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INTERACTION OF CANNABIS AND GENERAL ANAESTHETIC AGENTS IN MICE

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1 A cannabis extract (I) (in a concentration equivalent to 10 mg Δ^9-tetrahydrocannabinol(THC)/kg) prolonged pentobarbitone anaesthesia in mice maximally 20 min to 2 h after medication. The effect was still significant after 8 h, but less than at 2 hours.

2 The cannabis extract (I) (equivalent to 10 mg Δ^9-THC/kg) prolonged both pentobarbitone and ether anaesthesia in mice when administered 20 min before the anaesthetic. After eight consecutive daily doses of cannabis, the pentobarbitone anaesthesia was still significantly longer than a control group, while ether anaesthesia was not significantly prolonged.

3 A second cannabis extract (II) with a different ratio of cannabinoids (also administered in dosage equivalent to 10 mg Δ^9-THC/kg) failed to affect pentobarbitone anaesthesia in mice. This extract presented about 4% the dose of cannabidiol as extract I.

4 Δ^8-THC, Δ^9-THC and cannabidiol prolonged pentobarbitone anaesthesia with cannabidiol being generally more active than Δ^9-THC. Cannabinol (10 mg/kg) was inactive.

5 The effects of cannabidiol and Δ^9-THC were found to be additive, and there was a consistent trend for cannabinol to reduce the effectiveness of Δ^9-THC and cannabidiol when given in combination.

6 Premedication with phenoxybenzamine, phentolamine, propranolol, iproniazid, protriptyline, desipramine, reserpine, α-methyl tyrosine or parachlorophenylalanine did not affect the extract I-induced prolongation of pentobarbitone anaesthesia.

7 It is concluded that cannabis may affect pentobarbitone and ether anaesthesia in mice at least partially by a direct depressant effect, and that the cannabis-induced prolongation of anaesthesia is probably unrelated to any effect on central 5-hydroxytryptamine or catecholamine neurones.

Introduction

One of the earliest reported properties of cannabis extracts and of pure cannabinoids is their ability to prolong barbiturate anaesthesia in mice (Loewe, 1944; Garriott, Forney, Hughes & Richards, 1968; Gill, Paton & Pertwee, 1970; Paton & Pertwee, 1972) and in rats (Kubena & Barry, 1970). Some controversy exists as to whether this effect is due to a direct depressant action of cannabis on the central nervous system or is due to an interference by cannabis in the metabolism of the barbiturate by the microsomal enzymes. Paton & Pertwee (1972) advanced the latter explanation when they reported the failure of a cannabis extract to prolong anaesthesia induced by ether, which is not metabolized by microsomal enzymes. On the other hand Kubena & Barry (1970) reported that Δ^9-tetrahydrocannabinol (Δ^9-THC) did prolong barbitone anaesthesia in rats. Barbitone, like ether, does not undergo microsomal degradation, and is excreted unchanged.

To elucidate these effects further we have made a comparative study of the effect of two cannabis extracts on both pentobarbitone- and ether-induced anaesthesia in mice. The effects of Δ^8-THC, Δ^9-THC, cannabinol and cannabidiol on the duration of pentobarbitone anaesthesia were also studied.

Cannabis affects the levels and turnover of endogenous catecholamines and of 5-hydroxytryptamine in brain (Bose, Saifi & Bhagat, 1963, 1964; Holtzman, Lovell, Jaffe & Freedman, 1969; Colombine, Westfall & McCoy, 1970; Christensen, Freudenthal, Gidley, Rosenfeld, Boegli, Testino, Brine, Pitt & Wall, 1971; Maître, 1971). Although there is some conflict in the reports of these effects, it seems that in moderate doses cannabis causes an increase in the levels of 5-hydroxytryptamine and a reduction in the turnover of both 5-hydroxytryptamine and noradrenaline. It was considered of interest, therefore, to determine

whether any relationship exists between those properties of cannabis which are responsible for the potentiation of barbiturate anaesthesia and those which are responsible for the changes in brain amine levels and turnover. This was done by studying the effect on potentiation of pentobarbitone anaesthesia by cannabis extracts in mice which had been pretreated with drugs known to modify noradrenergic, dopaminergic and serotoninergic mechanisms.

