Equianalgesic Doses of Subcutaneous but Not Intrathecal Morphine Alter Phenotypic Expression of Cell Surface Markers and Mitogen-induced Proliferation in Rat Lymphocytes

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Background: Surgical trauma and opioids are linked with suppression of immune function. Evidence suggests a probable supraspinal action of morphine in altering immune function, although the role of spinal systems have not been evaluated. Therefore, this study compared the effect of equianalgesic doses of subcutaneous and intrathecal morphine on lymphocyte proliferative responses and phenotypic expression of lymphocyte cell surface markers in rats.

Methods: Equianalgesic doses of subcutaneous (10 mg/kg) or intrathecal (30 µg, by a chronic intrathecal catheter) morphine were given twice for 5 h (time 0 and 2.5 h). Immediately after the 5-h period or 24 h after the initial injection, spleens were harvested and lymphocytes isolated. Mitogen-induced (phytohemagglutinin, concanavalin A, pokeweed, lipopolysaccharide) lymphocyte proliferation and monoclonal antibody labeling of cell surface markers (T cell, B cell, CD4+, CD8+) were then performed.

Results: Subcutaneous morphine acutely suppressed lymphocyte proliferation to the mitogens phytohemagglutinin, pokeweed, and concanavalin A by 37%, 21%, and 20%, respectively; however, proliferative responses returned to baseline within 24 h. Morphine treatment did not alter the response to lipopolysaccharide. The number of splenic lymphocytes also decreased, whereas the percentage of lymphocytes expressing the CD4+ marker (T helper/inducer cells) modestly increased. Intrathecal morphine did not alter lymphocyte proliferative responses, nor did it change phenotypic expression of cell surface markers.

Conclusions: Subcutaneous morphine inhibited lymphocyte proliferation, decreased splenic lymphocyte number, and altered phenotypic expression of cell surface markers, whereas equianalgesic doses of intrathecal morphine did not. Although these results suggest that spinal opioids may have theoretical benefits for the analgesic management of immunocompromised patients, further studies are clearly indicated. (Key words: Opioids: morphine, intrathecal. Immune function: lymphocyte.)

CONSIDERABLE evidence suggests that opioids interact with the immune system.[1–3] High dose opioid anesthesia (morphine, fentanyl, and sufentanil) suppresses natural killer (NK) cell activity in both mechanically ventilated and spontaneously breathing rats,[4,5] whereas intermittent morphine administration is associated with decreased resistance to bacterial infection and depressed phagocytic function in mice.[6] In addition, acute subcutaneous administration of morphine suppresses Concanavalin A (ConA)-stimulated peripheral blood lymphocyte proliferation in rats. This effect is partially reversed by naltrexone.[7] Chronic opioid administration (morphine, heroin) results in depressed lymphocyte proliferative responses and reduced expression of cell surface antigens specific to T-lymphocytes and T-helper/inducer (CD4+) and T-suppressor/cytotoxic (CD8+) lymphocyte subsets in mice.[8,9] Although there is surprisingly little data in humans regarding the effects of acutely administered opioids on immune function, chronic exposure to opioids results in a reduction in lymphocyte proliferative responses, decreased levels of T cells, and depressed levels of the ratio between their CD4+ and CD8+ cells.[10,11] In addition, 90–150 mg oral morphine for a 36–60 h period results in a significant decrease in antibody-dependent cell cytotoxicity by peripheral blood mononuclear cells in human volunteers.[12]

The mechanism by which opioids influence immune function is not clear. *In vitro*, morphine inhibition of lymphocyte proliferative responses occurs at concentrations that are two orders of magnitude greater than the plasma concentrations resulting from subcutaneous

administration, which suppress lymphocyte proliferation and produce analgesia *in vitro*.[13] In contrast to morphine, systemic administration of N-methyl morphine, a quaternary analog of morphine that does not cross the blood–brain barrier, has no effect on NK cell activity.[14] These findings suggest that brain opioid receptors are involved in the immunosuppressive effects of opioids, independent of direct drug effects on circulating lymphocytes. The thesis that opioids act centrally to alter immune function is directly supported by the observation that morphine injected into the lateral cerebral ventricle or the periaqueductal gray matter (PAG) of the mesencephalon in rats results in a powerful suppression of NK cell activity.[14,15]

Although opioids may have deleterious effects on immune function, surgical trauma leading to a pain state is also linked with suppressed immune competence.[16,17] Evidence suggests that postoperative immunosuppression may be induced neurogenically by the high intensity afferent input generated by injury.[18,19] The evidence that pain may suppress immune function emphasizes the need for aggressive control of both acute and chronic pain states. However, the accumulating body of data that suggests that opioids may negatively influence immune competency is disconcerting. Because no appropriate clinical outcome data exist, these observations raise the possibility of deleterious effects of effective analgesia that may be magnified in patient populations that have prior impairment of immune competency, such as patients with human immunodeficiency virus (HIV) or cancer.

It is known that opioids may exert their analgesic actions at several points along the neuraxis, including the brain and spinal cord in humans and animals.[20,21] Previous studies have shown a probable supraspinal action of morphine in altering immune function,[14,15] but the influence of spinal systems on immune competency have not been examined systematically. Therefore, this study compared the immunomodulatory effects of equianalgesic doses of subcutaneous and intrathecal morphine. To assess immune status and to differentiate between alterations in T and B cell function, lymphocyte proliferative assays were performed using the T cell mitogens, phytohemagglutinin (PHA) and ConA, a T and B cell mitogen, pokeweed mitogen (PWM), and a B cell mitogen, lipopolysaccharide. In addition, opioid administration reportedly alters the number of splenic and circulating lymphocytes and the number of lymphocyte subsets in some compartments of the immune system.[10,11,22] These changes may reflect alterations in lymphocyte trafficking and/or expression of receptors necessary for primary activation of the T cell in the immune response. Therefore, the effect of subcutaneous and intrathecal morphine on the total number of spleen lymphocytes and the number of T and B cells present in the spleen were assessed. In addition, expression of the CD4+ and CD8+ molecules were measured. The selection of these assays provides a broad assessment of lymphocyte function and/or activities.

Materials and Methods

Experimental procedures conformed to guidelines established by the Council of the American Physiologic Society and were approved by the Animal Care Committee of the University of California, San Diego.

Subjects

Male Sprague-Dawley rats (300–350 g; Harlan Industries, Indianapolis, IN) were maintained on a 12-h light-dark cycle, with free access to food and water. Rats were housed two per cage and allowed to acclimate for 1 week after arrival.

Animal Preparation

For intrathecal administration of a drug, chronic intrathecal catheters were implanted under halothane anesthesia, using a modification of the method described by Yaksh and Rudy.[23] Briefly, a polyethylene (PE-10) catheter was introduced through an incision in the atlanto-occipital membrane and advanced caudally, extending to the rostral edge of the lumbar enlargement, under aseptic conditions. After implantation, rats were housed individually and allowed to recover for 7–10 days. Animals with any sign of neurologic deficit were not used.

