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(+)-8-OH-DPAT and 5-MeODMT Induced Analgesia is Antagonised by Noradrenaline Depletion

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ARCHER, T., E. ARWESTRÖM, B. G. MINOR, M.-L. PERSSON, C. POST, E. SUNDSTRÖM AND G. JONSSON. *(+)-8-OH-DPAT and 5-MeODMT induced analgesia is antagonised by noradrenaline depletion.* PHYSIOL BEHAV 39(1) 95–102, 1987.—In experiments with both rats and mice the 5-HT agonists 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and 5-methoxy-N,N-dimethyl-tryptamine (5-MeODMT) were shown to produce reliable analgesic effects after acute administration (1 mg/kg SC) in the tail-flick, hot-plate and shock-titration tests of nociception. Prior treatment with the noradrenaline neurotoxin, N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP4), systemically administered to both rats and mice abolished the analgesic effects of both the 5-HT agonist compounds in all the tests of nociception used. Intrathecal 6-hydroxydopamine (6-OHDA) treatment also abolished the analgesic effects of 8-OH-DPAT and 5-MeODMT; in the tail-flick test the analgesia induced by 8-OH-DPAT was reversed to an hyperalgesia. Biochemical analyses confirmed notable noradrenaline depletions in the spinal cord. It is concluded that an important interaction between presynaptic noradrenergic terminals and serotonergic receptor sites, possibly 5-HT_{1A}, mediates spinal nociception processes.

8-OH-DPAT 5-MeODMT DSP4 Noradrenaline depletion

THERE is much evidence documenting the analgesic effects of acute administration of 5-hydroxytryptamine (5-HT), intrathecally, or systemic administration of various 5-HT agonists [8, 11, 25, 31, 32]. Schmauss *et al.* [27] combined putative 5-HT antagonists with intrathecal administration of 5-HT to characterise the spinal receptor system mediating the antinociceptive effect; their findings suggested that a 5-HT_1 receptor may be the predominant mechanism by which intrathecal 5-HT affects nociception. Recently we reported [6] that the analgesic effects of the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), were abolished in the hot-plate and tail-flick tests and reversed to a hyperalgesia in the shock titration test by prior treatment with the noradrenaline (NA) neurotoxin, N-2-chloroethyl-N-ethyl 1-2-bromobenzylamine (DSP4). DSP4 has been shown to cause permanent and severe NA depletions in the brain and spinal cord of rats and mice [5,17]. A new type of 5-HT receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), was shown recently to have a direct 5-HT receptor stimulating effect [7]. 5-HT receptors in the central nervous system have been classified into two types, 5-HT_1 and 5-HT_2, on the basis of the receptor binding affinity of labelled 5-HT and spiperone [23]. Middlemiss and Fozard [19] reported that 8-OH-DPAT discriminates between 5-HT_{1A} and 5-HT_{1B} binding partially confirmed by Roberts [26]. Furthermore, Peroutka [22] has demonstrated that 5-HT_{1A} binding sites are directly labelled with [^{3}H]8-OH-DPAT in bovine hippocampal membranes. Acute subcutaneous (SC) administration of 8-OH-DPAT in mice caused dose-dependent alterations of responding in the hot-plate test of nociception whereas tail-flick responding was unaffected at the doses employed [13]. The purpose of this investigation was (1) to compare the analgesic properties of 8-OH-DPAT with 5-MeODMT, since our previous work involving various techniques for depleting NA standardly utilised 5-MeODMT, and (2) to ascertain whether or not NA depletion, by systemic DSP4 or intrathecal 6-hydroxydopamine (6-OHDA) administration, could antagonise the antinociceptive properties of both these 5-HT agonists in both rats and mice.

METHOD

General: Rats

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 labora-

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tory conditions with a 12 hr on/12 hr off lighting schedule (lights on at 06.00 hr) 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 systemic administration of DSP4 and the intrathecal 6-OHDA operations, the animals were allowed a four-week and a one-week recovery period, respectively, in the same housing conditions. Behavioural testing was carried out during the hours of light (0800–1400 hr).

Shock-titration testing was carried out in a specially constructed test box (25×26×30 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 [6]. Shocks (0.75 sec) 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 50, 75, 100, 150, 200, 250, 300, 400 and 500 μA intensities in step-wise manner, following a 3-min habituation to the test box. The time-gap between shocks was 15–20 sec.

