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Blockade and Reversal of 5-Methoxy-N,N-Dimethyltryptamine-Induced Analgesia Following Noradrenaline Depletion

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The acute effects of the 5-hydroxytryptamine agonist, 5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT), upon pain sensitivity, using shock titration, tail-flick and hot-plate methods, in noradrenaline- and 5-hydroxytryptamine-depleted rats were examined. Noradrenaline depletion, following the systemic administration of N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP4, 2 × 50 mg/kg, i.p.), caused a reversal of the analgesic effect of 5-MeO-DMT on shock-titration from hypo- to hypersensitivity, and a total blockade of the antinociceptive effect of 5-MeO-DMT upon pain responses in the hot-plate and tail-flick tests. Pretreatment with either *p*-chloroamphetamine (2 × 10 mg/kg) or *p*-chlorophenylalanine (200, 100, 100 mg/kg), that depletes central 5-hydroxytryptamine stores, failed to alter the analgesia caused by acute 5-MeO-DMT. Strong evidence is provided for the effect of central noradrenaline depletion upon the analgesic effect of the 5-HT agonist. These findings suggest an important tonic influence of the noradrenaline system upon the descending spinal 5-HT pathway in rats.

INTRODUCTION

There is a considerable amount of evidence implicating a role of central serotonin mediation of pain mechanisms[27,38]. The response threshold to painful stimuli is heightened by treatments that elevate or mobilize the availability of central 5-hydroxytryptamine (5-HT)[29,40], or stimulate 5-HT receptors directly[32], whereas this threshold is lowered by treatments that remove the availability of 5-HT[25,34,35]. On the other hand, the precise effect of noradrenaline (NA) depletion upon the pain threshold has been the subject of much debate[28,41]. However, recent evidence using microinjections of the catecholamine neurotoxin, 6-hydroxydopamine, into central regions[26] or systemic injections of the selective NA neurotoxin N-2-chloroethyl-N-ethyl-2-bromo-benzylamine (DSP4) have indicated no alteration of pain thresholds with either the hot-plate and tail-flick[4], or foot shock[2] methods. The systemic administration of DSP4 (one or two injections, 50 mg/kg, i.p.) causes a sustained and severe blockade of NA accumulation in rat and mouse brain slices, an inhibition of dopamine-β-hydroxylase activity and a marked long-lasting depletion of endogenous NA concentrations in several regions, including the spinal cord, with terminals originating from the locus coeruleus[18,20,31]. Since the central effects of DSP4 upon NA neurons last several months, whereas the peripheral effects dissipate within 7–10 days, DSP4 has been found to be a valuable pharmacological tool for investigating the functional role of central NA pathways[3].

5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is a direct-acting 5-HT agonist[1,10,13] which has been found to cause hyperalgesia at low doses and analgesia at higher doses[8]. The purpose of this investigation was to test whether the analgesic effects of 5-MeO-DMT would be altered by either NA or 5-HT depletion. Thus, in addition to DSP4, the NA depletor, *p*-chlorophenylalanine, which inhibits 5-HT synthesis[23], and *p*-chloroamphetamine, which causes a long-term selective degeneration of 5-HT terminals in the raphe system[17], were used to deplete 5-HT.

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MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats, aged 65–75 days (AB Anticimex, Sollentuna, Sweden), were randomly assigned to the different treatment conditions. They were housed, on sawdust bedding material, in groups of 4 or 5 animals under laboratory conditions with a 12 h on/12 h off lighting schedule (light on at 06.00 h) in a room thermostatically maintained at 21 ± 1 °C for up to 6 weeks prior to shock-titration, tail-flick and hot-plate testing for pain sensitivity. The animals were given drug administrations at various different occasions as outlined in each of the two different experiments.

