Br. J. Pharmac. (1973), **49**, 243–252.

The effects of (-)-Δ^9-tetrahydrocannabinol on reserpine-induced hypothermia in rats

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Summary

1. An intravenous injection into rats of 1 mg/kg (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC) had no effect on rectal temperature and produced in the subcellular fractions of the brain a shift of 5-hydroxytryptamine (5-HT) from the particulate or 'bound' 5-HT to the supernatant or 'free' fraction, whereas the noradrenaline (NA) decreased in both fractions.

2. Pretreatment of rats by an intravenous injection of 1 mg/kg Δ^9-THC three times a week for four weeks, prevented the hypothermia and the reduction in brain 5-HT produced by an intraperitoneal injection of 15 mg/kg reserpine given 24 h after the last Δ^9-THC injection.

3. Pretreatment of rats by a single intravenous injection of 1 mg/kg Δ^9-THC prevented the hypothermia and reduction in brain 5-HT produced by an intraperitoneal injection of reserpine given 1 h before. The reduction in brain NA was not prevented except at the 18 h time interval.

4. An injection of 1 mg/kg Δ^9-THC intravenously into rats 3 h after an intraperitoneal injection of reserpine accentuated the reserpine hypothermia as well as the reduction of 5-HT but not of NA in the brain.

5. The reserpine hypothermia was not prevented by a single intravenous injection of 1 mg/kg Δ^9-THC when cinanserin, a 5-HT inhibitor, was injected 30 min before the reserpine.

Introduction

There have been many attempts to relate brain amine concentrations with thermoregulation. Based on the results obtained on injection of noradrenaline (NA) and 5-hydroxytryptamine (5-HT), into the cerebral ventricles, Feldberg & Myers (1963, 1964, 1965) suggested the involvement of catecholamines and 5-HT in the central mechanism of temperature control. The effects of brain amines on body temperature vary among different species; in rats 5-HT and NA produce hypothermia, but the effect of NA changes to hyperthermia as the concentration increases (Feldberg & Lotti, 1967). More recent evidence also suggests a role for dopamine in thermoregulation. The hypothermic response to 6-hydroxydopamine in rats has been related to the release of dopamine (Simmonds & Uretsky, 1970). Fuxe, Hökfelt & Ungerstedt (1970) related the hypothermia observed after administration of apomorphine to activation of dopaminergic receptors. In addition, the dopamine-β-oxidase inhibitor, FLA-63 (bis-(4-methyl-1-homopiperazinylthiocarbonyl)disulphide), has been shown to decrease the effect of dopamine on body temperature in mice (Svensson & Waldeck, 1969).

The role of brain monoamines in reserpine-induced hypothermia continues to be a controversial problem. In rats, imipramine-like drugs prevent the hypothermia produced by reserpine (Costa, Garattini & Valzelli, 1960; Garattini, Giachetti, Jori, Pieri & Valzelli, 1962; Garattini & Jori, 1967), but when given during the reserpine hypothermia they increase body temperature (Askew, 1963; Jori & Garattini, 1965). This hyperthermia may be due to potentiation of NA action because the imipramine-like drugs prevent the NA re-uptake, thus making more NA available to the receptor. An antagonism of the hypothermic effect of reserpine in rats by α-methyl-*m*-tyrosine, the precursor of a false transmitter metaraminol, has also been reported (Garattini & Valzelli, 1961). Recently, the effect of $\triangle^9$-tetrahydrocannabinol ($\triangle^9$-THC) on 5-HT metabolism has been studied in rats by Sofia, Dixit & Barry (1971). These authors found that the reduction of brain 5-HT induced by reserpine was retarded by pretreatment with $\triangle^9$-THC.

The present investigation deals with the effect of chronic and acute $\triangle^9$-THC pretreatment on the hypothermia and reduction of the brain monoamines produced by reserpine as observed on their subcellular distribution. We also examined whether $\triangle^9$-THC had an effect when given after reserpine at a time when the hypothermia and reduction of brain monoamines had already occurred, and whether the effect of acute $\triangle^9$-THC pretreatment could be influenced by cinanserin, an inhibitor of 5-HT.