The hot-plate and tail-flick methods have been developed from the techniques that have been described in detail elsewhere [9,12]. Hot-plate testing was conducted with an electrically heated and thermostatically controlled aluminium surface set at 58±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 5-MeODMT and 8-OH-DPAT. 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 Plexiglas 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). 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). The cut-off criterion was 20 sec for the tail-flick test. Each of these critical experiments was performed 'blind,' i.e., the experimenter that tested the rats was completely unaware of the treatments. Each rat was used for one drug treatment only but tested on all three tests.

General: Mice

White NMRI male mice (ALAB, Sweden) weighing 12–22 g were used; they were maintained under similar housing conditions to the rats, except that they were housed ten animals per cage. After DSP4 treatment the animals were allowed a two-week recovery period. Behavioural testing was carried out during the hours of light (0800–1400 hours).

The tail-flick and hot-plate tests for mice were performed in the same apparatus as for the rats (see above). For the tail-flick test no adaptation to the restraining tube was necessary as the mice were hand-held so that the experimenter's thumb and index finger secured the base of the tail. Test

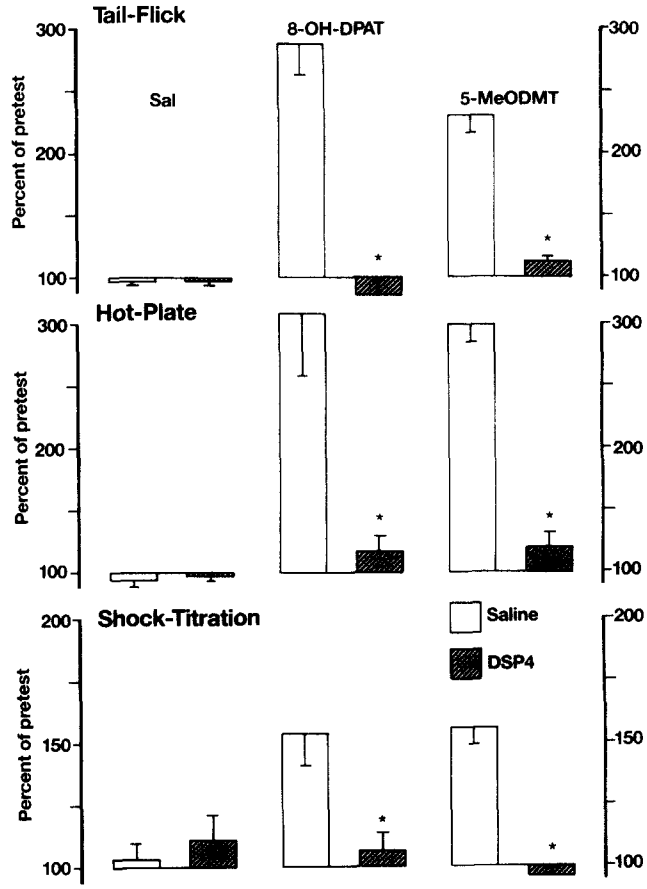

FIG. 1. The antagonism of 8-OH-DPAT- and 5-MeODMT-induced analgesia in rats by prior DSP4 treatment. DSP4 (2×50 mg/kg) was administered on two occasions 60 min after IP injections of zimeldine (2×20 mg/kg). 8-OH-DPAT (1 mg/kg SC) and 5-MeODMT (1 mg/kg SC) were administered 15 min before testing. Values are expressed as means±s.e.m. of 6 animals. *$p<0.01$, DSP4 condition vs. saline condition.

latencies were measured on two occasions 15 min before (Pre-test) and 30 min after (Tests) the acute administration of either 8-OH-DPAT, 5-MeODMT or saline. The testing procedure was maintained as for the rat experiment. Each mouse was used for one drug treatment only but tested on both tests.

