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Effect of 5-Hydroxytryptamine Precursors on Morphine Analgesia in the Formalin Test

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ABBOTT, F. V. AND S. N. YOUNG. *Effect of 5-hydroxytryptamine precursors on morphine analgesia in the formalin test.* PHARMACOL BIOCHEM BEHAV 31(4) 855–860, 1988.—The 5-hydroxytryptamine (5HT) precursors tryptophan and 5-hydroxytryptophan had no significant effect on the behavior of rats in the formalin test when given by themselves. However, both compounds significantly attenuated the analgesic effect of morphine in the formalin test. The 5HT antagonist methysergide enhanced the antinociceptive effect of morphine but systemic 5HT had no effect. Assays of whole brain and spinal cord indoles revealed different patterns as a result of tryptophan or 5-hydroxytryptophan loading. The effect common to both treatments was an increase in brain 5HT. There was no effect of morphine on any measure. Formalin injection by itself did not alter indole levels in the brain or spinal cord. Our results, taken in conjunction with previous work, suggest that 1) 5HT in the spinal cord does not influence pain perception in the formalin test and 2) 5HT in the brain can antagonize morphine analgesia in the formalin test. We conclude that there may be circumstances in which the use of 5HT precursors for clinical pain management may be contraindicated.

5-Hydroxytryptamine Morphine Pain Formalin test Tryptophan 5-Hydroxytryptophan Rats Antinociception

IN animal pain tests that involve measuring the latency for a withdrawal reflex (e.g., tail-flick test), the antinociceptive action of morphine is mediated, in part, by activation of descending brainstem and bulbar 5-hydroxytryptamine (5HT) systems [for reviews see (6, 16, 27)]. However, in the formalin test in which the response to a minor tissue injury is assessed, lesions of raphe nuclei involved in the descending 5HT systems or the dorsolateral funiculus have no effect on morphine antinociception (3, 4, 28). On the other hand, lesions of the median raphe nucleus enhance morphine antinociception (3) indicating that, in this pain model, 5HT appears to be antagonistic to morphine. Thus, while descending 5HT neurons may inhibit nociceptive afferents, 5HT neurons in the brain can, in some circumstances, antagonize morphine analgesia.

In humans, as in rats, administration of the 5HT precursors L-tryptophan and 5-hydroxytryptophan (5HTP) increases CNS 5HT synthesis (32). This, and the positive role for 5HT in reflex withdrawal tests, has led to clinical studies on the analgesic effect of tryptophan and 5HTP in chronic pain patients. In a placebo-controlled crossover study, five patients, mainly with pain from disc disease, were given 1.5–3.0 g 5HTP per day or placebo, while a further five patients received 7–10 g tryptophan per day instead of 5HTP. The 5HT precursors failed to alleviate pain significantly (30). Another study found negative results in a comparison of tryptophan (5 g per day given at bedtime) and chlorpromazine in patients with fibrositis syndrome (23). However, in five patients with deafferentation pain, 5HTP at a dose of 300–800 mg/day decreased pain over a 3-month period (10). Further encouraging results were obtained in a double-blind study of 30 patients with chronic maxillofacial pain who were given placebo or tryptophan (3 g per day). After four weeks there was a greater reduction in reported clinical pain and a greater increase in pain tolerance in the tryptophan group than in the placebo group (29).

Tryptophan has also been found useful in reversing the decline in potency over time of three different analgesic treatments. At a dose of 3 g/day for two months tryptophan reversed tolerance to brain stimulation in four patients (20). Tryptophan (4 g per day for 2–9 weeks) also reversed tolerance to opiates in five patients who had been given opiates chronically to treat low back and leg pain (21). Finally, tryptophan (2 g per day) was given to five rhizotomy

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and cordotomy patients in whom pain had resumed. Their sensory deficits reexpanded to the maximum extent initially recorded after surgery (22).

Whereas some of the reports summarized above are consistent with the animal data indicating that activation of descending 5HT neurons can promote analgesia, one clinical study indicates an increase in postsurgical analgesic requirements following 5HT precursor loading. We have recently reported preliminary results from a clinical trial of intravenous tryptophan loading on morphine requirements following major abdominal surgery (1). In these data, there is a trend for morphine requirements to be higher in patients receiving tryptophan during the first few hours after surgery. The data suggest that, as in the formalin test, 5HT can be antagonistic to morphine in acute clinical pain.

