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Intrathecal noradrenaline restores 5-methoxy-N,N-dimethyltryptamine induced antinociception abolished by intrathecal 6-hydroxydopamine

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Summary. Intrathecal administration of 6-hydroxydopamine (6-OHDA) abolished the antinociceptive effects of acute administration of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 1 mg/kg, s.c.) in the hot-plate, tail-flick and shock titration tests of nociception. The antinociceptive effects of 5-MeODMT, abolished by the prior intrathecal 6-OHDA treatment, were restored by intrathecal administration (2 or 1 μg) of noradrenaline (NA), immediately prior to 5-MeODMT, in all three tests of nociception. Biochemical analysis confirmed severe NA depletions (95 percent loss) in the lumbar and thoracic regions of the spinal and much lesser dopamine depletions (25–35 percent loss). Intrathecal 5,7-dihydroxytryptamine (5,7-DHT) attenuated 5-MeODMT induced antinociception in the tail-flick test and combined NA + 5-Me ODMT induced antinociception in the hot-plate and tail-flick tests. Intrathecal administration of 5,7-DHT caused a severe depletion of 5-hydroxytryptamine in the lumbar region of the spinal cord. The present findings demonstrate further the modulatory role of NA upon serotonergic systems in nociception and indicate the necessity of NA availability for induction of 5-MeODMT analgesia.

Keywords: Intrathecal 6-OHDA, 5-MeODMT, analgesia, blockade, intrathecal NA, restoration, NA depletion, rats.

Introduction

Descending 5-hydroxtryptamine (5-HT) pathways originating in the raphe obscurus, raphe pallidus and raphe magnus nuclei (Dahlström and Fuxe, 1965) have been implicated in the modulation of afferent nociceptive transmission (Belcher et al., 1978; Davis and Roberts, 1981; Oliveras et al., 1979; Yaksh and Wilson, 1979). Although much data indicate 5-HT involvement in nociception, the function of neuronal 5-HT systems in the spinal cord remains diffuse (Roberts, 1984). Activation of the dense noradrenergic innervation to

the spinal cord (Westlund et al., 1983) has been shown to produce an inhibitory effect on dorsal horn neurons in the spinal cord (Engberg and Marshall, 1971). Also, application of noradrenaline (NA) into the spinal cord subarachnoid space causes a notable antinociceptive effect (Howe et al., 1983; Kuraishi et al., 1979; Reddy et al., 1980), whereas blockade of noradrenergic receptors (Sagen and Proudfit, 1984) or destruction of NA terminals (Proudfit and Hammond, 1981) have resulted generally in an hyperalgesia effect, but not always.

It has previously been demonstrated that the analgesia produced by systemic administration of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) or intrathecal 5-HT or systemic administration of several other 5-HT agonists is dependent upon an intact spinal noradrenergic system (Archer et al., 1985; Archer et al, 1986a; Minor et al., 1985; Post et al., 1985). The purpose of the present investigation was to demonstrate whether or not the blockade of 5-MeODMT induced analgesia, resulting from prior intrathecal administration of 6-hydroxydopamine (6-OHDA) which drastically depletes NA in the spinal cord, could be restored by intrathecal administration of NA just prior to the subcutaneous injection of 5-Me ODMT. As a control for the possible involvement of spinal 5-HT systems in this regard the effects of spinal 5-HT depletion after intrathecal administration of 5,7-dihydroxytryptamine (5,7-DHT) upon 5-MeODMT and NA induced analgesia were assessed.

Material and methods

Subjects

Male Sprague-Dawley rats weighing 350–360 g (aged 90–95 days) at arrival were randomly allocated to the different treatment conditions and allowed a two week acclimatization to the laboratory. They were housed, on sawdust bedding material, in groups of 3 or 4 animals under laboratory conditions with a 12 h on/12 h off lighting schedule (lights on at 6.00 h) in a room thermostatically maintained at 21 ± 1 °C for up to four weeks prior to shock-titration, tail-flick and hot-plate testing for pain sensitivity. After the operations, the animals were allowed a 10-day recovery period in the same housing conditions. Behavioural testing was carried out during the hours of light (8.00–14.00 h).