Treatment Drugs

The treatment drugs included: 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Sigma Chemicals, St. Louis, MO), (+)-8-OH-2-(di-n-propylamino)tetraline hydrobromide (8-OH-DPAT) (obtained from the Unit of Organic Chemistry, Department of Pharmacology, University of Göteborg, Göteborg, Sweden), N-2-chloroethyl-n-ethyl-2-bromobenzylamine (DSP4) (Astra Läkemedel AB, Sweden), 6-hydroxydopamine hydrochloride (6-OHDA) (Sigma Chemicals), zimeldine hydrochloride (ZIM) (Astra Läkemedel AB), and pargyline hydrochloride (Saber Laboratories, Illinois). 8-OH-DPAT, ZIM and pargyline were dissolved in 0.9% saline, 5-MeODMT and DSP4 in distilled water, and 6-OHDA in 0.9% saline containing 0.1% ascorbic acid.

TABLE 1

PRE-TEST VALUES IN THE TAIL-FLICK, HOT-PLATE AND SHOCK-TITRATION TESTS FOR SALINE AND DSP4 PRETREATED RATS ASSIGNED TO THE Sal, 8-OH-DPAT AND 5-MeODMT GROUPS

	Pretreatment					
	Saline			DSP4		
	Sal	8-OH-DPAT	5-MeODMT	Sal	8-OH-DPAT	5-MeODMT
Tail-flick: (sec)	4.45 ±0.2	4.55 ±0.1	4.35 ±0.3	4.55 ±0.1	4.40 ±0.2	4.15 ±0.2
Hot-plate: (sec)	5.90 ±0.3	5.30 ±0.2	5.50 ±0.4	5.50 ±0.4	4.80 ±0.2	5.20 ±0.3
Shock-titration: (μAmp)	133 ±8	150 ±0	137.5 ±6	146 ±4	150 ±9	146 ±8

DSP4 (2×50 mg/kg) and saline were injected on two occasions, 60 min after zimeldine (20 mg/kg IP), four and five weeks prior to testing.

Values are expressed as means ± s.e.m. (n=6).

Two-way ANOVA was performed on the data from each test, no significant effects were obtained.

Statistical Analysis

The results are expressed as means±s.e.m. In each case (hot-plate, tail-flick and shock titration for rats; hot-plate and tail-flick for mice) the measures of nociception are expressed as a percentage of the pre-test response latency, i.e., Test/Pre-test × 100. Parametric statistics were used throughout as group size was constant within each experiment. Two-way ANOVA was performed according to the design outlined by Snedecor and Cochran [28] for the data from each experiment. Pairwise testing for differences between groups was performed using the Tukey HSD test [18]. The 1% level of significance was maintained throughout unless where otherwise stated.

Biochemical Assay

Endogenous monoamine concentrations were determined using high pressure liquid chromatography with electrochemical detection (l.c.e.c.) as described previously [17]. The catecholamine and 5-HT values were expressed as $ng \cdot g^{-1}$ wet weight of the tissue, based on internal standard measurements. Endogenous 5-HT was assayed using l.c.e.c. according to Ponzio and Jonsson [24]. Rats and mice were sacrificed within one to three weeks of testing. Lumbar spinal cord regions were rapidly dissected out on ice and stored at −70°C until analysis.

EXPERIMENT 1

It has previously been shown that NA depletion blocks the analgesic response to 5-MeODMT (e.g., [6]). The purpose of this experiment was to test the analgesia induced by the 5-HT_{1A} agonist, 8-OH-DPAT, in normal and DSP4-treated rats. Groups treated acutely with 5-MeODMT were also tested to provide a comparison with our previous experiments.

Procedure

Male Sprague-Dawley rats were injected IP either with DSP4 (2×50 mg/kg), or saline (5 ml/kg) 60 min after IP injections of the 5-HT uptake inhibitor zimeldine (2×20 mg/kg) on two occasions five days apart. Four weeks later, DSP4- and

