Br. J. Pharmac. (1982), **76,** 115–119

ALTERATION IN THE LEVEL OF ENDOGENOUS HYPOTHALAMIC PROSTAGLANDINS INDUCED BY Δ^9-TETRAHYDROCANNABINOL IN THE RAT

IAN M. COUPAR & DAVID A. TAYLOR

School of Pharmacology, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria 3052, Australia

1 Whole brain and regional brain levels of prostaglandin E_2 (PGE_2)-like material have been determined following administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) in rats.

2 Intravenous administration of Δ^9-THC 2 mg/kg, resulted in marked behavioural changes and hypothermia. The behavioural changes consisted mainly of catatonia (most apparent at 30 min after administration of Δ^9-THC), followed by sedation (most evident at 60 min). Hypothermia was marked from 30 min after administration of Δ^9-THC.

3 Δ^9-THC did not alter the whole brain levels of PGE_2-like material 30, 60 or 120 min after administration.

4 Δ^9-THC did not alter the levels of PGE_2-like material in the medulla oblongata/pons, midbrain, cortex and cerebellum, 30 min after administration. However, there was a significant reduction of PGE_2-like material in the hypothalamus, 30 min after Δ^9-THC.

5 It is suggested that the Δ^9-THC-induced decrease in hypothalamic PGE_2-like material may contribute to the hypothermia observed following Δ^9-THC administration.

Introduction

Recently it has been suggested that the sedative and cataleptic effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) depend on the availability of prostaglandin E_2 (PGE_2) (Fairbairn & Pickens, 1979; 1980; Pickens, 1981). The suggestion that Δ^9-THC mediates its sedative and cataleptic effects through PGE_2 is supported by the fact that PGE_2 itself causes sedation and catalepsy in mice, rats and cats (Horton, 1964; Poddubiuk, 1976; Pickens, 1981).

PGE_2, apart from causing sedation and catalepsy, also produces hyperthermia in several species (Clark & Clark, 1980) and an E-type prostaglandin is released in the central nervous system during pyrogen-induced fever (Feldberg & Gupta, 1973). The site of the prostaglandin release and action is presumed to be the hypothalamic region of the brain (Ferreira, 1977).

Δ^9-THC produces sedation, catatonia and hypothermia. From the work of Fairbairn & Pickens (1980), Δ^9-THC would be expected to increase prostaglandin levels in the brain, but this should be associated with hyperthermia. Because of the apparent paradoxical effects of Δ^9-THC, based on indirect evidence, we decided to measure the whole brain and regional brain levels of prostaglandins of the rat following Δ^9-THC. The dose of Δ^9-THC administered was chosen because it produced sedation, catatonia and hypothermia.

Methods

Animals

Male albino Wistar rats weighing 220 to 280 g were used. Food and water were available *ad libitum*. For intravenous administration, rats were surgically prepared by insertion of permanent polyethylene (PE 10) catheters into the external jugular vein under amylobarbitone and methohexitone anaesthesia. For the 48 h recovery period, following surgery and the experimental period, the animals were kept in individual cages. The rooms in which the animals were housed and the experiments conducted were maintained at an ambient temperature of 20 to 23°C with a 12 h light-dark cycle.

Preparation of Δ^9-tetrahydrocannabinol

Δ^9-THC was suspended in saline (0.9% w/v NaCl solution) by use of polyvinylpyrrolidone (PVP) following the method of Fenimore & Loy (1971). Δ^9-THC suspension or PVP in saline, as control, were injected intravenously in volumes of 1 ml/kg body weight.

Extraction and determination of prostaglandin-like material

The rats were killed by decapitation at 30, 60 and

0007-1188/82/050115–05 $01.00

120 min after the injection of Δ^9-THC or PVP. Their brains were rapidly removed, blotted free of blood, frozen in liquid N_2 within 45 s and then stored at −20°C. In another experiment, the brains of animals injected with Δ^9-THC or PVP 30 min before decapitation were dissected on an ice-chilled petri dish into 5 regional areas according to the method of Glowinski & Iversen (1966) before being frozen (within 2.5 min). The areas were the cerebral cortex, hypothalamus, cerebellum, medulla oblongata and pons, and the midbrain including the hippocampus and thalamus. Brain regions from 3 rats were combined for determination of prostaglandin-like material.

