Biochemical Pharmacology, Vol. 53, pp. 823–828, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00006-3

Inhibition of Swine Microglial Cell Phagocytosis of *Cryptococcus neoformans* by Femtomolar Concentrations of Morphine

Grzegorz Sowa, Genya Gekker,* Myriam M. Lipovsky,* Shuxian Hu,* Chun C. Chao,*† Thomas W. Molitor‡ and Phillip K. Peterson*†‡§*

*Neuroimmunobiology and Host Defense Laboratory, Minneapolis Medical Research Foundation, Minneapolis, MN; †University of Minnesota Medical School, Minneapolis, MN; and ‡College of Veterinary Medicine, University of Minnesota, St. Paul, MN, U.S.A.

ABSTRACT. Microglia are important immune effector cells within the brain. The phagocytosis of nonopsonized *Cryptococcus neoformans* by swine microglia was used as an *in vitro* model for studies on cellular mechanisms of opiate-mediated immunomodulation in the brain. Morphine inhibited potently ($IC_{50} \approx 10^{-16}$ M) the phagocytosis of C. *neoformans* by primary cultures of neonatal pig microglia. The μ opioid agonist Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol (DAMGO) also suppressed phagocytosis but with a much lower potency than morphine ($IC_{50} \approx 10^{-8}$ M). The inhibitory effects of morphine and DAMGO were blocked by equimolar concentrations of naloxone and by the selective μ opiate receptor antagonist β-funaltrexamine. Pertussis toxin but not cholera toxin reversed the inhibitory effects of both morphine and DAMGO. Our data suggest that morphine inhibits phagocytosis of C. *neoformans* by swine microglia via a mechanism involving μ opiate receptors coupled to a pertussis toxin-sensitive G_i/G_o protein signaling pathway. BIOCHEM PHARMACOL 53;6:823–828, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. morphine; μ opiate receptor; microglia; phagocytosis; *Cryptococcus neoformans*

Opiates are known to modulate a number of immune responses [1, 2]. Mononuclear and polymorphonuclear phagocytes play important roles in host defense against a variety of microorganisms. Morphine-induced alteration of the respiratory burst activity of human polymorphonuclear leukocytes and mononuclear phagocytes [3–6] and impairments of the antimicrobial activity of human [7] and murine phagocytes [8, 9] have been reported.

The CNS is the primary target of opiate action. Despite a growing body of evidence that opiates have neuroimmunomodulatory effects, little is known about the action of opiates on microglia. In the CNS, microglia are believed to be the functional equivalent of macrophages in somatic tissues [10, 11]. Because the brain is the primary target of opiates, it has been suggested that opiates may play an immunomodulatory role within the CNS via their effects on microglial cells [12]. Recent *in vitro* studies in our laboratory demonstrated that morphine stimulates the phagocytosis of nonopsonized *Mycobacterium tuberculosis* by human microglia [13]. In the present study, an interest in opiate effects on phagocytosis of nonopsonized microorganisms was extended to the fungus *Cryptococcus neoformans*. C. *neoformans* meningoencephalitis is an opportunistic fungal infection that affects 5–10% of AIDS patients [14, 15]. Cell-mediated immunity provided by T lymphocytes and activated macrophages is the main line of defense against C. *neoformans* infection [16].

In this study, we used primary cultures of neonatal swine microglia, which we have found share a number of functional properties of human fetal microglia [17]. Using a recently developed method that specifically identifies phagocytized (i.e. ingested) cryptococci,[‖] we found that this process is inhibited profoundly by extremely low (femtomolar) concentrations of morphine. We also examined the mechanism of morphine's suppressive effect and found that a G protein-coupled μ opiate receptor appears to be involved.

MATERIALS AND METHODS

Chemicals

Morphine sulfate was obtained from the Hennepin County Medical Center Pharmacy. The μ opioid receptor antago-

§ Corresponding author: Phillip K. Peterson, M.D., Department of Medicine, Hennepin County Medical Center, 701 Park Ave., Minneapolis, MN 55415. Tel. (612) 347-2877; FAX (612) 904-4299.

Received 15 August 1996; accepted 24 October 1996.

‖ Lipovsky MM, Gekker G, Anderson WR, Molitor TW, Peterson PK and Hoepelman A, Manuscript submitted for publication.

nist β-FNA¶ was provided by Dr. P. S. Portoghese (University of Minnesota). The selective μ opioid receptor agonist DAMGO, pertussis toxin, and cholera toxin were purchased from Research Biochemicals International (Natick, MA).

