Br. J. Pharmac. (1973), **49**, 588–594.

The effect of cannabinoids on intestinal motility and their antinociceptive effect in mice

G. B. CHESHER, C. J. DAHL,* M. EVERINGHAM, D. M. JACKSON, H. MARCHANT-WILLIAMS AND G. A. STARMER

Department of Pharmacology, University of Sydney, Sydney, New South Wales 2005, Australia
** Australian Government Analytical Laboratory, Department of Science, Melbourne, Victoria, Australia.*

Summary

1. After oral administration to mice, pethidine, $\triangle^8$-tetrahydrocannabinol (THC), $\triangle^9$-THC, a cannabis extract and cannabinol had a dose-dependent antinociceptive effect when measured by the hot-plate method. Cannabidiol was inactive at 30 mg/kg. $\triangle^8$-THC, $\triangle^9$-THC and pethidine did not differ significantly in potency, but $\triangle^9$-THC was 6·5 times more active than cannabinol.

2. After oral administration, three different cannabis extracts, $\triangle^8$-THC, $\triangle^9$-THC and morphine produced dose-dependent depressions of the passage of a charcoal meal in mice. $\triangle^8$-THC and $\triangle^9$-THC were equipotent and were about five times less potent than morphine. Cannabidiol was inactive up to 30 mg/kg. The effect of the three cannabis extracts on intestinal motility could be accounted for by their $\triangle^9$-THC content.

3. The antinociceptive effect of pethidine and the effect of morphine on intestinal motility were antagonized by nalorphine whilst the effects of the cannabis extracts and the pure cannabinoids were not.

4. From these results it is concluded that although cannabis and the narcotics share several common pharmacological properties, the mode of action of each is pharmacologically distinct.

Introduction

In pharmacological terms, cannabis and its derivatives are not considered to be narcotic analgesic drugs. There is evidence, however, that they do share with the narcotic analgesics the properties of analgesia and depression of intestinal motility. The analgesic effectiveness of cannabis derivatives has been reported in experimental animals (Bicher & Mechoulam, 1968; Buxbaum, Sanders-Bush & Efron, 1969; Buxbaum, 1972; Dewey, Harris & Kennedy, 1972) and in man (Walton, 1938). Several authors have reported that $\triangle^9$-tetrahydrocannabinol reduced defaecation in rats (Masur, Martz, Korte & Bieniek, 1971; Drew, Miller & Wikler, 1972) and Dewey *et al.* (1972) reported that this substance delayed passage of a charcoal meal in mice.

In the present study, we describe an investigation of the effects of extracts of cannabis leaf and hashish, $\triangle^9$-tetrahydrocannabinol ($\triangle^9$-THC), $\triangle^8$-THC, cannabidiol, cannabinol acetate, morphine and pethidine on the threshold of the hot-plate test and intestinal motility in mice.

Methods

Preparation of cannabis extracts and materials

Extracts of cannabis leaf or 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 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 transparent ' red oil ', a sample of which was silylated and assayed for cannabinoids by gas-liquid chromatography. Three ' red oil ' extracts, each from a different sample of cannabis were prepared and the assay results are shown in Table 1.

TABLE 1. *The composition of three cannabis extracts.*

Extract	Source	Content % of THC	CBD	CBN
I	Pakistan hashish	22	43	34
II	Australian Cannabis leaf	52	39	5
III	Australian Cannabis leaf	41	8	20

THC = $\triangle^9$-tetrahydrocannabinol; CBD = cannabidiol; CBN = cannabinol.

Cannabis extracts and the pure cannabinoids were dissolved or suspended in propylene glycol and kept at −20° C until required. Dilutions were made with a solution of Lissapol-Dispersol (ICI) (Whittle, 1964) to give a final concentration of 5% propylene glycol. Pethidine hydrochloride, morphine sulphate and nalorphine hydrochloride were dissolved in water or 0·9% w/v NaCl solution (saline) and doses given were calculated as the salt. All drugs were administered in a dose volume of 1 ml/100 g body weight.

Antinociceptive action

The mice (SW strain, males, 20–30 g) were allowed food and water *ad libitum* up to the time of the experiment. The method of Woolfe & MacDonald (1944) was used and the hot-plate was maintained at a constant temperature of 55 ± 1° C. Before dosing, all mice were tested individually and the time spent on the hot-plate before the animal elicited the end-point response (flicking of a hind paw) was recorded. Mice which failed to respond within 30 s were discarded. A mean reaction time and a critical reaction time (CRT, the mean pre-drug reaction time plus two standard deviations) were calculated for each group of mice used. The results were expressed as the number of mice in each group which remained after medication on the hot-plate beyond the CRT; the ED_{50} and its limits of error (P=0·05) were calculated by the method of Litchfield & Wilcoxon (1949). All drugs except pethidine were administered orally 60 min prior to testing. Pethidine was administered intraperitoneally 30 min before testing.