Drugs and Injections

The drugs were dissolved in sterile saline for both intrathecal and subcutaneous administration. Subcutaneous morphine sulfate (Merck, Rahwah, NJ) was administered in a dose of 10 mg/kg between the scapula, whereas intrathecal morphine sulfate was given as 30 μg in 10 μl saline. Intrathecal drugs were delivered in a total volume of 10 μl, followed by 10 μl saline to flush the catheter. Naltrexone hydrochloric acid (National Institute on Drug Abuse) was administered in a dose of 1 mg/kg subcutaneously.

Nociceptive Threshold Measurement

Nociceptive thresholds were determined using the paw withdrawal reflex to thermal stimulation.[24] Rats were individually placed in clear plastic chambers with a glass floor and allowed to acclimate for 30 min. Floor temperature was maintained at 30°C by a thermister-controlled heater unit. After the acclimation period, a radiant heat source was positioned under the glass floor directly beneath a hind paw. The trial was started by a switch that activated the radiant heat source and an electronic timer. When the rat briskly lifted its paw, the switch was released manually, stopping the timer. The radiant heat source was a high intensity projector lamp bulb located 40 mm below the glass floor and projecting through a 5 mm × 10 mm aperture in the top of a movable case. Lamp voltage was adjusted to give baseline latencies of 7–10 s. A cutoff of 20 s was assigned to prevent tissue damage.

Experimental Paradigms

Time Course of Analgesic Effects of Subcutaneous and Intrathecal Morphine. In the first experiment, equivalency of analgesia produced by intrathecal and subcutaneous morphine was defined. Groups of rats were given saline or morphine subcutaneously or intrathecally two times during a 5-h period (at time 0 and at 2.5 h), and nociceptive thresholds were measured as described above. Subcutaneous administration of 10 mg/kg morphine to rats results in a rapid rise in plasma morphine concentrations to 2 μg/ml, peaking within 30 min, with a half-life of approximately 2 h.[25] Therefore, this dosing regimen resulted in rapid systemic effect, which lasted for approximately 5 h.

Effect of Subcutaneous and Intrathecal Morphine on Immune Function Assays. Studies to investigate the effects of morphine on mitogen-induced lymphocyte proliferation and the phenotypic expression of lymphocyte cell surface markers were carried out with subcutaneous and spinal (intrathecal) drug delivery, using equianalgesic doses as defined in the time course study outlined earlier.

The effects of systemic morphine were examined in rats without implanted catheters randomly assigned on receipt into one of five groups: cage controls, subcutaneous saline, subcutaneous morphine, subcutaneous naltrexone, and coadministration of subcutaneous morphine and naltrexone. Rats received one injection at time zero and another 2.5 h after the first. Five hours after the first injection, the rats were killed by decapitation and the spleens aseptically removed. Lympho-

cytes were isolated, and immune functions assays were performed as described later. In a separate group of animals, the rats were sacrificed 24 h after the first injection and tissues isolated and processed as described.

The effects of intrathecal morphine were assessed in rats prepared with intrathecal catheters as described previously. Seven to 10 days after placement of intrathecal catheters, rats were assigned randomly to one of four groups: cage controls, intrathecal controls (no injection), intrathecal saline, and intrathecal morphine. Rats received one injection followed by the second injection 2.5 h later. As in the systemic studies, rats were killed 5 h after the first injection, and the spleens were removed for lymphocyte function analyses.

Physiologic Responses to Systemic Morphine

In a separate group of animals, the effect of subcutaneous morphine, injected as described previously, on heart rate, mean arterial blood pressure, and blood gases was assessed. Animals were anesthetized briefly with halothane (1.5%), and the tail artery was cannulated for systemic arterial blood pressure measurement and blood sampling. The rats were placed in rodent restrainers and allowed to recover from the anesthetic and acclimate for 45–60 min. The tail artery cannula was connected to a Grass polygraph (Model 7, Quincy, MA), and heart rate and mean arterial blood pressure were monitored for a 15-min baseline period and throughout the 5 h of drug treatment. Arterial blood samples (150 μl) were taken during the baseline period and at 0.5, 1, 2, 3, 4, and 5 h after the morphine injections, and arterial oxygen pressure, partial pressure of carbon dioxide, and pH were measured (by blood gas analyzer, Model 1306, Instrumentation Labs, Milano, Italy).

Lymphocyte Function Assays

Lymphocyte Proliferation Assay. Splenocytes were isolated using a modification of the method of Reynolds and coworkers.[26] Briefly, spleens were dissociated into a single cell suspension by teasing the spleens apart with two sterile needles into calcium and magnesium-free Hanks' balanced salt solution. The cells were centrifuged in 50-ml tubes at 400 × g for 30 min over 10 ml Ficoll-Hypaque (Histopaque, Sigma Chemical, St. Louis, MO) to produce mononuclear cells. Cells at the interface were collected and washed twice with Hanks' balanced salt solution and resuspended in RPMI 1640 tissue culture medium (supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine,

1% penicillin-streptomycin, 20 mM Hepes and 2×10^{-5} M 2-mercaptoethanol) at a concentration of 2×10^{6} cells/ml. Cells were counted on a Coulter cell counter (Model ZF, Hialeah, FL) and cell viability always exceeded 95%, as determined by trypan blue exclusion.

Lymphocyte proliferation was performed using a modification of a previously described method.[27] Briefly, 100 μl of the cell suspension (2×10^{5} cells/well) were incubated with increasing concentrations of the mitogens phytohemagglutinin, PWM, ConA, or E. coli lipopolysaccharide in a total volume of 200 μl in 96 well round-bottom microtiter plates. At each mitogen dose, cultures were plated in triplicate. Cultures were incubated for 4 days (phytohemagglutinin, PWM, ConA) or 6 days (lipopolysaccharide) in a humidified atmosphere of 5% CO_2/95% air. Eighteen hours before harvesting, the plates were pulsed with 1 μCi tritiated thymidine (New England Nuclear, Boston, MA) per well. Samples were harvested with a Skatron cell harvester (Model 11000, Sterling, VA) onto glass fiber filter paper and then counted on a beta-scintillation counter (model 1900CA, Packard, Downers Grove, IL). Results were expressed as counts per minute of mitogen-stimulated cultures minus counts per minute of unstimulated cultures from each animal.

Lymphocyte Cell Surface Marker Analysis. T cell, B cell, and T cell subset enumerations were identified by flow cytometry, using a modification of the method of Batuman and coworkers.[27] Fluorescein-labeled monoclonal antibodies directed at phenotypic cell surface markers (Pharmingen, San Diego, CA) were used. Mouse anti-rat antibodies were used to identify: T cells (clone OX52), B cells (clone OX33, anti-CD45), T helper cells (clone OX38, anti-CD4), and T cytotoxic/suppressor cells (clone OX8, anti-CD8). Optimal antibody concentrations were determined empirically. Cells (1×10^{6}) were incubated with appropriate concentrations of antibody in phosphate-buffered saline in a total volume of 300 μl on ice for 60 min. The cells were then washed with 3 ml cold phosphate-buffered saline, centrifuged at $400 \times g$, and resuspended in 0.5 ml cold phosphate-buffered saline. Fluorescence was analyzed using an Ortho cytofluorograph model 50-H (Ortho Pharmaceuticals, Raritan, NJ). Positive cells were calculated as a percentage above a threshold defined by the negative control (monoclonal antibody omitted). Average percent fluorescence of negative controls was 1–2%.

