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Opiate Pharmacology of Intrathecal Granulomas

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Background: **Chronic intrathecal morphine infusion produces intradural granulomas. The authors examined a variety of opioids infused intrathecal for analgesic activity and toxicity.**

Methods: **Two sets of experiments were undertaken in dogs with chronic intrathecal catheters: (1) Six-hour intrathecal infusions were used to determine the full analgesic dose and the maximum tolerated dose. (2) To establish toxicity, the maximum tolerated dose was given for up to 28 days by continuous intrathecal infusion. Drugs examined were morphine sulfate, hydromorphone, D/L-methadone, L-methadone, D-methadone, fentanyl, [d-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO), naloxone, or saline.**

***Results: Analgesia and tolerability*: Six-hour intrathecal infusion of agonists resulted in a time-dependent increase in thermal escape latency. At higher concentrations, dose-limiting motor dysfunction and sedation occurred, and hypersensitivity occurred. The concentrations, in mg/ml, for full analgesic dose/maximum tolerated dose were as follows: morphine, 0.9/12.0; hydromorphone, 1.0/3.0; D/L-methadone, 2.8/3; L-methadone, 1.0/> 1.0; fentanyl, 0.3/2.0; DAMGO, 0.1/> 2.0; D-methadone, > 1/> 1; naloxone, > 10/> 10. *Spinal pathology*: Chronic intrathecal infusion of the maximum tolerated dose revealed 100% intradural granuloma formation after morphine, hydromorphone, L-methadone, and naloxone. DAMGO induced a mass in only a single animal (one of three). D/L- and D-methadone produced intradural granulomas but were also associated with parenchymal necrosis. Saline and fentanyl animals displayed no granulomas.**

Conclusions: **Intrathecal opiate–induced granulomas are not strictly dependent on opioid receptor activation. Therefore, opiates at equianalgesic doses present different risks for granuloma formation. Importantly, D/L- and D-methadone also resulted in parenchymal necrosis, an affect associated with the *N*-methyl-D-aspartate antagonist action of the D-isomer.**

THE observation that chronic intrathecal infusion of morphine sulfate leads to the appearance of organized collections of inflammatory cells in the intrathecal space adjacent to the catheter tip has been reported in case studies[1–8] and in systematic reviews.[9] These inflammatory masses, consisting of granulation tissue, have generally been termed *granulomas* in the literature. Preclinical studies in large animal models have shown that these masses, arising from the meninges and leading to spinal cord compression, reflect a dose-dependent effect of the opiate leading to the intrathecal accumulation of these inflammatory cells.[10,11]

A variety of studies have demonstrated that these effects are not the result of a specific catheter material formulation or the infusion process itself. Therefore, multiple drug classes have been studied using intrathecal infusion in the dog model, and this meningeal granuloma has not thus far been observed except with morphine sulfate or a δ-opioid peptide (D-penicillamine2-D-penicillamine5 enkephalin [DPDPE]; see Yaksh *et al.*[12] and Horais *et al.*[13]). Moreover, in recent work with serial magnetic resonant imaging in dogs, substitution of saline for morphine after the development of a granuloma led to a regression of the granuloma and that formation was dependent on the concentration, not the dose, of morphine.[14]

An important question in these studies is whether the effects are mediated by an opioid receptor. If so, other opiate agonists should have a similar effect. Human clinical studies are not definitive because the patient frequently receives multiple drugs at widely varying doses for varying degrees of time.[9,12] Accordingly, we set out in the current studies to examine the effects of several intrathecally infused opioids in the dog model, including hydromorphone, methadone, fentanyl, and the opioid peptide [d-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO). The opiate antagonist naloxone was also studied. These compounds were chosen because of their structural similarities (hydromorphone and naloxone) or differences (methadone) with morphine. Fentanyl, a synthetic phenylpiperidine, and DAMGO, a peptide, are highly selective μ agonists with structures unrelated to the other compounds tested. Testing of methadone and its isomers allowed us to take advantage of the stereoselectivity of the μ-agonist effect. Methadone is generally available as an equal mixture of the D- and L-isomers. The L-isomer possesses the opioid activity of the racemic mixture, whereas the D-isomer has greater than 100-fold less opiate activity but possesses significant *N*-methyl-D-aspartate (NMDA) receptor antagonist activity.[15,16] It was hoped that this selection of a relatively small number of opiate active compounds would provide insight on the role of chemical structure, μ-receptor activation, or physiochemistry on granuloma formation. Importantly, in these initial studies, we sought to compare the effects of the behaviorally defined maximum tolerated dose (MTD) of each agent or the maximum usable doses if solubility was limited. In addition, we determined the minimal infusion dose, which resulted in a maximum analgesia (fully analgesic dose [FAD]) to a thermally evoked skin switch after a 6-h infusion. These two parameters, the MTD and the FAD, would permit us to assess the relative magnitude of the

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therapeutic ratio for that agent given by chronic intrathecal infusion.

Materials and Methods

All studies described were performed under protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Diego.

Animals

Male beagle dogs (Marshall Farms, New Rose, NY), aged 12–16 months and weighing 12–15 kg, were individually housed in approximately 2 × 3-m runs with wood shavings and given *ad libitum* access to food and water with lighting set on a daily 12-h light–dark cycle. Kennel temperatures were maintained within the range of 16.5°–27.8°C. Animals were adapted for a minimum of 5 days before surgery. A nylon vest was placed on each dog approximately 48 h before scheduled intrathecal catheter placement surgery for acclimation.

