EFFECTS OF SOME TETRAHYDROCANNABINOLS ON THE BIOSYNTHESIS AND UTILISATION OF CATECHOLAMINES IN THE RAT BRAIN

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Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC) is the active constituent of marijuana. It has variously been reported to accelerate (MAITRE *et al.*, 1970a; SCHILDKRAUT and EFRON, 1971), retard (TRUITT and ANDERSSON, 1971) or have no influence (LEONARD, 1971) on the turnover and metabolism of catecholamines in the rat brain. Other biochemical and histological investigations have indicated that Δ^9-THC and Δ^8-THC increase noradrenaline turnover in several brain regions and decrease the turnover of dopamine in the neostriatum (FUXE and JONSSON, 1971).

We examined the effects of Δ^9-THC, Δ^8-THC and $\Delta^{3,4}$-dimethylheptyltetrahydrocannabinol (DMHP) on the accumulation and disappearance of ^{3}H-catecholamines formed from ^{3}H-tyrosine. Determinations of the endogenous content of homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in the corpus striatum were made, to gain a better insight into the metabolism of dopamine in this region.

MATERIALS AND METHODS

The experiments were performed on male albino rats, weighing 180–230 g, which had been acclimatised for 2–3 weeks in an animal room kept at a constant temperature of 22–23°C under controlled lighting conditions consisting of 14 hr light followed by 10 hr darkness. Δ^9-THC, Δ^8-THC and DMHP were synthesised in our Chemistry Department by Dr. W. Bencze.

The cannabinols were suspended in Tween 80 with the aid of glass homogenisers. Physiological saline was added in small portions to give a final suspension containing 15 mg/ml of the cannabinols and 2% of Tween 80. In the experiments reported here the cannabinols were injected intraperitoneally in a dose of 30 mg/kg. Control rats received the vehicle alone. All the experiments were started between 7·00 and 8·00 a.m.

A detailed account of the estimation of the accumulation and disappearance of ^{3}H-catecholamines following the injection of ^{3}H-tyrosine has already been published (MAITRE *et al.*, 1970b and 1972). Briefly in the accumulation experiments the rats received the cannabinols 1 hr before and in the disappearance experiments 1 hr after 3,5-^{3}H-L-tyrosine (the Radiochemical Centre, Amersham, England). The radioactive tyrosine was diluted with cold L-tyrosine, so that the rats received 1 mCi and 200 μg L-tyrosine/ml/kg body weight.

In the accumulation experiments, the brains were removed 1 hr after the injection of ^{3}H-tyrosine (i.e. 2 hr after treatment with the cannabinols). In the disappearance experiments, the control brains were removed 1 and 3 hr after the ^{3}H-tyrosine injection. The 1-hr group showed the amounts of ^{3}H-catecholamines present in the

brain at the time of treatment with the cannabinols. The brains of the drug-treated rats were removed 3 hr after the injection of ^{3}H-tyrosine (i.e. 2 hr after treatment with the cannabinols).

In both types of experiment, the amounts of ^{3}H-catecholamines found 1 hr after the ^{3}H-tyrosine injection were taken as a measure of biosynthesis. The disappearance of ^{3}H-catecholamines between 1 and 3 hr after the tyrosine injection, i.e. between the cannabinol injection and the removal of the brains was taken as a measure of utilisation.

^{3}H-noradrenaline and ^{3}H-dopamine were extracted from the tissues and separated from ^{3}H-tyrosine or its other metabolites by adsorption onto alumina and subsequent paper chromatography, or by passage through Dowex 50WX4 columns as described in the paper mentioned above.

The determinations were made both in whole brain and in individual brain regions dissected out as described by GLOWINSKI and IVERSEN (1966). HVA and DOPAC were isolated according to the technique used by MURPHY *et al.* (1969). HVA was determined fluorometrically by an automated procedure based on the method described by ANDEN *et al.* (1963). DOPAC was estimated as described by SHARMAN (1971). These determinations were only carried out in the corpus striatum.

