Review paper

Opioid peptides and opiate alkaloids in immunoregulatory processes

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Abstract

Among the various non-neuronal cell types known to express and utilize neuropeptides, those of the immune system have received much attention in recent years. In particular, comparative studies in vertebrates and invertebrates have shown that endogenous opioid peptides are engaged in receptor mediated autoregulatory immune and neuroendocrine processes. The majority of these immune processes are stimulatory, as determined by their effects on conformational changes indicative of immunocyte activation, cellular motility, and phagocytosis. Endogenous opioid peptides form an effective network of messenger molecules in cooperation with cytokines, opiate alkaloids, and certain regulatory enzymes (neutral endopeptidase 24.11). Peptide-mediated immunostimulatory effects observed in this system are operationally counteracted by the inhibitory effects of morphine and related opiates. Opioid/opiate signaling processes are mediated by several types of receptors with different degrees of selectivity. Among them the recently identified, opioid insensitive μ_3 receptor deserves attention on account of its specificity for opiate alkaloids.

Key words: opioid peptides, morphine, leukocytes, immunoregulation, invertebrate immune cells, G protein, neutral endopeptidase.

Introduction

There is a growing realization in biological sciences that neuropeptides, formerly thought to be produced by a select group of neurons, are found in a variety of non-neuronal cells. Comparative studies in vertebrates and invertebrates [1-4] have highlighted the biological importance of immunoactive cells which make use of neuropeptides for autoregulatory control as well as the bidirection exchange of information between the immune system and the neuroendocrine system. Along with cytokines, long considered to be the primary messenger molecules of the immune system, endogenous opioid peptides form an effective network of communication. Previous studies demonstrated the presence of opioid peptides and opiate alkaloids in both invertebrate and vertebrate nervous and immune systems along with highly selective types of opioid receptors [5-9].

Functional aspects

Biological activities associated with proinflammatory processes that have been analyzed in detail in higher invertebrates as well as vertebrates

include cellular adherence, locomotory activity and conformational changes of immunocompetent cells (see Scharrer and Stefano, 1994). Adherence of mammalian neutrophils and invertebrate immunocytes is enhanced by opioid neuropeptides (see [10, 11]). In human neutrophils substance P (10-11 M) modulates the expression of two cell-surface adhesion molecules, Mo 1 and LAM-1 [12]. Polymorphonuclear leukocytes (PMN), lymphocytes, and monocytes as well as invertebrate immunocytes in addition to random movements (chemokinesis), show migratory behavior directed toward sites of inflammation or antigenic challenge (chemotaxis). In an vitro study by Heagy [13-18] and colleagues, T-lymphocytes exhibited chemotaxis in the presence of a concentration gradient of Met-enkephalin or b-endorphin. Synthetic enkephalin analogs, including DADLE (D-Ala2-D-Leu5-enkephalin), DPDPE (D-Pen2-D-Pen5-enkephalin), and DAG0 (D-Ala2-MePhe4Gly5-enkephalin) stimulated the T-cells to a lesser extent, a finding that will be discussed below. *In vitro* tests with immunocytes of *Mytilus* and *Leucophaea* showed chemotactic movements and the formation of large cellular clumps after opioid peptide exposure along with the same poor reaction to DADLE (see [19-21]).

Furthermore, changes in the activity of human and invertebrate immunocytes are preceded by characteristic conformational alterations. Prior to the onset of locomotory behavior, stimulated by opioid peptides, the following signs indicative of cellular activation are observed. Mammalian and invertebrate cells in the inactive condition are more or less rounded, upon activation show an increase in cellular size and surface area and/or the formation of pseudopodia. D'Ala2-Met5-enkephalinamide (DAMA) is most effective in inducing these changes. DADLE, the compound most closely related to DAMA, is not as potent in mammalian and invertebrate immunocytes (see [19-25]). The distinctly lower effectiveness of DADLE in both human and invertebrate immune reactions is in contrast to the situation in the mammalian nervous system where no discrepancy in the binding potency of Met-enkephalin and Leu-enkephalin has been observed (see [10]). Taken together, these studies demonstrate that opioid peptides exhibit, in general, immuno-stimulatory actions.

Immunocyte opioid receptors

Deltorphin I, a naturally occurring opioid peptide isolated from amphibian skin, has the ability to modulate both human and invertebrate immuno-regulatory activities in a manner quite similar to Met-enkephalin [26]. Its binding and pharmacological studies also have provided evidence for a special subtype of δ opioid receptor δ_2, sensitive to naltrindole antagonism [3, 4, 10, 27], on human and invertebrate immune cells [26]. The results obtained with deltorphin I support the view that the special role played by endogenous Met-enkephalin in immunobiological activities of vertebrates and invertebrates is mediated by a special subtype of delta opioid receptor, δ_2. It is also of interest to note that both the invertebrate immunocytes and human granulocytes thus have a δ_1 and δ_2 receptor.