C. neoformans

The encapsulated strain NIH 37 serotype A C. *neoformans* was obtained from the National Institutes of Health (NIH, Bethesda, MD) and was maintained on Saboraud Dextrose Agar (Merck, Darmstadt, Germany) at 4°.

Microglial Cell Cultures

One-day-old outbred Yorkshire pigs were obtained from the University of Minnesota Animal Facility under a protocol approved by the Animal Care, Use, and Research Committee. Microglial cell cultures were prepared as previously described [17]. Briefly, the meninges were removed from the cerebral cortex and tissues were dissociated following a 30-min trypsinization (0.25%). Isolated cells were layered on top of medium containing 9.6 mL of 100% Percoll, 4.8 mL of 1.25 M sucrose, and 25 mL of PBS and centrifuged at 30,000 *g* at 4° for 45 min. Dissociated cells (2.5×10^5 cells/mL) were then plated in 75-cm^2 culture flasks in complete medium, containing DMEM (Sigma Chemical Co., St. Louis, MO), 10% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT)), penicillin (100 U/mL), and streptomycin (100 μg/mL). Culture medium was changed every 3 days after plating.

Microglial Cell Treatments

Prior to constituting the phagocytosis assay, microglial cell cultures were exposed to opioids, opiate antagonists, or toxins for indicated periods of time in complete culture medium followed by washing and reconstituting the cells in culture medium devoid of fetal bovine serum but containing each of the indicated test reagents.

Phagocytosis Assay

Phagocytosis of nonopsonized C. *neoformans* by swine microglia was quantified microscopically, a procedure to be described in detail elsewhere.* Briefly, C. *neoformans* (the target cells) were heat-killed (56° for 30 min), and diluted to 10^8 yeast cells/mL. The yeast cells were labeled with FITC (Sigma) at 50 μg/mL in PBS at room temperature for 30 min. Microglia (the effector cells) were placed in four-chamber tissue culture slides (2.5×10^4 cells in 0.5 mL of complete medium per well). After 1 hr of incubation at 37°, the medium was replaced, and the cells were treated as indicated above. Thereafter, the complete medium was replaced by 0.5 mL of DMEM with FITC-labeled C. *neoformans* (2.5×10^6 yeast cells/mL; effector:target cells ratio of 1:50). After an incubation of 2 hr at 37° with gentle shaking (85 rpm), cells were washed three times with 1 mL of PBS and then incubated for 5 min at room temperature with the fluorescent dye diaethanol (Uvitex; 1% solution in PBS). After washing two times with PBS, the slides were dried and fixed with 100% methanol for 3 min. The slides were kept at 4° until examined for phagocytosis. Using an epifluorescent microscope equipped with a UV objective, 4–5 random microscope fields were examined under bright field illumination to determine the cells with associated yeast (100 cells per each slide were counted), and then examined with UV excitation to exclude the adherent yeast stained with Uvitex. Only fully internalized yeasts (i.e. not stained with Uvitex) were counted. The percentage of microglia that phagocytized at least one yeast cell (% phagocytosis), and the total number of yeast cells ingested by 100 microglial cells (yeast/100 microglia) were assessed. The slides were evaluated by a microscopist blinded to the treatment groups.

Statistics

Data are expressed as means ± SEM of values from 2 to 3 independent experiments using microglia isolated from different pigs. Where appropriate, data were compared by analysis of variance using Stat View II (Abacus Concepts, Inc., Berkeley, CA), and significant differences were assessed with Fisher's least significant difference test ($P < 0.05$).

RESULTS

Phagocytosis of C. neoformans *by Microglial Cells*

Phagocytosis of nonopsonized C. *neoformans* was first assessed using swine microglia that were not exposed to opiates. After 2 hr of incubation, the percentage of microglial cells that ingested one or more yeast cells (% phagocytosis) was 29 ± 3, while the total number of yeast cells ingested by 100 microglial cells (yeast/100 microglia) was 37 ± 6 (N = 6). Of the microglial cells that had ingested cryptococci, there were on average 1.29 ± 0.12 cryptococci/cell (range 1 to 5 yeast/cell) (N = 6).

Opiate Effects on Phagocytosis of C. neoformans

To determine if morphine affected phagocytosis of C. *neoformans*, microglial cells were preincubated with morphine at concentrations ranging between 10^{-20} and 10^{-6} M for 24 hr, and then challenged with cryptococci for an additional 2 hr. Morphine inhibited in a concentration-dependent manner both the percentage of cells that had internalized yeast (Fig. 1A) and the number of yeast cells ingested per 100 microglial cells (Fig. 1B). Phagocytosis was maximally

¶ *Abbreviations*: β-FNA, β-funaltrexamine; DAMGO, Tyr-D-Ala-Gly-*N*-Me-Phe-Gly-ol; DMEM, Dulbecco's modified Eagle's medium; and FITC, fluorescein isothiocynate.

