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Antinociceptive Effect of Intrathecal Morphine in Tolerant and Nontolerant Spinal Rats[1]

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SIUCIAK, J. A. AND C. ADVOKAT. *Antinociceptive effect of intrathecal morphine in tolerant and nontolerant spinal rats.* PHARMACOL BIOCHEM BEHAV **34**(3) 445–452, 1989.—The antinociceptive effect of intrathecal morphine on the tail-flick (TF) reflex of rats was significantly enhanced within one day after spinal transection ($ED_{50} = 0.125$ μg) relative to the effect obtained in intact rats ($ED_{50} = 5.9$ μg). By 20–30 days after spinalization the potency of intrathecally administered morphine had substantially declined. Intact rats, made tolerant to the antinociceptive effect of systemic morphine (3.0 mg/kg, SC on each of seven consecutive days), were not tolerant to intrathecal morphine ($ED_{50} = 6.5$ μg). In contrast, rats that were pretreated with either morphine alone, repeated TF tests alone, or both of these treatments, were tolerant to intrathecal morphine when tested one day after spinal transection. The results suggest first, that the antinociceptive effect of intrathecal morphine in intact rats is tonically inhibited by descending supraspinal input and that removal of this input is responsible for the enhanced antinociceptive effect of intrathecal morphine in spinal rats. Second, the data suggest that tolerance to the antinociceptive effect of intrathecal morphine in intact rats may also be tonically inhibited by supraspinal input, because spinal opiate tolerance is expressed after spinal transection.

Spinal opiate antinociception Morphine tolerance Spinal rats

PRESENT views of opiate action in the central nervous system originated with the classic experiments of Wikler, Martin and colleagues, who demonstrated that systemic morphine could suppress nociceptive reflexes in animals that had sustained a complete spinal transection. Their results showed that, in addition to a direct action in the brain, morphine exerted a direct effect at the spinal cord (42).

Subsequent research indicated, however, that the doses of systemic morphine required to suppress spinal reflexes in the spinal preparation were greater than those required to produce the same effect in intact animals (2, 8, 10, 17, 23, 46, 49). This observation that systemic morphine is more potent in the intact than in the spinal animal, led to the hypothesis that opiate analgesia is a function of both direct spinal and supraspinal actions and an increase in supraspinal inhibitory control of spinal reflex pathways (5,18).

We recently confirmed and extended this observation by demonstrating that the antinociceptive potency of systemic morphine on the tail-flick (TF) reflex was not only reduced within one day after spinal transection, but continued to decline during the posttransection interval, reaching asymptote three weeks after spinalization (2).

In contrast to systemic morphine, our results indicated that the antinociceptive effect of intrathecally administered morphine did not decrease after spinal transection. That conclusion was tentative, however, because intrathecal morphine produced a bimodel response distribution in spinally transected rats. That is, spinal rats showed either no response after intrathecal morphine injections or they gave a maximum antinociceptive reaction.

In subsequent (unpublished) experiments, it became evident that the intrathecal catheters of some rats were inadvertently punctured during spinal surgery. None of the spinal rats whose catheters were punctured subsequently responded to an intrathecal morphine injection. This result raised the possibility that our previous data underestimated the potency of intrathecal morphine in spinal rats. Therefore, the present experiments were conducted to reexamine the antinociceptive effect of intrathecal morphine administration in spinally transected rats. The results show that, in contrast to the systemic route, intrathecally administered morphine is more potent in acute, spinally transected rats than in intact rats. These data do not support the hypothesis that morphine produces analgesia at the spinal level by increasing descending supraspinal inhibition. Rather, the present results suggest that the antinociceptive action of spinally administered morphine is tonically inhibited by descending input and that removal of that input potentiates spinal opiate antinociception (36).

Further experiments were conducted to determine whether the effect of intrathecal morphine differed in other respects from the

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effect of systemic morphine in spinal rats. First, the posttransection time course of morphine-induced antinociception was determined by comparing the effect of intrathecal morphine in rats transected for 1 day, 20 days and 30 days. The results show that spinal opiate antinociception declines during the first three weeks after spinalization. The data are consistent with previous work demonstrating a similar time course for the reduction in systemic opiate antinociception in chronic spinal rats (2). Second, the effect of opiate tolerance on morphine-induced antinociception was assessed by comparing the dose-response function to intrathecal morphine in rats made tolerant before spinalization, with the dose-response function of nontolerant rats. Tolerance to systemic morphine did not alter the dose-response function to intrathecal morphine in intact rats. However, rats made tolerant to systemic morphine were tolerant to intrathecal morphine if they were tested one day after spinal transection. These data suggest that tolerance to spinal opiate administration is also inhibited, perhaps by descending supraspinal input, and that tolerance at the spinal level is expressed when such input is removed.

METHOD

Subjects

Male, albino Sprague-Dawley derived rats (King Labs, Oregon, WI) weighing 350–450 grams were used as subjects. After surgery they were housed individually so that their intrathecal catheters would not be damaged by cagemates. All animals had continuous access to food and water throughout the experiments.