Apparatus

Shock-titration testing was carried out in a test box (25 × 26 × 30 cm, Campden Instruments, London), wired to present scrambled footshock and balanced upon 4 strain gauges (one at each of the 4 corners). The test box was designed so that any sufficient force exerted by a rat's movements was translated into voltage and recorded on a voltimeter, as described previously[14,37]. 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, London). Titrations were continued upwards or downwards depending on non-response or response at the 50, 75, 100, 125, 150, 175, 200, 250 and 300 μA intensities in a step-wise, incremental manner, following a 3-min habituation to the test box. The hot-plate and tail-flick methods have been developed from the techniques that have been described in detail elsewhere[8,29]. Hot-plate testing was conducted with an IITC INC. Mod. 35 Analgesia-Meter 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 administration of acute 5-MeO-DMT. The test latencies were scored before (Pre-test) and after the 5-MeO-DMT injection. 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. The tail-flick test was conducted with an IITC INC. Mod. 33 Analgesia-Meter. The rats were adapted to the restraining tube on each of 3 consecutive daily sessions (lasting 10 min) on the 3 days prior to testing. The test latencies were scored, on two occasions, 15 min before (Pre-test) and after (Test) the 5-MeO-DMT injection. 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). Different rats were assigned to each of the treatment groups and test methods. Results are expressed as medians ± quartiles. Pairwise differences between groups were computed using the Mann–Whitney U-test[33].

The treatment drugs used included: DL-*p*-chloroamphetamine hydrochloride (PCA) (Sigma Chemicals, St. Louis, USA), 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) (Regis Chemical CO., USA), DL-*p*-chlorophenylalanine (PCPA) (Sigma St. Louis, USA), and N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP4) (Astra Läkemedel AB, Sweden). PCA was dissolved in 0.9% saline, 5-MeO-DMT and DSP4 in distilled water. PCPA was suspended in 1% methylcellulose in distilled water.

EXPERIMENT 1

Procedure

Thirty-four rats were administered DSP4 (2 × 50 mg/kg, i.p.) 5 weeks and 4 weeks before testing, and 34 rats were injected with saline (5 ml/kg, i.p.). At shock-titration testing groups of rats in the DSP4 and saline treatment conditions were administered either 5-MeO-DMT (0.5 mg/kg, n = 7; or 1.0 mg/kg, n = 10; or 2.0 mg/kg, n = 7) or saline (n = 10) 15 min before placement in the test box. The DSP4 and saline control rats were also tested for pain responses on the hot-plate test and for tail-flick responding on the tail-flick test. 5-MeO-DMT was injected subcutaneously, 15 min before testing.

Results

In the saline-treated rats, 5-MeO-DMT caused a dose-related increase in the shock intensity required to cause a flinch jump response detectable by the strain gauges, i.e. the 1.0 and 2.0 mg/kg 5-MeO-DMT groups in the saline pretreated condition required significantly higher shock intensities than the saline control group to cause flinch jumps. The median shock intensity for flinch-jump responses for the saline–saline control group was 137.5 μA which com-

pares very closely with that obtained from a subjective rating analysis[9]. NA depletion, as a result of DSP4 treatment (50 mg/kg) reversed the analgesic effect of 5-MeO-DMT (see Fig. 1). Thus, the median shock intensity to flinch jump responding was significantly lowered for the DSP4-treated rats that acutely received either 1.0 or 2.0 mg/kg 5-MeO-DMT, than for those rats that received saline. Table I presents the monoamine concentrations of the spinal cord following DSP4 treatment. As indicated, NA was severely depleted (9% of control) and 5-HT only slightly (85%) whereas DA was unaffected (104%). These results are consistent with those of Jonsson et al.[21] with the exception that these workers obtained a 40–50% depletion of 5-HT[21].

As for the shock-titration test, 5-MeO-DMT produced an analgesic effect upon the pain response threshold in the hot-plate test and the tail-flick latency in the tail-flick test. In these test situations, however, the DSP4 pretreatment caused a complete blockade rather than a reversal of analgesic effects of 5-MeO-DMT. Pain response (hot-plate) latencies for DSP4- and saline-treated rats are presented in Table II. During the test phase of the hot-plate test, the saline-treated (control) rats injected acute 5-MeO-DMT (1.0 mg/kg) showed significantly longer latencies than acute saline injected rats and DSP4-treated rats that were injected acute 5-MeO-DMT (1.0 mg/kg). For the tail-flick test, the control rats that received acute 5-MeO-DMT showed significantly longer latencies than DSP4-treated rats that were administered 5-MeO-DMT. Acute 5-MeO-DMT administered rats did not differ from the DSP4-treated animals that received saline acutely; this finding clearly suggests a complete blockade of the analgesic effects of the 5-HT agonist following spinal NA depletion with DSP4.