* Lipovsky MM, Gekker G, Anderson WR, Molitor TW, Peterson PK and Hoepelman A, Manuscript submitted for publication.

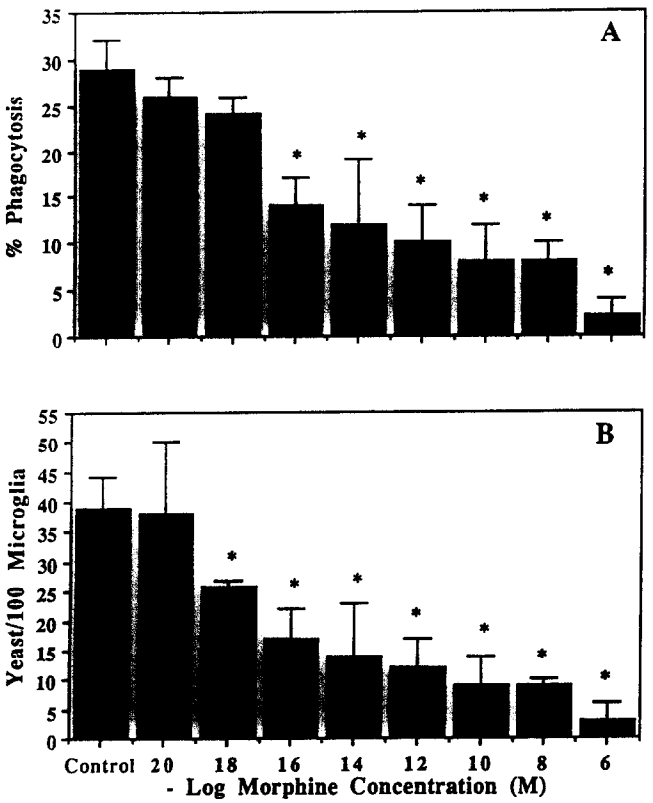

FIG. 1. Concentration-dependent effect of morphine on microglial cell phagocytosis of *C. neoformans*. Microglial cells (2.5×10^4) were preincubated for 24 hr in medium alone (control) or medium containing morphine at the indicated concentrations prior to adding yeast cells. After 2 hr of incubation with cryptococci, phagocytosis was assessed by determining: (A) the percentage of cells that ingested one or more cryptococci, and (B) the total number of cryptococci ingested by 100 microglia. Data are means ± SEM from three independent experiments. Key: (*)$P < 0.05$, compared with control.

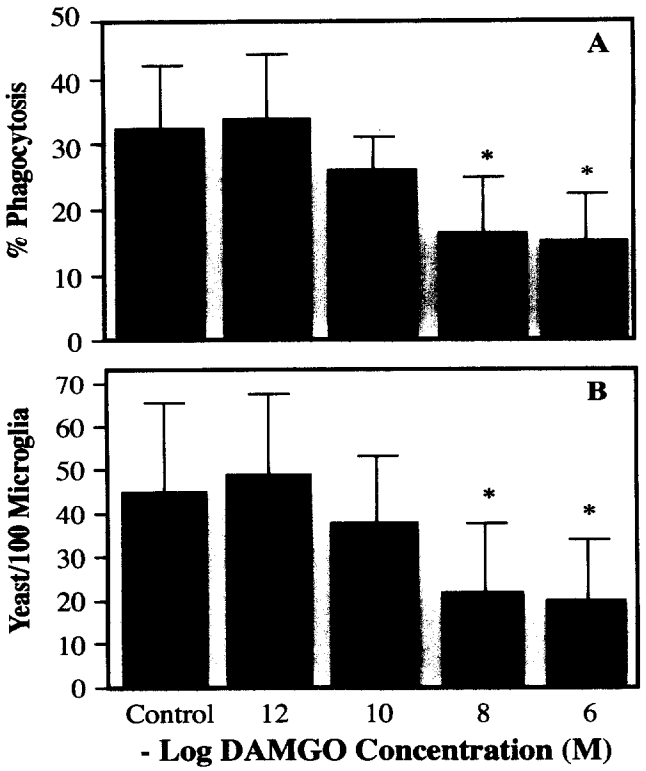

FIG. 2. Concentration-dependent effect of DAMGO on microglial cell phagocytosis of *C. neoformans*. Microglial cells (2.5×10^4) were preincubated for 24 hr in medium alone (control) or in medium containing DAMGO at indicated concentrations prior to adding yeast cells. After 2 hr of incubation with cryptococci, phagocytosis was assessed by determining: (A) the percentage of cells that ingested one or more cryptococci, and (B) the total number of cryptococci ingested by 100 microglia. Data are means ± range from two independent experiments. Key: (*)$P < 0.05$ compared with control.