Surgical Procedures

Spinal catheterizations were performed according to a modified version of the method of Yaksh and Rudy (48). The rats were placed in a stereotaxic frame under ether anesthesia. An incision was made behind the ears and the neck muscles were scraped to expose the back of the skull. An incision of the atlanto-occipital membrane allowed insertion of an 8-cm catheter of polyethylene tubing (PE10) filled with sterile saline into the spinal subarachnoid space. The catheter was held in place against the skull with adhesive. The incision was closed and the exposed tip of the catheter was heat sealed. When spinal injections were made the tip was cut and then resealed after drug administration. All animals were allowed to recover for at least 7 days before undergoing a spinal transection.

For spinal transection a laminectomy was performed under ether anesthesia between T6–T9. A 1–2 mm portion of the spinal cord was removed by excavation and replaced with Gelfoam® to reduce bleeding, after which the incision was sutured. For the first two weeks after surgery, spinally transected rats were bathed and their urine was expressed manually, once a day, by the application of pressure to their bladders. Their cages were placed under heat lamps to maintain body temperature. Within two weeks transected animals regained bladder and temperature control and the supportive postoperative measures were no longer required. At the end of each experiment the animals were sacrificed with an intraperitoneal injection of pentobarbital and the spinal column and the back of the skull were exposed. Animals were excluded from the experiment and their data were omitted from the analyses if: 1) the transection was incomplete, 2) the catheter was improperly placed (inside rather than outside of the cord) or 3) the catheter was punctured.

Behavioral Assessment

Reactivity to a noxious stimulus was evaluated with the tail-flick (TF) reflex, using a procedure derived from the method of D'Amour and Smith (15). Noxious stimulation was produced by a beam of high intensity light focused on the tail. The response was measured automatically and was defined as the interval between the onset of the heat stimulus and the abrupt flick of the tail. Each determination consisted of three trials; the mean score was taken as the response latency. In order to minimize tissue damage to the tail, animals not responding within 14 sec were removed from the apparatus and assigned a response latency of 14 sec. All rats were tested on the TF prior to and 40 min after each injection.

Drug Administration

For systemic administration, morphine sulfate (Merck, Rahway, NJ) was dissolved in 0.9% saline and administered subcutaneously such that the dose (3.0 mg/kg) was injected in a volume of 1 ml/kg. For intrathecal injections, solutions were made such that the injection volume of 10 μl contained the appropriate concentration (0.0625–30 μg). Each drug injection was followed by a 10-μl wash of saline. The injections were performed manually with a Hamilton microsyringe.

Statistical Analyses

The difference in latency between the pre- and postdrug test scores was determined for each rat and dose-response functions were constructed from these values. All analyses of variance and calculations of the regression lines were performed with the IBM Statistical Analysis System General Linear Model program (SAS, Cary, NC) provided by the University of Illinois Biostatistics Facility, or a commercial statistical program, the Portable Statistician. Post hoc comparisons were made using the Newman-Keuls test (39) to determine the significance of the differences among the group means.

Procedures

The first experiment evaluated the effect of spinal transection on spinal opiate antinociception. Separate groups of rats were spinally transected and allowed to recover for either 1, 20 or 30 days. Additional groups of intact rats were implanted with intrathecal catheters and maintained undisturbed for at least 20–30 days. At the appropriate time interval all rats were tested on the TF prior to and 40 min after intrathecal morphine administration.

The second experiment assessed the antinociceptive effect of intrathecal morphine in rats made tolerant to systemic morphine prior to spinal transection. Separate groups of rats received either morphine (3.0 mg/kg, SC) followed by a TF test (mor + test), saline (1 ml/kg, SC) followed by a TF test (sal + test) or morphine injections only (mor only, 3.0 mg/kg, SC), once a day, on each of seven consecutive days. Immediately after the seventh test session, one-half of the rats in the mor + test condition, and all of the rats in the other two treatment conditions, were spinally transected. On the eighth and final test session, 24 hr later, all rats were tested on the TF before and 40 min after an intrathecal challenge.

RESULTS

Effect of Spinal Transection on Tail-Flick Withdrawal Latencies of Nontolerant Rats

Table 1 summarizes the baseline scores of the nontolerant, intact and spinal groups. As previously noted by others (2, 10, 17, 46), a two-way analysis of variance indicated that baseline TF latencies were significantly decreased by spinal transection, $F(1,98) =$

TABLE 1

BASELINE TAIL-FLICK LATENCIES (±SEM) OF ACUTE (7-DAY) AND CHRONIC (20–30-DAY) INTACT AND SPINAL RATS

	Acute	Chronic
Intact	4.37 ± 0.15 (35)*	4.15 ± 0.15 (20)†
Spinal	3.34 ± 0.46 (12)	3.34 ± 0.25 (22) (20-day)
		3.34 ± 0.24 (10) (30-day)

Intact vs. spinal, *$p<0.05$, †$p<0.01$.
Number of subjects is indicated in parentheses.

