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OPIOID-SOMATOSTATIN INTERACTIONS IN REGULATING CANCER CELL GROWTH

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1. ABSTRACT

Opioids and somatostatin mediate their cellular effects through specific membrane receptors. Three major receptor classes (delta, mu and kappa) were identified for opioids, while for somatostatin, five different receptor classes (SSTR1-5) have been cloned. Through the interaction with their receptors, opioids and somatostatin exert their effects on cell growth, proliferation, differentiation and secretion. Specific actions of each receptor type have been reported, to be implicated in one or more of the cell functions referred above but have been mainly correlated with cell growth control. In several systems the effect of either neuropeptide is the reverse, inducing cell growth rather than antiproliferative and proapoptotic signals. In recent years, a growing number of reports indicate a possible interaction between opioid and somatostatin system. This could occur at the receptor level, through a cross-interaction of either neuropeptide with either receptor type, or receptor hetero-dimerization, and at a post-receptor level, via interaction with specific signaling molecules. These interactions provide new directions for the identification of specific molecules acting at the receptor and post-receptor level, mimicking the effects of both categories of agents.

2. INTRODUCTION

Endogenous opioids derive from three different precursor proteins, namely proenkephalin A and B (prodynorphin) and proopiomelanocortin (POMC) (1). These three proteins give rise to a number of opioid peptides with different affinities towards opioid receptors. Opioid receptors belong to the seven-loop transmembrane receptor superfamily (2-4), and are distinguished both pharmacologically and biochemically to three main categories: delta, mu and kappa. In addition, pharmacological evidences exist about further subdivision of delta receptors to delta 1 and 2, of mu receptors to mu1 and mu2 and finally of kappa receptors to kappa1-3 (5, 6).

Opioids have been initially discovered in the hypothalamic-pituitary portal circulation (7). Subsequent studies however revealed their presence in other areas of the central nervous system (CNS), the peripheral nervous system and autonomic ganglia, and in a number of normal and neoplastic tissues (8). Thus, the role of the opioid system, initially implicated in the transmission of nociceptive stimuli, has been extended in a number of functions including neuro-immunomodulation, cell growth and apoptosis, tissue remodeling and development, and secretion. In this respect, opioids appear to be a ubiquitous modulator of cell development, differentiation and function, acting, in most cases, as negative modulators.

In addition to endogenous opioid peptides, a number of food-derived opioids have been identified, from different proteins including hemoglobin, gluten, and caseins. Both alpha and beta caseins include peptides with a potent opioid activity (see 9, for a discussion). These peptides have been tested and found to decrease breast and prostate cell growth (10, 11). A very potent opioid pentapeptide, derived from human alpha$_{S1}$-casein (9), with remarkable antiproliferative activity in different systems, including breast (9, 12), prostate (11), bladder (in preparation), kidney (13, 14), and different cells of hemopoietic origin (in preparation), was identified by our group. It was named alpha$_{S1}$-casomorphin, and its structure is Tyr-Val-Pro-Phe-Pro-NH_2 (9). Furthermore, a number of peptides, identified mainly in the central nervous system, including endomorphins and nociceptin/orphanin FQ and a possible new opioid receptor, named (opioid receptor relared 1 (ORL-1) (reviewed in 15) opened new ways in opioid research.

Somatostatin (SST), a naturally occurring deka-tetrapeptide, is produced mainly in the hypothalamus and the pancreas, but it has also been identified in a variety of normal and cancer tissues (16). Two main forms of somatostatin are present in biological fluids: Somatostatin 14 and its precursor but active form somatostatin 28 (17). The biological action of somatostatin is mediated through specific receptors, belonging to the seven-loop transmembrane receptor superfamily (18). Five different

receptors (named SSTR1-5) have been cloned and pharmacologically characterized in a variety of tissues (18). Somatostatin, initially isolated also in the pituitary, was further identified in a number of CNS structures, and in peripheral organs. Its negative modulatory actions indicates also that somatostatin system (as the opioid system) is a major player in inhibiting a number of cellular processes including growth, differentiation, secretion and apoptosis, as well as antinociception (19). In addition to somatostatin, a novel dekatetrapeptide (cortistatin) has been isolated, providing an enlargement of the somatostatin system, while a specific receptor of this peptide has been proposed recently (20). Somatostatin therefore may be an important regulator of cell proliferation and differentiation (21-24).

