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SHORT COMMUNICATIONS

Perfusion of the isolated rat brain with [^{14}C]-Δ^1-tetrahydrocannabinol

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Considerable interest has focused on the biotransformation of Δ^1-tetrahydrocannabinol (Δ^1-THC), especially the metabolic pathway involving 7-hydroxylation [1]. It has been demonstrated that the 7-hydroxylated metabolite is as active as or more active than the parent compound [2–4] which has prompted suggestions that pharmacological effects arise only after Δ^1-THC has been metabolically activated [5–8]. It has been difficult testing this postulate, since Δ^1-THC is rapidly 7-hydroxylated in most animals species [2,9,10], a conversion that is not blocked by metabolic inhibitors such as SKF-525A [2]. However, recent evidence has shown that synthetic cannabinoids lacking the 7 position exhibit behavioral and cardiovascular effects typical of Δ^1- and Δ^6-THC which does not support the 7-hydroxy group as being a structural requirement for biological activity [11]. Several investigators have reported rather large ratios of Δ^1-THC to 7-OH-Δ^1-THC in brain at the time corresponding to peak behavioral activity [2,12,13], an additional fact which does not strengthen the metabolite theory. It would appear that psychoactivity could be attributed to both Δ^1-THC and its metabolites, because Δ^1-THC satisfies all the structural requirements for central nervous system (CNS) activity [14]. Furthermore, there is no direct evidence showing Δ^1-THC to be inactive. Since it has been shown that the isolated perfused rat brain is a suitable model for studying the effects of drugs on the CNS [15,16], direct evidence for effects of Δ^1-THC on the CNS could be obtained by using this technique. In order to obtain meaningful results, there must be no conversion of Δ^1-THC to 7-OH-Δ^1-THC. Therefore, the purpose of the first set of experiments was to determine whether rat brain could metabolize Δ^1-THC. A second set of experiments was designed to evaluate the effects of cannabinoids on the electroencephalograms (EEG) of the isolated rat brain.

Male Sprague–Dawley rats weighing 200–300 g were anesthetized with urethane (1.2 g/kg, intraperitoneally), and the isolated brain preparation described by Andjus *et al.* [17] was carried out without an interruption in the circulation to the brain. A closed circuit perfusion was done using apparatus I, as detailed by Fleck *et al.* [18]. The complete circuit consisted of a reservoir for the medium, piston pump, sintered glass filter, thermometer, manometer for controlling perfusion pressure, overflow with manometer, rat brain, and oxygenator. The medium was constantly oxygenated with 95% O_2 + 5% CO_2, and the temperature of the medium in the reservoir was kept at 37°. The perfusion pressure was maintained between 110 and 120 mm Hg, and the perfusion rate was between 2 and 4 ml/min. Each brain was perfused with 100 ml of freshly prepared medium which was composed of 30% well-washed bovine red blood cells (RBC), 4% (w/v) bovine serum albumin, and 22 mM glucose in Krebs–Henseleit solution [19]. In all experiments, 10 μl of an ethanolic solution of drug was added to the perfusion medium which made a final drug concentration of 10^{-5} M. The medium was oxygenated for approximately 20 min prior to perfusion of the brain. Bipolar electrodes were placed in the parietal region of the skull and EEGs were recorded at each 5 min interval throughout the perfusion period.

Radiolabeled Δ^1-THC was used for the investigation of metabolism *in situ*. [^{14}C]-Δ^1-THC (1 mCi/m-mole was purified by thin-layer chromatography (T.L.C.) using Silica gel F plates which were developed three times with petroleum ether–diethyl ether (19:1). The purification was repeated in order to achieve a radiopurity greater than 95 per cent. In duplicate experiments, isolated rat brains were perfused for 1 hr with medium containing [^{14}C]-Δ^1-THC (10^{-5} M). The spontaneous electrical activity indicated that the metabolic and functional condition of the brain was normal throughout the perfusion period [16]. There were alterations in the EEG which were apparently drug induced (discussed later). Control experiments were also carried out to determine whether or not the drug was altered by the experimental conditions. In duplicate experiments, [^{14}C]-Δ^1-THC was added to the medium and allowed to circulate for 1 hr and 20 min without the presence of a brain.

