Br. J. Pharmac. (1974), 52, 233-236

THE EFFECT OF Δ^1-TETRAHYDROCANNABINOL ON THE RELEASE OF [^{3}H]-(-)-NORADRENALINE FROM THE ISOLATED VAS DEFERENS OF THE RAT

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1 The amount of tritium released upon transmural stimulation of the isolated vas of the rat incubated with [^{3}H]-Δ^1-tetrahydrocannabinol (THC) (2.8 μM) was significantly greater than the non-stimulated overflow.

2 There was no difference between non-stimulated overflow and the effect of transmural stimulation of vasa equilibrated with [^{14}C]-sorbitol (6.5 mg/ml).

3 The difference between non-stimulated and stimulated efflux from vasa equilibrated with [^{3}H]-Δ^1-THC was abolished in rats pretreated for 24 h with 6-hydroxydopamine (250 mg/kg).

4 The amount of tritium released upon transmural stimulation of the vas incubated with [^{3}H]-noradrenaline (6 μM) was significantly greater than the non-stimulated overflow.

5 Δ^1-THC (560 nM and 14 μM) caused a significant dose-dependent reduction of both non-stimulated and stimulated tritium efflux, especially the latter.

Introduction

Δ^1-(*trans*)-Tetrahydrocannabinol (Δ^1-THC) has been shown to be the major psychoactive constituent of cannabis in man (Hollister, Richards & Gillespie, 1968; Mechoulam, Shani, Edery & Grunfeld, 1970). Intravenous administration of Δ^1-THC to anaesthetized rats and cats caused hypotension, bradycardia and potentiation of the pressor response to injected noradrenaline (NA); reflex vasoconstriction in the hindlimbs of cats in response to bilateral carotid occlusion was reduced (Graham & Li, 1973). The mechanisms by which these actions of Δ^1-THC were mediated have not been fully elucidated although it has been reported that sympathetic efferent activity is depressed following administration of this drug to cats (Oskoui, 1972; Vollmer, Cavero, Ertel, Solomon & Buckley, 1974). In the present study we investigated the accumulation of [^{3}H]-Δ^1-THC, [^{14}C]-sorbitol, and [^{3}H]-NA by the isolated vas of the rat and the changes in efflux during non-stimulated overflow and transmural stimulation. The effect of pretreatment of rats with 6-hydroxydopamine (6-OHDA) on [^{3}H]-Δ^1-THC was also examined.

Methods

Male Wistar rats of 100-200 g weight were killed by cervical dislocation, exsanguinated, the vasa removed, stripped and immersed in cold saline solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, NaH_2PO_4 1.0, KCl 5, $CaCl_2$ 2.5, glucose 11 and sucrose 10 (M.E. Holman, personal communication). The tissues were then blotted, weighed and incubated in Holman solution at 37°C, gassed with 5% CO_2 in O_2 and containing one of the following: (1) [^{3}H]-Δ^1-THC (2.8 μM) in Tween 80 (25 μg/ml). (A further six rats were injected with 6-OHDA (250 mg/kg i.v.) 24 h before incubation of the vasa with [^{3}H]-Δ^1-THC). (2) [^{14}C]-sorbitol (2.6 μg/ml) in carrier sorbitol to 6.5 mg/ml. (3) [^{3}H]-NA (6 μM) with ascorbic acid (20 μg/ml) and disodium edetate (10 μg/ml). Eighteen rats (36 vasa) were used for each compound. The vasa were then mounted in 5 ml Perspex organ baths between longitudinal platinum electrodes of 2 cm length and allowed to equilibrate at a tension of 0.3 g. The bath fluid was changed every 5 minutes. After 40 min the experiment began by transfer of 0.5 ml from each 5 ml bath to counting vials; the bath fluid was changed and field stimulation applied after 30 s (biphasic pulses, 150 V, 25 Hz, 1 ms for 10 s) by a Scientific Research Instruments Ltd, stimulator. Contractions were recorded with a force displacement strain gauge and recorded on a Devices Ltd, polygraph. These stimulation parameters had been found to be of supramaximal strength in preliminary experiments with vasa

from rats of the same colony and of similar body weight. After 5 min samples were collected as before, the bath was changed and 5 min periods of quiescence were alternated with 5 min periods containing 10 s stimulation, repeated four times. Scintillation medium (10 ml) consisting of 5 g of 2,5-diphenyloxazole and 0.5 g of 1,4-di(2-(5-phenyloxazolyl)) benzene in 1 litre toluene and 500 ml of Triton X-100, was added to each vial and the activity counted in a SL30 Intertechnique liquid scintillation counter, efficiency 25% for tritium and 80% for ^{14}C. Additionally an experiment was done on the vasa of three sets of nine rats incubated with [^{3}H]-NA as above, and then equilibrated in bathing fluid containing Tween 80 25 μg/ml; Δ^1-THC was also present in two sets in concentrations of 560 nM and 14 μM. The ct/min was converted to d/min and divided by the weight of the vas. Results are expressed as d min^{-1} mg^{-1} tissue.