decreased by morphine at a concentration of 10^{-6} M (93% inhibition), and the inhibitory activity of morphine was sustained at concentrations as low as 10^{-16} M. The μ receptor selective opioid agonist DAMGO also suppressed phagocytosis (maximally decreased by 55% at 10^{-6} M) (Fig. 2A) and the number of yeast cells ingested per 100 microglial cells (Fig. 2B). However, the suppressive activity of DAMGO was lost at concentrations of 10^{-10} M and below. Thus, although morphine and DAMGO were both capable of inhibiting phagocytosis of cryptococci by microglial cells, the inhibitory activity of morphine was orders of magnitude greater than that of DAMGO (IC_{50} values of about 10^{-16} M and 10^{-8} M, respectively). Overnight pretreatment of the microglial cells with opioids was required, since neither morphine (10^{-12} M) nor DAMGO (10^{-8} M) had any effect when added 3 hr before or at the time of the phagocytosis assay (data not shown).

Effects of Opiate Antagonists

To determine if the inhibitory effects of morphine and DAMGO were related to classical opioid receptors, microglial cells were pretreated with the relatively μ selective opiate receptor antagonist β-FNA for 30 min prior to treatment for 24 hr with morphine (10^{-12} M) or DAMGO (10^{-8} M). At equimolar concentrations, β-FNA completely blocked the inhibitory activities of morphine and of DAMGO, whereas β-FNA alone had no effect on phagocytosis (Fig. 3). This finding suggests that both opioids inhibit phagocytosis via a μ opiate receptor mechanism. Similar results were obtained when microglia were pretreated with the nonselective opiate receptor antagonist naloxone for 30 min prior to adding 10^{-12} M morphine to the microglial cell cultures, i.e. at an equimolar concentration, naloxone blocked by 94.7% the inhibitory activity of morphine on phagocytosis (mean of two separate experiments).

Involvement of G_i/G_o Proteins in Inhibitory Effects of Opioids

To determine if the inhibitory effects of morphine and DAMGO were mediated through a G protein signaling pathway, microglial cells were preincubated for 30 min with pertussis toxin (10 ng/mL) or cholera toxin (10 ng/mL),

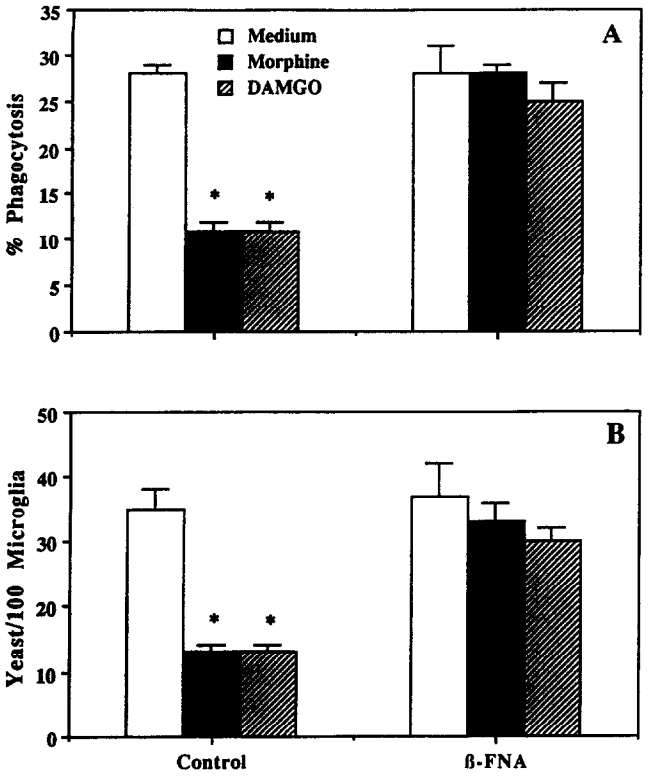

FIG. 3. Effects of β-FNA on morphine and DAMGO. Microglial cells (2.5×10^4) were incubated in medium alone (control) or were pretreated with β-FNA (10^{-12} M) for 30 min prior to incubation for 24 hr in medium alone or medium containing 10^{-12} M morphine or 10^{-8} M DAMGO. Yeast cells were added, and after 2 hr of culture, phagocytosis was assessed by determining: (A) the percentage of cells that ingested one or more cryptococci, and (B) the total number of cryptococci ingested by 100 microglia. Data are means ± SEM from three independent experiments. Key: (*)$P < 0.05$ compared with medium control.