At the end of perfusion, brains were homogenized in saline using a Teflon pestle tissue grinder. The perfusion medium was centrifuged at 1000 g for 20 min to obtain "plasma" and red blood cells. NCS solubilizer (Amersham/Searle) was used to digest whole brain homogenate (0.5 ml), plasma (0.1 ml), and red blood cells (0.1 ml) for liquid scintillation spectrometry. Radioactivity was extracted from plasma, red blood cells and whole brain homogenates by shaking with equal volumes of petroleum ether (three times) followed by equal volumes of diethyl ether (three times). All extracts (an amount containing 2000 dis./min) were co-chromatographed on T.L.C. plates with Δ^1-THC and 7-OH-Δ^1-THC standards, using two systems comparable to those previously described [20]. Δ^1-THC was quantitated on Silica gel F plates (impregnated with dimethylformamide) by developing twice with petroleum ether–diethyl ether (9:1). 7-OH-Δ^1-THC was quantitated on Silica gel F plates by developing once with petroleum ether–diethyl ether (1:1). The areas containing the standards were localized by spraying the plates with Fast Blue B (0.1% in 2 N sodium hydroxide). 1-cm sections of the Silica gel were scraped into vials containing 10 ml of scintillation mixture (0.4% diphenyloxazole and 0.01% 1,4-bis [2-(4-methyl-5-phenyl-oxazoly)] benzene) in toluene. The samples were counted utilizing external standardization for quench correction.

In the brain perfusion experiments, the radioactivity was distributed as follows: 10 per cent in brain, 46 per cent in plasma, and 44 per cent in red blood cells. In the control experiments 48 per cent of the radioactivity was in plasma and 52 per cent in red blood cells. The extraction removed 80 per cent of the radioactivity from brain, 96 per cent from plasma and only 20 per cent from red blood cells. Comparable amounts of radioactivity were extracted from the control plasma as well as the control red blood cells. The major portion of the plasma radioactivity was contained in the petroleum ether extract, whereas the radioactivity extracted from brain and RBCs was equally divided between the petroleum ether and diethyl ether fractions. Extraction with both petroleum ether and diethyl ether has been reported to effectively remove Δ^1-THC, 7-OH-Δ^1-THC, and other metabolites from brain homo-

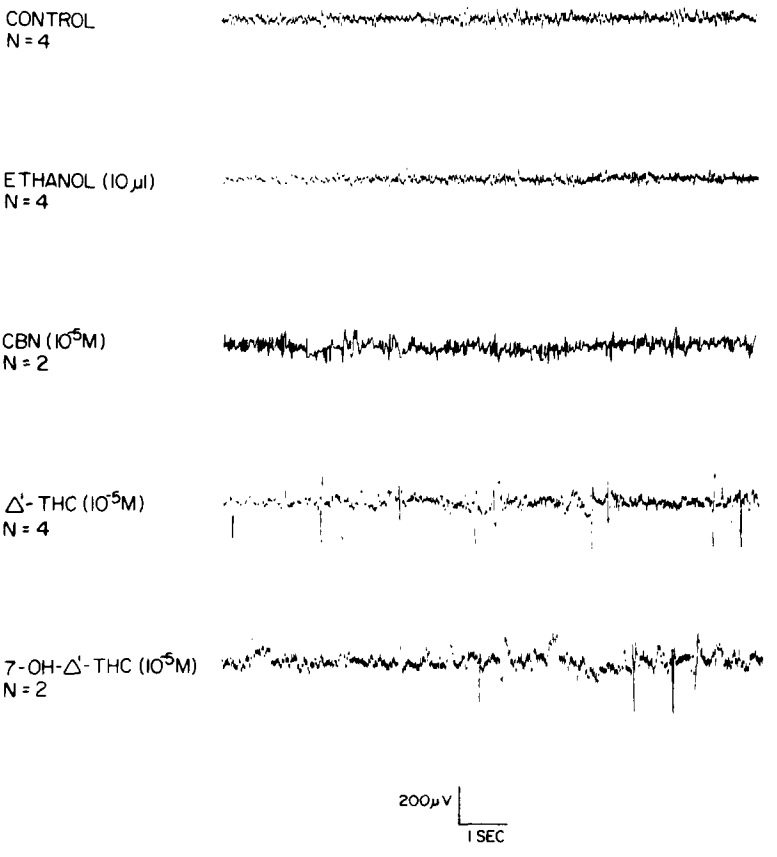

Fig. 1. Electroencephalograms of the isolated rat brain recorded after a perfusion of 20 min. Since tracings from bipolar leads (parietal region) are usually symmetrical and synchronous, only one EEG channel for each drug is shown. In the control experiment, no drug was added to the perfusion medium. N = the number of experiments carried out per group, and only the recording most representative of the group is presented. The results were consistent for each group with one exception. One EEG recording in the Δ^1-THC group was similar to control.

