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Serotonin Receptor Antagonists Induce Hyperalgesia without Preventing Morphine Antinociception

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BERGE, O.-G., O. B. FASMER AND K. HOLE. *Serotonin receptor antagonists induce hyperalgesia without preventing morphine antinociception.* PHARMACOL BIOCHEM BEHAV 19(5)873–878, 1983.—5-Hydroxytryptamine (5-HT) receptor blockade by administration of mianserin (1 mg/kg) or metergoline (0.25 mg/kg) shortened the response latencies of rats in the hot-plate (hind-paw lick response) and tail-flick tests, but did not consistently attenuate the antinociceptive effect of morphine (1.25–5.0 mg/kg). Injection of the opiate receptor antagonist naloxone (1 mg/kg) did not change tail-flick response latencies and did not interfere with the antinociceptive action of the 5-HT receptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT). The antinociceptive effect of morphine was reduced in chronically spinal rats, although significant increases in tail-flick latencies were observed after 2.5 and 5.0 mg/kg. Concomitant administration of 5-MeODMT failed to restore the effect of morphine in spinal rats. In the hot-plate test, morphine did not reliably prolong latencies to forepaw lick, indicating that this response is not a useful measure of pain sensitivity. The results suggest that different mechanisms underlie the analgesia induced by systemic administration of morphine and 5-HT mediated tonic inhibition of nociception.

Analgesia 5-Hydroxytryptamine Morphine Spinal rats

ANTINOCICEPTION may be induced by local injections of morphine into various regions of the central nervous system, such as the cerebral ventricles [21], mesencephalic central gray [25], raphe nuclei [14] or the subarachnoidal space of the lumbar spinal cord [50]. Although clinically defined analgesia is obtainable by morphine injections localized to the spinal cord [47], some experimental evidence suggests that the analgesic effect of systemically administered morphine may depend on concomitant activity at spinal and supraspinal sites [52,53].

Serotonergic neurotransmission at both spinal and supraspinal levels may be involved in morphine antinociception. A variety of studies have demonstrated attenuation of morphine effects after general depletion of 5-hydroxytryptamine (5-HT) by the synthesis inhibitor parachlorophenylalanine (PCPA) [28, 41, 45] or after electrolytic [16, 34, 38] and neurotoxic [12, 17, 44] lesions of 5-HT containing structures.

Other studies, however, have yielded contradictory results. Normal levels of morphine antinociception have been demonstrated by several test methods following general depletion of 5-HT in the central nervous system by PCPA [11, 20, 22] as well as after lesioning of ascending [9, 11, 23] and descending [32, 33, 36] serotonergic pathways. These results are difficult to reconcile with an indispensable role of 5-HT in morphine induced antinociception.

Interpretation of lesion effects in behavioral studies is, however, complicated by several factors. Recovery of apparently normal function in spite of biochemically verified persistent destruction has been demonstrated after neurotoxic lesions of the ascending [15] as well as the descending 5-HT systems [4]. In the latter case, recovery of tonic inhibition of nociception after 5,6-dihydroxytryptamine induced lesions of the descending 5-HT pathways seemed to depend on adaptive processes involving the remaining components of spinal serotonergic neurotransmission.

In addition, strain differences [42] as well as methods and conditions of testing [8, 10, 13, 30] may determine whether or not 5-HT depletion will attenuate morphine antinociception.

Thus the literature indicates that serotonergic mechanisms may participate in morphine antinociception, but neither the nature of these mechanisms nor the conditions under which they are active have been defined.

In the present study we have investigated whether treatments that reduce the tonic antinociceptive activity of serotonergic neurotransmission [3] attenuate morphine antinociception. We have also investigated the effects of morphine on spinally transected rats. Previous work has demonstrated significant although reduced morphine induced depression of the tail-flick response to radiant heat in rats 3 days after spinal transection [24] at which time the endogenous levels of spinal 5-HT may still be relatively high [1]. We have therefore allowed a minimum of 3 weeks between transection and experiments in order to assure a complete degeneration of the terminals of the descending 5-HT projections. Possible interactions between antinociception induced by the 5-HT receptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) and opiate mechanisms were studied in spinal and normal rats.

Parts of this study have been published as an abstract [5].