RESULTS

1. *Experiments on the whole brain*

The effects of Δ^9-THC, Δ^8-THC and DMHP on the accumulation and release of ^{3}H-catecholamines formed from intravenously injected ^{3}H-tyrosine are shown in Fig. 1. All three cannabinols had the same qualitative effect on catecholamine accumulation and release. The accumulation of ^{3}H-noradrenaline and of ^{3}H-dopamine was increased. Their effect on ^{3}H-noradrenaline was very marked whereas their effect on ^{3}H-dopamine was much less pronounced. The increases produced by Δ^9-THC and by Δ^8-THC were of the same order of magnitude and slightly smaller than those caused by DMHP.

The effects of the cannabinols on the disappearance of ^{3}H-catecholamines paralleled their effects on its accumulation. ^{3}H-noradrenaline utilisation was markedly accelerated to a similar extent by all three cannabinols. The disappearance rate of ^{3}H-dopamine, however, was not significantly altered by Δ^9-THC and Δ^8-THC. The mean values showed a trend towards a greater disappearance rate, but increased utilisation was only observed after treatment with DMHP ($P < 0{\cdot}05$).

2. *Experiments on different brain regions*

The accumulation of ^{3}H-noradrenaline was estimated in the hypothalamus, the pons-medulla and the remaining brain tissue, that of ^{3}H-dopamine in the same parts of the brain and in the corpus striatum. Δ^9-THC was not included in this series of experiments. The accumulation of ^{3}H-noradrenaline increased after treatment with Δ^8-THC by $82 \pm 10\%$ in the hypothalamus, by $41 \pm 9\%$ in the pons-medulla and by $52 \pm 7\%$ in the remaining tissue. The corresponding figures for DMHP were $165 \pm 30\%$, $39 \pm 12\%$ and $57 \pm 12\%$ respectively. The accumulation of ^{3}H-dopamine increased much less than that of ^{3}H-noradrenaline and only in the corpus striatum and the hypothalamus. In the corpus striatum, the increases averaged

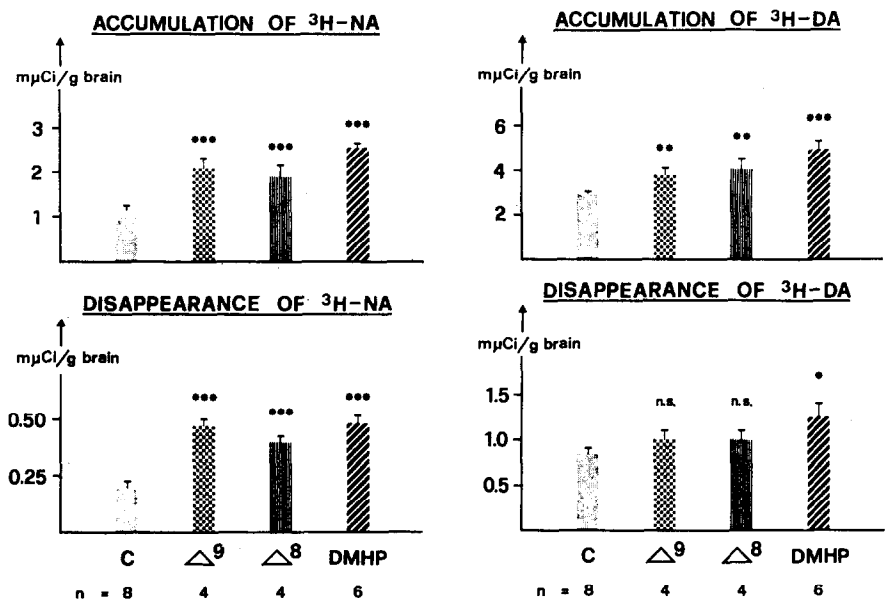

FIG. 1.—Effect of cannabinols on accumulation and disappearance of ^{3}H-noradrenaline (^{3}H-NA) and ^{3}H-dopamine (^{3}H-DA) formed from intravenously injected ^{3}H-L-tyrosine in the rat brain.

In *accumulation* experiments ^{3}H-tyrosine was injected 1 hr after the drugs. The brains were removed 1 hr after ^{3}H-tyrosine injection.

