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CANNABINOIDS AND SEROTONIN UPTAKE BY BLOOD PLATELETS: EVIDENCE FOR MULTIPLE SITES OF ACTION

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Abstract—The effect of cannabinoids on parameters of 5-hydroxytryptamine (5-HT) uptake by human platelets was examined. Natural and synthetic cannabinoids differing in the pyrene ring of the hydrophobic side chain or in steric configuration, inhibited differentially the saturation level of 5-HT uptake, the active uptake and the passive transport of the neurotransmitter, indicating multiple sites of action of the drugs. The pattern of inhibition did not correlate with the psychomimetic specificity of the cannabinoids.

Cannabinoids are known to affect cellular membranes of various systems: mitochondria [1], liposomes [2], lysosomes [3], red blood cells [4, 5], brain synaptosomes [6, 7] and blood platelets [8]. A differential effect of the cannabinoids studied, in apparent correlation with the psychomimetic activity, was demonstrated with respect to the inhibition of monoamine oxidase of brain mitochondria [9] and the inhibition of platelet aggregation [8]. The inhibition of platelet aggregation by cannabinoids is of particular interest in view of the marked specificity revealed: derivatives with a (+) steric configuration and with a dimethyl-heptyl side chain were most inhibitory [10]. Furthermore, the interest in the effects of cannabinoids on blood platelets stems from the recognition that platelets are instrumental for studies related to the central nervous system [11, 12]. Lauscher and Pletscher [13] concluded that platelets are models for differentiating the site of action of drugs interfering with 5-hydroxytryptamine (5-HT) uptake, as demonstrated recently by Rotman *et al.* [14] for schizophrenic patients. In the present study we examine the effect of several cannabinoids on parameters of 5-HT uptake by human blood platelets.

MATERIALS AND METHODS

Separation of blood platelets. Collection of human blood and separation of washed platelets were as described previously [15]. The washed platelets were suspended in Tyrode's buffer, fortified with apyrase (0.1 mg/ml). Experiments were completed within 2 hr thereafter.

5-HT uptake. Platelets (3×10^8/ml) were incubated with 5-HT (0.1 to 10 μM) in a final volume of 1 ml at 37°. For kinetic analyses (as in Fig. 4) the assay time was 4 min and was terminated by the rapid addition of 5 ml of ice-cold saline and filtration on cellulose nitrate filters (pore size 0.4 μM) followed by a rinse with additional 10 ml of cold saline. The radioactivity retained by the filters was counted by liquid scintillation. When the assay time was longer than 4 min, the assay was terminated by centrifugation, as described [15]. The values of 5-HT uptake were corrected for the corresponding uptake at 4°. 5-Hydroxytryptamine creatinine sulfate (sp. act. 54 mCi/mmole, radiochemical purity: 98%) was purchased from the Radiochemical Centre, Amersham, U.K.

Application of cannabinoids. Platelets were incubated for 1 min with the tested cannabinoid at 37° prior to the addition of 5-HT. Stock solutions of the cannabinoids were prepared in 70% ethanol, and an aliquot (5 μl) was added to the assay mixture. An identical quantity of ethanol was added to control. The addition of the vehicle caused a limited decrease in serotonin uptake (less than 5%). Comparison of the effects of cannabinoids on [^{14}C]-5-HT uptake was performed at drug concentrations of up to 9.4 μg/ml, since this is the highest concentration that could be used without inducing platelet aggregation under experimental conditions. The cannabinoids used are presented in Fig. 1.

RESULTS

A decrease in the saturation level of 5-HT uptake is typical for reserpine-treated platelets [17] and for platelets with storage pool disease [18]. CBD causes a similar effect, as shown in Fig. 2.