Surgical Preparation

For chronic intrathecal infusion, dogs were prepared with chronic intrathecal catheters. This preparation has been previously reported in detail.[11] In brief, after atropine (0.04 mg/kg intramuscular) and xylazine sedation (1.5 mg/kg intramuscular) and mask induction with isoflurane (4–5%), the dog was intubated, and anesthesia was maintained under spontaneous ventilation with 1.0–2.0% isoflurane and 50%:50% N_2O:O_2. Intraoperatively, animals were continuously monitored for oxygen saturation, inspired, and end-tidal values of isoflurane, carbon dioxide, nitrous oxide, and oxygen and heart and respiratory rates. The custom fabricated polyethylene catheter (0.61 mm OD) was inserted through the cisterna magna and passed to the level of the L1–L2 lumbar spinal cord. Dexamethasone sodium phosphate (0.25 mg/kg intramuscular) was administered just after catheter placement to lessen any inflammation due to implantation of the intrathecal catheter and to provide adjunctive postoperative analgesia. The external catheter was tunneled subcutaneously to exit at the left scapular region. The incision was closed, and butorphanol tartrate (0.04 mg/kg intramuscular) was administered upon recovery and as necessary to relieve postoperative discomfort. After anesthetic recovery, a nylon vest was placed on the animal, and an infusion pump (PANOMAT C-10; Disetronic Medical Systems, Saint Paul, MN; or equivalent) was secured in the vest pocket, where it was connected to the externalized end of the intrathecal catheter. Animals received intrathecal saline infusions for at least 3 days before initiation of drugs to allow recovery from the surgical procedure.

Necropsy

For euthanasia, dogs were sedated with acepromazine (10 mg intramuscular) and anesthetized with sodium pentobarbital (35 mg/kg intravenous or dose to effect). During deep anesthesia, cisternal cerebrospinal fluid (by percutaneous puncture) and plasma samples were obtained. After sample collection, animals were exsanguinated by perfusion with saline (approximately 4 l) followed by 10% formalin (approximately 4 l) delivered by roller pump at approximately 100 mmHg. The spinal column was exposed by laminectomy of the spinal canal and the lower brainstem. Methylene blue dye was injected through the catheter to visualize the position of the intrathecal catheter. The gross condition of the spinal cord and dura was noted. The spinal cord was dissected in blocks consisting of the cervical, thoracic, lumbar (catheter tip), and sacral regions, taking care to keep the dura intact and placed in fixative (10% neutral-buffered formalin). Tissue blocks were submitted for histopathologic analysis after embedding in paraffin, sectioning at approximately 4–8 μm, and staining with hematoxylin and eosin.

Drugs

Drug formulations were prepared from reagents of the highest available purity obtained from commercial sources. Some drugs used in these investigations, D/L-methadone hydrochloride, L-methadone, D-methadone, fentanyl hydrochloride, and DAMGO, were obtained from the National Institute of Drug Abuse. Naloxone hydrochloride was obtained from Sigma Chemical (St. Louis, MO). Preservative-free, Food and Drug Administration–approved formulations of morphine sulfate (Infumorph; Abbott, Abbott Park, IL), hydromorphone hydrochloride (Dilaudid-HP for Injection; Abbott), and saline vehicle (0.9% NaCl; Abbott) were used. Infusate drugs were prepared in a laminar flow hood using aseptic procedures. The diluents for compounds were either sterile, preservative-free, pharmaceutical-grade saline (0.9%) or water for injection. When necessary, high concentration solutions were prepared in sterile water, and osmolarity was adjusted to approximately 300 mOsm with sodium chloride. All solutions prepared from the powder were passed through a 0.22-μm sterilization filter. All batch solutions were dispensed into sterile, pyrogen-free, 10-ml vials for single use only and stored protected from light at 4°–22°C until used.

Observations

Behavior. Animals were observed a minimum of twice daily—morning and afternoon. Daily observations included temperature measured using a digital ear thermometer and general behavior. Specific behavioral indices of arousal (−3 to +3), muscle tone (−3 to +3), and coordination (0 to 3) were assessed. These measures have been discussed extensively elsewhere.[14,17] In brief, the scales represent mild, moderate, or severe deviations from normal, which is represented by a score of 0. Cumulative motor function scores are determined by

summing the absolute value of muscle tone and coordination scores, resulting in a maximum score of 6.

Nociception Response. The thermally evoked skin twitch response was measured using a probe with approximately 1-cm^2 surface area maintained at 62.5° ± 0.5°C. The probe was applied twice to the left and right shaved areas of the back at the thoracolumbar level of the spine. This resulted in a local cutaneous muscle contraction with a latency of 1–3 s.[18] The response is a C-polymodal–mediated, thermally evoked nociceptive reflex.[19] At the response, the probe was removed and the latency was recorded. If no response was seen within 6 s, the probe was removed and the maximal latency (6 s) was assigned for that trial.

