Br. J. Pharmac. (1977), **60**, 559–568

EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON THE RATES OF OXYGEN CONSUMPTION OF MICE

R.G. PERTWEE & R. TAVENDALE

Department of Pharmacology, University Medical Buildings, Foresterhill, Aberdeen, AB9 2ZD

1 Experiments with untreated mice confirmed that at ambient temperatures below 30°C, the oxygen consumption rate of mice normally kept at about 23°C varies inversely with ambient temperature.

2 At given ambient temperatures in the range 20 to 31°C the oxygen consumption rate was 32 to 43% greater for restrained than for unrestrained mice.

3 Hypothermia induced in restrained mice by Δ^9-tetrahydrocannabinol (Δ^9-THC) (1.0 to 4.0 mg/kg i.v.) was accompanied by marked falls in the rate of oxygen consumption. The size of these falls parallelled the degree of hypothermia and increased both with increases in dose and with decreases in the ambient temperature. The oxygen consumption rates of unrestrained mice were also lowered by hypothermic doses (10 to 40 mg/kg i.p.) of Δ^9-THC.

4 The maximum falls in oxygen consumption rate occurred at earlier times after drug administration than the maximum falls in rectal temperature.

5 At none of the ambient temperatures studied did the oxygen consumption rates ofΔ^9-THC-treated mice fall significantly below the basal levels (59 ± 3 ml 25 g^{-1} h^{-1}) of unrestrained, resting mice at 30°C.

6 The hypothesis that reduced rates of heat production contribute significantly towards the hypothermia induced by Δ^9-THC in our experiments is discussed. The possibility that biological processes responsible for increased heat production in response to cold are more sensitive to Δ^9-THC than those processes governing basal rates of heat production at thermally neutral environmental temperature is also raised.

Introduction

Cannabis and its psychically active constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) have been shown by several workers (see Paton & Pertwee, 1973) to affect the body temperature of a wide range of species. However, the mechanisms underlying this effect have yet to be elucidated. As a first step in the search for these mechanisms it was decided to investigate the effect of hypothermic doses of Δ^9-THC on the rate of heat production by mice. Changes in rates of heat production were detected indirectly by the measurement of rates of oxygen consumption.

Methods

Oxygen consumption rates of mice were measured by the use of a newly designed apparatus. The apparatus consisted of a closed system in which air enriched with oxygen was repeatedly circulated (500 ml/min) through an animal chamber, a container of silica gel, an oxygen analyser (Beckman OM-11), a carbon dioxide analyser (Beckman LB-2), a pump, a flowmeter and finally a container of soda lime. The animal chamber contained a single mouse. To maintain the gas pressure within the system constant, carbon dioxide generated by the mouse and absorbed by the soda lime was replaced by nitrogen. The nitrogen was stored in a spirometer and entered the system on demand through a one-way water valve. The temperature of the system could be held at any value in the range 9 to 40°C. In the experiments in which unrestrained mice were studied the total volume of the system was 505 ml (animal chamber 250 ml). In the remaining experiments the total volume was 905 ml (animal chamber 740 ml). The gas analysers provided a continuous measure of the partial pressures of oxygen and carbon dioxide in the system. At the start of each experiment, the system contained approximately 60 kPa oxygen and 40 kPa nitrogen. At no time was the partial pressure of oxygen allowed to fall below 21 kPa. The partial pressure of carbon dioxide in the system was maintained below 1.0 kPa.

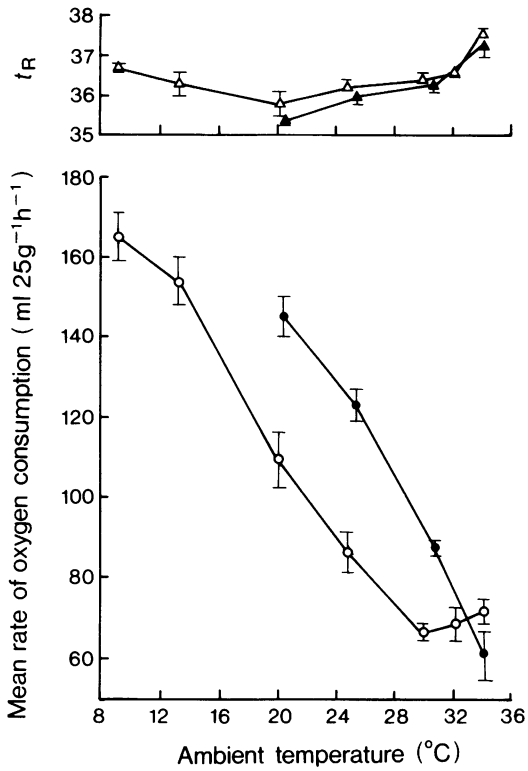

Figure 1 The effect of ambient temperature on the mean rectal temperature (t_R) and oxygen consumption rates of unrestrained (open symbols) and restrained (closed symbols) groups of 6 to 9 mice. Vertical lines show s.e.

