Studies in the Primate on the Analgetic Effects Associated with Intrathecal Actions of Opiates, α-Adrenergic Agonists and Baclofen

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The effects of intrathecally administered opiates (morphine sulfate and meperidine), α-adrenergic agonists (clonidine and ST-91) and baclofen were examined on the shock titration threshold of macaque monkeys chronically prepared with intrathecal (I) or epidural (E) catheters. Spinal opiates produced a long-lasting analgesia which was antagonized by naloxone. The order of potency was I morphine > I meperidine > E meperidine > E morphine. Clonidine and ST-91, also produced a dose-dependent, long-lasting elevation in the shock titration threshold, antagonized by phentolamine, but not naloxone. L-baclofen, but not d-baclofen, resulted in a dose-dependent elevation of shock titration threshold, which was not antagonized by naloxone. Repeated administration at 24-h intervals over a 7-day period of morphine, clonidine or baclofen, resulted in an significant reduction in the analgetic effects of each drug. Cross tolerance between the three classes of agents was not observed. Intrathecal co-administration of inactive doses of ST-91 and morphine resulted in a near maximal increase in the shock titration threshold, which failed to show any significant tolerance over 21 days.

Intrathecal ST-91 and morphine produced no change in either muscle strength, tendon reflexes, respiratory rate, urine formation, or the ability to locomote. Baclofen, in contrast, produced a dose-dependent decrease in muscle strength. That the intrathecal drugs did not produce anesthesia was demonstrated by their failure to block the avoidance response to ensuing ear shock cue by a light tactile stimulus applied to the hind paw. These results clearly indicate that a powerful analgesia can be produced by selectively activating adrenergic, opiate, and baclofenergic receptor systems in the spinal cord. (Key words: Analgesics: meperidine; morphine. Anesthetic techniques: epidural; spinal. Antagonists, narcotic; naloxone. Pain. Pharmacology: α-adrenergic agonists, baclofen. Receptors: adrenergic; baclofenergic; opiate.)

Evidence has begun to accumulate that there exists within the spinal cord several pharmacologically defined substrates whose activation is associated with the modulation of nociceptive information.

It has long been known that opiates exert a direct effect on spinal function. Administered systemically to spinal animals, opiates will inhibit spinal nociceptive reflexes1,2 and depress the discharge of dorsal horn nociceptors,3-5 while iontophoretic administration of opiates into the substantia gelatinosa will inhibit postsynaptic activation by small primary afferents.6,7 The observation of high levels of opiate binding in the substantia gelatinosa and its partial disappearance following rhizotomy8 is consistent with results obtained which show that opiates may act presynaptically to alter primary afferent excitability9 and attenuate the evoked release of substance P, a putative nociceptive transmitter from the spinal cord.10 The existence of enkephalin-containing neurons in the substantia gelatinosa11 and the ability to release enkephalin from the spinal cord12 offer further evidence that there exists an intrinsic opioid system whose activation may be associated with the modulation of the processing of nociceptive information. The intrathecal administration of opiates has produced a significant elevation in the behaviorally defined nociceptive threshold on a variety of tasks ranging from simple spinal nociceptive reflexes (tail flick and skin twitch) to more complex operant tasks such as hot plate and shock titration. The effect has been observed in a variety of species ranging from rats, rabbits, cats and primates to humans.13-17

The pharmacologic profile of the effect (e.g., structure activity relationship, antagonism, stereospecificity, dose dependence, and development of tolerance) indicates that the intrathecal action of opiates is on a receptor population which is functionally similar to that described for opiate binding and the antinociceptive effects of systemically-administered opiates.14,18-21

Activation of descending pathways which originate in the brainstem and which terminate in the spinal cord has been shown to reduce the discharge of nociceptive neurons22 and alter the animal's behavioral response to strong stimuli.23,24 Considerable evidence exists to support the idea that these descending pathways are monoaminergic in character. The supraspinal effects of opiates on spinal function are thought to be mediated through the activation of these descending systems. Direct evidence for this linkage derives from experiments in which the intrathecal administration of monoamine antagonists (such as phentolamine and methysergide) has antagonized the antreflexive effects of intracerebrally-administered opiates.25 That these descending monoamine termi-
nals do in fact alter spinal sensory processing is evidenced by the fact that the iontophoretic administration of noradrenaline and serotonin significantly inhibit the discharge of nociceptive neurons.\textsuperscript{29,27} The noradrenergic effect appears to be most specific for nociceptive neurons, with serotonin producing a more general inhibition of neuronal discharge in the dorsal horn. Significant dose-dependent elevations in the nociceptive threshold have been reported following the intrathecal injection of several α-adrenergic\textsuperscript{28-30} and serotonergic agonists,\textsuperscript{31,32} with no evidence of motor effects or any change in the animal's response to light brush and touch. The serotonin and noradrenalin effects are antagonized by methysergide and phentolamine, respectively, but not by the opiate antagonist naloxone. In addition, these analgesic effects are not antagonized by nonspecific vaso-dilators, thus arguing against the possibility of a local ischemia.\textsuperscript{30}

Wilson and Yaksh\textsuperscript{33} demonstrated that the intrathecal administration of baclofen β-(4)-chlorophenyl-γ-aminobutyric acid, produced a significant elevation in the nociceptive threshold at doses which were less than that required to produce a significant loss of muscle tone.\textsuperscript{34} D-baclofen is 1/100 times as active as the L-isomer, suggesting a specific locus of action on the cell membrane.

The following experiments extend these observations to the primate using a complex operant paradigm, namely the shock titration procedure, to assess changes in the nociceptive threshold produced when pharmacologically-defined terminals intrinsic to the spinal cord are activated by the intrathecal administration of their putative agonists. In addition, we sought to determine whether the agents have a specific effect on nociception or render the affected body regions anesthetic. We also examined whether the intrathecal action of these agents altered the animal’s urinary output or ability to exert muscle tension.