The frozen whole brains or brain regions were weighed and allowed to thaw in 10 ml of an ice cold 1:1 mixture of ethanol and saline. The tissues were homogenized with an Ultra Turrax or a Polytron homogenizer and the homogenate centrifuged at 12000 *g*, at 4°C, for 20 min. Prostaglandin-like material was extracted by a modification of a previously published method (Unger, Stamford & Bennett, 1971; Bennett, Stamford & Stockley, 1977). Neutral fats were removed from the supernatant by twice washing with two volumes of petroleum spirit (40–60°C boiling point). 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 evaporated off under reduced pressure and any formic acid that may have been carried over in the extraction procedure was removed by exposing the residue to a stream of dry N_2. The extract was dissolved in a minimum volume of Krebs-Henseleit solution. Prostaglandin-like activity was bioassayed against PGE_2 on the rat gastric fundus strip by a method similar to that described by Ferreira & De-Souza Costa (1976). The strips were suspended in liquid paraffin and superfused down their serosal surface with Krebs-Henseleit solution containing a mixture of antagonists (atropine, mepyramine, phenoxybenzamine 0.1 μg/ml; methysergide, propranolol 0.2 μg/ml and indomethacin 2 μg/ml). The Krebs-Henseleit solution was delivered via polyethylene (PE 10) tubing which was connected both to the tissue and to a strain gauge. Tissue responses were recorded using a Grass polygraph (model 79 C). The amount of prostaglandin-like material determined was expressed as PGE_2 equivalents per g of brain tissue.

Body temperature studies

The body temperature of unrestrained rats was recorded continuously by means of thermistor probes inserted 6 cm into the rectum and held in place, relative to the tail, by thin pieces of adhesive tape. The probes were inserted 1 h before administration of PVP or Δ^9-THC and remained in place for a further 2 h.

The statistical significance of differences between means was determined by Student's *t* test.

Drugs

The following drugs were used: amylobarbitone sodium (Eli Lilly); atropine sulphate (Sigma); indomethacin (Merck, Sharp & Dohme); mepyramine maleate (May & Baker); methohexitone sodium (Eli Lilly); methysergide hydrogen maleinate (Sandoz); 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

Behaviour and body temperature

Administration of Δ^9-THC, 2 mg/kg intravenously, produced characteristic changes in the behaviour of rats. The behavioural 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 in body temperature and brain prostaglandin level experiments. The behaviours were subjectively scored by one of us who was unaware of each animal's pretreatment. The catatonia was most significant 30 min after Δ^9-THC administration. The greatest level of sedation was observed at 60 min. By 120 min, the catatonia had all but disappeared although sedation persisted.

The body temperature of rats injected with Δ^9-THC fell during the 30 min following injection (Figure 1). The Δ^9-THC-induced hypothermia reached a maximum 45 to 60 min after administration and although the body temperature appeared to be returning to control level, the hypothermia was present at 120 min.

Brain prostaglandin-like material

The whole brain levels of prostaglandin-like material are tabulated in Table 1. The level of prostaglandin-like material of PVP-treated animals did not alter significantly during the 2 h period. Also, Δ^9-THC did not alter the whole-brain levels of prostaglandin-like material at 30, 60 or 120 min after administration.

The regional brain levels of prostaglandin-like material in control PVP-treated rats varied from 3.3 to 12.0 ng PGE_2 equivalents/g. The cerebellum con-

tained the lowest concentration of prostaglandin-like material, whereas the medulla oblongata/pons area had the highest concentration determined (Table 2). Δ^9-THC did not alter the levels of prostaglandin-like material in any of the brain regions except in the hypothalamus. Thirty min after drug administration the level of prostaglandin-like material determined in the hypothalamus of Δ^9-THC-treated rats was reduced to 67% of that determined in PVP-treated animals.

Discussion

The results of the present study show that administration of a dose of Δ^9-THC (2 mg/kg, intravenously) that produced marked behavioural changes, including sedation and catatonia, and hypothermia, did not alter the whole brain levels of prostaglandin-like material in rats. However, 30 min after administration of Δ^9-THC, the hypothalamic level of prostaglandin-like material was reduced.

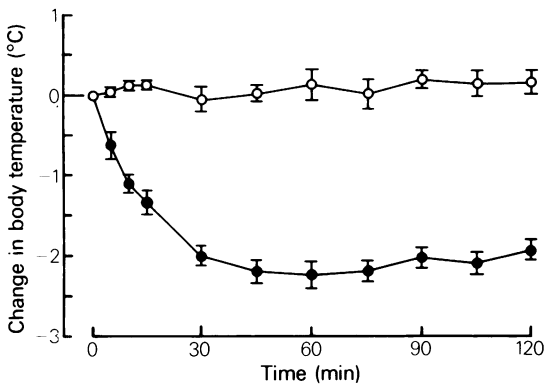

Figure 1 Effect of Δ^9-tetrahydrocannabinol (Δ^9-THC, 2 mg/kg; ●) and polyvinylpyrrolidone (PVP), as control (40 mg/kg; ○) on change in body temperature over a 2 h period following intravenous administration. Each point is the mean value from 6 rats; vertical lines show s.e.mean.