Both opioids and somatostatin have been reported to affect the growth of a variety of human cancers (see 8, for review, 25). A number of studies indicate that possible homo- and/or hetero-dimerizations of membrane receptors may occur (see 26, for an example). Based on that observation several studies have proposed possible interactions of the opioid and somatostatinergic system (27). In the present review, we will analyze the current knowledge concerning opioid and somatostatin receptors, signaling events occurring after ligand binding, and possible levels of interaction between the two systems. Possible implications of such interactions for cancer diagnosis and therapy will also be discussed briefly.

3. MOLECULAR BIOLOGY OF OPIOID AND SOMATOSTATIN RECEPTORS

Opioid receptors have been identified by radioligand binding in 1973 (28-30), while the first endogenous opioid peptides (Met^5- and Leu^5-enkephalins) have been isolated in 1975 (7), followed by the identification of β-endorphin (31), and the subsequent isolation of dynorphin (32, see 33, for a recent review). Soon after their discovery, opioid recptrors have been subdivided in to three classes delta, mu and kappa by pharmacological studies (34) and by the use of selective radioligands (35). Opioid receptors have distinct brain regional distributions (36) and dissimilar pharmacological properties (34). A common finding for all opioid receptors is the change in receptor densities and post-receptor adenylate cyclase activity, after agonist application, which could be responsible for the development of tolerance and dependence (37-40). Delta -opioid receptors were cloned in 1992 (41, 42), followed by the cloning of mu and kappa receptors (43-47). Sequence analysis of these cloned opioid receptors demonstrated that they belong to the superfamily of G protein-coupled receptors (GPCR), and share about 60% homology (48).

Somatostatin acts through high-affinity plasma membrane receptors (termed SST receptors, SSTRs), which were first described in 1978 (49). Subsequent studies using a variety of techniques showed SSTR expression in various densities in brain, gut, pituitary, endocrine and exocrine pancreas, adrenals, thyroid, kidneys, and immune cells (reviewed in 50, 51-55). The existence of more than one SSTR class was first proposed based on differential receptor binding potencies and actions of SST-14 and SST-28 in brain, pituitary, and islet cells (56, 57), a discovery confirmed, by molecular cloning of the five distinct SSTRs (reviewed in 18, 51, 53, 58), encoding proteins of 356 to 391 amino acid residues, with a sequence homology between 39–57%. Somatostatin receptors belong to the GPCR superfamily as opioid receptors. As many others GPCR (see 59, for a review), SSTRs undergo agonist ligand-induced internalization (see 25, for a recent review). In general, the mechanism and route of internalization of SSTR-agonist complexes involve aggregation of the hormone receptor complex in specialized areas of the membrane, followed by internalization of the hormone-receptor complex via clathrin-coated, as well as uncoated, pits.

Opioid and somatostatin receptors share a structural homology and were proposed to derive from the same ancestor gene (3, 54).

4. SIGNALING OF OPIOID AND SOMATOSTATIN RECEPTORS

As stated above, both opioid and somatostatin receptors belong to the GPCR superfamily. They are both linked to Go/i inhibitory subunit of adenylate cyclase (reviewed in 60, 61). Opioids as well as somatostatin were reported to inhibit cAMP formation, after ligand binding, and to decrease Ca^{+2} influx, through membrane slow Ca^{+2} channels activation (62). In recent years, however, a better understanding of the post-receptor events of opioids and somatostatin has emerged.

Opioid post-receptor signaling involves activation of MAPK/ERK and PI3K/Akt pathways (63-66). Morphine was found to induce the expression of the Fas protein, a receptor on the cell surface that triggers the cell's suicide by apoptosis when it binds to its ligand, FasL leading to caspase activation (67). Morphine was also found to promote apoptosis by tilting the ratio balance of Bax and Bcl-2 (68, 69). Apoptosis induced by morphine in cells of the immune systeme, is mediated by Fas/FasL, and over-expression of p53 and Bax (70). Opioid- induced nitric oxide (NO) production which in turn promotes apoptosis through accumulation of p53 was also described (71), however in other systems, opioids inhibit, in a direct manner, the activity of NOS (72).