METHOD

Animals

Male Wistar rats (Møllegård, Denmark, weight 250 g at the start of the experiments) were housed in conventional cages with free access to food and water. Animals used in the tail-flick experiments were kept in individual cages while rats tested with the hot-plate method were housed in groups of six. Room temperature was kept at 23±1°C. All testing took place in the middle of the light phase of a 12/12 hr light/dark cycle.

Drugs

Morphine hydrochloride (1.25–10.0 mg/kg), naloxone (0.5–4.0 mg/kg, donated by Endo Laboratories) and 5-MeODMT (1 mg/kg, Sigma) were given subcutaneously in the neck. Mianserin (1 mg/kg, Organon) and metergoline (0.25 mg/kg, donated by Farmitalia) were injected intraperitoneally. Dosages and injection schedules were derived from a previously reported study [3]. All drugs were dissolved in saline immediately prior to testing and injected in a volume of 1 ml/kg. The 5-MeODMT and metergoline solutions contained 0.2 and 5.0 mg/ml ascorbic acid respectively.

Surgery

Spinal transections were carried out under anesthesia of pentobarbital (40 mg/kg) and chloral hydrate (130 mg/kg) given intraperitoneally. The spinal cord was exposed through vertebra Th10 and transected by excavation. The transected rats received penicillin (150,000 IU/day) for the first 5 postoperative days. At least 21 days were allowed for recovery. Only animals that appeared healthy were used. At the end of the experiments, all transections were verified by visual inspection.

Nociceptive Tests and Handling Procedures

Tail-flick latencies were obtained by means of an IITC Export Inc. Model 33 Analgesia Meter. The animals were restrained by a perspex tube, and radiant heat focused on a spot 1–2 cm from the tip of the tail. The beam intensity was adjusted to produce control response latencies between 5 and 7 sec. Since the higher doses of morphine used in these experiments depress the tail-flick response sufficiently for tissue damage to occur, a cutoff time of 14 sec was employed. For at least 2 weeks prior to experiments, and between experimental sessions, the animals were handled and trained in the test situation. The tail-flick testing was conducted in the room where the animals were housed. During each session, 5 tests were carried out at 25 min intervals. A pre-injection latency was obtained as the mean of trials 2 and 3. The test compounds were injected immediately following trial 3, and the latency of trial 5 was taken as the test response. In the experiment were naloxone was used, this compound was injected 25 min prior to determination of drug effects.

The hot-plate tests were conducted with an IITC Export Inc. Model 35-D Analgesia Meter with a copper surface maintained at 55±0.2°C, enclosed by a lidded perspex box. For one week prior to testing and between test sessions, the rats were given daily 1 min exposures to the non-functional hot-plate. During the test sessions, latencies to forepaw and hind-paw licks were determined. Regardless of response, each rat was kept on the hot-plate for 45 sec. Hot-plate testing was conducted in a sound-attenuated room adjacent to the animal room, and each rat was brought to the test room immediately prior to testing. The animals were tested once only in each session, 50 min after saline or drug administration.