genates and plasma [13,21]. The radioactivity remaining in both control and experimental RBCs was probably due to a protein–cannabinoid complex, since blood apparently lacks the ability to metabolize Δ^1-THC to polar metabolites.

The T.L.C. data showed no evidence for brain metabolism since the plasma and RBC extracts from the brain experiments were identical to those from the control experiments. Greater than 90 per cent of the radioactivity on each T.L.C. plate corresponded to the Δ^1-THC region, while the remaining areas contained approximately 1 per cent each. Thin-layer chromatography of the brain extracts did not indicate the presence of metabolites. Approximately 90 per cent of the radioactivity in both petroleum ether and diethyl ether extracts corresponded to the Δ^1-THC region which showed that not all of the Δ^1-THC was removed by the petroleum ether extraction. The remainder of the radioactivity on the T.L.C. plates was not confined to a particular area but appeared to be due to retention of [^{14}C]-Δ^1-THC by other material. To further insure that trace amounts of 7-OH-Δ^1-THC were not present in the brain extracts, the petroleum ether and diethyl ether extracts from both brains were pooled and chromatographed on Sephadex LH-20 columns [22]. Ninety-five per cent of the radioactivity applied to the column was eluted in the Δ^1-THC region (40–50 ml) and no radioactivity appeared in the region (60–150 ml) of monohydroxylated metabolites [22].

There was no evidence for metabolism of Δ^1-THC by rat brain *in situ*, which agrees with that previously reported for preparations *in vitro* [7,23]. However, our data disagree with that reported for the intact monkey brain [24]. These investigators reported that Δ^1-THC could be 7-hydroxylated in the intact monkey brain on the basis that, following an intraventricular injection of radiolabeled Δ^1-THC, the ratio of 7-OHΔ^1-THC to Δ^1-THC was higher in blood samples taken from the left internal jugular vein than in samples from the right common carotid artery. Lack of agreement between the isolated rat brain and the intact monkey brain could be due to a species difference or to methodology.

In a second set of experiments, isolated rat brains were perfused with simplified blood that contained either Δ^1-THC, 7-OH-Δ^1-THC, cannabinol (CBN) or ethanol (10 μl), whereas control experiments were performed without the addition of a drug to the medium. The EEGs were evaluated visually and without knowledge of the treatment. Electroencephalograms recorded after 20 min of perfusion are presented in Fig. 1. The control, ethanol and CBN groups were similar in that they exhibited predominantly beta activity and were generally devoid of particular wave forms. The ineffectiveness of ethanol was probably due to its initial low concentration which was probably reduced further by evaporation during the oxygenation period preceding perfusion. Clear EEG changes were found throughout the perfusion period when Δ^1-THC and 7-OH-

Δ^1-THC had been added to the perfusion medium. These changes were characterized by high amplitude bursts and a decrease in frequency which agreed with the EEG activity of intact animals treated with Δ^1-THC [25]. The EEG pattern produced by Δ^1-THC and 7-OH-Δ^1-THC may be associated with psychoactivity since CBN, a psychotomimetically inactive cannabinoid[26], failed to produce these distinct alterations. However, an extensive series of experiments with a quantitative automatic evaluation of the EEGs would be necessary in order to determine the potency ratios of these cannabinoids.

In conclusion, we have shown that, even in the absence of metabolism, Δ^1-THC is capable of altering the EEG of rat brain which supports the hypothesis that both Δ^1-THC and its metabolites contribute to the psychoactivity of cannabis. Furthermore, a disproportionate amount of CNS activity in the rat cannot be attributed to 7-OH-Δ^1-THC on the basis that it is formed at or near its locus of action.

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