## Changes in rat erythrocyte membrane induced by $\Delta^1$-tetrahydrocannabinol, scanning electron microscope study

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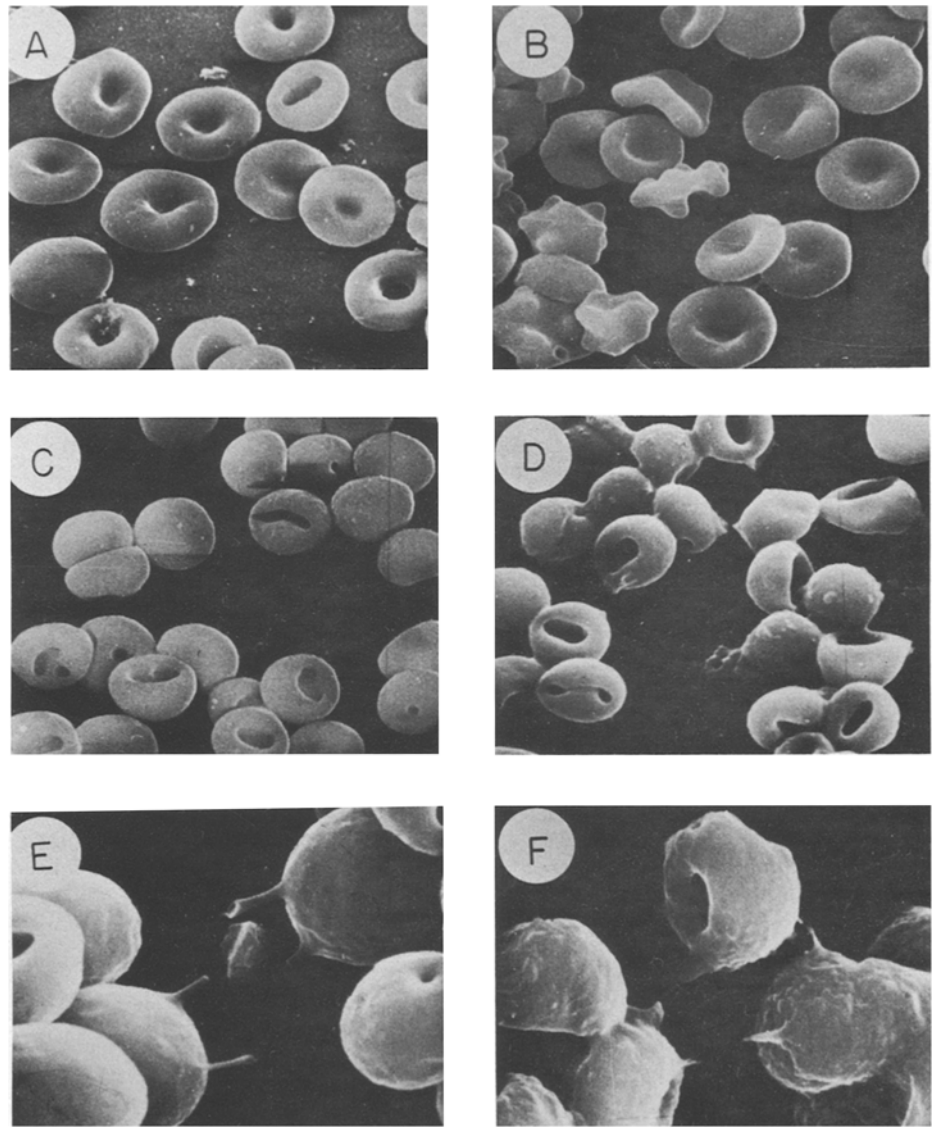
*Summary.* Although some biochemical dose-dependent effects are revealed in erythrocytes exposed to $\Delta^1$-tetrahydrocannabinol (THC) already at concentrations well below 10 µM, marked morphological changes of the erythrocyte membrane become evident, by scanning electron microscopy, only at THC concentrations beyond 15 µM. These observations provide evidence additional to previous chemical and physical studies, in which 15 µM is found to be a critical concentration with respect to the effects of TCH on erythrocyte membrane.

Previous studies have shown that TCH has various pronounced dose-dependent effects on red blood cells in vitro already at concentrations below 10 µM: a) protection against hypotonic hemolysis[2], b) inhibition of $K^+$-influx and c) inhibition of ($Na^+$-$K^+$) ATPase[3]. These 3 dose-dependent curves exhibit a rather abrupt change in slope at a THC concentration of about 15 µM. Furthermore, when measuring the adsorption of THC to human erythrocyte membrane, a marked drop (by a factor of 2) in the dose-dependent curve for the erythrocyte-membrane/buffer partition coefficient[4] has been demonstrated in the vicinity of about 15 µM.

In view of this data, it seemed of interest to examine the dose-dependent effects of THC on the morphology of the exposed erythrocytes. The observations were carried out with the aid of a scanning electron microscope.