Table 1 Whole brain levels of prostaglandin-like material 30, 60 and 120 min after intravenous administration of polyvinylpyrrolidone alone (PVP, 40 mg/kg) or Δ^9-tetrahydrocannabinol in the PVP vehicle (Δ^9-THC, 2 mg/kg)

	Prostaglandin E_2 (equivalents ng/g)	
Time (min)	*PVP*	*Δ^9-THC*
30	4.72 ± 0.50	5.53 ± 0.55
60	4.63 ± 0.16	5.55 ± 0.62
120	4.47 ± 0.32	4.15 ± 0.10

Each value represents the mean concentration (ng/g) ± s.e.mean of PGE_2 equivalents from 5 determinations.

The regional distribution of prostaglandin-like material in the rat brain is not as varied as that seen for more traditional neurotransmitters. For example, noradrenaline (Glowinski & Iversen, 1966) and histamine (Lewis, Fennessy, Laska & Taylor, 1980) have up to a 10 fold difference in the levels determined in the hypothalamus compared to the levels determined in the cerebellum. The wide distribution of prostaglandins without localized concentrations was first reported to occur in dog brain (Holmes & Horton, 1968). This is in keeping with the view that prostaglandins in the brain serve a function as neuromodulators rather than as neurotransmitters.

It is probable that PGE_2 is the major contributor to the prostaglandin-like activity determined. The

Table 2 Regional brain levels of prostaglandin-like material 30 min after intravenous administration of polyvinylpyrrolidone alone (PVP, 40 mg/kg) or Δ^9-tetrahydrocannabinol (Δ^9-THC, 2 mg/kg) in PVP

	Prostaglandin E_2 (equivalents ng/g)		
Brain region	*PVP*	*Δ^9-THC*	*% Control*
Medulla oblongata/Pons	12.01 ± 0.70	11.07 ± 0.64	92.2 ± 7.6
Midbrain	9.68 ± 0.95	9.33 ± 0.25	96.4 ± 10.1
Hypothalamus	8.06 ± 1.11	5.44 ± 0.22*	67.5 ± 14.0*
Cortex	5.30 ± 0.47	5.48 ± 0.22	103.4 ± 10.0
Cerebellum	3.31 ± 0.09	3.57 ± 0.17	107.8 ± 6.1
Whole Brain	6.94 ± 0.26	6.73 ± 0.14	97.1 ± 4.3

Each value represents the mean concentration (ng/g) ± s.e.mean of PGE_2 equivalents from 5 determinations.
The % control is the level determined in Δ^9-THC-treated animals expressed as a percentage of the level determined in PVP-treated animals ± relative s.e.
*$P < 0.05$, compared with PVP-treated control (Student's t test)

reasons for this opinion are that PGE_2 and $F_{2\alpha}$ are the most abundant in the brain of several species (Horton & Main, 1967; Holmes & Horton, 1968; Fumagalli, Folco & Longiave, 1977) and PGE_2 is 17.5 times more potent in producing a contraction of the rat fundus strip than $PGF_{2\alpha}$ (Bergstrom, Carlson & Weeks, 1968). The relatively low levels of PGE_2-like material determined in this study, may indicate the basal levels at death, since the whole brains were frozen within 45 s of decapitation and the brain regions were dissected on an ice-chilled petri dish. Although prostaglandins are released when cells die, the estimates of cortical PGE_2-material obtained in this study are actually lower than rat cortical levels reported following focused microwave irradiation (approximately half) and considerably lower than levels in tissue slice preparations (approximately one tenth; Fumagalli *et al.*, 1977).