Methods

Mice used were QS males (20-30 g) in all experiments other than the experiment to determine the duration of action of cannabis when inbred C57 males were used. Mice received food and water *ad libitum* up to the time of the experiment. Duration of anaesthesia was determined at the thermoneutral zone for mice of 31°C (Herrington, 1940).

Extracts of cannabis leaf or of hashish were prepared with light petroleum at room temperature. After concentration under reduced pressure at 40°C, the soft extract was taken up in methanol and stored at −20°C for 24 hours. Filtration of this solution at −20°C effected a satisfactory separation of solidified waxes. Other impurities were removed successively by adsorption chromatography on alumina (activity 1) from a chloroform solution and on Florisil (60-100 mesh) from a benzene solution. Removal of solvent produced a 'red oil', a sample of which was silylated and assayed for cannabinoids by gas-liquid chromatography.

Two extracts were used: Extract I, prepared from hashish, contained Δ^9-THC, 22.8%, cannabidiol, 42.7%, cannabinol, 34.0%. Extract II, prepared from a sample of cannabis leaf, contained Δ^9-THC, 52.8%, cannabidiol, 3.9%, cannabinol, 20.3%. The red oil extracts were stored at 2°C and assayed at regular intervals to ensure that decomposition had not occurred. Extract I and II, cannabinol, cannabidiol, Δ^9-THC and Δ^8-THC were dissolved (and suspended) in propylene glycol, stored in the deep freeze and suspended immediately before use in a lissapol-dispersol (ICI) solution (Whittle, 1964) to give a final propylene glycol concentration of 5%. Cannabis extracts thus prepared, or a vehicle control, were administered in all cases by gavage.

Pentobarbitone sodium was dissolved in water and administered by intraperitoneal injection. The dose used (50 mg/kg; expressed as the salt) was sufficient to induce sleep in all animals.

Ether was administered as 1 : 5 v/v solution in olive oil and a dose of 0.4 ml of this mixture per 20 g mouse was administered intraperitoneally.

Duration of anaesthesia was taken as the interval between the time of administration of the anaesthetic and the recovery of the righting reflex. In each instance the recovery of righting reflex was checked by placing the mouse on its back once again and the end point recorded only if the animal regained a position with all feet on the floor within 30 seconds.

Drugs were obtained from commercial sources, and were dissolved in distilled water to produce a dose volume of 1 ml/100 g. The doses mentioned refer to the salts. Reserpine was dissolved in a 20% solution of ascorbic acid and diluted with water before use. *p*-Chlorophenylalanine was suspended in lissapol-dispersol.

Results

Role of hypothermia on duration of anaesthesia

At 31°C, the hypothermia produced by pentobarbitone was not significantly different from that produced when pentobarbitone and cannabis extract I were given together.

Duration of action of cannabis extract I

Mice were pretreated with a single dose of extract I equivalent to 10 mg Δ^9-THC/kg (46 mg extract/kg) at various times before a dose of pentobarbitone. Prolongation by cannabis of pentobarbitone anaesthesia was maximal from 20 min to 2 h after the cannabis administration. After 4 h the effect declined, and after 8 h, although significant potentiation was evident, this effect was much less than at 4 hours (Table 1).

Effect of cannabis on duration of ether anaesthesia

A single dose of extract I (equivalent to 10 mg Δ^9-THC/kg), administered 20 min before an ether : olive oil mixture, significantly potentiated the duration of anaesthesia. A tolerance to this effect was observed in mice which had received eight daily doses of cannabis extract (equivalent to 10 mg Δ^9-THC/kg). These results, and the results of a parallel study using pentobarbitone anaesthesia are shown in Table 2. A similar multiple dosage schedule of cannabis extract I, while not abolishing the potentiation of pentobarbitone anaesthesia, did suggest that some tolerance occurred since the percentage prolongation of anaesthesia was reduced from 41% with a single dose to 30% after multiple dose pretreatment. In both cases, however, the prolongation was significant ($P < 0.05$).

