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ANTAGONISM OF THE EFFECTS ON THERMOREGULATION OF Δ^9-TETRAHYDROCANNABINOL BY CLOMIPRAMINE IN THE RAT

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1 The effect of pretreatment with clomipramine hydrochloride (15 mg/kg, i.p.) on the (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC)-induced changes in body temperature and brain amines of the rat was investigated.

2 A dose of 0.05 mg/kg of Δ^9-THC produced hyperthermia and a decrease in whole brain concentration of 5-hydroxyindoleacetic acid (5-HIAA). Doses of 2 and 5 mg/kg produced hypothermia and increases in brain 5-HIAA whereas 0.5 mg/kg did not affect either parameter. Δ^9-THC, at any of the doses, did not affect the whole brain concentrations of dopamine, noradrenaline or 5-hydroxytryptamine.

3 Clomipramine modified these responses of Δ^9-THC in that the dose-response curves appeared to be shifted to the right.

4 It is concluded that clomipramine acts as an antagonist to these actions of Δ^9-THC by interfering with entry of Δ^9-THC into tryptaminergic neurones.

Introduction

Since Δ^9-tetrahydrocannabinol (Δ^9-THC) has been shown to alter the brain levels of 5-hydroxytryptamine (5-HT) (Holtzman, Lovell, Jaffe & Freedman, 1969; Schildkraut & Efron, 1971; Sofia, Dixit & Barry, 1971) and 5-hydroxyindoleacetic acid (5-HIAA) (Fennessy & Taylor, 1977) it has been suggested that Δ^9-THC may exert some of its effects, such as hypothermia, through modification of tryptaminergic mechanisms. Evidence from previous studies indicates that clomipramine, a tricyclic antidepressant, preferentially inhibits the neuronal uptake of 5-HT (Lidbrink, Jonsson & Fuxe, 1971; Sugrue, Goodlet & Mireylees, 1976). Consequently, the present study was undertaken to examine the effect of clomipramine on the Δ^9-THC-induced changes in body temperature and brain amines in an attempt to test the hypothesis of tryptaminergic involvement in the actions of Δ^9-THC.

Methods

Animals

Male albino Wistar rats weighing between 240 and 280 g were used. Food and water were available *ad libitum*. For intravenous administration, rats were surgically prepared by insertion of permanent polyethylene (PE10) catheters into the external jugular vein under amylobarbitone and methohexitone sodium (i.p. anaesthesia). Prior to surgery, animals were housed in group cages, but for the 48 h recovery and for the duration of the experimental period, they were kept in individual cages. The room in which rats were housed and the experiments conducted was maintained at an ambient temperature of 21 ± 1°C with a 12 h light–dark cycle. Rats were used either for subjective behavioural observations and body temperature studies or brain amine determinations.

Preparations of Δ^9-THC and drug administration

For intravenous administration an injectible dispersion of Δ^9-THC in saline (0.9% w/v NaCl solution), using polyvinylpyrrolidone (PVP) following the method of Fenimore & Loy (1971) was used. Clomipramine hydrochloride (15 mg/kg, i.p.) dissolved in saline was injected 30 min before Δ^9-THC or PVP (i.v.). Solutions of clomipramine, Δ^9-THC and PVP were injected in a volume of 1 ml/kg body weight.

Body temperature studies

The body temperature of individually caged, unrestrained rats was recorded continuously for at least 3 h by means of a thermistor probe inserted 60–70 mm into the rectum 1 h before drug administration. Three thin pieces of adhesive tape held the probe in position relative to the tail. Temperatures were recorded continuously with an Offner type R pen recorder.

Brain amine determinations

One hour after the intravenous injection of Δ^9-THC or PVP, rats were killed by decapitation. Their brains were rapidly removed, blotted free of blood, frozen in liquid N_2 and stored at -20°C. For amine determinations, the frozen brains were weighed and, after thawing in a solution of 0.4 M perchloric acid containing 0.2% disodium edetate, homogenized in an Ultra-Turrax homogenizer. The amines dopamine, noradrenaline (NA) and 5-HT were extracted from the homogenate using Bio-Rex 70 (Bio-Rad Laboratories) according to the column procedure of Barchas, Erdelyi & Angwin (1972). 5-Hydroxyindoleacetic acid (5-HIAA) was further extracted from the first effluent off the column and the extracted amines were assayed spectrophotofluorimetrically as previously described (Fennessy & Taylor, 1977). The fluorescence developed was determined with an Aminco-Bowman spectrophotofluorimeter. The concentrations of brain amines are expressed in terms of the free base or acid.

