The Effect of Cannabinol on Δ^1-Tetrahydro-Cannabinol Clearance from the Blood

Δ^1-Tetrahydrocannabinol (Δ^1-THC) is the main biologically-active constituent of marihuana[1] and it is thought that the presence of this molecular species in the brain plays a major role in marihuana intoxication[2,3]. Cannabinol (CBN), another naturally occurring constituent of marihuana has not been found to produce any marked biological effects[1] and as a possible contributor to marihuana intoxication it has therefore been largely ignored. Three instances of modification of the behavioural effects of Δ^1-THC by CBN have been reported. On the one hand, it has been found that the Δ^1-THC depressant effect can be blockaded by CBN in both rats[4] and mice[5] and on the other, it has been suggested that large proportions of CBN in marihuana can potentiate the activity of Δ^1-THC also present[6]. Since CBN is generally present in administered Δ^1-THC as well as in marihuana, further investigation into the role of CBN in marihuana intoxication appeared to be called for.

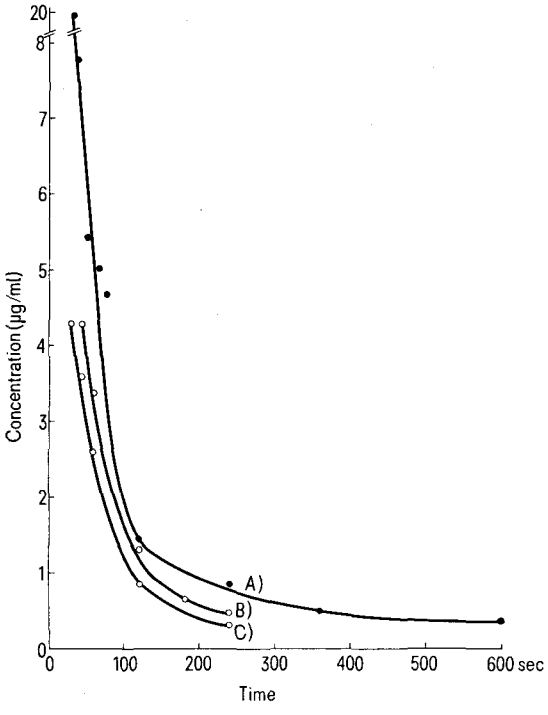

Fig. 1. The disappearance of Δ^1-THC from the blood of rats. A) 1 mg pure Δ^1-THC injected i.v.; B) 1 mg Δ^1-THC and 0.13 mg CBN injected i.v.; C) 1 mg Δ^1-THC and 1.3 mg CBN injected i.v.

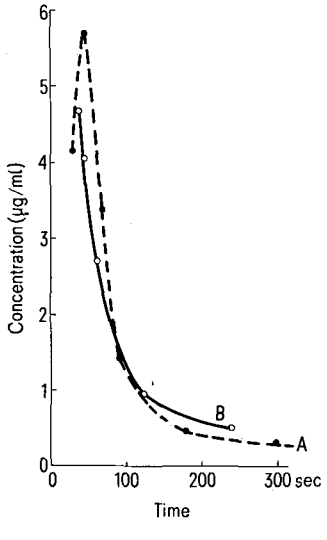

Fig. 2. The disappearance of CBN from the blood. A) 1.3 mg pure CBN injected i.v.; B) 1.1 mg CBN and 1 mg Δ^1-THC injected i.v.

Materials and methods. Male rats were conveniently used with the methods of i.v. administration and analysis reported previously[7]. In these experiments (and in other unpublished results), the amount of biological variation was found to be surprizingly low for rats of the same sex and similar weights. Except in the case where levels of pure Δ^1-THC fall extremely rapidly from 20 to 3 µg/ml the variation appeared to be no greater than normal experimental errors which were within $\pm$ 5% for determinations of spiked samples.

The cannabinoids were repurified using preparative layer chromatography, the Δ^1-THC containing 0.1% CBN.

Results. Figure 1 illustrates the disappearance of Δ^1-THC from the blood after injection of 1 mg Δ^1-THC. Results obtained prior to 35 sec show that the peak level from Δ^1-THC administered alone is 20 µg/ml and occurs at 35 sec. Observable behavioural effects (sluggishness, lack of grooming etc.) appeared within 3 min after injection (c.f. GILL et al.[8] who found a similar rapid intoxication with mice).

Figure 2 shows the disappearance of CBN from the blood after the injection of 1.3 mg CBN. The CBN administered by itself reaches a maximum of only 5.6 µg/ml and disappears considerably more rapidly than Δ^1-THC administered alone.

When injected together, Δ^1-THC and CBN disappeared at the same rate (Figures 1 and 2) with the ratio between them staying the same as it was in the injection. Thus, in the presence of CBN, the rate of clearance of Δ^1-THC was found to be considerably enhanced (Figure 1), whereas in the presence of Δ^1-THC, the rate of disappearance of CBN remained virtually unchanged (Figure 2). A 10-fold increase in the amount of CBN (0.13 to 1.3 mg) introduced with the Δ^1-THC produced no qualitative change in the observed effect (Figure 1) although a further smaller depression of Δ^1-THC blood levels was noticeable with increasing amounts of administered CBN (Figures 1 and 3).