**Methods**

Male and female *Macaca mulatta* (n = 5) or *Macaca fuscata* (n = 6), were trained on the operant shock titration paradigm (see below) and/or the tactile avoidance task (see below) and implanted with intrathecal catheters.

**Surgical Protocol**

To implant an intrathecal catheter, animals were anesthetized with sodium pentobarbital (50 mg/kg, iv). The head and back of the neck were depilated and the animal mounted with head flexed forward in a stereotaxic instrument. Following aseptic procedures, the cisternal membrane was exposed and the dura cut rostrocaudally on the midline approximately 2–3 mm from the posterior rim of the foramen magnum. The catheter, consisting of a 50-cm length of polyethylene-10 tubing (PE-10: 0.75 mm, o.d.), sterilized by a 1-h immersion in acetone, was then inserted into the subdural space in a two-step procedure. The axis of the cord was usually straightened by supporting the animal with sand pillows. In the first step, a PE-90 guide catheter (1 mm, o.d.) with a rounded hollow metal head was inserted through the cisternal membrane, with the tip pointing away from the surface of the cord, and the catheter aligned with the visualized axis of the spinal cord. The PE-90 tubing was gently pushed forward, while being rotated between the thumb and forefingers approximately 20–30° to the left and right. After approximately 4–8 cm, resistance was often felt. It is sometimes of value to rotate the catheter 90° in either direction such that the natural bend of the catheter places it pointing to the left or right and then the catheter is moved slightly forward. In the second step, once the obstruction had been passed, the PE-10 tubing filled with sterile saline, was inserted through the guide catheter and gently pushed forward with a slight rotation to aid its progression. Insertions are generally not carried out under x-ray or fluoroscopic guidance. It has been our experience in a series of more than 100 cats and 11 primates, that the catheter will smoothly track forward, on occasion along the lateral or ventral surface of the spinal cord. This has not presented any difficulty, as the volume of the injection assures that the dorsal and ventral surfaces of the caudal cord will be affected (see below). After inserting the catheter 25–30 cm from the cisterna magna, a level which from measurement corresponds to the level of the L2–L6 vertebrae, the guide catheter (PE-90) was “threaded” back over the PE-10 catheter. The PE-10 catheter was then gently irrigated with 0.2 ml of normal sterile saline and the external tip of the catheter closed with a stainless steel plug. The catheter was passed through a length of 19-gauge stainless steel tubing, blunted at the end, bent to lie closely to the skull. This served to protect the catheter as it passed through the musculature on the back of the neck and on to the top of the skull. Orthopedic bone screws were then placed in the skull and the metal protective tubing was brought up through a plastic "pedestal", approximately 1 cm in diameter. The pedestal and tubing were affixed to the skull and skull screws by cranioplast cement. The pedestal was covered with a cap secured to it by means of a screw embedded in the cranioplast cement. The muscle layers were sutured to eliminate void space.

In 3 animals, an epidural catheter (PE-10) was in-
sany alone was injected. With few exceptions, such injections alone had no detectable effect on the long-term shock titration thresholds. It should also be noted that the presence of a dose-dependent effect of the drugs on the several behavioral measures also argues against a nonspecific injection effect. Secondly, we wished to determine whether the spinal drug effect was mediated by a peripheral action. In these experimental sequences, drugs were dissolved in saline and administered intramuscularly in the thigh. In addition, as these experiments were carried out over an 8-month period, control intrathecal injections of morphine were made between experimental series and compared to the response to the first injections made after implantation to assess changes in responsiveness.

**Behavioral Testing**

*Shock titration.* To assess the animal's nociceptive threshold, the discrete trial shock titration paradigm was employed. The animal was placed in a primate restraining chair. Constant current shock (0–6 mA in 25 equal steps; 60 Hz) was delivered through foot plates to which the animal's feet were strapped with Velcro tapes. Shock was given during 5-s trial periods separated by 3-s intertrial intervals when no shock was presented. Failure to respond to the shock during the trial interval by pressing an available lever caused the shock to remain on for 3 s and resulted in the shock level being elevated one step in the subsequent trial. If the bar was depressed, during the shock interval, the shock was immediately terminated, and in the following trial, the shock came on one step lower. The animal was thus able to modulate the level of shock received. The level of shock maintained by the animal during the experimental session was recorded by a potentiometric pen writer.

The titration threshold was defined as the highest average shock level maintained by the animal during any given 5-min interval. The stability of the titration response permitted this threshold to be estimated by visual inspection of the titration records. Session lengths were from 3–12 h, although longer intervals were occasionally used to ascertain the time course of various treatments. As the prolonged exposure to shock might alone affect the shock threshold, in several experiments, animals were injected and 8–14 h were allowed to pass prior to placing the animal in the restraining chair.

*Non-noxious tactile stimulation.* To assess whether the monkey was able to detect a non-noxious tactile stimulus applied to the foot pad, a region rendered analgetic by the intrathecal drug, the animal was placed in a primate restraining chair and its left foot strapped...
to a plate. A blunted rod (2 mm, o.d.) was pressed against the plantar surface by an air pressure source such that a pressure of 20 g was exerted. The stimulus was presented for 5 s prior to the delivery of a 2-s, 60-Hz, 1-mA shock to the ear. Momentary depression of a lever during the tactile stimulus aborted the presentation of the shock. Pressing the lever during the shock, turned the shock off. Once trained, animals showed a reliable avoidance of the shock in 80–95 per cent of the trials. In these experiments, 75 trials were presented at pseudorandom intervals of 15–40 s. To prevent cues from nontactile stimuli, the animal was situated in a small cubicle isolated from the programming equipment. White masking noise was present during trials. The intrathecal drug was injected and the animal was tested again after an interval corresponding to the time when maximum analgesia would be observed.