Apparatus

Shock-titration testing was carried out in a test box ($25 \times 26 \times 30$ cm, Campden Instruments Ltd, London), wired to present scrambled footshock and balanced upon four strain gauges (one at each of the four corners). The test box was designed so that any sufficient force exerted by a rat's movement was translated into voltage and recorded on a voltimeter, as described previously (Archer et al., 1985). Shocks (0.75 s) were delivered to the grid floor of the test box by a shock generator and shock scrambler (Models 521/e and 521/s, Campden Instruments Ltd, London). Shock titrations were continued upwards or downwards depending upon nonresponse or response at the 150 μA intensity (starting shock level), by utilizing the 50, 75, 100, 150, 200, 250, 300, 400 and 500 μA intensities in a step-wise manner, following a 3-min habituation to the test box. Shock intensities were measured before (Pre-test) and after (Test) the injection of the test drug. The time-gap between shocks was 15–20 secs.

The hot-plate and tail-flick methods have been developed from the techniques that have

been described in detail elsewhere (D'Amour and Smith, 1941; Eddy and Leimbach, 1951). Hot-plate testing was conducted with an electrically heated and thermostatically controlled aluminium surface set at 58 ± 0.2 °C.

The animals were adapted to the test procedure by a prior exposure to the test apparatus, 15 min before the acute administration of the test drugs (NA and 5-MeODMT). The test latencies were scored before (Pre-test) and after (Test) the injection of the test drug. During testing the rats were confined to the hot-plate by a plexiglass chamber (27 × 28 × 26 cm). The latency to a licking of the paws or the vigorous shaking of a paw was recorded (pain response latency). Pre-test latencies of the different groups in each of the experiments varied within the range 4.0 to 6.5 secs for the pain response latencies. The tail-flick test was conducted by directing a concentrated light beam producing heat on the surface of the tail. The rats were adapted to the restraining tube on each of three consecutive daily sessions (lasting 10 min) on the three days prior to testing. The test latencies were scored, on two occasions, 15 min before (Pre-tests) and after (Test) the acute drug injections. During testing a rheostat controlled light beam was focussed on the tip of the tail and the time interval from the onset of the heat stimulus to the flick of the tail was recorded (Tail-flick latency). Pre-test latencies varied within the range 3.2 to 4.8 secs for the tail-flick latencies. Each of these critical experiments was performed "blind" i.e. the experimenter that tested the rats was completely unaware of the treatments.

Operations

In each of the animals an in-dwelling polyethylene catheter (PE 10), previously stretched to double length, was inserted 8.5 cm into the spinal subarachnoidal space through a slit in the atlanto-occipital membrane, with the tip of the catheter in the lumbar subarachnoidal space (Yaksh and Rudy, 1976). The catheters were inserted 10–14 days before antinociception testing. For Experiments 1 and 2, 6-OHDA (20 μg as hydrochloride in 10 μl vehicle, 0.05% ascorbic acid in 0.9 saline) was injected over one min, 10 min after systemic administration of pargyline (20 mg/kg, i.p.). The sham operated rats were operated with in-dwelling catheters, as above, except that the vehicle only was administered intrathecally (15 μl) 10 min after pargyline. For Experiment 3, 5,7-DHT (20 μg as creatinine sulphate in 10 μl 0.9% saline) was injected over a period of 15 sec, 30 min after systemic administration of DMI (20 mg/kg, i.p.). The sham operated rats were operated with in-dwelling catheters, as above, except that saline only was administered intrathecally (15 μl) 10 min after DMI. Any rats showing motor dysfunction after surgery were rejected. To ascertain correct placement of the catheters all the rats were tested for motor impairment by intrathecal injection of 15 μl of 50 mg/ml Xylocain (Astra Läkemedel AB, Södertälje, Sweden) 2–3 days after the operations. If a motor blockade of the hind legs was not observed, these rats were rejected (Minor et al., 1985).

Treatment drugs

The treatment drugs used included: 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Sigma Chemicals, St. Louis, U.S.A.), 6-hydroxydopamine hydrochloride (6-OHDA) (Sigma Chemicals), 5,7-dihydroxytryptamine (5,7-DHT) (Sigma Chemicals), pargyline hydrochloride (Saber Laboratories, Illinois, U.S.A.), and desipramine hydrochloride (DMI) (Ciba-Geigy, Basle, Switzerland). 5-MeODMT was dissolved in distilled water, pargyline and DMI in 0.9% saline, 6-OHDA and 5,7-DHT as above.