Methods

Male Sprague-Dawley rats weighing 150 to 170 g were used. Their rectal temperature was taken every 30 or 60 min by means of a rectally inserted probe with a telethermometer (Yellow Spring Instrument Company) calibrated at 0·1° C intervals. The rats were used in groups of six animals and for each time interval their mean temperature was calculated and the S.E. was determined.

For the chronic $\triangle^9$-THC pretreatment groups of four rats were used. Each rat received intravenous injections of 1 mg/kg $\triangle^9$-THC three times a week for four weeks and at least 24 h after the last injection, reserpine (15 mg/kg) was given intraperitoneally; 18 h later the rats were killed for determination of brain 5-HT and NA.

For the acute $\triangle^9$-THC pretreatment three groups of four rats were used. Each rat was given a single intravenous injection of 1 mg/kg $\triangle^9$-THC 1 h before the intraperitoneal injection of 15 mg/kg reserpine, and one group was killed 3 h, another 6 h, and the third 18 h later for determination of the brain 5-HT and NA. The same number of reserpine-control animals were killed at each time interval. Groups of rats receiving only the intravenous $\triangle^9$-THC injection were killed 4, 7 and 19 h after the injection so that the time intervals coincided with those of the rats given both compounds.

In the experiments in which the effect of $\triangle^9$-THC was studied after an intraperitoneal injection of 15 mg/kg of reserpine, the intravenous injection of 1 mg/kg $\triangle^9$-THC was given 3 h later. This time interval was chosen because 3–6 h after the injection of reserpine the rate of fall in body temperature was found to be greatest. To determine the 5-HT and NA in the brain the rats, again in groups of four, were killed 3, 6 and 18 h after the reserpine injection.

Subcellular distribution of 5-hydroxytryptamine and noradrenaline in brain homogenates

The rats were killed by decapitation under light ether anaesthesia and the brains removed and frozen until assayed. They were homogenized in 2 parts 0·025 M sucrose containing 1·5 mM ethylenediamine tetraacetic acid and 2 mM tranylcypromine. The homogenate was centrifuged at 100,000 *g* for 20 min as described by Giarman, Freedman & Schanberg (1964).

The supernatant and particulate fractions were separated and the pH adjusted to 2 with dilute HCl to give a total volume of 3 ml. Both 5-HT and NA were first extracted into *n*-butanol and then returned to 1·5 ml of 0·1 N HCl containing 1% cysteine according to a modified procedure of Curzon & Green (1970). Duplicate 0·5 ml aliquots were taken and assayed separately for 5-HT and NA. Internal standards of 5-HT and NA were added to the whole brain homogenates and run through the extraction procedure.

5-HT was determined by the *o*-phthalaldehyde(OPT) reaction and the fluorescent product measured on an Aminco-Bowman Spectrophotofluorometer (activation λ: 360 nm; fluorescence λ: 470 nm). NA was oxidized by the procedure of Laverty & Taylor (1968). This method utilizes oxidation with iodine, alkaline rearrangement and subsequent measurement of the fluorescence at an acidic pH (activation λ: 380 nm; fluorescence λ: 480 nm).

The concentration of the amines in the supernatant and particulate fractions are expressed as μg/g (mean of 4 assays ± S.E.M.). Total brain values were estimated from the two fractions. Percentage values are the difference between the Δ^9-THC group and either the reserpine or vehicle controls. The statistical significance was calculated by Student's *t* test.

Drugs

Δ^9-Tetrahydrocannabinol (Δ^9-THC) was synthesized in our laboratories; it contained less than 10% of unidentified impurities as determined by gas chromatography (Idänpään-Heikkilä, Fritchie, Englert, Ho & McIsaac, 1969). As its absorption on intraperitoneal injection is poor and erratic (Ho, Fritchie, Englert, McIsaac & Idänpään-Heikkilä, 1971) it was injected in a 4% Tween-80–0·9% NaCl solution into the tail vein. A dose of 1 mg/kg in a volume of 0·1 ml was injected each time. In the experiments in which the drug was injected for four weeks the solutions were prepared fresh daily, and the dose was adjusted for the increase in body weight. Reserpine (Regis Chemical Company) was dissolved in 30% propylene glycol and a dose of 15 mg/kg was injected intraperitoneally in a volume of 0·5 ml. Cinanserin (Nutritional Biochemical Corporation) an inhibitor 5-HT was dissolved in 0·9% w/v NaCl and 10 mg/kg was injected intraperitoneally in a volume of 0·5 ml.

