THE EFFECT OF Δ^1-TETRAHYDROCANNABINOL ON THE UPTAKE OF [^{3}H]-(—)-NORADRENALINE BY THE ISOLATED PERFUSED HEART OF THE RAT

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Forty-eight isolated rat hearts were perfused with Krebs solution and with [^{3}H]-(−)-noradrenaline ([^{3}H]-NA) containing 4.5 nCi/ml in 50 ng/ml, as described by Iversen (1963). To groups of six, cocaine was added to [^{3}H]-NA in concentrations of 400 nM, 2 and 10 μM. To the rest Tween 80, 25 μg/ml was added to [^{3}H]-NA and in three groups Δ^1-THC additionally in concentrations of 560 nM and 2.8 or 14 μM. Cocaine caused a linear concentration-related inhibition of uptake; Tween caused a 38.7% inhibition; THC caused additionally a linear concentration-related inhibition.

Δ^1-Tetrahydrocannabinol (Δ^1-THC) is the major psychoactive constituent of *Cannabis sativa* (Mechoulam, Shani, Edery & Grunfeld, 1970). Intravenous administration of Δ^1-THC to anaesthetized rats, cats and dogs causes bradycardia, hypotension, potentiation of the pressor response to injected noradrenaline and inhibition of the reflex vasoconstriction in the hindlimb of the cat which follows bilateral carotid occlusion (Graham & Li, 1973). The mechanism of the peripheral actions has not been fully elucidated. This report is concerned with the effect of Δ^1-THC on the retention of [^{3}H]-noradrenaline by the perfused heart of the rat.

Methods The isolated hearts from 48 female Wistar rats (220-240 g wt) which had been stunned and bled were perfused for 20 min with modified Krebs solution as described by Iversen (1963), then with Krebs containing [^{3}H]-(−)-noradrenaline ([^{3}H]-NA) (Radio Chemical Centre, Amersham) diluted with non-radioactive (−)-NA to a final concentration of 4.5 nCi/ml and 50 ng/ml for 15 min and finally drug-free Krebs for 2.5 minutes. These concentrations and times were selected from Iversen (1963) as being likely to give rise to a moderate retention of isotope label on which drug effects may be demonstrated. Cocaine was added to the [^{3}H]-NA in concentrations of 400 nM and 2 or 10 μM in three groups of six hearts. In a further four groups of six, Tween 80 was added to [^{3}H]-NA in a concentration of 25 μg/ml and in three of these groups it contained Δ^1-THC in concentrations of 560 nM and 2.8 or 14 μM. The perfusate was measured and the isotope activity of the input and output measured in samples. This permitted an estimate of the efficiency of the counting method. After perfusion each heart was removed, blotted, and a sample excised from the wall of each chamber. Each sample was weighed, added to 1.0 ml Protosol solution (New England Nuclear Pilot Chemical Division) and incubated for 12 h at 50° C. To each vial when cool was added 10 ml of a solution of 2,5-diphenyloxazole 5 g and 1,4-di(2-(5-phenyloxazolyl) benzene 0.1 g in toluene 1 l; each sample was counted in a SL 30 Intertechnique Liquid Scintillation Counter, efficiency 25%. Activity was expressed as d/min per mg wet wt and the mean of the four samples as d/min per mg 'whole heart'. The effects of drugs are expressed as differences as a percentage of the uptake of [^{3}H]-NA for cocaine and Tween 80. The mean inhibition attributed to Tween 80 was subtracted from the percentage inhibitions recorded for [^{3}H]-NA-Tween-THC and the remainder attributed to Δ^1-THC, for each chamber wall (see Table 1).

Results As described by Iversen (1963) cocaine caused a concentration-related inhibition of the isotope-label recovered from the heart, 1.2 μM being 50% effective (see Table 1). Tween 80 also proved to be an active inhibitor, 38.7% inhibition occurring with a concentration of 25 μg/ml. Δ^1-THC dissolved in Tween 80 solution produced an additional inhibition which was linear, concentration-related and 87.3% effective at 14 μM (see Table 1) i.e. Δ^1-THC inhibited an additional 50% of the possible uptake at this concentration, and may be approximately one tenth as active as cocaine.

Discussion If one accepts that [^{3}H]-noradrenaline is taken into the heart from a perfusing fluid as Iversen (1963, 1967) and others report and that perfusion for 15 min with a concentration of 50 ng/ml neither saturates the uptake mechanism (see Fig. 2(a) Iversen, 1963) nor is likely to be substantially involved in non-specific intracellular uptake such as that described by Gillespie & Muir (1970); and if one accepts further that a washout period of 2.5 min is

Table 1 Percentage inhibition of the accumulation of [^{3}H]-(−)-noradrenaline by the myocardium of the several chambers of the rat heart and the 'whole heart' induced by cocaine, by Tween 80 (25 μg/ml) and by Δ^1-tetrahydrocannabinol (THC) in Tween 80

Drug	*Concentration*	*RA*	*LA*	*RV*	*LV*	*'Whole heart'*
Cocaine	400 nM	39.8 ± 15.2	5.3 ± 5.1	32.6 ± 7.3	28.7 ± 7.6	26.6 ± 6.9
	2 μM	52.0 ± 12.4	52.0 ± 10.9	63.5 ± 7.5	76.3 ± 4.2	61.0 ± 6.9
	10 μM	73.3 ± 3.5	74.9 ± 10.9	87.5 ± 2.8	87.3 ± 3.1	80.7 ± 4.9
Tween 80	25 μg/ml	34.2 ± 13.0	18.6 ± 9.3	50.3 ± 4.3	51.5 ± 8.4	38.7 ± 8.6
Δ^1-THC in Tween 80	560 nM	34.4 ± 4.4	36.3 ± 3.9	18.7 ± 4.1	15.3 ± 1.4	26.2 ± 5.4
less values for	2.8 μM	44.2 ± 4.8	59.9 ± 2.9	25.6 ± 2.1	23.3 ± 3.8	37.5 ± 7.9
Tween 80 alone	14 μM	59.2 ± 1.3	69.9 ± 3.8	35.4 ± 3.8	29.8 ± 4.8	48.6 ± 8.5

The figures are the means of 6 hearts with s.e. mean. R = Right, L = Left, A = Atrium, V = Ventricle and 'whole heart' is the mean of these.

sufficiently long to clear the major portion of extracellular label from a contracting perfused heart (Morgan, Henderson, Regen & Park, 1961) the present experiment shows that Tween 80 reduced by a substantial amount the accumulated d/min per mg wt of myocardium. It may not, therefore, be the best medium for this type of experiment. Nevertheless Δ^1-THC produced an additional concentration-related linear inhibition. The IC_{50} for cocaine on whole heart was 1.2 μM, for Δ^1-THC in Tween 80 14 μM, a potency ratio of 12. Drugs such as desmethylimipramine (Sigg, Soffer & Gyermek, 1963; Parsons, 1965) and cocaine (Bhagat, Bovell & Robinson, 1967) potentiate the pressor response to injected noradrenaline and inhibit the accumulation of [^{3}H]-NA in perfused heart. They are believed to block the uptake process in adrenergic nerves. Δ^1-THC which acts in a similar fashion may well do so also. Carmichael & Israel (1973) correlated the octanol : water partition coefficient of several psychoactive drugs with their IC_{50} for the inhibition of uptake of NA in isolated brain slices of mouse. The coefficient for Δ^1-THC is too high to measure (Gill, Paton & Pertwee, 1970). According to Carmichael & Israel's nomogram the IC_{50} for cocaine should be about 1 μM but that for Δ^1-THC 1000-fold less, not more.

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