Study Protocols

The following studies were undertaken in this report:

Assessment of Fully Analgesic Dose and Maximum Tolerated Infusion Dose. Dogs prepared with lumbar intrathecal catheters received an intrathecal infusion of different concentrations of test drug delivered by continuous infusion for 6 h at a rate of 40 μl/h, producing a total infused volume of 240 μl. Preliminary studies with several infusion intervals indicated that 6 h was the minimum time required for these opiates to show a stable nociceptive response. Catheters were filled with the test solution before initiation of infusion to eliminate any time delay associated with the dead volume of the catheter. Dogs were tested for thermal escape latency and behavior assessment before and at 1, 6, and 24 h after infusion initiation. A minimum of three dogs was examined for each drug. Each animal typically completed one complete set of doses (typically three or four doses) to construct a dose–response curve before being tested with another compound. A dose–response curve was thus generated for each animal. Two drugs were examined with each animal. Animals were tested with a single dose on any given day with a minimum of 3–4 days between any two sequential infusions. Doses were assigned in different animals to minimize any systematic ordering effect (*e.g.*, low to high dose or medium to low or high dose). Based on preliminary dose-ranging studies, the infusion concentration ranges selected were 0.01, 0.1, and 1 mg/ml morphine; 0.1, 0.3, 1.0, 3.0, and 6.0 mg/ml hydromorphone; 0.3, 1.0, 3.0, and 10 mg/ml D/L-methadone; 0.01, 0.03, 0.1, and 1 mg/ml DAMGO; and 0.03, 0.1, 0.3, 1, and 2 mg/ml fentanyl. The maximum doses for D-methadone (0.3 and 1 mg/ml) and L-methadone (0.1, 0.3, and 1 mg/ml) were limited by the solubility of the two chemical forms available of the two isomers. The FAD was defined as the minimum dose needed to produce a skin twitch latency of at least 6 s at 6 h. In determining the dose ranges for inclusion in the studies on analgesic dosing, disruptive side effects were often observed at the highest doses. These side effect profiles were used to generate the data leading to an estimation of the MTD. The MTD of a given drug for any dog was defined as that dose producing at least one of the following observations: (1) drug treatments that led to moderate or severe sedation (arousal scores of −2 or −3, respectively) or agitation (arousal scores of +2 or +3) for a period of greater than 1 h during or after the period of infusion; or (2) hind limb motor dysfunction (defined as a combined score of 4 or greater out of a possible 6 using the absolute values of motor coordination and hind limb muscle tone) for greater than 1 h during or after the period of infusion. The lowest dose associated with those signs in two or more dogs was defined as the MTD.

Table 1. 28-Day Intrathecal Infusion Treatments

Intrathecal Drug	Test Dose, mg/ml	Number of Animals
Saline	0.9% NaCl	3*
Morphine sulfate	12.5	9*
Hydromorphone HCl	3	3
D/L-Methadone HCl	3	3
D-Methadone	1	3
L-methadone	1	3
Fentanyl HCl	2	3
DAMGO	2	3
Naloxone HCl	10	2

* Indicates animals reported in Allen *et al.*[14]

Assessment of Chronic Infusion Drug Toxicity. To assess the effects of the chronically delivered intrathecal agent, separate animals prepared with lumbar intrathecal catheters were assigned to receive a 28-day infusion of the MTD of one of the several opiates as outlined in table 1. The model and paradigm used here is identical to that reported previously with intrathecal morphine.[11,14] Accordingly, for analysis purposes, results from animals receiving morphine in several groups reported in that study and included for comparison in these studies are indicated in table 1. At 28 days, or if there was an earlier onset of behavioral signs that was deemed unacceptable, animals were deeply anesthetized and necropsies were performed as described above.

Statistics

Between-group comparisons for behavioral indices and CSF chemistries were undertaken with one-way analysis of variance. *Post hoc* comparisons *between* groups comparisons were accomplished with the Dunnett *t* test. Dose–response curves for the analgesic effects were analyzed with least-square linear regression. The significance of the regression slope for each drug and the lowest concentration that blocked the skin twitch along with the 95% confidence intervals were calculated. All statistics were accomplished using GraphPad Prism, version 4.03, for the Macintosh (Graph Pad Software, San Diego, CA).

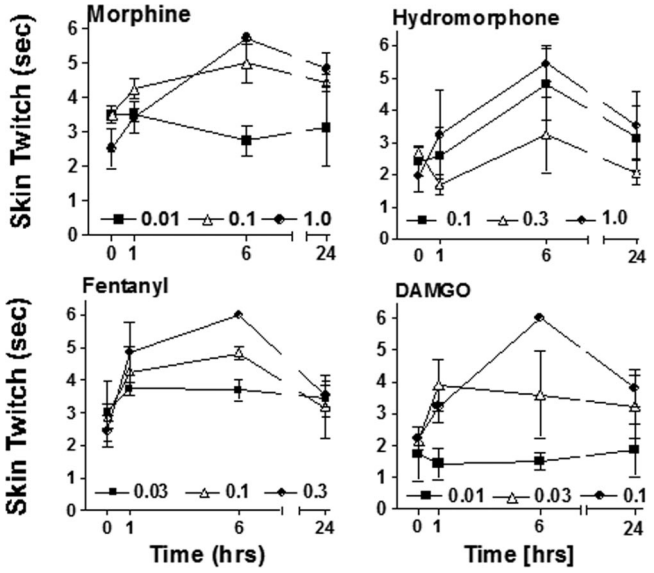

Fig. 1. Antinociceptive effects (skin twitch response latency expressed in seconds with a 6-s maximum latency) measured before (baseline) and at 1, 6, and 24 h after beginning a 6-h intrathecal infusion (40 μl/min) of three concentrations of morphine, hydromorphone, fentanyl, or DAMGO. Each curve presents the mean and SEM of 3 dogs. All doses are given as mg/kg.