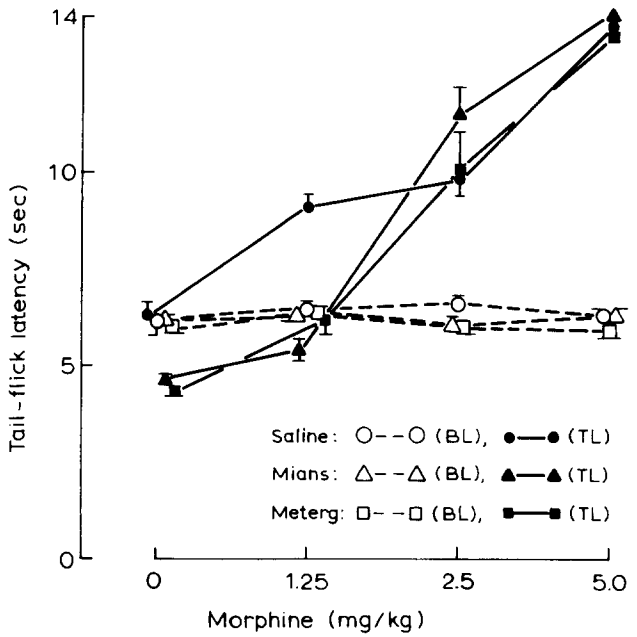

FIG. 1. The effect of 5-HT receptor antagonists on tail-flick latencies and morphine antinociception (mean±SEM; n=9 for each point. SEM is not indicated for the postinjection latencies after 5 mg/kg morphine due to employment of cutoff time). Mians=mianserin, 1.0 mg/kg. Meterg=metergoline, 0.25 mg/kg. BL=basal level, the mean of two preinjection test values. TL=test level, the latencies obtained 50 min after injection of the drugs. For further details see methods.

Each rat was used for up to 3 sessions, with a minimum of 7 days between sessions. The various drug-combinations and doses of morphine were given in random order. All testing was conducted by an observer ignorant of the drug treatment of the animals.

Statistics

The data were examined by analysis of variance (ANOVA) as detailed in the results, or by Student's *t*-test whenever the analysis was restricted to two means. Unless otherwise stated, 5% was taken as the level of significance.

RESULTS

In the tail-flick test, morphine induced a dose-related prolongation of latencies whether given together with saline or either of the two 5-HT blockers mianserin or metergoline (Fig. 1). In the absence of morphine, the 5-HT antagonists shortened the response latencies. Similarly, the latencies obtained after administration of 1.25 mg/kg morphine where shortened by concomitant administration of the blockers.

Statistical analysis revealed no difference between preinjection latencies (ANOVA, completely randomized design, all groups included). Analysis of the post-injection trials (3×3 ANOVA, the data obtained after 5 mg/kg of morphine were not included since employment of cutoff time caused truncation of the scores) demonstrated significant main effects of morphine (0, 1.25, 2.5 mg/kg; $F(2,72)=97.91$,

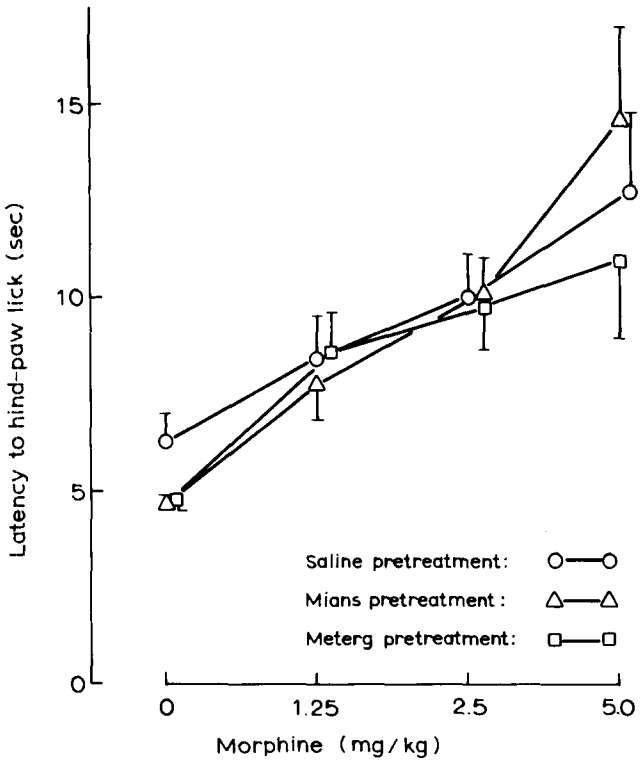

FIG. 2. The effect of 5-HT receptor antagonists and morphine on latencies to hind-paw lick in the hot-plate test (mean±SEM; n=9 for each point). Mians=mianserin, 1.0 mg/kg. Meterg=metergoline, 0.25 mg/kg. Testing was performed 50 min after drug injection.

