Pharmacology Biochemistry and Behavior, Vol. 45, pp. 871–879, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Intrathecal Coadministration of Serotonin and Morphine Differentially Modulates the Tail-Flick Reflex of Intact and Spinal Rats

CLAIRE ADVOKAT

Department of Psychology, Louisiana State University, Baton Rouge, LA 70803

Received 2 September 1992

ADVOKAT, C. *Intrathecal coadministration of serotonin and morphine differentially modulates the tail-flick reflex of intact and spinal rats.* PHARMACOL BIOCHEM BEHAV **45**(4) 871–879, 1993.—In a previous study, we found that the antinociceptive effect of IT-administered morphine on the tail-flick (TF) reflex of rats was potentiated within 1 day after spinal transection. This suggested that the analgesic effect of spinal morphine in the intact animal was tonically suppressed, presumably by the release of a transmitter(s) from descending supraspinal pathway(s), and that the potency of IT morphine was increased because these inputs were removed by spinalization. Because spinally projecting serotonin [5-hydroxytryptamine (5-HT)] fibers are known to be involved in modulating nociception at this site, the present studies examined the possibility that 5-HT might be the proposed "antiopiate" at the spinal cord. Separate groups of intact and spinal rats were pretested on the TF and then injected IT with either morphine (intact: 0.25–5.0 μg, spinal: 0.0312–0.5 μg) or 5-HT (1–200 μg), or combinations of these two agents, in a single solution. All rats were then retested 15 min later and the difference in latency was used to compare the effect of these treatments. The results confirmed that the antinociceptive effect of IT morphine was significantly increased by spinalization, whereas the antinociceptive effect of 5-HT was essentially abolished. In intact rats, morphine-induced analgesia was potentiated by a low (10 μg) dose of 5-HT but not by higher doses. However, in the spinal rat morphine-induced antinociception was antagonized by the same (10 μg) dose. The data suggest that IT 5-HT promotes antinociception in intact rats but acts pro-nociceptively in spinal rats. It is speculated that this difference may be due to an intervening mechanism(s) in the interaction between spinal morphine and serotonin that requires supraspinal mediation.

Spinal opiate antinociception Intrathecal serotonin Intrathecal morphine Tail-flick

WE have previously shown that the antinociceptive effect of spinally administered morphine on the tail-flick (TF) test is greatly potentiated within 24 h after spinal transection (53). This result suggested that in the intact animal the analgesic effect of spinal morphine was inhibited or antagonized by descending, supraspinal input and that spinalization increased opiate potency by removing this input (1,53).

This interpretation implies that the postulated descending inhibition of spinal opiate analgesia is due to the tonic release of a transmitter(s) from supraspinal pathway(s). If correct, it might be expected that exogenous administration of this same transmitter would a) further reduce the analgesic effect of spinal morphine in intact rats and b) reduce the antinociceptive effect of spinal morphine in spinal rats such that the morphine dose–response relationship in spinal rats would be similar to that obtained in intact rats. Because there is extensive evidence that spinally projecting serotonergic fibers play an important role in the modulation of nociception at the spinal level, the present studies were designed to assess the possibility that serotonin [5-hydroxytryptamine (5-HT)] might be the hypothesized "antiopiate."

At first glance, this proposal seems unlikely because most of the data supporting the role of 5-HT in spinal nociceptive processing indicates that 5-HT itself promotes analgesia at the level of the spinal cord, with respect to the thermally elicited TF reflex of intact and spinal rats. First, it has been reported that IT administration of 5-HT antagonists such as methysergide (48) and metergoline (21) reduced baseline withdrawal latencies (produced hyperalgesia) in intact rats. Second, neurochemical lesions of descending serotonergic pathways by IT administration of 5,6-dihydroxytryptamine (5,6-DHT) also reduced latencies, although this effect waned after the first week, presumably because of the development of supersensitivity (7,22,24). Third, numerous studies have shown that IT administration of 5-HT itself produces an antinociceptive increase in TF latency in mice and rats (3,11,12,17,18,27, 31,33,52,54,61,63) that is antagonized by IT administration of 5-HT antagonists (33,52,54,63). Further, the antinociceptive action of spinal 5-HT was potentiated in rats pretreated with 5,6-DHT, again suggesting the development of postsynaptic supersensitivity (30,49). Finally, 5-HT blocked the presumptive nociceptive response of biting, elicited by IT substance P, in mice (15,31).

There is some evidence that 5-HT might promote nocicep-

tion at the spinal cord. For example, in two studies IT injection of the 5-HT_{1A} agonist 8-hydroxy-*N,N*-dipropyl-2-aminotetralin (8-OH-DPAT) (12,54) reduced TF latencies in rats. However, this could be due to a specific facilitating action of 8-OH-DPAT on motorneurons rather than sensory input (26,32,35,36). Yet, in mice IT 5-HT itself produces a biting and/or scratching response (15,17,25,31,60) that was also elicited by IT injection of a specific 5-HT_2 agonist (17).