During each experiment, analyser readings were recorded at intervals of 2 to 10 minutes. This procedure allowed the mean rate at which oxygen was being consumed in each of these intervals to be calculated. Oxygen consumption rate ($\dot{V}O_2$) was expressed as volume consumed per unit body weight per unit time (ml 25 g^{-1} h^{-1}) after adjustment for s.t.p.

Adult male mice (LACA strain) weighing 28–35 g were used. The mice were maintained on a circadian cycle of 12 h light (09 h 00 min to 21 h 00 min) and 12 h darkness and received food and water *ad libitum*. Experiments were carried out both in the morning and in the afternoon. Replicates of each experimental treatment were made at both these times. The mice were injected either intraperitoneally before entry into the animal chamber or intravenously 20 to 40 min after they had been sealed in the chamber. Mice were kept in the chamber for periods of 1 to 2.5 hours. Intravenous injections were made through cannulae which had been inserted into the lateral tail veins of mice held in a restraining apparatus (Pertwee, 1970, 1974). Use of the restraining apparatus also allowed the thermistors that were used to measure body temperature to remain in position throughout an experiment. Both rectal and paw temperatures were monitored. The former was measured with a thermistor probe (Y.S.I. 402) inserted 3 cm into the rectum. Paw temperature was measured by attaching a thermistor with adhesive tape to the plantar surface of a hind paw. Measurement of paw temperature can provide an index of change in peripheral vasomotor tone (Pertwee, 1970), changes in which reflect changes in heat loss. Rectal, paw and ambient temperatures were recorded by the use of a modification of the method described by Pertwee (1970). Each of three thermistors formed in turn one arm of a Wheatstone bridge circuit. A single channel pen recorder was connected across this circuit. By the use of an electric motor and a set of microswitches (Sealectro Ltd.) the readings of the three thermistors were recorded in sequence. The readings from any one of the thermistors was registered once every minute.

For injection, Δ^9-THC was mixed with two parts of Tween 80 by weight and then dispersed in 0.9% w/v NaCl solution (saline). Control injections contained doses of Tween 80 equal to or greater than those used in the corresponding drug injections. In all experiments, the volume injected was either 0.25 ml/25 g (i.p.) or 0.20 ml/25 g (i.v.).

Differences between the means of experimental data were evaluated by Student's t test ($P >$ or < 0.05) and limits of error have been expressed as standard errors. In cases where large differences in variance ruled out the use of Student's t test, Cochran's approximation to the Behrens-Fisher test described by Snedecor & Cochran (1973) was used.

Results

Oxygen consumption rates of untreated mice

Figure 1 shows how the oxygen consumption rates ($\dot{V}O_2$) and rectal temperatures of mice which had not received any drug treatment varied with ambient temperature. The $\dot{V}O_2$ of unrestrained mice decreased progressively with increase in ambient temperature over the range 9 to 30°C. At 30°C, the $\dot{V}O_2$ was minimal and was not significantly different from values observed at 32 or 34°C. The $\dot{V}O_2$ was also found to be significantly affected by the degree of motor activity of the mice. At 30°C, the $\dot{V}O_2$ during periods of activity was 85 ± 6 ml 25 g^{-1} h^{-1} whereas the value of $\dot{V}O_2$ during periods of rest was 59 ± 3 ml 25 g^{-1} h^{-1}. The mean $\dot{V}O_2$ for the whole of the experimental period at 30°C was 66 ± 2 ml 25 g^{-1} h^{-1}.