Statistical Analysis

Experiments for immune function were analyzed by a one-factor or two-factor analysis of variance and posthoc Tukey's for specific comparisons. Experiments that involved physiologic parameters and nociceptive thresholds were analyzed using repeated measures analysis of variance and linear contrasts for specific comparisons. Significance was assigned at the $P < 0.05$ level.

Results

Time Course of Analgesia

Both subcutaneous and intrathecal administration of morphine resulted in maximal paw withdrawal latency values over the majority of the 5-h period (fig. 1) and were significantly increased when compared with subcutaneous or intrathecal saline over all times (n = 4–5, $P < 0.001$). However, latencies after subcutaneous morphine began to return to baseline 2.5 h after both injections, whereas latencies decreased after the first intrathecal morphine injection only. Coadministration of subcutaneous morphine and naltrexone demonstrated a complete blockade of the analgesia, as
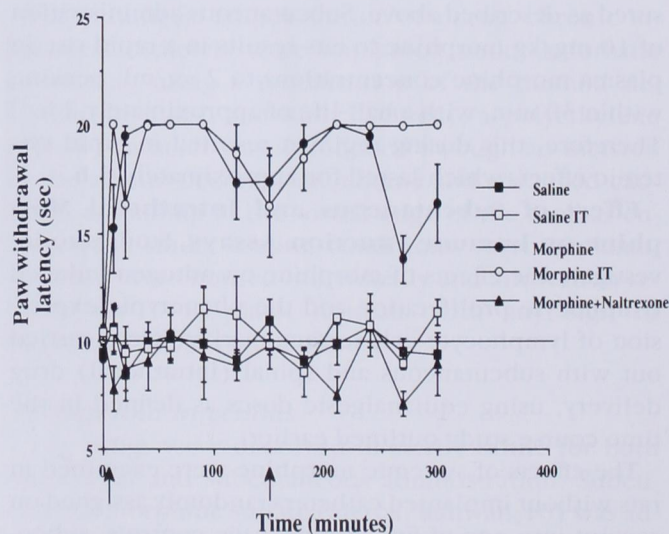

Fig. 1. Time course of paw withdrawal latencies after subcutaneous saline, subcutaneous morphine, subcutaneous morphine + naltrexone, intrathecal (IT) saline, and intrathecal morphine. Arrows indicate time of injections. Both subcutaneous and intrathecal morphine were significantly different from subcutaneous and intrathecal saline, respectively ($P < 0.001$). Subcutaneous morphine + naltrexone also differed significantly from subcutaneous morphine ($P < 0.001$).
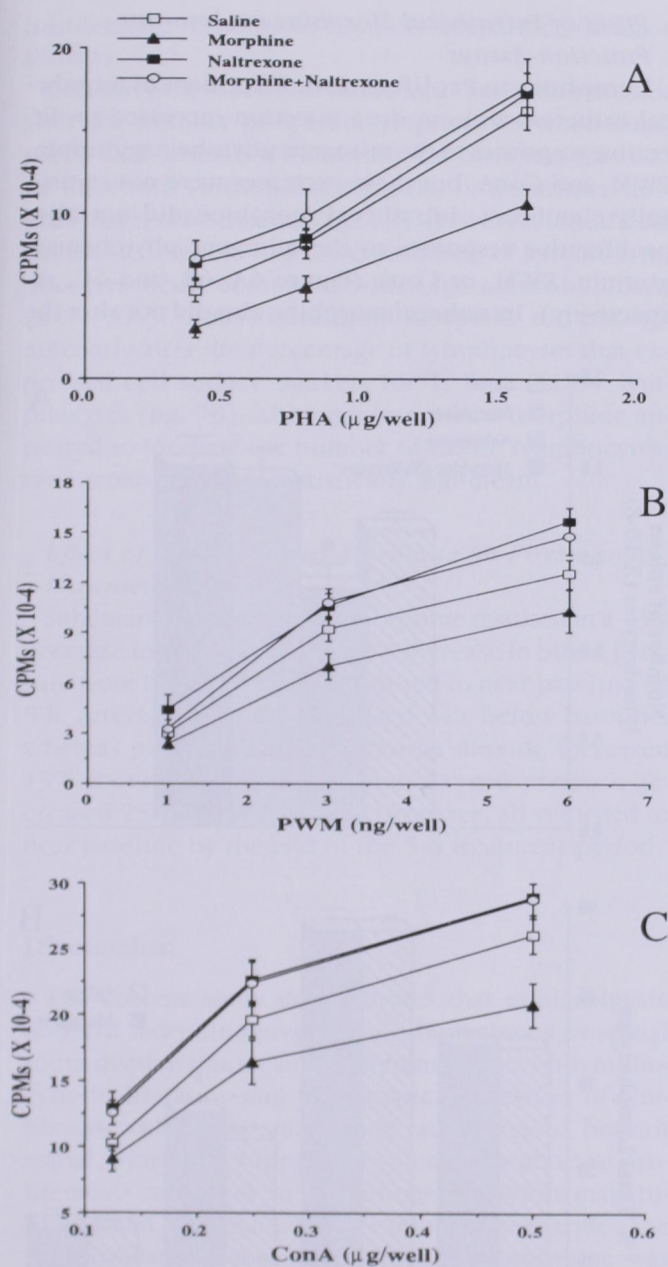
Fig. 2. Lymphocyte proliferative responses to the mitogens phytohemagglutinin (*A*), pokeweed mitogen (*B*), and Concanavalin A (*C*) after systemic saline, morphine, and/or naltrexone. The proliferative responses to all three mitogens after systemic morphine differed significantly from systemic saline, naltrexone, and morphine + naltrexone ($P < 0.001$). Naltrexone significantly increased the response to pokeweed mitogen (*B*; $P < 0.05$).

Effect of Subcutaneous Morphine on Immune Function Assays

Lymphocyte Proliferation. The effects of subcutaneous morphine and/or subcutaneous naltrexone on the proliferative response to the mitogens phytohemagglutinin, PWM, ConA and lipopolysaccharide were examined. Morphine significantly suppressed the response to phytohemagglutinin, and naltrexone reversed this effect (fig. 2A; n = 9, $P < 0.001$). Previous studies in our laboratory showed that saline injections alone do not alter proliferative responses when compared with home cage control animals (data not shown). Naltrexone alone had no effect on phytohemagglutinin-stimulated lymphocytes. Morphine also suppressed the proliferative response to PWM, and this effect also was reversed by naltrexone (fig. 2B; n = 9, $P < 0.001$). However, naltrexone significantly increased the proliferative response to PWM when given alone (n = 9, $P < 0.05$). Lymphocyte proliferative responses to ConA were suppressed by morphine, and again, these effects were blocked by coadministration of naltrexone (fig. 2C; n = 9, $P < 0.001$). Naltrexone had no effect on ConA-stimulated lymphocyte proliferation. Systemic morphine had no effect on lipopolysaccharide-stimulated lymphocyte proliferation (fig. 3). Proliferative responses to phytohemagglutinin, PWM, and ConA returned to control values within 24 h of treatment (fig. 4A, 4B, and 4C).

