〔Review〕

Trends in Physiological Role of Opioids in Psychoneuroendocrine-Immune Network

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Abstract: Extensive evidence suggests that opioid influences on the immune response are mediated through opioid receptors on the surface of immune cells. In addition, the binding of opioid agonists to centrally located opioid receptors appears also to alter immune activity through interactions with the neuroendocrine and the nervous systems. Finally, other findings summarized in this review provide evidence consistent with the hypothesis that under stressful stimuli, the immune system is altered through the interrelations among the neuroendocrine system, the sympathetic nervous system and central opioidergic pathways.

Key words: Immune system, Opioids, Central nervous system, Neuroendocrine system, Sympathetic nervous system, Stress

It is now accepted that the immune response cannot be considered anymore as an isolated entity separated from the psychosomatic web that governs the entire body. Evidence is accumulating which indicates that the immune system creates a triangle of reciprocal interactions with the endocrine system and the nervous system that contributes to the maintenance of the organism's homeostasis. Opiates have been shown to participate as messengers between this triad of interrelated networks. Thus, the relationship between opiates and the immune system have attracted the interest of the scientific community in the past few years.

The ultimate test to find out whether the function of a given immune system remains intact, consists of measuring its capability to protect the body from pernicious effects caused by exogenous pathogens. Intravenous opiate use has long been related with higher susceptibility to infectious diseases. Early studies by Hussey and Katz (1950), Louria et al (1967) and Cherubin (1971) noted increased incidence of a variety of infectious diseases (i.e., bacterial endocarditis, tetanus, sepsis, malaria, and hepatitis) in drug addicts but those effects were exclusively

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Abbreviations **AFC**: antibody forming cells, **AHT**: anterior hypothalamus, **ATP**: adenosine triphosphate, **CNS**: central nervous system, **ConA**: concanavalin A, **CRF-ACTH**: corticotropin releasing factor-adrenocorticotropin hormone, **CRH**: corticotropin releasing hormone, **CTAP**: D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2, **DAMGO**: [D-Ala, N-MePhe, Gly-ol] enkephalin, **dBcAMP**: dibutyril cAMP, **DP**: double positive, **DPAT**: 2-(di-n-propylamino) tetralin, **DTH**: delayed-type hypersensitivity, **FS**: foot shock, **GTP**: guanosine triphosphate, **GVH**: graft vs. host, **HPA** axis: hypothalamus-pituitary-adrenal axis, **IL**: interleukin, **LC**: locus coeruleus, **NE**: norepinephrine, **NK**: natural killer, **nor-BNI**: norbinaltorphimine, **PAG**: periaqueductal gray matter, **PHA**: phytohemagglutinin, **PSY**: psychological, **RU-486**: mifepristone, **SIA**: stress-induced analgesia, **SNS**: sympathetic nervous system, **SRBC**: sheep red blood cells, **U-50, 488H**: trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl) cyclohexyl)-benzenacetamide

attributed to the special conditions derived from the drug paraphernalia. We had to wait for more than a decade to find a report in which opioids and immunological phenomena were related: Miller et al (1979) suggested that the higher incidence of chronic hepatitis in drug addicts was caused by certain immune disorders. A recent report by Savona et al (1985) connected drug abuse with thrombocytopenia. Moreover, with the onset of the epidemic of HIV infection, a concern about the immunologic abnormalities observed in parenteral drug abusers has grown dramatically. As the development of opportunistic infections related with the partial inhibition of cell-mediated immunity is a distinctive feature of AIDS, studies on the effects of opiates, a possible cofactor, in the pathogenesis of infections caused by opportunistic microorganisms, have become a priority in the context of the intravenous drug abusing population. Because there are numerous difficulties in reporting controlled studies of opiates in humans, there is a need for additional animal models to explore the alterations due to opioid administration upon the immune system.

Since several review articles (Schuster, 1988; Bryant et al 1990; Lee, 1990; Bryant and Holaday, 1993) have dealt extensively with the regulatory effects on the immune response exerted by exogenous opioids, we have limited the present review to the underlying mechanisms that can be implicated in such alterations. Although different interpretations can be stated referring to the mechanisms involved in the immune changes derived from the presence of opioids, all of them can be included in two main types: direct and indirect mechanisms.

When opioid binding sites were first demonstrated, it was generally thought that opioid receptors represent an homogenous group. This simplistic idea was soon abandoned when different endogenous opioid peptides were found, each of which has a distinct pharmacological profile towards opioid receptors. Since various experiments *in vitro* have shown evidence of immunological variations when opioids were added, the search for opioid receptors on the surface of immune cells have become of primary interest. On the other hand, a number of effects upon the immune system, not reproduced in *in vitro* experiments, observed after central administration of opioids, lead to the conclusion that opioids, through the interaction with central receptors, will activate other neurological pathways which may ultimately modify the immune response.

However, modern research not only deals with the mechanisms involved in opioid-mediated immunoregulation but is also trying to elucidate other questions of paramount importance: I) the role played by the several opioid systems activated under certain kinds of stress in the alteration of immunocompetence; II) the types of opioid receptors implicated in the immunomodulatory action of opiates; and III) how far other neurochemical pathways and neurotransmitters participate in the effects of opiates upon the immune system. The answers are far from clear, and further research is required. An attempt to present the state of current knowledge is the subject of this review.

DIRECT MECHANISMS

The occurrence of opioid-induced immunosuppression raises the possibility of the existence of opiate receptors on the surface of immune cells. In view of the importance of membrane receptors to explain the relationships among opioids and the immune system, several experiments were conducted to find evidence of direct opioid interaction with immune cells. Opioid binding sites on immune cells will not only explain the immunomodulatory role of opioids but also facilitate the search of explanatory reasons of other physiological phenomena derived from opioids administration. Moreover, since endogenous opioids are produced constitutionally or in response to stimulation by cells of the immune system, the search for the reciprocal connections is of primary interest.