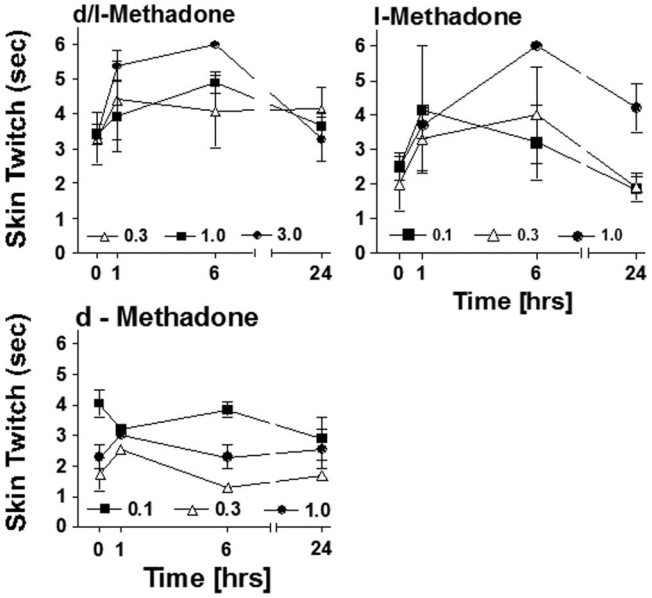

Fig. 2. Antinociceptive effects (skin twitch response latency expressed in seconds with a 6-s maximum latency) measured before (baseline) and at 1, 6, and 24 h after beginning a 6-h intrathecal infusion (40 μl/min) of three concentrations (mg/ml) of D/L-methadone (n = 3), L-methadone (n = 3), or D-methadone (n = 2).

Results

Fully Analgesic and Maximum Tolerated Infusion Doses

Analgesic Dose Response. Dogs with chronic intrathecal catheters received different doses of drugs as a 6-h continuous fixed rate infusion. They were examined for their skin twitch response latency at 1, 6, and 24 h after the initiation of the infusion. These antinociceptive effects evoked by continuous infusion of the opiate agonists over 6 h were time and concentration dependent (figs. 1 and 2). Plotting the dose–effect curves at 6-h curves revealed positively inflected slopes, which were statistically significant ($P < 0.05$; fig. 3 and table 2). Calculating the ED_{100} (blocking) dose showed that the rank ordering of decreasing analgesic potency was DAMGO, fentanyl > morphine, hydromorphone, L-methadone, D/L-methadone > D-methadone, naloxone = 0 (table 2). At the FAD, there were only minor effects on motor function, arousal indices, or physiologic parameters.

Maximum Tolerated Dose. As infusion concentrations were increased, the MTD was typically observed by the appearance of signs within 2–3 h of the initiation of infusion. These signs included hind limb weakness and reduced hind limb coordination and an extreme hypersensitivity to light stroking of the flank (morphine, hydromorphone, and D/L-methadone). Fentanyl doses were limited by emesis and behavioral depression. All of these effects resolved with decreasing infusate concentrations. Appearance of these endpoints precluded examination of higher doses. Limiting side effects were not associated with the highest dose of DAMGO examined. Higher doses of DAMGO were not examined because of limitation of peptide availability. D- and L-methadone were available only in the free base form, and the maximum concentration (1 mg/ml) was limited by solubility of the bases. The MTD based on these studies is summarized in table 2. Given these observations, the approximate therapeutic ratio (MTD/FAD) in the dog model based on the 6-h infusion at 40 μl/h was calculated and is also presented in table 2. The highest therapeutic ratio was

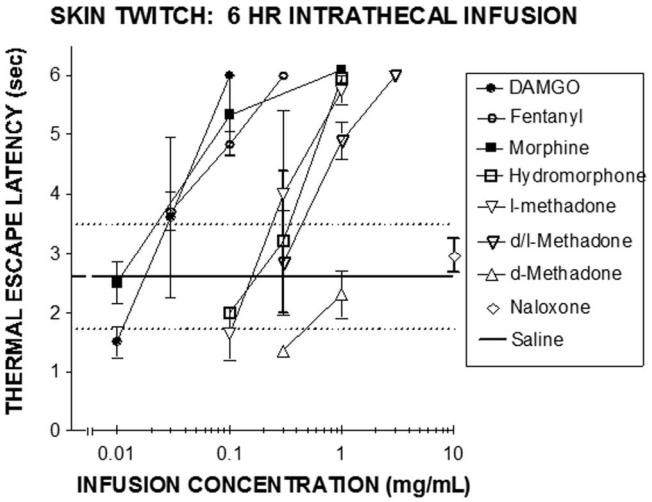

Fig. 3. Dose–response curve plotting mean ± SEM of the response latency in seconds measured after 6 h of infusion of the agents indicated *versus* infusion concentration. Data derived from studies in figures 1 and 2. The *solid horizontal line* represents saline response, and the *dotted lines* are the 95% confidence intervals for that saline effect.

Table 2. Summary of the Intrathecal Infusion Concentration Resulting in a Block of the Skin Twitch and the Maximum Tolerated Dose

Intrathecal Drug	FAD at 6 h,* Mean (95% CI)	MTD at 6 h, Median	Therapeutic Ratio (MTD/FAD)
Saline	No effect	No effect	—
Morphine sulfate	0.9† (0.2–3.8)	12‡	13
Hydromorphone HCl	1.0† (0.5–3.3)	3	3
D/L-Methadone HCl	2.8† (1.4–7.2)	3	1.1
L-Methadone	1.0† (0.5–16)	1	1
D-Methadone	> 1	1	—
Fentanyl HCl	0.3 (0.2–0.4)	2	6.7
DAMGO	0.1† (0.05–0.2)	> 2	> 20
Naloxone HCl	> 10	> 10	—

Fully analgesic dose (FAD) and maximum tolerated dose (MTD) are given as mg/ml; dogs received 0.240 ml over the 6-h infusions.