22.2, $p<0.0001$. Post hoc comparisons showed that the difference between intact and spinal rats was statistically significant for both the Acute ($p<0.05$) and Chronic conditions ($p<0.01$). There was no difference in the respective latencies of either intact or spinal rats as a function of time (Acute vs. Chronic).

Effect of Spinal Transection on the Antinociceptive Response to Intrathecal Morphine

The dose-response functions of Intact and Acute (1 day) spinal rats are shown in Fig. 1. Analysis of the regression lines indicated an ED_{50} of 5.9 μg (95% confidence limits, 3.8–9.1 μg) and 0.125 μg (95% confidence limits 0.109–0.145 μg) respectively. A two-way ANOVA performed on the scores from those doses that were common to the two conditions indicated a significant difference, $F(1,22)=47.7$, $p<0.001$, between intact and spinal rats. These data show that the effect of intrathecal morphine is significantly enhanced one day after spinal transection.

To determine whether the effect of intrathecal morphine changed during the posttransection interval, separate groups of rats were injected at 20 and 30 days following spinal transection and the results compared with the dose-response function of rats tested one day after spinalization. These data, summarized in Fig. 2, show that the antinociceptive effect of intrathecal morphine decreases between 1 and 20–30 days after spinalization. Analysis

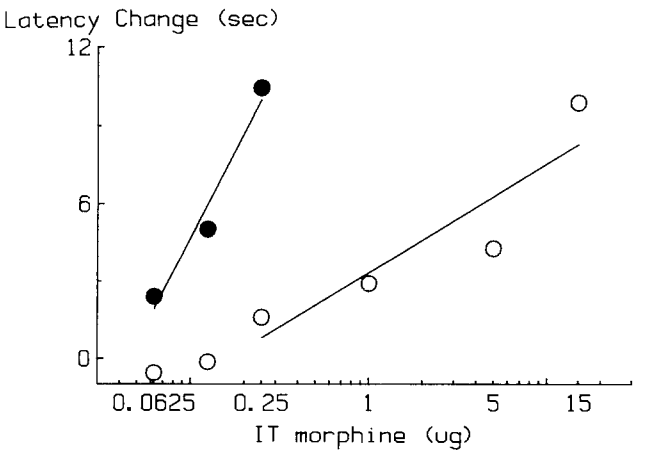

FIG. 1. Dose-response functions to intrathecal morphine in intact rats (open circles, n=3–5 per dose; SEM=±1.2, 1.0, 1.5 and 0.5 sec for 0.25, 1.0, 5.0 and 15 μg, respectively) and rats that were spinally transected 24 hr previously (filled circles, n=4 per dose; SEM=±0.9, 1.2 and 0.7 sec for 0.0625, 0.125 and 0.25 μg, respectively). Rats were tested on the tail-flick before and 40 min after an acute intrathecal injection.

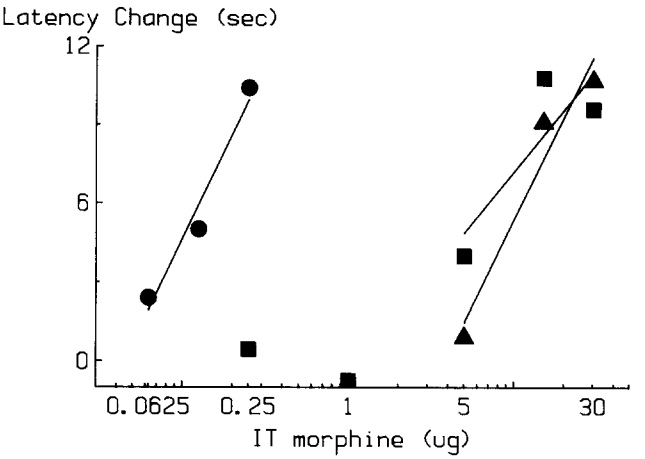

FIG. 2. Dose-response functions to intrathecal morphine in rats that were spinally transected one day (filled circles, same as Fig. 1) 20 days (filled squares, n=2–6 per dose) or 30 days (filled triangles, n=2–4 per dose) previously. Rats were tested on the tail-flick before and 40 min after an acute intrathecal injection.

of variance indicated that, although there was a significant dose effect, $F(2,20)=18.6$, $p<0.001$, the two dose-response functions of 20- and 30-day spinal rats did not differ from each other ($p>0.4$). This result suggests that the decreased potency of intrathecal morphine reaches an asymptote within approximately 3–4 weeks after transection. This conclusion may be premature, however, because the effect of intrathecal morphine was not assessed at longer (or shorter) posttransection intervals.

The data summarized in Fig. 2 suggest that opiate antinociception in chronic spinal rats may return to the levels observed in intact rats. Unfortunately, this possibility could not be quantitatively confirmed. The scores of both 20- and 30-day spinal rats reached the maximum cut-off score at doses of 15 and 30 μg. As a result, it was not possible to calculate an accurate ED_{50} value for these two conditions and to determine whether it was the same as that of intact rats.