1. Experimental evidence in vitro

There is accumulating evidence that certain populations of opioid receptors may be present on lymphocytes as shown by Wybram et al (1979) who demonstrated that the number of T lymphocytes forming rosettes rapidly with

SRBC (sheep red blood cells), a functional measure of T cell function used to quantitate the number of circulating T cells, was reduced by incubation with morphine, met-enkephalin, and dextromoramide. Naloxone, an opioid antagonist, reversed the decrease in the number of rosettes caused by opioids, indicating that these effects are mediated by opioid receptors. A clear indication for direct effects of exogenous opioids on lymphocyte proliferative responses has not yet been found. While proliferation responses have been shown to be inhibited in some studies (Rogers et al, 1992), others have reported stimulated or unaffected responses (Bocchini et al, 1983; Bayer et al, 1992). According to the report of Bryant and Holaday (1993) concentrations in the 100 μM range are required for consistent inhibition. In addition, Bussiere et al (1990) observed that μM concentrations of morphine directly suppress in a time-dependent manner the production of IgM by antibody-forming cells (AFC) in response to SRBC *in vitro* in a Mishell-Dutton culture system. Co-culture experiments demonstrated as well, that morphine did not inhibit antibody responses through the induction of suppressor cells of factors. In a similar study, Taub et al (1991) showed that not only morphine but also DAMGO, a μ-selective agonist, and U50,488 or U69,593, κ-selective agonists, when added with antigen (SRBCs) to immune splenocytes at the initiation of a 5-day Mishell-Dutton culture, inhibited antibody formation.

Besides lymphocytes, other immune cells appear to be affected by a direct opioid action. Roy et al (1991a) studying the effects of chronic morphine treatment on macrophage colony formation on cultured bone marrow cells, observed a reduced response of the macrophage progenitor cells towards macrophage colony stimulating factor. Additionally, Perez-Castrillon et al (1992) described an inhibition of human monocytes chemotaxis activity in the presence of opioids. Peterson et al (1987a) reported that chronic exposure of cultured peripheral blood mononuclear cells to morphine produced a marked suppression of respiratory burst activity, a microbicidal function involved in a physiologic response in which oxygen is rapidly metabolized to yield a series of highly toxic intermediates, in response to opsonized exogenous compounds zymosan and phorbol myristate acetate and a minimal but statistically significant increase in resting oxygen metabolism.

Peterson et al (1987b) observed that cultures of human peripheral blood cells when treated with morphine released lower quantities of interferon-γ, which ultimately, might be responsible for their cytotoxicity. Pasotti et al (1989) reported inhibition of human granulocyte aggregation and ATP liberation in the presence of morphine and Marcoli et al (1988) suppression of granulocyte chemotaxis.

2. Opioid receptors on immune cells

1) Binding studies

The evidence for an opioid link to immune cells is based on the demonstration of the alteration of their characteristics by opiates and their ability to bind opioids stereospecifically. Whether lymphocyte receptor sites are identical to neural ones or whether, at least, they share characteristics with them, including molecular size, immunogenicity, and the use of specific intracellular signaling pathways are other relevant questions.

Lopker et al (1979) have described stereospecific binding of opioids to both granulocytes and monocytes and suggested that there were opioid receptors on phagocytic leukocytes. Their data yielded a Kd of 10 nM for granulocytes and 8 nM for monocytes. The number of binding sites is approximately 3000 per monocyte and 4000 per granulocyte. Moreover, Carr et al (1988a) have developed an antibody that recognizes and specifically binds δ-opioid receptors from NG108-15 cells, a neuroblastoma x glioma hybrid cell line. The use of cell lines has been advantageous in the characterization of binding sites. Cell lines, as opposed to mixed cells isolated from immune organs, are homogeneous populations of cells which can be cultured under controlled conditions. In further studies, the same authors observed that the antibody blocks the binding of dihydromorphine (which interacts with the μ_2

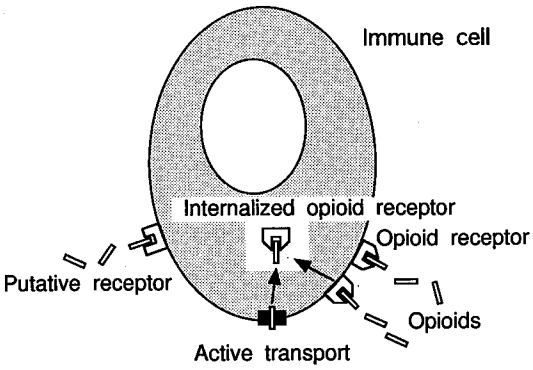

Fig. 1 A schematic diagram of an immune cell summarizing the models proposed for the direct interactions exerted by opioids: 1) Stereospecific binding of opioids to surface sites which shares the properties of classical opioid receptors, 2) Stereospecific binding of opioids, introduced in the interior of the cell by active transport, to inner classical opioid binding sites, 3) Internalization of opioid receptor binding sites, 4) Opioid binding sites different from the classical opioid receptors found in neural tissue and 5) Direct interaction between opioids and immune cells without the mediation of receptors.

opioid receptor of neural tissue) to splenocytes and competes with met-enkephalin, β-endorphin and naloxone for binding to the surface of splenocytes, indicating that the antibody recognizes an antigen on the surface of the splenocytes which shares the properties and configuration of a classical opioid receptor (Wolozin and Pasternak, 1981). Savino et al (1990) showed a naloxone reversible stimulatory effect of opioids on thymulin, a chemically defined thymic hormone known to stimulate some steps of T-cell differentiation, in thymic epithelial cells cultures.

Other experiments that supports the presence of classical opioid receptors on cells of the immune system were displayed using the δ-selective ligand [^{3}H]-cis-(+)-3-methyl-fentanyl isothiocyanate which binds to a site on leukocytes (Carr et al, 1987) shown to have characteristics similar to those of the opioid receptors from neuroblastoma x glioma NG 108-15 hybrid cells possess (Klee and Nirenberg, 1974; Hazum et al, 1979a; Law et al, 1985).

Ovadia et al (1989), investigating the existence of opioid binding sites on membranes of rat lymphocytes, discovered that mitogen-activated spleen cells but not thymocytes had specific and displaceable binding sites for [^{3}H]-naloxone. Naloxone-reversible inhibition of both granulocyte aggregation and ATP liberation when granulocytes were exposed to morphine *in vitro*, suggests a classical opioid receptor mediation in the mechanism (Pasotti et al, 1989). Additionally, Yamasaki et al (1982) and Casale et al (1984) reported the implication of opioid receptors in the opioid-induced cutaneous mast cell degranulation.