Statistical analysis

All the results are expressed as means ± s.e.m. In each test the measures of nociception from each rat are expressed as a percentage of the pre-test response latency, i.e.

$\frac{\text{Test}}{\text{Pre-Test}} \times 100$. For each nociception test in each experiment (hot-plate, tail-flick or shock titration) the data were subjected to two-way ANOVA (Snedecor and Cochran, 1967). Pairwise testing between groups within tests (1, 2 or 3) and between tests within groups was performed with the Tukey HSD test (Kirk, 1968). The 1% level of significance was used throughout unless where otherwise stated.

Biochemical assay

Endogenous monoamine concentrations were determined using high pressure liquid chromatography with electrochemical detection (l.c.e.d.) according to Keller et al. (1976) as modified by Jonsson et al. (1980). Endogenous 5-HT was assayed using l.c.e.d. (Ponzio and Jonsson, 1979). The catecholamine and 5-HT values are expressed as $ng \cdot g^{-1}$ wet weight of the tissue, based on internal standard measurements. Rats were sacrificed within one week of testing. Lumbar and thoracic spinal cord regions were rapidly dissected out on ice and stored at —70 °C until analysis.

Experiment 1

For each nociception test (tail-flick, hot-plate or shock titration) 3 tests were performed, common to both the 6-OHDA and SHAM rats. Test 1: Analgesia testing after either saline + MeODMT or saline + saline to establish the blockade of 5-MeODMT induced analgesia by 6-OHDA. Test 2: Testing after either saline + 5-MeODMT or NA + 5-MeODMT to establish the recovery of 5-Me ODMT induced analgesia by intrathecal NA administration. Test 3: Testing after either NA + saline or saline + saline to establish the effects of NA on measures of nociception.

Procedure

Fourteen 6-OHDA rats and fourteen SHAM rats were divided into two groups (n = 7) for the three tests. Test 1, the 6-OHDA and SHAM groups received intrathecal saline (10 µl) immediately before subcutaneous injection of either 5-MeODMT (1 mg/kg) or saline (5 ml/kg). One week later, Test 2, these groups received either intrathecal saline or NA (2 µg), respectively, immediately before 5-MeODMT (1 mg/kg). One week later, Test 3, these groups received either intrathecal NA (2 µg) or saline, respectively, immediately before saline. For each test, the pre-tests were performed 15 min before the drug administration and testing was carried out 10 min later.

Experiment 2

Although all three tests, tail-flick, hot-plate and shock titration, clearly indicated a reinstatement of 5-MeODMT analgesia after NA injection, in the tail-flick test NA, by itself, caused an analgesic effect. In Experiment 2, therefore, a lower dose of NA (1 µg) was used and a direct comparison between the NA + sal and NA + 5-MeODMT groups was made.

Procedure

Sixteen 6-OHDA rats were divided into two groups (n = 8) for two tests. In Test 1, both groups received 5-MeODMT (1 mg/kg, s.c.). In Test 2, one group received NA (1 µg) immediately prior to 5-MeODMT (1 mg/kg) and the other group received NA (1 µg) immediately prior to saline. Tail-flick, hot-plate and shock titration testing were performed 10 min after the administrations in each case.

Experiment 3

In order to ascertain whether or not spinal serotonergic mechanisms were involved in the blockade and reinstatement of 5-MeODMT induced analgesia, 5,7-DHT and SHAM rats

were tested, first with 5-MeODMT, and second with either NA + 5-MeODMT or NA + saline.

Procedure

Sixteen 5,7-DHT and sixteen SHAM rats were divided into two groups (n = 8) for the two tests. Test 1; all four groups received 5-Me ODMT (1 mg/kg, s.c.) 10 min before testing. Test 2: one 5,7-DHT and one SHAM group received NA (1 μg) intrathecally immediately before 5-MeODMT (1 mg/kg), the other groups received NA immediately before saline, and nociception testing occured 10 min later.

Results

Experiment 1

Tail-flick test

ANOVA indicated a significant Groups × Tests interaction effect, $F(6, 66) = 30.2$; subsequent Tukey tests revealed the following differences: In the SHAM rats for Test 1 5-Me ODMT caused reliable analgesia (see Fig. 1A, top panel); this effect was blocked for the 6-OHDA rats. For Test 2, administration of intrathecal NA in 6-OHDA rats caused a significant and considerable analgesic response to 5-MeODMT (see Fig. 1A, middle panel).