It was also conceivable that the response of intact rats to intrathecal morphine might change as a function of time following catheter implantation. We, therefore, compared dose-response functions to intrathecal morphine (5, 15 and 30 μg) in additional groups of intact rats that had sustained intrathecal catheters for either 7 or 20–30 days. The analysis indicated no effect of the posttransection interval on opiate antinociception, $F(1,33)=2.18$, $p<0.124$.

These results demonstrate that the potency of intrathecal morphine declines between 1 and 20–30 days after spinal transection. It is not yet clear whether 1) this decrease is complete within 30 days or 2) whether the potency of intrathecal morphine in chronic spinal rats is the same as that of intact rats.

Effect of Spinal Transection on the Antinociceptive Response to Intrathecal Morphine in Tolerant Rats

Daily administration of 3.0 mg/kg SC morphine, in conjunction with the TF test, induced tolerance within one week. These data are shown in Fig. 3, which summarizes the TF scores of rats injected with either morphine (mor + test) or saline (sal + test) 40 min prior to TF assessment on each of seven consecutive test sessions. The development of tolerance was confirmed by a repeated measures ANOVA, $F(6,35)=47.9$, $p<0.0001$, and the

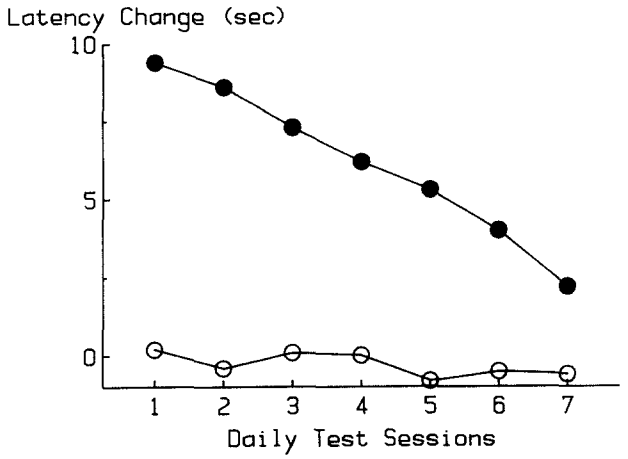

FIG. 3. Development of tolerance to morphine-induced analgesia on the tail-flick test. Rats were tested on the tail-flick before and 40 min after a single daily injection of either morphine (mor + test, 3.0 mg/kg, SC, n=36, filled circles) or saline (sal + test, 1 ml/kg, SC, n=12, open circles) on each of seven consecutive days.

fact that there was a significant decrease in the scores of the mor + test group between the first (9.4 ± 0.2 sec) and seventh (2.2 ± 0.4 sec) session, $t(35)=14.4$, $p<0.0001$.

On the next (eighth) day morphine was administered intrathecally to one-half of the tolerant rats (mor + test). The dose-response function of this group was compared with the function of nonpretreated, intact rats (Fig. 4) and a two-way ANOVA was performed on the scores of the three doses that these groups received in common. The results indicated that there was a significant dose effect, $F(2,27)=29.7$, $p<0.0001$, but no difference between the nonpretreated and morphine-tolerant (mor + test, $ED_{50}=6.5$ μg, 95% confidence limits 4.7–9.1 μg) rats ($p<0.38$). These data show that intact rats made tolerant to daily injections of 3.0 mg/kg SC morphine are not tolerant to intrathecal morphine.

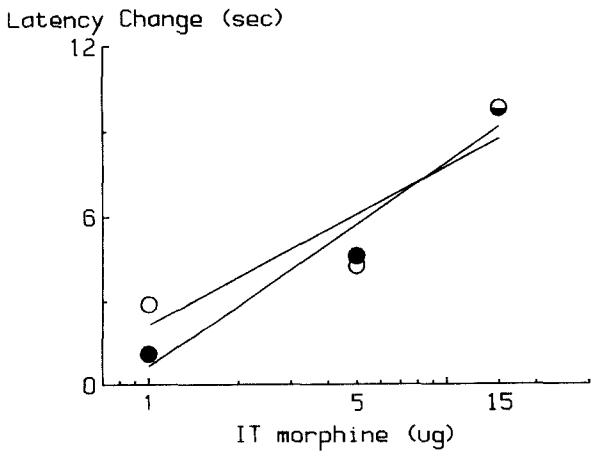

FIG. 4. Dose-response functions of intact rats to intrathecal morphine injections. One curve (mor + test, n=4–6 per dose, filled circles; SEM = ± 1.6, 0.8 and 0.2 sec for 1.0, 5.0 and 15.0 μg, respectively) was obtained from rats made tolerant to the analgesic effect of systemic morphine on the TF test (3.0 mg/kg, SC, on each of seven previous daily sessions). The second curve (no pretreatment, open circles, shown in Fig. 1) was obtained from rats that had no prior exposure to morphine or the TF test.

Immediately after the last tolerance session on day 7, the second half of the morphine-tolerant group (mor + test), and all of the saline-pretreated (sal + test) and morphine-pretreated (mor only) rats, were spinally transected. On the next day, the response of each of these pretreated groups to intrathecal morphine was determined.