Results

Figure 1 shows that an intravenous injection of 1 mg/kg Δ^9-THC had little or no effect on rectal temperature, but that an intravenous injection of 5 mg/kg produced hypothermia which lasted for approximately 3 hours. The intravenous injection of 1 mg/kg Δ^9-THC affected the brain monoamines, 5-HT and NA,

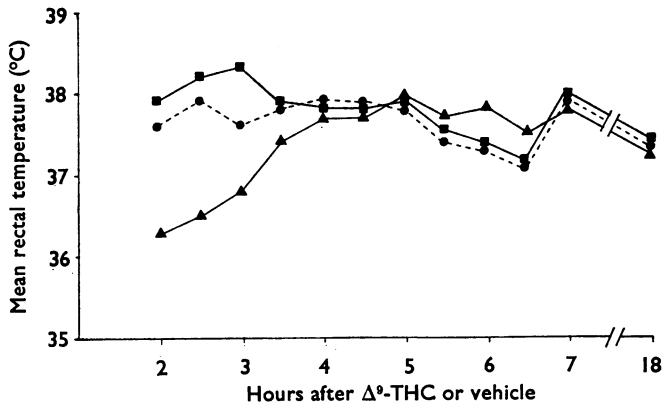

FIG. 1. Effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the rectal temperature of rats. (●- - - -●), vehicle control; (×——×), Δ^9-THC (1 mg/kg, i.v.); (▲——▲), Δ^9-THC (5 mg/kg, i.v.). Each value represents the mean of six animals.

TABLE 1. *Effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the subcellular distribution of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in the rat brain*

Treatment	Amine concentration (μg/g±S.E.) Total	Particulate (P)	Supernatant (S)	% Change from controls P	S
	5-HT				
Control (vehicle)	0·49	0·34±0·01	0·15±0·01	—	—
Δ^9-THC (1 mg/kg)					
4 h	0·42	0·24±0·01†	0·18±0·01	−29·3	+20·0
7 h	0·51	0·30±0·01	0·21±0·01†	−11·8	+40·0
19 h	0·49	0·29±0·03	0·20±0·02	−14·7	+33·3
	NA				
Control (vehicle)	0·34	0·21±0·01	0·13±0·01	—	—
Δ^9-THC (1 mg/kg)					
4 h	0·22	0·12±0·01*	0·10±0*	−43·0	−23·1
7 h	0·25	0·15±0·01†	0·10±0·01*	−28·6	−23·1
19 h	0·29	0·19±0·10	0·10±0·01*	−9·5	−23·1

Each value represents the mean of four animals. *$P<0·01$; †$P<0·05$.

differently. As shown in Table 1, the total level of brain 5-HT remained unchanged but there was a shift of the 5-HT from the particulate or 'bound' fraction to the supernatant or 'free' fraction. On the other hand, the total level of brain NA was reduced and both the particulate and supernatant fractions were lower than the reserpine controls.

Since the intravenous injection of 1 mg/kg Δ^9-THC caused very little change in the rectal temperature, this dose was chosen in the following experiments for studying the effect of Δ^9-THC on the reserpine-induced hypothermia and reduction of the brain monoamines in whole brain, as well as on its subcellular distribution.

Effect of chronic Δ^9-tetrahydrocannabinol pretreatment on the reserpine-induced hypothermia and reduction of brain monoamines

The long-lasting hypothermia which developed a few hours after an intraperitoneal injection of 15 mg/kg reserpine and was still evident 18 h later was prevented by chronic Δ^9-THC pretreatment. This is illustrated in Figure 2.