When mice were secured in a restraining apparatus, there was an approximately parallel shift to the right in the ambient temperature—oxygen consumption rate curve (Figure 1), so that in the temperature range 21

Table 1 Effects of Δ⁹-tetrahydrocannabinol (Δ⁹-THC, i.p.) on the oxygen consumption rates and rectal temperatures of unrestrained mice at 13°C

Drug	*Dose* (mg/kg)	n	*Minimum rate of oxygen consumption* (ml 25 g^{-1} h^{-1} ± *s.e.*)	T_1 (min)	n	*Rectal temperature* Initial (°C_R ± *s.e.*)	Minimum	T_2 (min)	P_1	P_2	P_{0-2}
Δ⁹-THC	5	–	–	–	8	37.2 ± 0.2	36.1 ± 0.3	30	–	<0.05*	<0.05
Tween 80	80	–	–	–	9	37.4 ± 0.2	36.9 ± 0.1				<0.001
Δ⁹-THC	10	5	129 ± 5	15	8	37.7 ± 0.2	35.2 ± 0.3	30	<0.05*	<0.001	<0.01
Tween 80	80	5	183 ± 19	–	9	37.4 ± 0.2	36.9 ± 0.1				<0.001
Δ⁹-THC	20	6	120 ± 8	30	8	37.5 ± 0.2	33.0 ± 0.6	30	<0.05	<0.001*	<0.001
Tween 80	80	5	160 ± 13	–	9	37.4 ± 0.2	36.9 ± 0.1				<0.001
Δ⁹-THC	40	6	103 ± 6	20	8	37.4 ± 0.1	31.4 ± 0.4	30	<0.05	<0.001	<0.001
Tween 80	80	5	173 ± 17	–	9	37.4 ± 0.2	36.9 ± 0.1				<0.001

Measurement of rectal temperature t_R and of oxygen consumption rate (Vo_2) were made on different mice. The earliest times after injection of Δ⁹-THC at which maximum falls in mean Vo_2 or t_R were observed are listed under T_1 and T_2 respectively. Digits listed under P_{0-2} are *P* values (paired *t* test) for differences between initial (preinjection) and minimum values of t_R. *P* values (unpaired *t* test) for differences between Δ⁹-THC and Tween treatments are listed under P_1 (Vo_2 values at T_1) and P_2 (t_R values at T_2). The asterisk denotes use of Cochran's approximation to the Behrens Fisher test. Digits listed under *n* refer to the number of mice used for each treatment.

Table 2 Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC, i.v.) on the rectal temperatures of restrained mice at an ambient temperature of 22°C

Drug	*Dose* (mg/kg)	n	*Mean rectal temperature* Initial (°C ± s.e.)	at T_1	at T_2	T_1 (min)	T_2	P_1	P_2	P_{0-1}	P_{0-2}	P_{1-2}
Δ^9-THC	1.0	5	36.1 ± 0.3	35.5 ± 0.3	35.0 ± 0.2	6	18	NS	<0.05	<0.001	<0.01	<0.05
Tween 80	4.0	6	36.1 ± 0.3	35.9 ± 0.3	35.9 ± 0.3					<0.01	NS	NS
Δ^9-THC	2.0	6	36.2 ± 0.3	34.3 ± 0.2	33.1 ± 0.2	10	28	<0.01	<0.001	<0.01	<0.001	<0.01
Tween 80	4.0	6	36.1 ± 0.3	36.0 ± 0.3	35.9 ± 0.3					NS	NS	NS
Δ^9-THC	4.0	5	36.0 ± 0.4	32.6 ± 0.3	30.8 ± 0.6	14	36	<0.001	<0.001	<0.01	<0.01	<0.01
Tween 80	8.0	6	36.7 ± 0.1	36.6 ± 0.1	36.7 ± 0.1					NS	NS	NS

The earliest times (minutes) after injection of Δ^9-THC at which maximum falls in mean oxygen consumption rate or in mean rectal temperature were observed are listed in the right hand columns under T_1 and T_2 respectively. Digits listed under P_1 and P_2 are P values (unpaired t test) for differences between Δ^9-THC and Tween treatments at the times T_1 and T_2 respectively. P values (paired t test) for differences between preinjection values and values obtained at the times T_1 and T_2 are listed under P_{0-1} and P_{0-2} respectively. P values (paired t test) for differences between values obtained at T_1 and T_2 are listed under P_{1-2}. The abbreviation NS indicates a P value >0.05. Digits listed under n refer to the number of mice used for each experimental treatment.