In Test 3, intrathecal NA was found to cause a significance analgesic effect (see Fig. 1A, bottom panel).

Hot-plate test

ANOVA indicated a significant Groups × Tests interaction effect, $F(6, 66) = 14.4$ and the following effects were obtained: In Test 1, the analgesic effects of 5-Me ODMT (1 mg/kg) were abolished by 6-OHDA treatment (see Fig. 1B, top panel). In Test 2, the analgesic effects of 5-Me ODMT were reinstated completely by the prior administration of NA (2 μg) and in Test 3 it was revealed that the dose of NA used did not cause any analgesic effects in this test of nociception (see Fig. 1B, middle and bottom panels, respectively).

Shock titration test

ANOVA indicated a significant Groups × Tests interaction effect, $F(6, 65) = 7.6$, as a result of the following significant pairwise comparisons: In Test 1, no significant analgesic effect of 5-MeODMT was obtained in the SHAM rats. However, the Sal + 5-MeODMT group in SHAM condition showed significantly more analgesia than the Sal + 5-MeODMT group in the 6-OHDA condition ($p < 0.05$) (see Fig. 1C, top panel). In Test 2, once again the analgesic effects of 5-MeODMT in 6-OHDA rats was reinstated by intrathecal NA administration (see Fig. 1C, middle panel). Test 3 confirmed that the data from the hot-plate test 3 in that no effect of NA (2 μg) per se was obtained (Fig. 1C, bottom panel). All three tests of nociception provided strong evidence that intrathecal administration of NA could reinstate the blockade of 5-MeODMT induced analgesia resulting from 6-OHDA treatment.