Opiate alkaloids

While opiate alkaloids, e.g., morphine, are not opioid peptides they do deserve special attention within the context of this review for several reasons. First, unlike antinociceptive mechanisms, opiate alkaloids and opioid peptides initiate different immunocyte behaviors [10]. As noted above, opioid peptides may be generally regarded as immunocyte stimulatory and/or activating ligands whereas morphine, noted first by Wybran *et al.* [28], is inhibitory. Secondly, confusion exists in the scientific literature as to the proper terminology for these ligands, e.g., opioid alkaloid and opiate peptide. Thirdly a novel opiate alkaloid and opioid peptide insensitive receptor, namely μ_3, has been demonstrated which does not recognize μ-opioid ligands [6, 29-37]. Lastly, opiate alkaloids appear to be naturally occurring substances found both in mammals and invertebrates (see [6-10, 29, 32, 33, 35-42]).

The above reports demonstrate that morphine and codeine substances were found in the pedal ganglia, hemolymph and mantle tissues of the mollusc *Mytilus edulis* [38, 39]. The pharmacological activities of the endogenous morphine material resemble those of authentic morphine. Both substances were found to counteract, in a dose dependent manner, the stimulatory effect of tumor necrosis factor (TNF)-α or interleukin (IL)-1α on human monocytes and *Mytilus immunocytes*. The immunosuppressive effect of this opiate material expresses itself in a lowering of chemotactic activity, cellular velocity and adherence as well as making active immunocytes inactive (rounded). These pharmacological effects of morphine on immunocytes are consistent with those actions attributed to opiates reported in the literature (see [10]). Indeed, it has been surmised that morphinergic transmission may regulate the down regulation of immune activation (see [6-8, 31, 32, 35-37, 40, 41, 43-49]).

Along with the opiate substances found in animal tissues came the recent discovery of a specific high-affinity and novel receptor site (μ_3) for opiate alkaloids on human monocytes as well as *Mytilus immunocytes* [29, 30, 50]. A variety of opioid peptides, tested by two methods, were found to be ineffective in displacing specifically bound

3-dihydromorphine. By contrast, the opiate alkaloid m ligands were potent and κ ligands dynorphin 1-17 and ethyl-keto-cyclazocine (EKC) were weak. Based on this novel displacement information we assigned this opioid peptide insensitive and opiate alkaloid sensitive site the name μ_3 [29]. Studies demonstrate that human granulocytes also contain the μ_3 subtype opiate receptor mediating inhibition by morphine and other opiates of cytokine-induced activation and chemotaxis [51-53]. Furthermore, in the presence of NaCl (50 mM) plus the GTP analog GppNHp (100 μM), there was a significant decrease in specific high-affinity binding of the agonist ligand ^{3}H-morphine. The influence of the GTP analog GppNHp on binding indicated that the granulocyte receptor was linked to a G protein [52]. The discovery of this receptor site mediating opiate effects was first found in an invertebrate and then in man, again demonstrating the value of the comparative approach [54].

It is important to note that the cloning of delta, mu and κ receptors has now been accomplished [55-58]. As a result of these and other studies now published it will be possible to study individual receptors regarding their effector coupling, pharmacological characteristics, regulation of expression as well as their regional distributions. Important information will also become available regarding their evolvement.

Biomedical significance

The biomedical importance of a well balanced immunoregulatory system is illustrated by the consequence of interference with its normal operation (see [27, 59]). Recent studies have shown that immunosuppression effected by neuropeptides may determine the course of certain diseases caused by parasites or viral infection [60-66]. There is experimental evidence supporting the concept that in schistosomiasis the parasite escapes detection and an effective immune reaction in the host by using the same signal molecules operating in the human immune and autoimmunoregulatory system. The release of ACTH by the adult parasite, and its conversion to a-MSH by NEP on human PMNs, inactivates specific defense cells and thus interferes with proper surveillance. Furthermore, the human immunodeficiency virus appears to have the ability to stimulate the production of ACTH by human immune cells [65] thus creating a scenario similar to that described for the parasitic worm. It is becoming quite clear that these peptides play important immunoregulatory roles, actions that include neuroimmune as well as autoimmunoregulatory mechanisms.

Furthermore, recent work has elucidated the enzymes and other regulatory phenomena involved with morphine biosynthesis and their regulation [31, 34-40, 44, 45, 67-73]. It is important to note that substances of abuse impact this system and appear to work, in part, by releasing morphine from cells that make it [7, 31, 34-36, 74-76].

Conclusions

I surmise that we are just scratching the surface of the involvement of neuropeptides and opiate alkaloids in immune and vascular regulation. This review has mainly emphasized the roles of opioid and related peptides, clearly leaving out many other types of peptidergic signaling compounds. For the most part, it is the opioid/opiate "story" that has emerged in recent years. Thus, we will undoubtedly look forward to the activities and presence of other peptidergic signaling molecules being used both in autoimmunoregulation and neuroimmunoregulation. Given the presence of many of these signaling molecules in neuroendocrine structures the field of neurosecretion will grow to include, if it hasn't already done so, neuroimmunology.

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