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THE EFFECT OF CONDITIONS INFLUENCING ENDOGENOUS PROSTAGLANDINS ON THE ACTIVITY OF Δ'-TETRAHYDROCANNABINOL IN MICE

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1 The cataleptic effect of Δ'-tetrahydrocannabinol (THC) depends upon the availability of the precursors of prostaglandins and the response is reduced in mice maintained on a diet deficient in arachidonic acid (AA) and restored by exogenous AA given intraperitoneally, or by feeding a normal diet.

2 In yeast-induced fever, which is accompanied by an increase in the synthesis of prostaglandins, THC shows an enhanced cataleptic effect.

3 Exposure to cold which results in depletion of prostaglandins reduces the effect of THC.

Introduction

In our previous paper (Fairbairn & Pickens, 1979) we showed that Δ'-tetrahydrocannabinol (THC) is considerably more active orally than intraperitoneally in the mouse, and that aspirin reduces the cataleptic effect of THC, probably by interference with prostaglandin E_2 (PGE_2) biosynthesis. To support this suggestion we have investigated whether those conditions which alter production of PGE_2 affect the response to THC in a corresponding manner. Firstly, we have kept the mice on a fat-free diet and thus deprived them of arachidonic acid (AA) which is the precursor of PGE_2. Secondly, we have induced yeast fever which increases the rate of biosynthesis of PGE_2 (Feldberg, 1974) and tested the effect of THC in these mice, and thirdly, we have examined the effect of THC on mice exposed to severe cold, which depletes the prostaglandin stores.

Methods

Cataleptic activity

LACA Tuck No. 1 strain albino female mice weighing 18 to 22 g were used throughout. The determination of cataleptic activity by the ring test (Pertwee, 1972) was carried out as described in our previous paper (Fairbairn & Pickens, 1979). Cataleptic responses were measured at 60 min unless stated otherwise. Potencies were calculated as cataleptic dose fifty (CD_{50}) as previously described.

Preparation of material for oral administration

Δ'-Tetrahydrocannabinol was suspended in 2.5% solution of Tween 80 as previously described. It was always given orally.

Fat-free diet

Mice, born at the School of Pharmacy, were weaned 3 weeks after birth and then fed a fat-free diet of brown bread and water. The effect of THC in these mice was investigated in the ring test at 18 and 28 days and the effect of exposure to severe cold at 3 months after starting the diet.

Yeast-induced fever

A 5% suspension of dried yeast in 2.5% Tween 80 was injected intraperitoneally and the fever measured as an increase in rectal temperature.

Exposure to severe cold

The animal house is normally kept at 23 to 25°C and the cabinet for the ring test at 30 to 32°C. In most experiments mice are transferred to the cabinet 24 h before carrying out the test. In the first series of experiments mice were exposed to a temperature of 6°C for 2 h immediately before the ring test, which was carried out at 30 to 32°C; others were kept at 15 to 17°C for 10 days before testing. To confirm the

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importance of pretesting temperatures, some were kept at 30 to 32° for 3 days before testing.

Results

Δ'-Tetrahydrocannabinol in mice fed a fat-free diet

Figure 1 shows that there was a reduction in the cataleptic response to THC when mice were maintained on a fat-free diet. After 18 days the response to 8 mg/kg (oral) THC was reduced from 89.5 ± 3.38 ($n = 8$) percentage catalepsy to 30.0 ± 3.92 ($n = 8$) and after 28 days the response was reduced from 84.2 ± 2.01 ($n = 6$) to 4.20 ± 2.41 ($n = 10$). In both experiments the response was restored by giving arachidonic acid (40 mg/kg) intraperitoneally and repeating the test 20 min later. The cataleptic response was also restored by feeding a normal diet for 7 days and retesting.

Δ'-Tetrahydrocannabinol in mice with a yeast-induced fever

Two hours after an intraperitoneal injection of yeast (200 mg/kg) the rectal temperatures of mice were increased by 2.1 ± 0.20°C ($n = 10$). This yeast fever was blocked by aspirin, 100 mg/kg orally. Twenty-four mice were randomized into 2 groups of 12. Mice in group 1 received a low dose of THC (0.25 mg/kg) and those in group 2 were given yeast together with THC (0.25 mg/kg), and this resulted in an increase in cataleptic activity from 26.4 ± 6.99% ($n = 12$) to 69.8 ± 5.93% ($n = 12$); $P < 0.001$ by Student's t test.

Δ'-Tetrahydrocannabinol in mice exposed to severe cold

In mice which were housed at 30 to 32°C for 3 days before and during the test, THC had a CD_{50} = 1.10 ± 0.07 mg/kg. However, mice which were housed at 15 to 17°C for one week and transferred to the cabinet (30 to 32°C) only 2 h before the test showed a much reduced activity (CD_{50} = 24.1 ± 0.7 mg/kg). Mice which were exposed to severe cold (6°C) for 2 h before receiving THC showed no response to the top dose of THC (25 mg/kg). We also exposed some mice which had been on a fat-free diet for 3 months to 6°C for 2 h and found a marked drop in rectal temperature of 5.2°C ±1.1 ($n = 7$); no such hypothermia was seen in mice fed on the normal diet (Oxoid 44B). Mice generally increase the generation of heat when placed in an extremely cold environment in order to maintain a normal body temperature. This they are unable to do when prostaglandins cannot be synthesized due, for example, to lack of precursors such as occurs in mice on a fat-free diet.

