Abstract—Low doses of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), quipazine and cyproheptadine produced facilitation of jumping in mice using the hot plate method. Higher doses produced severe motor disturbances which precluded the assessment of effects on nociception. The observed hyperalgesia might be a consequence of diminution of serotonergic tone resulting either from triggering of presynaptic serotonergic receptors in the case of 5-MeODMT and quipazine or from the blockade of postsynaptic serotonergic receptors in the case of cyproheptadine. The 5-MeODMT-induced hyperalgesia was not attenuated by buprenorphine, which under similar conditions antagonized completely the hyperalgesic effects of naloxone; thus, the hyperalgesic effects of 5-MeODMT do not seemingly involve opioidergic receptors.

It is generally believed that serotonin (5-HT) plays an important role in the regulation of nociception, and this opinion is based on the following observations: 5-HT has been shown to produce antinociception (1–5). Lesions of serotonin-rich raphe nuclei which were followed by a decrease in 5-HT content produced hyperalgesia (6, 7). Depletors of serotonin also produce hyper-reactivity to nociceptive stimuli (8–11), although unexplained differences exist between the various 5-HT depletors such as parachlorophenylalanine, parachloroamphetamine and 5,7-dihydroxytryptamine (12). Possibly, serotonergic systems mediate at least partly the antinociceptive effects resulting from triggering of opioidergic systems either by morphine or by diverse manipulations as revealed by lesion experiments, use of depletors and 5-HT antagonists (13–15).

However, the effects of 5-HT agonists, releasers antagonists or depletors are controversial (16). The purpose of this investigation was to examine in mice the effects of some serotonin modifiers with a test that had allowed us to measure the hypoalgesic and hyperalgesic effects of opioidergic agonists and antagonists, respectively (17). An additional aim of these experiments was to analyze whether the serotonergic system might trigger the enkephalinergic neurons as cautiously suggested by Basbaum and Fields (18) and Besson et al. (19).

MATERIALS AND METHODS

Male Swiss OF₁ mice (obtained from IFFA CREDO, 20–28 g) were maintained in a constant environment with a 12 hr dark and 12 hr light cycle. The animals had free access to commercial food (Extralabo) and tap water ad libitum. At least one week was
Table 1. Time interval in hr between the administration of modifying drugs and the hot plate test

<table>
<thead>
<tr>
<th>Modifying drug</th>
<th>Route</th>
<th>Time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MeODMT*</td>
<td>s.c.</td>
<td>0.25</td>
</tr>
<tr>
<td>dl-5-HTP**</td>
<td>i.p.</td>
<td>0.5</td>
</tr>
<tr>
<td>Quipazine**</td>
<td>i.p.</td>
<td>0.5</td>
</tr>
<tr>
<td>Cyproheptadine***</td>
<td>s.c.</td>
<td>1.25</td>
</tr>
<tr>
<td>Buprenorphine*</td>
<td>s.c.</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Routes of administration and time intervals chosen according to previous publications of this laboratory*, already used in the literature**, or pilot experiments***. All solutions were freshly prepared and administered in a volume of 0.5 ml/20 g (s.c. or i.p.).

allowed for acclimatization to the environment before experimentation. The hot plate test was usually carried out between 2.00 p.m. and 6.00 p.m.

The hot plate technique employed in this study has been described earlier (17). The apparatus consisted of a hot plate on which was placed a restraining cylinder (height 17 cm, diameter 13 cm). The temperature of the hot plate was 64±0.5°C. The “cut-off” time was 1 min. The latency to jump responses was determined by an experienced observer who was blind to treatment-schedules.

Independent groups of 10 mice were used in each experiment. Control groups received at appropriate timing saline (0.9% sodium chloride solution) or the respective solvent. Mice were exposed individually to the hot plate, and one mouse from each group was alternately tested. Each mouse was used only once. The routes and timings of administrations are listed in Table 1.

The statistical significance was calculated by the Student’s t-test. Results are expressed as percentages of concomitant controls with standard error of the means (S.E.M.).