In spinal rats, systemic administration of 5-HT agonists also elevated the TF latency, although these effects were modest and transient [waning after approximately 1 week (6,65)]. In view of the previously noted development of supersensitivity to IT 5-HT produced by neurotoxic lesions, this loss of antinociceptive activity after complete transection is surprising. However, serotonergic agents also produced a supersensitive facilitation of nonnociceptive hindlimb reflexes in chronic spinal rats (5) and it may be the case that supersensitive reactions in motor function are incompatible with the expression of antinociceptive responses.

Zemlan and associates extensively examined the effects of systemic serotonergic agents on several nociceptive reflexes in spinal rats (45,64,66) within 2 days to 2 weeks after transection. In their latest study (43), both 5-HT_{1A} and 5-HT_{1B} compounds increased the sensitivity of spinal rats to mechanical stimulation (indicating hyperalgesia) yet still increased TF latency, indicating perhaps a specific decrease in response to noxious thermal stimulation. Because only 5-HT_1 type agents were examined, it is not clear whether or not this antinociceptive action would be elicited by other receptor selective agents in spinal rats.

In summary, there is a great deal of evidence favoring an antinociceptive action of 5-HT itself at the spinal cord with respect to the tail-flick response and this appears to be the case after spinal administration in intact rats and systemic administration in spinal rats.

It might be expected, therefore, that manipulations of spinal serotonergic function would modify (i.e., increase) the analgesic effect of spinal morphine. However, the relevant data are inconsistent. Neurotoxic lesions of descending 5-HT pathways did not change the analgesic effect of spinal morphine on the paw pressure test (59) or on the TF (51). It has been anecdotally mentioned that IT 5-HT did not change morphine-induced analgesia on the TF in mice (31); however, Arts et al. (4) saw an additive effect of spinal 5-HT and morphine in this species. In addition, it has been shown that spinal morphine-induced analgesia is antagonized in rats by spinal 5-HT antagonists of all major receptor types (13) although in mice at least one dose of pindolol (a 5-HT_1 antagonist) did not affect this response (4).

Although these data suggest that 5-HT might be involved in morphine-induced analgesia at the spinal cord, it is possible that this relationship is indirect. For example, the apparent antagonism of morphine produced by 5-HT antagonists in intact rats (13) may be due to a direct reflex facilitation produced by blockade of 5-HT rather than an interference with the local action of morphine. Even though that study reported that the antagonists alone did not reduce TF latencies, the predrug baselines were so low that further decreases may not have been detectable.

A second possible confound concerns the effects of the experimental manipulations on tail-skin temperature. It has been reported that the TF response latency is inversely related to tail-skin temperature (20,58). Because skin temperature was increased by neurotoxic lesions of descending serotonergic pathways, as well as systemic and IT administration of 5-HT antagonists, the apparent hyperalgesic effects of these treatments has been questioned (19,20,58). In contrast, antinociception produced by 5-HT agonists, including 5-HT, was not related to changes in tail-skin temperature (18).

Therefore, although the evidence supports an antinociceptive action of 5-HT on the TF of rats the possible role of 5-HT on the spinal action of morphine is not yet clear. It is conceivable that 5-HT may independently modulate nociceptive input without altering the spinal effect of morphine or may also influence morphine-induced antinociception, either in a positive or negative manner. We decided to investigate this question because our previous research indicated that the antinociceptive effect of morphine on the spinal cord is less potent in intact than in spinal rats. The aim of these studies was to see if 5-HT might be responsible for the reduced potency of spinal morphine in the normal, intact rat. Therefore, the following experiments assessed the effect of 5-HT and morphine, separately and in combination, on the TF of intact and acute spinal rats.

METHOD

Subjects

The data from 288 male Sprague-Dawley-derived rats (Holtzman Laboratories, Madison, WI), weighing 350–500 g, were used in these studies (179 intact and 109 spinal). They were housed individually in suspended, stainless steel cages in a colony room maintained on a 12 L : 12 D cycle, with dark onset at 1700 h. Food and water were available ad lib.

Surgical Procedures

All rats were implanted under ether anesthesia with intrathecal catheters (PE-10) that terminated at the lumbar enlargement (8 cm). Any rat exhibiting signs of impairment, that is, evidence of crippling in any limb following surgery, was eliminated from the study. In addition to the catheter implantation, several groups of rats also sustained a spinal transection. A laminectomy was made between thoracic vertebrae 6 and 9 and the incision was packed with gel foam to reduce bleeding, after which the wound was sutured and the cages placed on heating pads to maintain body temperature. On the morning after surgery, the hindquarters of each rat were washed with warm water and their urine was expressed manually by the application of pressure to their bladders (53).