Further complications have been added when opioid binding sites that do not share characteristics with classical opioid receptors were characterized. Early experiments by Hazum et al (1979b) showed that ^{125}I-labeled β_H-(D-Ala) endorphin binds significantly and specifically to cultured lymphocytes in a manner which is not blocked by enkephalins or opioids, indicating the existence of an specific, non-opioid type of binding site for β-endorphin.

Roy et al (1991b, 1992) reported that an activation of thymocytes with interleukin (IL)-1 or phytohemagglutinin (PHA) resulted in a large increase in [^{3}H]morphine binding to these cells and demonstrated that those sites were clearly different from neural opioid receptors due to: a) poor selectivity; b) relatively low affinity and high capacity; and c) resistance to trypsin strongly inhibited by millimolar concentrations of Mg^{++}, Ca^{++}, Mn^{++} and Cl^-. In addition, Mendelsohn et al (1985) showed an absence of classical opioid receptors on human mononuclear cells.

Fiorica and Spector (1987) tried to characterize opioid receptors on the EL-4 thymoma cell line, very similar to T cells. EL-4 cells, for example, produce IL-2 on stimulation (Farrar et al, 1982). Binding studies on the EL-4 thymoma cell line suggest the presence of a κ-type opioid receptor similar to those found in neuronal tissues, although κ receptors in neuronal tissues have shown stereospecificity in contrast with the results obtained from the EL-4 cells.

Other investigations have related opioids with immune cells without a direct implication of any receptors. Simpkins et al (1985) showed that high concentrations of naloxone inhibits the release of oxygen from human neutrophils; however, the finding that morphine and nalorphine also inhibit oxygen release indicates that naloxone does not interact through an opioid receptor but more likely there is a direct interaction of opioids with the membrane.

Up to this point, the surface of immune cells has been considered to be the most suitable location for opioid receptors. Nevertheless, studies by Madden et al (1987, 1991) suggested that naloxone binding to T lymphocytes seems to occur both at the outer cell surface and in the interior of the cell. While binding sites in the outer membrane seem to be of low affinity (naloxone being displaced by morphine only at high concentrations), permeable cells apparently express high affinity binding sites intracellularly which might be responsible for the blockade of morphine actions by naloxone on human T lymphocytes. If so, one could speculate upon the need for opioids to enter the lymphocyte and bind to a proper receptor to develop their immunological activity. However, as the procedures used in isolating the lymphocytes involve several washings that may eliminate the receptors on the surface, the so-called *internal receptors* may represent new receptors being transported to the cell surface or used receptors remaining after endocytosis. Another possibility is suggested by the fact that certain lymphoid cells seem to internalize opioid ligands following binding (Schweigerer et al, 1985).

All these controversial observations call into question the role played by opioid receptors. Moreover, radioligand binding methods have been unable to demonstrate unequivocally the presence of opioid receptors on immune cells (Sibinga and Goldstein, 1988; Srisuchart et al, 1989). Further research is required to find a definite answer.

2) Selectivity profile

It has been well established that opioid receptors occur in the brain as independent and pharmacologically distinct μ, δ and κ receptors (Wolozin and Pasternak, 1981; Simon, 1987; Goldstein and Naidu, 1989). Considering the existence of classical opioid binding sites on immune cells, several studies have addressed the question of which type of receptors is present on the surface of these cells.

Agonist and antagonist competition studies by Taub et al (1991) using two different strains of mice and also mice with a selective defect in the μ opioid receptor showed that opioid receptors, similar to those present in the nervous system, exist on the surface of murine splenocytes. μ agonists and κ agonists were implicated through the mediation of a different receptor and agonist binding to κ opioid receptors, and to a lesser extent to μ receptors on splenocyte populations provoked immunosuppression. Szabo et al (1993) found that μ receptors mediated the inhibition of macrophage phagocytosis towards *Candida albicans in vitro*. Whereas CTAP, a μ-receptor antagonist, blocked morphine-induced immunosuppression, δ and κ antagonists showed no effect at all. In addition, Madden et al (1987) and Radulescu et al (1991) have also demonstrated biochemically and functionally the presence of μ opioid receptors on cells of the immune system.

Bidlack et al (1992) have characterized κ opioid binding sites on membranes from R1.1 murine lymphoma cell line using [^{3}H]naloxone and [^{3}H]U69,593. The R1.1 cell line possess neither μ nor δ binding sites. Further, Carr et al (1989) gave evidence of an specific binding of [^{3}H]U69,593 to lysed cells of the macrophage cell line P388d.

Studies held to clarify the type of receptor involved in the inhibition of humoral response have not been fully successful. Whereas Carr et al (1990) found that both naltrindole, a δ opioid receptor antagonist, and oxymorphindole, a δ-class opioid receptor agonist, significantly suppress ConA-stimulated Peyer's patch lymphocyte immunoglobulin production of IgM isotype and reduce interleukin-2 receptor expression. Taub et al (1991) discovered that μ and κ agonists (morphine and U50,488H, respectively) inhibit the ability of murine lymphocytes to generate antibodies to sheep

erythrocytes *in vitro*. Further, Arakawa et al (1993) observed some immunosuppresive effects of naltrindole on T-cell immunity in mixed lymphocyte reaction. δ opioid binding sites were characterized microscopically. Pasotti et al (1989) in *in vitro* stereospecific studies related μ receptors with inhibition of granulocyte aggregation.

The opioid receptors to which exogenous opioids interact may be part of an unknown autoregulatory pathway in the immune system mediated by the endogenous opioid peptides.