* Calculated using linear regression. † Regression slope (fig. 3) significantly greater than 0 ($P < 0.05$). ‡ Indicates data previously reported in part by Yaksh *et al.*[11]

CI = confidence interval.

DAMGO (> 20) and the lowest were D/L- and L-methadone.

Chronic Infusion Drug Toxicity

Necropsy and Pathology. In the current studies, we examined the effects of up to 28-day infusions of doses of the several opioids that were defined as being the MTD for the respective drug. The infusion of morphine, hydromorphone, D/L-methadone, D-methadone, L-methadone, or the opiate antagonist naloxone typically resulted in an intradural granuloma (table 3). At necropsy, the dura overlying the catheter tip of animals displaying a granuloma was frequently thickened and discolored and had adherent epidural fat on the superficial aspect. After dissection, a large space-occupying mass was observed with the catheter tip at its center. The mass typically extended approximately two or three segments rostrally and up to one or two segments caudally with a total length of up to 5 cm. Histopathologic examination of the tissue proximal to the catheter tip typically revealed a large space-occupying mass that was associated with local cord compression (fig. 4). The histopathology of the mass, described previously in detail,[11] consisted of large intradural aggregates of neutrophils, granulocytes, and macrophages. In well-developed masses, a necrotic center was frequently observed (figs. 4B and D). The morphology and rate of occurrence of the masses were generally indistinguishable between the animals receiving any opiate compounds, including the antagonist naloxone. However, dogs receiving D/L- or D-methadone displayed a more complicated pathology (see below). In addition, the occurrence of mass formation in animals receiving intrathecal infusions of the μ-opioid peptide DAMGO was significantly lower, with only one of three dogs forming a granuloma. No dogs receiving fentanyl or saline displayed granuloma formation (table 3).

Infusion of D/L-methadone and D-methadone produced mass formation in two of three and three of three dogs, respectively. Moreover, these compounds led to not only to a granulomatous mass proximal to the catheter tip, but also to local necrosis of the adjacent spinal parenchyma. Infusion of L-methadone, the opioid-active isomer, resulted in a typical pattern of granuloma formation around the catheter tip, with no evidence of parenchymal necrosis in any animal (fig. 5).

Behavior. During the course of the infusion, animals showed modest sedation and hind limb coordination and muscle weakness at the outset of the infusions of opiate agonists, but this typically resolved in 2–3 days. Over an interval of 14–21 days, some animals with morphine,

Table 3. Summary of Granuloma Formation and Incidence of Motor Dysfunction at Necropsy

Intrathecal Drug	Intrathecal Infusion Concentration, mg/ml	Animals with Granulomas Identified by Histopathology, n/Total	Animals with Motor Dysfunction at Death, n/Total	Animals Killed at < 28 Days because of Behavioral Signs, n/Total
Saline*	—	0/5	0/5	0/5
Morphine sulfate*	12	9/9	4/9	2/9
Hydromorphone HCl	3	3/3	1/3	1/3
D/L-Methadone HCl†	3	3/3	1/3	1/3
L-Methadone	1	3/3	1/3	1/3
D-Methadone†	1	3/3	1/3	1/3
Fentanyl HCl	2	0/3	0/3	0/3
DAMGO	2	1/3	0/3	0/3
Naloxone HCl	10	2/2	1/2	0/2

* Indicates data from animals previously reported in part by Allen *et al.*[14] † Spinal necrosis also present.