Discussion. The blood levels from CBN administered alone were at all times less than those of the injected

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equivalent amount of Δ^1-THC administered by itself. The subsequent decline of CBN concentrations was considerably more rapid than those of Δ^1-THC. These facts allow reconciliation of the findings that although CBN has very low activity by injection[1,9-11] it can cause intoxication when infused i.v. at 6 times the dose rate required by Δ^1-THC for intoxication[12]. However a more rapid disappearance of CBN compared to Δ^1-THC also means that equilibrium blood concentrations achieved at any given dose rate will be lower for CBN than Δ^1-THC. It follows that the apparently low activity of CBN is due to its relatively rapid clearance rather than to a low intrinsic activity.

When 0.13 mg CBN was injected with 1 mg Δ^1-THC, the Δ^1-THC blood levels from the mixture were already as low at 3.5 min as they were with Δ^1-THC alone, after 10 min (Figure 1). This suggests that an antagonism for the effects of Δ^1-THC in rats should be observable provided the assumption that there is rapid equilibration of Δ^1-THC between the blood and brain can be made. Such an assumption is not unreasonable since it has been noted that there is not such a blood-brain barrier for Δ^1-THC in mice[13]. Two groups[4,5] have noted an antagonism but detailed comparisons with our work are not possible.

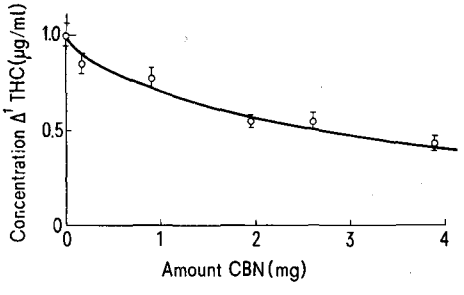

Fig. 3. Δ^1-THC blood levels determined at 3 min after i.v. injection of 1 mg Δ^1-THC and variable amounts of CBN.

Increasing the amounts of CBN introduced with the Δ^1-THC decreased Δ^1-THC blood levels only slightly but increased CBN blood levels in direct proportion. It might be expected therefore, that the CBN in CBN-rich mixtures could contribute significantly to the over-all activity, and relative to mixtures low in CBN but with the same Δ^1-THC, these mixtures could even exhibit an improved activity. This could explain the finding that the amounts of Δ^1-THC in marihuana containing an high proportion of CBN do not fully account for its observed activity[6].

CBN is generally present in 'pure' Δ^1-THC (as an aerial oxidation product), in marihuana, and is an artefact of smoking Δ^1-THC[14]. Our results are therefore of importance for in vivo and behavioural studies.

Summary. The co-administration of cannabinol with Δ^1-tetrahydrocannabinol accelerates the rate of clearance of Δ^1-tetrahydrocannabinol from rat blood. This increased rate of clearance appears to follow that of cannabinol. The implications of these findings are discussed.

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Effect of Ethyl m-Aminobenzoate (MS-222) on Ampullae of Lorenzini and Lateral-Line Organs

Methane sulphonate of meta-aminobenzoic acid ethylester[1] (MS-222, ethyl-m-aminobenzoate, tricaine methanesulphonate, metacaine methanesulphonate, $C_9H_{11}O_2N + CH_3SO_3H$) is widely used for general anesthesia and sedation of fish and amphibia. (For references see BOVÉ[2]). Belonging to the pharmacological group of local anesthetics, the substance can be expected to act not only on the central but also on the peripheral nervous system. Previous observations (HENSEL and NIER, unpublished) have shown that in cods (*Gadus gadus*) anesthetized with MS-222, the spontaneous activity of lateral-line organs was depressed. The present study was undertaken to investigate more systematically the effect of MS-222 on isolated preparations of ampullae of Lorenzini and lateral-line organs.

Materials and methods. Isolated preparations of the mandibular group of the ampullae of Lorenzini of dogfishes (*Scyliorhinus canicula*) were used. The ampullae were stimulated thermally and electrically as described previously (BROMM, HENSEL and NIER[3], HENSEL[4]). The electric stimuli were applied by means of a microelectrode inserted into the opening of an ampullary canal. Isolated preparations of lateral-line organs were obtained from the mandibular canal of dogfishes (*Scyliorhinus canicula*) and the lateral-line canal of cods (*Gadus gadus*). The preparations were placed on a water-circulated thermode, and afferent single-fibre activity was recorded. Usually the preparations were bathed with saline for selachians or teleosts, respectively, flowing with a constant velocity of ca. 0.02 cm^3 sec^{-1}. MS-222 was added in concentrations of 10^{-3} to $5 \cdot 10^{-5}$ g cm^{-3} to the bathing fluid.

Results. The isolated mandibular ampullae were kept at 19 °C. At this temperature the static discharge has its maximum (HENSEL and NIER[5]). The continuous application of 10^{-3} to $5 \cdot 10^{-5}$ g cm^{-3} MS-222 led to a gradual decrease in the resting discharge of the whole nerve as well as of single fibre preparations, the impulse rate reaching the zero level after 4 to 9 min. No systematic correlation was seen between the concentration applied and the time required for complete inhibition. This might be due to the fact that the size of ampullae, length of ampullary canals, thickness of connective tissue, and location of receptors within the ampullae varied with each preparation. When

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