Materials

The Δ^1-THC used in this study was imported by Digby Chemicals Co. from Makor Chemicals Ltd, Israel. It was suspended in 1% solution of Tween 80 in saline (0.9% w/v NaCl solution) in a concentration of 2 mg/ml. Before use it was added in appropriate amount (in terms of THC) to Holman solution containing Tween 80 (25 μg/ml) which therefore remained approximately constant. The [^{3}H]-Δ^1-THC was supplied by the Medical Research Council from N.I.M.H., D.H.E.W. (sample 1480-75-1), specific activity 109 μCi/mg, and a concentration of 1 mg/ml in 95% ethanol. It was diluted in Holman solution without Tween 80. Other drugs included (−)-[7-^{3}H]-NA, specific activity 33 mCi/mg and D-sorbitol-[1-^{14}C], specific activity 20 mCi/mg, both supplied by Radiochemical Centre, Amersham, England; and 6-OHDA (Koch-Light).

Results

Release of [^{3}H]-Δ^1-tetrahydrocannabinol

The spontaneous overflow of ^{3}H from the vasa incubated with [^{3}H]-Δ^1-THC and the effect of transmural stimulation is shown in Fig. 1 (upper line). No attempt was made to examine the proportions of parent compound and metabolites, but the amount of tritium released by stimulation was significantly greater ($P < 0.05$) than the mean level during non-stimulated overflow. This difference was abolished when rats were pretreated with 6-OHDA (Fig. 1, lower line), in an amount which reduced the contractions caused by transmural

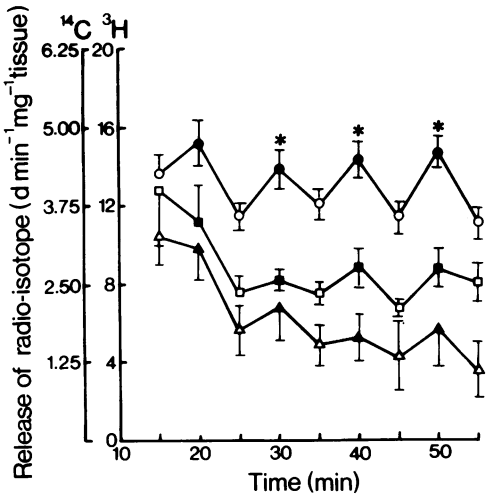

Figure 1 Overflow of isotope from rat isolated vas deferens incubated for 30 min with: (1) [^{3}H]-Δ^1-tetrahydrocannabinol (THC) 2.8 μM (○); (2) [^{3}H]-Δ^1-THC as in (1) but rats were injected with 6-hydroxydopamine (250 mg/kg i.v.) 24 h previously (□); (3) [^{14}C]-sorbitol (2.6 μg/ml) in 6.5 mg/ml carrier (△). Non-stimulated, open symbols; stimulated, closed symbols. * denotes $P < 0.05$ measured between stimulus point (●) and the mean of the previous and succeeding levels of spontaneous overflow (○). Each point is the mean of 12 measurements; vertical bars indicate s.e. mean. Ordinates: d min^{-1} mg^{-1} tissue; abscissae: time after incubation finished (10 min equilibration).

stimulation to 75% of control values and which Furness, Campbell, Gillard, Malmfors, Cobb & Burnstock (1970) demonstrated effectively destroys adrenergic neurones. The efflux of radioactivity from vasa incubated in [^{14}C]-sorbitol, which is distributed extracellularly, revealed no difference between the non-stimulated and the stimulated preparation (Figure 1). This efflux was low but significantly higher than background. These results imply that Δ^1-THC is distributed in a different manner from sorbitol, and that 6-OHDA abolishes that difference.

