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EFFECTS OF ACUTE Δ^1-TETRAHYDROCANNABINOL TREATMENT, OF HYPOTHERMIA AND OF AMBIENT TEMPERATURE ON CHOLINE INCORPORATION INTO MOUSE BRAIN

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Abstract—[Me-^{14}C]-choline was injected intravenously in mice after acute i.p. treatment with Δ^1-THC and the uptake of radioactive label by brain was measured in its aqueous and lipid extracts. The endogenous plasma choline level of 4.96 μg/ml was not affected by Δ^1-THC treatment. At an ambient temperature of 22° only the higher dose of Δ^1-THC (15 mg/kg) influenced the appearance of label in the brain: incorporation into the lipid fraction fell by 52 per cent, and into the brain as a whole by 23 per cent. Phenobarbitone (300 mg/kg) showed similar effects. Both drugs, when administered to mice housed at 22°, caused a marked lowering of rectal temperature for the duration of the radioactive studies. At an ambient temperature of 33·5°, most or all of the hypothermic effects of Δ^1-THC (15 mg/kg) and phenobarbitone were abolished. At this temperature, Δ^1-THC (15 mg/kg) inhibited radioactive incorporation into the brain lipid fraction by 19 per cent. Phenobarbitone also had a smaller effect but also inhibited incorporation into the whole brain in both its aqueous and lipid fractions (by 19% and 23% respectively). At 33.5° the lower dose of Δ^1-THC (3.75 mg/kg) caused an increase in incorporation into the brain aqueous (26%) and lipid (25%) fractions. These different responses are discussed in relation to the effects of (a) hypothermia, which caused decreased radioactive incorporation into brain, (b) ambient temperature, which increased incorporation when high, and (c) the spontaneous motor activity of the mice.

After administration of Δ^1-tetrahydrocannabinol (Δ^1-THC), the major psychoactive constituent of cannabis, it and its metabolites become widely distributed throughout the body, including the brain [1–5]. It is a highly lipid soluble drug and a considerable number of its actions have been described involving cell membranes, including effects on cell motility and division, on uptake of metabolic precursors, and on lysosomes, microsomes and red cells [6]. One report has shown that changes in the lipid content of rat-brain subcellular fractions occur following intraperitoneal administration of Δ^1-THC [7]. The effects were more marked in the acute state but some persisted during chronic treatment. Changes in the total phospholipid fraction were particularly notable and these included effects on the content of phosphatidylcholine, one of the major membrane phospholipids.

Phosphatidylcholine is one of the phospholipids found in brain that are dependent on choline as a precursor, as is the neurotransmitter, acetylcholine [8], whose metabolism may be influenced by the action of Δ^1-THC [9]. It is generally agreed, however, that virtually no *de novo* synthesis of choline occurs in the brain [8, 10, 11] and that it must be transported there by means of the bloodstream, either as free choline [10] or in a lipid-bound form [12]. It is also known that the base turns over rapidly in plasma with a half-life of only 1–2 min [13]. An investigation of the effects of Δ^1-THC on choline incorporation into brain therefore seemed warranted. In a study with phenobarbitone, in an anaesthetic dose, significant effects on choline incorporation in the mouse brain have been shown [14]. In view of the strong hypothermic action of both Δ^1-THC and phenobarbitone, gross effects of these drugs in mice on the incorporation of radioactivity into the brain after intravenous injection of [Me-^{14}C]choline were studied initially. The effect of removing hypothermia by raising the ambient temperature was then studied so that the relative contributions of the indirect effects, associated with changes in body temperature, and direct effects of the drugs could be compared.

METHODS

Injections

Male albino mice of the CD-1 strain were obtained from Charles River, U.K. Ltd., Manston Road, Margate, Kent and weighed 20–30g at the time of the experiments.

Administration of Δ^1-THC and phenobarbitone was by intraperitoneal injection. The dose of Δ^1-THC was either 3.75 mg/kg body wt or 15 mg/kg body wt and was injected in a volume of 0.02 ml/g body wt in a Tween 80-saline (0.9% w/v aq. NaCl) suspension with a ratio, by weight, for Tween 80:Δ^1-THC of 2:1; control animals received Tween 80-saline only, by the same procedure. In separate experiments phenobarbitone sodium (British Drug Houses Ltd., Poole, Dorset) was used at 300 mg/kg body wt as a solution in saline and injected in a volume of 0.02 ml/g body wt; control animals received saline only.