Analysis of results

In the body temperature studies, results are expressed in 'Thermic Index' values as defined by Jori, Paglialunga & Garattini (1967). The thermic index is a measurement of the cumulative change in body temperature from the original which is calculated 5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 min after the injection of Δ^9-THC or its vehicle. The thermic index values at 60 and 120 min have been calculated. For testing the statistical significance of differences between means, Student's t test was used.

Drugs

The following drugs and chemicals were used: amylobarbitone sodium (Amytal, Eli Lilly), clomipramine hydrochloride (Anafranil, Ciba-Geigy), dopamine hydrochloride (3-hydroxytyramine, Sigma), 5-hydroxyindoleacetic acid cyclohexyl ammonium salt (Sigma), 5-hydroxytryptamine creatinine sulphate (Koch-Light), methohexitone sodium (Brietal Sodium, Eli Lilly), (−)-noradrenaline hydrochloride (Levarterenol, Sigma), polyvinylpyrrolidone (Kollidon 25, BASF) and (−)-*trans*-Δ^9-THC (Batch SSC 75814, SSC81896, NIMH). All reagents used for extraction and assay were of analytical grade.

Results

Subjective behaviour

Behavioural biphasic effects of Δ^9-THC in rats have been described elsewhere (Fennessy & Taylor, 1977; Taylor & Fennessy, 1977). Clomipramine (15 mg/kg, i.p.) appeared to produce mild sedation manifested as a decrease in motor activity. Although these changes in behaviour were not quantified, pretreatment with clomipramine did not appear to alter greatly the Δ^9-THC-induced excitation and depression. If anything, Δ^9-THC-treated rats appeared to be less depressed when treated with clomipramine.

Effect of clomipramine on Δ^9-THC-induced changes in body temperature

The mean body temperature of 31 rats at the time of injection of clomipramine was 37.96 (±0.12)°C. Thirty minutes later, the temperature was significantly reduced to 37.56 (±0.12)°C ($P < 0.05$). In another 10 rats, saline did not alter body temperature during a 30 min observation period. The effect of clomipramine on changes in body temperature and thermic index values 1 and 2 h following PVP or the various doses of Δ^9-THC are shown in Figure 1. These values are obtained from the body temperatures measured at the time of injection of PVP or Δ^9-THC (i.e., 30 min after clomipramine). Consequently the differences obtained between PVP-treated rats, with and without clomipramine pretreatment, are artificial because the body temperature changes after PVP are measured from the lowered level. The maximal fall in body temperature of 0.40°C induced by clomipramine occurred after 30 min, and a further 1 h elapsed before body temperature returned to the pre-clomipramine level.

The effect of Δ^9-THC on body temperature is dependent on dose. At both 1 and 2 h after the Δ^9-THC injection the low dose of 0.05 mg/kg, compared to PVP controls, produced hyperthermia, whereas the higher doses of 2 and 5 mg/kg caused hypothermia. The intermediate dose of 0.5 mg/kg had no effect on body temperature. These responses are modified by pretreatment with clomipramine. Compared to PVP controls, the low dose did not change body temperature after clomipramine. The dose of 0.5 mg/kg resulted in an increase in body temperature 2 h after Δ^9-THC whereas with 2 mg/kg there was no change after clomipramine. The highest dose of 5 mg/kg produced hypothermia at both 1 and 2 h after Δ^9-THC. These changes in body temperature are