Failure of cannabis extract II to prolong pentobarbitone anaesthesia

Cannabis extract II (equivalent to 10 mg Δ^9-THC/kg) failed to prolong pentobarbitone anaesthesia (Table 3). This extract was tested in two strains of mice (QS and C57) and in neither case was there any significant potentiation of anaesthesia.

Effect of cannabinoids alone or in combination on duration of pentobarbitone anaesthesia

Δ^8-THC, Δ^9-THC and cannabinol (each 10 mg/kg) were administered 1 h prior to pentobarbitone. Δ^8-THC and Δ^9-THC significantly potentiated pentobarbitone anaesthesia whilst cannabinol was without effect (Table 4, experiment 1).

In view of the considerable difference in the ratio of cannabinoids in the two extracts tested, the possibility of interactions between the cannabinoids was investigated. Δ^9-THC, cannabinol and cannabidiol were administered, in a constant dosage of 10 mg/kg, 1 h prior to pentobarbitone. The results (Table 4, experiment 2) show that cannabidiol was more effective in prolonging anaesthesia than Δ^9-THC at the dose level tested, whilst cannabinol again was inactive. It should be noted that the prolongation by Δ^9-THC in this experiment was not significant. However, the combination of cannabidiol and Δ^9-THC (each 10 mg/kg) produced a greater prolongation of pentobarbitone anaesthesia than did cannabidiol alone ($P < 0.1$).

To clarify this interaction further, a third experiment was performed in which mice received either Δ^9-THC (20 mg/kg), cannabidiol (20 mg/kg) or a mixture of the two (each 10 mg/kg, total cannabinoid dose, 20 mg/kg). In this experiment (Table 4, experiment 3) as in experiment 2, cannabidiol was more potent than Δ^9-THC in prolonging sleeping time. However, the

Table 1 The duration of cannabis extract I-induced potentiation of pentobarbitone anaesthesia in C57 male mice. The dose of extract was constant and equivalent to 10 mg Δ^9-THC/kg

Premedication time (h)	*Duration of anaesthesia (min ± s.e. of mean (n)**)* *Control*	*Treated*	*% Prolongation*
0	60 ± 2 (17)	72 ± 3* (16)	20
0.33	68 ± 2 (20)	106 ± 4* (20)	58
1	65 ± 3 (20)	101 ± 5* (20)	55
2	60 ± 5 (17)	99 ± 6* (17)	64
4	58 ± 3 (21)	74 ± 3* (19)	27
8	46 ± 2 (30)	51 ± 2* (28)	11

* $P < 0.05$, the treated group is compared to the appropriate control group.
** (n), number of animals.

Table 2 Effect of cannabis extract I pretreatment on ether and pentobarbitone-induced anaesthesia in QS strain mice. In all cases the dose of cannabis extract was equivalent to 10 mg Δ^9-THC/kg, and mice received either one or eight daily doses, followed by the anaesthetic 20 min after the last dose of cannabis extract I

Number of doses	*Duration of anaesthesia (min ± s.e. of mean (n)**)* *Control*	*Treated*
	(a) *Ether anaesthesia*	
1	16 ± 3 (27)	27 ± 3 (25)*
8	16 ± 1 (24)	17 ± 3 (26)
	(b) *Pentobarbitone anaesthesia*	
1	64 ± 6 (19)	91 ± 8 (19)*
8	55 ± 5 (20)	72 ± 6 (21)*

* $P < 0.05$.
** (n), number of animals.

combination of the cannabinoids induced a sleeping time somewhere between those for the pure Δ^9-THC and cannabidiol, suggesting the interaction was additive rather than synergistic.

