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Effect of Δ^9-tetrahydrocannabinol on prostaglandin concentrations and fluid absorption rates in the rat small intestine

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Δ^9-Tetrahydrocannabinol (Δ^9-THC) has been shown to alter prostaglandin synthesis and this has led to speculation about the mechanism by which cannabis exerts some of its pharmacological effects (Burstein & Raz 1972; Burstein et al 1973; Burstein & Hunter 1978; White & Tansik 1980). It has also been suggested from indirect evidence that the sedative and cataleptic effects of Δ^9-THC depend on the synthesis of prostaglandin E_2 (PGE_2) in the gut (Fairbairn & Pickens 1979, 1980; Pickens 1981). Although Δ^9-THC generally inhibits defaecation (Masur et al 1971; Drew et al 1972), PGE_2 induces diarrhoea mainly by its inhibition of fluid absorption from the intestine (Rask-Madsen & Bukhave 1979). In spite of these conflicting reports there are none concerned with the direct effect of Δ^9-THC on the prostaglandin content of the intestine. We have, therefore, determined the content of PGE_2-like material in the small intestine of the rat following a dose of Δ^9-THC that causes marked catatonia and sedation, and also measured fluid absorption.

Materials and methods

Male albino Wistar rats 220 to 280 g were housed and the experiments conducted in rooms maintained at an ambient temperature of 20 to 23 °C with a 12 h light-dark cycle. Food and water were freely available.

Preparation of Δ^9-tetrahydrocannabinol. Δ^9-THC was suspended in 0·9% w/v NaCl (saline) with polyvinylpyrrolidone (PVP) according to Fenimore & Loy (1971). Δ^9-THC suspension, or PVP in saline as control, were injected intravenously in volumes of 1 ml kg^{-1}. Δ^9-THC was administered at 2 mg kg^{-1} since this dose causes catatonia, sedation and other effects (Coupar & Taylor 1982).

Extraction and determination of prostaglandin-like material. For intravenous administration, a permanent polyethylene (PE 10) catheter was inserted into the external jugular vein under amylobarbitone and methohexitone anaesthesia. For the 48 h recovery and for the experiment animals were kept in individual cages.

The rats were decapitated at 30, 60 and 120 min after injection of Δ^9-THC or PVP. Segments of jejunum (approximately 20 cm distal from the ligament of Treitz) and ileum (approximately 20 cm proximal from the ileocaecal junction) were removed immediately, rinsed with ice-cold saline, blotted, frozen in liquid N_2 and stored at −20 °C.

Prostaglandin-like material was extracted by modification of previous methods designed to recover basal quantities in tissues (Unger et al 1971; Bennett et al 1977). Briefly, frozen segments of intestine were weighed (range 1–1·5 g) and allowed to thaw in 10 ml of ice-cold ethanol-saline (1:1), homogenized for 40 s (Ultra Turrax) and centrifuged at 24 000 × *g* at 4 °C for 20 min. Neutral fats were removed from the supernatant by washing with two volumes of light petroleum (40–60 °C), the pH of the supernatant was adjusted to 3·5 with formic acid and the prostaglandin-like material was twice extracted into one volume of chloroform. The chloroform was removed and the dried extract dissolved in 10 ml of Krebs-Henseleit solution and prostaglandin was bioassayed against PGE_2 using the rat gastric fundus strip by a method similar to that of Ferreira & DeSouza Costa (1976). The strips were suspended in liquid paraffin and superfused with Krebs-Henseleit solution containing a mixture of antagonists (atropine, mepyramine, phenoxybenzamine 0·1 μg ml^{-1}; methysergide, propranolol 0·2 μg ml^{-1} and indomethacin 2 μg ml^{-1}). The amount of prostaglandin-like material determined is expressed as PGE_2 equivalents per g of wet tissue.