Cell Surface Markers. The effect of 5 h of subcutaneous morphine analgesia on total number of splenic
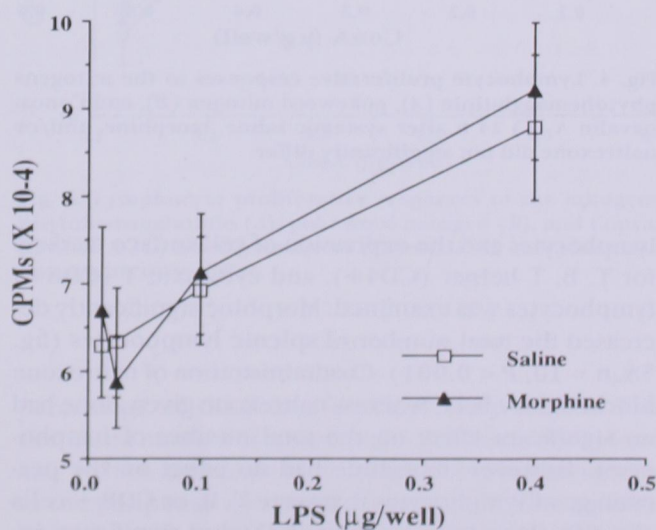

Fig. 3. Lymphocyte proliferative responses to the mitogen lipopolysaccharide acutely after systemic saline or morphine injections did not significantly differ.
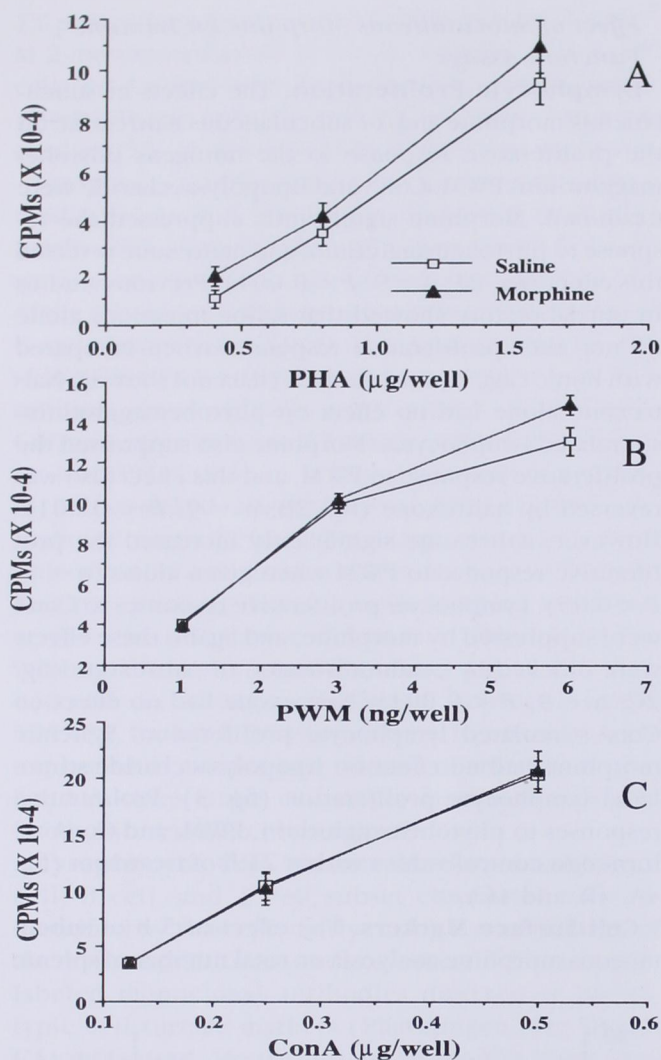
latencies in this group differed significantly from subcutaneous morphine (n = 5, $P < 0.001$) but not subcutaneous saline.

Fig. 4. Lymphocyte proliferative responses to the mitogens phytohemagglutinin (*A*), pokeweed mitogen (*B*), and Concanavalin A (*C*) 24 h after systemic saline, morphine, and/or naltrexone did not significantly differ.

lymphocytes and the expression of cell surface markers for T, B, T helper (CD4+), and cytotoxic T (CD8+) lymphocytes was examined. Morphine significantly decreased the total number of splenic lymphocytes (fig. 5A, n = 10, $P < 0.001$). Coadministration of naltrexone blocked this effect, whereas naltrexone given alone had no significant effect on the total number of lymphocytes. However, morphine had no effect on the percentage of lymphocytes that were T, B, or CD8 + cells (fig. 5B). Morphine caused a slight, but significant, increase in the number of CD4+ lymphocytes (n = 6, $P < 0.05$).

Effect of Intrathecal Morphine on Immune Function Assays

Lymphocyte Proliferation. Placement of intrathecal catheters without drug injection increased proliferative responses to the mitogens phytohemagglutinin, PWM, and ConA, but these increases were not statistically significant. Intrathecal morphine did not alter proliferative responses to the mitogens phytohemagglutinin, PWM, or ConA (Figure 6A, 6B, and 6C, respectively). Intrathecal morphine also did not alter the
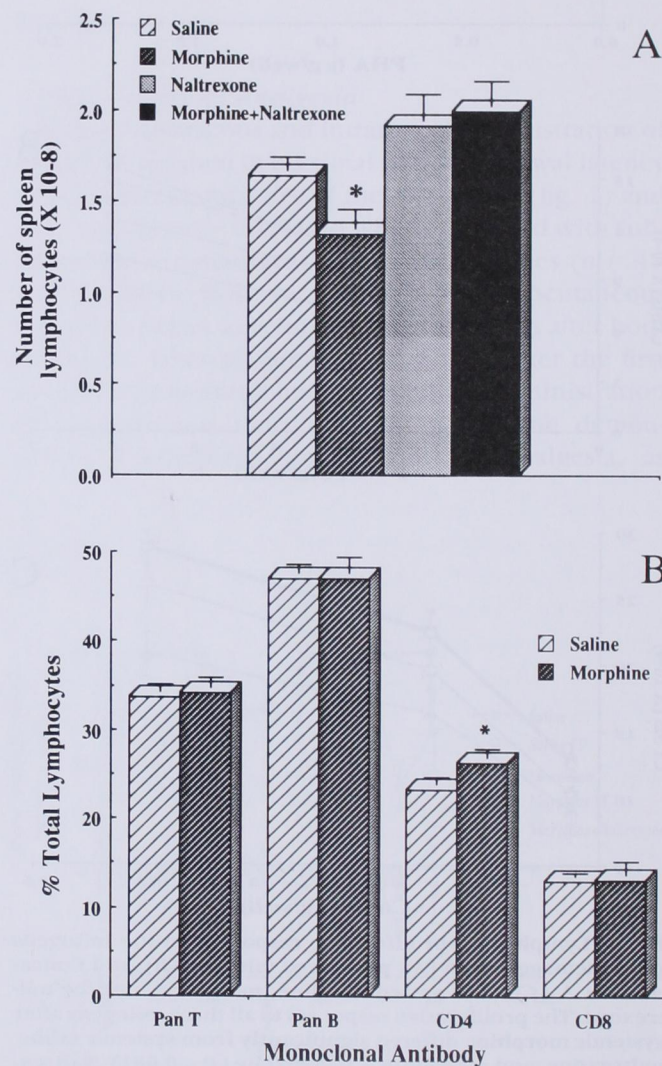

