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STEREOSPECIFIC ACTIONS OF 2,5-DIMETHOXY-4-METHYLAMPHETAMINE (DOM) ON COLONIC TEMPERATURE IN THE RAT AT VARIOUS AMBIENT TEMPERATURES

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1 The R(−) and S(+)-isomers of 2,5-dimethoxy-4-methylamphetamine (DOM) produce a dose-dependent hypothermia in rats kept in the cold (6°C).

2 This hypothermia was linearly dependent upon ambient temperature and the R(−)-isomer was considerably more potent than the S(+)-isomer.

3 A statistically significant tachyphylaxis was observed when R(−)-DOM was administered on two successive days. The response seven days after the second injection was similar to that on the first day of injection.

4 The hypothermia induced by R(−) and S(+)-DOM was antagonized by methysergide but not by *p*-chlorophenylalanine (PCPA) or pimozide. Methysergide, PCPA or pimozide alone did not elicit hypothermia at the doses used. The results indicate that R(−) and S(+)-DOM act at post-synaptic 5-hydroxytryptamine receptors.

Introduction

Amphetamine causes a dose-dependent hyperthermia when administered parenterally to rats at room temperature (Morpugro & Theobald, 1965). In addition, Yehuda & Wurtman (1972a) have found that amphetamine causes hypothermia when given to rats placed in the cold (4–15°C). The hypothermic effect appears to be a result of an amphetamine-induced release of dopamine from brain neurones (Yehuda & Wurtman, 1972b). This conclusion was supported by the suppression of the amphetamine-induced hypothermia by drugs such as pimozide, which block dopamine receptors. In the case of amphetamine, the (+)-isomer is more potent than the (−)-isomer in the perturbation of body temperature or behaviour. In terms of absolute configuration, these isomers are known to exist as S(+) and R(−) (Schrecker & Hartwell, 1957). With regard to the hallucinogenic derivatives of amphetamine such as 2,5-dimethoxy-4-methylamphetamine (DOM), Benington, Morin, Beaton, Smythies & Bradley (1973) found that R(−)-DOM was much more potent than S(+)-DOM in disrupting conditioned avoidance in the rat. This reversal in stereospecificity between amphetamine and DOM strongly suggests a different mode of action. Furthermore, the most potent isomer of lysergic acid diethylamide (LSD) is (5R;8R)-(+)-LSD (Stadler & Hofmann, 1962). If in fact there is a common mode of action for these hallucinogens, then it is conceivable that the phenyl moiety of DOM could be congruent with the A ring of (5R;8R)-(+)-LSD and the α-carbon of DOM would equate with the C5 position of LSD at which location both molecules exhibit the R configuration. Kuhlemeier, Beaton & Bradley (1975) have observed that like amphetamine, DOM induces hypothermia in rats housed in the cold. However, R(−)-DOM was more potent than S(+)-DOM in contrast to the amphetamine-induced hypothermia where the R(−)-isomer is less potent than S(+). It therefore appears that the hallucinogenic effect and the hypothermia induced by DOM may share common mechanisms as indeed they share stereospecificity. In order to delineate further the relative potency of the DOM isomers and their mode of action, we have extended our investigation of the hypothermic effects of this hallucinogen.

Methods

Adult male brown hooded rats were used in all experiments. Colonic temperature was measured 5.5 to 6.0 cm past the anal sphincter with a copper-constantan thermocouple and a Model 112, Honeywell multipoint temperature recorder. All experiments were carried out in an environmental chamber maintained at 6 ± 1°C except the studies relating ambient temperature to responses to DOM when ambient temperatures were 10, 20 and 30°C. In

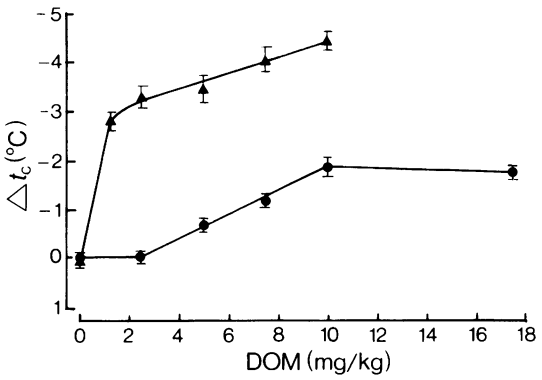

Figure 1 Dose-response curves showing the relation between change in colonic temperature (Δt_c) of rats after one hour in a cold (6 ± 1 °C) environment and various dosages of R(−)-2,5-dimethoxy-4-methylamphetamine (DOM) (▲) and S(+)-DOM (●). Points are means for 25 animals. Vertical lines show s.e. mean.

some experiments the rats were placed in cylindrical restraining cages during the experiments while in other experiments the animals were unrestrained in their usual cages. Colonic temperature was noted immediately before DOM administration and again after 1 h in the environmental chamber. All tests were carried out at the same time of day and at least one week was allowed between successive injections to the same animal except in the studies on tolerance.