Drug modification of brain amines and the effect on cannabis prolongation of pentobarbitone anaesthesia

Groups of mice were premedicated with one of a number of drugs known to affect catecholamine or 5-hydroxytryptamine kinetics in the central nervous system: desipramine 25 mg/kg, phentolamine 10 mg/kg, propranolol 10 mg/kg, protriptyline 25 mg/kg, phenoxybenzamine 5 mg/kg (all 0.5 h premedication time prior to pentobarbitone), α-methyl tyrosine (αMT) 500 mg/kg (4 h), iproniazid 100 mg/kg (24 h), parachlorophenylalanine 500 mg/kg (72 h), or reserpine 0.5 mg/kg (48 and 24 h), while parallel groups were premedicated with the vehicle. Twenty minutes before pentobarbitone, mice received either cannabis extract I (equivalent to 10 mg Δ^9-THC/kg) or the vehicle alone. Although some of the drug pretreatments themselves exerted an effect on pentobarbitone anaesthesia (αMT, desipramine, protriptyline and phenoxybenzamine prolonged pentobarbitone anaesthesia), none of them had a significant effect on the cannabis-induced prolongation of pentobarbitone anaesthesia.

Table 3 Effect of cannabis extract II on pentobarbitone anaesthesia in two strains of mice. A dose of extract equivalent to 10 mg Δ^9-THC/kg was administered 20 min prior to pentobarbitone

	*Duration of anaesthesia (min ± s.e. of mean (n)**)*	
Strain of mice	*Control*	*Treated*
C57	69 ± 3 (12)	74 ± 11 (17)
QS	54 ± 5 (9)	50 ± 4 (8)

** (n), number of animals.

Discussion

Many studies have reported the interaction which occurs between cannabinoids and barbiturates in mice and rats (Phillips, Brown & Forney, 1971; Paton & Pertwee, 1972). This interaction has been shown to occur with extracts of cannabis (Paton & Pertwee, 1972), and with Δ^9-THC (Garriot, King,

Table 4 The effects of Δ^8-THC, Δ^9-THC, cannabinol and cannabidiol and some combinations on the duration of pentobarbitone anaesthesia in QS strain mice

Drug and dose (mg/kg)	*Duration of anaesthesia (min ± s.e. of mean (n)**)*
Experiment 1	
Control	89 ± 4 (46)
Δ^9-THC (10)	114 ± 4 (49)*
Δ^8-THC (10)	120 ± 4 (44)*
Cannabinol	80 ± 4 (44)
Experiment 2	
Control	45 ± 4 (35)
Δ^9-THC (10)	52 ± 5 (20)
Cannabidiol (10)	71 ± 8 (20)*
Cannabinol (10)	50 ± 7 (20)
Cannabidiol + Δ^9-THC (10 + 10)	92 ± 9 (20)*
Cannabinol + Δ^9-THC (10 + 10)	58 ± 6 (20)
Cannabinol + cannabidiol (10 + 10)	61 ± 7 (19)*
Cannabidiol + Δ^9-THC + cannabinol (10 + 10 + 10)	85 ± 8 (15)*
Experiment 3	
Control	35 ± 3 (39)
Δ^9-THC (20)	64 ± 5 (38)*
Cannabidiol (20)	93 ± 7 (39)*
Cannabidiol + Δ^9-THC (10 + 10)	80 ± 9 (38)*

* $P < 0.05$.
** (n), number of animals.

Forney & Hughes, 1967; Kubena & Barry, 1970). The results described here support these findings and further report that Δ^8-THC possesses similar activity. Of considerable interest was the failure of cannabis extract II to prolong pentobarbitone anaesthesia in mice. Although both extracts were administered to present the same dose of Δ^9-THC, only extract I was active at the dose tested. The major difference between these two extracts is in their cannabidiol content, with extract I containing 42.7% and extract II only 3.9%. Although Δ^9-THC does potentiate pentobarbitone anaesthesia in mice, it has been suggested that cannabidiol is more active in this respect (Loewe, 1944). Our results are in agreement with this finding. In two of the three experiments in which these cannabinoids were directly compared, cannabidiol was more effective than Δ^9-THC. Notable also was the marked additive effect of Δ^9-THC and cannabidiol. That this effect was merely additive, rather than synergistic was shown by the finding that the response to the combination of Δ^9-THC and cannabidiol (each 10 mg/kg) was approximately equivalent to that observed with 20 mg/kg of each drug alone. In fact, the mean duration of anaesthesia of the mice dosed with the mixture of cannabinoids fell in between the means of the groups dosed with each of the cannabinoids alone. These findings may explain in part the failure of extract II (with a very low proportion of cannabidiol) to prolong significantly pentobarbitone anaesthesia, whilst extract I (with a high proportion of cannabidiol) was effective.