Fig. 5. Number of splenic lymphocytes after subcutaneous saline, morphine, and/or naltrexone (*A*) and percent of lymphocytes expressing cell surface markers for T lymphocytes (Pan T), B lymphocytes (Pan B), T helper cells (CD4), and cytotoxic T cells (CD8) (*B*). Morphine significantly decreased the total number of splenic lymphocytes (*A*; *$P < 0.001$). Morphine significantly increased the number of CD4+ lymphocytes (*B*; *$P < 0.05$).

proliferative response to lipopolysaccharide (data not shown).

Cell Surface Markers. Intrathecal morphine did not alter the number of spleen lymphocytes when compared with animals with intrathecal catheters that had no injections (intrathecal controls) or intrathecal animals that received saline (fig. 7A). However, intrathecal animals as a whole had lower numbers of splenic lymphocytes when compared with cage control animals (n = 7, $P < 0.05$). Intrathecal morphine did not significantly alter the percentage of lymphocytes that expressed cell surface markers for T, B, or CD8+ lymphocytes (fig. 7B). Although intrathecal morphine appeared to increase the number of CD4+ lymphocytes, the increase was not statistically significant.

Effect of Subcutaneous Morphine on Physiologic Parameters

Subcutaneous injection of morphine resulted in a 13% decrease in heart rate and a 16% increase in blood pressure from baseline, which returned to near baseline by 5 h. Arterial blood pH decreased 24% below baseline, whereas partial pressure of carbon dioxide increased 13% above baseline and arterial oxygen pressure decreased 23% below baseline. However, all returned to near baseline by the end of the 5-h treatment period.

Discussion

The current study demonstrates that equianalgesic doses of morphine given by a subcutaneous or spinal route display significantly different effects on lymphocyte proliferation and phenotypic expression of lymphocyte cell surface markers in rats. Systemic, but not spinal, morphine suppressed splenic lymphocyte proliferative responses to the mitogens phytohemagglutinin, PWM, and ConA, but not lipopolysaccharide. The most robust response to subcutaneous morphine was observed with the T cell mitogen phytohemagglutinin, with a smaller response noted with the T and B cell mitogen PWM. However, no response to morphine administration was observed with the B cell mitogen, lipopolysaccharide. These data suggest that subcutaneous morphine modifies T cell, but not B cell, proliferative responses in the rat. In addition, subcutaneous morphine resulted in a decrease in the number of splenic lymphocytes, although the percentages of T, B, and cytotoxic T lymphocytes did not change. Subcutaneous morphine, however, modestly increased the
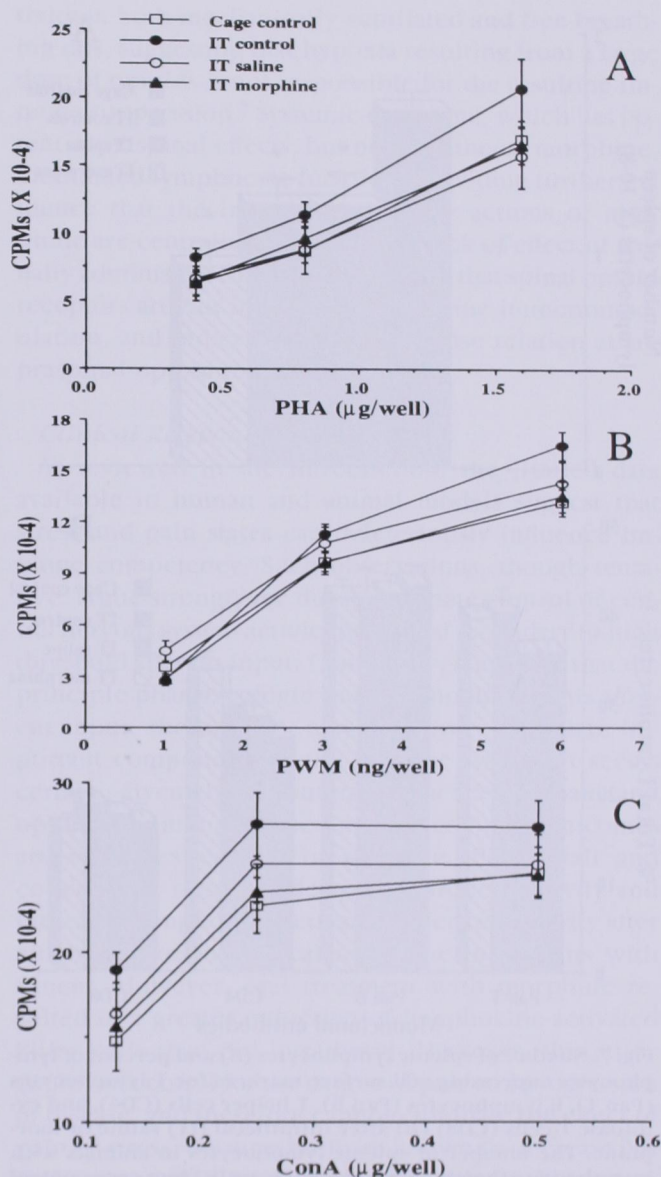

Fig. 6. Lymphocyte proliferative responses to the mitogens phytohemagglutinin (*A*), pokeweed mitogen (*B*), and Concanavalin A (*C*) after intrathecal (IT) saline or morphine did not significantly differ.