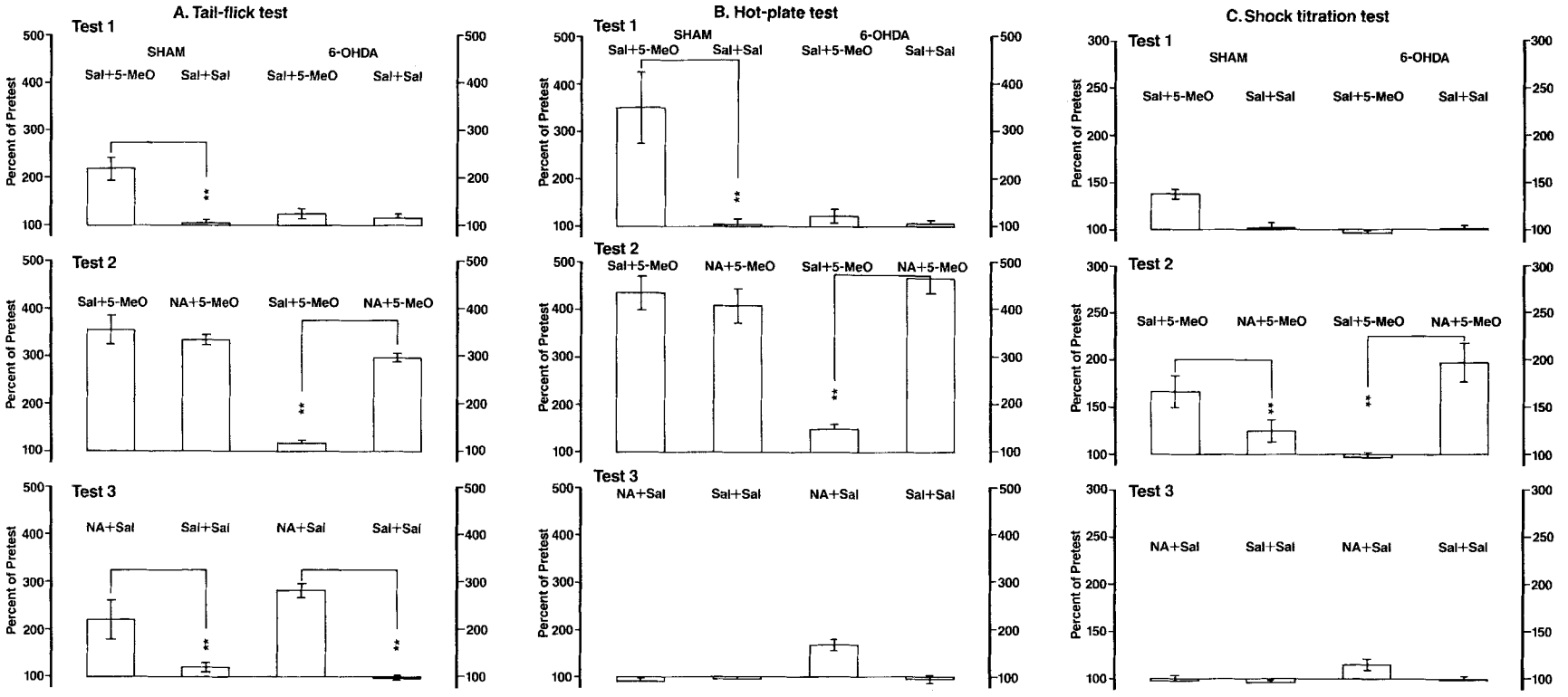

Fig. 1. Blockade of Me ODMT induced analgesia by intrathecal 6-OHDA treatment. Reinstatement of 5-MeODMT induced analgesia by intrathecal NA administration. Test 1: Intrathecal saline was administered immediately before either 5-MeODMT (1 mg/kg) or saline. Test 2: Intrathecal NA (2 μg) or saline were administered immediately before 5-MeODMT (1 mg/kg). Test 3: Intrathecal NA (2 μg) was administered immediately before saline. **A** Tail-flick data, **B** hot-plate, and **C** shock titration. In each case tail-flick, hot-plate, and shock titration tests were performed 10 min after the drug administrations. ** $p < 0.01$, Tukey HSD test

Experiment 2

Tail-flick test

ANOVA indicated a significant Groups × Tests interaction effect, [$F(1,28) = 75.1$] as a result of significant and considerable analgesia following administration of NA + 5-MeODMT. This result was obtained in the hot-plate test [$F(1,28) = 60.7$] and in the shock titration test [$F(1,28) = 8.8$], too. In neither of the nociceptive tests did the 5-MeODMT administration at Test 1 cause any analgesic effect (see Fig. 2, Test 1, left-hand panel for each figure: top, middle, and bottom). Thus, once again it was shown that 6-OHDA abolished the 5-MeODMT induced analgesia and prior administration of NA (1 μg) reinstated the analgesic effect itself. However, no indication of any significant analgesic effect of NA by itself was obtained in any of the nociception tests.

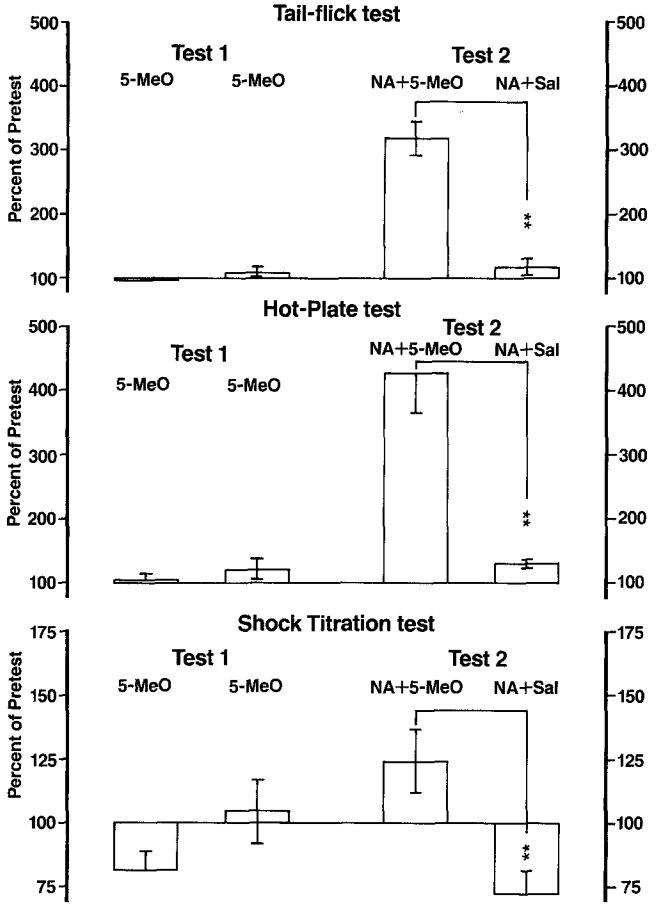

Fig. 2. Reinstatement of 5-MeODMT induced analgesia by intrathecal NA administration. All the rats were administered 6-OHDA (20 μg in 10 μl) intrathecally two weeks before testing. Test 1: 5-MeODMT (1 mg/kg) was administered to both groups. Test 2: Intrathecal NA (1 μg) was administered immediately prior to either 5-MeODMT (1 mg/kg) or saline. Tail-flick, hot-plate, and shock titration testing were performed 10 min after the drug administrations. ** $p < 0.01$, Tukey HSD test