The present study was initiated in order to obtain more insight into the clinical situations in which 5HT precursor loading might be either helpful or contraindicated in pain patients. We examined the effects of tryptophan loading on the dose-effect relation for morphine in the formalin test and on CNS indoles. In addition, since tryptophan loading could potentially act by conversion to other neuroactive metabolites such as tryptamine (19) or kynurenine products (31), we used 5HTP loading and methysergide, a 5HT receptor-blocker, to confirm that effects were due to conversion of tryptophan to 5HT.

METHOD

Subjects

Male Long Evans rats (Charles River, Que.) weighing 175–200 g were housed in group cages of 3–5 for 6–8 days prior to testing in the colony room. Food and water were available ad lib. A 12:12 light:dark schedule (on 7 a.m., off 7 p.m.) was maintained. All testing took place between 9 a.m. and 5 p.m.

Drugs

Morphine sulphate (gift from Sabex, Canada) and L-tryptophan (Sigma Chemicals, St. Louis, MO) were dissolved in distilled water. 5HTP and 5HT (Sigma Chemicals, St. Louis, MO) were dissolved immediately before use in distilled water containing 0.1% ascorbate. Methysergide maleate (gift from Sandoz, Canada) was prepared fresh each day by dissolving it in 10 N HCl, titrating to pH 6.3 with NaOH and then diluting to its final volume with distilled water. The appropriate vehicle was used in control rats. Injection volumes, routes of administration and injection times were as follows: morphine, 1 ml/kg, SC 50 min before testing; tryptophan and 5HTP, 10 ml/kg, IP 90 min before testing; 5HT 10 ml/kg, IP 60 min before testing; methysergide, 1 ml/kg, IP 30 min before testing.

Procedures

Rats were habituated to the laboratory and the formalin test chambers (30×30×30 cm Plexiglas boxes with an inclined mirror below the floor) by bringing them to the laboratory, weighing them and placing them in the test chambers in pairs for 10 min on 3 days. On the test day, they were given morphine or its vehicle and one other agent or its vehicle according to the schedules described above. They were always placed in the formalin test chamber at the time of morphine injection.

Formalin (2.5%, 0.05 ml) was injected SC into the plantar surface of one rear paw. This produces pain which decreases after 5–10 min, rises again after 10–15 min, and remains relatively stable for the next 30 min (11). Pain rating was carried out during the stable period from 30–50 min after formalin. An observer used a small computer to record the amount of time the rat spent in each of the following behavioral categories: "0"—weight is born evenly on both rear paws; "1"—limps during locomotion or rests with injected paw favoured; "2"—injected paw is elevated with at most the nails touching the floor; "3"—injected paw is groomed or bitten.

A pain score was calculated using the following formula:

$$\text{Pain score (E)} = \frac{(0 \times t_0) + (1 \times t_1) + (2 \times t_2) + (3 \times t_3)}{t_0 + t_1 + t_2 + t_3}$$

Where $t_0 - t_3$ represents the number of seconds spent in each behavioral category. Pain scores were converted to % maximal possible effect (%MPE):

$$\%\text{MPE} = \frac{E - E_{min}}{E_{max} - E_{min}} \times 100$$

where E is the pain score of a rat, E_{min} is the mean score of the control group and E_{max} is defined as a pain score of 0. Control pain scores (E_{min}) for various vehicle-treated groups ranged from 1.8 to 2.2. MPE_{50}s were calculated from a linear regression and variance estimates obtained by jacknifing the regression lines (24,25). Two tailed Student's *t*-tests were used to determine statistical significance.

Indole Assays

A separate group of rats were habituated to the laboratory in the same way rats for pain testing were. On the test day they received tryptophan, 5HTP and morphine or vehicle injections. Sixty min after morphine, they were anesthetized with IM ketamine-zylazine (90 mg/kg and 9 mg/kg) and killed by decapitation in another room. To ensure that formalin injection did not alter CNS indoles, brain and spinal cord tissue for rats with and without formalin injection were included. The brains and a 1.3–1.5 cm section of spinal cord (T10-L3-4) were removed and frozen at −70° for indole assays. Tryptophan, 5HT, and 5-hydroxyindoleacetic acid (5HIAA) in the tissues were measured using high performance liquid chromatography with fluorometric detection (5). Biochemical data were analyzed with the Kruskal-Wallis test followed by Wilcoxon tests instead of ANOVAs because variances were extremely nonhomogenous. The *p* values in the test have been adjusted using the Bonferoni method to reduce experimentwise error when multiple comparisons were performed.