Muscle tension. To assess the muscle strength following intrathecal drugs, a spring tension scale was attached to either foot while the animal was in a primate restraining chair. We defined muscle strength as the maximum scale reading which could be obtained during a 3 to 4-min period. To make the animal withdraw the attached foot, experimenters made loud noises, tickled the abdominal area vigorously, and pulled on the animal’s arms. These treatments resulted in vigorous threat gestures on the part of the animal and efforts to withdraw the hindlimbs up under the buttocks to achieve a sitting posture.

Histology

To assess whether the intrathecal administration of these agents had any detectable effect on spinal cellular morphology, cats were prepared with intrathecal catheters and injected at 24-h intervals for 7 days, with doses of morphine, ST-91 (an α-adrenergic agonist) or baclofen which yielded analgesia. A fourth cat received an equivalent amount of saline vehicle under similar circumstances. On the eighth day, the animals were killed with sodium pentobarbital and perfused transcardially with saline and formalin. After embedding the spinal cords, transverse sections were cut at 30 μm, and alternate slides were stained with cresyl violet or nissl. The conditions of the cords were assessed by an anatomist who was unaware of the drug treatment received by each animal. While the exact comparability of cat to primate gross spinal anatomy might be questioned, the purpose of this series of studies was to examine the effect of drugs on cellular morphology and myelination, both of which would have comparable biochemistry in either species. We felt the purpose of the histologic ex-

periments permitted the substitution of cats for the more valuable and scarce primates.

Statistics

Statistical comparisons were made using a Student t test for independent samples or for repeated measures where appropriate. Differences reaching significance at the level of P < 0.05 single tail were described as being statistically significant. In the intrathecal morphine experiments, naloxone was administered systemically in three doses to produce a dose-dependent antagonism of the opiate effect. Employing these data, an apparent pA2 value for naloxone was estimated. Normally, the pA2 is estimated using the ED50 values of the agonist attained with increasing doses of the antagonist. A principle assumption is that the antagonist produces parallel shifts in the dose-response curves, the magnitude of these shifts being proportional to the log dose of the antagonist. In other experiments, the interaction of intrathecal opiates and naloxone have been shown to meet this criteria.19 It was assumed that a similar competitive interaction holds in the primate cord. The dose of naloxone (m/kg, im) which reduced the magnitude of the response of a just maximally effective dose of intrathecal morphine to 50 per cent was calculated. Though not a classical method of calculating p(A)2,36 this approach has the advantage of reducing the number of experiments required to estimate the dose of naloxone which doubles the ED50 of the intrathecal agonist.

Results

Intrathecal and Epidural Morphine

Intrathecal morphine produced a dose-dependent elevation in the shock titration threshold over a range of 200–1000 μg (fig. 1B), and this effect was antagonized by systemic administration of the opiate antagonist naloxone. Figure 1A presents records from representative experiments carried out in a single animal. The top record presents the effects of 160 μg of morphine sulfate administered intrathecally. As can be seen, there was a gradual and controlled increase in the level of shock tolerated by the animal over a 3-h period. Naloxone (0.1 mg/kg, im) caused the animal to reduce the level of shock. A dose-dependent antagonism of the effect produced by a given dose of intrathecal morphine sulfate (1000 μg) was achieved by im naloxone in doses of 0.03 mg/kg (n = 4, t = 1.842, P < 0.10); 0.1 mg/kg (n = 3, t = 8.441, P < 0.05) and 1 mg/kg (n = 4, t = 36.87, P < 0.01). Naloxone alone in doses of 0.1–1 mg/kg, (im), had no
Fig. 1A. Shock titration records obtained in one animal in separate experiments following the injection at time 0 of morphine sulfate, 160 μg, intrathecally (record 1); morphine, 1 mg, epidurally (record 2); morphine sulfate, 12 mg, intramuscularly (record 3); meperidine, 10 mg, epidurally (record 4); and meperidine, 10 mg, intravenously (record 5). The vertical axes for each record indicates shock titration step. Details of the injection and the titration paradigm are as described in the text. Record 1 shows the effect of intramuscular naloxone (0.1 mg/kg) given at the arrow. The animal weighed 6.0 kg.

Effect on the titration threshold of non-morphine treated animals. Calculations based on the data presented in figure 1B indicate that for naloxone against intrathecal morphine on the shock titration paradigm, the pA₂ = 6.8. Intrathecal meperidine was approximately one-tenth as active as intrathecal morphine (fig. 1B). This effect was also antagonized by naloxone.

The animal whose records are shown in figure 1A also had an epidural catheter in place during the series of intrathecal experiments. Morphine administered epidurally (1 mg), produced only a minimal effect

Fig. 1B. The graph presents the log dose response curves for intrathecal morphine (INT MOR: •); intrathecal meperidine (INT MEP: ▲); epidural meperidine (EPI MEP: △); and epidural morphine sulfate (EPI MOR: ○). The open squares (◇) indicate the effect produced by the intramuscular administration of 0.01, 0.03, or 1.0 mg/kg naloxone hydrochloride during an interval when the shock titration threshold was maximally elevated following 1000-μg intrathecal injection of morphine sulfate. All points represent the mean ±SE of 3–11 animals.
in contrast to its activity when given intrathecally. Epidural meperidine was about ten times as active as a similar dose of epidural morphine. A consistent finding was that while an epidural dose of meperidine did not produce as large a shift in the titration threshold as a bolus intravenous dose of meperidine, the duration of action associated with the given dose of epidural meperidine was longer than the effect produced by the same dose administered intravenously.