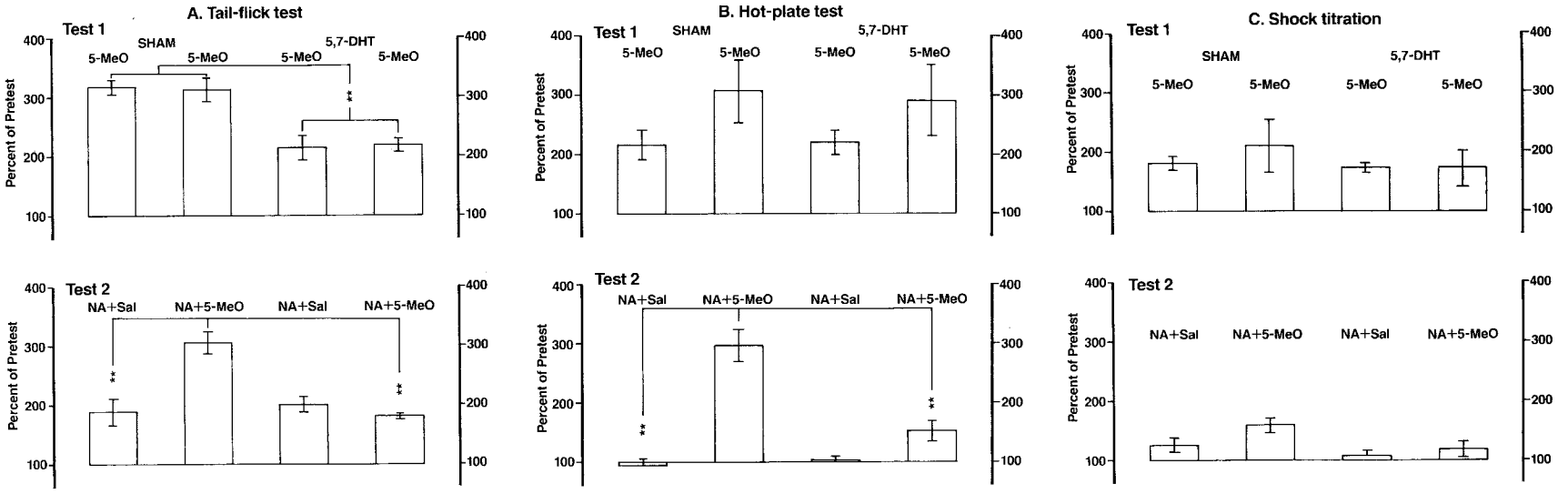

Fig. 3. Test-dependent attenuation of 5-MeODMT induced analgesia and NA + 5-MeODMT induced analgesia by intrathecal 5, 7-DHT treatment. Test 1: 5-MeODMT (1 mg/kg) was administered to all four groups. Test 2: Intrathecal NA (1 μg) was administered immediately prior to either 5-MeODMT (1 mg/kg) or saline, for both SHAM and 5,7-DHT rats. Tail-flick, hot-plate, and shock titration testing were performed 10 min after the drug administrations. ** $p < 0.01$, Tukey HSD test

Experiment 3

Tail-flick test

ANOVA indicated a significant Groups × Tests interaction effect, $F(3, 51) = 4.9$, resulting from a significantly lower percent of pre-test by the 5,7-DHT rats during Test 1. During Test 2, the SHAM-NA + 5-Me ODMT group indicated a greater analgesia than the SHAM-NA + Saline group and also the 5,7-DHT — NA + 5-MeODMT group (see Fig. 3A, top and bottom panels, respectively).

Hot-plate test

ANOVA revealed a significant Groups × Tests interaction $F(3, 50) = 7.8$, resulting from a significantly greater analgesic effect by the SHAM-NA + 5-MeODMT group compared with the SHAM-NA + Saline and the 5,7-DHT — NA + 5-MeODMT group during Test 2, which confirms the result of the tail-flick (see Fig. 3B, bottom panel).