Nociceptive Assessment

All rats were assessed with the TF test. Noxious stimulation was produced by a beam of high-intensity light focused on the tail. The response time was measured automatically and was defined as the interval between the onset of the thermal stimulus and the abrupt flick of the tail. Each determination consisted of three trials; the mean score was taken as the response latency. Animals not responding within 8 s were removed from the apparatus and assigned a response latency of 8 s.

Drug Administration

Morphine sulfate (Penick Corp., Lyndhurst, NJ) and 5-HT (Research Biochemicals, Inc., Natick, MA) were dissolved in 0.9% saline such that the injection volume of 10 μl contained the appropriate drug dose or dose combination. Each drug injection was followed by a 10-μl wash of saline. Injections

were performed manually using a 50-μl Hamilton syringe (Hamilton Co., Reno, NV) over 2–3 min.

Experimental Procedures

Separate groups of intact and spinal rats were pretested on the TF and then injected intrathecally with either morphine, 5-HT, or combinations of these two agents, coadministered in a single solution. Previously published reports indicated that the peak antinociceptive effect of intrathecal 5-HT occurred approximately 15 min after injection. In preliminary studies, we confirmed this result in intact rats. Therefore, all postdrug assessments were performed 15 min after the respective drug injections. When the results indicated that the antinociceptive effect of 5-HT was lost in spinal rats (see below), we tested 5-HT at 30 min in an additional group of spinal rats. We saw no change in the effect of 5-HT when spinal rats were tested 30 min rather than 15 min after injection. The doses of morphine were 0.25, 0.5, 1.0, 2.5, and 5.0 μg for intact rats and 0.0312, 0.05, 0.125, 0.25, and 0.5 μg for spinal rats. The doses of 5-HT were 1.0, 5.0, 10, 25, 50, 100, and 200 μg for intact rats and 10, 25, 50, 100, and 200 μg for spinal rats. Additional studies in separate groups of intact and spinal rats confirmed our previous experience that IT saline injections did not affect the response in either preparation. Intact rats were tested between 5–7 days after catheterization; spinal rats were tested 24 h after surgery. For each rat, the latency obtained before drug administration was subtracted from the latency obtained after drug administration to obtain a difference score for each subject.

Statistical Analyses

Statistical analyses were performed on the predrug baseline latencies and on the difference scores of the individual groups. Differences among groups were determined with either one-way or two-way analyses of variance (ANOVAs) and appropriate posthoc comparisons (Newman-Keuls or Dunnett's tests) or *t*-tests with the aid of a computer program (CRUNCH Interactive Statistical Program). Results were considered significant at $p < 0.05$ or less.

RESULTS

Intact Rats

Among all 28 separate groups of intact rats, mean baseline latencies ranged from 4.2–5.5 s. Because the maximum possible score was 8.0 s, the maximum possible (positive) difference score was 3.8–2.5 s.

The antinociceptive effect of IT morphine and 5-HT is summarized in Fig. 1, which shows the change in latency 15 min after administration of the indicated doses. With respect to morphine, ANOVA showed a significant overall effect [$n = 41$, $F(4, 40) = 7.37$, $p = 0.0002$], although posthoc Newman-Keuls tests indicated that only the response to the lowest dose (0.25 μg) was statistically different from the response to the four higher doses. Within-subject (paired) *t*-tests confirmed that each of these latter doses (0.5, 1.0, 2.5, and 5.0 μg) produced a significant increase in latency.

With respect to 5-HT, ANOVA similarly showed an overall significant effect among the seven doses [$n = 41$, $F(6, 40) = 5.28$, $p = 0.0006$], although posthoc Newman-Keuls tests showed that the response to each of the four lowest doses (1.0, 5.0, 10, and 25 μg) was significantly less than the response to the three higher doses (50, 100, and 200 μg). Within-subject

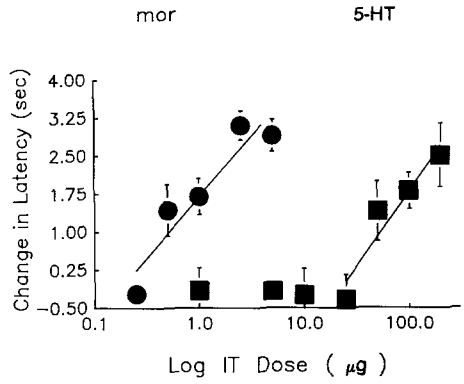

FIG. 1. Dose-response functions (±SEM) of intact rats to intrathecal administration of morphine (0.25, 0.5, 1.0, 2.5, or 5.0 μg) or 5-hydroxytryptamine (5-HT, 1.0, 5.0, 10, 25, 50, 100, or 200 μg). Each rat was tested on the tail-flick before and 15 min after the respective drug injection and the mean difference (±SEM) in latency between the two tests is shown for each group.

(paired) *t*-tests indicated that the 50-μg dose of 5-HT did not reliably increase response latencies ($n = 7$, $t = 2.3$, $p = 0.06$) although the two highest doses (100 and 200 μg) did significantly increase the TF latency over baseline values.