Somatostatin receptors elicit also their cellular responses through G-protein-linked modulation of multiple second-messenger systems including adenylyl cyclase, Ca^{2+} and K^+ ion channels, Na^+/H^+ antiporter, guanylate cyclase, phospholipase C, phospholipase A2, MAP kinase (MAPK), and serine, threonine, and phosphotyrosyl protein phosphatase (PTP) (reviewed in 51, 52-54). Native SSTRs are coupled to several subsets of K^+ channels rectifying inward K^+ current (73). Receptor activation of K^+ channels induces hyperpolarization of the membrane, rendering it refractory to spontaneous action potential activity, leading to a secondary reduction in intracellular Ca^{2+} due to inhibition of the normal depolarization-induced Ca^{2+} influx (53). In addition to this indirect effect, SSTRs act directly on

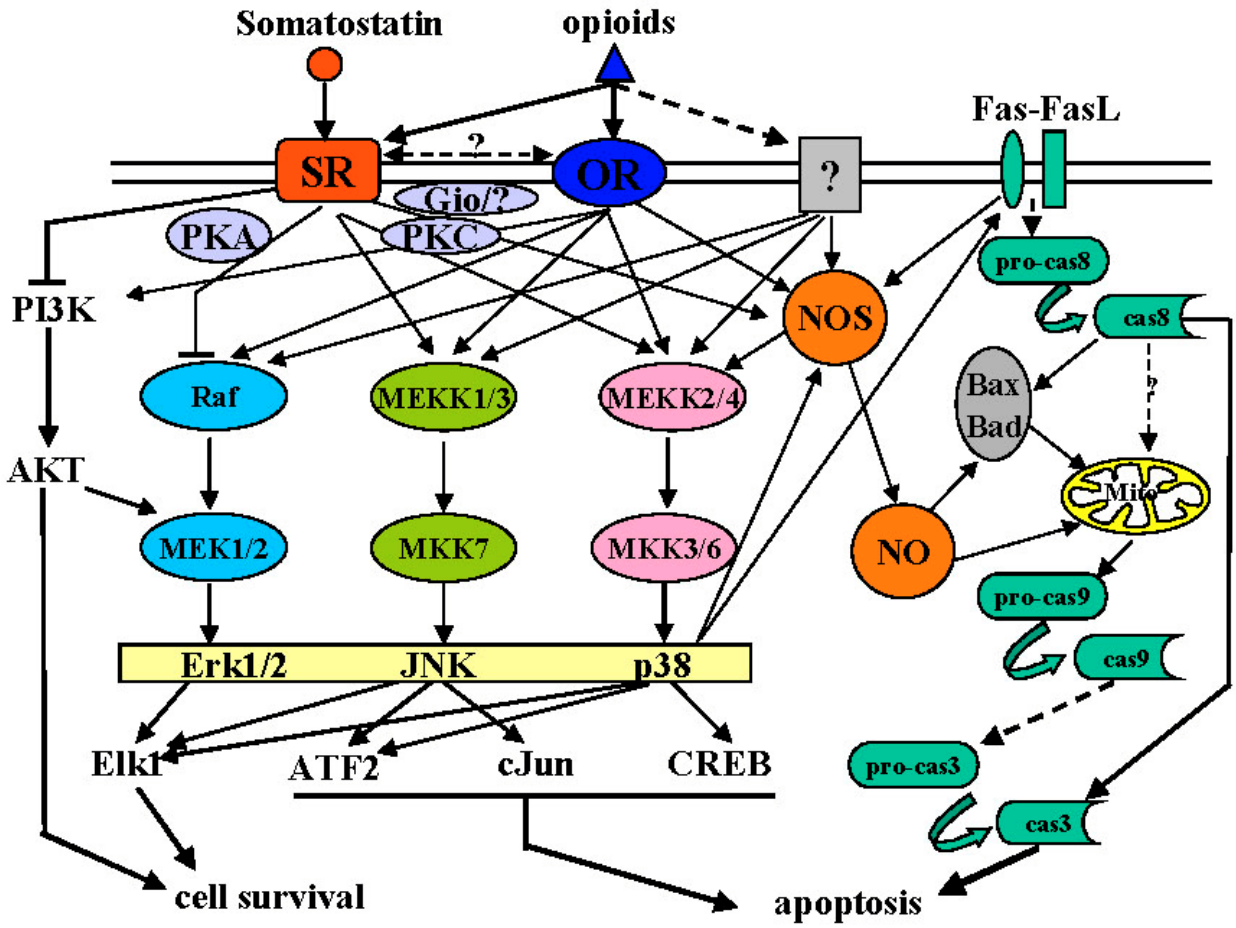

Figure 1. Signaling pathways triggered after binding of opioid or somatostatin to membrane receptors. As shown, binding can occur to selective cognitive receptors as well as in cross-reactive manner. ? indicate non-identified membrane binding sites that may be involved in receptor-mediated interactions.