Intestinal transport. In a separate group of rats, net transport of fluid by the small intestine was measured by a previously described recirculation technique (Coupar 1978). Food was withdrawn overnight and the animals were anaesthetized with pentobarbitone and the external jugular vein cannulated. The lumen of the jejunum or ileum was perfused with an isosmotic solution containing (g $litre^{-1}$) NaCl (8·57); KCl (0·37); dextrose (1·0), and phenolsulphonphthalein (0·02) to act as a non-absorbable marker for water transport. Recirculation of this solution through the lumen was initiated by gas lift 30 min after intravenous injection of either Δ^9-THC or PVP and continued for 20 min. Results are expressed as the net amount of water absorbed per g wet tissue perfused.

Statistics. Values are expressed as means ± s.e.m. and

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Table 1. Amounts of extracted PGE_2-like material (PGE_2-LM) and values of net water absorption in the small intestine following intravenous administration of polyvinylpyrrolidone alone (PVP, 40 mg kg^{-1} in saline) or Δ^9-tetrahydrocannabinol (Δ^9-THC, 2 mg kg^{-1}).

Time after inj. (min)		PGE_2-LM (ng g^{-1})		Water absorbed (μl g^{-1} in 20 min)	
		Control	Δ^9-THC	Control	Δ^9-THC
Ileum	30	39 ± 12	28 ± 5	444 ± 48	622 ± 43*
	60	25 ± 7	34 ± 8		
	120	49 ± 8	56 ± 5		
Jejunum	30	1·2 ± 0·2	0·6 ± 0·1*	75 ± 37	68 ± 61

Values are expressed as the mean ± s.e.m. (n = 5).
*$P < 0.05$ compared with control (Student's t-test).

the statistical significance of the difference between two means was determined using the Student's unpaired t-test.

Drugs. The drugs used were: amylobarbitone sodium (Eli Lilly); atropine sulphate (Sigma); indomethacin (Merck, Sharp & Dohme); mepyramine maleate (May & Baker); methohexitone sodium (Eli Lilly); methysergide hydrogen maleate (Sandoz); pentobarbitone sodium (Abbott); phenoxybenzamine HCl (Smith, Kline & French); propranolol HCl (ICI); prostaglandin E_2 (PGE_2, Upjohn) and Δ^9-THC (National Institute of Drug Abuse, U.S.A.).

Results

Administration of Δ^9-THC, 2 mg kg^{-1} intravenously, produced characteristic changes in the behaviour of rats. These changes consisted of pronounced catatonia, splayed rear legs, excitation followed by sedation, and vocalization to touch. The presence and severity of sedation or catatonia were determined in groups of animals (n = 6) other than those used for prostaglandin determinations. The behaviours were subjectively scored by one of us who was unaware of each animal's pretreatment. The catatonia was greatest 30 min after Δ^9-THC administration, and sedation was greatest at 60 min. By 120 min, the catatonia had almost disappeared, but sedation persisted.

Δ^9-THC did not significantly alter the amount of PGE_2-like material extracted from the ileum 30, 60 or 120 min after Δ^9-THC administration (Table 1). At the time of peak catatonia (30 min after Δ^9-THC administration), less PGE_2-like material was extracted from the jejunum.

At 30 min after injection, Δ^9-THC had a different effect on net water transport in different regions of the small intestine (Table 1), Δ^9-THC enhanced net water absorption in the ileum, but not in the jejunum.

Discussion

The average content of PGE_2-like material found is similar to radioimmunoassay estimates (Fitzpatrick & Wynalda 1976) and to bioassayed amounts from human ileal mucosa (Bennett et al 1977). Although compared with the ileum the jejunum releases more PGE-like material on stimulation (Collier 1974) and is more sensitive to its anti-absorptive effect (Coupar 1978; Bunce & Spraggs 1982), the basal content is lower in the jejunum than in the ileum. This suggests that release into the lumen may be a more important index of function than tissue content.