Results of the IT coadministration of 5.0 μg morphine and the various doses of 5-HT are summarized in Fig. 2. The open circles, which represent the effect of 5-HT alone, and the broken line, which represents the effect of morphine alone, are replotted from Fig. 1. The solid circles show the change in latency produced by combined administration of 5.0 μg morphine and each dose of 5-HT. ANOVA indicated that the effect of morphine was not significantly altered by the addition of any dose of 5-HT [$n = 32$, $F(4, 31) = 0.918$, $p = 0.473$]. It is clear from these data that the analgesic effect of 5.0 μg IT morphine in intact rats was not reduced by the addition of IT serotonin. If anything, the results suggested that spinal opiate antinociception might be potentiated by 5-HT. However, because the 5.0-μg dose of morphine produced a maximal response even in the absence of 5-HT it was not

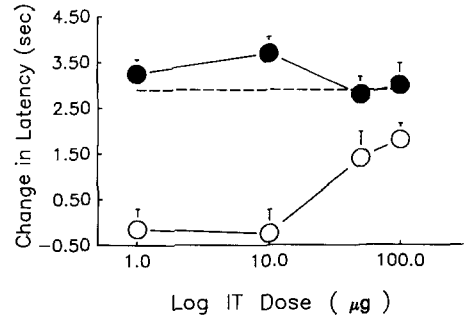

FIG. 2. Effect of intrathecal coadministration of morphine (5.0 μg) and 5-hydroxytryptamine (5-HT) on the tail-flick latency of intact rats. The broken line indicates the mean change in response latency 15 min after IT injection of morphine (replotted from Fig. 1). (○), mean change in latency after 5-HT (replotted from Fig. 1); (●), mean change in latency after coadministration of morphine and each dose of 5-HT. For graphic presentation, the SEM is indicated for one direction only.

possible to detect facilitation between these two drugs. Therefore, in the next study a lower dose, 0.5 μg, of morphine was coadministered with 5-HT.

The results of that study are summarized in Fig. 3. As in Fig. 2, the open circles and broken line, respectively, indicate the change in latency produced by 5-HT and morphine alone, replotted from Fig. 1. The solid circles represent the effect of 0.5 μg morphine in combination with each dose of 5-HT. In this case, an ANOVA indicated a significant overall effect [n = 68, $F(7, 67) = 2.52$, $p = 0.024$]. However, posthoc comparisons (Newman-Keuls and Dunnett's tests) showed that none of the groups injected with the combination of 0.5 μg morphine and 5-HT differed from the group injected with morphine alone. Further analysis of the data revealed that the overall statistical significance was due to the difference between the two groups injected with 1 and 5 μg 5-HT and the two groups injected with 10 and 25 μg 5-HT [n = 34, $F(3, 33) = 5.725$, $p = 0.0032$].

These data confirm the implication from the previous study that the addition of 5-HT does not reduce the analgesic effect of spinal morphine and may, in fact, potentiate the effect of morphine on the TF at intermediate doses of 10 and 25 μg. This conclusion was examined further with the addition of several groups of rats that were injected with either 1.0 or 2.5 μg morphine in combination with different doses of 5-HT. These groups provided sufficient data for the construction of several dose-response functions, such that the effect of different doses of 5-HT could be assessed in combination with several doses of morphine. A summary of these data is shown in Fig. 4, which presents the dose-response function (solid line) to morphine alone (open circles; replotted from Fig. 1) as well as the results from the groups injected with the various drug combinations.

Analysis of these data indicated a significant overall difference among the four dose-response functions [n = 87, $F(3, 86) = 4.61$, $p = 0.005$]. Further comparison showed that only those groups coinjected with morphine and 10 μg 5-HT were significantly different (had greater increases in latency) than the groups injected with morphine alone [n = 47, $F(1, 46) = 8.85$, $p = 0.005$]. The combined administration of either 1 or 50 μg 5-HT and morphine did not significantly alter TF latencies.

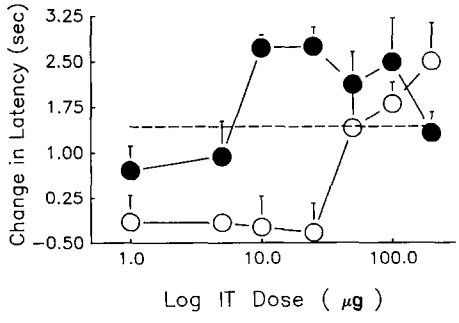

FIG. 3. Effect of intrathecal coadministration of morphine (0.5 μg) and 5-hydroxytryptamine (5-HT) on the tail-flick (TF) latency of intact rats. The broken line indicates the mean change in response latency 15 min after IT injection of morphine (replotted from Fig. 1). (○), mean change in latency after 5-HT (replotted from Fig. 1); (●), mean change in latency after coadministration of morphine and each dose of 5-HT. For graphic presentation, the SEM is indicated for one direction only.