high-voltage-dependent Ca^{2+} channels via Ga_{o2} protein (74), activation of a serine–threonine phosphatase (75), induction of cGMP-protein kinases (76) and a number of phosphatases (reviewed in 53). Other signaling pathways for endogenous SSTRs that have been described, include phospholipase A2-dependent stimulation of arachidonate production in hippocampal neurons, phospholipase C-mediated stimulation of IP3 formation in astrocytes and intestinal smooth muscle cells (77-79), and ERK signaling via a SHP-1-SHP-2-PI3K/Ras-Rap1/B-Raf/MEK pathway (80).

An overview of the current knowledge concerning opioid and somatostatin signaling pathways is presented in Figure 1.

5. OPIOIDS AND SOMATOSTATIN IN CANCER CELL PROLIFERATION

Endogenous opioids and opiate alkaloids have been implicated in a wide variety of pharmacological and physiological functions. In addition to their use as analgesics, opioids, appears to be important in the growth regulation of normal and neoplastic tissue. Opioid peptides and opioid receptors have been identified in a great diversity of human tumors (8). The effect of opioids on tumor growth is quite controversial since they inhibit as well as promote cell proliferation. Concerning solid tumors the effect of opioids is mainly inhibitory.

A number of studies have shown that opioids have a significant antiproliferative effect on cancer cells. Opioid alkaloids, endogenous opioid analogues and food derived opioid peptides inhibit in a dose dependent manner the proliferation of the human breast cancer cell lines T47D (10, 13) and MCF7 (81, 82), the hormone sensitive (LNCaP) and insensitive (PC3 and DU-145) prostate cancer cell lines (11), the opossum kidney cell line (13), the hepatoma cancer cell line HepG2 (unpublished data), small and non small lung cancer cells lines (83-86), KATO III and BALB/3T3 cells (87). B16/BL6 melanoma cells growth and metastases were also inhibited by morphine, while no effect was observed on HT-29 colon cells (88). Increased apoptosis and necrosis was observed on HT-29 colon adenocarcinoma (89), and lung cancer cells (85). A decade ago, we have further identified a potent pentapeptide named a_{s1}-casomorphin (9) that showed a remarkable inhibitory action on different cancer cell lines (breast, prostate and hepatoma). In addition we have reported the identification of another pentapeptide, named

receptorphin, with the sequence Tyr-Ile-Phe-Asn-Leu, conserved in the second transmembrane loop of the opioid receptor. This pentapeptide showed a potent antiproliferative activity, and specific opioid receptor binding characteristics (90). Opioids decrease cell proliferation and increase apoptosis in different cell systems (85, 87, 91). In such cases, after intra- and extracellular domains of opioid receptor destruction by a number of liberated proteolytic enzymes, and rupture of the membrane structure, receptorphin could be liberated, acting as a paracrine mediator of opioid actions. If such a mechanism occurs, opioid action could be potentiated after an opioid- and/or other inducers-related cell death. Indeed, it is tentative to assume that after an opioid mediated cellular death, receptorphin liberation might trigger a positive feedback loop, propagating the opioid effects to a number of adjacent opioid receptor-positive cells. This mechanism could possibly explain the time-lag found in opioid-related cell proliferation and apoptosis, reported in a number of malignant cell lines (see 10, 11, 13, 92, for examples). It is further interesting that, at the same position (second transmembrane segment) of the different types of the somatostatin receptor (SSTR-1 to –5) which present the greater homology with opioid receptors (2, 3), in different species, a peptide with the sequence Tyr-Ile(or-Leu)-Leu-Asn-Leu exists, presenting an homology in structure with receptorphin.