Maximal levels of 5-HT uptake were obtained within 30 min and this period was used for a comparison of the effect of several cannabinoids. Δ^1-THC, CBD and DMH–CBD were tested in view of

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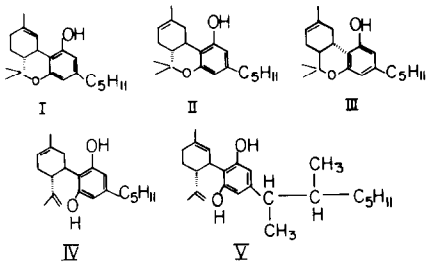

Fig. 1. Structures of cannabinoids used in this study corresponding to: (I) Δ^1-Tetrahydrocannabinol (Δ^1-THC); (II) (−)Δ^6-Tetrahydrocannabinol ((−)Δ^6-THC) $(\alpha)_D$ − 360°; (III) (+)Δ^6-Tetrahydrocannabinol ((+)Δ^6-THC) $(\alpha)_D$ + 230°; (IV) Cannabidiol (CBD); (V) The 1″,2″-dimethylheptyl homolog of cannabidiol (DMH–CBD) α_D − 150°.

their established effects in platelets [8, 10]. Small differences were found in the inhibitory effects of the cannabinoids tested (Fig. 3). Noteworthy is the exceeding potency of CBD.

Kinetics studies. At a concentration range of 5 μM and above, 5-HT uptake by platelets is apparently passive, while at a lower concentration range 5-HT uptake is considered active [19]. Accordingly, the effect of the cannabinoids on 5-HT uptake was tested at a wide concentration range of 5-HT. Figure 4 illustrates that DMH–CBD apparently impaired the passive 5-HT transport but hardly affected the active uptake. On the contrary, CBD and Δ^1-THC inhibited the active but not the passive component of 5-HT uptake.

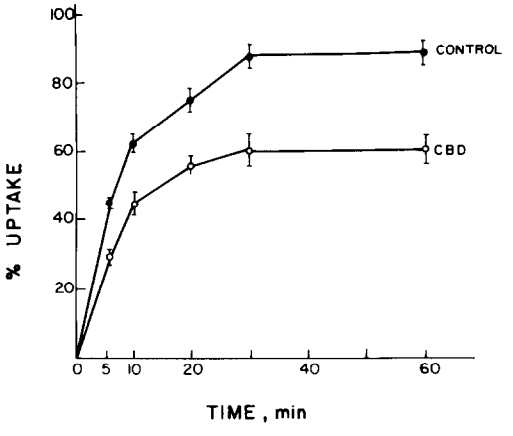

Fig. 2. Effect of CBD on 5-HT uptake by human platelets.

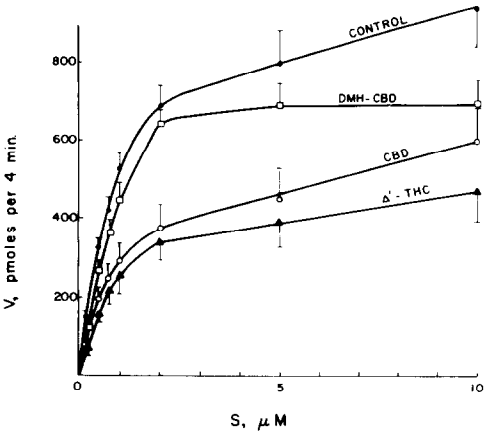

Fig. 4. Effect of cannabinoids on the kinetics of 5-HT uptake by human platelets. S denotes 5-HT concentrations.

When the uptake data were corrected for the passive transport, according to Tuomisto [19], this distinction in the cannabinoid effect is further illustrated (Fig. 5). K_I values for cannabinoid effect in active 5-HT uptake, derived from the double reciprocal plots of Fig. 5, were 30 μM, 41 μM and >1 mM for Δ_1-THC, CBD and DMH–CBD, respectively. Thus, in both brain synaptosomes and blood platelets the inhibition of 5-HT uptake by cannabinoids is non-competitive, with similar K_I values.