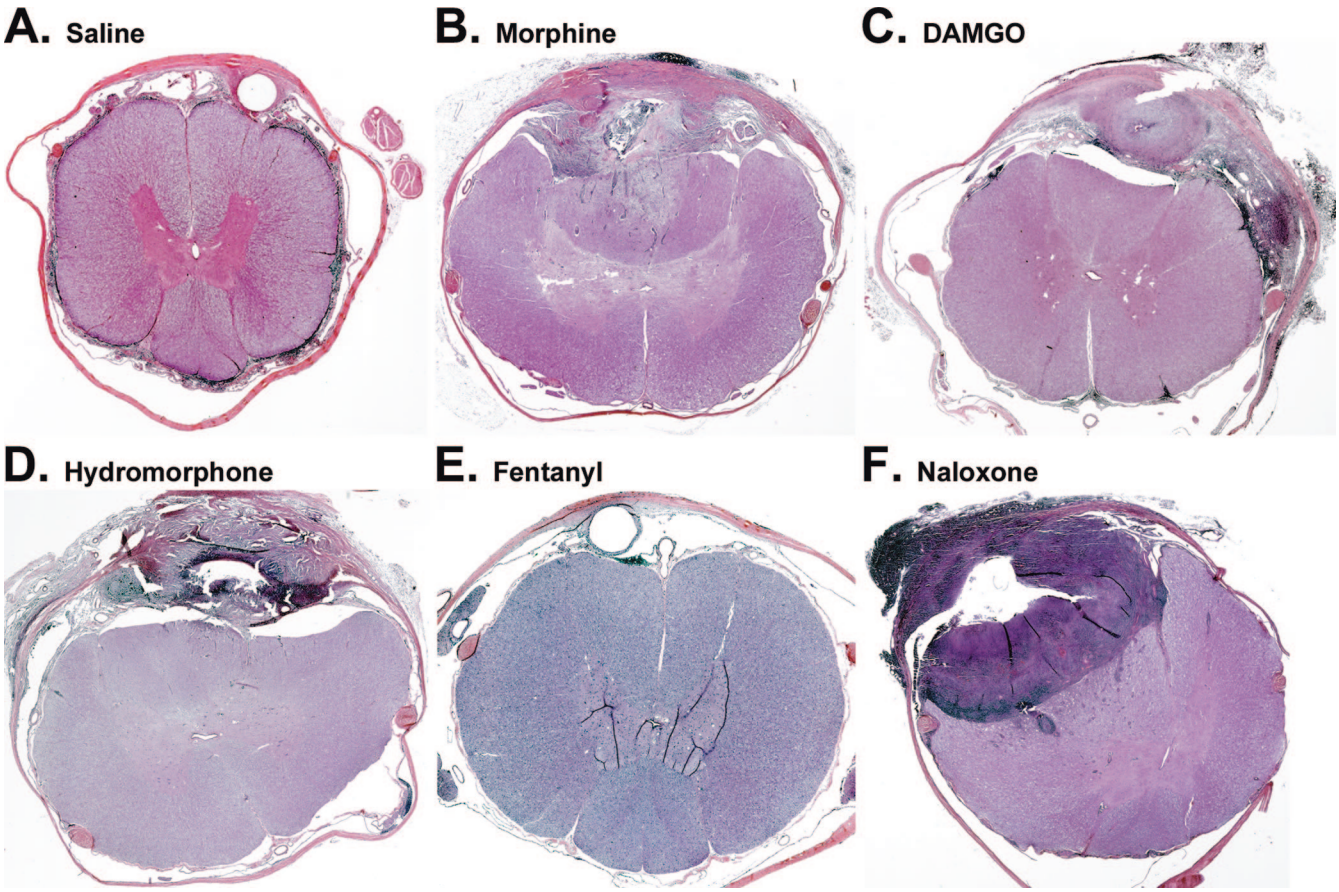

Fig. 4. Representative histochemistry in sections from lumbar spinal cord proximal to the infusion catheter tip of dogs receiving 28-day infusion of saline (*A*), morphine sulfate (12.5 mg/ml; *B*), DAMGO (2 mg/ml; *C*), hydromorphone HCl (3 mg/ml; *D*), fentanyl HCl (2 mg/ml; *E*), or naloxone HCl (10 mg/ml; *F*). Note size differential and lack of spinal cord compression, dural thickening, cellular infiltration, or development of a necrotic center as seen in *B*, *D*, and *F*.

hydromorphone, D/L-methadone, D-methadone, L-methadone, or naloxone infusion began to display progressive signs of motor dysfunction (*e.g.*, motor dysfunction score absolute values of 2 or greater). This dysfunction was characterized by development of progressive hind limb muscle stiffness, loss of hind limb coordination, and tactile hypersensitivity. Unilateral or bilateral hind limb paralysis also developed in some of these animals. These deficits were deemed to be sufficiently severe in some animals as to mandate early sacrifice (*e.g.*, $<$ 28 days). Table 3 summarizes the incidence of such observations by treatment. Animals receiving intrathecal infusions of saline, fentanyl, or DAMGO reliably displayed motor scores of 1 or less, and no animal receiving these treatments required early sacrifice.

CSF Chemistry at Sacrifice. As indicated in table 4, after long-term intrathecal infusion of the MTD of the several agents, there were prominent increases in cisternal cerebrospinal fluid protein sampled at necropsy for all of the agents with the exception of fentanyl and DAMGO. For morphine sulfate, hydromorphone hydrochloride, D/L-methadone hydrochloride, and L-metha-

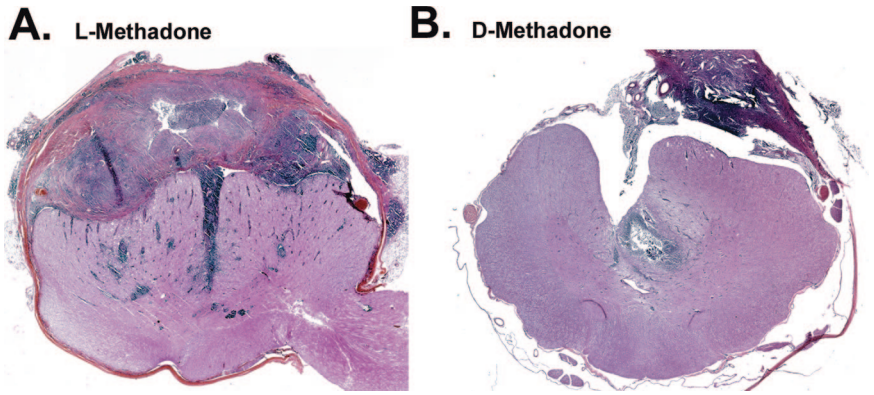

Fig. 5. Representative histochemistry in sections from lumbar spinal cord of dogs receiving 28-day intrathecal infusion of D/L-methadone (3 mg/ml; *A*), L-methadone (1 mg/ml; *B*), or D-methadone (1 mg/ml). Note the direct involvement of spinal parenchyma in *B*, as compared with *A* and those in figure 4.