The inhibitory effect of morphine on T47D, MCF7 and MDA-MB-231 cells was not reversed by the opioid receptor antagonists such as naloxone (88, 92). In addition, in MCF7 and MDA-MB-231 cells, pertussis toxin and forskolin did not antagonise the effect of morphine indicating that there was no involvement of a typical receptor coupled signaling cascade involving Gi adenyl cyclase and protein kinase A (88). Furthermore, the action of opioids could be mediated through an interaction with intracellular (93) or membrane (94) steroid receptors.

Different synthetic opioid peptides and alkaloids exhibit also an inhibitory effect on cancer cell growth. D-Ala^2,D-Leu^5-enkephalin (DADLE), D-Ser^2,Leu^5-enkephalin-Thr^6 (DSLET) and ethylketocyclazocine (EKC) inhibited in a dose-dependent manner cell proliferation of breast (T47D) (92) and prostate cancer cells (LNCaP, PC3 and DU-145) (11). D-Ala^2,MePhe^4,Gly-ol^5-enkephalin (DAMGO), inhibited significantly MIA PaCa-2 pancreatic adenocarcinoma, HT-29 colon adenocarcinoma, and CAL-27 squamous cell carcinoma of the head and neck(89). Kappa opioid agonists also inhibited the growth of PC12 rat pheochromocytoma cell line (95).

The group of Zagon provided another interesting possibility of opioid-tumor cell interaction: Met^5-enkephalin, a naturally occurring opioid pentapeptide, named opioid growth factor (OGF) by this group, was found to decrease the growth of head and neck squamous cell carcinoma (96, 97), human neuroblastoma SK-N-SH (98), and human renal cancer cells (caki-2) (99). Met^5-enkephalin also depressed the growth of N115 murine neuroblastoma, SK-N-MC human neuroblastoma, and HT-1080 human fibrosarcoma (100). An inhibitory effect of OGF was also observed on pancreatic cancer cells (BxPC-3), colon (HT-29) and head and neck (CAL-27) cancer cells (89), due to an effect on cell cycle, an increase in the cells in the G_0/G_1 phase with a compensatory reduction in cells in S and G_2/M phases. The effect was antagonised by the opioid antagonist naltrexone. It is interesting to note, at this point that growth arrest in G1 phase is not a constant effect of opioids on the cell cycle, as we have reported that, in the breast, opioid agonists increase the number of cells arrested in the G2/M phase (101). It seems therefore, that the cell cycle effect of these agents is tissue- (and perhaps receptor-) specific. The same group reported the identification of a new receptor, mediating the action of OGF, which was termed Opioid Growth Factor Receptor (OGFR) (reviewed in 102). This new site binds selectively Met^5-enkephalin, but not other opioid peptides or somatostatin analogs. It has a specific cellular distribution, representing a transcription factor rather than a membrane receptor, and processes no homology to other opioid or orphanin receptors, while it acts as a specific growth modulator of normal and neoplastic cells. Therefore, this new receptor could represent a new way of action of some endogenous opioids.

In contrast to the inhibitory effect of opioids on different cancer cells as described above, there are several cases mainly concerning cells of the immune or nervous system in which growth is stimulated by opioids. Met^5 enkephalin has dual effects on tumor cell growth. This peptide exerted an inhibitory effect in SK-N-MC human neuroblastoma cell line; in contrast, it stimulates the growth of U-373 MG human astrocytoma cell line (103). Beta casomorphine-5, and DAMGO stimulated neurite outgrowth, while DPDPE and U-50488, a kappa agonist, had no effect (104). In addition, beta endorphine and beta endorphine–like peptide increased the growth of the Jurkat T lymphoblastoid cell line, or of myeloid cell lines (105) and T lymphocytes (106). The immunoregulatory processes of opioids include cellular adhesion, migration, and interaction with cytokines. Those actions are mediated through specific opioid receptors expressed in different development stages and immune cell subpopulations. In multiple works those effects are not mediated via classical opioid receptors. Several studies suggest the interaction of opioids with adrenergic receptors (107-116). Physical interaction between opioid and adrenergic receptors, provide a novel mechanism for modulation of receptor function. Evidence suggests that somatostatin may also influence the immune system and its receptors are expressed in human lymphoid organs and cells (117). Somatostatin can regulate various immune functions including inhibition of lymphocyte proliferation, immunoglobulin synthesis, and cytokine production (118).