The time required for the titration threshold to return to baseline after intrathecal morphine was examined in four animals, following 1200 μg of morphine given over a 2-hour period; the mean time for return to the preinjection baseline was 21 ± 2 h. To assess the possibility that the duration of effect might be influenced by the prolonged exposure to shock, the preinjection titration baseline for 4 animals was assessed (4 ± 2 steps) prior to the intrathecal injection of morphine (1200 μg). The animals were then returned to their cages. Eighteen hours later, the animals were replaced in the restraining chairs and the titration thresholds assessed. Return to within 1 SE of the preinjection baseline occurred at 26 ± 3 h after the intrathecal injection.

**Effects of repeated injections of morphine.** Over an 8 to 10-month period, during which time these experimental sequences were carried out, 2–3 weeks elapsed between drug sequences. At the end of each drug-free interval, test doses of morphine, which in the drug-naive animal produced a maximum effect, were given. In 8 of 11 animals, no systematic change in sensitivity was observed in the analgetic response over the 8–month period. In two animals, loss of evoked response at 3 and 4 months was the result of a leak produced by excessive flexion of the catheter at the back of the skull. This was repaired and the animals again displayed a normal sensitivity to intrathecal morphine. In two animals, a loss of responsiveness to intrathecal morphine (for indeterminate reasons) was observed at 6 and 10 months, respectively. Injection of intrathecal morphine at 7-day intervals failed to produce any systematic evidence of tolerance.

**Fig. 2A.** Titration threshold records obtained in one animal following the daily intrathecal injection of 1200 μg of morphine sulfate, intrathecally, at the time indicated by the solid line. As described in the text, injections were made daily over a 7-day period; the representative changes in the titration threshold for days 1, 2, 3, 6 and 7 are presented. On day 6 the animals received intrathecal clonidine (2000 μg) and on day 7 intrathecal L-baclofen (60 μg), at the times indicated by the arrows. The animal weighed 5.1 kg.
More frequent intrathecal injections produced significant signs of tolerance. Figure 2A presents the effects of intrathecal morphine given daily in a single animal over a 7-day period. In this case, 1200 µg of morphine produced a near maximal effect within the first 3.5 h. On subsequent days, however, the magnitude of the threshold change diminished progressively. The mean results obtained in three animals are presented in figure 2B. By day 3, the evoked change in the titration threshold was significantly less than the effect produced on day 1 (n = 3; t = 9.250, P < 0.05). On day 6, following the failure of intrathecal morphine to produce a change in the threshold, the animal was administered intrathecal clonidine (2000 µg) and a significant elevation in the shock titration threshold was observed. On day 7, intrathecal baclofen (60 µg) was administered following the failure of 1200 µg of morphine to produce a detectable change in the threshold, and baclofen was also able to evoke a significant effect (fig. 2B). Both treatments resulted in a highly significant elevation in the titration threshold (baclofen: n = 3, t = 7.18, P < 0.05; clonidine: n = 3, t = 7.57, P < 0.05). These results suggest that in animals made tolerant to morphine, intrathecal α-agonists and baclofen remain capable of producing a significant change in the nociceptive threshold. Absence of cross tolerance strongly suggests that these two agents are not acting at an opiate receptor.

In addition, in experiments not presented here, doubling the intrathecal dose of morphine to which the animal was tolerant (1200 µg–2400 µg) resulted in a near maximal elevation in the titration threshold. These experiments were not carried further in view of the high concentration of morphine, though no untoward effects were observed.

One hour after the intrathecal morphine on the eighth sequential day of opiate injection, animals received an intrathecal injection of naloxone (100 µg). Within 30 s, there were clear signs of hyperreflexia. Animals showed an extreme hypersensitivity to light touch applied to the hind limbs, manifested by agitation and alarm vocalization. These signs were accompanied by an increased respiratory rate, piloerection, and urination, though the pupils remained normal and there was an absence of salivation. Though difficult to assess, there seemed to be little hypersensitivity to touch of the head and of the arms. This behavioral syndrome lasted approximately 30–45 min.

**Intrathecal Baclofen**

The intrathecal administration of l-baclofen produced a dose-dependent increase in the shock titration threshold (fig. 3A and B). Injection of 60–100 µg of baclofen resulted in a progressive increase in the nociceptive threshold. The duration of this effect was prolonged, lasting from 14–18 h. As shown in the bottom shock record (fig. 3A) baclofen was far less active when comparable doses were administered by the systemic route. Unlike morphine, intrathecal l-baclofen resulted in a dose-dependent degree of lower limb flaccidity.

Intrathecal d-baclofen, ten times the maximal analgesic dose of l-baclofen, resulted in only a slight increase in the nociceptive threshold, suggesting that the effect was stereospecific. As indicated in the individual record following 100 µg of l-baclofen and in the group means for these animals following 50 µg of l-baclofen (fig. 3B), naloxone administered systemically (2 mg/kg, im) had no effect on the elevated threshold produced by l-baclofen (n = 3, t = 0.312, P > 0.10). In figure 3A the delayed reduc-
tion in the threshold beginning approximately 45 min after naloxone is due to a gradual reduction in the baclofen effect and not to naloxone. As shown in figure 1, the naloxone effect is extremely rapid, occurring within minutes of its administration.

Effects of repeated injections of intrathecal L-baclofen.

To determine whether tolerance could be obtained, a paradigm similar to that for morphine was followed. Baclofen, 60 μg, was injected once daily in two animals. By day 7, the elevation in the titration threshold for the two animals was 56 per cent and 48 per cent of the effect produced on day 1. No change in the muscle weakness produced by baclofen was observed during the injection sequence period (see below). The intrathecal injection of 1200 μg morphine (on day 8) or 2000 μg clonidine (on day 9) produced nearly maximal elevations in the shock titration threshold comparable to those observed in the non-baclofen tolerant animal (see figs. 1 and 4B).