Table 4. Summary of Cisternal CSF Clinical Chemistry Assessed at Time of Necropsy after Approximately 28-Day Intrathecal Infusion

Intrathecal Drug	n	CSF Protein,* mg/dl	CSF Glucose, mg/dl	CSF Leukocytes, cells/ml	CSF Erythrocytes, cells/ml
Saline†	5	53 ± 26	67 ± 6	62 ± 43	180 ± 171
Morphine sulfate†	9	642 ± 346‡	67 ± 2	201 ± 85	17,391 ± 9,298
Hydromorphone HCl	3	294 ± 57‡	66 ± 6	442 ± 513	7,072 ± 5,281
D/L-Methadone HCl	3	205 ± 61‡	62 ± 4	166 ± 77	956 ± 165
L-Methadone	3	203 ± 35‡	60 ± 5	1,376 ± 1,517	2,532 ± 2,920
D-Methadone	2	139 ± 35	70 ± 2	78 ± 46	12,000 ± 3,083
Fentanyl HCl	3	38 ± 3	71 ± 3	51 ± 45	587 ± 621
DAMGO	3	72 ± 16	62 ± 2	400 ± 356	283 ± 79
Naloxone HCl	2	134 ± 42	64 ± 1	129 ± 65	338 ± 4,084

All data are presented as mean ± SEM.

* One-way analysis of variance across treatments is statistically significant ($P < 0.05$). † Indicates some animals previously reported in Yaksh *et al.*[11] ‡ Indicates significant difference from saline control using Dunnett *t* test ($P < 0.05$).

CSF = cerebrospinal fluid.

done, these increases were statistically greater than the vehicle control ($P < 0.05$). Cerebrospinal fluid glucose concentrations were not different from vehicle for any treatment, suggesting the absence of a gross infectious process. Cerebrospinal fluid leukocytes were observed in saline-treated animals and, these cell counts seemed to be increased in the several infusion groups, although these changes were not statistically significant.

Discussion

Previous work has indicated that chronic intrathecal delivery of morphine leads to a concentration- and time-dependent increase in the appearance of meningeally derived collections of inflammatory cells in the intrathecal space proximal to the infusion site in dogs[10,11] and in humans.[9,20] The current studies serve to extend our insights into the pharmacology of this granuloma-inducing effect. Although it would be ideal to produce complete dose–effect curves by examining the effects of multiple doses of different opioid agonists, we reasoned that an alternative effort would be to examine the effects of infusing the maximum concentration of each which could be behaviorally tolerated. If this dose had no effect on granuloma formation, we could calculate a minimum therapeutic ratio (MTD/FAD). We believed this was a scientifically efficient way to provide an estimate of the likelihood that a given agent would produce a granuloma at potentially usable doses. If the agent produced a granuloma at this dose, we could not exclude the likelihood that a yet lower dose would be equally at risk.

Opioid Pharmacology of Intrathecal Granuloma

We demonstrated in dogs that the μ-opiate agonists morphine, hydromorphone, D/L-methadone (racemic mixture), L-methadone, the opiate antagonist naloxone, and in other studies the δ-opioid agonist DPDPE,[13] given at their maximally tolerated intrathecal infusion concentration, all resulted in a similar incidence of intrathecal granuloma. Unexpectedly, DAMGO resulted in only one small granuloma in three dogs, whereas fentanyl, at even a substantially greater than analgesic dose, did not produce any granulomas. These discrepancies argue against a simple μ-opioid effect. In recent work, Johansen *et al.*[21] examined the effects of hydromorphone in a chronic sheep model. In their work, infusion up to 12 mg/day (6 mg/ml) evoked a mild spinal inflammation, but no evidence of a granuloma. On the other hand, Coombs *et al.*[22] reported that chronic epidural administration of hydromorphone in the sheep model produced large compressive granulomatous inflammatory masses. These results suggest that additional studies may be required to define the reasons for this apparent difference.

To further address the potential role for the opiate receptor, we examined separately the methadone isomers L-methadone (opioid agonist with no NMDA antagonism) and D-methadone (> 100 less opioid activity but with NMDA antagonist) and naloxone (opiate antagonist). Here, like the D/L-isomer, both D- and L-methadone produced an intrathecal granuloma. Interestingly, the D- and D/L-isomers also resulted in a complex picture that included severe parenchymal damage along with formation of a granuloma. These results with the D-isomer seem to be similar to the effects previously observed with intrathecal infusion of NMDA antagonists in sheep (dextrorphan, dextromethorphan, and memantine[23]) and in dogs (MK801, memantine, and amitriptyline; unpublished data, 2001: Tony L. Yaksh, Ph.D., and Kjersti Horais, B.S., Department of Anesthesiology University of California, San Diego) that yielded parenchymal injury. The mechanism of this apparently NMDA-antagonist-mediated injury is unknown. In frog oocytes, electrophysiologic studies with different NMDA subunit constituents found that the stereoisomers of methadone displayed little stereoselectivity with the exception of inhibiting currents in NMDA channels constructed from the

NR1/2A combination. These currents were preferentially inhibited by the D-methadone isomer.[24]