To investigate the possibility of cannabis-pethidine interactions, two experimental schemes were used. (a) Mice received cannabis extracts at various dose levels and some, in addition, received pethidine (6 mg/kg); the remainder, dosed with the vehicle only, served as controls. Cannabis extracts were administered 1 h and pethidine 0·5 h before testing. (b) The animals received pethidine at various dose

levels and some also received cannabis extract (60 mg/kg); the remainder, dosed with vehicle only, served as controls.

To determine if the antinociceptive effect produced by the cannabis extract could be antagonized by the narcotic antagonist, nalorphine, a group of mice was given a dose of cannabis extract (60 mg/kg) 1 h before testing on the hot-plate. These mice were then divided into two groups, one of which was given nalorphine (5 mg/kg) and the other saline, both by the intravenous route. A similar procedure was used to study the interaction of pethidine (6 mg/kg, given 0·5 h before testing on the hot-plate) and nalorphine or saline.

Intestinal Motility

The effect of drugs on intestinal motility was determined by measuring the rate of passage of a charcoal meal (Macht & Barba-Gose, 1931). Mice received 0·2 ml of a meal, consisting of animal charcoal 12 g, tragacanth 2 g and water 130 ml by lavage and were killed 15 min later. The length of the small intestine from pylorus to the ileo-caecal junction was measured and the distance which the charcoal meal had travelled was expressed as a percentage of the total length of the small intestine. The ED_{50} and the limits of error ($P=0·05$) were calculated by the method of Litchfield & Wilcoxon (1949). The cannabis extracts, $\triangle^8$-THC and $\triangle^9$-THC were administered by lavage, 45 min before the charcoal meal.

To determine whether the activity of cannabis extracts on intestinal motility was of a morphine-like nature, a comparison was made of the effect of the narcotic antagonist, nalorphine, on the depression of intestinal motility produced by morphine and by cannabis extracts. Groups of mice were dosed with either cannabis extract, 1 h before they were killed, morphine 0·5 h before they were killed, or a vehicle control. Nalorphine (8 mg/kg) or a vehicle control was administered intraperitoneally to these animals 0·5 h before they were killed.

Results

Antinociceptive effects

Pethidine, $\triangle^8$-THC, $\triangle^9$-THC, cannabis extract, and cannabinol all exhibited dose-dependent antinociceptive activity (Table 2). Pethidine did not differ significantly in potency from $\triangle^9$-THC. $\triangle^8$-THC and $\triangle^9$-THC did not differ significantly in potency and $\triangle^9$-THC was estimated to be 6·5 times more active than cannabinol. Cannabis extract I had 70% of the potency of cannabinol and 11% of that of

TABLE 2. *The effects of pethidine, $\triangle^8$-tetrahydrocannabinol (THC), $\triangle^9$-THC, cannabinol acetate and cannabis extract I on antinociceptive activity measured by the hot-plate method.*

Drug (Doses, mg/kg)	ED_{50} (mg/kg) (limits of error for $P=0·05$)	Slope Function	No. of observations
Pethidine	7·0	7·58	220
(2, 4, 6, 8)	(4·8–10·3)	(1·46– 39·26)	
$\triangle^9$-THC	5·0	7·07	300
(4, 7·5, 15, 30, 60)	(2·9– 8·8)	(1·96– 25·45)	
$\triangle^8$-THC	5·0	11·06	240
(4, 9·5, 15, 30)	(2·4–10·5)	(0·64–190·20)	
Cannabinol acetate	32·5	3·74	240
(10, 20, 40, 60)	(22·4–47·1)	(1·68– 8·30)	
Cannabis extract I	47·0	13·74	300
(7·5, 15, 30, 60, 100)	(30·9–71·4)	(3·57– 52·90)	

Δ^9-THC. In terms of Δ^9-THC present, the extract had approximately half the potency of Δ^9-THC. Cannabidiol was inactive at a dose of 30 mg/kg. All the dose-response curves were parallel.