Intrathecal Adrenergic Agonists

The intrathecal administration of norepinephrine (500 μg) produced a short-lasting elevation in the nociceptive threshold (data not shown). Figure 4A presents the shock titration threshold records observed with ST-91 and clonidine. The intrathecal administration of these two compounds produced a powerful, long-lasting elevation in the shock titration threshold. The clonidine effect was more immediate in onset. In contrast, ST-91, a compound having a lower lipid partition coefficient, showed a delayed onset similar in character to that observed with morphine. The systemic administration of clonidine produced a clear elevation in the shock titration

† Dr. Robert Ruffolo, personal communication.
threshold, whereas ST-91 was completely inactive when administered systemically. The dose-response curves for clonidine and ST-91, each obtained in three animals, are presented in figure 4B.

The systemic administration of phentolamine produced an approximate 50 per cent reduction in the elevated nociceptive threshold produced by 2000 μg ST-91 (n = 4, t = 6.78, P < 0.05) or 2000 μg clonidine (n = 4, t = 10.18, P > 0.05). In contrast, naloxone (2 mg/kg) had no effect on the elevated threshold produced by α-agonists (n = 3, t = 0.124, P > 0.10) (fig. 4B).

**Effects of Repeated Injections of Intrathecal α-Agonists**

To determine whether tolerance developed, three animals were injected once a day with a dose of ST-91 which produced a just maximal effect (2000 μg). By the sixth day, the threshold elevation evoked by intrathecal ST-91 had fallen from 23 ± 2 observed on day 1, to 14 ± 4 (n = 3, t = 3.791, P < 0.05). This maximum, though statistically reduced as compared to day 1, was still significantly elevated as compared to the preinjection baseline (n = 3, t = 3.426, P < 0.05). On days 7 and 8, morphine (1200 μg) and baplofen (60 μg) injected intrathecally produced a near maximal elevation of the shock titration threshold (24 ± 1; 21 ± 2, respectively).

**Interactions Between Opiates and α-Adrenergic Agonists**

In view of the significant antinociceptive effects of morphine and the α-adrenergic agonists, we sought to determine whether or not these agonists interacted with one another. The lack of cross tolerance between opiates and α-adrenergic agonists (see fig. 2), and the inability of naloxone to antagonize the effects of ST-91 and clonidine, suggested that the materials do not act
The repeated daily injection of these drugs failed to produce a significant degree of tolerance (fig. 5). Although there was fluctuation in the animals' response, the daily administration of these doses of drugs consistently produced a significant elevation in the nociceptive threshold, over a period of 21 days. This is in marked contrast to the results observed with daily administration of a near maximal effective dose of either drug alone (see fig. 2). To determine whether this was a nonspecific effect, animals were injected with phentolamine (16 mg/kg, iv) or naloxone (1 mg/kg, im) after 15 or 16 h on day 1 and 16, respectively. The injection of antagonist on the sixteenth
consecutive day of combined morphine-ST-91 administration was followed by the usual decrease of the nociceptive threshold; but, the behavioral syndrome associated with naloxone reversal of an opiate effect appeared considerably less intense than that evoked in animals made tolerant to high doses of intrathecal morphine (see above).

The duration of the intrathecal effect of ST-91 and morphine was not examined in every experiment. However, on selected days, the duration of the combined effect was examined by leaving the animal in the restraining chair, but attaching his feet to the shock pedals during the intervals indicated on the graphs (fig. 5A). There continued to be a long-lasting effect even after 16 daily injections of the drug combination.

**Observations on Behavioral Effects of Intrathecal Analgetic Agents**

In the 11 animals employed in this series of experiments, the median number of intrathecal injections (both drug and vehicle) made during the 8- to 10-month period in which this series of experiments were carried out was 46 (range 22–79). A single animal spontaneously displayed a left hind limb weakness at 6 months, but continued to respond to morphine. The catheter was withdrawn 2 cm and considerable recovery of normal strength was observed. No signs of neurological deficit were observed in the other ten animals at any time, indicating the viability and innocuousness of an intrathecal catheter inserted to the lumbar level from the monkey cisterna magna.

**Neurological signs.** The intrathecal injection of any of the opiates, α-adrenergic agonists or baclofen, at doses which could produce maximum analgesia failed to produce signs of behavioral suppression. During these intervals, animals continued to consume fluids, take bananas, and respond with threat or fear grimaces to the presence of people.

The intrathecal administration of morphine in doses which produced a maximum analgesia (1200–2400 μg) as measured by the shock titration procedure, failed to produce blunting of tendon reflex activity, or impairment of the ability of the animal to right or locomote in its cage or in the room. Such injections of morphine (at doses ≥1200 μg) were observed to evoke scratching behavior directed at the stomach and lower back. This occurred usually within 5 min of the injection and lasted approximately 10–20 min. Intrathecal administration of 1200 μg morphine (fig. 6) had no effect on muscle tension (n = 5, t = 0.413, P > 0.10).

The intrathecal injection of ST-91 in doses that produce maximum changes in the nociceptive threshold failed to produce any significant change in behavioral tone, reflex function, or as shown in figure 6, in the elicitable flexion tension (n = 4, t = 0.612, P > 0.10). In contrast, baclofen, 100 μg, a dose which produced a maximum change in the nociceptive threshold, was invariably associated with an absence of tendon reflexes and muscle weakness (n = 4, t = 9.55, P < 0.05). A lower dose (60 μg), however, yielded a significant increase in the analgetic threshold (see fig. 4B) and had only a moderate effect on muscle strength (n = 5, t = 1.962, P < 0.10). Neither baclofen nor ST-91 were observed to elicit scratching behavior. Retching or vomiting was never observed following the intrathecal or epidural administration of these agents.