Animals pretreated with *p*-chlorophenylalanine (PCPA, Regis Chemical Co., Morton Grove, Il) were given a first dose of 300 mg/kg (i.p.) 72 h before testing and a second and third dose of 100 mg/kg at 48 and at 24 h before testing. Pimozide, 8 mg/kg (s.c.) (kindly supplied by Dr. Paul Janssen) and methysergide, 1 or 2 mg/kg (s.c.) (Sandoz Pharmaceuticals, Hanover, NJ) were given to the rats 30 min before testing. DOM and methysergide were dissolved in sterile 0.9% w/v NaCl solution (saline) while PCPA and pimozide were suspended in sterile saline. DOM was injected subcutaneously. Colonic temperature measurements were made immediately before DOM administration and again after 60 min in the environmental chamber. The R(−) and S(+)-isomers of 2,5-dimethoxy-4-methylamphetamine hydrochloride were prepared as described by Nichols, Barfknecht, Rusterholz, Benington & Morin (1973). In most experiments the animals were placed in restraining cages but in the last experiments completed (see Table 1) and in later unpublished experiments unrestrained animals were used so that exposure periods longer than 1 h could be used if desired. Colonic temperatures of saline-treated restrained rats decrease continually for the duration of restraint.

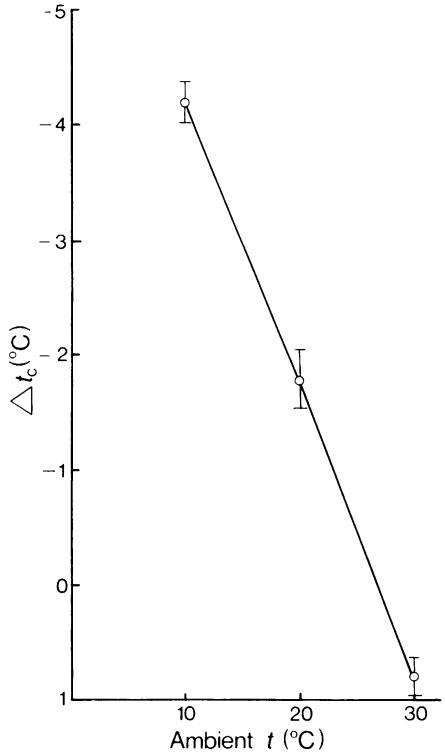

Figure 2 Relationship between ambient temperature and change in colonic temperature (Δt_c) of rats given 10 mg/kg of R(−)-2,5-dimethoxy-4-methylamphetamine (DOM). Points are means for 18 animals. Vertical lines show s.e. mean.

Results

Hypothermic effects of DOM isomers

The dose-response relationships between colonic temperature and the R(−) and S(+)-isomers of DOM at an environmental temperature of + 6 °C are shown in Figure 1. The R(−)-isomer is clearly more potent than S(+). R(−)-DOM induced hypothermia when given at 1.25 mg/kg while S(+)-DOM had no effect at 2.5 mg/kg. S(+)-DOM at 17.5 mg/kg gave the same response as S(+)-DOM at 10 mg/kg. Doses of R(−)-DOM above 10 mg/kg were not given because it was feared that the resulting hypothermia might be fatal. The relation between ambient temperature and colonic temperature is illustrated in Figure 2 for a group of rats given 10 mg/kg R(−)-DOM. The equation of this line is $Y = 0.25\ X - 6.7$ where Y is colonic temperature change (corrected for saline control) in °C 1 h after the injection and X is the ambient temperature in °C. The hypothermia induced by R(−)-DOM is

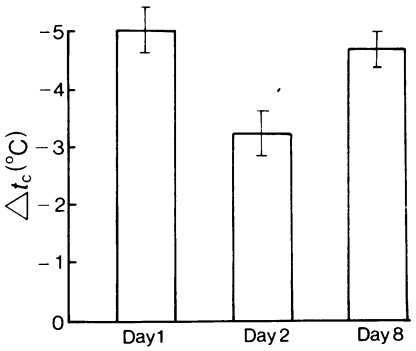

Figure 3 Mean and standard error of change in colonic temperature (Δt_c) of nine rats given 10 mg/kg R(−)-2,5-dimethoxy-4-methylamphetamine (DOM) and placed in a cold (6 ± 1 °C) environment for 1 h on successive days and again seven days after the second injection. Temperature measurements were made immediately before injection and placement in the environmental chamber and after 1 h in the chamber.

undoubtedly controlled by ambient temperature within this range. Slight hyperthermia is evident at 30°C.

Tachyphylactic effect on DOM administration

A tachyphylactic effect on the colonic temperature response was seen when R(−)-DOM (10 mg/kg) was given to the animals on two consecutive days as shown in Figure 3. The mean and standard error of the response on the first day was −5.02 ± 0.39°C; the response to the same dose given 24 h later was −3.22 ± 0.39°C, and the response seven days after the second injection was −4.65 ± 0.31°C. There was no significant difference between the response on the first day of this study and the response seven days after the second test day (paired *t*-test). However, the response on the second test day was significantly less ($P < 0.02$) than the response in the experiments conducted 24 h earlier.