Δ^9-THC at a dose which causes marked behavioural effects including catatonia and sedation, reduced the jejunal content of PGE_2-like material. This result confirms earlier in-vitro findings that Δ^9-THC can inhibit prostaglandin synthesis (Burstein & Raz 1972; Burstein et al 1973). Similarly, Δ^9-THC reduces the content of PGE_2-like material extracted from rat hypothalamus (Coupar & Taylor 1982). However, Fairbairn & Pickens (1979, 1980) have suggested that the cataleptic and sedative effects of Δ^9-THC depend on PGE_2 synthesis in the gut. This implies that the central actions of Δ^9-THC depend on an intermediate released from the gut under the influence of PGE_2. Such a mechanism in the jejunum is unlikely since at 30 min after Δ^9-THC administration, the yield of PGE_2-like material from the jejunum was less than in controls. However, Δ^9-THC may act this way in the ileum where the level of PGE_2-like material was not depressed by Δ^9-THC or in another region of the gut not investigated.

The Δ^9-THC-induced reduction of jejunal PGE_2-like material was not associated with enhanced net water absorption. This was expected since E-type prostaglandins are more important modulators of intestinal fluid transport in certain pathological states than under normal physiological conditions (Rask-Madsen & Bukhave 1979; Lee & Coupar 1980).

Δ^9-THC inhibits defaecation in rats and this action has been attributed to its tranquillizing effect (Masur et al 1971). The present results show that Δ^9-THC enhances net water absorption from the ileum, which is the major water-absorbing region of the small intestine. Since this effect is not associated with a reduced content of PGE_2-like material, we suggest that Δ^9-THC increases ileal water absorption, and that the reduced hydration of the colonic contents may contribute to inhibition of defaecation.

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Anti-allergic properties of pirquinozol (SQ 13,847) an orally effective agent. Evaluation in an anti-IgE-induced pulmonary function model in rats

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It has been reported previously that pirquinozol (SQ 13,847) is an orally effective anti-allergic agent as demonstrated in animal model systems (Free et al 1979; Casey et al 1980). Further, pirquinozol is a prodrug which requires conversion to the oxidative metabolite, SQ 12,903, for maximum expression of activity both in-vivo and in-vitro. In-vitro, SQ 12,903 inhibits histamine release from rat mast cells in a manner similar to that observed for disodium cromoglycate (DSCG) and doxantrazole (Free & Hall 1980). Prophylactically administered pirquinozol inhibits immunologically-induced bronchospasm in rats passively sensitized with whole rat serum containing IgE anti-egg albumin, as measured by changes in both airway conductance and dynamic compliance (Casey et al 1980).

We have reported the development of a model of reversed active lung anaphylaxis induced in rats by intravenous challenge with an anti-serum prepared against rat IgE myeloma protein (Casey & Abboa-Offei 1979). The technical advantages of this system include the lack of a necessary presensitization of the test animals and the ease of anaphylactic challenge; these changes resulted in increased consistency of responsiveness of the subject animals. Pharmacologically, the major advantage was the ability to observe duration of drug action, similar to that reported in the clinic for disodium cromoglycate (Kolotkin et al 1974; Orr 1974).

The results reported here confirm and extend the originally reported inhibition of bronchoconstriction by pirquinozol in an alternative rat model. Furthermore, the duration of action and time to peak efficacy have been determined at various doses.

Materials and methods

Anti-IgE-induced bronchoconstriction. The procedure for the reversed active lung anaphylaxis in rats has been prevously described (Casey & Abboa-Offei 1979). Briefly, antiserum against the purified rat IgE myeloma protein was prepared by immunization of rabbits and was quantitated by its ability to inhibit the induction of a passive cutaneous anaphylactic (PCA) reaction in rat skin. The highest dilution of rabbit anti-rat IgE antiserum capable of completely inhibiting the PCA reaction was considered the end point. The anti-rat IgE antiserum used in this investigation completely inhibited the rat PCA reaction at a dilution of 1/800 and is, therefore, defined as containing 800 PCA inhibition units ml^{-1}. In the studies reported here, rats were challenged with 40 units of anti-IgE antiserum. When normal rabbit serum was substituted for anti-IgE antiserum, there was no effect on pulmonary function.

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