Table 3 Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC, i.v.) on the oxygen consumption rates of restrained mice at an ambient temperature of 22°C

Drug	*Dose* (mg/kg)	n	*Mean oxygen consumption rate* Initial (ml 25 g^{-1} h^{-1} ± s.e.)	at T_1	at T_2	T_1 (min)	T_2	P_1	P_2	P_{0-1}	P_{0-2}	P_{1-2}
Δ^9-THC	1.0	5	140 ± 1	115 ± 7	126 ± 4	6	18	<0.01	<0.05	<0.05	<0.05	NS
Tween 80	4.0	6	150 ± 4	166 ± 10	146 ± 7					NS	NS	<0.05
Δ^9-THC	2.0	6	152 ± 4	84 ± 7	115 ± 6	10	28	<0.01	<0.001	<0.001	<0.05	<0.05
Tween 80	4.0	6	150 ± 4	136 ± 13	153 ± 6					NS	NS	NS
Δ^9-THC	4.0	5	145 ± 6	58 ± 6	89 ± 9	14	36	<0.001	<0.01	<0.001	<0.01	<0.01
Tween 80	8.0	6	148 ± 3	148 ± 10	148 ± 5					NS	NS	NS

The values of mean oxygen consumption rate in this table were obtained in the same experiments as the values of mean rectal temperature given in Table 2. See also footnote to Table 2.

to 31°C, the VO_2 at any given ambient temperature was 32 to 43% greater for restrained mice than for unrestrained animals. At 34°C, the VO_2 of restrained mice was 60 ± 6 ml 25 g^{-1} h^{-1}, a value which was not significantly different from the minimum VO_2 values of unrestrained mice.

Effects of Δ^9-THC on the oxygen consumption rates of mice

The first experiments were carried out on unrestrained mice at 13°C and the results are summarized in Table 1. Administration of Δ^9-THC (10 to 40 mg/kg i.p.) produced significant, reversible falls in VO_2. Experiments with different groups of mice showed that the above drug treatments also induced a significant degree of hypothermia. Small but statistically significant falls in rectal temperature were also observed in mice that received only Tween 80. However, at all the doses of Δ^9-THC that were studied, the hypothermia following the injection of the drug was significantly greater than that following the injection of Tween 80. Maximum falls in VO_2 ranged from 29 to 40% of VO_2 values of a Tween-treated control group. At the same doses of Δ^9-THC, maximum falls in rectal temperature ranged from 2.5°C to 6.0°C.

Subsequent experiments were carried out with mice secured to a restraining apparatus and fitted with an intravenous cannula and with temperature probes. This procedure, unlike the one used in the preliminary experiments, allowed measurements of VO_2 to be made (1) simultaneously with the measurement of body temperature and (2) before, during and immediately after drug administration. The experiments were carried out at 22°C. At this ambient temperature, Δ^9-THC (1.0 to 4.0 mg/kg i.v.) produced significant falls both in VO_2 and in rectal temperature. The results are summarized in Tables 2 and 3. A dose of 1.0 mg/kg produced a peak fall in VO_2 of $17 \pm 5\%$ of the mean pre-injection value whereas doses of 2.0 and 4.0 mg/kg produced peak falls of $44 \pm 5\%$ and $60 \pm 5\%$ respectively. The degree of hypothermia produced by Δ^9-THC also increased progressively with dose. Tween 80 produced a slight, transient fall in rectal temperature in one group of animals (Table 2). In all the other experiments at 22°C, administration of Tween 80 alone produced no significant decreases either in VO_2 or in rectal temperature.

The falls in VO_2 produced byΔ^9-THC significantly affected the volume of oxygen consumed by mice during the onset of hypothermia. A dose of 1.0 mg/kg produced a fall in the volume of oxygen consumed over the period in which rectal temperature fell to a minimum value that was 21% of the volume consumed in the same interval of time (18 min) by a Tween-treated group of mice. Doses of 2.0 and 4.0 mg/kg produced falls in the volume of oxygen consumed during the onset of hypothermia which were respectively 32% and 49% of control values.

The time taken after intravenous administration of Δ^9-THC for VO_2 and rectal temperature to reach minimum values was not constant and increased progressively with dose. At each dose, the maximum fall in VO_2 occurred at an earlier time than the maximum fall in rectal temperature. Falls in rectal temperature were maximal 18 to 36 min after injection whereas maximal falls in VO_2 took place in 6 to 14 minutes. At the latter times rectal temperatures, although already significantly below pre-injection values, were still significantly above the minimum values observed 18 to 36 min after injection. Furthermore, in groups of mice that had received Δ^9-THC at doses of 2.0 or 4.0 mg/kg, a significant recovery of VO_2 was observed at the times of peak hypothermia.

Effects of ambient temperature on changes in oxygen consumption rate induced in restrained mice by Δ^9-THC at a dose of 2.0 mg/kg (i.v.)