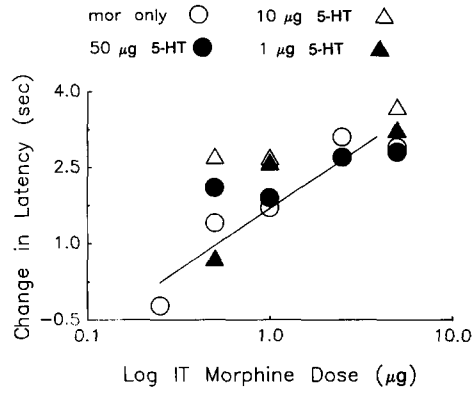

FIG. 4. Effect of the intrathecal coadministration of morphine and 5-hydroxytryptamine (5-HT) in intact rats. (○), dose-response effect of morphine alone (replotted, with the corresponding regression line, from Fig. 1); (●), dose-response function of morphine in combination with 50 μg 5-HT; (△), morphine in combination with 10 μg 5-HT; (▲), morphine in combination with 1.0 μg 5-HT.

Spinal Rats

As expected from numerous earlier reports [see (53)], baseline latencies were decreased after spinal transection. This is due to the removal of descending inhibitory input normally exerted on spinal nociceptive circuits. Among the 18 separate groups of spinal rats, mean baseline TF latencies varied from 3.5–4.7 s, which provided a maximum range of 4.5–3.3 s between baseline and the cut-off score. A t-test comparing the two sets of group means (28 intact vs. 18 spinal) confirmed a significant decrease in spinal vs. intact rats [n = 46, $F(1, 44) = 9.26$, $p < 0.0001$]. It should be noted that statistical comparisons among the groups were always made within the respective intact and spinal conditions.

The antinociceptive effect of spinal morphine in intact and spinal rats is shown in Fig. 5. As reported previously, despite the decrease in baseline latencies IT morphine was more potent in the spinal than in the intact rat. In this study, the difference in potency was approximately 10-fold. ANOVA indicated an overall effect of dose in spinal rats [n = 40, $F(4, 39) = 10.74$, $p < 0.0001$]. Posthoc, Newman-Keuls tests indicated that the four highest doses [0.05, 0.125, 0.25, and 0.5

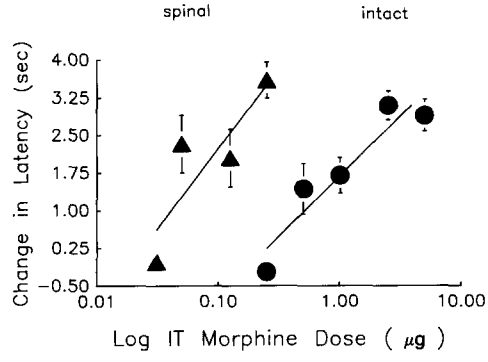

FIG. 5. Dose-response function (± SEM) to intrathecal morphine in intact (replotted from Fig. 1) and spinal (0.0312, 0.05, 0.125, and 0.25 μg) rats on the tail-flick (TF) reflex.

μg (data not shown)] produced response increases that were significantly greater than that of the lowest dose (0.0312 μg). Each of the four highest doses significantly increased the TF latency above baseline.

Because the potency of IT morphine was significantly increased after spinal transection, it was necessary to reduce the dose of morphine that was coadministered with 5-HT in spinal rats. Therefore, a dose of 0.05 μg was chosen for combined administration with 5-HT. The results of that study are shown in Fig. 6, which summarizes the effects of 5-HT alone (open triangles), 0.05 μg morphine alone (broken line), and their combined administration (solid triangles) in spinal rats.

Intrathecal administration of various doses of 5-HT to spinal rats produced an inverted U-shaped function, with an overall significant effect of dose [$n = 35$, $F(4, 34) = 3.25$, $p = 0.025$]. However, the results of the Newman–Keuls tests showed no significant differences among the groups. Inspection of the data suggested that reflex latencies were increased by the intermediate doses of 25, 50, and 100 μg but not by the lowest (10 μg) or highest (200 μg) dose of 5-HT. However, individual within-subject t-tests indicated that although response latencies were (statistically) increased by 25 μg 5-HT ($n = 9$, $t = 2.79$, $p = 0.0237$) the other doses produced no significant change.

Although the shape of the dose-response function to 5-HT in spinal rats was unusual, a similar relationship was obtained when morphine was coadministered with the same doses, that is, addition of 0.05 μg morphine also produced an inverted U-shaped function. An ANOVA was performed to determine whether coadministration of 5-HT altered the effect of morphine. The results indicated that there was a significant overall effect among the morphine groups [$n = 43$, $F(5, 42) = 2.71$, $p = 0.034$]. None of the groups coinjected with morphine and 5-HT were different from those injected with morphine alone. However, in this case the response of those rats injected with 0.05 μg morphine and 10 μg 5-HT was significantly less than the response of rats injected with 50 or 100 μg 5-HT.

