and below this value (Albert 1985). It is likely that for drugs and chemicals which have a non-specific membrane stabilizing action in acute overdose, this rule will also apply. It is recommended therefore that testing of new chemicals, whether pharmaceutical, industrial or agricultural, at the acute toxicity stage, should include the determination of the n-octanol/water partition coefficient under physiological conditions (pH 7·4 temperature 37°C) in addition to any determinations performed under environmental conditions for industrial or agricultural chemicals.

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Inhibition of the cataleptic effect of tetrahydrocannabinol by other constituents of *Cannabis sativa* L.

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Abstract—Tetrahydrocannabinol (THC) induced catalepsy in mice, whereas a cannabis oil (6·68% w/w THC), four cannabinoids and a synthetic mixture did not. Cannabinol (CBN) and olivetol inhibited THC-induced catalepsy in the mornings and the evenings, but cannabidiol (CBD) exhibited this effect only in the evenings. A combination of CBN and CBD inhibited THC-induced catalepsy equal to that of CBN alone in the mornings, but this inhibition was greater than that produced by CBN alone in the evenings.

The psychotropic potency of cannabis is believed to be dependent upon its content of THC (Fairbairn & Pickens 1981). Cannabis has been shown to induce catalepsy in experimental animals (Paton & Pertwee 1973a) and man (Paton & Pertwee 1973b), and this response has been presumed to correlate with psychotic effects. Cannabis oil contains a mixture of cannabinoids and other substances and is generally more potent than the herb or the resin. Whilst THC produces catalepsy in mice, at least one other cannabinoid, CBD, is inactive (Pertwee 1972).

Previous reports have postulated a link between prostaglandin (PG) levels and the central effects of cannabis (Fairbairn & Pickens 1979), however we have shown a close similarity in the effects of cannabinoids on PG release from cell culture (Barrett et al 1985) and cell free assays for enzymes of arachidonate metabolism (Evans et al 1987a). Clearly if THC is unique in possessing central activity, PG metabolism is an unlikely target. It was therefore important to re-examine the cataleptic response to cannabis, including an evaluation of the natural oil, synthetic mixtures of cannabinoids, and the pure cannabinoids.

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Materials and methods

Drug preparation. Stock solutions were prepared (10 mg mL^{-1}) in redistilled ethanol and stored at −4°C. Serial dilutions were made with 0·9% w/v sodium chloride containing 2·5% w/v Tween 80. The final concentrations of ethanol did not exceed 1% v/v and this concentration had no effect in the cateleptic tests performed.

Animals. Male albino CD1 mice (Charles River), 22–30 g, were maintained at 30–32°C.

Cannabinoids (THC, Δ^1-tetrahydrocannabinol; CBN, cannabinol; CBD, cannabidiol; CBG, cannabigerol; olivetol) were purchased from Makor Chemicals Ltd, Israel. Cannabis (strain UNC 335) was cultivated and an oil produced as previously described (Barrett et al 1985).

Drugs were administered (1 mL/100 g weight) by gavage; controls received only the vehicle. Two hours later, animals were gently placed on a wire ring (Pertwee 1972) and the sum of the time in seconds during which the animal remained immobile over a 5 min period was recorded. Catelepsy was expressed as the

Table 1. Effect of cannabinoids and olivetol on THC-induced catalepsy. The results are the mean, s.e. mean of (n) animals per group. Student's *t*-test was used to evaluate significant differences. *Significant difference ($P < 0.05$) in response between THC alone and in combination with other cannabinoids. P_m demonstrates the significane of difference between the response in the morning and the evening. NS is not significant ($P > 0.1$).

Treatment		%Mean catalepsy (± s.e.mean) Morning		Evening	P_m
12·5 mg kg^{-1}	THC	64·5±6·5 (6)		76·4±4·5 (7)	NS
12·5 mg kg^{-1} +25 mg kg^{-1}	THC CBD	62·5±3·5 (7)	NS	33·5±5* (11)	<0·05
12·5 mg kg^{-1} +16 mg kg^{-1}	THC CBN	32·6±4·2 (7)*		39·5±9* (11)	NS
12·5 mg kg^{-1} +16 mg kg^{-1} +25 mg kg^{-1}	THC CBN CBD	41·0±7·0 (9)*		20·9±7* (8)	<0·1
12·5 mg kg^{-1} +100 mg kg^{-1}	THC CBD	61·4±6·1 (6)	NS	31·9±4·2* (7)	<0·05
12·5 mg kg^{-1} +100 mg kg^{-1}	THC olivetol	36·6±7·0 (6)*			
12·5 mg kg^{-1} +100 mg kg^{-1}	THC CBN	21·82±7·6 (5)*			
1·25 mg kg^{-1}	THC	12·7±2 (6)			
1·25 mg kg^{-1} +2·5 mg kg^{-1} +1·6 mg kg^{-1}	THC CBD CBN	4·2±1 (9)*			

percentage of the total time spent on the ring during which the animal remained motionless. Data are expressed as the mean with s.e. mean.

Antagonism was measured against a fixed dose of THC (12·5 mg kg^{-1} or 1·25 mg kg^{-1}) administered as before together with cannabinoids or their mixtures. Mornings are defined as 0600 h to noon, and evenings as 1800 h to midnight.

Analysis of cannabis. The oil prepared from cannabis was analysed by means of gas-liquid chromatography (GLC) on a 1 m OV-17 column at 245°C and a flow rate of 40 mL min^{-1} of nitrogen.

Results

THC induced catelepsy in mice at doses of 0·625 mg kg^{-1} to 25 mg kg^{-1}. Olivetol, CBG, CBN and CBD were inactive in doses of up to 100 mg kg^{-1} (Fig. 1). Cannabis oil was inactive in this test up to a dose of 12·5 mg kg^{-1}, as was a synthetic mixture prepared in an identical ratio to the natural product.