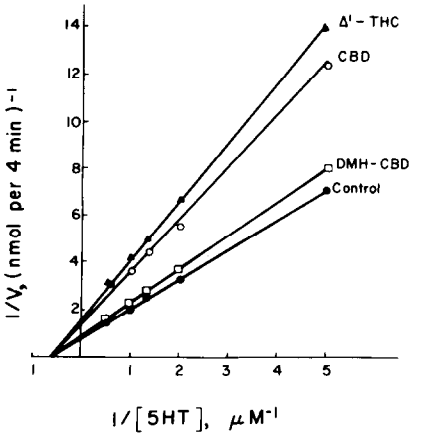

Fig. 5. Double reciprocal plot of 5-HT uptake by human platelets as affected by cannabinoids.

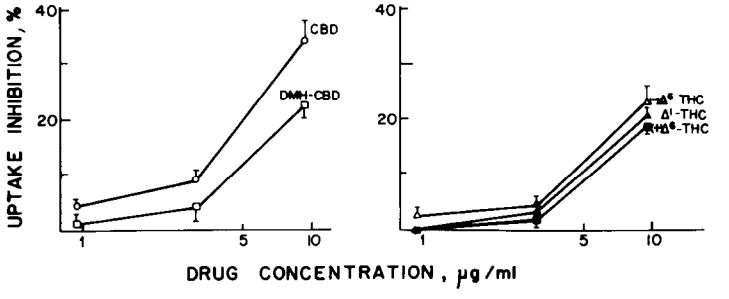

Fig. 3. 5-HT uptake by human platelets: extent of inhibition by cannabinoids.

DISCUSSION

Uncertainties have been raised concerning the actual concentration of the aqueous solutions of cannabinoid due to their limited solubility [7, 20]. Schurr [21] measured the partition of CBD and THC in cellular suspensions and observed that 97 per cent of the cannabinoids added to the suspension at 50 μg/mg protein, were associated with the cellular elements, and 3 per cent was found in the suspending medium. This implies that the effective concentration of the cannabinoid in cellular suspensions is not determined by the solubility in the aqueous solution *per se* but mainly by its partition coefficient between the cellular elements and the suspending medicine. Furthermore, it seems therefore justified to express the dose of the cannabinoid in a practical term of the quantity added to the assay system, rather than in assumed molar concentrations.

The major conclusion stemming from our study is that multiple platelet sites are involved in the effects of the cannabinoids on 5-HT accumulation. Since the cannabinoids, particularly CBD, exerted a reserpine-like effect and reduced the saturation level of 5-HT uptake (Figs 2 and 3), the likely site affected is the granule. The higher potency of Δ^1-THC, relative to CBD, in reducing V_{max} of 5-HT uptake (Fig. 4), indicates yet another cellular mechanism of the cannabinoid effect, possibly involving the plasma membrane. Furthermore, since the hydrophobic dimethylheptyl-CBD inhibited the passive uptake of 5-HT most effectively (Fig. 4), an additional site of effect is likely.

The structure–activity relationship of cannabinoids in inhibition of aggregation [10] is distinctly different from that observed for the inhibition of serotonin uptake. The impairment of ADP-induced platelet aggregation by cannabinoids was clearly specific, and derivatives with a (+) steric configuration and with a dimethyheptyl side chain were most inhibitory. However, unlike CBD, the dimethylheptyl CBD did not affect the active 5-HT uptake. Conceivable different cellular sites of interaction are involved in the effect of cannabinoids on platelet with respect to aggregation and 5-HT uptake. Such diversified effects of cannabinoids should be considered when the overall cellular impact of cannabinoids is assessed.

As indicated, the cannabinoids were applied along with ethanol, which by itself exerted limited effect. Yet, the inhibition of glucose efflux from human erythrocytes by cannabinoids was amplified by alcohols [5]. It is therefore possible that some of the inhibitory responses observed in the present study actually reflect interacting effects of ethanol and the cannabinoids, possibly due to an increase in the effective drug concentration in the membrane by the alcohol.

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