Tables 4 and 5 show how changes in ambient temperature in the range 22 to 34°C influenced the effect of Δ^9-THC on VO_2 and rectal temperature. Significant, reversible falls in both VO_2 and rectal temperature were induced by the drug throughout this range. In contrast, at none of the ambient temperatures studied was any significant reduction in VO_2 produced by Tween 80. Figures 2 and 3 show the time courses of the effects of Δ^9-THC and of Tween 80 both on the rectal temperature and on the VO_2 of groups of mice at an ambient temperature of 27°C.

In the range 22 to 34°C, the size of the fall in VO_2 that followed injection of Δ^9-THC decreased progressively with increase in ambient temperature. At 22°C the maximum fall in VO_2 was 68 ml 25 g^{-1} h^{-1} whereas at 34°C the maximum fall was only 23 ml 25 g^{-1} h^{-1}. Minimum values of VO_2 following Δ^9-THC ranged from 84 ± 7 ml 25 g^{-1} h^{-1} at 22°C to 52 ± 5 ml 25 g^{-1} h^{-1} at 30°C. The minimum values of VO_2 at 32 and 34°C were not significantly different from the value at 30°C. The degree of hypothermia produced by Δ^9-THC also varied inversely with ambient temperature. Minimum values of rectal temperature ranged from 33.1 ± 0.2°C at 22°C to 37.3 ± 0.1°C at 34°C.

As in the experiments carried out at 22°C, the maximum fall in VO_2 following the injection of Δ^9-THC at each of the higher ambient temperatures studied, occurred at an earlier time than did the maximum fall in rectal temperature. Furthermore, at the times at which the maximum fall in VO_2 occurred, the degree of hypothermia although already significant was far from maximal. In addition, at 27 and 32°C, a significant recovery of VO_2 was observed at the times of peak hypothermia.

Table 4 The influence of ambient temperature on the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC, 2.0 mg/kg i.v.) and Tween 80 (4.0 mg/kg i.v.) on the rectal temperature of restrained mice

Drug	*Ambient temperature (°C)*	n	*Mean rectal temperature* *Initial*	*at T_1*	*at T_2*	T_1	T_2	P_1	P_2	P_{0-1}	P_{0-2}	P_{1-2}
				(°C ± s.e.)		*(min)*						
Δ^9-THC	22	6	36.2 ± 0.3	34.3 ± 0.2	33.1 ± 0.2	10	28	<0.01	<0.001	<0.01	<0.001	<0.001
Tween 80		6	36.1 ± 0.3	36.0 ± 0.3	35.9 ± 0.3					NS	NS	NS
Δ^9-THC	27	6	36.9 ± 0.1	36.2 ± 0.2	34.9 ± 0.3	6	18	NS	<0.05	<0.01	<0.001	<0.001
Tween 80		5	36.2 ± 0.2	36.1 ± 0.2	36.1 ± 0.3					NS	NS	NS
Δ^9-THC	30	6	37.3 ± 0.2	36.0 ± 0.1	35.9 ± 0.1	12	14	<0.001	<0.001	<0.001	<0.001	<0.05
Tween 80		6	37.2 ± 0.2	37.4 ± 0.2	37.4 ± 0.2					<0.05	NS	NS
Δ^9-THC	32	6	37.5 ± 0.2	36.8 ± 0.2	36.6 ± 0.2	16	20	<0.05	<0.01	<0.01	<0.01	<0.05
Tween 80		6	37.3 ± 0.2	37.4 ± 0.2	37.5 ± 0.2					NS	NS	NS
Δ^9-THC	34	6	38.1 ± 0.1	37.6 ± 0.1	37.3 ± 0.1	14	24	NS	<0.001	<0.01	<0.01	<0.05
Tween 80		6	37.9 ± 0.2	37.9 ± 0.2	38.0 ± 0.1					NS	NS	<0.05

For details see footnote to Table 2.