CBN inhibited THC (12·5 mg kg^{-1})-induced catalepsy at a dose of 16·0 mg kg^{-1}, both in the mornings and the evenings, whilst olivetol was also inhibitory at 100 mg kg^{-1}. CBD (25 mg kg^{-1}) would only produce an inhibition in the evenings. A combination of CBD and CBN (Table 1) produced inhibition of THC-induced catalepsy equal to that of CBN alone in the mornings, but this inhibition was greater in the evenings. When the dose of CBN was increased to 100 mg kg^{-1}, inhibition of THC greatly increased whilst the inhibition produced by CBD remained the same.

By means of GLC analysis the proportions of cannabinoids in the natural oil were calculated to be: THC, 6·68%; CBN, 9·08%; CBD, 14·13%.

Discussion

Our results demonstrate that olivetol, CBN and CBD inhibited THC-induced catelepsy in mice, but were not capable of producing catalepsy alone (Fig. 1), or in potentiating the effect of THC. This result is in agreement with the report of Jones & Pertwee (1972) in that these workers found that the action of THC could be antagonized by CBD. However, we have shown a diurnal variation in the activity of that cannabinoid, whilst CBN was active both in the mornings and the evenings (Table 1). In combination the effect of CBN and CBD was shown to be synergistic when given in the evenings but in the mornings their effect could be attributed to CBN alone. The THC receptor may have more than one form, or possibly these antagonists act at different sites centrally.

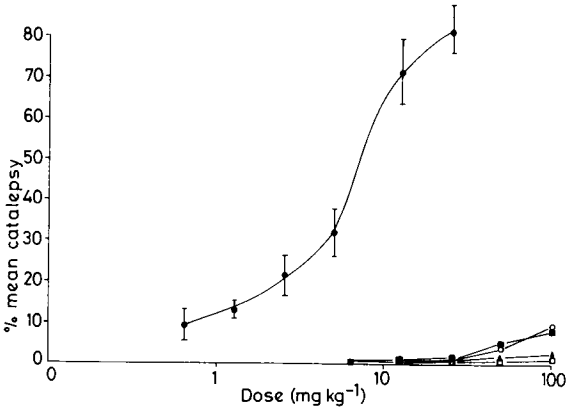

FIG. 1. Induction of catalepsy by cannabiboids; percentage catalepsy following oral administration of THC (●), CBD (○), CBN (■), CBG (□) and olivetol (▲). The values are the mean and the vertical lines represent the s.e. mean where n = 7 animals per group.

A cannabis preparation high in THC content was shown to have no cataleptic effect in mice and neither did a synthetic mixture of cannabinoids prepared in similar proportions to the natural product, whereas THC alone was shown to have an ED50 of 6·95 mg kg^{-1} (Fig. 1). The activity of cannabis therefore is not related solely to its content of THC. It has been previously shown (Fairbairn & Pickens 1981) that cannabis oil can be more potent than pure THC in this test. The cannabis used in these experiments is a direct descendant of that plant grown in the same botanical gardens over 15 generations, suggesting that the strain has changed its chemovariety. Earlier specimens may have contained unknown synergists possibly acting via modulation of PG levels centrally.

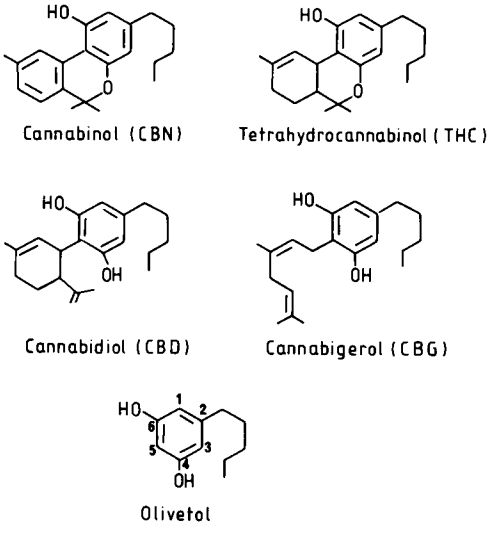

FIG. 2. Constituents of cannabis used in mouse cataleptic tests.

The actions of cannabis are complex and the receptor to THC is unknown. However the direct antagonism of the action of THC in-vivo by other cannabinoids would suggest that differences in the actions of these closely related drugs is not due to differences in their ability to cross the blood/brain barrier. Fairbairn & Pickens (1979) demonstrated that PG levels are significant in the actions of THC, and Burnstein et al (1982) have shown that THC will stimulate phospholipase A_2 in-vitro. However, Evans et al (1987b) were unable to demonstrate significant differences between the activities of CBN and THC in a cell-free assay involving that enzyme. There are similarities beteween the cannabinoids in their ability to affect the enzymes of arachidonate metabolism including phospholipase A_2 and to prevent PG secretion from cell culture (Barrett et al 1985; Evans et al 1987a). Of the five constituents of cannabis investigated here, compounds possessing a free C-4 hydroxyl group (Fig. 2) were potent peripheral analgesic and anti-inflammatory agents (Formukong et al 1986), possibly due to their effects upon arachidonate release and metabolism (Evans et al 1987a, b). In the case of THC, cyclization of the C-4 hydroxyl to the epoxide configuration, conferred unique cataleptic activity, whilst aromatization of the ring produced a compound which was an antagonist to that central activity. Although PG levels centrally may be significant in terms of the response to THC, the receptor is unlikely to be located in that pathway, but is more likely to be sensitive to or dependent upon PG concentrations. Diurnal variations may also be associated with fluctuations in hormone levels.

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