RESULTS

As illustrated in Fig. 1A and B, L-tryptophan, 100 mg/kg, and 5HTP, 40 mg/kg, both shifted the dose-effect relation for morphine antinociception in the formalin test to the right, raising the MPE_{50} by a factor of 1.6 [tryptophan: $t(27)=3.84$, $p<0.001$; 5HTP: $t(28)=3.66$, $p<0.01$]. In neither case was the slope altered [tryptophan: $t(27)=1.18$, $p>0.1$; 5HTP: $t(28)=1.04$, $p>0.1$]. Neither tryptophan nor 5HTP had a significant effect on baseline pain [$t(10)=1.11$ and 0.08 respectively]. The nonselective 5HT antagonist, methysergide (10

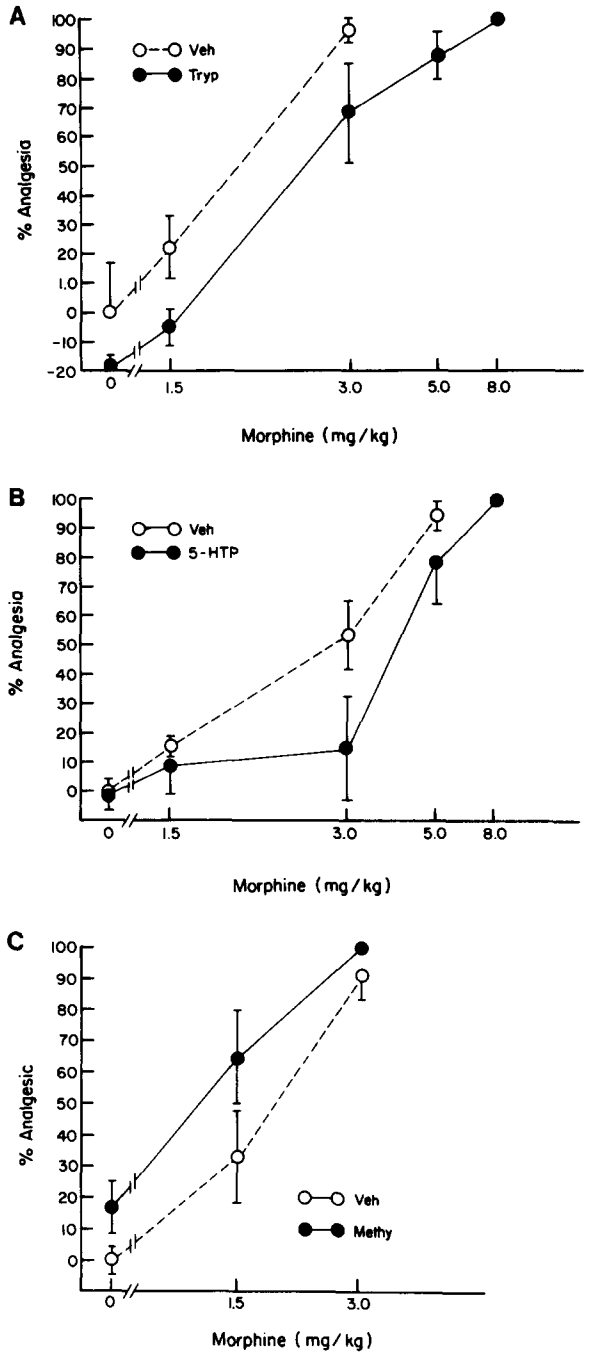

FIG. 1. Dose-effect relationships for morphine in the formalin test after pretreatment with 100 mg/kg L-tryptophan (A), 40 mg/kg 5HTP (B), or 10 mg/kg methysergide (C).

mg/kg), enhanced the antinociceptive effects of morphine by a factor of about 2 [Fig. 1C; $F(1,17)=10.73$, $p<0.01$; jacknife not used because of incomplete dose-response data] without altering baseline pain levels significantly, $t(10)=1.86$. Although methysergide-treated rats were somewhat hyperactive, neither tryptophan nor 5HTP had any marked behavioral effects at the doses used.

Table 1 shows the effects of formalin injection on indole levels in rats which were habituated to the formalin test chambers. The lack of effect indicates that the pain produced by formalin injection 30 min prior to sacrifice does not constitute a stressor as severe as 30 min of restraint [cf. (15)]. This is consistent with the fact that formalin injection also does not induce adrenal ornithine decarboxylase to the extent that 60 min exposure to loud noise does (K. Missala and T. L. Sourkes, personal communication).

The effects of loading with the two precursors on brain and spinal cord indoles are clearly different as can be seen in Fig. 2A–C (p values indicated in Fig. 2). Tryptophan pretreatment did not alter brain tryptophan levels at 90 min after loading but produced a marked increase in spinal cord tryptophan. Brain levels of 5HT were elevated by both tryptophan and 5HTP but in the spinal cord only 5HIAA was significantly elevated. There was no effect of morphine on any measure. Thus the only effect common to both tryptophan and 5HTP loading was to increase brain 5HT.