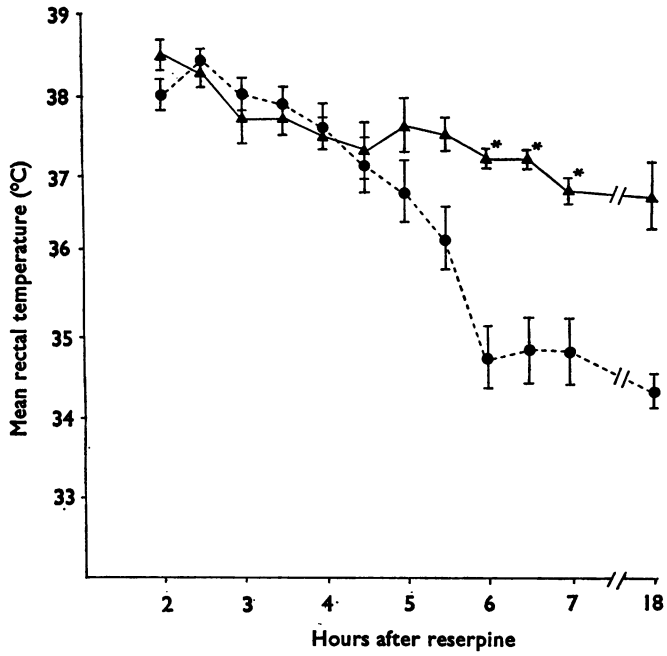

FIG. 2. Effect of chronic Δ^9-tetrahydrocannabinol (Δ^9-THC) treatment on reserpine-induced hypothermia in rats. (●- - - -●) reserpine (15 mg/kg, i.p.); (▲——▲), same dose of reserpine injected 24 h after the last chronic dose (1 mg/kg, i.v.) of Δ^9-THC. Vertical bars indicate S.E. obtained from six animals. *$P<0.01$.

TABLE 2. *Effect of chronic Δ^9-tetrahydrocannabinol (Δ^9-THC) treatment on the subcellular distribution of brain 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in reserpine-treated rats*

Treatment	Amine concentration (μg/g±S.E.) Total	Particulate (P)	Supernatant (S)	% Change from controls P	S
	5-HT				
Reserpine controls	0·21	0·10±0·01	0·11±0·01	—	—
Reserpine to chronic Δ^9-THC animals	0·32	0·17±0·01*	0·15±0·01†	+70·0	+36·3
	NA				
Reserpine controls	0·24	0·12±0	0·12±0	—	—
Reserpine to chronic Δ^9-THC animals	0·26	0·13±0·01	0·13±0·01	+ 8·3	+ 8·3

Reserpine (15 mg/kg; i.p.) was injected 24 h after the last chronic dose of Δ^9-THC, and the animals were killed 18 h after the reserpine. Each value represents the mean of four animals. *$P<0.01$; †$P<0.05$.

The effect of chronic Δ^9-THC pretreatment on the reduction of the brain monoamines which occurred after the reserpine injection differed for 5-HT and NA. The 5-HT reduction was blocked, whereas the NA reduction was not affected. These results are summarized in Table 2.

Without the Δ^9-THC pretreatment, both the 5-HT and NA levels in the brain were greatly reduced 18 h after the intraperitoneal reserpine injection. Since the reduction was more pronounced in the particulate than in the supernatant fraction, the levels of both 5-HT and NA became approximately equal in the two fractions. The reversal of the 5-HT reduction produced by the Δ^9-THC pretreatment was

found in both fractions, but more significantly in the particulate fraction where it resulted in an increase of 70% over the reserpine control. It is possible that this reversal of the 5-HT reduction might account for the prevention of the reserpine hypothermia by the chronic Δ^9-THC pretreatment as illustrated in Figure 2.