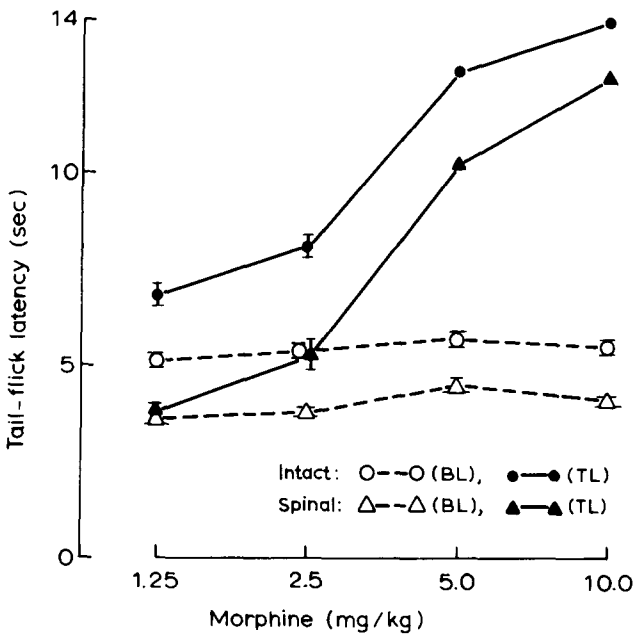

FIG. 3. The effect of morphine on tail-flick response latencies of intact and spinal rats (mean±SEM; n=9–10 for each point. SEM was not calculated for means including cutoff values). BL=basal level. TL=test level, obtained 50 min after injection of morphine.

$p<0.001$) and pretreatments (saline, mianserin, metergoline; $F(2,72)=8.18$, $p<0.001$). A significant interaction, $F(4,72)=8.61$, $p<0.001$, was primarily due to the different slopes of the dose-response curves between 0 and 1.25 mg/kg morphine and between 1.25 and 2.5 mg/kg morphine. Further analysis (2×2 ANOVA) revealed significant interaction between morphine (0, 1.25 mg/kg) and mianserin (0, 1.0 mg/kg; $F(1,32)=11.47$, $p<0.002$) but not between the same doses of morphine and metergoline (0, 0.25; $F(1.32)=1.98$, $p>0.10$).

Randomized ANOVA demonstrated significant difference between the groups that received saline or antagonists only, $F(2.24)=20.84$, $p<0.001$, and between the groups that received saline or antagonists in combination with 1.25 mg/kg morphine, $F(2,24)=28.38$, $p<0.001$. In each case the mianserin or metergoline treated animals responded with significantly shorter latencies than the corresponding saline injected rats ($p<0.001$, t-ind. for each of the 4 comparisons made between saline and antagonist injected groups). The difference between the groups that received 2.5 mg/kg morphine was not significant, $F(2.24)=2.18$, $p>0.10$.

In the hot-plate test, latencies both to forepaw lick and to hind-paw lick were recorded, since both criteria are frequently referred to in the literature.

The hind-paw lick scores (Fig. 2) resembled the results from the tail-flick test in that the mean latencies after mianserin and metergoline were significantly shorter than the latencies following saline only ($p<0.025$, t-ind. for each antagonist treated group compared to the saline group). There was no apparent interference with the antinociceptive effect of morphine. 3×4 ANOVA demonstrated significant main effect of morphine, 0, 1.25, 2.5 and 5.0 mg/kg; $F(3,96)=16.16$, $p<0.001$, but not of pretreatments (saline, mianserin, metergoline; $F<1$). No significant interaction was present $F<1$).

The forepaw lick scores differed from the other test-results in that morphine failed to increase the latencies of the saline pretreated rats (ANOVA, randomized design $F<1$, data not shown).

Possible interactions between serotonergic and opiate mechanisms of antinociception were investigated further by means of the tail-flick test. Chronically spinalized rats were introduced in order to study the response in absence of descending serotonergic influence [3]. As previously found [3,6] spinal transection reduced the tail-flick latencies by approximately 25%.

The effects of morphine on the tail-flick response of spinal and intact rats are compared in Fig. 3. In both groups, a dose-related prolongation of latencies was found, the dose-response curve of the spinal rats being shifted to the right. No exact determination of the difference in dose response was attempted, but it appears that roughly twice the dose of morphine was required in spinal rats to obtain an effect similar to that seen in intact animals.

The results summarized in Table 1 show that administration of the opiate receptor antagonist naloxone failed to block the antinociceptive action of the 5-HT agonist 5-MeODMT in either spinal or intact rats. Naloxone given alone in doses between 0.5 and 4 mg/kg (data for 1 mg/kg only shown) was furthermore without any effect in the tail-flick test. Administration of naloxone (1 mg/kg) strongly attenuated the effect of morphine (10 mg/kg) in both spinal and intact animals (data not shown).

