Short communications

A metabolic interaction *in vivo* between cannabidiol and Δ^1-tetrahydrocannabinol

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The effect of pretreatment with cannabidiol (CBD; 50 mg/kg i.p.) on the distribution of radioactivity in chromatograms of ethyl acetate extracts of the brains of mice injected with tritiated Δ^1-tetrahydrocannabinol (^{3}H-Δ^1-THC; 1·0 mg/kg i.v.) was determined. The pretreatment with CBD produced significant increases in the levels of radioactivity in the brain assigned to Δ^1-THC and its centrally active metabolite 7-hydroxy-Δ^1-THC: the changes produced were respectively 1·4 and 2·0 fold.

Pretreatment with CBD did not bring about any detectable change in the degree of 'catalepsy' produced by Δ^1-THC. This negative finding may have been due to the wide limits of error that were obtained in the bioassay.

Δ^1-Tetrahydrocannabinol (Δ^1-THC) is generally acknowledged to be the main centrally active principle of cannabis, while cannabidiol (CBD), another major naturally occurring cannabinoid is much less active centrally (Mechoulam, Shani, Edery & Grunfeld, 1970; Pertwee, unpublished observation). However, unlike Δ^1-THC, CBD is a potent inhibitor of hepatic drug metabolism (Paton & Pertwee, 1972) and this raises the question of whether CBD can inhibit the metabolism of Δ^1-THC *in vivo* thereby affecting the concentration in the central nervous system of Δ^1-THC and its metabolites. Of particular interest would be an effect on the concentration of 7-hydroxy-Δ^1-THC, a metabolite reported (Christensen, Freudenthal, Gidley, Rosenfeld, Boegli, Testino, Brine, Pitt & Wall, 1971) to be even more active centrally than Δ^1-THC itself. The existence of such an interaction would then raise the related question of whether the inhibitory effect of CBD on the metabolism of Δ^1-THC modifies the central action of Δ^1-THC. The hypothesis was investigated by evaluating the behavioural response to ^{3}H-Δ^1-THC of mice which had previously been injected with CBD or its vehicle, and by determining the brain levels of Δ^1-THC and its metabolites immediately after the bioassay had been completed.

Methods.—The experiment was performed at room temperature using non-fasted adult male white mice weighing 25 to 28 g supplied by A. J. Tuck. The CBD, which was generously supplied by Professor R. Mechoulam, was dispersed in a mixture of Tween 80 and a 0·9% NaCl solution, the dispersion containing, by weight, 2 parts of Tween 80 to 1 part of CBD. The CBD was administered intraperitoneally to 6 mice. The volume injected was 0·2 ml/25 g while the dose of CBD used was 50 mg/kg, a dose known to prolong sleep induced in mice by pentobarbitone (Paton & Pertwee, 1972). A control group of 6 mice received intraperitoneal injections of Tween 80 in NaCl solution. Thirty minutes after the injection of CBD or its vehicle, each mouse received an intravenous injection of ^{3}H-Δ^1-THC. The Δ^1-THC was labelled in positions 1 and 2 of the pentyl side chain and when used had a specific activity of 584 mCi/mmole ($4{\cdot}13 \times 10^6$ dpm/μg); its synthesis is described elsewhere (Gill & Jones, 1972a). The Δ^1-THC was dispersed in a mixture of Tween 80 and a 0·9% NaCl solution, the dispersion containing by weight 5 parts of Tween to 1 part of THC. The dose and volume injected were respectively 1 mg/kg and 0·2 ml/25 g.

The pharmacological response to the ^{3}H-Δ^1-THC was evaluated 15 min after the injection of Δ^1-THC by means of the ring test, a bioassay which provides a measure of the 'cataleptic' effect of Δ^1-THC. In the bioassay a mouse is placed across a horizontal wire ring of 5·5 cm diameter for periods of up to 6 minutes. The percentage of this time during which the mouse remains completely still is recorded and is called the 'immobility index'. Immediately after completion of the ring test, the mice were killed by administration of carbon monoxide. The method employed for the determination of brain levels of Δ^1-THC and its metabolites has been described in detail by Gill & Jones (1972b). Individual whole brains were removed and then rinsed and homogenized in 0·1 M phosphate buffer (pH 7·4). An aliquot of each homogenate was digested with methanolic hyamine hydroxide and

the total radioactivity was measured by liquid scintillation counting. The remainder of the homogenate was extracted with purified ethyl acetate. The dried extract was counted and, after concentration under a stream of nitrogen, was chromatographed on silica-impregnated paper. The distribution of radioactivity in the chromatogram was determined by division into strips followed by elution of each strip with scintillator solution directly in the counting vials.

Results.—Radioactive spots were observed with the R_f values of authentic Δ^1-THC and 7-hydroxy-Δ^1-THC, the primary metabolite, and the activity was provisionally assigned to these two compounds. In addition there was a minor extractable component, chromatographically more polar than 7-hydroxy-Δ^1-THC, which has not been identified and which may well consist of a mixture of compounds. This has been loosely termed the 'polar metabolite'. A fraction of the radioactivity was not extractable. This fraction may consist of Δ^1-THC and its metabolites strongly bound to lipoprotein: Agurell and his colleagues (Wahlqvist, Nilsson, Sandberg, Agurell & Granstand, 1970) have shown that such binding occurs with plasma lipoproteins.

