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THE UPTAKE OF TRITIATED Δ^1-TETRAHYDROCANNABINOL BY THE ISOLATED VAS DEFERENS OF THE RAT

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1 Weighed stripped vasa deferentia were incubated in Holman's solution containing (a) [^{14}C]-sorbitol 0.014 mM, (b) [^{3}H]-noradrenaline ([^{3}H]-NA) 12.31 nM, (c) [^{3}H]-tetrahydrocannabinol ([^{3}H]-Δ^1-THC) 1 μg/ml for 5, 10, 20 and 30 minutes.

2 Tissues were washed, dissolved in Protosol, counted by standard scintillation counting technique and 'drug space' expressed as ct min^{-1} mg^{-1} tissue/ct min^{-1} μl^{-1} bathing fluid.

3 Vasa incubated for 30 min with [^{14}C]-sorbitol were washed for varying lengths of time; 82% clearance had taken place after 2 washes of 5 minutes.

4 The uptake of [^{3}H]-NA was inhibited by the presence of desmethylimipramine (DMI) 10 nM in the bath or by pretreatment of rats with 6-hydroxydopamine (6-OHDA).

5 The uptake of [^{3}H]-Δ^1-THC was not inhibited by the presence of DMI. It was reduced but not abolished by 6-OHDA pretreatment.

Introduction

Δ^1-(*trans*)-Tetrahydrocannabinol (Δ^1-THC) is generally held to be the major psychoactive principal of *Cannabis sativa* L. Previous work has shown that Δ^1-THC suspended in 4% Tween 80 caused hypotension in rats and cats and reduction of the reflex vasoconstriction in the hindlimbs which normally follows the systemic action of a vasodilator drug (Graham & Li, 1973). Analysis of the peripheral activity of Δ^1-THC has so far shown that Δ^1-THC inhibits the uptake of [^{3}H]-noradrenaline ([^{3}H]-NA) into the isolated heart of the rat (Graham, Lewis & Li, 1974a) and that it inhibits in a dose-related manner the release (efflux of ^{3}H) from the isolated vas deferens of the rat which has been equilibrated with [^{3}H]-NA and then electrically stimulated (Graham, Lewis & Li, 1974b). Moreover, if the rat vas was equilibrated with [^{3}H]-Δ^1-THC in Tween 80 and then washed, transmural stimulation released significantly more ^{3}H than during the non-stimulated state and pretreatment of the animals with 6-hydroxydopamine (6-OHDA) abolished this difference. The implication was that Δ^1-THC or a metabolite was released from the adrenergic neurone on appropriate stimulation. In order to complete the evidence about this process studies of the uptake of [^{3}H]-Δ^1-THC into rat vas have been undertaken. We do not extrapolate our findings from vas to brain, even as a hypothesis.

Methods

Uptake studies

Male Wistar rats weighing 100–120 g were killed by a blow on the head. Both vasa were removed and immersed in cold Holman's solution of the following composition (mM) NaCl 120, $NaHCO_3$ 25, NaH_2PO_4 1, KCl 5, $CaCl_2$ 2.5, glucose 11, sucrose 10, ascorbic acid 0.11, disodium edetate 0.04. Each vas was stripped of mesentery, blotted on filter paper and weighed. Pairs of vasa were incubated for 5, 10, 20 and 30 min in 2.5 ml of Holman's solution at 37°C oxygenated with 5% CO_2 in O_2 and containing one of the following: (a) [^{14}C]-sorbitol 0.014 mM in carrier sorbitol to 35.68 mM, (b) [^{3}H]-(–)-NA 12.31 nM in carrier NA to 2.96 μM, (c) [^{3}H]-(–)-NA 12.31 nM in carrier NA to 2.96 μM and 10% v/v ethanol or (d) [^{3}H]-Δ^1-THC 1 μg/ml and 10% v/v ethanol. The concentration of THC is expressed in μg/ml for lack of precise knowledge of the bioavailability of this drug. Incubations for 5–30 min in media (a), (b) and (d) were repeated with the addition of desmethylimipramine (DMI) in a concentration of 10 nM which was found by previous trial to be an optimally effective concentration for preventing the uptake of [^{3}HT-NA in the vas. Finally, the experiment was replicated at the 10 and 30 min incubation times for [^{3}H]-Δ^1-THC and at 30 min for [^{3}H]-NA and [^{14}C]-sorbitol on vasa

from rats pretreated with 6-OHDA according to the 14 day schedule of Thoenen & Tranzer (1968).