At 29.6 min (± 0.5, S.E.M.) after the drug injection [Me-^{14}C]choline chloride (specific activity 53 mC/mmol; the Radiochemical Centre, Amersham, Bucks.)

was injected into the tail vein using 37.5 mμC/g body wt (equivalent to 74 ng choline/g) in a volume 5–6 μl/g body wt, with the mouse restrained but without added anaesthesia. At 26.7 min ($\pm$0.4, S.E.M.) after the tail-vein injection the mice were killed by decapitation, blood was collected in small heparinized tubes in ice, before being centrifuged for the collection of plasma, and the brain was dissected out as quickly as possible and frozen until extracted.

Brain-extractions

Brains were homogenized in ice-cold trichloroacetic acid (10%, w/v) [15] for the extraction of water-soluble components, and the insoluble residue was washed twice with water. Lipids were then extracted and washed by the method of Folch *et al.* [16]. By this method choline plasmalogen, an acid-labile choline-containing lipid, would not be extracted and any associated labelled choline would appear in the water-soluble fraction. A control experiment (see below) indicated that even after a prolonged period following [Me-^{14}C]choline administration, very little label was associated with the choline plasmalogen fraction.

Assay of radioactivity

The ^{14}C-labelled samples from the plasma and from the aqueous and lipid extracts of the brain were counted in a Phillips PW 4510/01 Automatic Liquid Scintillation Analyser. The scintillator used was toluene: Triton X-100 (2:1, v/v; B.D.H. scintillation grade (containing 4g PPO/L. The lipid samples were evaporated to dryness before addition of the scintillator. The counts were corrected to 100% efficiency by the external standard channels-ratio method.

Control experiments for choline study

Distribution of choline-label in the brain-lipid fraction. 3.5 μl [Me-^{14}C]choline chloride (200 μC/ml with specific activity 53 mC/mmol) were injected into the third ventricle of the brain, while the mouse was held in a restraining device [17], using a micrometer screw gauge coupled to a fine needle. Accuracy of injection site was checked by using ink and noting its appearance in the ventricular system when the brain was sectioned. Mice were killed 17 hr after the intracerebral injection of [Me-^{14}C]choline and lipids were extracted and washed from the brain by the method of Folch *et al.* [16]. The choline-containing phospholipids were isolated by chromatography on a column of alumina [18] and were separated into an alkali-labile fraction (phosphatidylcholine), an alkali-stable acid-labile fraction (choline plasmalogen) and an alkali- and acid-stable fraction (sphingomyelin) by the hydrolytic procedures of Dawson [19]. At least 95 per cent of the radioactivity associated with the lipid fraction was from phosphatidylcholine in the alkali-labile fraction, the remainder being in the choline plasmalogen and sphingomyelin fractions. Identification of phosphatidylcholine was confirmed by thin layer chromatography.

Effect of saline-perfusion of brain. The degree of radioactive contamination of the brain by blood in the routine experiments following the intravenous injection of [Me-^{14}C]choline was assessed by lightly anaesthetizing the mice with diethyl ether immediately prior to their normal time of sacrifice, and perfusing the brain *in situ* with 5 ml saline by way of the aorta. A mean reduction in radioactivity of only 7 per cent was found in the total recovery from the perfused brains and so, in the routine experiments, brains were not perfused and no correction was made. The ratio of radioactivity between the aqueous and lipid fractions was also not significantly affected, indicating, in addition, that the short period of ether anaesthesia for the perfusion was without effect on this parameter.

Endogenous plasma choline. Since it is assumed that the net uptake and incorporation of choline by the brain is proportional to the radioactivity recovered, it is essential to show that the plasma specific activity of choline after injecting [Me-^{14}C]choline is identical for test and control mice, i.e. that the drug administration did not affect endogenous plasma choline levels. Blood samples were taken from control and Δ^1-THC treated mice (15 mg/kg), kept at 22°, at the normal time for intravenous [Me-^{14}C]choline injection. Choline was extracted from the plasma and acetylated, with acetylchloride, according to Bligh [20] and the acetylcholine was estimated by a bioassay method using the guinea-pig ileum [21]. A value for the plasma choline level in six control mice of 4.96 $\pm$ 0.47 (S.E.M.) μg/ml was obtained and this was not significantly different from 5.07 $\pm$ 0.28 (S.E.M.) μg/ml obtained in the six drug-treated mice.

Measurement of body temperature and motor activity

Body temperatures were measured using a rectal probe coupled to an electric thermometer (Light Labs., Brighton, Sussex).

