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NOCICEPTION IS ENHANCED AFTER LOW DOSES AND REDUCED AFTER HIGH DOSES OF THE SEROTONIN RECEPTOR AGONIST 5-METHOXY-N,N-DIMETHYLTRYPTAMINE

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SUMMARY

The effects on pain sensitivity of intracerebroventricular injections of 5-methoxy-N,N-dimethyltryptamine were tested by the tail-flick method. Following administration of 1.6, 3.1, 6.3, 12.5 and 25 μg (n = 8 for each dose), tail-flick latencies were reduced by 13–24%. Fifty and 100 μg caused a biphasic response (hyperalgesia followed by analgesia), whereas 400 μg increased mean latencies by 28–39%. The hyperalgesia observed after low doses was most likely due to reduced activity in descending serotonergic neurons following presynaptic stimulation. Higher doses caused analgesia, probably by stimulating spinal postsynaptic serotonergic receptors as well.

There is considerable evidence for the existence of presynaptic regulation of ascending serotonergic neurons. Iontophoretic application of 5-hydroxytryptamine (5-HT) agonists [1, 3] as well as systemic administration [7] inhibits the firing of serotonergic neurons in the dorsal raphe nucleus. Iontophoretic and intravenous administration of the 5-HT receptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) revealed that this drug has a preference for presynaptic receptors [3]. Low systemic doses of the drug caused depression of serotonergic dorsal raphe cell activity with concomitant behavioral changes similar to those seen after LSD administration [9]. On the other hand, higher doses of the drug given systemically caused behavioral effects probably mediated by postsynaptic 5-HT receptors [4–6]. In a recent investigation [2], we concluded that there is a tonic descending serotonergic inhibition of the tail-flick (TF) response to radiant heat in rats. This

inhibition could be blocked by 5-HT receptor antagonists, as well as by spinal transection. On the other hand, 5-HT receptor agonists like 5-MeODMT increased the inhibition of the TF response, spinal animals being supersensitive to this stimulation of postsynaptic receptors.

On the basis of these investigations, it may be expected that stimulation of postsynaptic 5-HT receptors in the spinal cord will inhibit the TF response, while stimulation of putative presynaptic 5-HT receptors of the descending 5-HT system may have the opposite effect due to inhibition of firing of raphe-spinal neurons. If 5-MeODMT in low doses preferentially affects presynaptic receptors, and activates postsynaptic receptors when given in higher doses, it may be possible to show both increases (low doses) and decreased (higher doses) in sensitivity to radiant heat, depending on the dose of 5-MeODMT used. In the present experiment this hypothesis was tested, 5-MeODMT being administered by intracerebroventricular (i.c.v.) injections.

Thirty-two male Wistar rats weighing 260–290 g at the time of surgery were individually housed with free access to food and water. Testing took place in the middle of the light phase of a 12 : 12 h light : dark schedule.

Surgery was carried out under anesthesia of pentobarbital (40 mg/kg) and chloral hydrate (130 mg/kg) given i.p. A guide cannula prepared from a hypodermic needle (o.d. 0.6 mm) was inserted stereotactically through a drilled hole in the skull. The following coordinates were used: 0.5 mm caudal to bregma, 1.5 mm lateral to the midline, 2 mm ventral to the surface of the cortex. The skull was positioned with bregma and lambda in the same horizontal plane. The hub of the guide cannula was fastened to the skull by dental acrylic, retained by four screws in the bone.

For one week prior to surgery, during the 7–10-day recovery period, and throughout the time of testing, the animals were handled daily according to a schedule simulating the test-procedure.

Injections were performed by means of a stainless steel cannula (o.d. 0.35 mm), penetrating 1 mm beyond the tip of the guide cannula. The injection cannula was connected to a microsyringe via PE50 tubing (Clay Adams), and injections carried out by a perfusion pump. Twenty-five μl of solution were injected in 90 sec. The injection cannula was left in situ for a further 60 sec.

Tail-flick latency was tested using an IITC Inc. Mod. 33 Analgesiameter, in which radiant heat was focused on a spot 1–2 cm from the tip of the tail. Beam intensity was adjusted to give a reaction time of 4–5 sec in control rats. During each experiment, the procedure was repeated at 20 min intervals for a total of 8 trials. A preinjection basal level was established for each rat as the mean latency of trials 2 and 3. Ten minutes after the 3rd trial, 5-MeODMT was injected. Each rat received several doses of the drug. The doses were given in random order, with at least one week between injections. 5-MeODMT (Sigma) was dissolved in distilled water containing 0.2 mg/ml ascorbic acid. The solution was prepared immediately before injection.

To allow comparison of data obtained with slightly different beam intensities, the scores for each day of testing were transformed to percentages of the mean of all basal level scores of that day. Analysis of variance was carried out in order to test for differences between trials for each dose. When a significant trial effect was present, after-drug trials were compared with basal levels using Student's *t*-test for dependent measures.