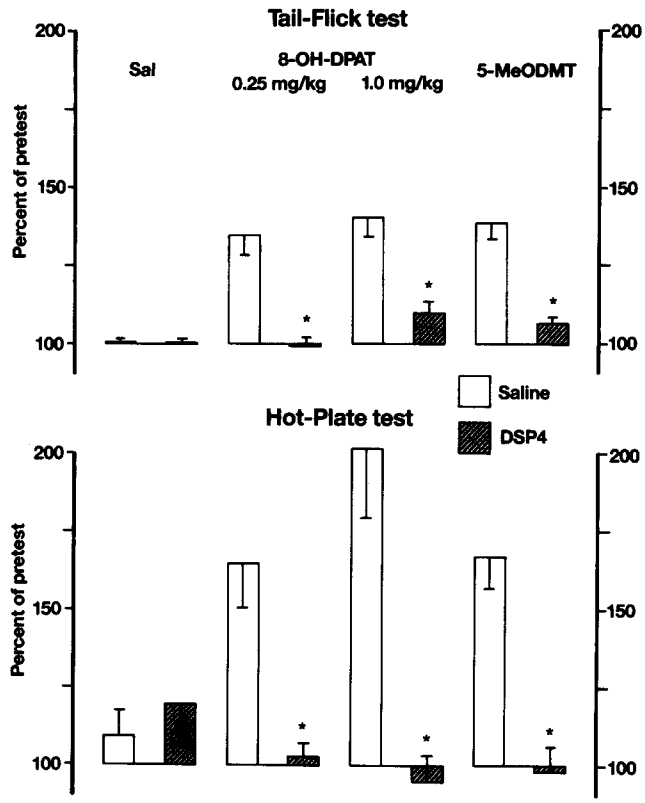

FIG. 2. The antagonism of 8-OH-DPAT- and 5-MeODMT-induced analgesia in mice by prior DSP4 treatment. DSP4 (1×50 mg/kg) was administered on one occasion 60 min after IP injection of zimeldine (1×20 mg/kg). 8-OH-DPAT (0.25 and 1 mg/kg SC) and 5-MeODMT (1 mg/kg SC) were administered 15 min before testing. Values are expressed as means±s.e.m. of 10 mice. *$p<0.01$, DSP4 condition vs. saline condition.

TABLE 2

PRE-TEST VALUES IN THE TAIL-FLICK AND HOT-PLATE TESTS FOR SALINE AND DSP4 PRETREATED MICE ASSIGNED TO THE Sal, 8-OH-DPAT (2 GROUPS) AND 5-MeODMT

	Pretreatment							
	Saline				DSP4			
	8-OH-DPAT				8-OH-DPAT			
	Sal	(0.25)	(1.0)	5-MeODMT	Sal	(0.25)	(1.0)	5-MeODMT
Tail-flick: (sec)	5.50 ±0.1	5.50 ±0.1	5.60 ±0.1	5.35 ±0.2	5.60 ±0.1	5.75 ±0.1	5.25 ±0.1	5.70 ±0.1
Hot-plate: (sec)	6.90 ±0.4	6.80 ±0.2	6.95 ±0.6	6.60 ±0.3	6.00 ±0.4	7.00 ±0.4	6.20 ±0.4	6.20 ±0.2

DSP4 (1×50 mg/kg) and saline were injected once, 60 min after zimeldine (20 mg/kg), two weeks prior to testing.
Values are expressed as means ± s.e.m. (n=6).
Two-way ANOVA was performed on the data from each test, no significant effects were obtained.