In separate experiments the motor activity of the mice was monitored under the conditions of the radioactive study using an Aktograph Type B (Life Science Laboratories Ltd., Sarum Road, Leagrave, Luton, Bedfordshire). This was arranged to print out the activity recorded every 10 sec during the period of [Me-^{14}C]choline metabolism. Activity tended to come in bursts, which was assessed by calculating for ten consecutive 10-sec periods the mean activity $\pm$ S.D. In turn, ten such 100-sec values for mean activity and standard deviations were normally obtained for the duration of one experiment. Four experiments have been carried out for each set of conditions, divided equally between mid-morning and mid-afternoon sessions. A "grand" mean $\pm$ S.E.M. of the mean activities and a "grand" mean $\pm$ S.E.M. of the standard deviations are quoted in the results.

For all experiments involving radioactive choline uptake by brain, and measurement of body temperature and motor activity, mice had been maintained in their stock quarters under a normal light–dark schedule (*i.e.* light from 7.45 hr to 20.00 hr). Approximately 1 hr before the first mouse was due to be given an intraperitoneal injection, they were removed from their stock-quarters to the experimental room, which was sound-proofed, illuminated and temperature-controlled. For the duration of the experiments, test or control mice were housed, four to a cage (unless stated otherwise).

Statistics

Mean values are given $\pm$ S.E.M. (except where

Table 1. Effect of Δ^1-THC on ^{14}C-levels in plasma and brain after intravenous injection of [Me-^{14}C]choline*. Dose responses at 22° ambient temperature

	d.p.m. × 10^3				d.p.m. Ratio Aqueous/lipid (in brain)
	Total/ml_{Plasma}	Total/g_{Brain}	Aqueous/g_{Brain}	Lipid/g_{Brain}	
15 mg Δ^1-THC/kg (13)	20.44 ± 1.10	18.58 ± 0.93	16.34 ± 0.75	2.25 ± 0.19	7.60 ± 0.36
Tween 80-saline Control (11)	17.45 ± 0.58	24.28 ± 2.86	19.64 ± 2.32	4.65 ± 0.58	4.33 ± 0.17
% effect of drug	+ 17	− 23	− 17	− 52	+ 76
P (by analysis of Variance)	< 0.01	< 0.05	N.S.	< 0.001	< 0.001
3.75 mg Δ^1-THC/kg (7)	19.41 ± 0.91	23.23 ± 1.14	18.82 ± 1.10	4.42 ± 0.29	4.39 ± 0.40
Tween 80-saline Control (5)	18.41 ± 1.04	20.18 ± 0.39	16.09 ± 0.37	4.09 ± 0.27	4.02 ± 0.36
% effect of drug	+ 5	+ 15	+ 17	+ 8	+ 9
P (by analysis of Variance)	N.S.	N.S.	N.S.	N.S.	N.S.

* In Tables 1–3 inclusive and in Table 5, tissues were sampled 57 min after Δ^1-THC injection (= 27 min after choline injection).

Other experimental details in Methods.

stated) with the number of mice in parenthesis. The significance of the difference between two means was tested by Student's '*t*' test, except in Tables 1–3 inclusive, for which, because there appeared to be differences between experimental runs, an analysis of variance was carried out, based on non-orthogonal two-way tables [22], to allow for "between-run" effects. A value for $P \leqslant 0.05$ was taken as significant.

RESULTS

Δ^1-*THC*

Table 1 shows that there was a decrease of 52 per cent in radioactivity incorporated into the brain lipid fraction in mice dosed with 15 mg Δ^1-THC/kg. This was not accompanied by a significant decrease of label in the aqueous fraction of the brain. In these mice slightly higher levels (+ 17%) of radioactivity were retained in the plasma. In the lower-dosed group no significant differences were observed.

The experiments of Table 1 were carried out at an ambient temperature of 22°. The effects of Δ^1-THC on body temperature under these conditions are shown in Fig. 1a. It can be seen that at 15 mg Δ^1-THC/kg there was a marked lowering of body temperature which persisted for the period during which [Me-^{14}C]choline was being metabolized in the above