The antinociceptive effect of cannabis alone did not differ significantly from that produced by cannabis plus pethidine (Table 3). Another group of mice, in which the testing procedure was carried out 1·5 h after cannabis and 1 h after pethidine administration gave similar results. In the experiment where the pethidine

TABLE 3. *The effect of interaction on the antinociceptive effects of cannabis and pethidine in mice.*

Drug Administration (mg/kg) First	Second	Time after cannabis before testing (min)	ED_{50} (mg/kg) (limits of error for $P=0.05$)	Slope function	No. of observations
			(a) Cannabis		
Cannabis (5, 7·5, 15, 30)	Pethidine (6)	60	18·0 (10·3–31·5)	9·08 (1·85– 44·49)	120
		90	23·0 (13·9–38·2)	10·00 (1·60– 62·50)	160
Cannabis (5, 7·5, 15, 30)	Vehicle Control	60	21·2 (10·8–43·0)	15·12 (0·66–346·2)	118
		90	12·7 (7·9–20·3)	9·30 (3·72– 23·25)	160
			(b) Pethidine		
Cannabis (60)	Pethidine (2, 4, 6, 8)	60	4·6 (3·1– 6·9)	9·21 (1·33– 63·73)	230
Vehicle Control	Pethidine (2, 4, 6, 8)	60	7·0 (4·8–10·3)	7·58 (4·76– 10·3)	230

dose was varied and the cannabis dose remained constant, the results were essentially similar to those reported above. Cannabis possibly potentiated the antinociceptive effect of pethidine, but the effect was statistically not significant.

The antinociceptive effect of pethidine but not that of the cannabis extract was antagonized by nalorphine (Table 4).

TABLE 4. *The effect of nalorphine (5 mg/kg) on the antinociceptive effects of pethidine and cannabis extract I.*

Treatment	Pethidine (6 mg/kg) Mean Time on hot-plate ± S.E.M. (s)	Cannabis Extract I (60 mg/kg) Mean Time on hot-plate ± S.E.M. (s)
Before nalorphine	13·8±3·4 (41)	22·1±1·5 (37)
After nalorphine	8·1±0·9 (17)	22·8±2·0 (18)
After saline	16·0±1·4 (20)	25·3±1·7 (19)

Pethidine and cannabis extract were administered 35 and 65 min respectively before administration of nalorphine or saline. The numbers in brackets indicate number of observations.

Intestinal Motility

All cannabis extracts, Δ^8-THC and Δ^9-THC had a dose-dependent effect on the passage of the charcoal meal (Table 5). Cannabidiol was inactive at all of five dose levels (6–30 mg/kg) tested. All the cannabis extracts and cannabinoids tested were significantly less potent than morphine but the regression lines were parallel. Δ^8-THC and Δ^9-THC were not significantly different in potency. When the ED_{50} values for the three cannabis extracts were converted into their equivalent Δ^9-THC contents (Tables 1 and 5), the ED_{50} values calculated for Δ^9-THC were:

TABLE 5. *The effects of morphine, Δ^8-tetrahydrocannabinol (THC), Δ^9-THC and cannabis extracts I, II and III on the passage of a charcoal meal in mice.*

Drug treatment & dose (mg/kg)	ED_{50} (mg/kg) (limits of error for $P=0.05$)	Slope function	Potency (morphine=1)
Morphine (0·25, 0·5, 1·0, 2·0, 4·0, 8·0)	3·4 (1·7– 6·7)	0·186 (0·066–0·520)	1·0
Δ^8-THC (2·0, 5·0, 10·0, 20·0, 40·0)	13·5‡‖ (10·9– 16·7)	0·072 (0·007–0·756)	0·25 (0·12–0·53)
Δ^9-THC (2·0, 5·0, 10·0, 20·0)	20·0 (12·9– 31·0)	0·370 (·250–0·55)	0·17 (0·07–0·39)
Cannabis Extract I (4·5, 11·4, 22·7, 45·5, 91·0, 182·0)	86·0‡‖ (46·0–160·8)	·164 (·059–0·459)	0·028 (0·015–0·1)
Cannabis Extract II (4·8, 9·7, 19·4, 38·8, 77·6)	21·5‡‖ (13·6– 34·0)	·217 (0·095–0·494)	0·16 (0·07–0·36)
Cannabis Extract III (2·9, 7·25, 14·5, 29·0, 58·0)	68·0‡‖ (29·8–155·3)	0·042 (·003–0·671)	0·05 (0·017–0·15)

‡‖Significantly less potent than morphine at $P<0.05$.
The ED_{50} is that dose of compound required to slow the passage of a charcoal meal by 50% when compared with control animals which received the vehicle only.

I. 18·92 mg/kg; II. 11·18 mg/kg; III. 27·88 mg/kg. Thus, allowing for experimental error, the effects of the extracts can probably be attributed to the content of Δ^9-THC.

Whilst nalorphine effectively antagonized morphine-induced inhibition of gastrointestinal motility (Table 6), it had no effect on the action of either cannabis extracts I or II.