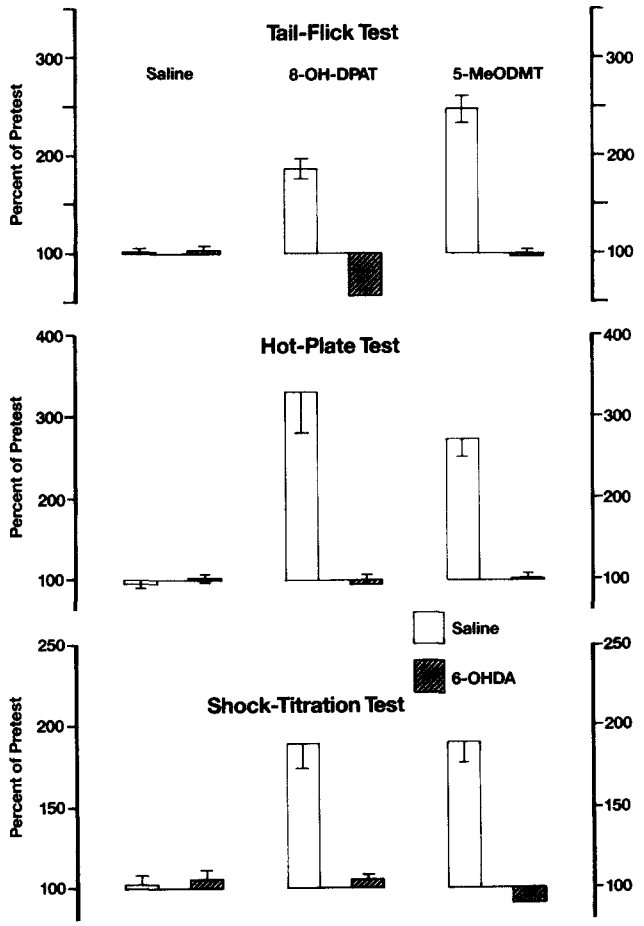

FIG. 3. The antagonism of 8-OH-DPAT- and 5-MeODMT-induced analgesia in rats by prior intrathecal 6-OHDA treatment. 6-OHDA (20 μg in 10 μl) was administered 10 min after pargyline (20 mg/kg) one week before testing. 8-OH-DPAT (1 mg/kg SC) and 5-MeODMT (1 mg/kg SC) were administered 15 min before testing. Values are expressed as means±s.e.m. of 7 animals. *$p<0.01$, 6-OHDA condition vs. saline condition.

saline-treated rats were tested for antinociceptive responses, using the tail-flick, hot-plate and shock-titration tests, following either acute 8-OH-DPAT (1 mg/kg SC), 5-MeODMT (1 mg/kg SC) or saline (5 ml/kg SC). At testing, each rat was given a pre-test for pain responses on the hot-plate, two pre-tests on the tail-flick test, and a flinch jump pre-test for shock titration. Fifteen min later, each rat was injected either saline or the test drug and hot-plate, tail-flick and shock titration testing was performed 15 min after the acute administrations. The total N for each group was 6 rats. The parameters measured were: Pain response latency for the hot-plate test, tail-flick latency and shock intensity (μAmp) for the shock titration test. One week after nociception testing spinal cord tissue was dissected out and stored at −70°C until analysis of monoamine concentrations.

Results and Discussion

The analgesic effects of acutely administered 8-OH-DPAT and 5-MeODMT to both normal and DSP4-treated rats in the hot-plate, tail flick and shock titrations test are presented in Fig. 1.

Two-way ANOVA was performed on the precent of pre-test data from each test, and Tukey HSD tests were used for pairwise comparisons. The two-way ANOVA indicated a Groups × Tests interaction: $F(10,90)=24.9$. Tukey HSD tests indicated the following differences:
Tail-flick test (Fig. 1, top panel), *Hot-plate test* (Fig. 1, middle panel), *Shock titration test* (Fig. 1, bottom panel). Both 8-OH-DPAT (1 mg/kg SC) and 5-MeODMT (1 mg/kg SC) caused clear analgesic effects on tail-flick responses (unshaded blocks) in the saline condition. In the DSP4 treatment condition (shaded blocks), the analgesic effects of these compounds were abolished completely.

Table 1 presents the pre-test tail-flick and pain response latencies as well as the pre-test shock intensities required to produce a flinch jump response for all the groups in this experiment. DSP4 treatment did not affect responding in any of the tests.

These results indicate unambiguously that NA depletion antagonises 8-OH-DPAT induced analgesia and confirm our earlier results concerning 5-MeODMT induced analgesia and NA depletion.

TABLE 3

PRE-TEST VALUES IN THE TAIL-FLICK, HOT-PLATE AND SHOCK-TITRATION TEST FOR 6-OHDA AND SALINE PRETREATED RATS ASSIGNED TO THE SALINE, 8-OH-DPAT AND 5-MeODMT GROUPS

	Pretreatment					
	Saline			6-OHDA		
	Saline	8-OH-DPAT	5-MeODMT	Saline	8-OH-DPAT	5-MeODMT
Tail-flick: (sec)	5.10 ±0.1	4.75 ±0.2	5.00 ±0.1	4.85 ±0.1	5.10 ±0.2	4.90 ±0.2
Hot-plate: (sec)	5.60 ±0.3	4.90 ±0.4	5.40 ±0.4	4.75 ±0.2	5.90 ±0.5	5.30 ±0.6
Shock-titration: (μAmp)	146 ±10	129 ±7	143 ±7	143 ±12	125 ±6	139 ±12

6-OHDA (20 μg in 10 μl containing 0.05% ascorbic acid) and saline (15 μl containing 0.05% ascorbic acid) were administered intrathecally one week before testing.