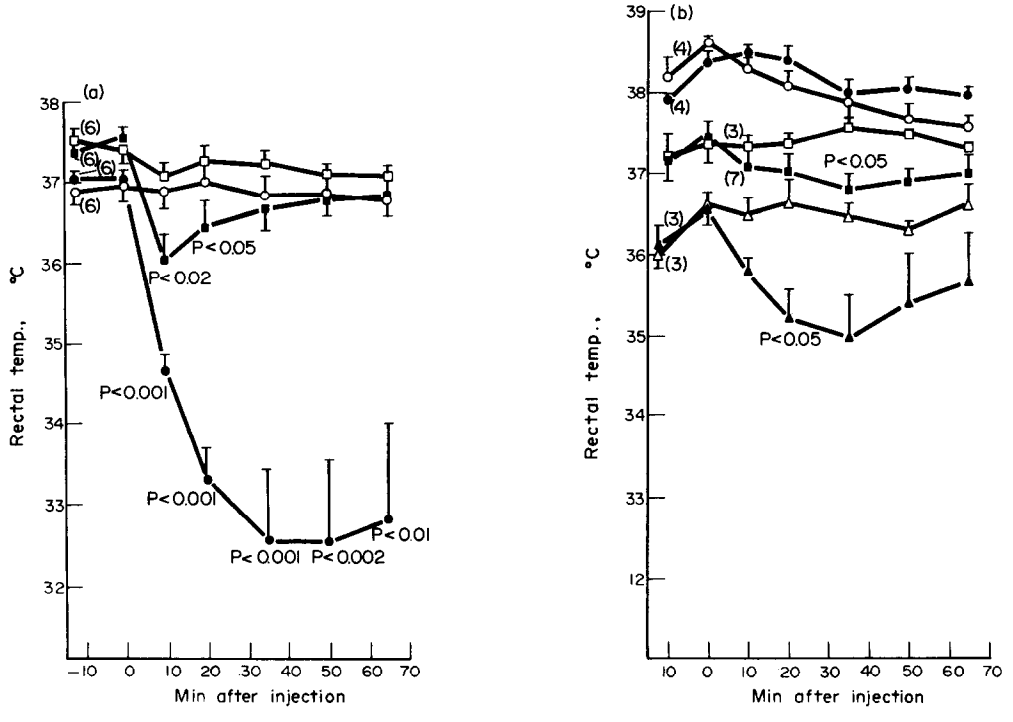

Fig. 1. Effect of Δ^1-THC on rectal temperature*.

* Injections (i.p.) at time zero (a) Dose responses at 22° ambient temperature (■, 3.75 mg Δ^1-THC/kg; □, Tween-80-saline control; ●, 15 mg Δ^1-THC/kg; ○, Tween-80-saline control) (b) Responses at 30° and 33.5° ambient temperatures (▲, 15 mg Δ^1-THC/kg at 30°; △, Tween-80-saline control; ■, 3.75 mg Δ^1-THC/kg at 33.5°; □, Tween-80-saline control; ●, 15 mg Δ^1-THC/kg at 33.5°; ○, Tween-80-saline control).

Table 2. Effect of Δ^1-THC on ^{14}C-levels in plasma and brain after intravenous injection of [Me-^{14}C]choline. Dose responses at 33.5° ambient temperature

	d.p.m. × 10^3				d.p.m. ratio Aqueous/lipid (in brain)
	Total/ml_{Plasma}	Total/g_{Brain}	Aqueous/g_{Brain}	Lipid/g_{Brain}	
15 mg Δ^1-THC/kg (9)	13.03 ± 0.72	25.50 ± 1.16	20.11 ± 0.96	5.39 ± 0.33	3.79 ± 0.19
Tween 80-saline Control (9)	12.51 ± 0.72	28.21 ± 1.48	21.53 ± 1.18	6.68 ± 0.39	3.25 ± 0.13
% effect of drug	+ 4	− 10	− 7	− 19	+ 17
P (by analysis of Variance)	N.S.	N.S.	N.S.	< 0.01	*
3.75 mg Δ^1-THC/kg (6)	19.39 ± 1.33	34.28 ± 2.08	26.62 ± 1.40	7.66 ± 0.70	3.56 ± 0.20
Tween 80-saline Control (7)	18.20 ± 0.82	26.58 ± 1.12	21.08 ± 0.99	6.13 ± 0.40	3.39 ± 0.20
% effect of drug	+ 7	+ 29	+ 26	+ 25	+ 5
P (by analysis of Variance)	N.S.	< 0.001	< 0.001	< 0.05	N.S.

* Significant interaction between treatment and experimental runs.

experiments. Although there was a temporary lowering of body temperature with the lower dose of Δ^1-THC it had recovered to near the control value by the time when the [Me-^{14}C]choline would have been injected.

Since hypothermia itself might interfere with choline metabolism, it was necessary to test whether the effects of the higher dose of Δ^1-THC on [Me-^{14}C]-choline incorporation could be repeated in the absence of a lowered body temperature. To do this, it was required first to find an environmental temperature at which the hypothermia was negligible. Figure 1b shows that even at 30° the Δ^1-THC-treated mice show a slight but prolonged lowering of body temperature but that at 33.5° there are virtually no significant differences between control and test groups.