Combined injections of 1.25 mg/kg morphine and an optimal dose [3] of 5-MeODMT (1 mg/kg) significantly prolonged the response latencies of both groups but a difference

TABLE 1

EFFECT OF NALOXONE AND MORPHINE ON 5-MeODMT-INDUCED ANTINOCICEPTION

Drug treatment (mg/kg)	Group*	Tail-flick latency† pre-injection	Tail-flick latency† post-injection
5-MeODMT (1)	C	100 ± 5%	127 ± 5%§
	ST	76 ± 2%	125 ± 7%¶
Naloxone (1)	C	100 ± 3%	98 ± 3%
	ST	74 ± 3%	77 ± 3%
Morphine (1.25)‡	C	100 ± 4%	132 ± 5%¶
	ST	71 ± 2%	75 ± 3%
5-MeODMT (1) + naloxone (1)	C	100 ± 3%	127 ± 4%¶
	ST	70 ± 2%	124 ± 3%¶
5-MeODMT (1) + morphine (1.25)	C	100 ± 3%	162 ± 6%¶
	ST	72 ± 2%	129 ± 14%§

*C=intact rats (n=10–12), ST=chronically spinalized animals (n=8–11).

†Mean ± SEM in percent of preinjection scores of intact rats. The actual preinjection means were between 6.0 and 6.9 sec (intact rats) or between 4.4 and 5.2 sec (spinal animals).

‡Same data as in Fig. 3.

§Significantly greater than preinjection scores, $p<0.01$.

¶Significantly greater than preinjection scores, $p<0.001$.

in latencies between transected and intact rats was observed similar to that after morphine alone. In spinal rats, the combined treatment was no more effective than 5-MeODMT given alone.

DISCUSSION

In the present experiments, administration of the 5-HT receptor antagonists metergoline and mianserin reduced the response latencies of intact rats in the tail-flick and hot-plate tests. Administration of the opiate receptor antagonist naloxone did not change tail-flick latencies of either normal or transected rats. Thus, under the conditions employed, serotonergic systems, but not opioide systems, mediate tonic inhibition on nociceptive responses.

Neither of the two 5-HT antagonists consistently attenuated the effect of systemically administered morphine, and, conversely, naloxone failed to interfere with the antinociceptive effect of the 5-HT agonist 5-MeODMT as tested by the tail-flick method. Finally, an optimal dose of 5-MeODMT did not restore the antinociceptive effect of concomitantly administered morphine in spinal animals.

Using the tail-flick test, we have consistently been able to demonstrate tonic antinociceptive activity of the descending serotonergic system [3, 4, 7]. In the present experiments, the 5-HT receptor antagonists mianserin and metergoline also reduced the latencies to hind-paw lick in the hot-plate test, suggesting that 5-HT mediated inhibition of nociception is of consequence not only for reflex responses, but also for more complex behavioral reactions to noxious stimulation.

The forepaw lick latencies were not significantly reduced by the 5-HT antagonists and not reliably prolonged by administration of morphine. It was noted that forepaw lick frequently occurred when the animals were rearing and the forepaws had not been in contact with the hot surface for several seconds. The response was therefore not directly elicited by the noxious stimulus. Forepaw lick may be related to heat exposure in general [37] and the present findings suggest that it is less suitable as a measure of nociception.

Behavioral studies have previously suggested a tonic antinociceptive role of 5-HT, most likely mediated by the descending systems. For instance, lesions of the descending pathways by 5,6-DHT [49] or intrathecal injection of the putative 5-HT antagonist methysergide [35] have been reported to produce hyperalgesia in the tail-flick and hot-plate tests, although the findings related to methysergide have been inconsistent [19,51]. Systemic administration of the 5-HT antagonist cyproheptadine has been reported to lower response thresholds in the paw compression test [18] but a series of antagonists including metergoline and mianserin failed to affect response latencies in the hot-plate test [29]. In the latter report no distinction was made between forepaw and hind-paw lick responses, which may, in view of our present findings, explain the lack of effect of the antagonists.