Somatostatin and somatostatin receptors have also been identified in a number of tumors, and tumor cell lines. Pituitary and neuroendocrine tumors, renal, breast, lymphomas, meningiomas, gliomas, neuroblastomas and pheochromocytomas express different subtypes of somatostatin receptors (reviewed in 25). $SSTR_2$ is the subtype more constantly expressed in these tumors. This predominant expression of SSTR2 in human tumors forms the basis for the successful clinical application of

octapeptide SST-analogs such as octreotide and lanreotide (synthetic SST analogs) in controlling symptoms related to hormonal hypersecretion in patients with GH-secreting pituitary adenomas, islet cell tumors, or carcinoid tumors (119, 120), and rases the possibility to visualize SSTR-positive tumors using radiolabeled analogs. SSTR subtype expression patterns in human neuroendocrine tumors may be very important for the development of SSTR-targeted radiotherapy or chemotherapy.

Many tumor cell lines have been shown to be a rich source of membrane SSTRs, e.g., AtT-20 pituitary tumor cells, hamster insulinoma, Rin m5F islet tumor cells, AR42J and Mia PaCa pancreatic tumor cells, human breast cancer, neuroblastoma, glioma, and leukemic and myeloma cell lines (see 50, 51, 52, 54, for reviews). In general, activation of SSTRs results in a growth inhibition of lymphocytes, inflammatory, intestinal mucosal, and bone precursor cells (reviewed in 53). As indicated above, the pronounced ability of SST to block regulated secretion from many different cells is due in part to SSTR-induced inhibition of two key intracellular mediators, cAMP and Ca^{2+}. This effect appears to be mediated via a G-protein-dependent inhibition of exocytosis and is induced through SST-dependent activation of the protein phosphatase calcineurin (121). In contrast to the antisecretory properties of SST, its antiproliferative effects were recognized lately (122). The antiproliferative effects of SST have since been demonstrated in normal dividing cells, e.g., intestinal mucosal cells, activated lymphocytes and inflammatory cells, as well as *in vivo* in solid tumors, e.g., DMBA-induced or transplanted rat mammary carcinomas, and cultured cells derived from both endocrine and epithelial tumors (pituitary, thyroid, breast, prostate, colon, pancreas, lung, and brain) (reviewed in 53).

The antiproliferative effects of SST involve cytostatic (growth arrest) and cytotoxic (apoptotic) actions and are mediated (i) directly by SSTRs present on tumor cells and (ii) indirectly via SSTRs present on nontumor cell targets to inhibit the secretion of hormones and growth factors that promote tumor growth and to inhibit angiogenesis, promote vasoconstriction, and modulate immune cell function (reviewed in 53). Several SSTR subtypes and signal transduction pathways have been implicated, with SSTR2 being the major subtype implicated in growth arrest (27). The mechanism of the direct antiproliferative action of SSTR activation implies the activation of a number of intracellular mediators, including PTP activation (123-125), modulation of MAPK activity (24, 123, 126-129), and up-regulation of the cyclin-dependent protein kinase inhibitor p21 cip1/WAF1 (130). In addition to its cytostatic effect, SST induces apoptosis, in a dose-dependent manner (131), through PTP activation (132). SSTR3 is the subtype mainly involved in this phenomenon (133), and could be blocked by pre-treatment with pertussis toxin or orthovanadate, suggesting the mediation of pertussis toxin-sensitive G proteins, PTP, and activation of the proapoptotic bax protein (133).

Somatostatin may also directly control cell growth by inhibiting the synthesis and/or the secretion of autocrine growth factors, cytokines and hormones involved in the proliferation of tumour cells. It may influence the synthesis and/or the secretion of these factors and/or down-regulate the expression of their receptors leading to disruption of proliferative autocrine loops. At the cellular level, blockade of secretion by somatostatin is mediated through inhibition of Ca^{2+} and cAMP production. Additionally, somatostatin can directly interfere with the exocytotic machinery by inhibiting the protein phosphatase calcineurin (revewed in 134). The specific SSTR subtypes involved in these processes and the underlying mechanisms remain to be investigated. Recent results using the patch-clamp technique indicate that in human neuroendocrine gut tumour cells, somatostatin and octreotide inhibit L-type voltage-dependent calcium channels with the same amplitude suggesting that at least SSTR2 and 5 may be involved in inhibition of Ca^{2+} influx and thereby inhibition of tumour-produced neurotransmitters and hormone (135). For example, insulin-like growth factor-1 (IGF-1) produced by hepatocytes through GH-dependent and -independent mechanisms is an important modulator of many neoplasms (136) and the octreotide negatively controls serum IGF-1 level as a result of an effect on GH secretion, probably via SSTR2 and SSTR5, and a direct effect on IGF gene expression (137, 138).