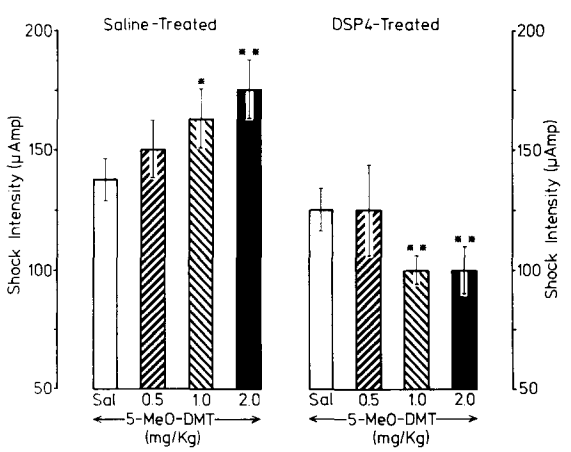

Fig. 1. The reversal of 5-MeO-DMT-induced analgesia by pretreatment with DSP4. Acute 5-MeO-DMT (0.5–2.0 mg/kg, i.p.) was injected 15 min prior to placement in the test-box. DSP4 (50 mg/kg, i.p.) was administered, twice, two weeks and one week prior to the shock-titration testing. For statistical analysis, the 5-MeO-DMT groups were compared to the appropriate saline control in each pretreatment condition, using the non-parametric Mann–Whitney U-test[23]. Values are expressed as medians ± quartiles. $*P \leq 0.05$; $**P \leq 0.02$.

TABLE I

Monoamine assays (ng/g tissue) on the spinal cords of DSP4- and saline-treated rats. DSP4 (50 mg/kg, i.p.) was injected 3 weeks before sacrifice

Values are expressed as medians ± quartiles. The methods of Keller et al.[22] (NA and DA) and Ponzio and Jonsson[30] (5-HT) were used for the amine determination.

Groups	*Noradrenaline*	*Dopamine*	*5-Hydroxytryptamine*
DSP4	31 ± 7*	33.5 ± 6	271.5 ± 32
Control	361 ± 25	32 ± 3	323 ± 19
(%)	(9)	(104)	(85)

* $p \leq 0.001$.

EXPERIMENT 2

Procedure

For the second experiment, 20 rats were injected with PCA (2 × 10 mg/kg i.p.) 8 and 7 days before testing, 20 rats were injected with PCPA (200, 100, 100 mg/kg i.p.) 72, 48 and 24 h before testing and 20 rats were injected with saline (5 ml/kg) one week prior to testing. For shock-titration measurements, groups of rats (n = 10) in each condition (PCA, PCPA and saline) were administered either 5-MeO-DMT (1.0 mg/kg) or saline 15 min before placement in the test box. The purpose of this experiment was to test whether the reversal and blockade of 5-MeO-DMT-induced analgesia could be attributed to the small, but nevertheless quite consistent, depletion of 5-HT that is caused by DSP4 treatment.

Results

Pretreatment with either PCA (2 × 10 mg/kg) or PCPA (200, 100, 100 mg/kg), which deplete 5-HT stores in the spinal cord quite considerably, did not affect the analgesic effects of acute 5-MeO-DMT

TABLE II

The pain response (hot-plate test) and tail-flick (tail-flick test) latencies of rats treated with either DSP4 (50 mg/kg, i.p.) or saline on two occasions, 4 and 5 weeks before testing

After the pre-test, rats were injected with either 5-MeO-DMT (1.0 mg/kg) or saline 15 min before the test. For hot-plate testing, the surface temperature of the plate was set at 58 °C (IITC Mod. 35-D). A pain response was defined as a licking or kicking of the hind-legs. The cut-off point was 20 s. For tail-flick testing, a rheostat controlled light beam was directed on the tip of the tail (IITC INC. Mod. 33). Latency to flick was recorded. Note that the pre-tests were carried out before the administration of acute 5-MeO-DMT (1.0 mg/kg) or acute saline.