3. Underlying pathways

The presumed existence of opioid binding sites on cells of the immune system implies the additional problem of finding the post-receptor coupling events. McDonough et al (1980) measured the lymphocyte number and function in 44 street opioid addicts and 28 controls, showing that *in vivo* administration of opioids produces a reduction in the number and function of T lymphocytes and a concomitant increase in null cells. Naloxone effectively blocked both T-cell depression and null-cell increase by allowing the null cells to express SRBC receptors. cAMP and dibutyryl cAMP (dBcAMP) also converted null cells to T cells. In neuronal cells, both naloxone and dBcAMP but not cAMP, reverse the effects of opioid binding because cAMP is not taken up by these cells. Lymphocytes, on the other hand, incorporate cAMP directly (Parker, 1976), explaining their ability to affect opiate actions in lymphocytes but not in neural cells. The concentrations of naloxone, dBcAMP and cAMP found to be effective in lymphocytes are similar to those used for reverse opioid effects in neuronal cells, indicating that the effects of the lymphocytes are as specific as those found in neuronal opioid receptors.

Administration of opioids inhibits adenylate cyclase activity decreasing at the same time the levels of cAMP in the neuroblastoma x glioma NG 108-15 hybrid cells. The removal of morphine resulted in a compensatory increase in adenylate cyclase that became evident as a rebound elevation in cAMP levels (Klee and Nirenberg, 1974; Sharma et al, 1977). The existence of opioid receptors on the surface of lymphocytes that modulate their function through the modulation of cAMP, supports the hypothesis that implicates cyclic nucleotides in the underlying mechanism of the immunoregulatory actions of opioids. In this regard, the specific antibody to the opioid receptor in the NG 108-15 hybrid cells developed by Carr et al 1988a (see above) was shown to be agonistic compared with β-endorphin in that it suppressed adenylate cyclase activity in splenocytes. Its agonistic property was also blocked by naloxone, suggesting the presence of opioid receptors.

Ovadia et al (1989) reported that specific binding sites for naloxone in mitogen-activated cells showed a significant specificity for GTP analogues, which is consistent with the hypothesis that a GTP-binding regulatory protein may couple with receptors to adenylate cyclase during the process of opioid binding to lymphocytes. In addition, Lawrence and Bidlack (1992, 1993a) have shown that κ-opioid binding sites activity on R 1.1 cell membranes is sensitive to cations and guanine nucleotides. In a recent study, it was reported that the κ-opioid receptor in the R1.1 thymoma cell line is coupled to adenylate cyclase through a GTP-binding protein sensitive to the pertussis toxin. Moreover, the interaction of a κ agonist U50,488 with κ-opioid receptors in the R1.1 thymoma cell line inhibits both the basal and forskolin-stimulated cAMP production (Lawrence and Bidlack, 1993b).

Other possibilities have also been considered. Carr et al (1988b) reported that β-endorphin diminished the K^+ channel conductance in a time-dependent fashion. This effect was partially reversed upon the addition of naloxone, indicating the involvement of opioid receptors. Roy et al (1991b) proposed that opioid binding sites in immune cells could act as a counterbalance to the possible feedback system provided by up-regulation of IL-2 receptors.

INDIRECT MECHANISMS

When some of the immunosuppressive effects elicited by opioids in *in vivo* studies were not reproduced in *in vitro* (Kay et al, 1984;

Wybram, 1985a, 1985b, 1985c; Bayer et al, 1990), indirect mechanisms were taken into account. That implies, first, the binding of opioids to centrally located receptors and second, as a consequence of such interaction, the activation of other neurochemical pathways that induce various immunological alterations. The effects of opioids on neuroendocrine function and the sympathetic nervous system are of particular relevance in considering the role of these processes in the alteration of the immune response.

1. CNS, immune system and opioids

1) Involvement of central opioid receptors

Several reports strongly suggest that some of the effects provoked by the opioids on the immune system are mediated through receptors located in the central nervous system (CNS).

Shavit et al (1986c) found evidence that implicates brain opioid receptors in the immunomodulatory effects of morphine. In this way, morphine injection into the lateral ventricle suppressed natural killer (NK) cell activity to the same degree as a systemic dose higher by three orders. This effect was blocked by naltrexone. However, when N-methylmorphine, a morphine analogue that does not penetrate the blood-brain barrier, was administered systemically, no effects on NK activity were elicited, indicating that morphine-induced immunosuppression was mediated through neural opioid receptors.

Weber and Pert (1989) found within the brain the specific area where morphine, by interacting with opioids receptors, elicits a suppressive effect on NK activity. They showed that a direct microinjection of morphine into the periaqueductal gray matter (PAG) of the mesencephalon, but not other brain regions, can inhibit NK function within 3 h in the rat.

On the other hand, Hernandez et al (1993) showed that microinjection of morphine in the anterior hypothalamus resulted in a significant suppression of blood lymphocyte activity, suggesting that independent immune cell activities are under the influence of specific and different central pathways.

2) Type of opioid receptors

Accepting that central opioid receptors regulate some immune parameters, research regarding the opioid receptor subtype is of primary interest.

Following the microinjection of opioid receptor-selective agonists into the lateral ventricle of rats, Band et al (1992) showed that whereas DAMGO (a μ-selective agonist) reduced the NK cytotoxicity response, U50,488, a selective κ-receptor agonist, did not affect NK cytotoxic activity. In contrast, DPDPE increased NK cytotoxicity, suggesting that δ receptors in the CNS may contribute to immunoenhancement. These data are consistent with those obtained by Arakawa et al (1993) who observed *in vivo* the ability of naltrindole, a δ-receptor antagonist, to prolong the survival of rat kidney allografts. Moreover, Carr et al (1993) found a substantial inhibition of NK activity after acute administration of morphine. The facts that pretreatment with β-funaltrexamine, a μ_1- and μ_2-receptor antagonist, reversed the morphine-induced immunosuppression and the μ_1-selective antagonist, naloxonazine, had no effect, suggest the involvement of the μ_2-receptor subtype in morphine-mediated suppression of splenic NK activity. In addition, pretreatment with naltrindole and nor-BNI, δ and κ receptors antagonists, respectively, showed no involvement of such receptors.

2. Neurochemical pathways

Given that morphine can alter immune status by activating central opioid receptors, the next question is by what means this activation can be referred to peripheral effects on the immune system. Generally speaking, two plausible pathways can be implicated in the centrally mediated suppression of immune response following opioid administration.