Release of [^{3}H]-noradrenaline

Incubation in [^{3}H]-NA caused an accumulation of labelled material in the vas. The release of this was always significantly greater during stimulation than non-stimulation (Figure 2). Tween 80 in a concentration of 25 μg/ml had no significant effect on the d/min during either state. The effect of two concentrations of Δ^1-THC in 25 μg/ml of Tween 80 was to reduce the levels in the bath fluid

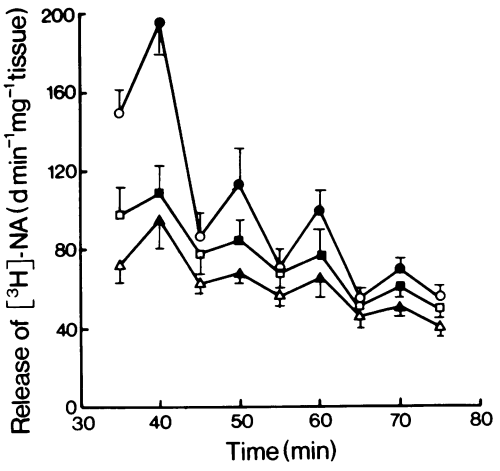

Fig. 2 Overflow of ^{3}H from rat isolated vas incubated for 30 min in [^{3}H]-(−)-noradrenaline (NA) 6 μM. Non-stimulated, open symbols; stimulated closed symbols. (○) Control [^{3}H]-NA levels; (□) Δ^1-tetrahydrocannabinol (THC) 560 nM; (△) Δ^1-THC 14 μM, both in Tween 80 (25 μg/ml). Each is the mean of 12 measurements with s.e. mean. Ordinates: d min^{-1} mg^{-1} tissue; abscissae: time after incubation finished (35 min equilibration).

during the stimulated and the non-stimulated states, the former to a greater extent than the latter (Figure 2). This occurred after incubation in [^{3}H]-NA and after equilibration, i.e. it could not be an effect on uptake of ^{3}H. On account of the apparent non-normality of the distributions, statistical tests involving ranks were used for analysis of probability. These data showed consistently that increasing concentrations of Δ^1-THC lowered levels of tritium in the bath and that this effect remained significant ($P < 0.04$) when the larger inter-unit variability was allowed for. This inhibition was concentration related and was true whether the peaks (stimulated efflux), the troughs (non-stimulated efflux) or both were considered. Statistical analysis also indicated that the amount of tritium released on stimulation was significantly greater than the non-stimulated level for all stimulation values and each stimulation period ($P < 0.000005$, sign test for median for controls), that the two measures in the control group were significantly larger than in the presence of Δ^1-THC ($P < 0.05$) when compared with inter-unit variability and that this change was concentration related (Figure 2).

Discussion

The present experiments showed that rat isolated vas which had been incubated with [^{3}H]-Δ^1-THC released significantly more tritium into the bath fluid in a given period of time when stimulated than not stimulated; and that pretreatment of the rats with 6-OHDA in a dose which destroys adrenergic nerves (Furness *et al.*, 1970) abolished this difference and caused the pattern of overflow to resemble that given by the extracellularly distributed substance, [^{14}C]-sorbitol. We conclude that the release of Δ^1-THC from the stimulated vas came at least in part from the adrenergic nerve. Langer (1970) showed that rat isolated vas deferens pre-incubated with [^{3}H]-NA and stimulated transmurally at supramaximal voltage, 4 Hz, for 1 ms released tritium. Fifty per cent of this was shown to exist as [^{3}H]-(−)-NA; as the frequency increased so did the proportion of [^{3}H]-NA. Relying on this careful report and in view of the fact that the frequency parameter in our experiment was 25 Hz it was surmised that the major portion of the efflux would be noradrenaline. In anaesthetized animals intravenous administration of Δ^1-THC or of extract of cannabis gave rise to systemic hypotension and bradycardia (Graham & Li, 1973). These haemodynamic effects probably resulted in part from diminished sympathetic outflow from the central nervous system (Vollmer *et al.*, 1974). Hypotension may also be in part due to a diminution in the spontaneous and stimulated efflux of transmitter from the peripheral adrenergic nerves, i.e. Δ^1-THC is an adrenergic neurone blocker.

We are grateful to Mr A. Richards, Department of Medical Physics, and Mr R. Newcombe, Department of Medical Statistics, Welsh National School of Medicine for their advice and assistance. This work was supported by the Medical Research Council of Great Britain: Δ^1-THC was supplied by N.I.M.H., U.S.A. via M.R.C., G.B.

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(Revised May 22, 1974)