It has been suggested that descending 5-HT pathways may affect nociception via an enkephalinergic link in the spinal cord [2]. The failure of naloxone to shorten the latencies or to block the effect of 5-MeODMT in the tail-flick test indicates that such a link is of little importance for 5-HT mediated antinociception as presently tested.

In the hot-plate test, injection of 5-HT antagonists failed to interfere with morphine antinociception. In the tail-flick test, significantly shorter latencies were found in the rats receiving 1.25 mg/kg morphine combined with either of the 5-HT antagonists as compared to the animals that received morphine only. The observed difference was partly due to the hyperalgesia induced by the antagonists when given alone, but statistically significant interaction was found between mianserin and the 1.25 mg/kg dose of morphine. The antagonists did not reduce the effects of higher doses of morphine.

Thus, the 5-HT antagonists employed did not consistently block morphine analgesia in the present experiments. It should be noted that the antagonists under similar conditions consistently blocked the antinociceptive effect of 5-MeODMT [3]. Previous reports have shown that 5-HT receptor antagonists may reduce the antinociceptive effect of morphine microinjected into the brain stem [14,48]. Direct evidence for involvement of the descending 5-HT pathways was demonstrated by intrathecal injection of methysergide which blocked the antinociceptive effect of morphine microinjected into the periaqueductal gray [46]. The local concentrations of morphine obtained in these studies were probably considerably greater than after systemic administration of analgesic doses of the drug [40]. The relevance to morphine analgesia induced by systemic administration is therefore uncertain. Attenuation of the analgesic effect of systemically injected morphine by administration of the 5-HT receptor antagonist cyproheptadine has been found in the paw-compression test [18] while no consistent effect of several blockers was evident in the hot-plate test [29] or by methysergide in the hot-plate and tail-flick tests [13]. These reports tend to support the contention that the effect of morphine is largely independent of 5-HT receptors sensitive to classical antagonists, at least as measured by the tail-flick and hot-plate tests.

Some evidence suggests that 5-HT may modulate nociception by actions on non-serotonergic receptors or via 5-HT receptors less sensitive to the antagonists used in these studies. Inhibition of 5-HT synthesis, but not administration of receptor blockers, has been reported to cause an

enhancement of shock induced analgesia [43]. Recently we found that the potent antinociceptive effect of p-chloroamphetamine-induced 5-HT release was attenuated by 5-HT depletion, but not by metergoline pretreatment (Ögren and Berge, in preparation). It is therefore conceivable that interaction with similar 5-HT systems may contribute to the analgesic effect of morphine.

It has been proposed that morphine-induced release of spinal 5-HT may be of importance for the analgesic effects of the drug [27]. Several studies have demonstrated that morphine analgesia is enhanced by inhibition of the neuronal reuptake of 5-HT, even by doses of reuptake inhibitors that have no direct effect on nociception [26, 31, 39]. Although these experiments demonstrate that 5-HT release may enhance the analgesia observed after injection of morphine, there is little evidence to suggest that this is the case unless the normal reuptake system is impeded. In the present study, significant prolongation of tail-flick latencies by morphine was observed in chronically spinal rats where sufficient time had been allowed between lesioning and experiments to ascertain complete degeneration of serotonergic terminals in the spinal segments below the transection. The effects were similar to those reported after shorter survival times [24].

Some evidence indicates that normal levels of morphine analgesia after systemic injection requires concomitant action at the spinal level and activation of descending systems [52,53]. Assuming that the reduced effect of morphine in spinal rats is due to a loss of descending 5-HT activity, this might be compensated by administration of a 5-HT receptor agonist. In the present study, however, an optimal dose of 5-MeODMT failed to restore the effect of a low dose of morphine.

The results do not rule out an involvement of 5-HT at sites not sensitive to the drugs employed in the present experiments. In fact, we have recently found that serotonergic connections within the brainstem may be important for the expression of morphine-induced analgesia under certain conditions [8]. It may be concluded, however, that there is a dissociation between a tonically active antinociceptive system which is sensitive to 5-HT receptor blockade by low doses of metergoline and mianserin, and the systems mediating morphine antinociception which are not consistently affected by identical doses of these drugs.

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