Drugs: Quipazine hydrogen maleate* (Miles, U.S.A.) was dissolved in saline. dl-5-Hydroxytryptophan (dl-5HYP, Sigma, U.S.A.) was dissolved in warm saline. Buprenorphine* (Reckitt & Colman Ltd, England), cyproheptadine hydrochloride* (Merck Sharp & Dohme, U.S.A.) and 5-MeODMT (Serva, France) were dissolved in a few drops of N/10 hydrochloric acid and diluted appropriately with distilled water. The doses were given in terms of the salts.

RESULTS

5-MeODMT (0.1, 0.3, 1, 3, 10 and 30 mg/kg) produced dual effects on nociceptive reactions: low doses (0.1, 0.3 and 1 mg/kg) shortened the latencies to jump. Higher doses (3, 10 and 30 mg/kg) prolonged the latencies to jump. No overt signs were observed after 0.1 mg/kg but reddening of the ears and head twitches appeared after the administration of all higher doses. Further, body and head tremors, stereotyped sniffing and spastic paralysis of the hind limbs followed after 3, 10 and 30 mg/kg. The motor disturbances were dose related and might be at the origin of the increases in latencies to jump. Head twitches, side-to-side head weaving, head tremor, body tremor and splayed hind limbs were already described in mice and rats (20–22).

The doses of quipazine studied were 0.003, 0.03, 0.1, 1, 3, 10 and 30 mg/kg. Quipazine produced clear-cut hyperalgesic effects at low doses (0.03, 0.1 and 1 mg/kg). At higher doses (10 and 30 mg/kg) hyperalgesic
effects were no longer observed. No other signs were noticed after 0.03 and 0.1 mg/kg, whereas reddening of the ears and head twitches were observed in approximately 50% of the mice after higher doses (1, 3, 10 and 30 mg/kg). Vetulani et al. (23) also reported the variability of head twitches produced by quipazine.

dl-5-HTP also produced hyperalgesia at low doses (1 and 3 mg/kg), but they did not reach statistical significance. A high dose (200 mg/kg) induced a moderate but significant increase in latencies to jump, concomitantly with head twitches, hind paw shakes, writhing and autonomic disturbances such as diarrhoea (Fig. 1).

One of the putative 5-HT antagonists, cyproheptadine (studied doses: 0.01, 0.03, 0.3, 1, 10 and 30 mg/kg), had dual effects on the latencies to jump depending on doses (Fig. 1). At lower doses (0.01, 0.03, 0.3 and 1 mg/kg), facilitation of jump occurred. No other overt signs were noticed at these doses. Higher doses (10 and 30 mg/kg) significantly prolonged the latencies to jump, but important motor disturbances such as ataxia, reduced

![Figure 1](image-url)

Fig. 1. Facilitation of a nociceptive reaction in mice by 5-HT agonists (5-MeODMT, dl-5-HTP and quipazine) or by a putative 5-HT antagonist (cyproheptadine). Ordinates: latency of jumping reactions in percentage of control mice (c). Abscissae scale: doses of the drugs in mg/kg, log scale. Vertical bars represent the standard error of the means. Hatched area, mean±standard error of the means for the corresponding control mice (C). *P<0.05, significant difference from controls. § Motor disturbances such as ataxia, reduced muscular tone, etc. Autonomic disturbances such as diarrhea. Groups of 10 mice in each experiment; values are means of at least two distinct experiments. The absolute values of the mean latencies to jump (in sec)±standard error of the mean for the concomitant control mice for 5-MeODMT, quipazine, dl-5-HTP and cyproheptadine, respectively were 28±1, 33±4, 27±6 and 34±5.
muscular tone and flat posture concomitantly appeared; ptosis was also present. That no real antinociceptive effect was produced by these doses was indicated by vigorous paw shakes on the hot plate.

To assess if the hyperalgesic effects of 5-MeODMT involved the opioidergic system, we employed buprenorphine, a ligand of opioid receptors with a slow dissociation rate (24–26), which completely suppressed the pronociceptive effects of naloxone (27, 28). Under similar experimental conditions, buprenorphine did not attenuate the hyperalgesic effects of 5-MeODMT (Fig. 2). Furthermore the antinociceptive effect of morphine (3 mg/kg) was not abolished by 5-MeODMT (0.3 and 1 mg/kg) (unpublished experiments), whereas the former was completely antagonized by naloxone (0.03 mg/kg) under these experimental conditions (28).