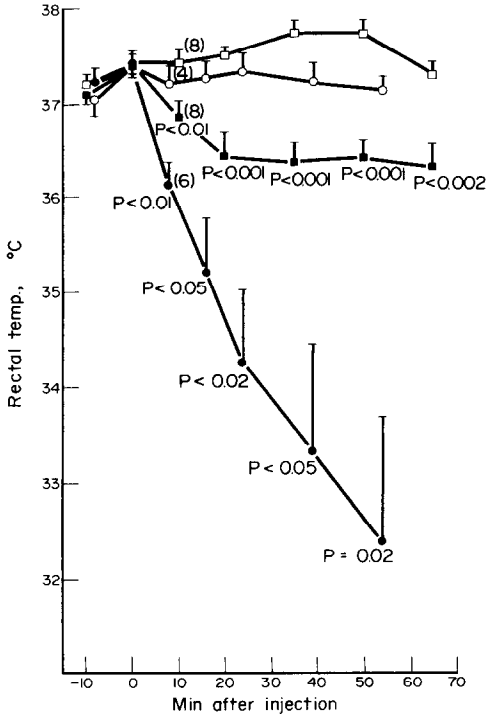

Fig. 2. Effect of phenobarbitone sodium (300 mg/kg) on rectal temperature at 22° and 33.5° ambient temperatures*.
* Injections (i.p.) at time zero. (●, phenobarbitone at 22°; ○, saline control; ■, phenobarbitone at 33.5°; □, saline control.)

Effects of Δ^1-THC on [Me-^{14}C]choline incorporation were therefore investigated at an ambient temperature of 33.5° and the results are shown in Table 2. At the higher dose of Δ^1-THC the radioactivity incorporated in the lipid fraction of the brain was again significantly lower than in control mice although the effect (− 19%) was smaller than at the 22° ambient temperature (Table 1). There was again no effect in the aqueous fraction of the brain, and, now, no effect of the drug on plasma levels of radioactivity. At the lower dose of Δ^1-THC, however, increased incorporation of label into both the aqueous and the lipid fractions of the brain (+ 26% and + 25% respectively) was found when the ambient temperature was 33.5°. This resulted in a corresponding increase in total radioactivity found in the brains of these mice.

Phenobarbitone

Clearly the greater part of the effects of Δ^1-THC at 15 mg/kg on [Me-^{14}C]choline incorporation is related to a lowered body temperature. Since Diamond [14], using phenobarbitone at 300 mg/kg, also found a marked inhibition of incorporation of choline into brain lipids in the mouse, the effect of this dose on body temperature was investigated at the different ambient temperatures. Figure 2 shows that at an ambient temperature of 22° there is a progressive fall in body temperature after the injection of phenobarbitone. At 33.5°, the body temperature was again significantly lower in the phenobarbitone-treated group but the difference was small and at no time did the mean body temperature fall below 36.3°.

A comparison of the effects of phenobarbitone at 300 mg/kg on [Me-^{14}C]choline incorporation at these two ambient temperatures is shown in Table 3. At the lower temperature there was a marked inhibition (− 57%) of incorporation into the lipid fraction of the brain, in qualitative agreement with Diamond [14]. With lower values obtained in the aqueous fraction in the phenobarbitone-treated mice, the total radioactivity found in the brains of these mice was also significantly lower than in the controls.

At the higher temperature, phenobarbitone caused a decreased incorporation of label into both the aqueous and lipid fractions of the brain, though the

Table 3. Effect of phenobarbitone sodium (300 mg/kg) on ^{14}C-levels in plasma and brain after intravenous injection of [Me-^{14}C]choline

Ambient Temp.	Treatment	d.p.m. × 10^3 Total/ml$_{Plasma}$	Total/g$_{Brain}$	Aqueous/g$_{Brain}$	Lipid/g$_{Brain}$	d.p.m. Ratio Aqueous/lipid (in brain)
33.5°	300 mg Phenobarbitone sodium/kg (8)	12.01 ± 0.39	22.68 ± 1.38	18.47 ± 1.26	4.21 ± 0.22	4.43 ± 0.26
	Saline Control (6)	14.47 ± 0.67	28.15 ± 1.76	22.68 ± 1.45	5.47 ± 0.38	4.16 ± 0.16
	% effect of drug	− 17	− 19	− 19	− 23	+ 6
	P (by analysis of Variance)	< 0.01	< 0.001	< 0.01	< 0.02	N.S.
22°	300 mg Phenobarbitone sodium/kg (7)	15.54 ± 1.12	16.67 ± 0.95	14.84 ± 0.91	1.83 ± 0.15	8.38 ± 0.66
	Saline Control (6)	14.79 ± 1.17	22.52 ± 0.55	18.26 ± 0.23	4.26 ± 0.36	4.41 ± 0.28
	% effect of drug	+ 5	− 26	− 19	− 57	+ 90
	P (by analysis of Variance)	N.S.	< 0.001	*	< 0.001	= 0.001