Potential Factors Contributing to Intrathecal Morphine-induced Granuloma

The current study suggests a lack of a specific role for μ-opiate receptors in granuloma formation and leaves open the question of mechanism. We have previously excluded several possibilities: (1) Reaction to catheter or vehicle infusion: At least partial regression of the granuloma occurs rapidly with removal of the opiate and the lack of effect of many other agents.[12] (2) Infection: There have been no CSF samples positive for pathogenic bacteria and no changes in CSF glucose levels.[11] Histochemical staining for bacteria in previous studies was negative. (3) Catheter/infusion solution: Catheters and the infusion of saline had no effects. With regard to the drugs, solutions were prepared as to have osmolarity in the range of 300 mOsm $\pm$ 10%. Solution pH was in the range of approximately 5.5–7 for these agents and for the vehicle. All solutions were prepared aseptically and passed through 0.22-μm filters into sterile containers. Although we did not specifically test the role of the sulfate moiety from morphine sulfate, granulomas were observed with other salts and with bases that were prepared in saline. The experience with intrathecal morphine emphasizes that these effects are concentration dependent.[11,14]

Given the current structure activity series, what can be said regarding the pharmacology of the mechanism leading to the intradural accumulation of inflammatory cells? Given the granuloma-producing activity of morphine, hydromorphone, methadone, and naloxone, the modest effect of DAMGO, and the lack of effect of fentanyl at the dose used, the simple role of a μ-opiate receptor activation is not evident. The important link is the movement of inflammatory cells from the local meninges. We present two hypotheses: (1) Nitric oxide release: μ opioids acting through μ receptors can initiate release of nitric oxide in human endothelial cells.[25] These observations lead to a working hypothesis that morphine at increased concentrations and persistent exposure may activate nitric oxide synthase in meningeal vasculature and initiate a cascade that serves to increase local capillary permeability to these activated cells. The reported pharmacology of a μ-opioid effect, however, would suggest that the ordering of "granuloma-producing" activity should be fentanyl > morphine, which it is not. (2) Mast cells: There are large numbers of mast cells that are in residence in brain and spinal meninges.[26,27] These appear to have the phenotype of cutaneous, rather than mucosal, mast cells. Degranulated mast cells release numerous compounds including proteolytic enzymes (tryptase and chymase), vasodilators (histamine and serotonin), compounds that increase vascular and blood–brain barrier permeability (tumor necrosis factor α) and act as chemoattractants.[28] In skin, opiates produce skin mast cell degranulation and mediator release, but the ordering of activity is not representative of a μ receptor: morphine = hydromorphone > fentanyl.[29–31] This ordering of activity for mast cell degranulation resembles what we have found for granuloma induction. Ongoing experiments are targeting these potential alternatives.

Activity of Spinally Infused Opiates

The FAD was determined here by using an acute (6-h) infusion paradigm instead of the more commonly studied bolus delivery. The overall rank ordering of activity observed here is identical to that which has been previously reported for several agents after bolus delivery in rats[32,33] and primates.[34,35] Importantly, D-methadone, an isomer lacking opiate agonist activity, was without analgesic effect at the concentrations we were able to test in this model of acute thermal escape. This is consistent with the activity profile of intrathecal NMDA antagonists.[36]

In these infusion studies, we noted that the estimates of relative agonist potency assessed in dogs by bolus and by infusion yielded different apparent potencies for the two drugs that we have studied in this model by intrathecal bolus and infusion delivery, morphine and fentanyl. Therefore, previous work with skin twitch and bolus delivery indicated that the morphine/fentanyl potency ratio was approximately 5 mg/0.05 mg = 100. In the current study with continuous infusion, this ratio was approximately 0.9 mg/ml/0.3 mg/ml = 3. Interestingly, a similar finding has been reported in rats. On a hot plate test, the intrathecal potency ratio for sufentanil and morphine after bolus delivery was 28.[32] In contrast, with continuous infusion, the ratio was 15.[37] We hypothesize that this difference in the intrathecal potency ratio observed after bolus and continuous delivery reflects the tendency for polar agents with a low octanol–water partition, such as morphine (log P of approximately 0.7), to show accumulation relative to more lipid-soluble compounds, such as fentanyl, with a high log P (approximately 2–3), which show a more rapid clearance.[38,39] Our work thus predicts that agents with a rapid clearance (*e.g.*, high log P and low molecular weight) will seem less potent after continuous infusion than after bolus delivery when compared with a more slowly cleared agent (*e.g.*, low log P and high molecular weight). It is interesting to note that in these studies, intrathecal infusion showed hydromorphone and morphine to be essentially equiactive. In the literature, hydromorphone is often considered to be several times more potent.[40] Again, it is interesting to consider, given the above reasoning, that the log P of hydromorphone (log P of approximately 1.2)[39] would give a higher relative activity when delivered as a bolus than for a lower lipid-soluble agent such as morphine.

Conclusion

The current and accompanying work[14] suggest that formation of intrathecal inflammatory masses are induced a constant infusion of a variety of, but not all, opiate agonists, and also an antagonist. For agents that do produce masses, the concentration, rather than total dose, appears to be an important determinate of formation. This suggests formation is not simply a result of opioid receptor activation given that fentanyl, a potent agonist, produced no masses, even at very high concentrations. These observations raise the possibility that fentanyl may possess some therapeutic advantage as compared with morphine regarding granuloma formation. Its high lipid solubility, however, leading to rapid clearance and significant systemic exposure, makes it a less-than-optimal drug for spinal delivery. Other phenylpiperidines with lower lipid solubility, a slower clearance after spinal delivery, and longer duration of action might be more suitable as a continuously delivered intrathecal drug. The mechanisms of this effect are uncertain, although the structure–activity relation provides support for the role of local meningeal mast cells, the activation of which might lead to a local increase in dural vessel permeability.

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