percentage of CD4+, or T helper lymphocytes. The decreased number of lymphocytes present in the spleen may reflect migration of lymphocytes from the spleen to the peripheral circulation in response to morphine treatment. In addition, the increased percentage of CD4+ cells may result from either preferential movement of other lymphocyte subsets (*i.e.*, CD8+, B cells)
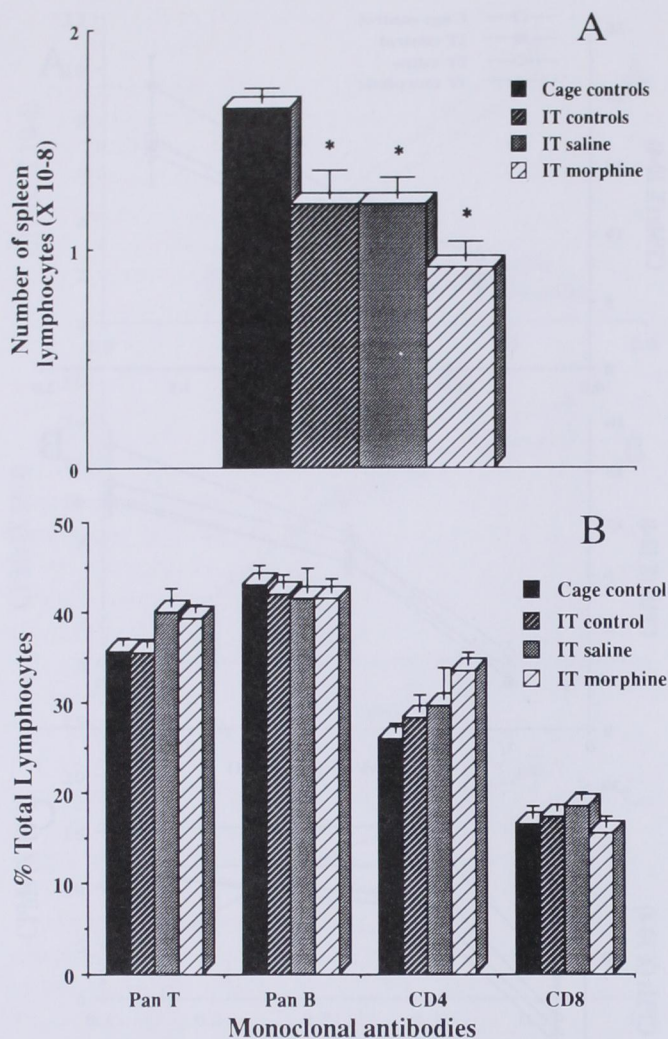
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Fig. 7. Number of splenic lymphocytes (A) and percent of lymphocytes expressing cell surface markers for T lymphocytes (Pan T), B lymphocytes (Pan B), T helper cells (CD4), and cytotoxic T cells (CD8) (B) after intrathecal (IT) saline or morphine. The number of splenic lymphocytes in animals with intrathecal catheters differed significantly from cage control animals (A; *P < 0.05). Intrathecal morphine did not significantly alter the percentage of T, B, CD4+, or CD8+ cells (B).

out of the spleen or increased expression of the CD4 molecule which is required for antigen recognition.

Importantly, these effects were reversed by naltrexone, an opioid receptor antagonist, suggesting that the effects of subcutaneous morphine were mediated through opioid receptors. Naltrexone alone significantly enhanced the response to the mitogen PWM while also affecting the response to phytohemagglutinin and ConA, although without statistical significance. These data are consistent with others[28] that point to a

tonic inhibitory effect of endogenous opioids on lymphocyte proliferation which is blocked by opioid antagonists. In addition, the effects of morphine were acute and did not persist much longer than the period of analgesia as lymphocyte proliferative responses were normal 24 h after morphine treatment.

In marked contrast to the actions of subcutaneous morphine, intrathecal morphine at doses that produced a comparable antinociceptive action for an equivalent time period or longer did not significantly alter lymphocyte proliferative responses or cell surface marker expression when compared with intrathecal saline treatment. However, animals that were implanted with intrathecal catheters but received no treatment had elevated proliferative responses, although not statistically significant, and decreased numbers of lymphocytes in the spleen. These responses reflect inflammatory responses to the presence of a chronic intrathecal implant (1–2 weeks) and presumably would not be noted in acute intrathecal injections. In addition, it is important to note that morphine did not alter either proliferative responses or cell numbers when compared with animals receiving intrathecal saline.

The present results are consistent with others that report suppressed proliferative responses after acute systemic administration of morphine in rats and humans.[4,7,12] The changes noted in the number of splenic lymphocytes are consistent with those studies reporting decreased spleen cellularity after chronic morphine administration[8]; however, both decreased and increased numbers of CD4+ cells have been observed.[9,29] Although systemic morphine reportedly suppresses splenic NK cell activity in rats, Bayer and coworkers[13] demonstrated suppression of proliferative activity of peripherally circulating, but not splenic, rat lymphocytes, whereas Lysle et al.[30] report suppression of mitogen-induced proliferation in both splenic and blood lymphocytes, but not in lymphocytes from the mesenteric lymph nodes. However, our results demonstrated suppression of T cell-mediated proliferation of splenic lymphocytes. The differences between these studies may result from differences in the dosing regimen and from compartment-specific effects. In this study, the rats received two subcutaneous injections of morphine 2.5 h apart and were sacrificed 2.5 h after the second injection. Bayer and coworkers, in contrast, injected the rats once and sacrificed the animals 2 h after the injection. Extended periods of exposure to subcutaneous morphine may, therefore, result in significant effects on not only pe-

ripherally circulating lymphocytes, but also those sequestered in the spleen.

Intervening Systems Mediating Opioid Effects on Immune Response

The mechanisms whereby subcutaneous morphine, acting through opioid receptors, alters immune function is not known. Increasing evidence indicates that the *in vivo* effects of morphine are mediated through central opioid receptors.[13-15] Two possible pathways that have been implicated in the mediation of the immunomodulatory effects of morphine are the hypothalamic-pituitary-adrenal axis and/or the sympathetic nervous system. Although the hypothalamic-pituitary-adrenal axis appears to be partially involved in the suppression of immune responses after chronic morphine treatment,[8] the suppression of lymphocyte proliferation after acute morphine administration is apparently glucocorticoid independent.[7] Recent evidence implicates the sympathetic nervous system in the suppression of NK cell activity, primarily through activation of alpha adrenoceptors.[31] Indeed, endogenous catecholamines are associated with alterations in lymphocyte trafficking and suppression of T cell function.[32,33] In addition, morphine and related opioid agonists were found to increase concentrations of plasma catecholamines after central administration.[34,35] Additional evidence has shown that stimulation of opioid receptors at discrete hypothalamic and brainstem sites increase sympathetic outflow.[36] As the spleen is directly innervated by noradrenergic fibers,[37] morphine immunomodulation may occur through local release of catecholamines in the spleen.

Opioids have potent effects on respiratory and cardiovascular function, resulting in hypoxia, hypercarbia, and acidosis.[38] In addition, hypoxia reportedly stimulates increases in catecholamine concentrations.[39] Because such changes are not typically observed after the spinal delivery of opioids in humans or in the present rodent model,[20,40] these physiologic alterations may account for the differences observed between these two routes of administration in the current experiments. Mild hypercarbia and hypoxia were observed for short intervals immediately after subcutaneous injections. Given that morphine has potent central effects that result in stimulation of sympathetic outflow, which can directly affect cells in the spleen, it seems unlikely that the effects noted in this study were a result of increases in plasma catecholamines resulting from mild hypoxia. In addition, opioid anesthesia suppresses NK cell ac-

tivity in both mechanically ventilated and free-breathing rats, suggesting that hypoxia resulting from a large dose of opioids is not responsible for the resulting immune suppression.[5] Systemic morphine, which has potent supraspinal effects, but not intrathecal morphine, modulated lymphocyte function, providing further evidence that the immunomodulatory actions of morphine are centrally mediated. The lack of effect of spinally administered morphine suggests that spinal opioid receptors are not involved in morphine immunomodulation, and indicates a dose–response relation at supraspinal opioid receptors.