1) Activation of the hypothalamus-pituitary-adrenal (HPA) axis

The relationships between opioids and the neuroendocrine system are well established. Depending on the specific hormonal axis, opioids can stimulate or inhibit the release of hypothalamic releasing factors and pituitary and target gland hormones. The hypothalamus-pituitary-

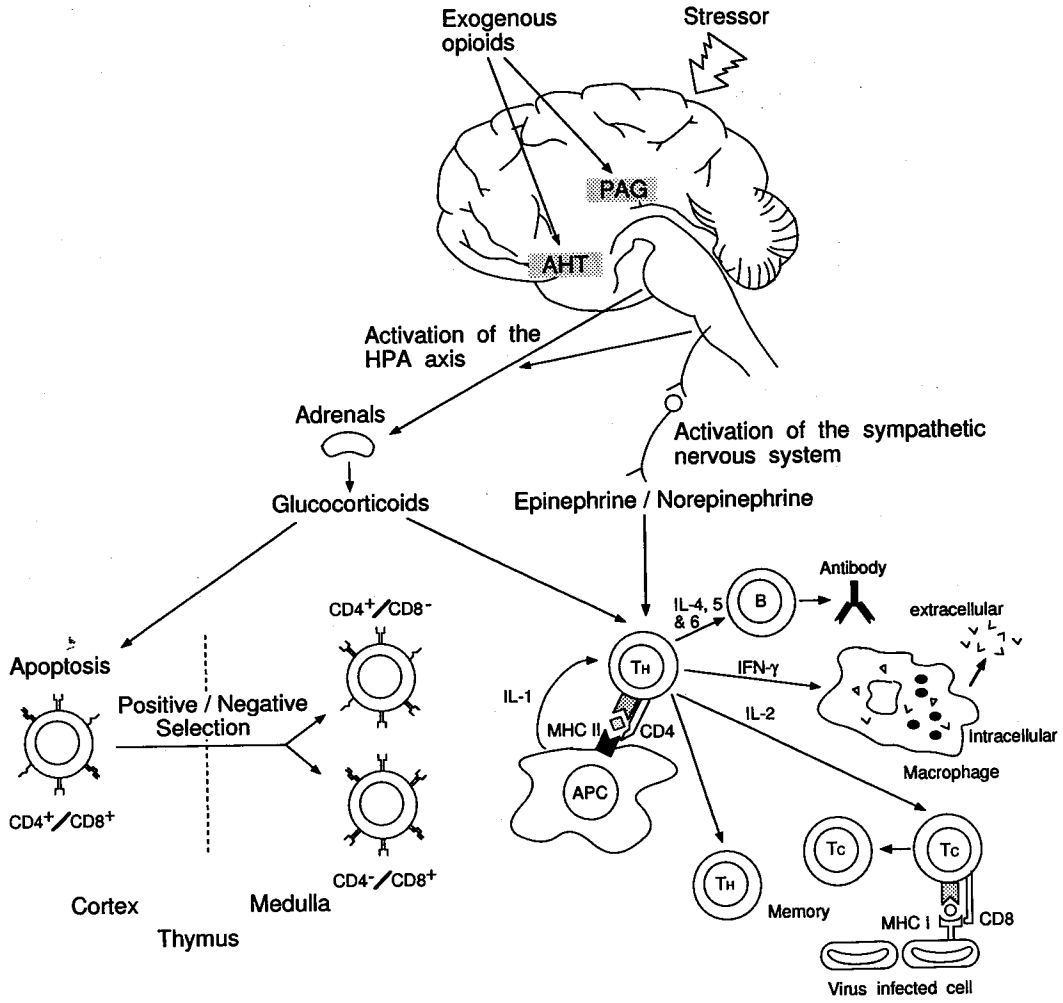

Fig. 2 Proposed model for the interrelations among opioids, neuroendocrine, nervous and immune systems. Morphine-induced immunomodulation is mediated indirectly through CNS receptors located at least in the periaqueductal gray matter (PAG) and anterior hypothalamus (AHT). Opioid-induced activation of the neuroendocrine or the sympathetic nervous systems represent potential pathways that could influence immune cell activity. Stressors as well, by activating endogenous opioidergic pathways in the CNS, and subsequently, neuroendocrine or autonomic neurochemical mechanisms, exert an indirect influence on certain immune parameters. For more detailed explanations refer to the text. **MHC**: major histocompatibility complex. $\mathbf{T_H}$: helper T cell. $\mathbf{T_C}$: cytotoxic T cell. **B**: B cell.

adrenal (HPA) axis, in particular, is essential for the preservation of homeostasis, linking changes in central neuronal activities to modifications in target gland functions. Early findings by George and Way (1955) showed a morphine-induced activation of the HPA axis. Later reports revealed that acute administration of morphine stimulates the HPA axis only at a relatively high dose (20 mg/kg) (Simon et al, 1975a ; George and Kokka, 1976 ; Pechnick et al, 1985). Although tolerance to the effects of opioids develops when administered chronically, adrenal hypertrophy is also observed (Simon et al, 1975b), indicating continuous and excessive

adrenal stimulation. At the same time, corticoid release from the HPA axis has been associated with immunosuppressive states both in *in vitro* and *in vivo* experiments (see Parrillo and Fauci (1979) for review; Almawi et al, 1991). Therefore, the links among opioids, HPA axis and the immune system, are of particular interest in understanding the underlying mechanisms in opioid-induced immunoregulation.

Implantation of a 75-mg morphine pellet, a regimen commonly used in the study of opioid tolerance and dependence, produced splenic and thymic atrophy, decreased thymic cellularity and suppressed mitogen-induced proliferative responses in mice (Bryant et al, 1987, 1988a, 1988b; Arora et al, 1990; Sei et al, 1991). Flow cytometric analysis of thymocytes stained with monoclonal antibodies to CD4 and CD8 revealed that the $CD4^+/CD8^+$ "double positive" (DP) thymocytes, phenotypically an immature cortical T-cell subset, were depleted to a greater extent than was any other subpopulation (Arora et al, 1990; Sei et al, 1991; Fuchs and Pruett, 1993). Considering that: 1) morphine administration as a pellet implantation shows an increase in the levels of circulating corticosteroids (Bryant et al, 1988a, 1988b, 1990; Bayer et al, 1990; Sei et al, 1991), 2) glucocorticoids are known to deplete mainly $CD4^+/CD8^+$ DP thymocytes (Blomgren and Anderson, 1971; Boersma et al, 1979; Reichert et al, 1986) and 3) naloxone blocked morphine-induced depletion of DP in the thymus, but had no effect when administered alone (Sei et al, 1991; Fuchs and Pruett, 1993), it can be suggested that, in at least one model, activation of the HPA axis is a critical contributor to the immunosuppressive effects of morphine. Moreover, blockade of morphine-induced suppression of lymphocyte proliferation in adrenalectomized or in mice treated with RU-486, a glucocorticoid receptor antagonist (Bryant et al, 1990) and abolition of the morphine-induced decrease in $CD4^+/CD8^+$ DP thymocytes by adrenalectomy related to a sham-operated group (Sei et al, 1991), give additional support to the previous hypothesis.