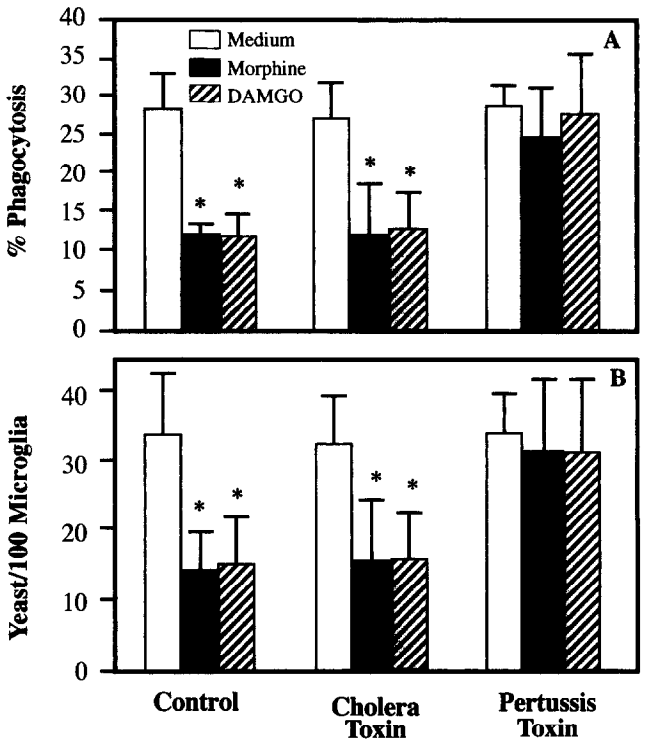

FIG. 4. Effects of pertussis toxin on morphine and DAMGO. Microglial cells (2.5×10^4) were incubated in medium (control) or were pretreated with cholera toxin (10 ng/mL) or pertussis toxin (10 ng/mL) for 30 min prior to incubation for 24 hr in medium alone or medium containing 10^{-12} M morphine or 10^{-8} M DAMGO. Yeast cells were added, and after 2 hr of culture, phagocytosis was assessed by determining: (A) the percentage of cells that ingested one or more cryptococci, and (B) the total number of cryptococci ingested by 100 microglia. Data are means ± range from two independent experiments. Key: (*)$P < 0.05$ compared with medium control.

and then treated with morphine (10^{-12} M) or DAMGO (10^{-8} M) for 24 hr. While pertussis and cholera toxin themselves did not affect phagocytosis, pertussis toxin but not cholera toxin completely reversed the inhibitory effects on phagocytosis of morphine and DAMGO (Fig. 4).

DISCUSSION

In the present study, we found that morphine almost completely inhibited the phagocytosis of nonopsonized C. *neoformans* by swine microglial cells. Moreover, astonishingly low concentrations (10^{-16} M) of morphine were sufficient for this inhibitory effect. In sharp contrast to these findings, the phagocytosis of M. *tuberculosis* by human microglia was shown in previous studies to be enhanced by morphine and only by concentrations in the nanomolar range [13]. The reason for these opposing effects of morphine on microglial cell phagocytosis (i.e. inhibition of ingestion of cryptococci vs stimulation of ingestion of M. *tuberculosis*) is unknown but could be related to differences in the nature of the target (large encapsulated yeast vs small unencapsulated bacilli) or to the animal species or age from which the effector cells were derived (neonatal swine vs fetal human microglia).

The phagocytosis of nonopsonized C. *neoformans* by swine microglia seems to be mediated via CD14 receptors,* as is also the case for phagocytosis of nonopsonized tubercle bacilli by human microglia [18]. Thus, modulation of CD14 receptors may be involved both in the stimulatory and inhibitory effects of morphine on phagocytosis of nonopsonized microorganisms by microglia. Higher (nanomolar) concentrations of morphine have been reported to inhibit the phagocytosis by murine peritoneal macrophages of sheep erythrocytes opsonized with immunoglobulin IgG [19] and nonopsonized *Candida albicans* [20, 21]. However, the maximal inhibitory effects of morphine in these earlier studies were lower (below 40%) than those observed in this study. Possibly the different mechanisms of phagocytosis of these targets, i.e. Fc receptors for IgG opsonized erythro-

* Lipovsky MM, Gekker G, Anderson WR, Molitor TW, Peterson PK and Hoepelman A, Manuscript submitted for publication.

cytes and lectin-like receptors for nonopsonized C. *albicans* vs CD14 receptor-mediated phagocytosis of nonopsonized C. *neoformans* could be the reason for the observed differences in the potency of the inhibitory action of morphine in these experimental models. Differences due to animal species (rodent vs swine) and the type of mononuclear phagocyte (peritoneal macrophages vs microglia) could be other explanations.