From the work of Fairbairn & Pickens (1979; 1980), it was expected that administration of Δ^9-THC would increase the level of brain prostaglandins. However, the results of the present study indicate that the whole brain levels of PGE_2-like material do not alter, but the hypothalamic levels were actually reduced following Δ^9-THC administration. The indirect methods of Fairbairn & Pickens (1979; 1980) suggested that Δ^9-THC increased the availability of arachidonic acid, possibly from the gut, with a subsequent increase in the level of prostaglandins. They postulated that PGE_2 was involved with the catatonia produced by Δ^9-THC. The results of the direct methods employed in the present study do not support the suggestions that Δ^9-THC increases the level of PGE_2-like material. However, this does not exclude the possibility that prostaglandins are involved in the catatonia produced by Δ^9-THC. For instance, it is possible that an increase in the level of prostaglandins induced by Δ^9-THC may occur in a more discrete region of the brain than was dissected in the present study. The observation that Δ^9-THC reduced the level of PGE_2-like material in the hypothalamus is evidence that Δ^9-THC may have differential effects on the levels of prostaglandins in various brain regions. Another factor that may complicate investigation of the action of Δ^9-THC on the level of prostaglandins is the variable effect of Δ^9-THC on prostaglandin synthesis. Δ^9-THC has been reported to increase the availability of arachidonic acid and the synthesis of PGE_2 and $F_{2\alpha}$ (Burstein & Hunter, 1978; White & Tansik, 1980). On the other hand, there are reports that Δ^9-THC inhibits prostaglandin synthesis (Burstein & Raz, 1972; Burstein, Levin & Varanelli, 1973).

Prostaglandins of the E series have been implicated in fever, and it has been proposed that the antipyretic action of aspirin, indomethacin and paracetamol is due to inhibition of prostaglandin synthesis (Milton & Wendlandt, 1971; Vane, 1971; Feldberg & Gupta, 1973). Δ^9-THC has also been shown to have antipyretic activity (Paton, Pertwee & Temple, 1972). The antipyretic agents appear to act by inhibiting the formation of prostaglandins in response to a pyrogen. The observation that other prostaglandin synthesis inhibitors do not produce hypothermia, whereas Δ^9-THC does, may be explained by the multitude of actions of Δ^9-THC on various neurotransmitters. Previously, the effect of Δ^9-THC on body temperature has been associated with changes in the tryptaminergic (Taylor & Fennessy, 1977; Fennessy & Taylor, 1978) and the noradrenergic (Singh & Das, 1976) systems. It is our hypothesis that Δ^9-THC possesses hypothermic activity by virtue of its action on different neurotransmitters. The decrease in the level of hypothalamic prostaglandins induced by Δ^9-THC may not be sufficient to produce hypothermia by itself but may be contributory to this effect.

To examine this hypothesis further, it may be of interest to determine the effect of prostaglandin synthesis inhibitors and inactive cannabinoids on the brain levels of prostaglandins. However, it is our hypothesis that the Δ^9-THC-induced hypothermia is the result of actions on a number of neurotransmitters, such as noradrenaline and 5-hydroxytryptamine, affected uniquely by Δ^9-THC.

We wish to thank the National Institute of Drug Abuse (U.S.A.) for the supply of Δ^9-THC; Upjohn for PGE_2 and other Drug Companies mentioned in the Methods section for their generous gifts. This work was partly supported by The National Health & Medical Research Council of Australia.

References

BENNETT, A., STAMFORD, I.F. & STOCKLEY, H.L. (1977). Estimation and characterization of prostaglandins in the human gastrointestinal tract. *Br. J. Pharmac.*, **61**, 579–586.

BERGSTRÖM, S., CARLSON, L.A. & WEEKS, J.R. (1968). The prostaglandins: a family of biologically active lipids. *Pharmac. Rev.*, **20**, 1–48.

BURSTEIN, S. & HUNTER, S.A. (1978). Prostaglandins and cannabis -VI: Release of arachidonic acid from HeLa cells by Δ^1-tetrahydrocannabinol and other cannabinoids. *Biochem. Pharmac.*, **27**, 1275–1280.

BURSTEIN, S., LEVIN, E. & VARANELLI, C. (1973). Prostaglandins and cannabis — II: Inhibition of biosynthesis by the naturally occurring cannabinoids. *Biochem. Pharmac.*, **22**, 2905–2910.

BURSTEIN, S. & RAZ, A. (1972). Inhibition of prostaglandin

E_2 biosynthesis by Δ'-tetrahydrocannabinol. *Prostaglandins*, **2,** 369–374.

CLARK, W.G. & CLARK, Y.L. (1980). Changes in body temperature after administration of acetylcholine, histamine, morphine, prostaglandins and related agents. *Neurosci, Biochem. Rev.*, **4,** 175–240.

FAIRBAIRN, J.W. & PICKENS, J.T. (1979). The oral activity of Δ^1-tetrahydrocannabinol and its dependence on prostaglandin E_2. *Br. J. Pharmac.*, **67,** 379–385.

