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The interaction of hashish components with human erythrocytes

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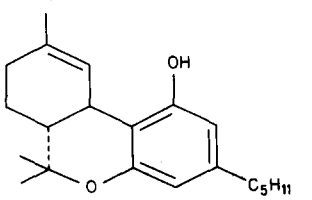
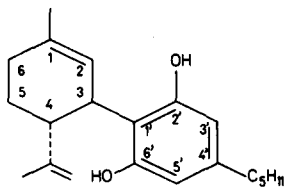
SUMMARY

At 10^{-5} M the hashish components, Δ^1-tetrahydrocannabinol and cannabidiol, stabilize human erythrocytes reversibly against hypotonic hemolysis. Δ^1-Tetrahydrocannabinol expanded the erythrocyte membrane in a hypotonic medium more effectively than did cannabidiol.

Δ^1-Tetrahydrocannabinol is the major psychoactive compound of hashish[1]. Cannabidiol, however, is not psychoactive but serves as a precursor in the synthesis and probably in the biogenesis of Δ^1-tetrahydrocannabinol in *Cannabis sativa* L.[1]. The structural formulae of these compounds are as follows:



Δ^1-Tetrahydrocannabinol



Cannabidiol

It is known that at low concentrations a variety of lipid-soluble compounds, such as anaesthetics, fatty acids, steroids and detergents stabilize several types of membranes[2]. The stabilization of erythrocytes against hypotonic hemolysis is of particular interest[2,4,9-11].

The clinical potency of phenathiazine tranquilizers correlated with their stabilizing potency against hypotonic hemolysis[3]. Since the mode of action of the hashish compounds is not known, it was of interest to study their interaction with erythrocyte membranes[5], as an approach to study on nerve cells.

Experiments were conducted at room temperature (20–21°C). Human blood was drawn from fasting adult males and stock suspensions of washed red blood cells were prepared as described[6]. For osmotic fragility measurements, $6 \cdot 10^7$ cells were added with rapid mixing to a 5-ml solution of 69 mM NaCl containing 2 mM sodium phosphate (pH 7.2) and the tested hashish component. After incubation for 10 min, the suspensions were centrifuged at 2000 × *g* for 5 min, and the supernatant was analysed for hemoglobin content (Klett-Summerson colorimeter, 54 filter). For the control, without hashish components, 65% of the cells were hemolyzed under these conditions. Δ^1-Tetrahydrocannabinol and cannabidiol were kindly supplied by Prof. R. Mechoulam from the School of Pharmacy, the Hebrew University, Jerusalem.

Fig. 1 shows that both Δ^1-tetrahydrocannabinol and cannabidiol exert antihemolytic effects at similar concentrations to those of tranquilizers[2]. Δ^1-Tetrahydrocannabinol is more effective in stabilizing erythrocytes at the lower concentration range. Unlike cannabidiol, which caused the commonly found biphasic effect (decreasing stabilization at superoptimal concentrations), the stabilization by Δ^1-tetrahydrocannabinol was apparent at even the highest concentrations, up to 10^{-4} M. Δ^1-Tetrahydrocannabinol affected rat erythrocytes similarly, as reported by Chari-Bitron[7].

The reversibility of the stabilization effect by the hashish compounds (added at

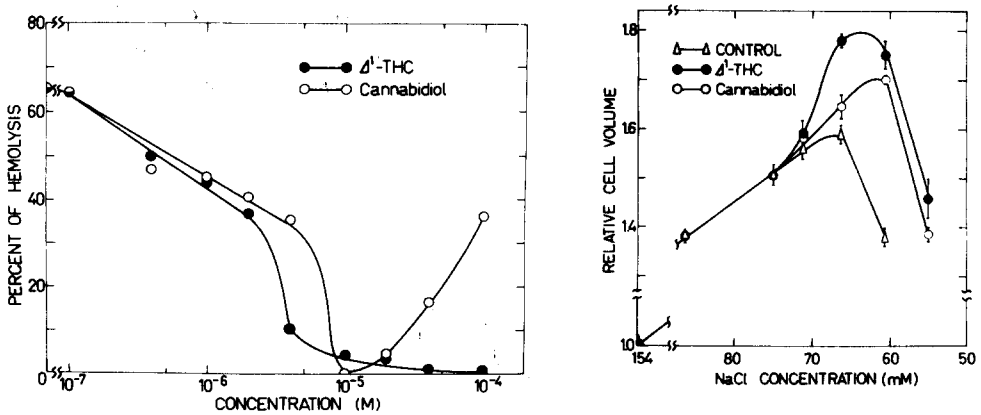


Fig. 1. Osmotic fragility of human erythrocytes as affected by hashish components. Essentially the same results were obtained in six additional experiments. Δ^1-THC = Δ^1-tetrahydrocannabinol.

Fig. 2. Relative cell volume of human erythrocytes as affected by medium tonicity and hashish components (10^{-4} M) and corrected for hemolysis[14]. The bars represent the full range of variation obtained in six experiments.

10^{-5} M) was tested after cycles of washing and centrifugation[3]. A single cycle reduced the stabilization effect of both Δ^1-tetrahydrocannabinol and cannabidiol by 50% and a second cycle eliminated it altogether. In contrast, Chari-Bitron[7] indicated that the binding of Δ^1-tetrahydrocannabinol to the rat erythrocyte membrane was irreversible.

The reduced osmotic fragility of erythrocytes caused by anaesthetics has been related to a drug-induced expansion of the membrane area[3], the membrane expansion being characterized by an increase in the critical hemolytic relative volume (V_c)[9]. Hence we studied the relative volume of human erythrocytes, as affected by the hashish components. The procedure for these experiments was as described[8]. Fig. 2 shows that while the added components did not affect the cell volume in an isotonic NaCl medium, they did increase the critical hemolytic value. Significantly, Δ^1-tetrahydrocannabinol elevated the V_c value more than cannabidiol.

Several factors may account for the greater membrane expansion by Δ^1-tetrahydrocannabinol: Δ^1-Tetrahydrocannabinol is more hydrophobic than cannabidiol, which favors its interaction with hydrophobic regions of the membrane. Furthermore, the structure of Δ^1-tetrahydrocannabinol is planar (the rotation around carbon 3 is not feasible as it is in the cannabidiol molecule), thus occupying a greater area and enabling the greater membrane expansion by Δ^1-tetrahydrocannabinol. These factors, in turn, may also contribute to the psychomimetic potency of Δ^1-tetrahydrocannabinol. It may be significant that the effective concentrations of the drug in stabilizing erythrocytes (Fig. 1), correlate with the doses leading to psychomimetic reactions in hashish smokers[12,13].

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