Experiments at 22° and 33.5° ambient temperatures.
* Significant interaction between treatment and experimental runs.

effect on the latter (− 23 %) was less than at the 22° ambient temperature. There was also a slight decrease (− 17 %) in plasma levels of radioactivity.

Motor activity

During the experiments with Δ^1-THC-treated animals at 33.5° considerable hyperactivity was noted. It was also found that, when mice were individually housed, so that they could not huddle together or interact in other ways, there was a hypothermic effect of 15 mg Δ^1-THC/kg even at an ambient temperature of 33.5° (Fig. 3). This should be contrasted with the lack of hypothermia when the mice were housed together (Fig. 1b). Since the hyperactivity represented an unusual aspect of Δ^1-THC action, it was investigated in detail. Table 4 shows that, at 15 mg Δ^1-THC/kg and at 33.5°, the drug-treated mice grouped together have both a higher spontaneous activity, and a much larger standard deviation of the activity in each 10 sec period, i.e. a different behavioural pattern, with activity more liable to come in bursts than with untreated grouped controls at the same temperature. Thus the 'cataleptic' effect of Δ^1-THC seen with individual mice [23] is lost under these conditions. There were no significant differences in activity at the lower temperature with 15 mg Δ^1-THC/kg although the higher standard deviation in the drug-treated animals approached significance.

At the lower dose of Δ^1-THC there were no significant differences in activity or behaviour between test and control groups at either ambient temperature. The phenobarbitone-treated mice were anaesthetized by the drug in all the experiments reported, and the varying ambient temperature did not obviously alter the depth of anaesthesia.

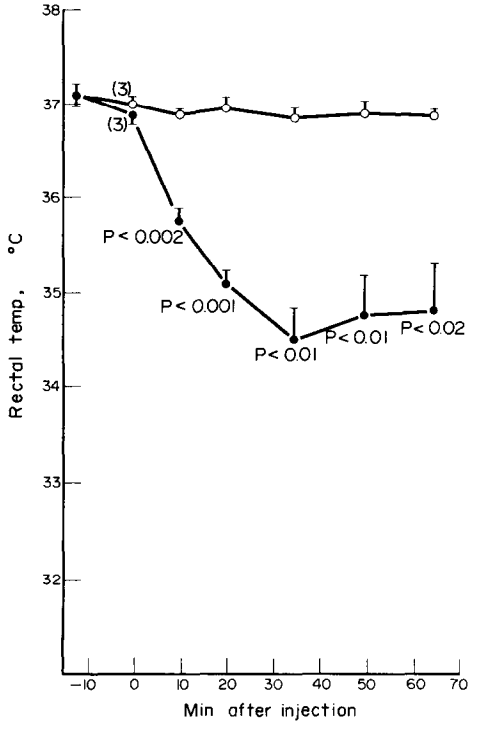

Fig. 3. Effect of 15 mgΔ^1-THC/kg on rectal temperature of individually-housed mice at 33.5° ambient temperature*.
* Injections (i.p.) at time zero. (●, Δ^1-THC; ○, Tween-80-saline control).

DISCUSSION

Choline incorporation into brain

Incorporation of choline into the lipid fraction of the brain was of primary interest in this study in view of the reported effects and properties of Δ^1-THC (see Introduction) and the time chosen for this measurement, namely 26.7 min after the intravenous injection of [Me-^{14}C]choline, was one at which total incorporation into lipid was still increasing linearly with time and several minutes before reaching a plateau [14].

