-coupled transmembrane receptors (OTR). In vitro, an antiproliferative effect of OT was demonstrated in neoplastic cells of either epithelial (mammary and endometrial) or nervous or bone origin, all expressing OTR. Furthermore, the growth-inhibiting effect of OT was also tested and confirmed in mouse and rat mammary carcinomas in vivo. In neoplastic cells from another OT target tissue, trophoblast, the OT effect was to promote proliferation, the opposite of what previously observed in all the other neoplastic OT responsive cells. The signal transduction involved in the OT biological effect was different in OT growth-inhibited or growth-stimulated cells. In the former, the OT effect was mediated by the activation of the cAMP-PKA pathway, a non-conventional OT signaling, whereas in the latter by the increase of intracellular calcium and tyrosine phosphorylation, which are the 'classical' OT transducers. The unexpected role of OT (and OT analogues) in regulating cell proliferation, as well as the diffuse expression of OTR in neoplastic tissue of different origin, open new perspectives on the biological role of the OT-OTR system in cancer.

Key words: biological effects, cancer, neoplastic pathology, oxytocin, oxytocin receptors

The expression of oxytocin receptors (OTR):
From physiology to neoplastic pathology

OTR are G-protein coupled receptors, with the typical seven transmembrane domains [1]. Through these receptors, OT exerts many different biological functions, mostly related to reproduction and behavior [2-4]. OTR are therefore expressed in different tissues such as endometrium [5], myometrium [6], trophoblast [7] and ovary [8], where they are involved in different functions, mainly related to parturition; in the breast (where OTR mediate the myoepithelial cell contraction required for milk ejection) [9]; and in the central nervous system (where activation of OTR participates in the control of various behavioral functions) [4]. In the last years, OTR have also been described in different tissues, which were not considered conventional targets for the peptide, such as vasculature [10, 11] and bone [12]. In the above listed tissues, OTR expression and activation are related to physiological or para-physiological functions. However, few years ago the presence of OTR in tumors arising from some of these OT target tissues, physiologically expressing OTR, has been investigated. In breast carcinomas, OTR were demonstrated in the large majority of primary breast lesions both at mRNA level [by reverse transcriptase-polymerase chain reaction (RT-PCR)] and protein level (by immunohistochemistry) by our group and others [13, 14]. The presence of OTR has also been described in breast cancer cell lines, regardless of their estrogen dependency or independency [15]. Similarly, OTR are present in primary adenocarcinomas of the endometrium [16], a tissue which is known to express OTR under physiological conditions, as well as myometrium. Therefore, the presence of the specific OTR mRNA in endometrial carcinomas evidenced by RT-PCR required confirmation by an alternative technique such as in situ hybridization (ISH) which could determine the effective localization of the OTR mRNA. By ISH it was possible to recognize that the neoplastic cells from endometrial origin were themselves hosting the OTR mRNA and that the RT-PCR results were not dependent on the presence of a myometrial component in the extracted tissue [16]. The ISH positivity was intense and diffuse in all the primary endometrial carcinomas examined. Interestingly, the pattern of protein expression as evidenced by immunohistochemistry (IHC) was variable according to the degree of tumor differentiation [16]. More precisely, poorly differentiated carcinomas were diffusely OTR immunoreactive, whereas well differentiated tumors presented a clonal pattern of OTR immunoreactivity [16]. It still remains to be defined if the overexpressed OTR in poorly differentiated endometrial tumors have still conserved their functionality or if they are defective, inactive receptors.
Remaining within the ‘reproductive area’, in a recent study we demonstrated that OTR are also present in a human choriocarcinoma cell line, as well as in two normal trophoblast cell lines [17].

Finally, OTR are present in tumors of glial and neuronal origin of both the central and peripheral nervous system. In fact, primary glioblastomas and neuroblastomas, as well as human cell lines originating from these tumors, express OTR both at mRNA and protein level [18]. As a very recent observation, it has been reported that bone cells express OTR [12]. According to what was previously observed in all the other neoplasms derived from tissues expressing OTR under physiological conditions, human osteosarcoma cell lines express OTR as well, both at the mRNA and protein level [19].

Biological roles of OT in OTR-expressing tumors and their signaling

There are some traditional roles for OT, which have been known for a very long time and are mostly related to reproductive and behavioral functions. Besides these roles, it has become evident that OT may participate in other biological activities in the neoplastic context. In experiments set in vitro, we demonstrated a unique effect of inhibition in the proliferation of breast [15], endometrial [16], glial [18], neuronal [18] and bone [19] neoplastic cells. The entity of such inhibition ranged from 30% to 50% for all the different cell lines and had been evident since the first 48 hours of OT treatment. The lower concentrations of the peptide which could significantly reduce the cell proliferation were 10 nM or 100 nM, depending on the cell line studied. The inhibition of breast tumor growth was also confirmed by in vivo experiments on Balb-c mice injected with the mammary TS/A tumor and treated by subcutaneous pulsatile administration of the peptide [20]. Although the inhibiting effect of OT on cell proliferation was reproducible in all the above-described cells, in the BeWo human choriocarcinoma cell line OT produced an opposite effect on cell proliferation [17]. In fact, 10 nM to 1 µM OT treatment had significantly increased the cell number since the earliest time (48 hours). Furthermore, when a selective OT antagonist (OTA) was added to the medium together with OT, the mitogenic effect was removed; moreover, OTA alone was able to significantly inhibit cell proliferation [17]. The opposite effects of OT on cell proliferation (inhibition vs. stimulation) are coupled to different signaling pathways. When we looked at the signaling involved in the antiproliferative effect in breast, endometrial, bone and nervous tumors, we could uniformly observe that the cAMP-PKA pathway was involved, the intracellular cAMP levels were increased and that a selective inhibitor of the PKA eliminated the antiproliferative effect of OT, when added to the cell medium [16, 18, 21].

This observation was in contrast to the traditional signaling related to the activation of OTR in physiological contexts, which usually involves intracellular Ca increase and the inositol pathway. Some authors suggested that the activation of the cAMP-PKA pathway could depend on the binding of OT to the vasopressin receptor type 2, as cAMP-PKA are signal transducers of this receptor subtype, for which OT maintains a certain affinity [22]. In the experiments on choriocarcinoma cells, the mitogenic effect of OT was coupled to intracellular Ca increase, getting back to the ‘traditional’ OT signal transduction. Moreover, the specificity of this response was demonstrated by the removal of such effect by the simultaneous infusion of the selective OT antagonist (OTA), the lack of Ca increase after vasopressin infusion and the Ca increase induced by the OT agonist Thr4-OT. In these cells, the proliferative effect was also associated with the tyrosine phosphorylation of three major proteins of 125, 60 and 45 kDa [17].

The OT/OTR system in neoplastic pathology: Future applications

It has become more and more evident that the distribution of the OTR within neoplastic tissues of different origin is wider than expected. Moreover, through these specific binding sites, OT may play a regulatory role in the tumor growth. Further studies would clarify if the different biological action of OT may depend on a cell specificity or on the existence of OTR subtypes coupled to different signaling pathways. What can be speculated by now is that the expression of OTR could find possible future applications in the radio-imaging of different neoplasms, as well as in their therapy. The presence of some OT analogs devoid of contractile activity but still conserving a high OTR affinity could be relevant in both applications.

References

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