Dose-response functions to intrathecal morphine obtained from the three pretreatment conditions were compared with the dose-response function of spinally transected rats that were not previously exposed to either morphine or the TF test (spinal, no pretreatment, shown in Fig. 1). For clarity of presentation the results are summarized for each of the pretreatment conditions in Parts A, B and C of Fig. 5, respectively.

Part A of Fig. 5 compares the dose-response curves to intrathecal morphine of nonpretreated spinal rats and spinal rats made tolerant as a result of prior morphine administration and TF tests (mor + test). The curve of the mor + test rats is shifted to the right ($ED_{50}=1.0$ μg, 95% confidence limits 0.77–1.3 μg) of the curve of nonpretreated rats. The data show that rats made tolerant to systemic morphine are also tolerant to intrathecal morphine, when evaluated one day after spinalization. These data contrast with the results obtained in intact rats, which indicated that tolerance to systemic morphine did not induce tolerance to intrathecally administered morphine. Because of this difference between intact and acute spinal rats in the expression of "cross-tolerance" to intrathecal morphine, additional experiments were performed to assess the respective effect of the nociceptive TF test and opiate pretreatment on tolerance to intrathecal morphine.

Part B of Fig. 5 summarizes the dose-response function to intrathecal morphine of sal + test rats and nonpretreated rats, one day after spinalization. The dose response curve of sal + test rats is shifted to the right ($ED_{50}=1.6$ μg, 95% confidence limits 1.14–2.24 μg) of the dose-response curve of nonpretreated rats. These data show that prior exposure to the TF test procedure alone also reduces the antinociceptive effect of intrathecal morphine administered within one day after spinal transection.

Part C of Fig. 5 compares the dose-response function to intrathecal morphine of morphine-pretreated (mor only $ED_{50}=0.7$ μg, 95% confidence limits 0.45–1.08 μg) and nonpretreated rats, one day after spinal transection. These data show that systemic morphine administration alone also reduces the antinociceptive effect of intrathecal morphine in acute spinal rats. Analysis of variance, performed on the three dose-response curves of pretreated rats (mor + test, sal + test and mor only), indicated that there was a significant dose effect, but no difference among these three conditions.

Finally, Table 2 compares the baseline TF latencies obtained under each of these four experimental conditions, prior to the final, respective morphine injections. There was a significant difference among the four groups, $F(3,58)=14.6$, $p<0.001$. As indicated by post hoc comparisons, the baseline TF latencies of all three, pretreated, spinally transected groups were significantly less than the latencies of the pretreated, intact group. This showed that, in spite of the various pretreatment conditions, withdrawal latencies of spinal rats were still lower than those of intact rats. In addition, the latencies of those rats that had repeatedly performed the TF response (sal + test) before the transection, were also significantly lower than those of the other two groups of spinal rats.

DISCUSSION

Effect of Acute Spinal Transection on Morphine-Induced Antinociception

The first significant finding of these experiments is that the antinociceptive effect of intrathecal morphine on the TF reflex is