Clinical Relevance

As reviewed in the introduction, the limited data available in human and animal models suggest that stress and pain states can deleteriously influence immune competency. Such observations, though tentative, argue strongly for the appropriate control of central nervous system activity produced secondary to high threshold afferent input. Conversely, it appears that the principle pharmacologic route of modifying this afferent input, the opioids, may negatively modulate important components of the immune system. It seems certain, given these contrary influences of pain and opioids on immune function, that complex outcomes are to be expected. For example, Provinciali and colleagues[41] observed decreased NK cell activity and increased lymphokine-activated killer cell activity after either oral or intrathecal morphine in patients with cancer. However, oral treatment with morphine resulted in a greater induction of lymphokine-activated killer cells than did intrathecal. However, this study could not control for the degree of pain and progression of disease, and therefore cannot eliminate the effect of pain or stress, because both are known to decrease NK cell activity.[42-44] In addition, morphine analgesia attenuates surgery-induced enhancement of metastases,[19] suggesting that the negative immunomodulatory effects of postoperative pain may outweigh those of morphine alone. These data are supported by Yeager and Colacchio,[45] who observed decreased tumor burden in rats given daily subcutaneous morphine injections the day before and 2 days after colon cancer cell inoculation. In contrast, acute but not chronic administration of morphine in mice augments mortality associated with Friend virus[46] and Toxoplasma gondii[47] infections.

Though not clear, the lack of effect on lymphocyte function after intrathecal morphine, as indicated in this study, suggests that the spinal administration of mor-

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phine may have some theoretical advantages in patients where immune competency is of importance. Morphine was shown to promote the growth of HIV in human peripheral blood mononuclear cells[48] and reactivate HIV reproduction in human Kupffer cells.[49] In addition, it was suggested that chronic exposure to morphine may predispose to HIV infection.[50] However, other factors are clearly relevant to this issue, including the incidence of catheter- or injection-related infections in immunocompromised patients.[51–53] In addition, epidural opioids have been associated with an apparent increase in the reactivation of Herpes simplex after Caesarean section.[54,55] Again, neither the pharmacology nor the mechanism of this effect is known. These issues are pertinent to the use of spinal analgesics in the pain patient and require ongoing consideration. The need to manage pain has long been acknowledged to be important for the psychological wellbeing of the patient. However, continued work in this area is particularly significant because of deleterious effects that pain-evoked afferent input may have on outcome after tissue trauma in healthy patients and, more importantly, in patients with compromised immune systems.

References

1. Blalock JE: A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol Rev 1989; 69:1–32

2. Carr DJJ: The role of endogenous opioids and their receptors in the immune system. Proc Soc Exp Biol Med 1991; 198:710–20

3. Morley JE, Kay NE, Solomon GF, Plotnikoff NP: Neuropeptides: Conductors of the immune orchestra. Life Sci 1987; 41:527–44

4. Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC: Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. Brain Behav Immun 1989; 3:129–37

5. Beilin B, Shavit Y, Cohn S, Kedar E: Narcotic-induced suppression of natural killer cell activity in ventilated and nonventilated rats. Clin Immunol Immunopathol 1992; 64:173–6

6. Tubaro E, Borelli G, Croce C, Cavallo G, Santiangeli C: Effect of morphine on resistance to infection. J Infect Dis 1983; 148:656–66

7. Bayer BM, Daussin S, Hernandez M, Irvin L: Morphine inhibition of lymphocyte activity is mediated by an opioid dependent mechanism. Neuropharmacology 1990; 29:369–74

8. Bryant HU, Bernton EW, Kenner JR, Holaday JW: Role of adrenal cortical activation in the immunosuppressive effects of chronic morphine treatment. Endocrinology 1991; 128:3253–8

9. Kimes AS, Smith WJ, Winchell CJ, London ED: Effects of morphine on the phenotypic expression of cell surface markers on murine lymphocytes. Life Sci 1992; 51:807–14

10. Brown SM, Stimmel B, Taub RN, Kochwa S, Rosenfield RE: Immunological dysfunction in heroin addicts. Arch Intern Med 1974; 134:1001–6

11. Donahoe RM, Falek A: Neuroimmunomodulation by opiates and other drugs of abuse: Relationship to HIV infection and AIDS, Psychological, Neuropsychiatric and Substance Abuse Aspects of AIDS. Edited by Bridge TP, Mirsky AF, Goodwin FK. New York, Raven Press, 1988, pp 145–58

12. Yeager MP, Yu CT, Campbell AS, Moschella M, Guyre PM: Effect of morphine and beta-endorphin on human Fc receptor-dependent and natural killer cell functions. Clin Immunol Immunopathol 1992; 62:336–43

13. Bayer BM, Gastonguay MR, Hernandez MC: Distinction between the in vitro and in vivo inhibitory effects of morphine on lymphocyte proliferation based on agonist sensitivity and naltrexone reversibility. Immunopharmacology 1992; 23:117–24

14. Shavit Y, Depaulis A, Martin FC, Terman GW, Pechnick RN, Zane CJ, Gale PG, Liebeskind JC: Involvement of brain opiate receptors in the immune-suppressive effect of morphine. Proc Natl Acad Sci U S A 1986; 83:7114–7

15. Weber RJ, Pert A: The periaqueductal gray matter mediates opiate-induced immunosuppression. Science 1989; 245:188–90

16. Abraham E: Host defense abnormalities after hemorrhage, trauma, and burns. Crit Care Med 1989; 17:934–9

17. Stevenson GW, Hall SC, Rudnick S, Seleny FL, Stevenson HC: The effect of anesthetic agents on the human immune response. ANESTHESIOLOGY 1990; 72:542–52

18. Koenig A, Koenig UD, Heicappel R, Stoeckel H: Differences in lymphocyte mitogenic stimulation pattern depending on anaesthesia and operative trauma: I. Halothane-nitrous oxide anaesthesia. Eur J Anaesthesiol 1987; 4:17–24

19. Page GG, Ben-Eliyahu S, Yirmiya R, Liebeskind JC: Morphine attenuates surgery-induced enhancement of metastatic colonization in rats. Pain 1993; 54:21–8

20. Yaksh TL, Al-Rodhan NRF, Jensen TS: Sites of action of opiates in production of analgesia. Prog Brain Res 1988; 77:371–94