Somatostatin and analogues can also indirectly control tumour development and metastasis by inhibiting angiogenesis *in vitro* and *in vivo* (139). Overexpression of peritumoral vascular somatostatin receptors with high-affinity for somatostatin and octreotide has been reported in human primary colorectal carcinomas, small cell lung carcinoma of the lung, breast cancer, renal cell carcinoma and malignant lymphoma and this expression appears to be independent of receptor expression in the tumor (140). Somatostatin can act as an antiangiogenic factor by inhibiting endothelial cell growth and monocyte migration invasion and SSTR2, SSTR3 or SSTR5 might be involved in these effects (141, 142).

Reviewing the current literature, it is obvious that SSTR1, 2, 4, and 5, play an important role on cell secretion and proliferation while SSTR3 shows unique cytotoxic effects (130, 132, 133). Many tumor cells co-express SST or cortistatin along with SSTRs (143, 144), suggesting that the presence of a high density of SSTRs as well as SST may reflect an attempt by the tumor to activate the endogenous SST system for autocrine/paracrine modulation of the neoplastic response (145).

6. INTERACTION OF OPIOID AND SOMATOSTATIN SYSTEM

6.1. Receptor-mediated actions

The above brief review indicates that the opioid and the somatostatin systems are involved in the modulation of several physiological processes such as the neuroendocrine function, the immune response, the stress response and analgesia. There are several studies in which the action of somatostatin is modulated by opioids and vice-versa indicating an interaction of the two systems: i. A paracrine effect of beta-endorphin on gastric mucosa was

observed. Indeed, the somatostatin release in to the gastric lumen, followed by the suppression of gastric acid secretion, was increased by beta endorphin (146), an effect that was inhibited by naloxone and therefore mediated by opioid receptors. ii. The dose-related somatostatin effect on body temperature of the rat was found to be inhibited by opioid antagonists. Hyperthemia was induced by low doses of somatostatin and was mediated via mu opioid receptor while hypothemia was induced by high doses of somatostatin via kappa opioid receptors (147). iii. During insulin tolerance test, oxytocin release was found to be inhibited by somatostatin (148), an effect abolished by naloxone and therefore either mediated by opioid receptors or being the result of independent pathways possibly interacting down stream. iv. The cyclic somatostatin analog D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (CTP) also was found to interact with mu opioid receptor (149) and to antagonize the analgesic effect of selective mu agonists in the guinea pig ileum and mouse vas deferens (150). The above interactions suggest that somatostatin could act via opioid receptors. For the first time an interaction of opioids with the somatostatin receptor system was described by our group, concerning the inhibitory effect of morphine on the growth of T47D cells (27), that was found to be mediated via the SSTR2. Binding to SSTRs was also found to mediate partially the effect of a number of casomorphins (alpha and beta) (10) in the same cell line, as well as the effect of a_{s1}-casomorphin (9) and receptorphin (90). We have also found such interactions in different cancer systems such as the renal OK cells, where, in the absence of mu opioid receptors the inhibitory effect of morphine was mediated by somatostatin receptors (13), and in hepatocellular cancer-derived cells (unpublished data). Recently, Stirweiss *et al*. published that the cyclic pentapeptide, cCD-2 (Tyr-cyclo[d-Orn-Tyr(Bzl)-Pro-Gly]), derived from beta-casomorphin-5, inhibited the growth of SH-SY5Y cells independently of opioid receptors. cCD-2 possessed only low affinity for mu- receptors and its action was exerted by specific binding to somatostatin receptors (SSTR1) and stimulating the activity of protein tyrosine phosphatases (151). Finally, two years ago it was reported that the SSTR2 and the mu opioid receptor (MOR1) heterodimerize in human embryonic kidney 293 cells without altering the binding or coupling properties of the receptors (26) but affecting the phosphorylation and desensitization of the two G protein-coupled receptors.