Hot-plate test:	*Saline-treated*		*DSP4-treated*	
Pre-test (s)	7.0 ± 0.5	7.0 ± 1.0	6.0 ± 1.0	6.0 ± 0.75
	Sal	5-MeO-DMT (1 mg/kg)	Sal	5-MeO-DMT (1 mg/kg)
Test (s)	6.0 ± 1.0	20.0 ± 0*,**	6.25 ± 0.5	6.0 ± 0.75
Tail-flick test:				
Pre-test (s)	4.5 ± 0.25	4.4 ± 0.3	4.3 ± 0.2	4.2 ± 0.3
	Sal	5-MeO-DMT (1 mg/kg)	Sal	5-MeO-DMT (1 mg/kg)
Test (s)	4.3 ± 0.15	9.8 ± 0.4*,**	4.3 ± 0.3	4.6 ± 0.25

Values are expressed as medians ± quartiles. * $p < 0.001$ vs Sal (saline-treated); ** $p < 0.001$ vs 5-MeO-DMT (DSP4-treated).

(1.0 mg/kg). Table III presents the shock-titration, hot-plate and tail-flick measures of 5-MeO-DMT-induced analgesia following pretreatment with either PCPA, PCA or saline. Mann–Whitney U-tests indicated significantly greater response thresholds (μA for shock titration and seconds for hot plate and tail-flick) in the acute 5-MeO-DMT condition as compared to the acute saline condition for all 3 pretreatment conditions. This result (see Table III) provides strong evidence to preclude the involvement of 5-HT depletion in the DSP4-induced blockade and reversal of 5-MeO-DMT analgesia. Subsequent work at our laboratory (Archer, Jonsson, Minor and Post, submitted) indicated that a complete protection from the 5-HT depleting effects of DSP4 by pretreatment with a 5-HT uptake inhibitor, zimeldine gave exactly the same reversal and blockade, as for DSP4, of the analgesic effect of 5-MeO-DMT.

TABLE III

Shock-titration, tail-flick and hot-plate measures of 5-MeO-DMT-induced analgesia following pretreatment with either p-chlorophenylalanine (PCPA) or p-chloroamphetamine (PCA).

PCPA (200, 100 and 100 mg/kg) was injected 72, 48 and 24 hours before testing. PCA (2 × 10 mg/kg) was injected 8 and 7 days before testing. For the shock-titration method, rats were injected with either 5-MeO-DMT (1.0 mg/kg) or saline 15 min before placement in the test box. For the hot plate and tail-flick tests, rats were given a pre-test and then injected with 5-MeO-DMT (1.0 mg/kg) or saline 15 min before the measurement of pain response latencies and tail-flick latencies. Note than the pre-tests were carried out before the administration of acute 5-MeO-DMT (1.0 mg/kg) or acute saline.

	Saline		*PCPA*		*PCA*	
	Sal n = 10	5-MeO-DMT (1 mg/kg) n = 10	Sal n = 10	5-MeO-DMT (1 mg/kg) n = 10	Sal n = 8	5-MeO-DMT (1 mg/kg) n = 12
Schock-Titration Test:						
Test (μA)	137.5±12.5	175.0±12.5**	125.0±12.5	175.0±12.5**	125.0±10.0	175.0±6.25**
Hot-Plate Test:						
Pre-test (s)	7.5±1.5	7.0±2.0	5.0±0.5	6.0±1.0	7.0±1.5	7.0±1.0
Test (s)	5.0±2.0	20.0±0*	7.0±1.0	20.0±0*	6.0±1.5	20.0±0*
Tail-Flick Test:						
Pre-test (s)	4.45±0.4	4.25±0.5	5.0±0.4	4.5±0.7	3.2±0.6	4.1±0.4
Test (s)	4.1±0.6	9.8±0.7*	4.2±0.8	10.0±0.5*	3.65±0.7	8.0±1.5*

Values are expressed as medians ± quartiles. * $p < 0.001$ vs Sal (acute saline); ** $p < 0.01$ vs Sal (acute saline).