The inhibitory effect of morphine in this study appeared to be mediated via μ opiate receptors, since it was reversed by the relatively selective μ opioid receptor antagonist β-FNA as well as by the nonselective opioid receptor antagonist naloxone. These data are consistent with the observation of Szabo *et al.* [21], who also demonstrated such a μ opioid receptor selectivity of morphine. Since our experiments with antagonists suggested that the μ opiate receptor was involved in the inhibitory effect of morphine, we also used the selective μ opiate receptor agonist DAMGO. To our surprise, DAMGO was about eight orders of magnitude less potent than morphine, as well as less effective in terms of its maximal inhibitory effect. In contrast to our findings, DAMGO was more effective than morphine in inhibition of phagocytosis of nonopsonized C. *albicans* by murine peritoneal macrophages [21]. However, in both cell types, namely swine microglia and murine peritoneal macrophages, the potencies and the maximal inhibitory effects of DAMGO on phagocytosis were similar. The effect of DAMGO at a nanomolar concentration in inhibiting a variety of different immune functions is consistent with K_d values reported for μ opioid receptor specific ligands in different cell types of both nervous and immune tissue origin [22–24], and supports the involvement of the classical μ opioid receptor in regulation of these functions.

The different potencies of morphine and DAMGO in inhibiting microglial cell phagocytosis of C. *neoformans* could be explained by the involvement of different subtypes of μ opiate receptors activated by these ligands. For example, the existence of a μ_3 opioid receptor sensitive to morphine and other opiate alkaloids but not opioid peptides, including DAMGO, has been reported [23, 25, 26]. Using [^{3}H]morphine in binding assays, a μ_3 receptor subtype has been characterized in different cell types, including cat microglia, with a K_d of 14 nM [26]. Moreover, exposure of cat microglia to morphine or etorphine but not to opioid peptides caused changes in the morphology of these cells, suggesting that these functional effects of morphine were mediated exclusively by μ_3 but not by the classical μ_1 or μ_2 opioid receptors. Involvement of μ_3 receptors in functional effects of morphine on other phagocytic cell types also has been reported [25, 27].

Because μ_3 receptors appear to be sensitive to morphine only at nanomolar and higher concentrations [23, 25, 27] and are not activated by DAMGO, the findings in the present study would argue against μ_3 receptor involvement in the inhibitory effect of morphine on swine microglial cell phagocytosis of C. *neoformans*. Also, current knowledge about the classical μ_1 and μ_2 opioid receptors cannot provide a satisfactory explanation of the extremely high potency of morphine observed in this study. Interestingly, comparable potency of morphine in the modulation of other immune functions has been observed in previous *in vitro* studies in our laboratory. Picomolar concentrations of morphine were capable of potentiating lipopolysaccharide-induced tumor necrosis factor-α production by human [28] and murine [29] microglial cells. Also, we have found in studies of human peripheral blood mononuclear cell cultures that picomolar concentrations of morphine promote the growth of human immunodeficiency virus-1, and that this effect was both naloxone inhibitable and stereospecific, i.e. (+)-morphine had no effect [30].

Thus, our observations suggest that microglial cells have an ultra high affinity μ opiate binding site that is unlikely to be identified by classical pharmacological tools, such as ligand binding assays. Rather, novel approaches like studies on coupling of receptors to G proteins and related intracellular signaling pathways may be more fruitful. We initiated studies on the involvement of G proteins in mediating the effects of morphine on microglial cell phagocytosis in our earlier study of M. *tuberculosis* [13] and extended this assessment to the phagocytosis of C. *neoformans* in the present study. It appears that the activation of G_i/G_o but not G_s proteins by morphine is essential for its effect on phagocytosis, since this effect was fully reversed by the pretreatment of cells with pertussis toxin but not cholera toxin. This observation suggests that like classical μ opioid receptors [31], the microglial cell μ opioid receptor is coupled to pertussis toxin-sensitive G_i/G_o proteins.