These data suggested that although intrathecal administration of 10 μg 5-HT alone did not alter the TF latency of spinal rats this dose antagonized the latency increase produced by 0.05 μg morphine. This was examined further by assessing the effect of 10 μg 5-HT in combination with 0.125 and 0.25 μg morphine. The results of that study are summarized in Fig. 7,

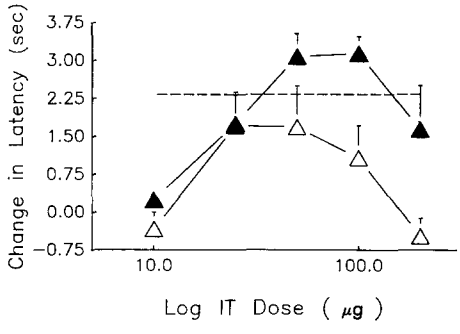

FIG. 6. Effect of intrathecal coadministration of morphine (0.05 μg) and 5-hydroxytryptamine (5-HT) on the tail-flick latency of spinal rats. The broken line indicates the mean change in latency after morphine alone (replotted from Fig. 5). (△), mean change in latency after 5-HT; (▲), response to coadministration of morphine and each dose of 5-HT. For graphic presentation, the SEM is indicated for one direction only.

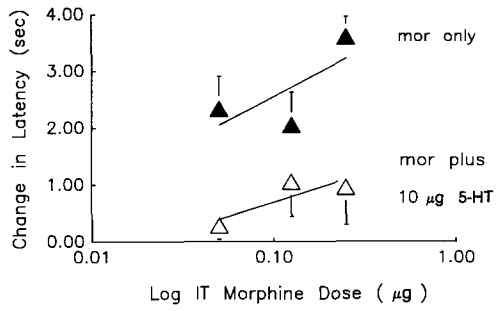

FIG. 7. Effect of intrathecal coadministration of morphine and 5-hydroxytryptamine (5-HT) on the tail-flick latency of spinal rats. (▲), mean change in latency after morphine; (△), response to the same doses of morphine combined with 10 μg 5-HT. For graphic presentation, the SEM is indicated for one direction only.

which shows the response function of morphine alone (solid triangles, replotted from Fig. 5) and morphine plus 10 μg 5-HT (open triangles) in spinal rats. ANOVA indicated a significant overall effect of 5-HT on opiate antinociception [$n = 42$, $F(1, 41) = 20.73$, $p \leq 0.0001$].

In summary, the results of the present study indicate that coadministration of 10 μg 5-HT produces a modest but statistically significant potentiation of IT morphine-induced antinociception in intact rats, whereas the same dose significantly antagonizes this response in spinal rats. In the absence of morphine, 10 μg 5-HT did not significantly alter the latency of this spinal reflex in either preparation.

DISCUSSION

Antinociception to IT 5-HT and Morphine in Intact and Spinal Rats

The effect of intrathecally administered morphine and serotonin on the TF of intact rats was generally in agreement with the literature. With regard to morphine, TF latency was significantly increased by the four highest doses (5.0, 2.5, 1.0, and 0.5 μg) but not by the lowest dose (0.25 μg). There was no difference among the doses in analgesic effect. In this study, morphine appeared to be more potent than previously indicated (53). However, this difference was most likely due to the facts that in the present study the maximum score was 8 s rather than 14 s and animals were tested 15 min after injection rather than 40 min. Although these parameters were adequate for the production of analgesia, they did not allow a distinction to be made among the doses. Nevertheless, because the effect of IT 5-HT was known to be more rapid, transient, and less efficacious than morphine, the effect of morphine was examined under conditions that would allow a comparison between the two drugs.

In fact, the results obtained with 5-HT are consistent with the literature in that doses of 50, 100, and 200 μg significantly increased latencies relative to lower doses. Although there was no difference among these doses, it was found, as reported by Yaksh and colleagues (52), that 50 μg did not significantly increase latencies above baseline.

While these studies point to an antinociceptive effect of spinal 5-HT, there is conflicting data with regard to the relevant receptor subtypes. For example, in mice the evidence supports the conclusion that 5-HT_1 receptors mediate antinociception [(16–18), although not always (23)]. By contrast, in

rats it has been reported that an increase in TF latency was mediated by an effect on 5-HT_2 (54) and 5-HT_3 receptors (27). However, in a recent article neither 5-HT_{1A}, 5-HT_{1B}, 5-HT_2, nor 5-HT_3 agonists increased TF latency in intact rats, although the increase in latency produced by 5-HT itself was reversed by antagonists specific for each of these receptor subtypes (12).