In control mice, receiving Tween-80-saline, an endogenous plasma choline concentration of 4.96 μg/ml (= 48 μM) was found. This is at the higher end of the range of values (0.5–5.2 μg/ml) found by Bligh [20] for other laboratory animals. The injected choline

Table 4. Effect of Δ^1-THC on motor activity in grouped mice*. Dose responses at 22° and 33.5° ambient temperatures

	33.5°		22°		33.5°		22°	
	15 mg Δ^1-THC/kg	Tween 80-saline control	15 mg Δ^1-THC/kg	Tween 80-saline control	3.75 mg Δ^1-THC/kg	Tween 80-saline control	3.75 mg Δ^1-THC/kg	Tween 80-saline control
Activity units	8.20 ± 0.99	1.79 ± 0.25	0.88 ± 0.17	0.74 ± 0.13	1.33 ± 0.17	1.21 ± 0.21	1.58 ± 0.20	1.17 ± 0.17
P	< 0.001		N.S.		N.S.		N.S.	
Standard deviation of activity units	11.33 ± 1.10	1.95 ± 0.27	1.63 ± 0.34	0.96 ± 0.13	2.29 ± 0.33	1.43 ± 0.22	1.69 ± 0.13	1.27 ± 0.17
P	< 0.001		N.S. ($< 0.10 > 0.05$)		N.S.		N.S.	

* Activity was recorded between 35 min and 52 min after Δ^1-THC injection.
Recording of activity and other experimental details in Methods.

may be calculated to increase further the plasma level by approximately 35 per cent, but this is well below the 100 μM concentration at which saturation of choline transport into mouse brain seems to occur [14]. Since plasma choline levels were not affected by Δ^1-THC (15 mg/kg) at the time (29.6 min later) that [Me-^{14}C]choline was intravenously injected, specific activities of plasma choline were initially the same in both test and control animals. Assuming immediate homogeneous distribution of the injected label in the plasma, the initial level of radioactivity would be approximately 2.1×10^6 d.p.m./ml plasma. The final measured radioactivity in the plasma in some situations, however, showed slight but significant differences between the test and control groups implying a difference in specific activity of the plasma choline at that time. When comparing test and control groups in this discussion, nevertheless, it is assumed that the same incorporation of radioactivity into a fraction indicates the same incorporation of choline (or its radioactive products) into that fraction. We have, however, assumed that a difference (of not more than 17 per cent, e.g. Table 1) in the specific activity of the plasma choline finally would introduce only a negligible error because, apart from other reasons, (a) in all mice the plasma level of radioactivity finally was approximately two orders of magnitude less than initially, and (b) plasma choline has a rapid half-life [13] and most of the net incorporation of radioactivity into the brain would have occurred in the first few minutes at similar specific activities for test and control animals.

Ansell and Spanner [15] showed that the label in [Me-^{14}C]choline remained confined to the choline base during its metabolism in rat brain. When [Me-^{14}C]choline was injected intracerebrally in the mouse in this study, more than 95 per cent of label in the lipid fraction of the brain was found in phosphatidylcholine many hours later. In the main experiments in this study, labelled choline in brain lipid will again be found chiefly as phosphatidylcholine [14]. The aqueous fraction of the brain, which was not investigated, would contain free choline, phosphorylcholine, CDP-choline and small amounts of acetylcholine and betaine [14, 15].

Hypothermia

Conditions of marked hypothermia, whether brought about by Δ^1-THC or phenobarbitone, were characterized by a decreased incorporation of labelled choline into the whole brain. Furthermore there was a selective inhibition of incorporation of brain choline into the lipid fraction causing an elevation of the ratio of radioactivity between the aqueous and lipid fractions of the brain, by 76 per cent for Δ^1-THC and by 90 per cent for phenobarbitone. These effects of hypothermia could be important in the interpretation of Diamond's results [14] if during that study the ambient temperature was such as to allow hypothermia. Similarly in the work of Kewitz and Pleul [24], the possibility exists that hypothermia played a rôle in the inhibition of choline incorporation into brain lipid at anaesthetic doses of urethane.

The effects of hypothermia on choline uptake and metabolism by brain is likely to reflect a general depression of metabolism. Haavik *et al.* [25] have indeed shown that oxygen consumption in the mouse was reduced to little more than half that in control mice during the hour after administration of Δ^1-THC at 16 mg/kg, the effect being much reduced at 4 mg/kg. Δ^1-THC also caused a dose-related decrease in oxygen consumption in homogenates of brain tissue [26].

Δ^1-THC

With grouped mice at 33.5° hypothermic effects of Δ^1-THC were eliminated. Under these conditions at the 15 mg/kg dose of Δ^1-THC, there was a specific inhibition of choline incorporation into the lipid fraction of the brain. In this respect the drug therefore had a similar but smaller (– 19%) effect as when the body temperature was allowed to fall. However, these drug-treated mice showed marked spontaneous hyperactivity at 33.5° which is likely to be associated with a rise in metabolic rate. If, as seemed likely from the effects of hypothermia, a decreased incorporation of choline into brain lipid resulted from a decreased metabolic rate, the converse may also hold. Thus the apparently small inhibitory effect of Δ^1-THC in the absence of hypothermia may mask greater inhibition of choline incorporation into brain lipids if a higher metabolic rate due to hyperactivity was effectively increasing such incorporation in those conditions.