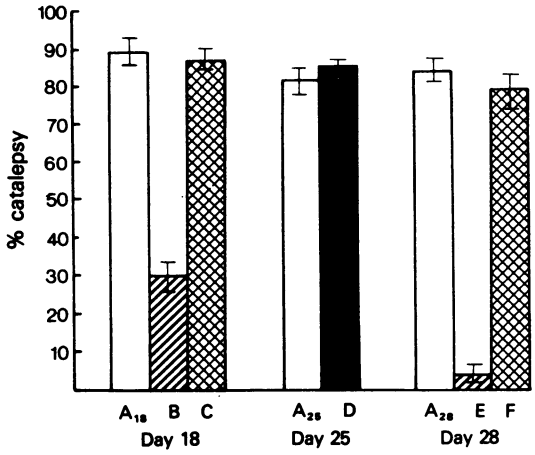

Figure 1 The effect of a fat-free diet (FFD) on the cataleptic response to 8 mg/kg of Δ'-tetrahydrocannabinol (THC) given orally to Tuck No. 1 strain, female mice weaned 21 days after birth. Day 0 = day of weaning. Height of histogram is mean % catalepsy, vertical lines are the s.e. mean. Control mice (A) were fed oxoid diet 44B from day 0 to 28 and tested for catalepsy to the THC on days 18 (A_{18}), 25 (A_{25}) and 28 (A_{28}). Test mice (B) were fed a FFD from day 0 to 18 and tested on day 18, they were then given either arachidonic acid (40 mg/kg i.p.) (AA) and retested 20 min later (C), or fed diet 44B from days 18 to 25 and retested on day 25 (D). Test mice (E) were fed a FFD from day 0 to 28 and tested on day 28. They were then given AA and retested 20 min later (F).

Discussion

Our first paper suggested that the oral activity of THC depended on the availability of endogenous PGE_2. Conditions affecting the latter should therefore affect the action of THC. We have altered endogenous prostaglandins in 3 ways.

(a) Firstly, we have reduced them by keeping mice on a fat-free diet and this resulted in a reduced response. Mice on a fat-free diet are deficient in essential fatty acids and thus also of arachidonic acid which is a precursor of prostaglandin biosynthesis. Our next experiment showed that small doses of arachidonic acid restored the cataleptic response to THC. The response was also restored when the mice were fed on a normal diet.

(b) Secondly, we have shown that the cataleptic effect of THC is potentiated in mice with yeast-induced fever. The synthesis of prostaglandins is increased by bacterial pyrogens (Feldberg, 1974). Not all pyrogens in all species, however, act via the prostaglandin mechanism and we have confirmed that the yeast-induced fever in our mice is due to an increase in prostaglandin biosynthesis, by showing that the

fever was abolished by aspirin. It is interesting to recall Feldberg's work (1974) which showed that the bacterial pyrogen *Shigella dysenteriae* which induced fever in cats, due to increased PGE biosynthesis, was always accompanied by somnolence and stupor during which the cat does not react to events in its environment. Both the fever and the stupor were reversed by aspirin. Horton (1964) also found that large doses of PGE produced catatonic stupor. It seems, therefore, that THC may act either as a modulator or a potentiator of the rate of prostaglandin biosynthesis. If this is so, high doses of THC will exhaust the precursors of prostaglandin biosynthesis and increased doses of the THC will then have no further effect. This is consistent with the fact that in all pharmacological experiments, there is a ceiling effect for THC, after which increasing the dose has no further effect on the magnitude of the response. For example, we have found in the ring test that the high oral doses of 25, 50 and 100 mg/kg of THC have approximately equal effects. We have also found that in the barbitone sleeping time experiment 1, 10 and 20 mg/kg of THC given orally increases barbiturate sleeping time in a dose-related manner, but 50 mg/kg has no greater effect than 20 mg/kg.

(c) Thirdly, we found that exposure to extreme cold which reduces the continued ability to synthesize prostaglandins reduces the cataleptic response to THC. The temperature of the animal house therefore plays an important part in the reproducibility of the response to THC and it is advisable to keep mice at 30 to 32°C for 3 days before carrying out the test for catalepsy.

The response to herbal cannabis in those who smoke it or ingest it is notoriously variable. This is due in part to the lack of standardization of reefers and the variability in the THC content of herbal cannabis (Fairbairn, Hindmarch, Simic & Tylden, 1974). Our results suggest that it may also be due to a poor diet, in which case herbal cannabis would have a lowered effect, not an enhanced effect as previously suggested by Carlini, Masur, Karniol & Leite (1972). The increased activity of THC in a raised room temperature may also account in part for the greater effect of cannabis in hot climates (Carlini, *et al.*, 1972) and crowded marihuana parties.

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