It is possible that the difficulty in making these conclusions is partly due to the role of 5-HT in nonnociceptive function, particularly in motor control. For example, systemic administration of 8-OH-DPAT (35), but not other 5-HT_{1A} agonists (36), produces spontaneous TFs in rats. Serotonergic neurons are known to project to the ventral horn of the spinal cord and synapse on α-motoneurons, and IV 8-OH-DPAT increases spinal motoneuron excitability in spinal rats (26,32), which may be pertinent to reports that IT administration may be pronociceptive (12,54). It has also been reported that in rats IT administration of 5-HT_2 agonists (and high doses of 5-HT itself) can produce back muscle contractions (26), which may interfere with performance of the TF. Because about half the 5-HT receptors in the rat spinal cord are of the 5-HT_1 type (34,64), it may be necessary to clarify the role of the 5-HT receptor subtypes in sensory vs. motor control to fully understand its function in nociceptive circuits.

The present studies are also consistent with earlier work in showing that IT morphine was more potent in spinal than in intact rats (53). Despite the lower maximum score and shorter postinjection interval, the pronounced effect of spinal transection on spinal opiate antinociception was replicated. Doses of IT morphine that were ineffective in intact rats, for example, 0.25 μg and less, produced a significant antinociceptive response in spinal rats within 1 day after transection. As a result of this increase in potency, it was necessary to reduce the dose of morphine used in combination with 5-HT in spinal rats (from 0.5 to 0.05 μg).

To the best of our knowledge, this is the first assessment of the effect of IT 5-HT in spinal rats. Previous studies of serotonergic function in spinal rats have been performed with systemic administration of 5-HT agonists and antagonists. In those studies that used the TF assay, reflex latencies increased with increasing doses of 5-HT_1 agents, including 8-OH-DPAT and the nonspecific compound 5-methoxydimethyltryptamine [5-MeODMT (43,65)]. In contrast to those data, this study found an inverted U-shaped function after IT injection of 5-HT. Although the overall analysis suggested that there was an antinociceptive response to 5-HT at intermediate doses, posthoc comparisons did not support this conclusion. Within-subject *t*-tests indicated that only the 25-μg dose statistically elevated TF latency. It is not clear why systemic administration of serotonergic agonists produced dose-dependent increases in TF latency in spinal rats whereas IT 5-HT did not. It is also not clear why the agonist 8-OH-DPAT produced a decrease in latency when given IT to intact rats (12,54) yet increased latencies when given systemically to spinal rats (43). It may be that the site at which the drugs produce their effects is not the same after IT and systemic administration (e.g., peripheral vs. central) or that the amount of drug that reached the relevant sites was not equivalent.

Another interpretation is that the effect of intrathecally administered serotonergic agents in intact rats may be modulated by supraspinal input, that is, descending input may mediate the "hyperalgesic" response to 8-OH-DPAT in intact rats and when this is removed an antinociceptive effect may be expressed. The present results are generally compatible with this interpretation in that spinal transection had different effects on the antinociceptive response to IT morphine and 5-HT. While the effect of morphine was significantly potentiated by spinalization, the same treatment abolished the modest dose–response relationship to 5-HT obtained in intact rats. Doses of 100 and 200 μg, which had been shown in numerous studies to increase TF latency in intact rats, did not do so in acute spinal rats. Therefore, the response to IT 5-HT in intact rats, like the response to morphine, may be mediated by an additional process, for example, by another transmitter that is also eliminated by spinalization. In this regard, Minor et al. reported that depletion of noradrenaline, by systemic (2) or IT pretreatment with neurotoxins, blocks the antinociceptive effect of IT 5-HT on the TF and that IT administration of noradrenaline restored the antinociceptive effect of 5-MeODMT (39,40). Delander and Hopkins reported that IT norepinephrine and 5-HT potentiated each others effect on the TF in mice (14). Although they saw no change in 5-HT-mediated antinociception after depletion of spinal norepinephrine, Sawynok and Reid did see a decrease in 5-HT-mediated antinociception after IT adrenergic blockade (50). These observations point to an adrenergic mediation of the antinociceptive effect of spinal 5-HT.

There are also other possibilities. For example, substance P (SP) is not only found in primary afferents but is colocalized with 5-HT in descending raphe–spinal pathways (9,10). Destruction of primary afferent SP (by IT capsaicin injection) produces antinociception (62) whereas destruction of descending 5-HT pathways produces hyperalgesia (see the introductory section). This difference has been interpreted to suggest that primary afferent and raphe–spinal SP systems exert opposite effects on pain. There is support for this conjecture with regard to mechanically induced nociceptive reflexes in spinal rats, where the addition of 5-HT agonists antagonized the hyperalgesic effect of IT SP (44). It is conceivable that SP and 5-HT, derived from descending pathways, interact in some way to reduce the nociceptive action of SP released from primary afferents and that exogenous administration of 5-HT alone in the spinal rat does not restore this function.