Effect of pretreatment with pimozide, PCPA, and methysergide

The data are combined in Table 1. Neither pimozide nor PCPA pretreatment had a significant effect on the fall in colonic temperatures of rats subsequently given saline, R(−)- or S(+)-DOM. Pretreatment with methysergide at a dose of 2 mg/kg significantly ($P < 0.001$) reduced the hyperthermia. The responses to the R(−) and S(+)-isomers of DOM were equally affected by methysergide pretreatment when the effects of both isomers alone were equalized by giving 0.625 mg/kg R(−)-DOM and 8.75 mg/kg of S(+)-DOM. When the animals were given equimolar quantities of R(−)- and S(+)-DOM (10 mg/kg) and pretreated with 1 mg/kg methysergide the response to the R isomer was significantly reduced ($P < 0.05$) but the response to the S isomer was not significantly affected ($P > 0.05$).

Discussion

These results show that the temperature lowering effect of DOM seen in rats placed in the cold is stereospecific and that the R(−)-isomer is more effective than the S(+)-isomer. In contrast, the hypothermia induced by amphetamine is more pronounced after injection of the S(+)-isomer. Although amphetamine and DOM both produce hypothermia the reversal of stereospecificity suggests

Table 1 Effect of pimozide, *p*-chlorophenylalanine (PCPA) and methysergide on colonic temperature of rats given 2,5-dimethoxy-4-methylamphetamine (DOM) and placed in an environmental chamber maintained at 6 ± 1 °C for one hour.

Pretreatment	*Treatment: Saline*	*R-DOM*	*S-DOM*	n
	Change in colonic temperature (Δt_c °C)			
Pimozide	−1.73 ± 0.27	−5.07 ± 0.35	−2.83 ± 0.30	15
PCPA	−1.49 ± 0.34	−4.66 ± 0.20	−2.36 ± 0.23	9
Methysergide 1 mg/kg	−0.49 ± 0.12	−2.83 ± 0.38	−1.06 ± 0.17	9
None	−1.53 ± 0.15	−5.36 ± 0.33	−3.02 ± 0.42	15
Methysergide 2 mg/kg	−0.05 ± 0.10	−0.25 ± 0.07	−0.61 ± 0.16	25
None	−0.29 ± 0.10	−1.20 ± 0.13	−1.29 ± 0.13	25

Rats pretreated with pimozide, PCPA and methysergide (1 mg/kg) were tested in restraining cages and given 10 mg/kg DOM or saline. Rats pretreated with methysergide (2 mg/kg) were given 0.625 mg/kg of R-DOM and 8.75 mg/kg of S-DOM and were unrestrained.

that the effect is produced by a different mechanism of action for each drug. The potency of R(−)-DOM in inducing hypothermia and in disrupting avoidance behaviour in the rat is higher than the potency of S(+)-DOM (Benington *et al.*, 1973). Snyder, Unger, Blatchley & Barfknecht (1974) have shown a higher potency of the R(−)-isomer of 2,5-dimethoxy-4-ethyl-amphetamine in the psychedelic activity in humans. The tachyphylaxis observed in DOM-induced hypothermia is similar to that reported for the effects of DOM on cat brain electrical activity (Wallach, Friedman & Gershon, 1972), for the increase in arterial blood pressure in the dog (Cheng, Long, Barfknecht & Nichols, 1973), and for hallucinogenic effects in man (Hollister, MacNicol & Gillespie, 1969). A detailed metabolic study with labelled DOM would be required in order to understand this development of behavioural and physiological tolerance. Wallach *et al.* (1972) have suggested that a metabolite of DOM might continue to act as an antagonist at the receptor involved. It is feasible that DOM itself might remain at the receptor or could induce a long term change in receptor function akin to desensitization or permanent depolarization. In the experiments described here, the DOM-induced hypothermia was effectively attenuated by methysergide but not by PCPA, as is also the case for the effect of DOM on the EEG in the cat (Wallach *et al.*, 1972). This finding implies that DOM-induced hypothermia may be due to a direct effect on post-synaptic 5-hydroxytryptamine receptors. Any action on dopamine receptors may be ruled out as the effect is not antagonized by pimozide. The 5-hydroxy-tryptamine-like effect of DOM may be related to its potency as an hallucinogen. Both effects are best achieved by the R(−)-isomer and may share a common site with (5R,8R)-(+)-LSD (Morin, Benington, Mitchell, Beaton, Bradley & Smythies, 1975) and other hallucinogenic indoles (Bradley & Johnston, 1970). The fact that hypothermia is induced in this way by a potent hallucinogen such as DOM may provide a reliable measure with which to explore both the mode of action of hallucinogenic drugs and the role of neurotransmitter systems in temperature regulation.

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