None of the intrathecal administrations produced any apparent effect on respiratory rate during the periods of titration threshold testing, not even intrathecal baclofen in doses which produced massive hind leg flaccidity. Failure to observe palpebral ears, tongues, or fingernails during the interval suggests that oxygenation was adequate. Systemic morphine, in doses which produced a similar change in the nociceptive
Effects of intrathecal agents on light touch perception. Two animals were trained in the tactile avoidance paradigm. The results with one animal, representative of both, are presented in figure 8. This figure indicates the percent avoidance responses (mean ± SE) made by one animal following intrathecal saline, intrathecal baclofen, intrathecal ST-91, and intrathecal morphine, at times of maximal analgesia. Each treatment was replicated three times on separate experimental days. Intrathecal morphine and the α-agonist failed to have any detectable effect on the ability of the animal to perceive the discriminative stimulus and avoid the shock to the ear. In contrast, following the higher dose of baclofen (60 μg), there was a slight decrease in the number of avoidance responses, suggesting that there may have been a slight reduction in the ability of the animal to detect the presence of the light pressure applied to the bottom of the foot. To control for nonspecific effects, lidocaine was infiltrated into the region of application of the tactile probe. As shown in figure 8, this reduced avoidance responses to less than 15 per cent of control. During trials when the animal failed to avoid the shock, a considerable agitation in response to the shock applied to the ear was still observed, in spite of significant analgesia manifested in the lower regions of the body. This escape responding was unaltered by any intrathecal treatment. Thus, of the trials in which avoidance did not occur, the mean level of escape re-

![Graph depicting muscle flexion strength](image)

**Fig. 6.** A histogram presenting the effects of intrathecal saline (Sal); morphine, 1200 μg (Mor 1200); ST-91, 4000 μg; t-baclofen, 100 μg (Bac 100), on the ability of the animal to exert a flexion force. The ordinate indicates the percent of control flexion strength obtained during the period when the antinociceptive effects of the indicated treatments were observed to be maximal. Each histogram is the mean ± SE of 3–8 separate experiments.

threshold, was invariably associated with some degree of respiratory slowing and significant signs of behavioral depression. Other than with systemic injections, the only instances where respiratory depression was noted occurred in two of three animals receiving epidural meperidine (10 mg). Respiratory depression accompanied by marked behavioral depression and somnolence was noted with an onset occurring from 15–30 min after the epidural injection. The intrathecal administration of meperidine was not accompanied by such a depressive effect. In one animal, inhibition of respiration required the administration of naloxone. Naloxone invariably reversed the respiratory and behavioral depression produced by the epidural meperidine.

**Urine formation.** Approximately 200 ml of urine was formed during an 8-h control period when the animals were titrating in the restraining chair but were not injected (fig. 7). Intrathecal injections of saline failed to alter this volume. Intrathecal injections of analgetic doses of morphine (1200 μg), ST-91 (2000 μg) or baclofen (60 μg) also had no effect. Specific gravity of urine averaged 1.05 ± 0.14. In contrast, the highest dose of morphine (2400 μg) was associated with a slight but nonsignificant reduction in the volume of urine. Urinary specific gravity rose to 1.15 ± 0.15. The observation of periodic urination throughout the day suggested that the reduced volume was not due to a block of the ability to void.

![Graph depicting urine formation](image)

**Fig. 7.** A histogram presenting the effects of intrathecal saline (Sal); morphine sulfate, 800 μg (Mor 800); morphine, 1200 μg (Mor 1200); ST-91, 4000 μg; or t-baclofen, 60 μg (Bac 60), on the volume of urine formed during an 8-h period in which the animal was in the primate restraining chair and undergoing titration threshold testing. Control (Cont) indicates the level of urine formation in animals receiving no treatment. Each histogram represents the mean ± SE of 4–10 animals.
response was between 91 ± 6 per cent and 99 ± 1 per cent. Though the shock levels to the ear and legs were not matched for motivational intensities, and the 1 mA ear shock may have been more imperative than the 6 mA foot shock, these results suggest that the intrathecal action of the drug was limited to the caudal dermatomes.

**Histological Examination of Cats Receiving Repeated Intrathecal Injections**

As described in the methods section, cats were injected intrathecally with either saline, morphine (1200 µg), ST-91 (2000 µg), or baclofen (60 µg), once a day for 7 days and sacrificed on the eighth day. No evidence of pathological change in the spinal cord could be detected as a result of treatments over this 8-day interval at the light microscopic level, either in the state of myelination or cellular morphology.

**Discussion**

In discussing these experiments two essential questions must be addressed. First, are the effects produced by these intrathecally and epidurally administered agents due to an action on spinal substrate? Secondly, are the effects produced by intrathecally-administered agents associated with the activation of the presumptive receptor population known to exist within the spinal cord and with which the action of these agents is commonly associated (e.g., morphine acting upon opiate receptor sites)?

**Considerations on the Anatomical Localization of the Spinal Drug Effect**

Several points argue that the effects produced by the intrathecal administration of the drugs in the volumes used in these experiments, were in fact due predominantly to an action on the spinal cord: 1) The analgesia produced by the intrathecal administration of all of these agents appeared to be limited to the caudal cord. Animals rendered analgetic by intrathecal injections to foot shock showed normal responsiveness to ear shock. 2) Behavioral effects which are commonly associated with high analgetic doses of opiates, such as respiratory depression and behavioral suppression, were not observed in these experiments. 3) If the effects produced by intrathecal administration are due to movement of the drug into the brain, there are two alternatives: redistribution via the blood, or diffusion into the brain via the cisterna magna. With regard to the first alternative, polar compounds such as morphine sulfate and ST-91 were more active both in terms of maximum effect and duration when given intrathecally than when given systemically. The converse would be expected if the drugs were acting after vascular absorption.