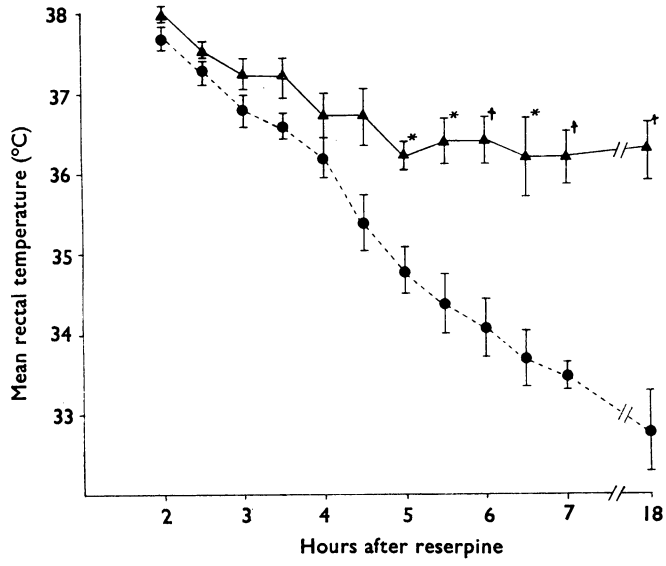

FIG. 3. Effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) pretreatment on reserpine-induced hypothermia in rats. (●- - - -●), effect of reserpine (15 mg/kg, i.p.); (▲——▲) Δ^9-THC (1 mg/kg, i.v.), one h prior to reserpine. Vertical bars indicate S.E. obtained from six animals. *$P<0{\cdot}01$; †$P<0{\cdot}001$.

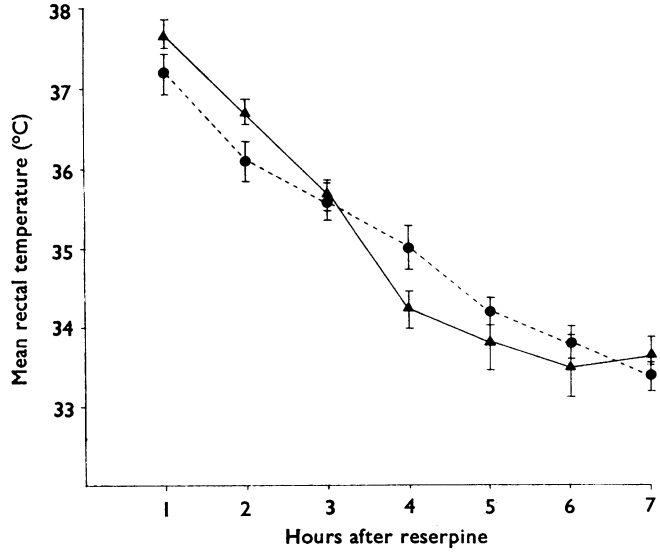

FIG. 4. Effect of cinanserin on the blockade of reserpine-induced hypothermia by Δ^9-tetrahydrocannabinol (Δ^9-THC) (●- - - -●), cinanserin (10 mg/kg, i.p.) injected 30 min prior to reserpine (15 mg/kg, i.p.); (▲——▲) Δ^9-THC (1 mg/kg, i.v.) injected 30 min before cinanserin and one h prior to reserpine. Vertical bars indicate S.E. obtained from six animals.

Effect of acute Δ^9-tetrahydrocannabinol pretreatment on the reserpine-induced hypothermia and reduction of brain monoamines

An intravenous injection of 1 mg/kg Δ^9-THC given 1 h before an intraperitoneal injection of 15 mg/kg reserpine prevented the reserpine hypothermia. The effect, as shown in Fig. 3 was significant from 5 h onwards during the 18 h time interval. However, this prevention of the reserpine hypothermia by Δ^9-THC did not occur when 10 mg/kg of cinanserin was injected 30 min before the reserpine. This is illustrated in Figure 4.

The effect of acute Δ^9-THC pretreatment on the reduction of the brain monoamines which occurred after the reserpine injection again differed for 5-HT and NA. The reversal of the 5-HT reduction was even more pronounced than with the chronic pretreatment while the NA reduction by reserpine was not consistently affected until 18 hours. These results are summarized in Table 3.