Values are expressed as means ± s.e.m. (n=7).

Two-way ANOVA was performed on the data from each test.

EXPERIMENT 2

Although the blockade of 5-MeODMT induced analgesia by NA depletion has been well confirmed in rats, this test of the modulatory role of the descending NA system has never been investigated in mice. The purpose of Experiment 2 was to ascertain whether DSP4 treatment of mice would antagonise the analgesia produced by the 5-HT agonists, 5-MeODMT and 8-OH-DPAT.

Procedure

Male NMRI mice were injected IP either with DSP4 (1×50 mg/kg) or saline (5 ml/kg) 60 min after an IP injection of zimeldine (20 mg/kg). Two weeks later, DSP4- and saline-treated mice were tested for analgesic responses to acute 8-OH-DPAT (0.25 and 1.0 mg/kg SC) and 5-MeODMT (1 mg/kg SC) or saline treatment. After the pre-tests, each mouse was injected either saline or the test drug and nociceptive testing was performed 15 min after the acute administration. Tail-flick and hot-plate tests performed in a similar manner to that of the rats.

Results and Discussion

The analgesic effects of acute administration of 8-OH-DPAT and 5-MeODMT to both normal and DSP4-treated mice in the hot-plate and tail-flick tests are presented in Fig. 2.

Two-way ANOVA indicated a significant Groups × Tests interaction, $F(7,139)=5.0$. Tukey HSD tests indicated the following pairwise differences:
Tail-flick test (Fig. 2, top panel), *Hot-plate test* (Fig. 2, bottom panel). 8-OH-DPAT caused reliable analgesic effects at both doses administered, 0.25 and 1 mg/kg SC, as did 5-MeODMT at the 1 mg/kg dose SC (unshaded blocks). In the DSP4 treatment condition (shaded blocks) the analgesic effects of both doses of 8-OH-DPAT as well as that of 5-MeODMT were abolished.

Table 2 presents the pre-test tail-flick and hot-plate responses from the mice tested in this experiment. Note that no effects due to the DSP4 treatment were obtained.

These results strongly confirm our data from experiments with rats. NA depletion with DSP4 in mice abolishes the analgesia produced by both 8-OH-DPAT and 5-MeODMT.

EXPERIMENT 3

The purpose of this experiment was to localize the region where NA depletion antagonizes analgesia induced by 8-OH-DPAT and 5-MeODMT. Intrathecal 6-OHDA administration depletes lumbar and thoracic NA quite selectively and we have previously shown that this blocks 5-MeODMT induced analgesia [4]. Thus, the effect of intrathecal 6-OHDA treatment upon 8-OH-DPAT induced analgesia was studied and 5-MeODMT was included as a reference compound, as well.

Procedure

Male Sprague-Dawley rats were injected intrathecally with 6-OHDA or saline one week before testing. A polyethylene catheter (PE10) 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. 6-OHDA (20 μg in 10 μl 0.9% saline containing 0.05% ascorbic acid) was injected over one min, 10 min after systemic administration of pargyline (20 mg/kg IP) to increase the efficacy of 6-hydroxydopamine in depleting NA. Saline (15 μl containing 0.05% ascorbic acid) was injected 10 min after pargyline, also, for the saline treatment condition. The catheters were removed soon after the 6-OHDA administration. Any rats showing motor dysfunction after surgery were rejected. At testing (one week after the intrathecal operations), the pre-tests were performed as above and fifteen min later each 6-OHDA or saline treated rat was injected either 8-OH-DPAT (1 mg/kg SC), 5-MeODMT (1 mg/kg SC), or saline (5 ml/kg SC). Tail-flick, hot-plate and shock-titration testing was performed 15 min later. Three weeks after nociception testing spinal cord tissue was dissected out and stored at −70°C until analysis of monoamine concentrations.