A phenomenon occurring after somatostatin and opioid application is the resistance of cells to their action, due to receptor internalization (reviewed in 25, 39, 60). This effect was recently reported to occur after heterodimerization of opioid and somatostatin receptors, providing new insights of an interaction between the two systems.

6.2. Non-receptor interactions

Opioids can enter into the cell by a number of different ways. Opiate alkaloids being hydrophobic can cross the cell membrane and enter directly into the cytoplasm. In contrast for neuropeptides receptor-mediated endocytosis is required. For example beta endorphin has be found to interact with intracellular binding sites even though it has been applied extracellularly (152) while enkephalin analogs were found to recycle to the culture medium after endocytosis of the ligand-receptor complex and lysosomal receptor degradation. (38). Studies that we performed on T47D cells showed a direct interation of opioids with the nitric oxide (NO) system. Kappa-opioid agonists (ethylketocyclazocine and a_{s1}-casomorphin) decreased the release of NO_2^- / NO_3^- in a time and dose dependent-manner by directly inhibiting NOS activity (72).

Although several lines of evidence have reported interactions between opioids and somatostatin receptors the basis for this has not been well explored. Direct receptor-receptor interactions could account, in part, for opioid-somatostatin cross-talk like for opioid-adrenergic interaction. Physical association between somatostatin and opioid receptors could modulate receptor function and its investigation is of great interest in tumor cell biology. The molecular mechanisms that mediate these synergistic interactions need also to be characterized. Figure 1 provides a current scheme of intracellular pathways involved in opioid and somatostatin signalling. As shown, both agents bear similar signalling pathways often resulting in common functions such as growth arrest, apoptosis and inhibition of secretion. It is therefore plausible to suggest that interaction between the two systems might occur at this level as well and investigations could lead to precious information.

7. CLINICAL IMPLICATIONS AND CONCLUSIONS

This review was designed to examine the role of opioids and somatostatin in cell survival, and the underlying cellular mechanisms of action, with an emphasis to possible interactions between opioid and somatostatin system. Opiate alkaloids, such as morphine, and their endogenous opioid peptide counterparts have been implicated in a variety of pharmacological and physiological functions. They appear to be important in the growth regulation of normal and neoplastic tissue. Opiates, such as morphine, have been used extensively in the clinical management of pain due to their potent analgesic effect. As the aged population is increasing so is the occurrence of cancer, and it appears that the use of morphine is likely to be increased. Thus, it is important to evaluate the biological effects of opiates on cancer cells. In addition, over the past decade, antiproliferative effects of somatostatin and analogs have been reported in many somatostatin receptor-positive normal and tumor cell types. Regarding the molecular mechanisms involved, somatostatin (or its analogues mediate their action through both indirect and direct effects. Somatostatin acts through five somatostatin receptors (SSTR1-5), which are variably expressed in normal and tumor cells. *In vitro* and *in vivo* data demonstrate anti-neoplastic (anti-proliferative) effects of somatostatin analogues on the cancer cells. Although SST-treatment was efficiently applied in cases of GH-secreting tumors, its application in other pathologies remains controversial and conflicting results have been reported (25), due to the same phenomena of receptor internalization and desensitization.

The current state of knowledge can not provide information about the possible use of both agents in

clinical applications. Extensive research focused on the influence of opioids and somatostatin on tumour growth, with emphasis on the biological mechanisms involved in the antineoplastic activity, could provide new molecular targets as analgesic adjuvants. Inhibition of cell growth and induction of apoptosis may be one mechanism of opioid and /or somatostatin cytotoxicity. In addition, the fact that opioids and somatostatin could be locally found, raises the possibility to modulate their local production and therefore interfere to cancer cell growth locally. Alternatively, by gaining knowledge on the signalling mechanisms implicated in the SST and opioid action, one could design specific agents, which could stimulate and/or inhibit specific signalling molecules, mimicking therefore the effects of either agent. Therefore, the following years, will be fruitful in this type of research, providing beneficial results for patients. Further, these studies will provide evidence of interaction between the opioid receptor subtypes and receptors of other G-protein-coupled receptor families. These various levels of interaction provide a mean by which the cellular response to opioids or somatostatin can be exquisitely controlled and modified by both physiological status and disease. Greater understanding of these interactions will provide the conceptual basis for future therapeutics with enhanced efficacy and with greater cellular and functional specificity.

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