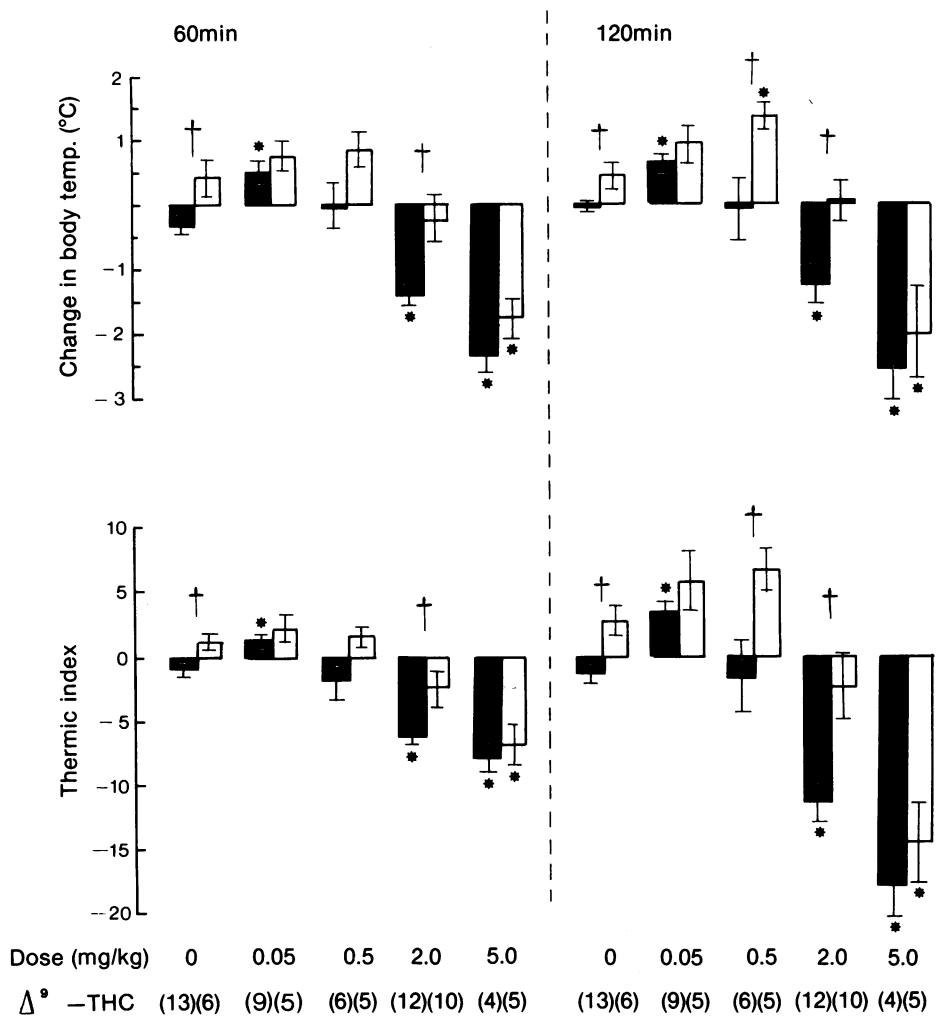

Figure 1 The effect of different doses of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) on body temperature and thermic indices, at 60 and 120 min, in rats receiving either no pretreatment (solid columns) or clomipramine hydrochloride (15 mg/kg i.p., open columns), 30 min before injection of Δ⁹-THC. The figures in parentheses are the number of rats in each group. The values are the means; s.e. means are shown by vertical lines. * $P < 0.05$ compared to PVP-treated controls (no Δ⁹-THC); † $P < 0.05$ comparing clomipramine pretreatment with no pretreatment at each dose of Δ⁹-THC.

reflected by the thermic index values as is the time course of the body temperature response (Figure 1).

The antagonism by clomipramine of the Δ⁹-THC-induced changes in body temperature is demonstrated further by comparison of the effect of Δ⁹-THC, at each dose, with and without pretreatment with clomipramine. With clomipramine pretreatment, at all doses of Δ⁹-THC and at both times, there was an increase in all values but this trend was significant at a dose of 0.5 mg/kg Δ⁹-THC at 2 h and with 2.0 mg/kg at both 1 and 2 h ($P < 0.05$) (Figure 1).

Effect of clomipramine on Δ⁹-THC-induced changes of brain amines

Pretreatment with clomipramine did not alter the whole brain concentrations of dopamine, NA, 5-HT and 5-HIAA of rats treated for 1 h with the control vehicle, PVP (Table 1). Compared with PVP-treated rats, Δ⁹-THC did not affect the whole brain concentrations of dopamine, NA or 5-HT in rats with or without clomipramine pretreatment except for an increase in the dopamine concentration of clomipra-

mine-pretreated rats that had received 5 mg/kg Δ^9-THC (Table 2). In addition, with each dose of Δ^9-THC, the concentrations of these amines were not modified by clomipramine pretreatment. However, the brain concentrations of 5-HIAA were markedly modified by Δ^9-THC in rats with or without clomipramine-pretreatment (Figure 2). Compared to PVP-treated rats, Δ^9-THC at the lowest dose decreased, whereas the two highest doses increased the concentration of 5-HIAA. Following pretreatment with clomipramine, Δ^9-THC at doses of 0.5 and 2 mg/kg decreased the brain concentration of 5-HIAA. At all doses of Δ^9-THC, pretreatment with clomipramine significantly modified the concentrations of brain 5-HIAA compared to animals that did not receive clomipramine ($P < 0.05$).