**DISCUSSION**

Serotoninergic structures are considered to have important functions in the regulation of nociception as reviewed by Messing and Lytle (14). However, effects of serotonin agonists and antagonists on nociception are still controversial (16). The first objective of this work was to examine some of them in mice with a method (hot plate) allowing the measurement of both reduction and prolongation of latencies to jump; the second aim was to find out if some of these effects might be mediated by the opioidergic system.

With regard to the first objective, we observed facilitation and retardation of nociceptive reactions, but their analysis revealed that they did not fit into a simple scheme of balanced effects. The hyperalgesic effects of low doses of 5-MeODMT described already by Barthelemy et al. (29) were confirmed. Similar effects of 5-MeODMT and tryptamine have been recently reported in the tail flick test in rats (30, 31). Hyperalgesic effects were also obtained after low doses of quipazine and cyproheptadine. These results might be concordant with the often-held opinion that manipulations which reduce brain or spinal cord serotoninergic transmission are associated with increased sensitivity and/or reactivity to nociceptive stimuli (14). Low doses of 5-MeODMT has been shown to inhibit the firing of serotoninergic raphe neurons by acting on presynaptic serotonin receptors (32–34). This agrees well with the observed reduction in jumping latencies. Higher doses of 5-MeODMT produced motor disturbances which resulted in an increase in jumping latencies. This might, in addition, correspond to triggering of postsynaptic serotoninergic receptors. Quipazine might also be presumed to trigger presynaptic 5-HT receptors as it favors the accumulation of 5-HT at the same sites (35, 36). Higher doses of quipazine did not produce sig-
significant reduction in jumping latencies, possibly as a result of a release of 5-HT triggering presynaptic as well as post-synaptic serotoninergic receptors. Low doses of dl-5-HTP did not produce any significant reduction in jumping latency. High doses prolonged the jumping latencies which might indicate triggering of post-synaptic serotoninergic receptors, but the simultaneous occurrence of autonomic and motor discomfort precluded such a speculation. Cyproheptadine is reputed to be an antagonist at postsynaptic 5-HT receptors, but not at presynaptic ones (37–39); thus, a preferential blockade of postsynaptic 5-HT receptors would be expected to enhance nociceptive reactions. The site of action of cyproheptadine might be at the spinal level in accordance with its antagonism of the antinociceptive effects of serotonin administered intrathecally (5). However other interpretations might be proposed: firstly, 5-MeODMT and quipazine also produced peripheral hyperemia which might be, at least partly, responsible for the hyperalgesia. Secondly, 5-MeODMT is also reputed to produce spinal excitatory effects (40, 41).

On the other hand, prolongation of jumping latencies were observed after high doses of 5-MeODMT and cyproheptadine. 5-MeODMT and cyproheptadine thus produced dual effects on nociception depending on the dose; similar biphasic effects of 5-MeODMT had been recently described in the tail flick test (30). However, the increases of latencies to jump by 5-MeODMT and cyproheptadine were concomitant with motor disturbances so that it is far from ascertained whether they corresponded to real antinociceptive properties. Similarly, some doubt might be casted upon the high doses of dl-5-HTP as the increases of latencies to jump might be consecutive to the discomfort produced by concomitant writhing and diarrhea. Additionally 5-HTP loading may not always lead to increased concentrations of the neurotransmitter (5-HT) at biologically active postsynaptic receptor sites. Johnson et al. (42) demonstrated that 5-HTP decarboxylation occurs in catecholamine neurons with a depleting action on these amines. Thus, in mice hyperalgesia induced by low doses of 5-MeODMT, quipazine and cyproheptadine might fit with the inhibitory function attributed to serotoninergic neurons; but higher doses of these substances produced important interfering side-effects, possibly related to the simultaneous triggering of various types of 5-HT receptors described (43). Under these conditions, it is difficult to distinguish clearly their effects on nociception itself. Both naloxone and buprenorphine are known to interact with opioid receptors. Thus the fact the hyperalgesic effects of 5-MeODMT are not antagonized by buprenorphine indicates that 5-MeODMT does not directly interact with opioid receptors. From that fact, however, it is difficult to say that serotoninergic pathways do not apparently impinge upon opioidergic structures.

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