In *disappearance* experiments, ^{3}H-tyrosine was injected 1 hr before the drugs. ^{3}H-NA and ^{3}H-DA were measured 1 hr and 3 hr after ^{3}H-tyrosine injection (controls) or 2 hr after drug treatment. The columns represent the amounts of ^{3}H-catecholamines which disappeared from the brain between 1 and 3 hr after ^{3}H-tyrosine injection.

$42 \pm 6\%$ after Δ^8-THC and $34 \pm 10\%$ after DMHP. In the hypothalamus, they were $26 \pm 9\%$ and $57 \pm 14\%$ respectively. In the other regions, no significant changes were observed. These data were published recently (MAITRE *et al.*, 1972).

The disappearance of ^{3}H-catecholamines from different brain regions was estimated in rats treated with DMHP. That of ^{3}H-noradrenaline was accelerated in all regions of the brain, but particularly in the hypothalamus and cerebellum (Fig. 2). In the other regions, the effects were slight and of the same order of magnitude. As an example, disappearance in the cortex is illustrated in Fig. 2.

The utilisation of ^{3}H-dopamine was slightly increased in some brain regions, but this accelerating effect of DMHP never reached statistical significance at a probability level of $P = 0{\cdot}05$.

The action of DMHP on striatal dopamine was further evaluated by measuring the endogenous content of its major metabolites, HVA and DOPAC. The values obtained 2 hr after a single injection are shown in Table 1. The content of HVA was not altered markedly, whereas that of DOPAC was increased. The latter finding is in keeping with the recent observation that treatment of rats with Δ^9-THC or Δ^8-THC roughly doubled the amount of ^{3}H-DOPAC found in the whole brain 1 hr after an intravenous injection of ^{3}H-tyrosine, while DMHP was even more active (MAITRE *et al.*, 1972).

The preferential increase in DOPAC has been confirmed by studying the time-course of the effect of DMHP on striatal dopamine metabolism. The maximum

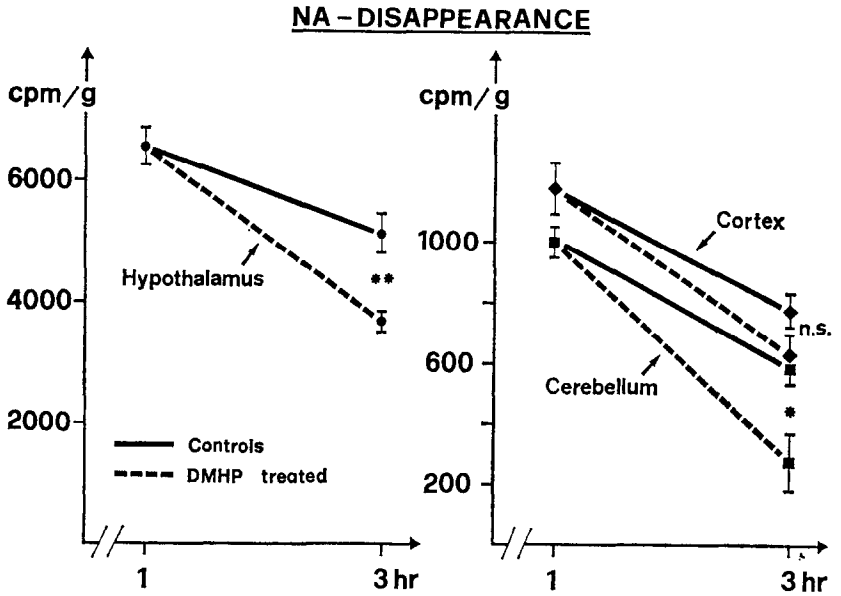

FIG. 2.—Effect of DMHP on noradrenaline (NA) disappearance from rat hypothalamus, cortex and cerebellum.
^{3}H-L-tyrosine was injected intravenously 1 hr before the administration of DMHP or its vehicle. ^{3}H-noradrenaline was estimated at the time of DMHP (or vehicle) injection (= 1 hr after ^{3}H-tyrosine) and 2 hr later.

increase in DOPAC was seen after 2 hr ($P < 0{\cdot}05$) and normalisation occurred between 4 and 16 hr. No concomitant alteration in HVA was detected.