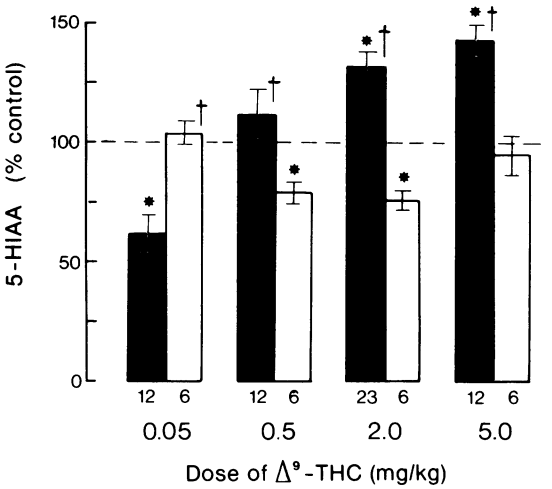

Figure 2 The effect of different doses of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the whole brain levels of 5-hydroxyindoleacetic acid (5-HIAA) in rats receiving either no pretreatment (solid columns) or clomipramine HCl (15 mg/kg i.p., open columns) 30 min before the injection of Δ^9-THC. The levels are expressed as percentage of control (PVP) together with their relative s.e. shown as the vertical bars. The figures at the bottom of each column indicate the number of rats in each group. * $P < 0.05$, compared to PVP-treated controls; † $P < 0.05$, comparing clomipramine pretreatment with no pretreatment at each dose of Δ^9-THC.

Discussion

The present study indicates that clomipramine modifies the effects of Δ^9-THC on body temperature and brain 5-HIAA concentrations in the rat. A dose of 2 mg/kg Δ^9-THC, which results in hypothermia, does not affect body temperature in rats pretreated with clomipramine, whereas a dose of 0.5 mg/kg, which normally does not affect body temperature, produces hyperthermia after clomipramine. This paradoxical effect of Δ^9-THC in clomipramine-pretreated rats is reminiscent of the results of Sofia (1972) and Taylor & Fennessy (1977) who showed that high doses of Δ^9-THC produced hypothermia while lower doses caused hyperthermia. Furthermore, the low dose of 0.05 mg/kg Δ^9-THC, after clomipramine, appears to be insufficient to modify body temperature significantly. Although clomipramine did not significantly alter the hypothermic response induced by 5 mg/kg Δ^9-THC, our results suggest that the trend is towards a reduction in this response. This high dose of Δ^9-THC may be large enough to overcome the inhibition resulting from clomipramine pretreatment. It is our opinion that the effect of clomipramine on the body temperature responses induced by Δ^9-THC, indicates a shift to the right of the dose–response curve, indicative of antagonism.

Although Δ^9-THC did not alter the whole brain concentrations of dopamine, NA or 5-HT, those of 5-HIAA were changed in a dose-dependent manner. These Δ^9-THC-induced changes in brain 5-HIAA are biphasic in that a dose of 0.05 mg/kg Δ^9-THC significantly reduces, whereas doses above 0.5 mg/kg significantly increase the 5-HIAA concentration (Taylor &

Table 1 Effect of clomipramine hydrochloride (15 mg/kg) on control whole brain concentrations of noradrenaline (NA), dopamine, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) measured 1 h after intravenous injections of polyvinylpyrrolidone (PVP, 40 mg/kg) in rats

	Whole brain concentrations (μg/g ± s.e. mean)			
Treatment	*NA*	*Dopamine*	*5-HT*	*5-HIAA*
Saline + PVP	0.407 ± 0.009	0.576 ± 0.034	0.393 ± 0.005	0.661 ± 0.019
Clomipramine + PVP	0.416 ± 0.029	0.484 ± 0.037	0.406 ± 0.013	0.688 ± 0.020
P value*	0.76	0.10	0.59	0.69

Clomipramine and saline were injected (i.p.) 30 min before the administration of PVP.
* *P* value, comparing saline + PVP with clomipramine + PVP.