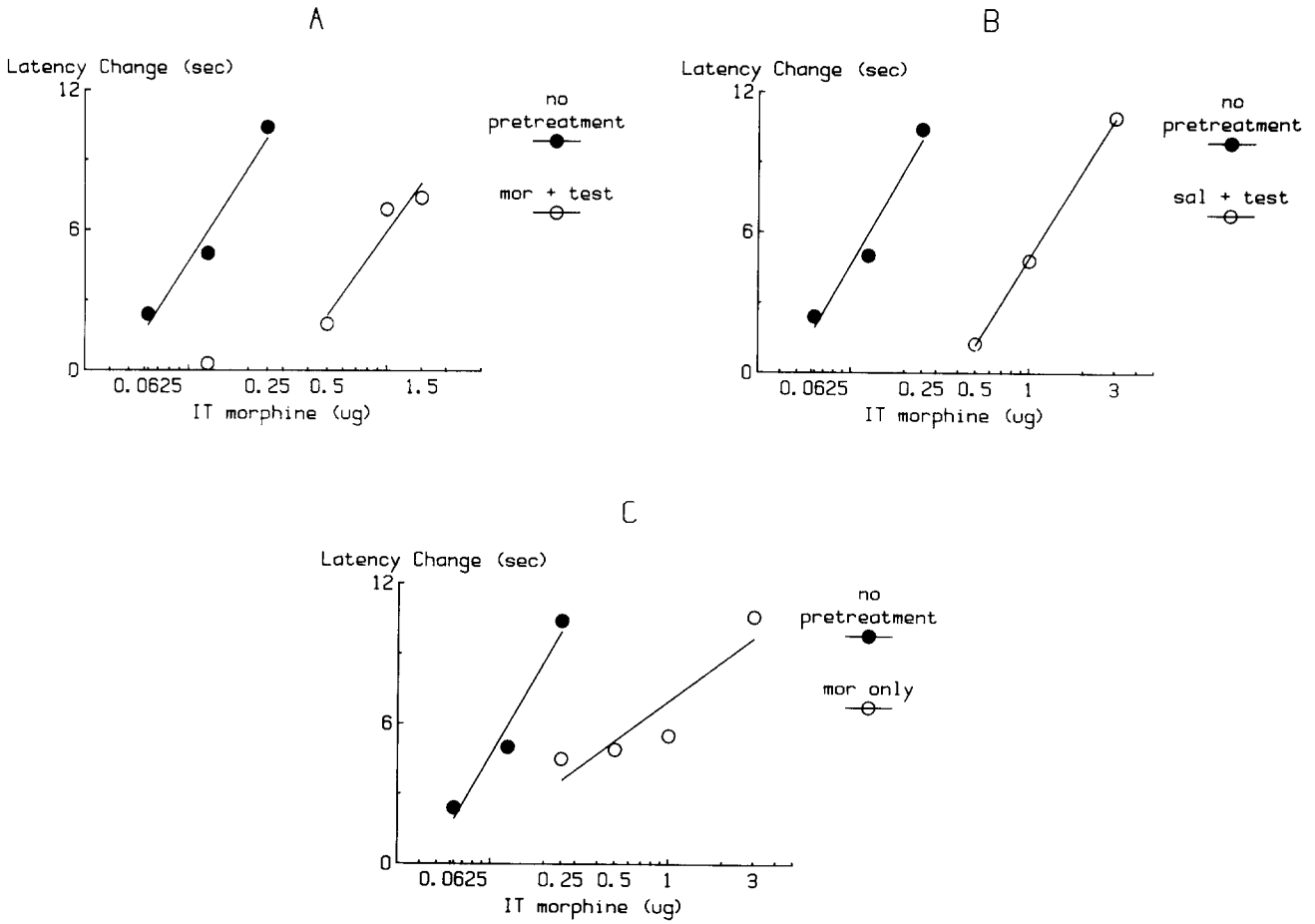

FIG. 5. Dose-response functions to intrathecal morphine in spinal rats. Part A compares the curves obtained from rats that had no prior exposure to morphine or the TF test (no pretreatment, filled circles, shown in Fig. 1) with the curve obtained in rats made tolerant to systemic morphine (mor + test, n = 5 or 6 per dose, open circles; SEM = ± 1.3, 2.0 and 1.4 sec for 0.5, 1.0 and 1.5 μg, respectively). Part B compares the curve obtained from nonpretreated rats with that of rats that were previously tested on the TF, in the absence of morphine (sal + test, n = 4 per dose, open circles; SEM = ± 1.9, 2.6 and 0.6 sec for 0.5, 1.0 and 3.0 μg respectively). Part C compares the curve obtained from nonpretreated rats with that of rats previously injected with morphine (mor only, n = 3 or 4 per dose, open circles; SEM = ± 1.6, 2.4, 2.6 and 0.6 sec for 0.25, 0.5, 1.0 and 3.0 μg, respectively). Spinal transections were performed approximately two hours after the last pretreatment session and intrathecal dose-response functions were obtained 24 hr later.

enhanced within 24 hr after spinal transection. This result is the opposite of what would be predicted from numerous studies showing that the antinociceptive effect of systemic morphine on this response is reduced within 24 hr after spinalization. The present studies do not provide an explanation for this difference in potency between systemic and intrathecal morphine. One possibility is that spinal surgery disrupts the pharmacokinetics of systemically administered morphine. As a result, the amount of drug that reaches the spinal cord after subcutaneous injection may be reduced in spinal rats relative to intact rats. The amount of morphine that reaches the spinal cord by the intrathecal route might not be altered by spinal transection.

TABLE 2

EFFECT OF PRETREATMENT CONDITION ON BASELINE TF LATENCY (± SEM) OF INTACT AND SPINAL RATS

Pretreatment	Tail-Flick Condition	Latency
Intact	Morphine + Test	4.12 ± 0.10 (14)
Spinal	Morphine + Test	3.27 ± 0.14 (21)†
	Morphine Only	3.49 ± 0.23 (15)*
	Test Only	2.42 ± 0.18 (12)†‡

Intact vs. spinal, *$p<0.05$, †$p<0.01$.
Spinal: Test Only vs. Morphine + Test and Morphine Only, ‡$p<0.01$.

One problem with this interpretation is that the analgesic effect of systemic morphine on the TF reflex is also reduced by either subtotal spinal lesions (4, 6, 21) or lesions of various sites in the brain (12, 16, 20, 29–31, 33, 34, 47). It is less likely that such lesions interfere with opiate pharmacokinetics. Furthermore, it has been shown that spinal transection does not alter blood flow to the spinal cord of rats (40). On the other hand, we are not aware of any studies which have examined the effect of either subtotal spinal lesions or specific brain lesions on the analgesic response to intrathecal morphine. It remains to be seen whether such lesions will also potentiate spinal opiate antinociception.

It is also possible that the reduced effect of systemic morphine in spinal animals may be due to an action of the opiate at peripheral sites. Even after spinalization, systemic morphine might exert an effect at sites outside of the central nervous system which are not reached by the intrathecal route and that such actions

may be responsible for the attenuated effect of systemic morphine on the TF.