Shock titration test

ANOVA did not indicate any significant Groups × Tests interaction nor any significant Groups effect for the shock titration data.

Table 1. Monoamine assays on the spinal cords (lumbar and thoracic regions) of the intrathecal 6-OHDA and saline treated rats in Experiment 2, and intrathecal 5,7-DHT and saline treated rats in Experiment 3

		ng/g wet Weight tissue	
Experiment 2:		Lumbar	Thoracic
	6-OHDA	5.5 ± 4**	5.6 ± 3**
Noradrenaline	(%)	(2)	(2)
	Saline	301 ± 38	296 ± 22
	6-OHDA	10.2 ± 4*	9.8 ± 3.5*
Dopamine	(%)	(77)	(65)
	Saline	13.2 ± 4.5	15.2
Experiment 3:			
	5.7-DHT	39 ± 8**	—
5-Hydroxytryptamine	(%)	(11)	
	Saline	361 ± 30	—

6-OHDA (20 µg in 10 µl) was injected intrathecally 10 min after pargyline (20 mg/kg, i.p.). 5,7-DHT (20 µg in 10 µl) was injected intrathecally 30 min after desipramine (20 mg/kg, i.p.)
Values are expressed as means ± s.e.m. of 6 animals
(%) Percent of control value
** $p < 0.001$, * $p < 0.01$ Students t-test

Thus, the results of Experiment 3 indicate an attenuation of the analgesic effects of 5-MeODMT in the tail-flick test and an attenuation of the analgesic effects of NA + 5-MeODMT in the tail-flick and hot-place test. In the 6-OHDA experiments the opposite result was obtained since NA treatment reinstated the analgesic effect of 5-MeODMT that was abolished by the previous administration of 6-OHDA.

Biochemical assays

The monoamine assays of the rats used in the above experiments are presented in Table 1. Intrathecal administration of 6-OHDA caused severe NA depletions (2% of control values) in both the lumbar and thoracic regions of the spinal cord whereas dopamine (DA) concentrations were reduced to a much lesser extent although significantly so (77 and 65% of control values, respectively). Intrathecal 5,7-DHT caused a severe depletion of 5-HT concentrations (11% of control values).

Discussion

The present experiments demonstrated that the analgesia induced by 5-MeODMT (1 mg/kg) was blocked completely by prior intrathecal administration of 6-OHDA in all three tests of nociception. Intrathecal administration of NA, at either the 2 μg or 1 μg dose level, restored the analgesic effects of 5-MeODMT in all three tests of nociception. However, in the tail-flick test, the higher dose of NA (2 μg) by itself caused a significant analgesic effect but not the lower dose (1 μg). Prior intrathecal administration of 5,7-DHT attenuated 5-MeODMT induced analgesia in the tail-flick test, as we have observed previously (Archer et al., 1986a). Finally, the 5,7-DHT treatment attenuated also the combined analgesic effect of NA + 5-MeODMT in both the tail-flick and hot-plate tests. No restoration of 5-MeODMT analgesia by intrathecal NA was obtained in the tail-flick, hot-plate or shock titration tests for the 5,7-DHT treatment experiment. The biochemical analyses confirmed severe NA depletions as a result of intrathecal 6-OHDA treatment and a lesser but significant DA depletion in the lumbar and thoracic regions of the spinal cord. Severe 5-HT depletions resulted from intrathecal 5,7-DHT administration.

The blockade of 5-MeODMT induced analgesia by NA depletion confirms our previous findings (e.g. Archer et al., 1985; Danysz et al., 1986). The restoration of 5-MeODMT induced analgesia by intrathecal NA administration cannot be explained on the basis of the antinociceptive properties of the 2μg dose of NA alone in the tail-flick test. Firstly, the hot-plate and shock titration results compromise this explanation, especially since the NA + saline test (Test 3) was made in a different groups and a week later than the NA + 5-MeODMT test (Test 2). Secondly, the results from Experiment 2 offer a direct comparison, NA + 5-MeODMT versus NA + saline, in the tail-flick test (Test 2). Taken together, these finding develop and reinforce the postulate that noradrenergic-serotonergic interactions in spinal nociception transmission un-