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Table 2 Effect of pretreatment with clomipramine hydrochloride (15 mg/kg, i.p.) on whole brain concentrations of noradrenaline (NA), dopamine and 5-hydroxytryptamine (5-HT) in rats treated for 1 h with different intravenous doses of Δ⁹-tetrahydrocannabinol (Δ⁹-THC)

	Amine concentration (% control)†					
Dose of Δ⁹-THC (mg/kg)	*NA*		*Dopamine*		*5-HT*	
	Δ⁹-THC	*Clomipramine + Δ⁹-THC*	*Δ⁹-THC*	*Clomipramine + Δ⁹-THC*	*Δ⁹-THC*	*Clomipramine + Δ⁹-THC*
0.05	107.42 ± 4.60 (12)	95.21 ± 5.20 (6)	95.45 ± 6.91 (12)	89.54 ± 4.90 (6)	93.39 ± 5.38 (12)	93.53 ± 4.78 (6)
0.5	87.96 ± 5.84 (12)	90.92 ± 4.81 (6)	84.27 ± 11.45 (12)	104.32 ± 5.20 (6)	87.65 ± 6.95 (12)	95.65 ± 3.92 (6)
2.0	97.15 ± 7.36 (35)	91.73 ± 4.49 (6)	101.95 ± 3.51 (36)	103.08 ± 12.48 (6)	101.60 ± 2.93 (36)	103.33 ± 5.35 (6)
5.0	91.11 ± 4.96 (12)	97.80 ± 5.52 (6)	108.91 ± 8.29 (12)	129.21 ± 14.32 (6)*	106.45 ± 3.89 (12)	101.11 ± 5.90 (6)

Clomipramine was injected 30 min before administration of Δ⁹-THC.

† Values of the amines are expressed as percentages of the levels determined in PVP-treated controls ± relative s.e. Figures in parentheses are the number of rats in each group.

* $P < 0.05$, compared with PVP.

Fennessy, 1977). Clomipramine (15 mg/kg), by itself, did not modify the brain concentrations of any of the amines or of 5-HIAA 1.5 h after intraperitoneal administration. This observation conflicts with those of Collard & Roberts (1974), Goodlet & Sugrue (1974) and Sugrue *et al.* (1976) who found clomipramine to reduce rat brain levels of 5-HIAA. However, different doses and different times after administration were used, so comparisons are difficult to make. Clomipramine pretreatment of rats receiving different doses of Δ^9-THC did not modify brain concentrations of dopamine, NA or 5-HT, except for that of dopamine which was increased after the highest dose of Δ^9-THC. On the other hand, clomipramine did significantly alter the concentrations of 5-HIAA at each dose of Δ^9-THC. At the lowest dose, the 5-HIAA concentration was increased back to the control level, whereas with the three higher doses of Δ^9-THC, the levels were significantly reduced either back to the control level or significantly below it. This effect of clomipramine on the Δ^9-THC-induced changes in brain 5-HIAA concentrations reflects a similar pattern to that seen in its effect on body temperature, i.e., a shift of the dose-response curve to the right. In the presence of clomipramine, low doses of Δ^9-THC had no effect, whereas doses of 0.5 and 2 mg/kg reduced and a dose of 5 mg/kg did not affect brain 5-HIAA.

Previously it has been suggested that there is a dose-dependent inverse relationship existing between the effects of Δ^9-THC on body temperature and changes in brain 5-HIAA of the rat (Taylor & Fennessy, 1977), although a greater range of doses of Δ^9-THC are needed, the present results confirm this observation and suggest that clomipramine, by apparently shifting the dose-response curves to the right, is acting as a type of antagonist to Δ^9-THC. Clomipramine has been reported to inhibit accumulation of exogenous 5-HT by brain tissues (Shaskan & Snyder, 1970; Lidbrink *et al.*, 1971) and to be a potent inhibitor of uptake of endogenous 5-HT from synaptic clefts (Carlsson, Jonason, Lindqvist & Fuxe, 1969; Meek, Fuxe & Andén, 1970; Collard & Roberts, 1974). It is our hypothesis that several of the actions of Δ^9-THC are mediated through tryptaminergic mechanisms whereby an increase or decrease in brain 5-HIAA indicates a corresponding increase or decrease in release of brain 5-HT. High doses of Δ^9-THC increase the release of brain 5-HT which is manifested as hypothermia and at low doses the decreased release is manifested as hyperthermia. This indicates a dual action of Δ^9-THC whereby release of 5-HT has been shown to be inhibited (Englert, Ho & Taylor, 1973) or increased (Ho & Johnson, 1976). If the effects of Δ^9-THC are mediated through release of neuronal 5-HT it would be expected that clomipramine potentiates some of the actions of Δ^9-THC. However, since antagonism does occur it is suggested that clomipramine blocks the uptake of Δ^9-THC into tryptaminergic neurones.

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