21. Yaksh TL: Spinal opiates: A review of their effect on spinal function with emphasis on pain processing. Acta Anaesthesiol Scand 1987; 31:25–37

22. Bryant HU, Berton EW, Holaday JW: Morphine pellet-induced immunomodulation in mice: Temporal relationships. J Pharmacol Exp Ther 1988; 245:913–20

23. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976; 117:1031–6

24. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32:77–88

25. Berkowitz BA, Cerreta KV, Spector K: The influence of physiologic and pharmacologic factors on the disposition of morphine as determined by radioimmunoassay. J Pharmacol Exp Ther 1974; 191:527–34

26. Reynolds CW, Timonen T, Herberman RB: Natural killer cell activity in the rat. I. Isolation and characterization of the effector cells. J Immunol 1981; 127:282–7

27. Batuman OA, Sajewski D, Ottenweller JE, Pitman DL, Natelson BH: Effects of repeated stress on T cell numbers and function in rats. Brain Behav Immun 1990; 4:105–17

28. Manfredi B, Sacerdote P, Bianchi M, Locatelli L, Veljic-Radulovic J, Panerai AE: Evidence for an opioid inhibitory effect on T cell proliferation. J Neuroimmunol 1993; 44:43–8

29. Arora PK, Fride E, Petito J, Waggie K, Skolnick P: Morphine-induced immune alterations in vivo. Cell Immunol 1990; 126:343–53

30. Lysle DT, Coussons ME, Watts VJ, Bennett EH, Dykstra LA: Morphine-induced alterations of immune status: Dose dependency, compartment specificity and antagonism by naltrexone. J Pharmacol Exp Ther 1993; 265:1071–8

31. Carr DJJ, Gebhardt BM, Paul D: Alpha adrenergic and mu-2 opioid receptors are involved in morphine-induced suppression of splenocyte natural killer activity. J Pharmacol Exp Ther 1993; 264:1179–85

32. Crary B, Hauser SL, Borysenko M, Kutz I, Hoban C, Ault KA, Weiner HL, Benson H: Epinephrine-induced changes in the distribution of lymphocyte subsets in peripheral blood of humans. J Immunol 1983; 131:1178–81

33. Heilig M, Irwin M, Grewal I, Sercarz E: Sympathetic regulation of T-helper cell function. Brain Behavior Immun 1993; 7:154–63

34. Van Loon GR, Appel NM, Ho D: B-endorphin-induced stimulation of central sympathetic outflow: B-endorphin increases plasma concentrations of epinephrine, norepinephrine and dopamine in rats. Endocrinology 1981; 109:49–53

35. Siren AL, Paakkari P, Goldstein DS, Feuerstein G: Mechanisms of central hemodynamic and sympathetic regulation by mu opioid receptors: Effects of dermorphin in conscious rat. J Pharmacol Exp Ther 1989; 248:596–604

36. Appel NM, Kiritsy-Roy JA, Van Loon GR: Mu receptors at discrete hypothalamic and brainstem sites mediate opioid peptide-induced increases in central sympathetic outflow. Brain Res 1986; 378:8–20

37. Felten DL, Felten SY, Carlson SY, Olschowka JA, Livnat S: Noradrenergic and peptidergic innervation of lymphoid tissue. J Immunol 1985; 135:755s–65s

38. Bailey PL, Streisand GB, East KA, East TD, Isern S, Hansen TW, Posthuma EFM, Rozendaal FW, Pace NL, Stanley TH: Differences in magnitude and duration of opioid-induced respiratory depression and analgesia with fentanyl and sufentanil. Anesth Analg 1990; 70:8–15

39. Hart BB, Stanford GG, Ziegler MG, Lake CR, Chernow B: Catecholamines: Study of interspecies variation. Crit Care Med 1989; 17:1203–22

40. Yaksh T, Nouihed R: The physiology and pharmacology of spinal opiates. Annu Rev Pharmacol Toxicol 1985; 25:433–62

41. Provinciali M, Di Stefano G, Raffaeli W, Pari G, Desiderio F, Fabris N: Evaluation of NK and LAK cell activities in neoplastic patients during treatment with morphine. Int J Neurosci 1991; 59:127–33

42. Tonnesen E, Brinklov MM, Christensen NJ, Olesen AS, Madsen T: Natural killer cell activity and lymphocyte function during and after coronary artery bypass grafting in relation to the endocrine stress response. ANESTHESIOLOGY 1987; 67:526–33

43. Pollock RE, Lotzova E, Standford SD: Surgical stress impairment of murine natural killer cell cytotoxicity involves pre- and postbinding events. J Immunol 1989; 143:3396–3404

44. Woodruff MFA: The cytolytic and regulatory role of natural killer cells in experimental neoplasia. Biochim Biophys Acta 1986; 865:43–57

45. Yeager MP, Colacchio TA: Effect of morphine on growth of metastatic colon cancer in vivo. Arch Surg 1991; 126:454–6

46. Starec M, Rouveix B, Sinet M, Chau F, Desforges B, Pocidalo JJ, Lechat P: Immune status and survival of opiate- and cocaine-treated mice infected with Friend virus. J Pharmacol Exp Ther 1991; 259:745–50

47. Chao C, Sharp B, Pomeroy C, Filice GA, Peterson PK: Lethality of morphine in mice infected with *Toxoplasma gondii*. J Pharmacol Exp Ther 1990; 252:605–9

48. Peterson PK, Sharp BM, Gekker G, Portoghese PS, Sannerud K, Balfour HH: Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures. AIDS 1990; 4:869–73

49. Schweitzer C, Keller F, Schmitt MP, Jaeck D, Adloff M, Schmitt C, Royer C, Kirn A, Aubertin AM: Morphine stimulates HIV replication in primary cultures of human Kupffer cells. Res Virol 1991; 142:189–95

50. Arora PK: Morphine-induced immune modulation: Does it predispose to HIV infection. NIDA Res Monogr 1990; 96:150–6

51. Crul BJ, Delhaas EM: Technical complications during longterm subarachnoid or epidural administration of morphine in terminally ill cancer patients: A review of 140 cases. Reg Anaesth 1991; 16:209–13

52. DuPenet SL, Peterson DG, Williams A, Bogosian AJ: Infection during chronic epidural catheterization: Diagnosis and treatment. ANESTHESIOLOGY 1990; 73:905–9

53. Tom DJ, Gulevich SJ, Shapiro HM, Heaton RK, Grant I: Epidural blood patch in the HIV-positive patient: Review of clinical experience. ANESTHESIOLOGY 1992; 76:943–7

54. Crone LA, Conly JM, Clark KM, Crichlow AC, Wardell GC, Zbitnew A, Rea LM, Cronk SL, Anderson CM, Tan LK: Recurrent herpes simplex virus labialis and the use of epidural morphine in obstetric patients. Anesth Analg 1988; 67:318–23

55. Valley MA, Bourke DL, McKenzie AM: Recurrence of thoracic and labial herpes simplex virus infection in a patient receiving epidural fentanyl. ANESTHESIOLOGY 1992; 76:1056–7