Sampling and counting

Following incubation, a 100 µl aliquot of the aqueous medium was taken and 10 ml of scintillation fluid containing Triton X-100 added. Vasa were blotted individually, dissolved in 1 ml Protosol solution at 54°C for 18 h and 10 ml scintillation fluid (without Triton X-100) added. All samples were counted by standard technique in an SL 30 liquid Scintillation Counter. The accumulation of each drug was expressed as ct min^{-1} mg^{-1} tissue/ct min^{-1} μl^{-1} medium and termed 'drug space'. Since quenching of samples and efficiency of counting did not vary significantly disintegrations per min were not calculated.

Clearance of [^{14}C]-sorbitol

After incubation of some vasa with [^{14}C]-sorbitol for 30 min the tissues were washed for 5–30 min in 50 ml of gassed Holman's solution at 37°C which was changed every 5 minutes. Based on the findings that 82% of [^{14}C]-sorbitol was cleared by two 5 min washes, this procedure was adopted in all experiments with [^{3}H]-NA and [^{3}H]-Δ^1-THC, in order to remove the majority of the NA and Δ^1-THC from the extracellular space. All results were calculated by ignoring the 18% of the drug remaining within the extracellular space.

Materials

Scintillation fluid: scintillation grade 2; 5-diphenyloxazole 5g; 1,4-di(2-(5-phenyloxazolyl) benzene 0.5 g; toluene 1 litre; Triton X-100. 500 ml was also added for aqueous samples. Protosol; New England Nuclear Chemical Co; desmethylimipramine, Geigy Pharmaceuticals; [^{3}H]-(–)-NA (Radiochemicals, Amersham) specific activity 48 mCi/mg; [^{3}H]-Δ^1-THC (N.I.M.H., D.H.E.W. samples 1480-75-1) specific activity 109 µCi/mg; [^{14}C]-D-sorbitol (Radiochemicals, Amersham) specific activity 9.3 mCi/mg.

Results

Uptake of [^{14}C]-sorbitol

Uptake of [^{14}C]-sorbitol increased with time, saturation occurring between 20 and 30 min (Table 1) where the sorbitol space increases in the time order $5 < 10 < 20$, each value being significantly different from its predecessor. Neither the presence of DMI 10 nM nor pretreatment of rats with 6-OHDA significantly affected the uptake pattern. Two wash periods of 5 min cleared 82% of the sorbitol from the extracellular space.

Uptake of [^{3}H]-noradrenaline

The uptake of [^{3}H]-NA into isolated rat vas is shown in Table 2. Inspection of a best fitting smooth curve applied to these data suggested that there was rapid accumulation during the initial 5 min followed by a slower phase, but saturation had not occurred at 30 minutes. The presence of 10% ethanol did not significantly alter the pattern of NA uptake. DMI 10 nM reduced uptake of [^{3}H]-NA at 30 min only ($P < 0.02$) but was not significantly effective at 5, 10 or 20 minutes. Pretreatment of rats with 6-OHDA also reduced the uptake of [^{3}H]-NA at 30 min ($P < 0.005$), the only time of incubation tested.

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

The accumulation curve of [^{3}H]-Δ^1-THC in rat isolated vas is shown in Figure 1. There was an initial rapid uptake followed by a slower phase. The amount of Δ^1-THC bound by the tissue was greater than that of NA at all incubation times. The presence of DMI in a concentration of 10 nM did not alter the accumulation curve. Pretreatment of rats with 6-OHDA

Table 1 Uptake of [^{14}C]-sorbitol by rat vas deferens

Time	Sorbitol 'space'	Sorbitol 'space' in the presence of DMI 10 nM	Sorbitol 'space' after pretreatment with 6-OHDA
5	0.347 ± 0.017 (5)	0.404 ± 0.024 (4)	—
10	0.497 ± 0.024 (5)	0.604 ± 0.470 (5)	—
20	0.848 ± 0.790 (5)	0.709 ± 0.073 (4)	0.794 ± 0.089 (4)

Sorbitol 'space' = ct min^{-1} mg^{-1} tissue/ct min^{-1} μl^{-1} medium. Each result is the mean of at least 4 observations (n) ± s.e. mean.
(Student's t test) There was no significant difference in the presence of desmethylimipramine (DMI) 10 nM or after pretreatment with 6-hydroxydopamine (6-OHDA) ($P > 0.2$ in all cases).