TABLE 6. *The effect of nalorphine (8 mg/kg) on the actions of morphine (8 mg/kg) and cannabis extracts I and II (equivalent to 10 mg/kg tetrahydrocannabinol) on the passage of a charcoal meal in mice.*

First treatment	Second treatment	Passage of charcoal meal expressed as % of total length of small intestine ±S.E.M.*
Morphine	Nil	9·0±0·6 (21)
Water	Nalorphine	35·8±3·4 (20)
Morphine	Nalorphine	24·7±1·9 (20)
Water	Water	44·6±1·8 (20)
Extract I	Water	33·2±2·2 (21)
Extract I	Nalorphine	22·4±2·3 (19)
Vehicle control	Water	48·6±2·5 (20)
Vehicle control	Nalorphine	37·5±3·5 (20)
Extract II	Water	29·4±2·0 (25)
Extract II	Nalorphine	23·3±2·1 (25)
Vehicle control	Nalorphine	43·6±3·3 (24)
Vehicle control	Water	55·5±3·0 (10)

*The values are the means and their standard errors. The numbers in brackets are the number of observations.

Discussion

There is still some doubt as to the nature of the response of cannabis-treated animals when tested by standard pharmacological methods for analgesia. Evidence for antinociceptive effects has been reported in a number of species following administration of Δ^9-THC (Bicher & Mechoulam, 1968; Bukbaum, 1972; Buxbaum *et al.*, 1969). Although Dewey *et al.* (1972) were unable to demonstrate a significant antinociceptive effect when using the tail flick method in mice (in

doses below 100 mg/kg), they reported a prolonged antinociceptive effect in the same species when using the hot-plate method.

In the present experiments we have found that Δ^8-THC, Δ^9-THC, a cannabis extract and cannabinol all produced a dose-dependent increase in reaction time when tested on the hot-plate, with Δ^8-THC and Δ^9-THC having equal potency and cannabinol approximately one sixth of the potency of Δ^9-THC. The results for Δ^8-THC and Δ^9-THC are thus in broad agreement with those of Dewey *et al.* (1972).

The analgesic dose-response curve for pethidine was parallel to those for the cannabinoids; both Δ^8-THC and Δ^9-THC being approximately equipotent with pethidine. These results agree more closely with those of Bicher & Mechoulam (1968) than those of Buxbaum (1972) who reported considerable deviation from parallelism between the dose-effect curves for cannabinoids and narcotic analgesics. These differences might have been due to the route of administration since Buxbaum (1972) injected mice subcutaneously and absorption of cannabinoids by this route is slower and less complete than by the oral route used in our studies (Ho, 1971).

The interaction between cannabinoids and pethidine in mice tested by the hot-plate method was only suggestive of an additive effect. This finding was quite unlike the interactions of cannabinoids with barbiturates or ether on the duration of anaesthesia in mice. Doses of cannabis extracts or Δ^9-THC which themselves are not hypnotic, significantly potentiate the sleeping times of mice induced by barbiturate or ether (Paton & Pertwee, 1972; Chesher, Jackson & Starmer, 1974).

A possible effect of cannabis on intestinal motility had been noted in the observation that Δ^9-THC reduced the incidence of defaecation in rats including those considered to be 'high defaecators' (Masur *et al.*, 1971; Drew *et al.*, 1972). A depressant effect of Δ^9-THC and Δ^8-THC administered subcutaneously on the passage of a charcoal meal in mice has been reported by Dewey *et al.* (1972), although a clear dose-response relationship was not apparent. In the present studies we have shown that oral administration of both Δ^8-THC and Δ^9-THC and three cannabis extracts produced parallel dose-dependent depressions of the passage of a charcoal meal. As with the results on the antinociceptive effects, Δ^8-THC and Δ^9-THC did not differ significantly in potency and the potency of the three cannabis extracts can reasonably be accounted for by their Δ^9-THC contents. It appears therefore that other cannabinoids are exerting little effect on intestinal motility; this concept is supported by our findings of the lack of activity of cannabidiol on intestinal motility and by the wide divergence in content of cannabidiol in the three extracts used. For the latter reason, cannabinol appears to be unimportant in the activity observed in these studies.

Although, when tested for the antinociceptive effects and the effects on intestinal motility, the dose-response curves of cannabinoids and narcotics were parallel, the response to nalorphine clearly suggests a different mode of action. For both effects, nalorphine antagonized the action of the narcotic analgesic but had no effect on the responses to the cannabinoids.

We wish to thank Professor R. H. Thorp for his constant encouragement and Miss Margaret Rutherford for her skilled technical assistance. This work was supported by a grant from the National Health and Medical Research Council. Samples of cannabis were obtained from seized material by courtesy of the N.S.W. Government. Δ^8-THC and Δ^9-THC were obtained from the WHO, Geneva, by courtesy of Dr. O. Braenden.

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(*Received March* 20, 1973)