The results are summarized in Fig. 1. It was found that the levels of the radioactivity in the brain assigned to Δ^1-THC and its 7-hydroxy metabolite were both significantly ($P<0.005$) elevated by pretreatment with CBD, being respectively about 1·4 and 2·0 times greater in the mice pre-treated with CBD than in the mice which had received instead a Tween–NaCl solution. In addition, CBD significantly ($P<0.02$) reduced the brain level of the unidentified polar metabolite. However, no change was detected ($P>0.1$) in the level of the non-extractable material. These changes in levels of radioactivity were not accompanied by a corresponding behavioural change: the mean immobility index (±S.E.M.) of the group of mice pretreated with CBD was 51·5 (±12·5) whereas the mean immobility index of the control group was 52·2 (±4·9).

Discussion.—The results suggest that *in vivo* (1) CBD inhibits not only the metabolism of Δ^1-THC but also that of the primary metabolite 7-hydroxy-Δ^1-THC,

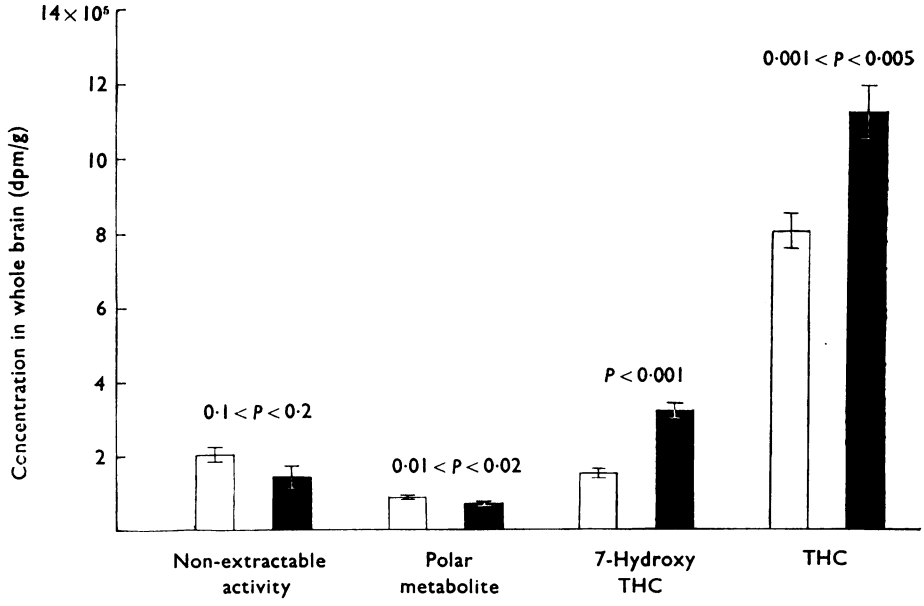

FIG. 1. Effect of cannabidiol (CBD) on the metabolism of Δ^1-tetrahydrocannabinol (Δ^1-THC) *in vivo*. Six mice were injected with CBD (50 mg/kg ; i.p.) while a control group received Tween 80 in NaCl solution. After 30 min the mice were injected with ^{3}H-Δ^1-THC (1 mg/kg i.v. ; specific activity $4·13\times10^6$ d.p.m./μg) and brain levels were determined after a further 20 minutes. Concentrations of radioactive components in the brain (mean±S.E.M.) are expressed as d.p.m. per gramme of brain tissue. (Control □ ; CBD ■).

and (2) the second stage of this metabolic pathway is inhibited to a greater extent than the first. It is of interest that SKF 525A, a better known metabolic inhibitor than CBD, produces qualitatively similar effects *in vivo* to those described here (Gill & Jones, 1972b). Experiments concerned with ^{3}H-Δ^1-THC alone (Gill & Jones, 1972b) have demonstrated the existence of a highly significant correlation between the immobility index and the levels in the mouse brain of radioactivity assigned to Δ^1-THC and 7-hydroxy-Δ^1-THC. The failure of CBD pretreatment to produce a detectable change in the immobility index is interesting in view of a current hypothesis that 7-hydroxy-Δ^1-THC may be solely responsible for the behavioural changes that follow administration of Δ^1-THC (Mechoulam, 1970; Christensen, *et al.*, 1971). Pretreatment with CBD produced a two-fold increase in the level of radioactivity assigned to 7-hydroxy-Δ^1-THC but proportionally a smaller change in the combined brain levels of Δ^1-THC and 7-hydroxy-Δ^1-THC. This suggests that the metabolite is not solely responsible for the behavioural changes that follow administration of Δ^1-THC and that Δ^1-THC itself contributes at least partly towards the effect. However, the wide limits of error that were obtained with the ring test prevent any definite conclusion from being reached. Both CBD and Δ^1-THC are natural constituents of cannabis and the metabolic interaction that occurs between the two cannabinoids may well influence the pharmacology of the crude material. Unequivocal evidence to support this possibility could only be provided however by an experiment in which CBD and Δ^1-THC were administered to animals at the same time, by the same route and in the same proportion to one another as the proportion in which they are found in cannabis.

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