Our studies have also indicated that cannabinol does not potentiate pentobarbitone anaesthesia, and there was a consistent trend to suggest that it might reduce the duration of anaesthesia especially when in combination with the other cannabinoids. This finding agrees with the finding of Krantz, Berger & Welch (1971) that cannabinol antagonizes the pentobarbitone potentiating properties of Δ^9-THC on sleeping time in rats.

It would appear therefore that apart from possible interactions with the anaesthetic used, interactions of the cannabinoids with each other should be considered. It is of importance therefore that in studies using cannabis extracts, the ratio of the various cannabinoids should be indicated.

The mechanism by which cannabis prolongs the duration of anaesthesia has not been satisfactorily elucidated. When administered alone, cannabis extracts exert a central nervous system depressant effect without anaesthesia even in high doses (Paton, 1968). Tincture of cannabis was frequently used in man for its sedative properties (Shafer, 1972) and both Δ^9-THC and cannabis extracts induce sedation in the conscious dog (Angus, Chesher, Cobbin, Einstein, Goodman, Jackson & Starmer, 1971). It has also been reported that Δ^9-THC and cannabidiol are effective inhibitors *in vitro* of the microsomal metabolism of phenazone (Paton & Pertwee, 1972), and these authors considered this to be a possible mechanism of action for cannabis-induced prolongation of pentobarbitone anaesthesia. Unlike these authors, we found that the duration of anaesthesia induced by a non-metabolized anaesthetic, ether, was prolonged by a cannabis extract. The possibility exists therefore that cannabis might exert a dual effect in these experiments, a direct depressant effect *per se*, and a drug interaction, perhaps at the microsomal level, to reduce the rate of metabolism of the pentobarbitone. Repeated administration of cannabis induces a tolerance to the ether potentiating effect, while the apparent absence of tolerance to cannabis when pentobarbitone was used as anaesthetic might be explained by the cannabis interfering with barbiturate metabolism and masking the tolerance which may still develop to its direct depressant effect.

The differences of our results from those of Paton & Pertwee (1972) with regard to cannabis potentiation of ether anaesthesia, may be a result of a strain difference in the mice used, or more likely to the nature of the cannabis extracts used because these authors used an alcoholic extract that differed markedly from the red oils used in this study. We do not believe that a hypothermic effect was involved, since under the conditions of our experiments, there was no significant difference in body temperature of mice in the cannabis treated and control groups. A possible basis for the differences observed might be in a relative proportion of other cannabinoids in the extracts used.

Because our findings suggested that cannabis was able to prolong both pentobarbitone and ether anaesthesia, we explored the possibility that cannabis might exert this action by interacting in some way with either catecholaminergic or serotoninergic neurones. The failure of αMT, a specific inhibitor of tyrosine hydroxylase (Spector, Sjoerdsma & Udenfriend, 1965; Corrodi & Hanson, 1966), and parachlorophenylalanine, a blocker of 5-hydroxyindole production (Koe & Weissman, 1966) suggests that an intact synthetic pathway for noradrenaline, dopamine and 5-hydroxytryptamine is not required for cannabis-induced prolongation of pentobarbitone anaesthesia. The necessity for intact stores of noradrenaline, dopamine and 5-hydroxytryptamine was disproved by the inactivity of reserpine. Neither α-adrenoceptor antagonists (phenoxybenzamine and phentolamine), nor the β-adrenoceptor antagonist (propranolol) exerted any effect. Finally, the possibility that cannabis might interfere with

noradrenaline uptake mechanisms in some manner appears unlikely since desipramine and protriptyline, both tricyclic antidepressants which are known to block selectively noradrenaline uptake (Carlsson, Fuxe, Hamberger & Lindquist, 1966; Ross & Renyi, 1967) were inactive in these tests. These findings led us to conclude that cannabis interaction with pentobarbitone-induced anaesthesia is independent of any effect cannabis may have on either turnover, or endogenous levels of noradrenaline, dopamine and 5-hydroxytryptamine.

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