Table 5 The influence of ambient temperature on the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC, 2.0 mg/kg i.v.) and Tween 80 (4.0 mg/kg i.v.) on the oxygen consumption rates of restrained mice

Drug	*Temperature (°C)*	n	*Mean oxygen consumption rate* *Initial*	*at T_1*	*at T_2*	T_1	T_2	P_1	P_2	P_{0-1}	P_{0-2}	P_{1-2}
				(ml 25 g^{-1} h^{-1})		*(min)*						
Δ^9-THC	22	6	152 ± 4	84 ± 7	115 ± 6	10	28	<0.01	<0.001	<0.001	<0.05	<0.05
Tween 80		6	150 ± 4	136 ± 13	153 ± 6					NS	NS	NS
Δ^9-THC	27	6	120 ± 3	61 ± 4	90 ± 5	6	18	<0.001	NS	<0.001	<0.001	<0.01
Tween 80		5	107 ± 5	115 ± 7	105 ± 9					NS	NS	NS
Δ^9-THC	30	6	95 ± 4	52 ± 5	57 ± 7	12	14	<0.001	<0.01	<0.01	<0.05	NS
Tween 80		6	86 ± 4	85 ± 4	94 ± 5					NS	NS	NS
Δ^9-THC	32	6	84 ± 4	50 ± 4	79 ± 9	16	20	<0.01	NS	<0.001	NS	<0.01
Tween 80		6	74 ± 3	75 ± 4	76 ± 6					NS	NS	NS
Δ^9-THC	34	6	71 ± 3	48 ± 5	57 ± 7	14	24	NS	NS	<0.01	NS	NS
Tween 80		6	71 ± 3	54 ± 7	65 ± 3					NS	NS	NS

The values of oxygen consumption rate in this table were obtained in the same experiments as the values of rectal temperature given in Table 4. See also footnote to Table 2.

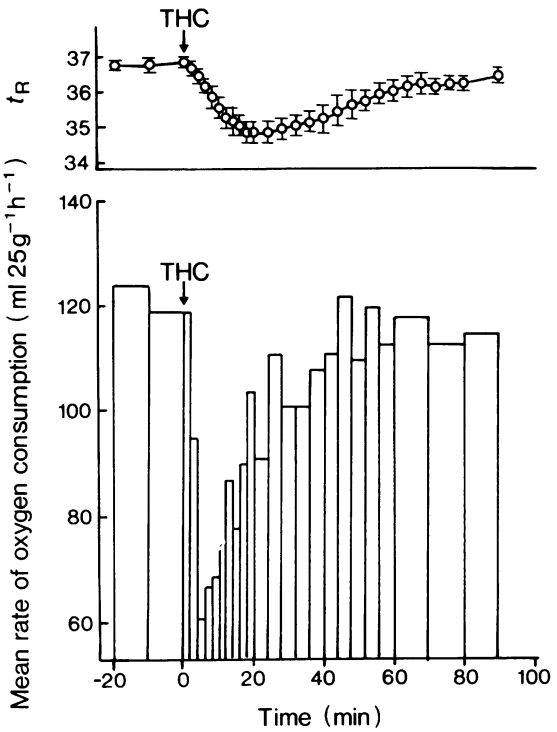

Figure 2 The effect of Δ⁹-tetrahydrocannabinol (THC, 2.0 mg/kg i.v.) on the mean rectal temperatures (t_R) and oxygen consumption rates of a group of 6 mice kept separately at an ambient temperature of 27°C. The arrow denotes the time of injection. Vertical lines show s.e.

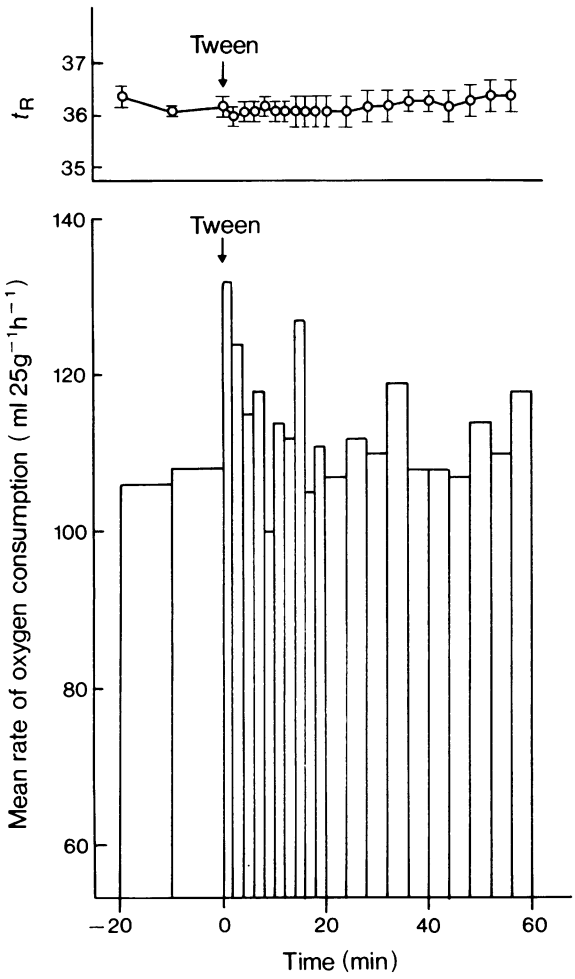

Figure 3 The effect of Tween 80 (4.0 mg/kg i.v.) on the mean rectal temperatures (t_R) and oxygen consumption rates of a group of 5 mice kept separately at an ambient temperature of 27°C. The arrow denotes the time of injection. Vertical lines show s.e.