TABLE 4

MONOAMINE ASSAYS ON THE SPINAL CORDS OF DSP4- AND SALINE-TREATED RATS AND MICE, AND INTRATHECAL 6-OHDA- AND SALINE-TREATED RATS

	ng/g tissue		
	Noradrenaline	Dopamine	5-Hydroxytryptamine
Experiment 1 rats:			
Saline	458 ± 18	27.5 ± 3	318 ± 23
DSP4	21 ± 1*	28 ± 4	341 ± 38
(%)	(5)	(102)	(107)
Experiment 2 mice:			
Saline	276 ± 12	37 ± 3	242 ± 8
DSP4	45 ± 4*	34 ± 6	233 ± 18
(%)	(16)	(92)	(96)
Experiment 3 rats:			
Vehicle	466 ± 41	31.5 ± 5.5	473 ± 72
6-OHDA	38 ± 13*	28.5 ± 12.5	521 ± 69
(%)	(8)	(91)	(110)

Values are expressed as means ± s.e.m.
Percent of control values are shown in parentheses.
*$p<0.01$, Students t-test.
For Experiment 1 (rats): DSP4 (2×50 mg/kg) was injected IP 60 min after ZIM (2×20 mg/kg IP).
For Experiment 2 (mice): DSP4 (1×50 mg/kg) was injected IP 60 min after ZIM (1×20 mg/kg).
For Experiment 3 (rats): 6-OHDA (20 μg in 10 μl) was injected intrathecally 30 min after pargyline (20 mg/kg IP).

Results and Discussion

The analgesic effects of acute administration of 8-OH-DPAT and 5-MeODMT to both sham-operated and intrathecal 6-OHDA administered rats in the hot-plate, tail-flick and shock titration tests are presented in Fig. 3.

Two-way ANOVA indicated a significant Groups × Tests interaction, $F(10,108)=6.2$. Tukey HSD tests indicated the following pairwise differences:
Tail-flick test (Fig. 3, top panel), *Hot-plate test* (Fig. 3, middle panel), *Shock titration test* (Fig. 3, bottom panel). Both 8-OH-DPAT (1 mg/kg SC) and 5-MeODMT (1 mg/kg SC) caused clear analgesic effects on tail-flick responses (unshaded blocks) in the saline condition. In the 6-OHDA condition (shaded blocks), the analgesic effect of 8-OH-DPAT was reversed to an hyperalgesia, whereas the analgesic effect of 5-MeODMT was abolished completely. Intrathecal 6-OHDA treatment itself did not produce any effects on the tests of nociception.

Table 3 presents the pre-test tail-flick and pain response latencies as well as the pre-test shock intensities for all the groups in this experiment. Intrathecal 6-OHDA treatment did not affect responding in any of the tests.

The results of the intrathecal 6-OHDA experiment confirm those of the previous two experiments. NA depletion abolished the analgesia induced by 8-OH-DPAT. Additionally, the results replicate our earlier findings of prior 6-OHDA treatment antagonising 5-MeODMT induced analgesia.

Biochemical Analysis

Monoamine assays are presented in Table 4. DSP4 treatment (2×50 mg/kg IP 60 min after zimeldine, 2×20 mg/kg IP) for rats and (1×50 mg/kg IP 60 min after 1×20 mg/kg zimeldine) for mice severely depleted NA in the spinal cord in both rats (5% of control values) and mice (16% of control values) whereas DA and 5-HT were almost unaffected (90+% of control values). Intrathecal 6-OHDA treatment (20 μg in 10 μl, 10 min after pargyline, 20 mg/kg IP) depleted NA concentrations drastically in the spinal cord, DA and 5-HT were unaffected.

DISCUSSION

The results of the present investigation are quite straightforward and may be summarised as follows: The 5-HT agonists, 8-OH-DPAT and 5-MeODMT, both caused a reliable analgesia at the 1 mg/kg dose in rats and mice (0.25 mg/kg dose of 8-OH-DPAT also). Prior treatment with the NA neurotoxin, DSP4, which caused severe NA but not DA or 5-HT depletions in the spinal cord, abolished the analgesic effects of these compounds in all the tests of nociception that were applied. Prior intrathecal administration of 6-OHDA abolished completely the analgesic effects of 8-OH-DPAT and 5-MeODMT in the hot-plate and shock-titration tests whereas the analgesic effect of 8-OH-DPAT in the tail-flick test was reversed to an hyperalgesia. The analgesia induced by 5-MeODMT in the tail-flick test was abolished also. The 6-OHDA treatment data were consistent with DSP4 treatment data of both rats and mice. These findings demonstrate that NA is necessary for 8-OH-DPAT induced analgesia to be obtained in rats and mice and for 5-MeODMT induced analgesia in mice; it is confirmed also that the abolition of 5-MeODMT induced analgesia by NA depletion in rats is a

consistent and reliable effect. The biochemical analyses confirmed NA, but not DA and 5-HT, depletions in the spinal cord.