In recent experiments (Yaksh, Kaiko and Inturissi, in preparation), following the intrathecal administration of morphine in unanesthetized cats, measurable levels of this alkaloid could be detected in cisternal CSF samples. However, the levels of opiate in the cisternal CSF (<10 ng/ml) produced by intrathecal injections of opiates in doses sufficient to produce analgesia, block of skin twitch reflexes (1 mg/kg, iv) were below the cisternal levels (>100 ng/ml) associated with analgesia achieved by systemic administration. Moreover, in those experiments alkaloids disappeared from the cisternal CSF before recovery from the analgesia produced by the intrathecal injections.

The prolonged duration of action of intrathecally-administered drugs is due to the high concentration of drug in the spinal space and the rate at which the drug is inactivated or cleared from the intrathecal space. As metabolism makes a minor contribution, the rate at which the drugs are redistributed into the peripheral circulation will be a limiting factor for duration of action. Lipid soluble agents such as clonidine, meperidine, or methadone exit and penetrate readily, while more polar drugs having a low
lipid coefficient such as morphine and ST-91, penetrate and exit more slowly.\textsuperscript{37} The role of lipid solubility in the distribution and action following the intracerebral\textsuperscript{37} or epidural\textsuperscript{38} administration of drugs has been emphasized, and such considerations apply equally well to the spinal action of these analgesic agents. As indicated in the dose-response data in figure 1B for meperidine and morphine, the more lipid soluble the drug, the less difference there is between its intrathecal and epidural effects. The effectiveness of epidural morphine at doses which are inactive systemically, clearly suggests that the drug is diffusing directly to morphine-sensitive sites in the cord and not by a redistribution from blood. It should be noted that the intrathecal administration of a drug having a low lipid partition coefficient does not absolutely exclude the presence of drug from peripheral or other central tissues. The observation of scratching behavior after the high dose of morphine is consistent with the peripheral release of histamine by morphine.\textsuperscript{39} The increased specific gravity and decrease in urine volume after high doses of intrathecal morphine also suggests a direct effect on the hypothalamo-pituitary axis.\textsuperscript{40} High doses of intrathecal ST-91 produce a transient hypertension as a result of its peripheral vasconstrictive effects.\textsuperscript{41}

Pharmacologic Characteristics of the Spinal Drug Effect

The ability of naloxone to antagonize the effects of intrathecal morphine strongly suggests that the intrathecal opiates in the primate are acting upon a specific receptor system. The use of the \textit{in vivo} pA\textsubscript{2} analysis of Schild\textsuperscript{42} has permitted us to determine that the characteristics of the receptor population acted upon by intrathecal opiates, is indistinguishable from that receptor population acted upon by systemic morphine. Thus, the p(A)\textsubscript{2} of naloxone against systemic morphine on the primate titration procedure is approximately 7.\textsuperscript{35} In the present experiments, the estimated \textit{in vivo} pA\textsubscript{2} of naloxone against intrathecal morphine is 6.8. These data, similar to those obtained in rats on the hot plate and tail flick,\textsuperscript{43,44} suggest that the analgesic effects of intrathecal morphine is through a receptor system which is functionally similar to that acted upon by systemic morphine. Details of the assumptions underlying these calculations are given elsewhere.\textsuperscript{36}

With regard to the \(\alpha\)-agonists, ST-91 and clonidine, we were able to antagonize the effect with systemically- and intrathecally-administered phentolamine. This suggests the likelihood that their action was through the activation of \(\alpha\)-adrenergic receptors in the spinal cord, as in the rat.\textsuperscript{45} While these agents are known to have vasoconstrictor activity, these intrathecal effects are not likely the result of a local ischemia of the spinal cord,\textsuperscript{46} as they are always completely reversible, and in the rat, intrathecal administration of a variety of vasodilator agents fails to influence the intrathecal effects of norepinephrine or their congeners.

Baclofen will antagonize dorsal-horn neuron activity evoked by small fiber input preferentially.\textsuperscript{46} Evidence suggests that this drug may serve to hyperpolarize primary afferent terminals\textsuperscript{47} and to release GABA.\textsuperscript{48}

Though intrathecally-administered agents presumably act on the binding sites \textit{within} the substantia gelatinosa, low but measurable amounts of opiate binding have been detected in the dorsal roots.\textsuperscript{49} That these receptors may be functionally active is suggested by the observation that serotonin, noradrenaline, and GABA agonists alter dorsal root ganglion cell activity by a direct effect.\textsuperscript{50-52} Additionally, recent experiments by Jurna and Grossman\textsuperscript{53} have indicated that opiates can reduce the long latency component of compound action potentials in peripheral sensory but not motor nerves. Antagonism of this effect by naloxone is presumptive evidence of an interaction with an opiate receptor. Such observations suggest the possibility that intrathecal or epidural opiates may exert some direct effect on primary afferent axons or ganglia in the epidural space or within the dura prior to entering the cord.

In previous experiments, we reported that the intrathecal administration of morphine in the rat yielded a rapid development of tolerance.\textsuperscript{46} The present experiments extend these observations to the primate. The daily administration of intrathecal morphine in a dose which initially produces a near maximal elevation in the shock titration threshold, produced a rapid development of tolerance during a 3–5 day period. Intrathecal ST-91 and baclofen were also observed to undergo a reduction in their effectiveness following repeated injection. The time course of the decline of the antinociceptive effectiveness of these two drugs was between 4–8 days. There appeared to be a residual antinociceptive effect which stabilized following the initial decrement. This was unlike morphine wherein a complete loss of the effect produced by a given dose was observed.