Effects of Δ⁹-THC on the paw temperatures of restrained mice

At ambient temperatures of 27, 30 and 34°C the hypothermia induced by Δ⁹-THC at a dose of 2.0 mg/kg (i.v.) was accompanied by significant falls in paw temperature. At each of these ambient temperatures the maximum fall in paw temperature occurred at a later time than the maximum fall in rectal temperature (see Tables 4 and 6). In contrast, at temperatures of 22 and 32°C, Δ⁹-THC had no significant effect on paw temperature. Administration of Tween 80 alone produced no significant falls in paw temperature at any of the ambient temperatures that were used.

Discussion

The results confirm earlier observations that Δ⁹-THC can lower the body temperature of mice and that the degree of hypothermia produced is dependent not only on dose level but also on the ambient temperature (Haavik & Hardman, 1973). The experiments also showed that hypothermia induced in mice by Δ⁹-THC is accompanied by marked falls in the rate of oxygen consumption. The results therefore provide indirect evidence for the concept that hypothermia induced in mice by Δ⁹-THC is associated with reduced rates of heat production. The results also support the hypothesis that there is a cause and effect relationship between reduced oxygen consumption and hypothermia, the latter resulting from an effect of Δ⁹-THC on oxygen utilizing processes of the body. Firstly, the effect of Δ⁹-THC on oxygen consumption correlates

Table 6 Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC, 2.0 mg/kg i.v.) and Tween 80 (4.0 mg/kg i.v.) on the paw temperature of restrained mice

Drug	*Ambient temperature (°C)*	n	*Mean paw temperature* (°C ± s.e.) *Initial*	*at T_{max}*	T_{max} (min)	P
Δ^9-THC	22	6	26.1 ± 0.5	25.2 ± 0.4	28	NS
Tween 80		6	27.9 ± 0.6	27.3 ± 0.5		NS
Δ^9-THC	27	6	29.1 ± 0.5	28.5 ± 0.4	24	<0.05
Tween 80		5	28.7 ± 0.5	28.9 ± 0.6		NS
Δ^9-THC	30	6	33.5 ± 0.5	32.0 ± 0.3	28	<0.05
Tween 80		6	32.6 ± 0.5	32.5 ± 0.6		NS
Δ^9-THC	32	6	34.3 ± 0.6	33.1 ± 0.1	32	NS
Tween 80		6	34.6 ± 0.5	35.1 ± 0.6		<0.05
Δ^9-THC	34	6	36.6 ± 0.5	34.5 ± 0.2	40	<0.05
Tween 80		6	35.6 ± 0.6	36.3 ± 0.5		NS

The earliest times (minutes) after injection of Δ^9-THC at which maximum falls in mean paw temperature were observed are listed under T_{max}. Digits listed under *P* denote the *P* values (paired *t* test) for differences between preinjection and postinjection values. Digits listed under *n* refer to the number of mice used for each treatment. The values of paw temperature in this table were obtained in the same experiments as the values of rectal temperature given in Table 4.

well with the hypothermia produced by the drug; both parameters increase with increase in dose of Δ^9-THC and with decrease in the ambient temperature. Secondly, the falls in oxygen consumption rate produced by the drug were always found to take place earlier than the falls in body temperature. Furthermore, in many of the experiments, a significant reversal of the effect of Δ^9-THC on oxygen consumption had already occurred at the time at which peak hypothermia was first observed. It should be noted however, that the latter findings may simply be a reflection of the tendency of rectal temperature to lag behind changes in the temperatures of other internal parts of the body.

Our measurements of paw temperature showed that the hypothermic doses of Δ^9-THC used in our experiments produced only falls in paw temperature. There was no sign therefore that increased rates of peripheral heat loss contributed significantly towards the hypothermia induced in mice by Δ^9-THC. However, this possibility cannot yet be ruled out since it is not clear whether paw temperature would necessarily become elevated as a result of peripheral vasodilatation in mice that were at the same time experiencing progressive decreases in deep body temperature. It should be noted that maximum falls in paw temperature occurred at later times than maximum falls in rectal temperature. It is possible therefore that the falls in paw temperature are caused by vasoconstriction of peripheral blood vessels and that this vasoconstriction contributes towards recovery from drug-induced hypothermia.