FAIRBAIRN, J.W. & PICKENS, J.T. (1980). The effect of conditions influencing endogenous prostaglandins on the activity of Δ'-tetrahydrocannabinol in mice. *Br. J. Pharmac.*, **69,** 491–493.

FELDBERG, W. & GUPTA, K.P. (1973). Pyrogen fever and prostaglandin-like activity in cerebrospinal fluid. *J. Physiol.*, **228,** 41–53.

FENIMORE, D.C & LOY, P.R. (1971). Injectible dispersion of Δ^9-tetrahydrocannabinol in saline using polyvinylpyrrolidone. *J. Pharm. Pharmac.*, **23,** 310.

FENNESSY, M.R. & TAYLOR, D.A. (1978). Antagonism of the effects on thermoregulation of Δ^9-tetrahydrocannabinol by clomipramine in the rat. *Br. J. Pharmac.*, **63,** 267–273.

FERREIRA, S.H. (1977). Inflammatory pain and fever. In *Prostaglandins and Thromboxanes.* ed. Berti, F., Samuelsson, B. & Velo, G.P. pp. 433–442. New York & London: Plenum Press.

FERREIRA, S.H. & DeSOUZA COSTA, F. (1976). A lamina flow superfusion technique with much increased sensitivity for the detection of smooth muscle-stimulating substances. *Eur. J. Pharmac.*, **39,** 379–381.

FUMAGALLI, R., FOLCO, G.C. & LONGIAVE, D. (1977). Influence of prostaglandins on central functions. In *Prostaglandins and Thromboxanes.* ed. Berti, F., Samuelsson, B. & Velo, G.P. pp. 383–421. New York & London: Plenum Press.

GLOWINSKI, J. & IVERSEN, L.L. (1966). Regional studies of catecholamines in the rat brain -I: The disposition of (^{3}H)-norepinephrine, (^{3}H)-dopamine and (^{3}H)-dopa in various regions of the brain. *J. Neurochem.*, **13,** 655–669.

HOLMES, S.W. & HORTON, E.W. (1968). The identification of four prostaglandins in dog brain and their regional distribution in the central nervous system. *J. Physiol.*, **195,** 731–741.

HORTON, E.W. (1964). Actions of prostaglandins E_1, E_2 and E_3 on the central nervous system. *Br. J. Pharmac. Chemother.*, **22,** 189–192.

HORTON, E.W. & MAIN, I.H.M. (1967). Identification of prostaglandins in central nervous tissues of the cat and chicken. *Br. J. Pharmac. Chemother.*, **30,** 582–602.

LEWIS, S.J., FENNESSY, M.R., LASKA, F.J. & TAYLOR, D.A. (1980). A modified method for the isolation and determination of brain-histamine using Bio-Rex 70. *Agents & Actions*, **10,** 197–206.

MILTON, A.S. & WENDLANDT, S. (1971). Effect on body temperature of prostaglandins of the A, E and F series on injection into the ventricle of unanaesthetized cats and rabbits. *J. Physiol.*, **218,** 325–336.

PATON, W.D.M., PERTWEE, R.G. & TEMPLE, D. (1972). In *Cannabis and its Derivatives: Pharmacology and Experimental Psychology.* ed. Paton, W.D.M. & Crown, J. pp. 50–75. London: Oxford University Press.

PICKENS, J.T. (1981). Sedative activity of cannabis in relation to its Δ'-*trans*-tetrahydrocannabinol and cannabidiol content. *Br. J. Pharmac.*, **72,** 649–656.

PODDUBIUK, Z.M. (1976). A comparision of the central actions of prostaglandins A_1, E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in the rat. *Psychopharmacology*, **50,** 89–94.

SINGH, P.P. & DAS, P.K. (1976). Role of catecholamines in the hypothermic activity of cannabis in albino rats. *Psychopharmacology*, **50,** 199–204.

TAYLOR, D.A. & FENNESSY, M.R. (1977). Biphasic nature of the effects of Δ^9-tetrahydrocannabinol on body temperature and brain amines of the rat. *Eur. J. Pharmac.*, **46,** 93–99.

UNGER, W.G., STAMFORD, I.F. & BENNETT, A. (1971). Extraction of prostaglandins from human blood. *Nature*, **233,** 336–337.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231,** 232–235.

WHITE, H.L. & TANSIK, R.L. (1980). Effects of Δ^9-tetrahydrocannabinol and cannabidiol on phospholipase and other enzymes regulating arachidonate metabolism. *Prostaglandins & Medicine*, **4,** 409–417.

(Received July 8, 1981.
Revised January 5, 1982.)