Recent experiments suggested that *apoptosis* might be the underlying mechanism that explains opioid-induced thymic atrophy. The term apoptosis was first used by Kerr et al (1972) and can be described as a selective process of cell deletion involving endogenous endonuclease activation. It is also part of the homeostatic mechanisms that function to control cell numbers in different kinds of tissues (Bursh et al, 1990) like the deletion of autoreactive cells in the thymus during the maturation process (McDonald and Lees, 1990; McConkey et al, 1989). Thymocytes that undergo apoptosis in the presence of glucocorticoids have exhibited endonuclease activation (Wyllie, 1980; Cohen and Duke, 1984), DNA fragmentation into characteristic lengths differing by 180 base pairs (Wyllie and Morris, 1982; Compton and Cidlowski, 1986) and cell surface changes related to phagocytosis (Morris et al, 1984). In this regard, Fuchs and Pruett (1993) discovered an increased level of fragmented thymocyte DNA following morphine pellet implantation. Electrophoresis of low molecular weight thymocyte DNA obtained from the lysates of morphine-treated mice showed that the fragments were sized in multiples of 180 to 200 base pairs, which is indicative of apoptosis. Whereas *in vitro* addition of morphine failed to demonstrate DNA fragmentation, administration of RU-486 selectively blocked the morphine-induced DNA fragmentation, suggesting the implication of glucocorticoids in the higher rate of morphine-mediated thymic apoptosis.

Morphine implantation has also been used as a model to determine whether immune function *in vivo* is altered by chronic opioid treatment. In this regard, Bryant and Roudebush (1990) determined *in vivo*: 1) antigen-induced T-cell proliferation by measuring the uptake of iododeoxyuridine by lymphoid tissue after antigen exposure, 2) a delayed-type hypersensitivity (DTH) response by measuring the degree of rear foot pad swelling induced by picryl chloride and 3) graft versus host (GVH) reaction by measuring the degree of splenomegaly of F_1 hybrid mice with splenocytes obtained from one of the parental strains. The immune system of the hybrid

(host) does not recognize the injected parental spleen cells (graft) as foreign, whereas the injected cells mount an immune response toward the host that leads to splenomegaly. In this study, they showed that morphine pellet implantation resulted in a marked inhibitory effect of *in vivo* lymphocyte proliferation, DTH and the GVH response, although the last one was dependent upon when the morphine pellet was implanted. The prevention of these effects on adrenalectomized mice suggests the involvement of glucocorticoids in the mechanism.

Alterations in the humoral response have also been related with the elevated levels of glucocorticoids caused by chronic morphine administration. Pruett et al (1992) ascertained that exposure to morphine *in vivo* for 12 to 24 h markedly inhibited the production of IgM AFC when stimulated by sheep erythrocytes in Mishell-Dutton cultures. Pretreatment with RU-486 significantly reversed morphine-induced suppression of AFC response, indicating the involvement of a glucocorticoid-mediated mechanism.

In contrast to the chronic effects of morphine, the immunosuppressive effects observed after acute administration of morphine appear to be glucocorticoid-independent. A recent report by Flores et al (1994) suggested that the HPA axis did not contribute significantly to the morphine-induced inhibition of ConA-stimulated blood lymphocyte proliferation. Pretreatment of animals with RU-486 as well as adrenalectomy failed to attenuate the inhibitory actions of morphine, indicating that glucocorticoids and other adrenal hormones are not essential in the suppression of lymphocyte responses.

2) Mediation of the sympathetic nervous system

The sympathetic nervous system (SNS) is another potential mechanism responsible for the immunosuppressive effects elicited by the administration of exogenous opioids. Felten et al (1985) found that both primary and secondary lymphoid organs were profusely innervated by noradrenergic and peptidergic terminals. In addition, several studies using selective radiolabeled ligands confirmed the presence of α- and β-adrenergic receptors on human lymphocytes (Hadden et al, 1970; Williams et al, 1976; Titinchi and Clarck, 1984; Sanders and Munson, 1985; Khan et al, 1986; Feldman et al, 1987; van Tits et al, 1990), on murine splenocytes (Loveland et al, 1981; Fuchs et al, 1988) and on mature medullary thymocytes in mice (Fuchs et al, 1988). Moreover, catecholamines *in vitro* have been shown to alter a variety of lymphocyte functions (Bourne et al, 1974; Joasoo and McKenzie, 1976). Furthermore, intracisternal administration of morphine, β-endorphin and DAMGO (a selective μ agonist) enhanced sympathetic outflow to both the adrenal medulla and the sympathetic nerve endings, resulting in an increase of circulating catecholamines (van Loon et al, 1981; Appel et al, 1986).

All these data taken together suggest a possible interconnection among opioids, the catecholaminergic system and the immune system. Carr et al (1993) demonstrated that α- and β-receptors played an important role in morphine-induced NK suppression. Administration of both α- and β-blockers, dose-dependently reversed morphine-induced inhibition of NK activity. However, the dose-response comparison of phentolamine (an α-blocker) and propranolol (a β-blocker) indicated mediation through α- rather than β-adrenoceptors. Moreover, the reversed suppression of NK activity by prazosin (a selective antagonist of α_1-receptor) suggested the mediation of the α_1-receptor which is consistent with the finding that yohimbine, an α_2-selective antagonist, had no effect on morphine suppression of NK activity. Further, chemical sympathectomy of lymphoid tissue has been reported to increase the values of NK activity (Livnat et al, 1985; Reder et al, 1989).