With the dose of Δ^1-THC at 3.75 mg/kg hypothermia was negligible at both ambient temperatures. At 33.5° the total radioactivity found in the brain was higher than in the controls, giving significant increases in *both* the aqueous and the lipid fractions, so there was no selectivity of incorporation between these fractions as was seen at the higher dose of Δ^1-THC. The total radioactivity found in the plasma and brain

Table 5. Effect of ambient temperature on ^{14}C-levels in brain after intravenous injection of [Me-^{14}C]choline into the control groups of mice*

	d.p.m. × 10^3		d.p.m. Ratio
Ambient temperature	Total/g_{Brain}	Lipid/g_{Brain}	Aqueous/lipid (in brain)
22° (22 mice)	22.87 ± 1.45	4.41 ± 0.31	4.28 ± 0.14
33.5° (22 mice)	27.67 ± 0.83	6.18 ± 0.24	3.54 ± 0.12
P	< 0.01	< 0.001	< 0.001

* Data from Tables 1–3 inclusive.

was higher than in any other situation, suggesting a more general systemic effect on choline metabolism under these conditions. At 22° no significant effects of 3.75 mg Δ^1-THC/kg were observed, although the mean values were generally higher in the drug-treated mice, consistent with the effects at 33.5°.

A differential effect of Δ^1-THC *in vivo*, dependent on the dose, may be possible since the high and low-dosed groups showed marked differences in their hypothermic and behavioural responses in this study, and therefore probably in their general metabolism. Thus at the 33.5° ambient temperature with grouped mice, the dose of 15 mg Δ^1-THC/kg was acting in conditions in which an eight-fold increase in spontaneous activity prevented the development of hypothermia. In contrast, the dose of 3.75 mg Δ^1-THC/kg at 33.5° was probably acting in conditions where the mice were adapting to the opposite phenomenon, namely the prevention of hyperthermia. Neither adaptation was necessary with the low dose of Δ^1-THC at 22°.

Phenobarbitone

At the 33.5° ambient temperature, hypothermic effects of phenobarbitone were very small, but an effect due to the drug of inhibition of radioactive incorporation into the whole brain persisted. This inhibition was reflected in proportionate decreases in incorporation into both the aqueous and lipid fractions of the brain, so that no significant effect on the ratio between them was found. This contrasts with the effects of hypothermia and those of Δ^1-THC, including the dose of 15 mg/kg, when significant inhibition of incorporation of radioactivity was specifically in the lipid fraction.

Ambient temperature

It is apparent from the results that differences in ambient temperature *per se* account for some changes in choline incorporation. When the individual data of all control mice are analysed, significant effects as shown in Table 5 were found. These indicate that at high ambient temperatures there is increased choline transport into the brain and that, once there, choline is preferentially incorporated into lipid.

CONCLUSIONS

The dominant effects of both Δ^1-THC and phenobarbitone were due to hypothermia and this caused a decreased uptake of radioactive choline by brain with a selective inhibition of incorporation into its lipid fraction. Thus, if body temperature is not controlled in a small animal the size and pattern of a direct effect on choline uptake due to a particular drug may be distorted. A high ambient temperature, used to correct hypothermia, was itself responsible for some increase in uptake of radioactive choline by brain with a stimulation of incorporation into its lipid fraction.

An inhibition of choline incorporation, specifically confined to the lipid fraction of the brain, was directly attributable to Δ^1-THC at 15 mg/kg. In contrast the inhibitory effect of phenobarbitone (at 300 mg/kg) on incorporation into brain was spread between both aqueous and lipid fractions. The specificity of the effect of Δ^1-THC was further shown by a stimulation of radioactive choline incorporation into brain at a lower dose of the drug (3.75 mg/kg) and this was dependent on ambient temperature.

These differential effects of Δ^1-THC indicate a complex relationship with choline-containing lipids, notably phosphatidylcholine, in the brain. Since the phosphatidylcholine content of the brain mitochondria, synaptosomes and myelin falls after acute treatment with Δ^1-THC at 10 mg/kg but rises in the microsomal fraction [7], the overall pattern of these effects in subcellular fractions of the brain may be dependent both on the dose of Δ^1-THC and the nature of the drug's interaction with the normothermic state of the animal.

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