In the present study, we found that only ~30% of the microglial cells in a given experiment participated in the phagocytosis of nonopsonized cryptococci. Although morphine profoundly inhibited the phagocytic activity of these cells, it is at present unknown whether they bear the putative μ opiate receptors. Alternatively, morphine could interact with a subpopulation of microglia, which may or may not be phagocytic, that release a factor that indirectly suppresses phagocytosis by neighboring cells within the microglial cell cultures.

The biological relevance of the *in vitro* finding that morphine is capable of inhibiting microglial cell phagocytosis of C. *neoformans* is unknown. If these cells play a role in the defense of the CNS against this fungus, then these findings suggest that opiate users would be at increased risk of developing cryptococcal meningoencephalitis. Because of the high incidence of this opportunistic infection in AIDS patients [14, 15], it would be of value to determine whether systemically administered opiates foster the pathogenesis of C. *neoformans*. Interestingly, morphine has been reported to be synthesized endogenously in animal tissues including the brain [32], and the concentration of morphine in the brains of mice has been shown to increase up to 10 pmol/g tissue after i.p. injection of the immunomodulator muramyl dipeptide [33]. Such concentrations of morphine seem suf-

ficient for the activation of μ opioid receptors on microglial cells and for the modulation of functional activities that are sensitive to such an activation. Thus, the observation that morphine can alter microglial cell function at concentrations in the femtomolar range suggests a physiological role for endogenous morphine within the brain during immunologic responses to infectious disease.

This work was supported, in part, by National Institutes of Health Grants DA-04351 and DA-08446.