TABLE 3. *Effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) pretreatment on the subcellular distribution of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in the reserpine-treated rats*

Treatment	Amine concentration (μg/g±S.E.) Total	Particulate (P)	Supernatant (S)	% change from reserpine controls P	S
	5-HT				
Reserpine (15 mg/kg)					
3 h	0·12	0·06±0·01	0·06±0	—	—
6 h	0·11	0·05±0·01	0·06±0	—	—
18 h	0·23	0·10±0·01	0·13±0·01	—	—
Δ^9-THC (1 mg/kg) 1 h before reserpine					
3 h	0·29	0·10±0·01*	0·18±0·02†	+ 66	+200
6 h	0·34	0·15±0·02†	0·19±0·01†	+200	+217
18 h	0·47	0·25±0·02†	0·22±0·02†	+150	+ 69
	NA				
Reserpine (15 mg/kg)					
3 h	0·20	0·10±0·01	0·10±0·01	—	—
6 h	0·20	0·10±0·01	0·10±0·01	—	—
18 h	0·24	0·13±0·01	0·11±0	—	—
Δ^9-THC (1 mg/kg) 1 h before reserpine					
3 h	0·24	0·17±0·01†	0·07±0·01	+70·0	−30·0
6 h	0·17	0·10±0·01	0·07±0 *	0	−30·0
18 h	0·37	0·20±0·01†	0·17±0·01†	+53·8	+54·5

Δ^9-THC was injected i.v. one h prior to the i.p. administration of reserpine, and the concentration of amine was determined 3, 6 and 18 h after reserpine. Each value represents the mean of four animals. *$P<0·01$; †$P<0·001$.

Without the Δ^9-THC pretreatment, both the 5-HT and NA levels were rapidly reduced after the injection of 15 mg/kg of reserpine, and their levels were approximately equal in both fractions. The depletion was produced as early as 3 h after the injection, however, at 18 h a slight increase was obtained in both the particulate and supernatant fractions. This could be due to the high dose of reserpine used which caused the maximum depletion to occur before 18 hours.

The reversal of the 5-HT reduction produced by acute Δ^9-THC pretreatment was found in both fractions and was highly significant at all of the time intervals studied, but was most pronounced at 6 hours. This coincides with the prevention of the reserpine hypothermia, which was significant as early as 5 h as illustrated in Figure 3.

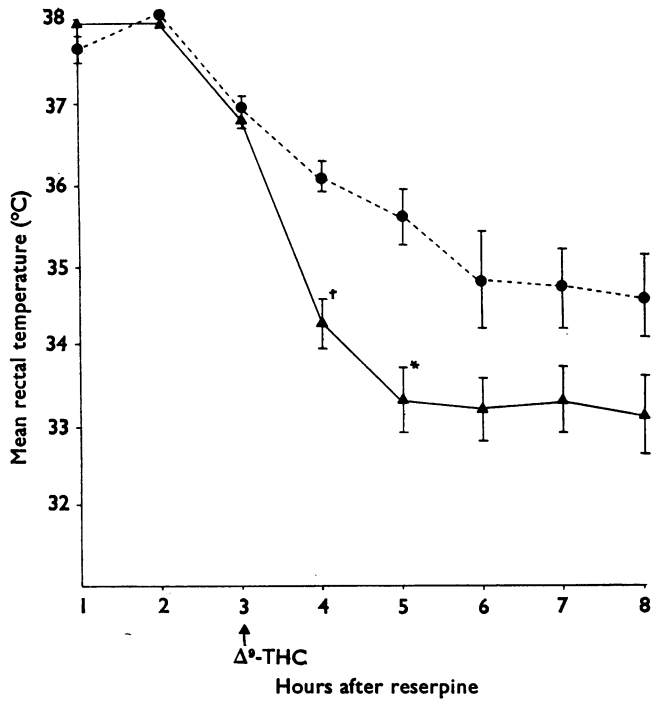

FIG. 5. Effect of △⁹-tetrahydrocannabinol (△⁹-THC) after reserpine-induced hypothermia in rats. (●----●) reserpine (15 mg/kg, i.p.); (▲——▲) △⁹-THC (1 mg/kg, i.v.) injected 3 h after reserpine. Vertical bars indicate S.E. obtained from six animals. *$P<0.01$; †$P<0.001$.