derly the analgesia responses to 5-MeODMT obtained from the various tests of nociception. The results of the 5,7-DHT lesion experiment were radically different from the 6-OHDA experiments in the demonstrations of 5-MeODMT and NA + 5-MeODMT induced analgesia that were obtained. The attenuation of the effects of 5-MeODMT by 5,7-DHT in the tail-flick test confirms previous findings (Archer et al., 1986a), whereas the attenuation of the combined effects of NA + 5-MeODMT in the tail-flick test may be explained on the basis of tail-flick data, i.e. the attenuation of 5-Me ODMT induced analgesia in the tail-flick test. However, this explanation cannot be used in the case of the hot-plate where previous findings indicate that spinal 5-HT depletion caused an increased analgesic response to acute 5-MeODMT or 5-HT administration (Archer et al., 1986a; Howe and Yaksh, 1982).

The major finding that intrathecal NA administration restored the antinociceptive effects of 5-MeODMT abolished by intrathecal 6-OHDA treatment appears to be consistent with other investigations. Hammond et al. (1985) found that stimulation of the nucleus raphe magnus and the nucleus reticularis paragigantocellularis increased the efflux of NA, but not 5-HT, after the animals had been pretreated with fluoxetine and desipramine. In this case the interaction is assumed to exist between a descending 5-HT system and endogenous NA. Sagen and Proudfit (1981) have proposed that the activation of nucleus raphe magnus neurons induces a hypoalgesia that is mediated, at least partially, by activation of spinally projecting NA neurons. Furthermore, the antinociception induced by focal electrical stimulation of the nucleus raphe magnus or the nucleus reticularis paragigantocellularis, or microinjections of either L-glutamate, morphine or phentolamine into these nuclei, is antagonised by intrathecal administration of NA antagonists or spinal NA depletion (Jensen and Yaksh, 1984; Sagen et al., 1983; Satoh et al., 1980). Much other evidence appears to support important central 5-HT — NA interactions: e.g., Rappaport et al. (1985) found that intracerebroventricular administration of 5,7-DHT increased α_1 and α_2 adrenoceptors in the cortex and hippocampus. Also, low doses of clonidine suppressed shock induced jumping behavior induced by 5-HT agonists (Nishikawa et al., 1983).

Some substantial evidence appears to suggest that the analgesic effects of nucleus raphe magnus (NRM) stimulation is mediated by both raphe-spinal serotonergic neurons and bulbospinal noradrenergic neurons (Hammond et al., 1980; Jensen and Yaksh, 1984; Sagen et al., 1983). Thus, the antinociception resulting from electrical stimulation of the NRM can be antagonised by intrathecal injections of either 5-HT or NA receptor antagonists (Hammond and Yaksh, 1984), and intrathecal injections of either 5-HT or NA can produce antinociception (Howe et al., 1983; Schmauss et al., 1983). Other evidence suggest cholinergic involvement in NRM activity (Behbehani, 1982), and it has been shown that microinjections of the cholinergic agonist carbachol into the NRM produce antinociception (Brodie and Proudfit, 1984). Recently, the role of noradrenergic and serotonergic innervation in the antinociception resulting

from microinjections of carbachol into the NRM was studied by application of various 5-HT and NA antagonists into the spinal cord (Brodie and Proudfit, 1986). It was shown that the α_2-adrenoceptor antagonist yohimbine attenuated carbachol-induced antinociception whereas the 5-HT antagonist methysergide had no effect. Brodie and Proudfit (1986) suggest that carbachol-induced analgesia following microinjection into the NRM is mediated by a selective activation of α_2-adrenoceptors located on bulbospinal noradrenergic neurons. This evidence appears to be supported by recent evidence indicating that the antinociception induced by 5-MeODMT is antagonised by intrathecally administered α_2-adrenoceptor antagonists (Archer et al., 1986b).

In conclusion, the further demonstration of important NA — 5-HT interactions may be caused by the modulation of 5-HT and 5-HT agonist induced analgesia by the descending NA projections. The findings demonstrated by Brodie and Proudfit (1986) appear to complicate any understanding of how NA application locally restores the analgesic effects of 5-MeODMT and until the interactive involvement of all the neurochemical and neurohormonal systems is adequately described no explanation can be forwarded. Certainly, any reconceptualisation of the modulation of ascending spinal sensory neurotransmission by the descending 5-HT system must postulate the function of the descending NA system.

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