To confirm that the precursor loading effects were due to alteration in central rather than peripheral 5HT metabolism, 5HT (3 mg/kg) was administered IP 60 min before testing. This treatment did not alter the effects of 3 or 6 mg/kg morphine, $F(1,19)=1.96$, $p<0.1$, despite the fact that cardiovascular side-effects such as erythema of the paws were present.

DISCUSSION

The data presented here support previous studies (3,12) which suggested that 5HT neuronal systems are antagonistic to morphine antinociception in the formalin test. The effects of precursor loading and receptor blockade with methysergide also suggest that the modulating effects of 5HT are relatively small. Tryptophan produced a modest increase in brain 5HT while 5HTP produced a much larger increase but both precursors shifted the morphine dose-effect relation to the right by about 1.6-fold. Methysergide, at a dose that produces general hyperreactivity, had a modest potentiating effect on morphine antinociception. Baseline pain scores were slightly higher in tryptophan-treated rats while methysergide produced a small decrease in baseline. These effects did not reach statistical significance. However they suggest that alteration of baseline pain may underlie the effects of the treatments on morphine antinociception. This conclusion is weakened by the fact that, in addition to the baseline effects of tryptophan and methysergide not reaching significance, 5HTP failed to alter baseline nociceptive scores. Thus, it is possible that the 5HT systems in the brain which modulate formalin pain play a significant role only when activated by the presence of morphine. On the other hand, previous evidence from lesion studies (3, 4, 28) indicates that spinal cord 5HT depletion does not play any role in modulating response to morphine in the formalin test although these lesions attenuate morphine effects in reflex withdrawal tests.

Brain tryptophan was not significantly elevated 90 min after 100 mg/kg tryptophan suggesting that the rats metabolized tryptophan relatively quickly. Whether this is a property of the strain of rats used or due to induction of metabolizing enzymes is not clear. However, the increase in brain 5HT indicates that the loading procedure did produce functional alterations in 5HT metabolism and confirm that brain tryptophan was elevated at an earlier time point. The increase observed in spinal cord tryptophan following tryptophan loading suggests that the decline in spinal cord tryptophan after tryptophan loading is slower than the decline in brain tryptophan.

TABLE 1

EFFECTS OF FORMALIN INJECTION ON BRAIN AND SPINAL CORD INDOLES IN THE ABSENCE OF ANY OTHER TREATMENT

	Tryptophan (μg/g)		5HT (ng/g)		5HIAA (ng/g)	
	Brain	Spinal Cord	Brain	Spinal Cord	Brain	Spinal Cord
Control	4.23 ±0.20	4.69 ±0.40	391.5 ±11.12	544.7 ±20.80	250.6 ±6.45	299.2 ±16.55
Formalin	4.66 ±0.10	5.65 ±0.69	387.7 ±11.81	507.98 ±34.49	268.0 ±8.99	319.8 ±12.98

Means and standard errors of 5 or 6 samples are shown. Formalin produced no statistically significant changes.

The lack of effect of systemic 5HT on formalin pain is interesting because there are two potential mechanisms whereby the precursor loading effects could be due to peripheral conversion to 5HT. First, 5HT increases the release of noradrenalin from terminals of sympathetic neurons (14). Since peripherally acting antisympathetic agents decrease formalin pain (8,9), it is possible that 5HT could enhance pain by increasing sympathetic activity. Second, 5HT produces pain and a wheal and flare reaction when applied to a blister base on human skin. This may be due to direct stimulation of sensory afferents (26). It is plausible to expect that circulating 5HT could gain access to the area of tissue damaged by formalin and increase pain by this mechanism since there is marked extravasation in the injected paw (9). The effects of methysergide indirectly support the conclusion that peripheral 5HT does not play a role in formalin pain because it is inactive at excitatory peripheral 5HT receptors (13). The present data, therefore, support lesion studies (3) which indicate that 5HT antagonizes morphine in inflammatory pain by a central mechanism rather than by increasing pain generated in the periphery.