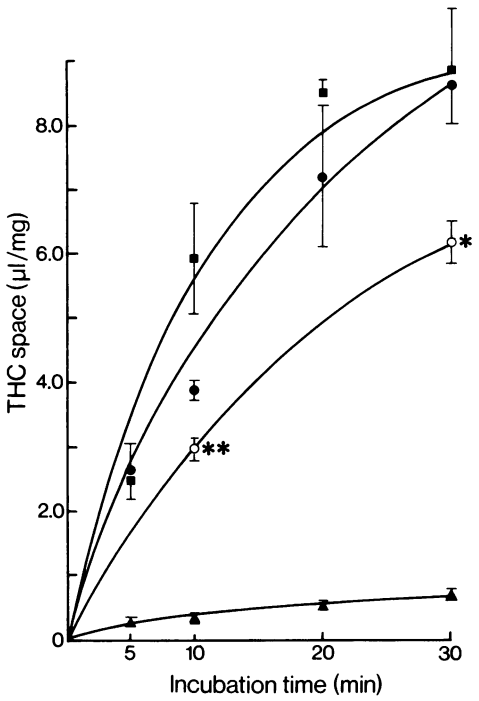

Figure 1 The accumulation of [^{3}H]-Δ^1-tetrahydrocannabinol (THC) in rat vas deferens *in vitro* (●) and the effect of 6-hydroxydopamine (6-OHDA) pretreatment (○) or of desmethylimipramine (DMI) 10 nM (■) on it. For comparison the accumulation of [^{3}H]-(−)-noradrenaline (NA) is also shown (▲). Abscissa scale: time of incubation in minutes. Ordinate scale—'drug space' = ct min^{-1} mg^{-1} tissue/ct min^{-1} µl^{-1} medium. Each result is the mean of at least 4 observations. DMI had no effect, while 6-OHDA reduced the accumulation of Δ^1-THC which was itself much greater than that of NA.
Student's *t* test, * = $P < 0.025$; ** = $P < 0.005$.

significantly reduced accumulation of Δ^1-THC at 10 min ($P < 0.005$) and 30 min ($P < 0.025$).

Discussion

If D-sorbitol is distributed extracellularly, the estimated values of 70–80% for 'sorbitol space' are large. However, these values were obtained consistently when vasa were incubated with [^{14}C]-sorbitol for 20 and 30 minutes. One explanation for the large extracellular volumes obtained may be that this carbohydrate is distributed to some extent intracellularly as well as extracellularly. This would appear unlikely for two reasons. First, rapid equilibration was obtained with sorbitol (20–30 min) and second, rapid clearance occurred after two 5 min wash periods. The vasa were not cut longitudinally for fear of extensive damage to the muscle and the variable volume of the lumen doubtless accounts for a proportion of the calculated sorbitol space.

The results show that this distribution is not significantly altered by the presence of DMI or pretreatment with 6-OHDA. In contrast, NA is actively accumulated by adrenergic neurones (Iversen, 1967) and this accumulation is significantly reduced by blocking uptake with DMI, or by pretreatment with 6-OHDA, a drug known to destroy adrenergic neurones (Thoenen & Tranzer, 1968).

Since the THC was in alcoholic solution, a control experiment was performed to observe the effect of alcohol on uptake of NA. No significant effect was found. It was assumed that the presence of 10% ethanol was unlikely to affect the accumulation of Δ^1-THC.

Previous work (Graham *et al.*, 1974b) showed that rat vas equilibrated with [^{3}H]-Δ^1-THC released labelled compound following transmural electrical stimulation and that the pretreatment of rats with 6-OHDA abolished this process; it was to be expected

Table 2 Uptake of [^{3}H]-noradrenaline (NA) by rat vas deferens

Time (min)	*NA 'space'*	*NA 'space' in presence of 10% ethanol*	*NA 'space' in presence of DMI 10 nM*	*NA 'space' after pretreatment with 6-OHDA*
5	0.326 ± 0.042 (4)	0.249 ± 0.027 (4)	0.187 ± 0.026 (4)	
10	0.327 ± 0.034 (5)	0.347 ± 0.078 (4)	0.397 ± 0.032 (4)	
20	0.532 ± 0.050 (5)	0.462 ± 0.033 (5)	0.467 ± 0.034 (5)	
30	0.753 ± 0.065 (4)	0.586 ± 0.062 (4)	0.510 ± 0.034 (4)	0.359 ± 0.012 (4)

NA 'space' = ct min^{-1} mg^{-1} tissue/ct min^{-1} µl^{-1} medium. Each result is the mean of at least 4 observations (*n*) ± s.e. mean.
Student's *t* test. There was no significant difference in the presence of 10% ethanol. Desmethylimipramine (DMI) 10 nM significantly reduced uptake at 30 min ($P < 0.02$). Pre-treatment with 6-hydroxydopamine (6-OHDA) also significantly reduced uptake at 30 min ($P < 0.005$).

that uptake of [^{3}H]-Δ^1-THC by rat vas should be demonstrable, and be reduced by 6-OHDA. This supposition is confirmed.

This route of accumulation of Δ^1-THC is unlikely to be the same as that for NA because the former was not reduced by DMI. The markedly greater affinity of THC for the tissue relative to NA implies other sites of binding for THC than are available for NA.

We are indebted for [^{3}H]-Δ^1-THC to N.I.D.A. and to the M.R.C. THC was held by one of us (J.D.P.G.) under H.O. licence no. L223.

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