5-MeODMT was injected in doses from 0.8 to 400 μg. Except for the two lowest doses tested, 0.8 and 1.6 μg, each injection was followed by a significant change in TF latency (Fig. 1). Low to moderate doses of the drug (3.1–50 μg) caused a rapid facilitation of the response in that the mean TF scores 10 min after start of the injection were significantly lower than basal levels ($P < 0.05$). The peak effect was observed after 10 min and was dose-dependent up to 12.5 μg where the mean TF latency was reduced to 76% of basal level ($P < 0.001$). Following 6.3 μg, the TF response was still facilitated 70 min after injection ($P < 0.05$), while higher doses produced only a short-lasting significant reduction in TF latency. At the highest doses, a dose-dependent increase in TF latencies was found. A dose of 100 μg 5-MeODMT caused a significant elevation of the latency to approximately 120% of basal level, at 30 min ($P < 0.01$), 50 min ($P < 0.05$), and 70 min ($P < 0.05$) after injection. Administration of 400 μg was followed by significant rise in TF latency

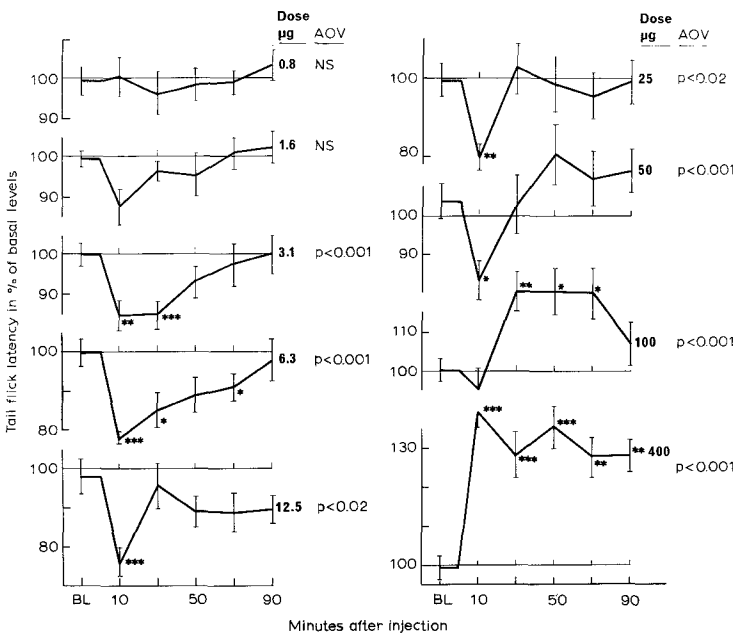

Fig. 1. The effect of 5-MeODMT on the tail-flick response to radiant heat (mean ± S.E.M., n = 8). AOV = Analysis of variance for repeated measures, p-values for differences between trials. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ vs basal level (Student's $t_{dep.}$).

after 10 min ($P < 0.001$), and the reflex remained significantly depressed throughout the 90 min test session.

In addition to modulating the TF response, 5-MeODMT caused motor disturbances probably due to postsynaptic serotonin receptor stimulation [6], and autonomic changes (increased urination and defecation). These effects were easily recognizable and progressively more intense when doses of 50 μg and higher were given.

Thus, low doses of 5-MeODMT increased, and high doses decreased sensitivity to radiant heat, while intermediate doses induced a biphasic response with an initial increase followed by a decrease in sensitivity. These data are best explained by postulating that low i.c.v. doses of 5-MeODMT enhance nociception by stimulating presynaptic 5-HT receptors, thereby inhibiting the firing of serotonergic neurons, while high doses in addition stimulate postsynaptic 5-HT receptors, resulting in reduced sensitivity. With intermediate doses there may be an initial stimulation of presynaptic receptors, which is later outweighed by postsynaptic stimulation. The reason for the different time courses for the stimulation of the two types of receptors is not known. Possibly the postsynaptic receptors are localized further away from the injection site (e.g. in the spinal cord) than the presynaptic receptors (e.g. on or near the soma of the raphe-spinal serotonergic neurons).

The possibility that both the increase and the decrease in sensitivity were due to stimulation of postsynaptic receptors has to be considered. Lesions of ascending 5-HT pathways have been reported to decrease squeal thresholds without changing TF latencies to noxious stimulation, implying that 5-HT receptors receiving input from these pathways may play a role in the expression of emotional reactions to noxious stimulation [12]. There is, however, no evidence in the literature to link stimulation of postsynaptic 5-HT receptors anywhere in the central nervous system with increased pain sensitivity as measured by TF or other reflex responses. It seems unlikely therefore that postsynaptic stimulation caused the increased sensitivity reported here although further investigations are warranted.

Even though the location of the pre- and postsynaptic receptors cannot be determined from the present data, other findings to be taken into consideration [2, 8, 10, 11] suggest postsynaptic spinal 5-HT receptors as likely mediators of the increased TF latency seen after high doses of 5-MeODMT. Reduced latency would be expected when presynaptic 5-HT receptors on the raphe-spinal 5-HT neurons are stimulated, reducing the activity of these neurons.

Further studies are needed to exclude the possibility that the reported effects may be caused by interactions between 5-MeODMT and systems other than serotonergic transmitter systems. The maximum depression of TF threshold found in this experiment (24%) was, however, very close to the reduction of approximately 25% seen under similar test conditions, when optimal doses of various 5-HT blockers were given systemically [2].

In conclusion, the data indicate that 5-MeODMT acts on pre- and postsynaptic

receptors of the descending 5-HT system in a manner similar to that known for the ascending system. The results further imply that the dose of serotonergic agonist given determines whether the drug will mimic or antagonize endogenous serotonin in a particular behavioral test.

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