Fecho et al (1993), examining the role of β-adrenergic receptors in morphine-induced immunosuppression, showed that nadolol, atenolol and ICI-118,551 (β-adrenergic antagonists) specifically and completely blocked morphine-mediated inhibition of the proliferative response of splenic leukocytes to the T-cell mitogens, ConA and PHA, the B-cell mitogen, lipopolysac-

charide and the combination of ionomycin and phorbol myristate acetate, which activate intracellular second messenger systems involved in the proliferation of T and probably B cells as well. Additionally, Baddley et al (1993) showed that the affinity of the β-agonist [^{125}I] cyanopindolol-sensitive binding sites in splenic lymphocytes is reduced following acute morphine administration.

STRESS, OPIOIDS AND IMMUNE SYSTEM : A PSYCHOSOMATIC NETWORK

Generally speaking, it is well understood that under conditions of severe stress, the hypothalamus and the adrenals release peptides (β-endorphin and met-enkephalin) which have the properties of opioid analgesics (Beluzzi et al, 1976). Endogenous opioids have also been implicated as mediators of stress-induced analgesia (SIA) ; (Akil et al, 1976 ; Lewis et al, 1980 ; Izumi et al, 1983). Moreover, both exogenous and endogenous opioids have been related as well with a number of effects on hormone release mediated by several stressors such as inhibition of luteinizing hormone, release of growth hormone and prolactin, and changes in the concentrations of oxytocin and vasopressin (Dupont et al, 1977 ; Bruni et al, 1977 ; Shaar et al, 1977; Briski et al, 1984; Lightman and Young, 1988). Overall, it seems that endogenous opioids play an important role in response to a certain number of stressors. Since opioids are directly involved in immunoregulation, it would be plausible to admit, a priori, interconnections among stress, opioids and the immune system.

Studies on the neuropharmacological basis of the analgesic effect following an environmental stressor has revealed that there are two main mechanisms, opioid and non-opioid, in the production of SIA (Izumi et al, 1983 ; Takahashi et al, 1987). While foot-shock (FS)-SIA was antagonized by naloxone, swimming-stress was insensitive to the antagonist, suggesting the mediation of opioid and non-opioid mechanisms, respectively. The involvement of μ-opioid receptors has also been suggested and, to a lesser extent, δ-receptors in the production of FS-SIA. On the other hand, Takahashi et al (1990) reported the direct involvement of κ-receptor and, to a lesser extent, μ-receptor mechanisms in the production of psychological (PSY)-SIA. That the analgesic effect produced by PSY-SIA was antagonized by naloxone implicates an endogenous opioid mechanism in this phenomenon.

Various immune parameters have been shown to be affected by certain types of stress. It was observed that in rats given a series of escapable shocks, identical inescapable shocks and no shocks, when reexposed to small amount of shock after 24 h, the proliferation of lymphocytes in response to the mitogens phytohemagglutinin and ConA was suppressed in the inescapable shock group but not in the escapable shock group. The blockade of these effects by naloxone indicates an endogenous opioid-mediated mechanism (Laudenslager et al, 1983). In addition, Lewis et al (1983a) showed that FS stress and high doses of morphine have permissive effects on the survival of rats inoculated with mammary tumor cells. In a further study, the same authors concluded that opioid peptides were implicated in stress-induced increase of tumor growth (Lewis et al, 1983b).

Studies investigating the cytotoxic activity of NK cells in rats subjected to one of two inescapable FS-stress paradigm, both of which induce analgesia, identical in shock intensity and total shock on time but differing in the time of application, showed that in only one, activation of opioid mechanisms can be implicated. Whereas intermittent footshock caused an opioid-mediated analgesia, continuous footshock resulted in the production of an equally potent but non-opioid analgesia. The suppression of splenic NK activity by the opioid form of stress, but not by the non-opioid form, suggests that endogenous opioid peptides mediate the effect of certain forms of stress on NK cell cytotoxicity. Supporting these results, it was observed that this suppression was blocked by naltrexone, and high doses of morphine (>10 mg/kg) mimicked the effects provoked by the opioid form of stress. That such high doses are required to alter NK activity implies either mediation by receptors

with low affinity for this drug or receptor binding for prolonged periods of time (Shavit et al, 1984, 1986a, 1986b). In addition, Greenberg et al (1984) found that stress induced by tail shock suppressed NK cytotoxicity and the effect was blocked by naltrexone. In this regard, while Weber and Pert (1989) demonstrated that the periaqueductal gray matter (PAG) mediated exogenous opioid-induced immunosuppression, Seeger et al (1984) showed that from the same brain location, endogenous opioids are released following exposure to FS-stress.

Exposure to inescapable but not escapable shock produces immunosuppression, suggesting that controllability of stressors plays a decisive role in controlling immune function (Laudenslager et al, 1983; Lewis et al, 1983a). Moreover, Shavit et al (1984, 1986a) reported that inescapable, but not escapable shock, produces *learned helplessness* which suppresses as well the immune function. When both stress procedures, continuous or intermittent FS exposure, were inescapable, only the intermittent footshock procedure caused learned helplessness (Shavit et al, 1986b).

Moreover, psychological depression related with learned helplessness in animals has also been associated with immunosuppression (Fox, 1981).

The potential mechanisms underlying the activation of the so-called *stress circuit*, implicate endogenous opioids, the HPA axis and the sympathetic nervous system. However, the interrelations among them are poorly understood.

Experimental evidence indicates that the functions of the immune system are regulated by endocrine and neuroendocrine signals (see Dunn (1989) for review). It has been fully demonstrated that glucocorticoids suppress the functions of immune cells through various mechanisms *in vitro* and *in vivo* (Parrillo and Fauci, 1979; Almawi et al, 1991). It has been reported as well that morphine, as well as κ- and σ-opioid receptor agonists (Eisenberg, 1985) elevate circulating levels of corticosterone. Similarly, traditional stress models are known to cause adrenal hypertrophy, thymic atrophy and increased circulating levels of corticosteroids (Keller-Wood and Dallman, 1984). Based on these findings there is ample opportunity for certain types of stressors to exert effects on some immune parameters through the release of glucocorticoids from the HPA axis, mediated by the release of endogenous opioids.