The lack of effect of DMHP on HVA levels and the small increase in DOPAC levels in the corpus striatum contrast with the huge increases seen after treatment with neuroleptic drugs such as chlorpromazine or haloperidol (Table 1), which also increase the accumulation of ^{3}H-dopamine formed from ^{3}H-tyrosine under the experimental conditions described here.

DISCUSSION

The cannabinols influence catecholamine metabolism in the rat brain. Judging from the data available so far, Δ^9-THC, Δ^8-THC and DMHP appear to produce qualitatively similar changes. For convenience, these changes will be discussed

TABLE 1. EFFECT OF DMHP ON HVA AND DOPAC LEVELS IN THE RAT CORPUS STRIATUM. COMPARISON WITH CHLORPROMAZINE AND HALOPERIDOL

Drug	Dose (mg/kg, route)	HVA % of control values	DOPAC % of control values
DMHP	30 i.p.	106 ± 13 n.s.	126 ± 6*
Chlorpromazine	10 p.o.	338 ± 39†	246 ± 15‡
Haloperidol	30 p.o.	650 ± 56‡	313 ± 36‡

* $P < 0{\cdot}05$ † $P < 0{\cdot}01$ ‡ $P < 0{\cdot}001$ $n = 4$

Results are expressed as per cent of the control experiments carried out on the same day. P values were also calculated against the controls of the same day.

Absolute values were 369 ± 20 ng/g for HVA (n = 24) and 559 ± 27 ng/g for DOPAC (n = 19)

without distinction between the cannabinols, on the assumption that they are in fact similar.

Both the biosynthesis and the utilisation of noradrenaline are increased markedly. This is the most obvious effect, being easily recognisable in each of the brain regions studied as well as in the whole brain. The greatest increases were observed in the hypothalamus. Recent studies (MAITRE *et al.*, 1972) had already shown that Δ^8-THC and DMHP have a great affinity for hypothalamic sites; it was found that the accumulation of ^{3}H-noradrenaline from ^{3}H-tyrosine was still marked 5 days after a single dose of the drug. In investigations based on histochemical techniques, MIRAS (1971) and CONSTANTINIDIS and MIRAS (1971) also reported an increase in the intensity of noradrenaline-induced fluorescence in the terminal varicosities of some hypothalamic nuclei after treatment of the rat with a standardised hashish-smoke sublimate. This seems to indicate that Δ^9-THC—the major active constituent of this extract—also exhibits a particular affinity for the hypothalamus. The biosynthesis and utilisation of dopamine are affected to a much smaller extent than those of noradrenaline. Interestingly enough, the accumulation of dopamine was more markedly influenced than its utilisation. This might reflect the fact that newly synthesised dopamine disappears from a pool with a relatively small specific radioactivity. Another possibility which could lead to erroneous disappearance values is that in the accumulation experiments the cannabinols were shown to enhance the amounts of ^{3}H-catecholamines in the tissue. This increase might play a role in the utilisation experiments (when the cannabinols are given after tyrosine), as the ^{3}H-tyrosine remaining in the blood and tissues at the time of the cannabinol injections may be more readily incorporated into ^{3}H-catecholamine in the drug-treated rats than in the controls. The disappearance rate might therefore have been greater than that actually measured. If such a process did occur, it must have taken place to an even greater extent in respect of noradrenaline, and the figures obtained for utilisation would consequently also represent minimum values. The increase in DOPAC levels without alteration in HVA in the corpus striatum is in striking contrast to the effects of typical neuroleptics on these dopamine metabolites. First, the effectiveness of the cannabinols is marginal, as compared with that of chlorpromazine or haloperidol, and secondly, the increase in HVA is greater than that of DOPAC after treatment with the neuroleptics. These results indicate that the cannabinols mainly increase intraneuronal dopamine metabolism.

SUMMARY

Δ^9-THC, Δ^8-THC and DMHP increased catecholamine biosynthesis and utilisation in the rat brain. Their effects on noradrenaline were marked, particularly in the hypothalamus. The effects on dopamine biosynthesis were more clearly demonstrable than those on dopamine utilisation. Endogenous levels of DOPAC were increased without alteration of HVA levels, indicating a preferential intraneuronal pathway of dopamine metabolism. No qualitative difference between the three cannabinols has been observed so far.

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