Experiments with untreated mice confirm earlier reports (Herrington, 1940) that at ambient temperatures below 30°C, the oxygen consumption rate of mice normally kept at about 23°C varied inversely with ambient temperature. The experiments also showed that at an ambient temperature of 30°C, rates of oxygen consumption during periods in which mice exhibited increased motor activity were 44% greater than during periods in which the animals were resting. Increases in oxygen consumption were also observed when mice were secured in a restraining apparatus. In the range 22–32°C, oxygen consumption rates at any given ambient temperature were 32 to 43% greater in immobilized mice than in unrestrained animals. Similar effects of restraint on oxygen consumption have been found to occur with both guinea-pigs (Bartlett, 1959) and rabbits (McEwen, 1975). The method of restraint used in our experiments has been shown previously (Pertwee, 1974) to be a stressful procedure as measured by its effect on plasma corticosterone concentrations in mice. It is therefore possible that the increase in oxygen consumption which takes place when the mice are immobilized may be induced by stress. In view of the increase in plasma corticosterone produced in mice by restraint it is important to note that hypothermic doses of Δ^9-THC were found to lower the oxygen consumption not only of immobilized mice but also of unrestrained animals. It should also be noted that the doses of Δ^9-THC found to produce hypothermia in unrestrained animals have themselves been shown in previous experiments (Pertwee, 1974) to elevate plasma corticosterone in mice. Consequently the question of whether or not the effects of Δ^9-THC on oxygen consumption and rectal temperature are influenced by the degree of stress experienced by the experimental animals cannot yet be answered.

Finally, it was observed that the size of the effect of Δ^9-THC on oxygen consumption rate was dependent on the rate of consumption before the drug injection was made. Both the size of the effect of Δ^9-THC on oxygen consumption rate and the pre-injection values of this parameter increased with decrease in ambient temperatures. It was also observed that the oxygen consumption rates of mice treated with hypothermic doses of Δ^9-THC never fell significantly below the basal levels observed in unrestrained, resting mice at 30°C. If reduced rates of heat production do indeed contribute significantly towards production of the hypothermia described in this paper, then the above findings support the hypothesis that the biological processes responsible for increased heat production in response to cold are more sensitive to Δ^9-THC than are the biological processes governing the basal rates of metabolism observed at thermally neutral environmental temperatures.

We thank Dr M.C. Braude of NIDA for supplies through the MRC of Δ^9-tetrahydrocannabinol. We also thank Mr Stewart Bourner (Aberdeen) and Mr P. Tolley (Oxford) for invaluable assistance in the design and manufacture of apparatus. One of us (R.T.) is the recipient of an MRC scholarship for training in research methods.

References

BARTLETT, R.G. (1959). Effects of restraint on oxygen consumption of the cold exposed guinea-pig. *J. appl. Physiol.*, **14**, 46–48.

HAAVIK, C.O. & HARDMAN, H.F. (1973). Evaluation of the hypothermic action of tetrahydrocannabinols in mice and squirrel monkeys. *J. Pharmac. exp. Ther.*, **187**, 568–574.

HERRINGTON, L.P. (1940). The heat regulation of small laboratory animals at various environmental temperatures. *Am. J. Physiol.*, **129**, 123–139.

McEWEN, G.N. (1975). Thermoregulatory responses of restrained versus unrestrained rabbits. *Life Sci.*, **17**, 901–906.

PATON, W.D.M. & PERTWEE, R.G. (1973). In *Marijuana: Chemistry, Pharmacology, Metabolism, and Clinical Effects*. Chapter 5, ed. R. Mechoulam, New York: Academic Press.

PERTWEE, R.G. (1970). The effects of anaesthetic gases at high pressure on thermoregulation. *D. Phil. Thesis, Oxford.*

PERTWEE, R.G. (1974). Tolerance to the effects of Δ^1-tetrahydrocannabinol on corticosterone levels in mouse plasma produced by repeated administration of cannabis extract or Δ^1-tetrahydrocannabinol. *Br. J. Pharmac.*, **51**, 391–397.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical Methods*. 6th Edition. pp. 114–116. Ames, Iowa: Iowa State University Press.

(Received November 19, 1976. Revised February 4, 1977.)