Finally, it is possible that systemically administered morphine may influence spinal reflexes indirectly, by acting at supraspinal sites to affect the release of an endogenous opiate antagonist. It has been shown, for example, that systemic administration of adrenocorticotropic hormone (ACTH) reduces the effect of systemic morphine on the TF in spinal rats (45) and that intrathecal administration of ACTH reduces the effect of intrathecal morphine on the TF in intact rats (7).

Our results, showing an enhanced potency of intrathecal morphine in spinal rats, are consistent with several recent reports in the literature. First, Sinclair (35) observed the same dichotomy between systemic and intrathecal morphine on the TF reflex in rats in response to a cold-block of the spinal cord. In that report, Sinclair showed that systemic morphine (1–4 mg/kg, IV) was less potent in the presence of the cold-block than in the absence of the cold-block. In contrast, intrathecal morphine was more potent in the presence, relative to the absence of the cold-block. His data are entirely consistent with the present results and mitigate the possibility that our finding may be related to the trauma of spinal surgery.

Second, recent reports of Herman and colleagues (22) demonstrate that the effect of intrathecal morphine on spinal reflexes in humans is also enhanced after spinal transection. These investigators have shown that muscle spasticity and hyperactive micturition reflexes, secondary to complete spinal cord lesions, are markedly suppressed by intrathecal morphine after an acute dose of 50 μg, or daily infusions of 0.5–0.9 mg. These doses are lower than acute doses of intrathecal morphine (0.25–20 mg) required for the relief of pain in intact humans (14). These data indicate that the increased potency of spinal morphine in spinal animals is not restricted to laboratory models, but also has clinical implications and relevance to the mechanisms of opiate action in humans.

Third, there is increasing evidence that the enhanced potency of spinally administered drugs in spinal animals is not limited to opiates. Several laboratories have reported that the response of spinal rats is enhanced, relative to intact rats, after intrathecal administration of several nonopiate agents. Both Stein *et al.* (38) and Jensen and Smith (24) showed that the increase in TF latency produced by intrathecal pentobarbital injection is significantly greater after spinal transection. Jensen has also shown a similar increase in the potency of intrathecal dopamine and apomorphine after spinalization. In that study, the increase in TF latency produced by the dopaminergic agonists was blocked by intrathecal administration of dopaminergic antagonists, but not by adrenergic or serotonergic antagonists.

Finally, potentiation of spinal drug action in spinal rats is not limited to the TF reflex. Similar results were recently reported by Mayer and colleagues with respect to the scratching response elicited by intrathecal administration of several neuroexcitatory compounds (11). The scratch response produced by intrathecal strychnine, picrotoxin and L-glutamic acid was potentiated by spinalization, while the response to substance P and kainic acid was not.

In summary, there is increasing evidence that the spinal action of a variety of pharmacological agents is enhanced within one to two days after spinal transection. These observations suggest that in the intact animal, the spinal action of morphine, as well as other nonopiate substances, is tonically suppressed by descending supraspinal input. When this input is eliminated by spinal transection the antinociceptive action of several compounds at the spinal cord is potentiated (1).

Effect of Chronic Spinal Transection on the Antinociceptive Response to Intrathecal Morphine

Although the potencies of intrathecal and systemic morphine are differentially affected one day after spinal transection, both routes of administration are less effective 3–4 weeks later. Regardless of whether morphine is administered spinally or subcutaneously, there is a substantial decline in the antinociceptive effect on the TF within three weeks. These data suggest that supersensitivity does not develop to the effect of morphine on the TF reflex in spinal rats.

This conclusion appears to conflict with other evidence which suggests that the effect of systemic morphine on spinal nociceptive reflexes is not reduced in chronic spinal animals. Martin demonstrated that spinal reflexes in chronic spinal dogs were suppressed by the same doses (0.25–8.0 mg/kg, IV) that were effective in intact dogs (25–27). Willer reported that the effect of systemic morphine (0.2 and 0.3 mg/kg, IV) was greater in spinal than in intact humans (42,43) and the results of Herman *et al.* indicate a similar increase in sensitivity of spinal reflexes to intrathecal morphine in chronic spinal humans. Although the reason for this discrepancy is not yet clear, it is possible that the effect of morphine on spinal reflexes may depend on the type of measurement used. In general, studies that show a decrease in opiate potency after chronic spinal transection have used response latency of withdrawal reflexes as the nociceptive endpoint. When reflex amplitude or intensity is used as the nociceptive index, the potency of morphine may, in fact, be increased after chronic spinal transection.

Effect of Spinal Transection on Spinal Opiate Antinociception in Tolerant Rats

The results of these studies indicate first, that intact rats made tolerant to the analgesic effect of systemic morphine on the TF test are not tolerant to a subsequent intrathecal injection. Similar results have been reported by several investigators using a variety of methods to induce tolerance in both laboratory animals (32,41) and humans (28). The fact that tolerance did not develop to intrathecal morphine in rats that were tested while under the influence of systemic morphine, implies that tolerance would also not be observed after chronic exposure to the TF test procedure in the absence of the drug. Although not examined in the present studies, we have previously shown this to be the case. Repeated performance of the TF reflex does not alter the analgesic effect of intrathecal morphine in intact rats (37).