Interactions between 5-HT and other neuromodulators at the spinal level, particularly adenosine, have also been demonstrated (14,50). Adenosine is presently believed to be preferentially localized in primary afferents, and there is evidence that 5-HT releases adenosine from this site (55,56). However, the fact that the antinociceptive effect of IT 5-HT was reduced after spinalization would suggest that the mechanism responsible cannot be restricted to primary afferent or intrinsic neuronal elements because these sites would still be accessible to exogenously administered 5-HT in the spinal rat. On the other hand, enkephalin has been located in descending pathways (29,37,38), and Kellstein et al. found that the antinociceptive effect of IT 5-HT was reduced by IT naloxone, implicating opioids in serotonergic spinal function (33).

Coadministration of IT 5-HT and Morphine in Intact and Spinal Rats

The combined administration of 5-HT and morphine did not reduce the analgesic effect of morphine alone in intact rats. On the contrary, low doses of 5-HT (10 and 25 μg), which alone did not change the TF latency, appeared to increase morphine-induced antinociception. When this was examined directly, it was found that (at least) 10 μg 5-HT potentiated IT morphine-induced analgesia. Although this potentiation, shown in Fig. 4, appeared modest, it should be noted that all 6 animals injected with 10 μg 5-HT and 0.5 μg

morphine reached the cut-off score whereas only 5 of 13 rats did so after morphine alone. A similar potentiation was indicated with 25 μg 5-HT but this was not examined further. It is surprising that this potentiation was not observed in response to the higher dose of 50 μg 5-HT, which alone had a borderline effect on TF latency. It seems that low doses of 5-HT, which by themselves do not affect latency, potentiate spinal opiate analgesia, but higher doses, which may independently, increase latency do not influence opiate analgesia. This dichotomy is consistent with reports that IT 5-HT had either no effect (31) or potentiated IT morphine (4) in mice. Intrathecal morphine may engage some mechanism involving 5-HT that is required for its antinociceptive effect at the spinal cord [even though it does not release 5-HT at this site (41,57)]. This is consistent with the results of Crisp and coworkers, showing that IT antagonists of 5-HT can reduce IT morphine analgesia (13).

If this is correct, such an interaction may be mediated by descending input because the same 10-μg dose of 5-HT that potentiated morphine in intact rats was found to antagonize morphine after spinal transection, even though it was ineffective by itself in both preparations. The facts that IT administration of serotonergic agonists does not significantly alter tail-skin temperature and that IT injection of 10 μg 5-HT has no significant cardiovascular effects (28) eliminates these possible confounding influences.

We are aware of only one other article that examined the combined effect of a serotonin agonist and morphine in spinal rats (8). In that study, the 5-HT agonist 5-MeODMT increased TF latencies after systemic administration and this response was unchanged after systemic injection of 1.25 mg/kg morphine. However, because this dose of morphine alone did not affect response latencies it was not possible to determine whether the 5-HT agonist reduced opiate antinociception. Moreover, animals were tested 21 days after spinalization, a time when serotonergic receptors would be supersensitive.

There are several reports involving depletion of spinal 5-HT that may appear inconsistent with our results. In rats with lesions of the dorsolateral funiculus (which contains the descending serotonergic fibers), systemic administration of the 5-HT antagonist methysergide blocked spinal morphine analgesia (11). Systemic methysergide in spinal rats also antagonized antinociception produced by a large (16 mg/kg) but not a small (8 mg/kg) dose of systemic morphine (47). However, it is not clear why a 5-HT antagonist would be effective at the spinal cord of rats after spinal 5-HT input was eliminated. In fact, one recent article did not show any effect of systemic pirenpirone (a 5-HT_2 antagonist, 0.24 mg/kg) on morphine antinociception (10 mg/kg) in spinal rats (46). It is possible that 5-HT might be released transiently by lesioned or severed raphe-spinal terminals or that methysergide may not be selective for 5-HT receptors (although this agent does not interact with opiate receptors (42)].

In conclusion, the present studies show first that the spinal actions of morphine and serotonin on the TF are differentially altered by spinalization—the antinociceptive effect of morphine is potentiated, while that of serotonin is eliminated. These results imply that the spinal action of both agents is modified by descending supraspinal input, although it remains to be seen whether these phenomena are mediated by a common mechanism or two independent processes. Second, the data show that a low (10 μg) dose of 5-HT, which was ineffective by itself, potentiated the antinociceptive effect of spinal morphine in intact rats but antagonized the antinociceptive effect of spinal morphine in spinal rats. In general, spinal transection appeared to shift the functional effect of 5-HT at the spinal cord from anti- to pro-nociceptive. These results implicate an intervening supraspinal process(es) in the interaction between spinal morphine and serotonin.

ACKNOWLEDGEMENT

This research was supported by U.S. Public Health Service Grant DA 02845 from the National Institute on Drug Abuse.

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