Supporting these findings, experiments using autoradiography techniques have shown that prolonged intermittent footshock or forced swimming causes a significant reduction in [^{3}H]-diprenorphine binding in the hypothalamus and other brain structures (Seeger et al, 1984). A decrease in high affinity binding of [^{3}H]etorphine after restraint stress in rat brain membranes (Hnatowich et al, 1986) and in the number of μ receptors in the midbrain after inescapable FS (Stuckey et al, 1989) have also been documented. These results indicate that a decrease in the binding of opioid receptor ligands following acute stress is due to a continuous activation of opioid receptors caused by endogenous opioid release. Moreover, β-endorphin is involved in the direct innervation of corticotropin releasing hormone (CRH-41) neurons by the arcuate nucleus and appears to be an important secretagogue in the corticotropin releasing factor-adrenocorticotropin hormone (CRF-ACTH) axis (Assenmacher et al, 1992). This activation includes production of CRF by the hypothalamus, production of ACTH in the pituitary and the production of glucocorticoids in the adrenals (Nikolarakis et al, 1987, 1989).

The sympathetic nervous system may as well be implicated in the opioid-induced immunoregulation after stress exposure. In this regard, acute stress, including footshock, cold environment and immobilization, affects the levels of norepinephrine (NE) in the *locus coeruleus* (LC). Among these changes are increases in NE turnover (Tanaka et al, 1983a, 1983b) and increases in the extracellular level of NE (Abercombrie et al, 1988; Finlay et al, 1990). Stress-induced enhancement in these indices of LC activation takes place in multiple brain areas that receive afferent inputs from this structure.

There is also evidence implicating cor-

ticotropin releasing factor (CRF) in the activation of the sympathetic nervous system during stress (Brown and Fisher, 1985; Fisher, 1989). Further, catecholaminergic release appears to activate the HPA axis. In the past few years, a number of relevant investigations have favored the concept that major cathecholaminergic pathways innervating the periventricular nucleus actually convey the most potent stimulatory signals to CRH-41 neurons which act as stimulatory components of the CRF-ACTH axis across postsynaptic α_1-, α_2- and β-adrenergic receptors (Assenmacher et al, 1987, 1992; Plotsky et al, 1989).

The next question that arises is: how do endogenous opioid systems communicate with catecholaminergic network and what is the evidence for this interaction? It has been reported that opioid binding to μ, but not to δ or κ receptors, inhibits the release of [^{3}H]NE from rat brain cortex slices (see Illes (1989) for review). Recent works by Schoffelmeer et al (1988, 1993) showed that the depolarization-induced release of NE from brain tissue is susceptible to inhibition by the interaction of endogenous β-endorphin and a presynaptic μ receptor. In addition, in the rabbit hippocampus, opioid binding to presynaptic κ receptors seems to decrease NE release (Jackisch et al, 1988). Moreover, the enhancement of opioid activity under stressful conditions appears to modulate the function of the NE system. Administration of naloxone enhances stress-induced increase in the levels of NE turnover (Tanaka et al, 1983b), potentiates stressor-induced conditioning of LC unit activity in freely moving cats (Abercombrie and Jacobs, 1988) and augments ether vapor stress-induced release of epinephrine and NE (Brown, 1992). Taken all these data into account, it appears that while the NE system remains under the inhibitory control of the opioidergic system, opioid antagonists elicit the contrary effects. How the opioidergic pathways, the neuroendocrine system and the SNS system interact under stressful situations and what kind of immunoalterations occurred as a consequence of such interactions still remain to be solved.

CONCLUSIONS

Experiments investigating the involvement of opioids in immunocompetence have examined the effects of many opioids on different immune cells or parameters. In considering the findings of these studies it is essential to bear in mind that:

1) the immune system is not a homogeneous entity susceptible to being altered in a unique way. On the contrary, it is comprised of multiple elements and influenced by numerous physiological as well as psychological or genetic factors; 2) opioids affect some but not all of its constituents; 3) the way by which opioids modify the immune response differs depending on the subject of study; 4) moreover, how those components are modified, varies depending on the animals, assays and protocols; and 5) the complex relationships existing among the brain, the neuroendocrine system, the autonomic nervous system and the immune status of the whole body makes it very difficult to ascertain whether the chronic effects on the immune system are specifically opioid-mediated or due to other determinants.

Since many of the effects of opioids have been demonstrated in experiments *in vitro* and various studies using radioligands have shown stereospecific binding of opioids to certain populations of immune cells, the presence of classical opioid receptors and subsequently a direct mechanism is likely to be one of the reasons that explain opioid-mediated immunoregulation. However, other studies have shown evidence for the existence of other putative opioid receptors non-sensitive to naloxone. The role of endogenous opioids is still controversial; the underlying mechanisms by which opioids exert their action on the immune cells have not yet been clarified. The opioid receptor types and, especially, subtypes involved in these processes are far from being determined. In any case, further research is required to reach a definite conclusion.

The immunoregulatory effects attributable to opioids can also be exerted through the binding to central receptors located at least in the peri-

aqueductal gray matter and the anterior hypothalamus, implying that diverse brain regions are involved in independent and specific immune alterations. The types of opioid receptors implicated in such changes are still the subject of inquiry. The HPA axis as well as the sympathetic nervous system have been considered possible mediators in the alteration of the immune response due to the administration of opioids. Other neurochemical pathways should also be considered as a matter of further research. An apoptotic process in certain populations of lymphocytes has been implicated as the ultimate consequence of opioid administration. Attempts to investigate stress-induced immunological alterations have yielded conflicting results. It is clear from the data obtained from different experiments that the type of stress employed, its duration, the frequency of the stressor, age and animal species, former stress experience, etc. are important variables in determining the signs of response. The role that central opioidergic neurons have in the modification of immunocompetence in individuals subjected to different stressors is unclear as well as the complex interelations that seem to be found among the opioidergic system, the sympathetic nervous system, the neuroendocrine system and the immune system. Psychological and genetic cofactors should be added to this complicated tissue of reciprocal influences.

Even though the present knowledge of the matter is still very limited, the recent findings are most provocative and should accelerate our understanding of the mechanisms involved in opioid-mediated immunoregulation.

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