References

1. Peterson PK, Molitor TW and Chao CC, Mechanisms of morphine-induced immunomodulation. *Biochem Pharmacol* **46:** 343–348, 1993.
2. Eisenstein TK, Hilburger ME and Lawrence DM, Immunomodulation by morphine and other opioids. In: *Drugs of Abuse, Immunity, and Infection* (Eds. Friedman H, Klein TW and Spector S), pp. 103–120. CRC Press, Boca Raton, 1995.
3. Sharp BM, Keane WF, Suh HJ, Gekker G, Tsukayama D and Peterson PK, Opioid peptides rapidly stimulate superoxide production by human polymorphonuclear leukocytes and macrophages. *Endocrinology* **117:** 793–795, 1985.
4. Peterson PK, Sharp B, Gekker G, Brummitt C and Keane WF, Opioid-mediated suppression of cultured peripheral blood mononuclear cell respiratory burst activity. *J Immunol* **138:** 3907–3912, 1987.
5. Peterson PK Molitor TW, Chao CC and Sharp B, Opiates and cell-mediated immunity. In: *Drugs of Abuse and Immune Function* (Ed. Watson RR), pp. 1–18. CRC Press, Boca Raton, 1990.
6. Fischer EG, Opioid peptides modulate immune function. *Immunopharmacol Immunotoxicol* **10:** 265–326, 1988.
7. Tubaro E, Avico U, Santiangeli C, Zuccaro P, Cavallo G, Pacifici R, Croce C and Borelli G, Morphine and methadone impact on human phagocytic physiology. *Int J Immunopharmacol* **7:** 865–874, 1985.
8. Tubaro E, Borelli G, Croce C, Cavallo G and Santiangeli C, Effect of morphine on resistance to infection. *J Infect Dis* **148:** 656–666, 1983.
9. Tubaro E, Santiangeli C, Belogi L, Borelli G, Cavallo G, Croce C, and Avico U, Methadone vs morphine: Comparison of their effect on phagocytic functions. *Int J Immunopharmacol* **9:** 79–88, 1987.
10. Streit WJ, Microglial cells. In: *Neuroglia* (Eds. Kentenmann H and Ransom BR), pp. 85–96. Oxford University Press, New York, 1995.
11. Gehrmann J, Matsumoto Y and Kreutzberg GW, Microglia: Intrinsic immuneffector cell of the brain. *Brain Res Rev* **20:** 85–96, 1995.
12. Peterson PK, Gekker G, Schut R, Hu S, Balfour HH Jr and Chao CC, Enhancement of HIV-1 replication by opiates and cocaine: The cytokine connection. In: *Drugs of Abuse, Immunity and AIDS* (Eds. Friedman H, Klein T and Spector S), pp. 181–188. Plenum Press, New York, 1993.
13. Peterson PK, Gekker G, Hu S, Sheng WS, Molitor TW and Chao CC, Morphine stimulates phagocytosis of *Mycobacterium tuberculosis* by human microglial cells: Involvement of a G protein-coupled opiate receptor. *Adv Neuroimmunol* **5:** 299–309, 1995.
14. Zuger A, Louie E, Holzman RS, Simberkoff MS and Rahal JJ, Cryptococcal disease in patients with the acquired immunodeficiency syndrome: Diagnostic features and outcome of treatment. *Ann Intern Med* **104:** 234–240, 1986.
15. Currie BP and Casadevall A, Estimation of the prevalence of cryptococcal infection among HIV infected individuals in New York City. *Clin Infect Dis* **19:** 1029–1033, 1994.
16. Murphy JA, Mechanisms of natural resistance to human pathogenic fungi. *Annu Rev Microbiol* **45:** 509–538, 1991.
17. Hu S, Chao CC, Khanna KV, Gekker G, Peterson PK and Molitor TW, Cytokine and free radical production by porcine microglia. *Clin Immunol Immunopathol* **78:** 93–96, 1995.
18. Peterson PK, Gekker G, Hu S, Sheng WS, Anderson WR, Ulevitz RJ, Tobias PS, Gustafson KV, Molitor TW and Chao CC, CD14 receptor-mediated uptake of nonopsonized *Mycobacterium tuberculosis* by human microglia. *Infect Immun* **63:** 1598–1602, 1995.
19. Casellas AM, Guardiola H and Ranaud FL, Inhibition by opioids of phagocytosis in peritoneal macrophages. *Neuropeptides* **18:** 35–40, 1991.
20. Rojavin M, Szabo I, Bussiere JL, Rogers TJ, Adler MW and Eisenstein TK, Morphine treatment *in vitro* or *in vivo* decreases phagocytic functions of murine macrophages. *Life Sci* **53:** 997–1006, 1993.
21. Szabo I, Rojavin M, Bussiere JL, Eisenstein TK, Adler MW and Rogers TJ, Suppression of peritoneal macrophage phagocytosis of *Candida albicans* by opioids. *J Pharmacol Exp Ther* **267:** 703–706, 1993.
22. Chen Y, Mestek A, Liu J, Hurley JA and Yu L, Molecular cloning and functional expression of a μ-opioid receptor from rat brain. *Mol Pharmacol* **44:** 8–12, 1993.
23. Makman MH, Morphine receptors in immunocytes and neurons. *Adv Neuroimmunol* **4:** 69–82, 1994.
24. Schoffelmeer ANM, De Vries TJ, Hogenboom F, Hruby VJ, Portoghese PS and Mulder AH, Opioid receptor antagonists discriminate between presynaptic *mu* and *delta* receptors and the adenylate cyclase-coupled opioid receptor complex in the brain. *J Pharmacol Exp Ther* **263:** 20–24, 1992.
25. Makman MH, Bilfinger TV and Stefano GB, Human granulocytes contain an opiate alkaloid-selective receptor mediating inhibition of cytokine-induced activation and chemotaxis. *J Immunol* **154:** 1323–1330, 1995.
26. Dobrenis K, Makman MH and Stefano GB, Occurrence of the opiate alkaloid-selective μ_3 receptor in mammalian microglia, astrocytes and Kupffer cells. *Brain Res* **686:** 239–248, 1995.
27. Stefano GB, Digenis A, Spector S, Leung MK, Bilfinger TV, Makman MH, Scharrer B and Abumrad NN, Opiate-like substances in an invertebrate, an opiate receptor on invertebrate and human monocytes, and a role in immunosuppression. *Proc Natl Acad Sci USA* **90:** 11099–11103, 1993.
28. Peterson PK, Gekker G, Hu S, Anderson WR, Kravitz F, Portoghese PS, Balfour HH and Chao CC, Morphine amplifies HIV-1 expression in chronically infected promonocytes cocultured with human brain cells. *J Neuroimmunol* **50:** 167–175, 1994.
29. Chao CC, Gekker G, Sheng WS, Hu S, Tsang M and Peterson PK, Priming effect of morphine on the production of tumor necrosis factor-α by microglia: Implications in respiratory burst activity and human immunodeficiency virus-1 expression. *J Pharmacol Exp Ther* **269:** 198–203, 1994.
30. Peterson PK, Sharp BM, Gekker G, Portoghese PS, Sannerud K and Balfour HH Jr, Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures. *AIDS* **4:** 869–873, 1990.
31. Minami M and Satoh M, Molecular biology of the opioid receptors: Structures, functions and distributions, *Neurosci Res* **23:** 121–145, 1995.
32. Stefano GB and Sharrer B, Endogenous morphine and related opiates, a new class of chemical messengers. *Adv Neuroimmunol* **4:** 57–67, 1994.
33. Horak P, Haberman F and Spector S, Endogenous morphine and codeine in mice—Effect of muramyl dipeptide. *Life Sci* **52:** PL255–260, 1993.