One explanation for this lack of tolerance concerns the relative concentration of morphine in the spinal cord produced by these two routes of administration. Studies that have determined morphine concentration in brain and spinal cord after intravenous or subcutaneous (unpublished) injection of analgesic doses (3–20 mg/kg) report values in the range of 100–300 ng/g for both sites (13). Recent analyses of spinal morphine concentration after intrathecal administration of analgesic doses of 3 and 5 μg obtained values of 1.1 μg/g and 3.0 μg/g, respectively (19). Therefore, even if the spinal cord became tolerant as a result of daily systemic morphine injections, it is conceivable that tolerance would be overcome by the high local concentration produced by the intrathecal challenge (28).

This interpretation, however, does not account for the results obtained in tolerant rats that were spinally transected. In contrast to intact rats, the response of spinal rats to intrathecal morphine was significantly reduced by pretreatment with either morphine

alone, TF assessment alone, or both of these manipulations, prior to the transection. All of these pretreatments produced a similar decrease in the potency of intrathecal morphine, relative to spinal rats that were not pretreated. This finding is consistent with previous results from our laboratory showing that the same pretreatment conditions also reduced the antinociceptive effect of systemic morphine on the TF reflex, one day after spinal transection (3).

This result indicates first, that the observed shifts in the respective dose-response functions were not due to the spinal transection alone. Second, these data are not consistent with an interpretation based on the relative difference in the spinal concentration of morphine produced by systemic and intrathecal morphine. If the high, local concentration of intrathecal morphine was responsible for overriding or masking tolerance to systemic morphine in intact rats, then spinal rats would also not be tolerant to an intrathecal challenge.

It is possible that the spinal transection disrupted the pharmacokinetics of morphine in opiate-pretreated rats. We are not aware of any data regarding the effect of spinal transection on the disposition of morphine in either tolerant or nontolerant animals. But, even if spinal surgery disturbed opiate metabolism, this would not account for the fact that the dose-response function of morphine-pretreated rats was comparable to that of rats pretreated with morphine plus TF tests. Nor would any change in opiate metabolism account for the reduced effect of spinal morphine in spinal rats that were only preexposed to the TF test procedure.

It is surprising that all three pretreatments produced a similar degree of tolerance in spinal rats. It might be expected that tolerance resulting from either morphine, or TF tests alone, would be potentiated when these manipulations were combined. Although difficult to explain, the present results presumably reflect the outcome of interactions between opiate and behavioral pretreatment at either spinal and/or supraspinal sites that occurred prior to spinal transection. For example, the data showed that baseline TF latencies of spinal rats are reduced when the TF response is repeatedly elicited prior to spinal transection (sal + test condition). Yet,all three treatments produced tolerance to spinal morphine. These observations suggest that systemic morphine administration in intact rats may 1) oppose or antagonize the tolerance-inducing effects of behavioral pretreatment on spinal nociceptive reflexes and 2) produce pharmacological tolerance at the spinal level. These actions could occur at either spinal or supraspinal sites in the intact animal. It may be possible to dissociate these hypothesized effects by administering the respective behavioral and opiate pretreatments to rats 3–4 weeks after spinal transection, i.e., in the absence of neural input from the brain.

We have proposed that the analgesic effect of intrathecal morphine in intact rats is tonically inhibited by descending supraspinal input. This hypothesis can account for the enhanced potency of intrathecal morphine in nontolerant rats after spinal transection. Our hypothesis is also consistent with the fact (discussed above) that to produce the same analgesic effect in intact rats, the concentration of morphine in the spinal cord must be much greater after intrathecal than after systemic administration. We suggest that the increased potency of systemic vs. intrathecal morphine at the spinal cord of intact rats is due to the fact that systemically administered morphine acts supraspinally to decrease or remove tonic descending inhibitory control of spinal opiate analgesia. For a more detailed discussion of this hypothesis, see (1).

Furthermore, this hypothesis provides a possible interpretation for the present results obtained in tolerant rats. When tolerance develops to morphine, the antinociceptive effect of the opiate at the spinal cord (and presumably the brain as well) is decreased. Therefore, the dose of morphine required to maintain a constant analgesic effect at both spinal and supraspinal sites, and to decrease descending inhibition, is greater. However, the intrinsic activity within descending inhibitory systems may also decrease during the development of tolerance. This produces less inhibition of spinal opiate action in the absence of supraspinal morphine. When morphine is applied intrathecally to intact tolerant animals, spinally mediated tolerance would then be counteracted by the decrease in supraspinal inhibition. As a result, behavioral tolerance would not be expressed. When the influence of descending inhibitory input is eliminated by spinal transection, spinal opiate tolerance is revealed. Although this proposal is purely speculative, it may be accessible to verification. Neurophysiological comparison of descending inhibitory input onto dorsal horn neurons (that mediate nociception) in tolerant and nontolerant rats, may represent one way of evaluating the present hypothesis.

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