The evidence supports the hypothesis that these three classes of drugs administered intrathecally are acting upon three separate receptor systems. Thus, naloxone failed to antagonize ST-91, clonidine, or baclofen. Animals made tolerant to morphine showed
a normal response to intrathecally-administered baclofen and \(\alpha\)-adrenergic agonists. Similarly, animals made tolerant to ST-91 and baclofen showed a normal responsiveness to the other agents. These data, therefore, strongly suggest that none of the effects were mediated through one or the other of these receptor systems, e.g., it is unlikely that the activation of an \(\alpha\)-adrenergic receptor was producing its effects via an intercalated enkephalin interneuron.

In the present experiments, we sought to determine whether minimal receptor activation of the opiate and \(\alpha\)-adrenergic system, at the same time, would in fact produce a significant effect. Previously, the systemic administration of clonidine and morphine resulted in a significant enhancement of the analgesia produced by either drug alone.\(^{24}\) From the present work it is clear that minimal activation of either spinal terminal system at the same time, does produce a near maximal behavioral effect. That this effect may be a synergy as opposed to simple additivity, is suggested first by the magnitude of the potentiation, and secondly, by the fact that naloxone or phentolamine alone is able to produce a nearly complete reversal of the antinociceptive effects produced by the combination of agents. In rat experiments,\(^{25}\) the concurrent intrathecal injection of a dose of morphine and ST-91 in doses which alone produce a 10–20 per cent increase in baseline, yielded a 4- to 7-fold decrease in the morphine ED\(_{50}\). Importantly, as in the present experiments with the primates, pretreatment of the rat with naloxone almost completely blocked the observed elevation in the nociceptive threshold.

Perhaps the most important aspect of the primate experiments is the clear attenuation of the rate of tolerance development in the combination treated animals. Though there were day to day variations, the tolerance development normally produced by a maximal activation of the opiate receptor system alone did not occur. In addition, the ability to antagonize the effects with naloxone at various time intervals, clearly indicated that the lack of tolerance development was not due to some nonspecific change in the animal's nociceptive threshold. In addition, it should be noted that the animal's thresholds were returning to near baseline levels at the end of the 24-hour interval after the preceding injection. Though the precise reason for this retarded development of tolerance is not known, we think that a probable cause may be the relatively low degree of activation of each of the independent receptor systems. The fact that the systems may functionally interact in a synergistic fashion produces the marked behavioral effects in spite of the low drug concentrations.

**Behavioral Consequences of the Intrathecal Actions of Opiates, \(\alpha\)-adrenergic Agonists and Baclofen**

In accord with the original experiments carried out in rats, rabbits, and cats, the results of the present experiments examining the effects of activating these specific receptor systems on non-nociceptive aspects of behavior reveal a high degree of functional specificity. Thus, systematic examination of the ability of the animal to exert a volitional flexion tension has indicated that morphine and the \(\alpha\)-agonists have no significant effect on muscle strength. In contrast, baclofen produced a dose-dependent degree of flaccidity.

The ability of the animal to detect the presence of light pressure applied to the sole of the foot suggests that in spite of the nonresponsiveness of the input associated with high-intensity stimulation, the animal remained capable of discriminating the presence or absence of a non-noxious, tactile stimulus. This agrees with observations that tactile thresholds in areas made analgesic by intrathecal morphine in humans are unaltered,\(^{28,29}\) and suggests that the intrathecal opiates and \(\alpha\)-adrenergic agonists are exerting their primary effects on throughout mediated by small but not large afferent fibers. On the other hand, baclofen appears to produce a more general effect. We would predict that anesthesia might result from the intrathecal action of this drug in humans.

In accord with most clinical reports,\(^8\) in these experiments low doses of intrathecal opiates did not appear to inhibit urine formation or retard urination. Daily urine specific gravities, were well within range of normal untreated animals. Food intake and body weight manifested little fluctuation during the long-term intrathecal administration of either the opiate or \(\alpha\)-adrenergic agonists.

The relatively selective influence on nociceptive behavior and the failure to alter several other physiological measures suggest that the receptor systems upon which these drugs acted are specifically associated with spinal cord substrates processing nociceptive stimuli. While the present study has focussed on three putative agonist systems, it is likely that future investigations will reveal the existence of additional modulatory systems, perhaps some having greater potency and specificity. Independent multiple opiate receptor systems in the spinal cord mediating analgesia in the rat and primate have in fact recently been de-

scribed. The ability to gain access to such neural substrates avoids the problems commonly encountered when drugs are administered via a systemic route. Thus, in recent experiments, we have demonstrated that intrathecal opiates failed to have any effect on the delivery or mortality of rats and rabbits. The long-lasting effect occurs by virtue of the slow rate at which these drugs having a low lipid partition coefficient exit from the spinal subarachnoid space. Predictably, drugs having a low lipid coefficient and a resistance to metabolism will possess the longer lasting effect and fewer peripheral side effects. Thus, the intrathecal administration of a large opioid peptide, e.g., β-endorphin produced a long-lasting analgesia.

The important consequence of the present trend in pain research and anesthesiology towards the intrathecal and epidural administration of pharmacologically-active substances is the recognition that the spinal cord is a complex of substrates having sensory functions which possess a differential pharmacology.

The ability to selectively activate spinal transmitter systems which are organized to influence the processing of noxious stimuli, offers a powerful means of controlling at the segmental level the content of the message generated by somatic stimuli, which upon reaching higher centers would otherwise give rise to the perception of pain.

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