It has previously been shown that 8-OH-DPAT is a potent and selective central 5-HT receptor agonist [1, 7, 15] with a reported affinity for the 5-HT_{1A} recognition site [19,32]. It has also been shown that 5-MeODMT has a high affinity for the 5-HT_{1A} recognition site [30], and that both 8-OH-DPAT and 5-MeODMT induce, to some extent, a similar behavioural syndrome consisting of reciprocal forepaw treading, a flat body posture, head weaving and hyperlocomotion [29]. Thus, one conclusion from the present results could be that NA terminals in the spinal cord may modulate 5-HT_1, possibly 5-HT_{1A}, postsynaptic receptor sites. Pazos and Palacios [21] found that the dorsal horn presented intermediate densities of sites, in the cervical region of the spinal cord, with 5-HT_{1A}-recognition site characteristics, whereas in the ventral horn, the concentration of specific binding was low or very low, and of the 5-HT_{1B} class. Any conclusion involving specific receptor sites must be only tentative in view of the complex 5-HT innervation. Alternatively, destruction of NA terminals may remove an inhibitory tonic influence and initiate some autoreceptor mechanism mediated via the 5-HT agonists. In this regard, Gozlan *et al.* [14] have found that in the rat striatum 8-OH-DPAT appears to bind to presynaptic receptors and it has been suggested that 8-OH-DPAT may act preferentially as an agonist at 5-HT autoreceptors [1].

The antagonism of the analgesia induced by 8-OH-DPAT and 5-MeODMT by spinal NA depletion is in agreement with the data from several other investigations. Analgesia induced by intrathecal 5-HT administration was abolished by DSP4 treatment in both rats [2,20] and mice [2]. Systemically administered p-chloroamphetamine, 5-hydroxytryptophan and quipazine each caused reliable analgesic effects that were antagonised by DSP4 treatment but only partially by the 5-HT antagonists, mianserin and metergoline [25], whereas the analgesic effects of 5-HT and 5-MeODMT were antagonised by the α_2-adrenoceptor antagonist, yohimbine [3]. In addition, both neonatally administered and intrathecally applied 6-OHDA abolished the analgesic effects of 5-HT, 5-MeODMT and p-chloroamphetamine in rats [4,10]. For a few days following spinal transection the acute administration of 5-HT agonists reliably elicits an analgesic effect [33], and this effect is reversed to an hyperalgesia one week after transection [34]. 5-HT depletion, whether via systemic p-chloroamphetamine or p-chlorophenylalanine or intrathecal 5,7-dihydroxytryptamine or 5,6-dihydroxytryptamine, had either no effect or caused a test-dependent attenuation or induced an increased analgesic effect of the 5-HT agonists [4, 6, 16]. It was also shown previously that 5-HT induced analgesia was abolished by prior DSP4 treatment in rats but only attenuated by DSP4 administration to mice [2]. The above results indicate that 8-OH-DPAT and 5-MeODMT induced analgesia was abolished by DSP4 treatment in both rats and mice, and by intrathecal 6-OHDA treatment in rats. This finding seems to support the involvement of a specific 5-HT recognition site in the noradrenergic-serotonergic interactions in antinociception. Thus, the blockade of 8-OH-DPAT and 5-MeODMT induced analgesia underlines the importance of an intact noradrenergic terminal projection in the control of pain thresholds that are elevated by 5-HT agonists.

In summary, the main findings concern noradrenergic involvement in 8-OH-DPAT analgesia in rats and mice as well as 5-MeODMT analgesia in mice. The ancilliary findings confirm previous results on noradrenergic involvement in 5-MeODMT analgesia in rats. The inter-species reliability is strong evidence in favour a critial NA-5-HT interaction in nociception.

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