The positive role of 5HT in opioid analgesia in pain tests involving reflex withdrawal from noxious heat (6, 16, 27) and the negative role in tissue injury induced pain have implications for clinical pain in man. In particular, it becomes important to understand in what circumstances treatments which enhance 5HT function may be either clinically useful or contraindicated. The present data are consistent with a study showing that acute administration of the 5HT uptake inhibitor amitriptyline in arthritic rats also antagonizes morphine antinociception (17). Together, these data suggest that negative effects occur when potentiation of 5HT function is associated with morphine treatment, at least in pain resulting from tissue injury and inflammation. On the other hand, pain-related behavior is reduced in arthritic rats (7) and chronic pain patients (18) treated chronically with 5HT uptake inhibitors without morphine. As discussed in the introduction chronic tryptophan and 5HTP loading have also been shown to produce positive effects in chronic pain and on pain tolerance. The reduction in formalin pain 3 days after 5HT depletion with 5,6-dihydroxytryptamine (12) and the potentiation of morphine following median raphe lesions (3) are not easily explicable in terms of acute vs. chronic 5HT manipulation. However, in these studies the compensatory processes following neural damage render comparisons difficult. The one clinical situation where preliminary studies indicate that acute tryptophan loading is beneficial even in the presence of morphine is in reversing morphine tolerance (21) and, surprisingly, no other studies have attempted to replicate this finding. It remains to be seen whether 5HT precursors antagonize morphine analgesia in types of pain other than that measured by the formalin test. Other problems that need investigation are the type of opiate receptors that must be activated in order for potentiation of 5HT function to reverse opiate analgesia and the interaction of 5HT with nonopiate analgesic drugs. The presence of two 5HT systems, one in the brain and one in the spinal cord, which can have opposite effects on pain perception in different circumstances, indicates the need for careful experimental work to determine the precise circumstance in which either (or both) can be activated.

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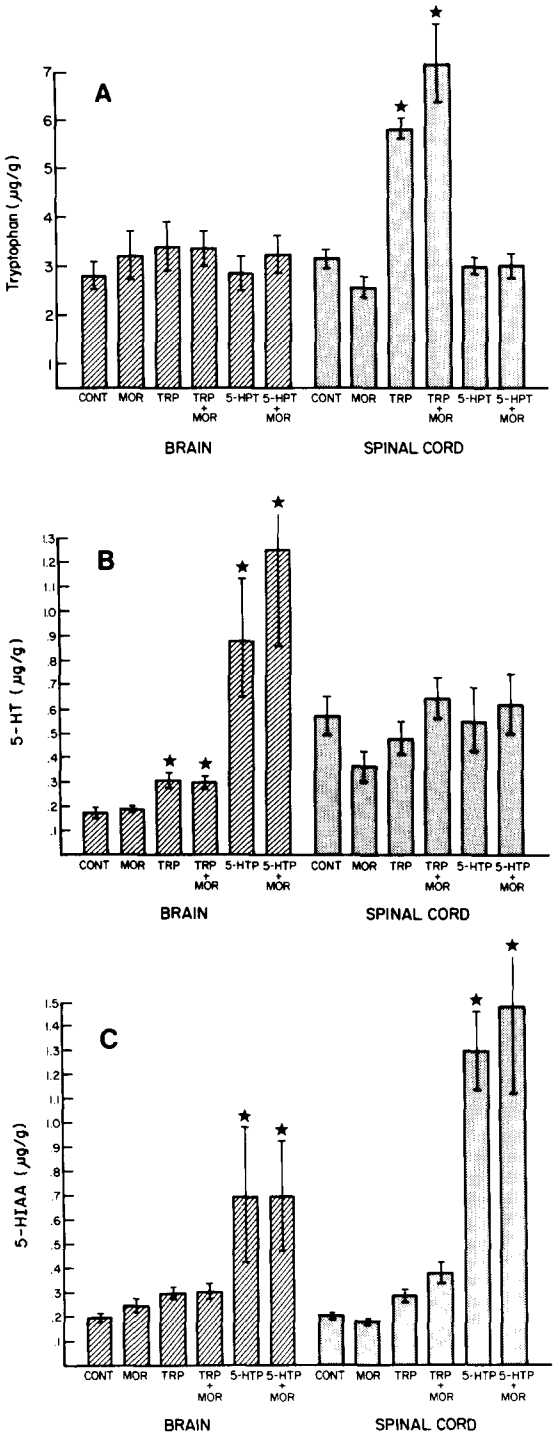

FIG. 2. L-tryptophan (A), 5HT (B) and 5HIAA (C) levels in whole brain and spinal cord. The treatment condition is indicated below each column: CONT=control: MOR=morphine; TRP=L-tryptophan; 5HTP=5-hydroxytryptophan. Significant difference from the control group ($p<0.05$, Wilcoxon with Bonferoni correction) is indicated by asterisks. The standard errors are shown only to illustrate the variability.

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