TABLE 4. *Effect of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) after reserpine on the subcellular distribution of brain 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in reserpine-treated rats*

Treatment	Amine concentration (μg/g±S.E.) Total 5-HT	Particulate (P)	Supernatant (S)	% Change from controls P	S
Reserpine (15 mg/kg)					
3 h	0·19	0·09±0·02	0·10±0	—	—
6 h	0·25	0·12±0·01	0·13±0·01	—	—
18 h	0·26	0·13±0·01	0·13±0·01	—	—
Δ⁹-THC (1 mg/kg) 3 h after reserpine					
3 h	0·18	0·10±0·01	0·08±0·01	+11	+20
6 h	0·15	0·07±0·01*	0·08±0·01†	−42	−39
18 h	0·20	0·10±0·01	0·10±0·01	−23	−23
	NA				
Reserpine (15 mg/kg)					
3 h	0·14	0·08±0·01	0·06±0	—	—
6 h	0·21	0·15±0·02	0·06±0·01	—	—
18 h	0·12	0·07±0·01	0·05±0	—	—
Δ⁹-THC (1 mg/kg) 3 h after reserpine					
3 h	0·15	0·10±0·01	0·05±0	+25	−16
6 h	0·23	0·14±0·01	0·09±0·01	− 7	+50
18 h	0·12	0·08±0	0·04±0	+14	−20

Δ⁹-THC was injected i.v. 3 h after the i.p. administration of reserpine, and the concentration of amine was determined 3, 6 and 18 h after reserpine. Each value represents the mean of four animals. *$P<0.01$; †$P<0.02$.

Effect of Δ^9-tetrahydrocannabinol after the hypothermia and reduction of brain monoamines produced by reserpine

When Δ^9-THC was injected 3 h after reserpine, the hypothermia was potentiated as illustrated in Figure 5. The effect was significant as early as 1 h after the Δ^9-THC and was still evident after 8 hours.

After the reduction of brain amines by reserpine, Δ^9-THC caused an enhancement in the depletion of 5-HT whereas NA reduction was essentially not affected. These results are summarized in Table 4.

Without the injection of Δ^9-THC the 5-HT and NA levels were reduced in both the particulate and supernatant fractions as early as 3 h after the reserpine. The injection of Δ^9-THC further depleted 5-HT in both fractions to approximately the same extent from 6 to 18 hours. These results were directly opposite to those obtained with either Δ^9-THC chronic or acute pretreatment. A possible correlation could exist between the further reduction of 5-HT in the brain and the potentiation of the reserpine-induced hypothermia by Δ^9-THC.

Discussion

Reserpine-induced hypothermia is prevented by both chronic and acute Δ^9-THC pre-treatment, and there is a concomitant blockade of the release of 5-HT in the 'free' and 'bound' forms in the rat brain. The reversal of reserpine action on total brain 5-HT by Δ^9-THC has also been demonstrated by Sofia *et al.* (1971).

When Δ^9-THC is injected after reserpine the potentiation of the hypothermia appears to coincide with an enhancement in the depletion of brain 5-HT. If the initial rate of release of 5-HT from the storage vesicles is responsible for the fall in rectal temperature as proposed by Brodie, Comer, Costa & Dlabac (1966), this could explain why the hypothermia is further augmented in reserpine-treated animals. The 5-HT, which has been released from the storage vesicles, stimulates the cold receptors and the temperature falls.

In the presence of cinanserin, a 5-HT inhibitor, Δ^9-THC pretreatment is no longer effective in blocking the reserpine hypothermia. This antagonism by cinanserin may be due to the loss of accessibility of 5-HT to the cold receptors. A similar effect of cinanserin on the hyperthermia produced by lysergic acid diethylamide in reserpine-treated animals has been reported by Rubin, Piala, Burke & Craver (1964).

Although the results of the present study do not preclude that the effects of Δ^9-THC on reserpine hypothermia could also be of peripheral origin there appears to be a correlation between the fall in rectal temperature and the release of brain 5-HT. There is, however, no obvious relation with brain NA. The involvement of central 5-HT neurones in thermo-regulation in rats has been proposed by Simmonds (1970) and 5-HT has been considered to be the more significant factor responsible for the sedative and hypothermic effects of reserpine (Brodie *et al.*, 1966).

We wish to thank Mrs. Ann Rougeaux, Mrs. Sara Reese and Mrs. Sherrel Jochen for their technical assistance.

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