DISCUSSION

NA depletion via systemic DSP4 (2 × 50 mg/kg) administered to adult rats reversed the analgesic effect of 5-MeO-DMT in the shock titration test and blocked the analgesic effects of 5-MeO-DMT completely in the hot-plate and tail-flick test, whereas 5-HT depletion via PCA (2 × 10 mg/kg) and PCPA (200, 200, 100 mg/kg) did not do so.

The present results suggest an important role for the tonic noradrenergic activity of the descending bulbo-spinal pathway upon the antinociceptive effects of 5-MeO-DMT since the removal of the presynaptic NA terminals with DSP4 (2 × 50 mg/kg) abolished the hypoalgesia produced by 5-MeO-DMT (1.0 mg/kg) in the hot-plate and tail-flick tests and reversed it to an hyperalgesia in the shock-titration test. DSP4 (2 × 50 mg/kg), by itself, produced no effects upon either measure of pain sensitivity[2,4]. The second experiment was performed to examine whether the small 5-HT depletion produced by DSP4 (Table I) could account for the effects obtained; this was not found to be the case. No evidence was obtained for any supersensitivity following 5-HT depletion with PCA or PCPA, and this discrepancy is not consistent with the measurable supersensitivity obtained by other investigators[15,16] following 5,7-Dihydroxytryptamine (5,7-DHT) and 5,6-Dihydroxytryptamine (5,6-DNT)-induced lesions of the descending 5-HT pathway. One possibility is that PCA and/or PCPA induce a qualitatively different spinal 5-HT depletion to 5,7-DHT and 5,6-DHT. Another, more likely explanation is that 'ceiling' effects masked the demonstration of any supersensitivity to the analgesic effects of 5-MeO-DMT.

Some account should be given of behavioural observations of the DSP4-treated rats administered the 5-HT agonist, 5-MeO-DMT. It was invariably noted that the rats injected 5-MeO-DMT (1.0 mg/kg) demonstrated at least some of the components of the 5-HT syndrome described previously[36]. DSP4-treated animals tended to show an increased incidence of the 5-HT syndrome, e.g. tremors, hindlimb abduction, reciprocal forepaw treading, circling, 'crawling', which one would expect to lead to elevated rather than lowered analgesic thresholds. The lowered threshold for incidence of the 5-HT syndrome following DSP4 has been noted in other circumstances[6], and must be taken into account in studies involving shock-motivated behaviours with 5-HT agonists and NA depletion[5]. It should be noted that the present results cannot be explained on the basis of the transient peripheral depletion of NA following DSP4. Fourteen days after DSP4 (50 mg/kg) treatment, there is a total recovery of peripheral NA[4,20] whereas the central depletion is permanent. In Experiment 1, the second DSP4 injection was administered 4 weeks before testing.

Some evidence exists to implicate a role of NA in pain mechanisms[39]. However, the relevance of the involvement of the descending NA system per se for the present findings is difficult to judge, and much further experimentation is in progress. It seems reasonable to assert that the evidence to demonstrate noradrenergic control of the analgesia induced by 5-HT agonists is irrefutable since both neonatal 6-hydroxydopamine (6-OHDA) administration (Minor, Post and Archer, submitted) and intrathecal 6-OHDA produce exactly the same reversal and blockade of analgesia as DSP4 treatment (Archeret al., submitted). At present, two alternatives are considered: (1) it is known that DSP4 treatment leads to a functional postsynaptic NA receptor supersensitivity[12] and this effect may be reflected in the hypersensitivity and/or blockade of hypoalgesia indicated with the 5-HT agonist, 5-MeO-DMT; (2) it is possible that the removal of an inhibitory NA tonic influence serves to trigger an autoreceptor 5-HT mechanism involving 5-HT agonists thereby resulting in a functional lowering of the availability of 5-HT at the postsynaptic receptor and this may result in an increased pain sensitivity. It seems certain that the eventual elucidation of this mechanism may provide a valuable insight to the interactions of the central NA and 5-HT pathways, which are implicated from the finding that NA terminals directly innervate 5-HT cells in the dorsal raphe[7,39]. Other evidence comes from studies suggesting a dual role of 5-HT and NA in morphine analgesia[11,19,24]. At present, it is suggested that an intact noradrenergic terminal projection is required to produce the analgesic effect of 5-MeO-DMT.

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