*Materials and methods.* Blood from male rats, average weight 150 g each, was drawn into heparin, and the buffy coat was removed from the blood by centrifugation. The erythrocytes were washed twice in 10 µM phosphate-buffer saline (pH 7), centrifuged (3000 × g/5 min) and resuspended in the same medium, giving a hematocrit of about 20%. A portion (0.1 ml) of this blood cell suspension was added to a series of test tubes, each containing 0.9 ml of buffered




Scanning electron micrograph of red blood cells exposed to THC. A: Untreated red blood cells. × 3300. B: Erythrocytes exposed to 13 M THC. Note: some crenated cells. × 3300. C and E: Deformed erythrocytes after exposure to 15 M THC. Note: roughness of cell membrane; interconnected crater-like invaginations and tube-like spikes. C: × 3300; E: × 5650. D and F: Erythrocytes showing pronounced damage after exposure to 35 M THC. D: × 3300; F: × 5450.

saline as well as different desired amounts of THC. The THC was initially dissolved in ethanol, and portions of this stock solution were added to the saline to yield final concentrations in the range of 0–35 µM. Ethanol alone in saline served as control, at a concentration not exceeding 0.5% in each tube. The cells were incubated at room temperature for 1 h.

In the preparation for the scanning electron microscope observations, the cells, after settling, were fixed in 2.5% glutaraldehyde for 30 min. Dehydration was accomplished in increments of ethanol and finally in acetone. The cells were dried by the critical point method[5] in a Polaron apparatus using liquid $CO_2$, attached to cover glasses previously coated with Polaroid fixative, and finally coated with 150–250 Å of gold (Polaron spattering unit). Observations were made using an ISI Superminiscan II at 15 kV.

*Results and discussion.* The scanning electron micrographs (figure, A–F) demonstrate no significant difference in cell membrane structure between untreated erythrocytes (figure, A) and those exposed to 10 µM THC. Only at a concentration of 13 µM THC, some of the cells become crenated (figure, B), while the majority still preserve their normal biconcave shape. At a concentration of 15 µM THC (figure, C and E), almost all the cells become invaginated, having one large crater. In some cells, the crater edges fuse at 1 or 2 points, producing small disconnected or interconnected cavities. Furthermore, the normally smooth cell surface becomes rough, and a few unequally distributed tube-like spikes are seen. At the dose of 35 µM THC, the changes remain essentially the same (figure, D and F) but become more pronounced.

These morphological observations provide additional evidence for the concept that 15 µM THC is a critical concentration with respect to the effects of THC on erythrocyte membrane. The mechanism underlying the extensive functional and morphological changes at this particular concentration is unknown. Presumably, some lipids of the membrane that are essential for preserving its configuration are primarily affected by the highly lipid-soluble THC, leading beyond a certain concentration to deformation of the entire membrane structure.

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## Electrophysiological actions of chlorimipramine on guinea-pig ventricular fibres

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*Summary.* Chlorimipramine (CMI, $1\times10^{-5}$M to $7\times10^{-5}$M) decreased the amplitude, overshoot and rate of rise of ventricular action potentials and abolished the Ca-mediated action potentials elicited in guinea-pig papillary muscles. These results indicates that CMI inhibits the rise in sodium and calcium conductances during the cardiac action potential.

Cardiovascular complications following the administration of tricyclic antidepressants are well-documented, both in accidental overdosage[1,2], and during chronic therapy of depressed patients[3–5]. However, little information is available on the effects of these drugs on the electrophysiological properties of isolated ventricular fibres. The present paper was undertaken to evaluate the effects of chlorimipramine (CMI) on the transmembrane potentials of isolated guinea-pig ventricular fibres.

*Methods.* Right ventricular papillary muscles from guinea-pig hearts were perfused with oxigenated and warmed (34 °C) Tyrode solution and stimulated at a basal rate of 60/min. Transmembrane potentials were recorded through glass microelectrodes filled with 3 M KCl (resistance 15–40 MΩ). The rate of rise of the action potential (dv/dt) was measured by electrical differentiation. Calcium-mediated action potentials were elicited by adding isoproterenol ($1\times10^{-6}$M) to high K (27 mM) Tyrode solution[6]. Statistical analisis was performed by using Student's t-test for paired data.

*Results.* The effects of CMI in concentrations between $1\times10^{-7}$ M (0.04 µg/ml) and $7\times10^{-5}$ M (25 µg/ml) were studied in ventricular fibres. Control values of the measured parameters and results obtained after 30 min exposure to the drug are summarized in the table. Results obtained with concentrations of less than $1\times10^{-5}$ M were omitted from this table, because they were not significantly different from the controls. At concentrations between $1\times10^{-5}$ M and $7\times10^{-5}$ M, CMI produced a concentration-dependent decrease on the amplitude, overshoot and the rate of rise of the action potential. No change was observed in the resting membrane potential. But the most apparent change produced by CMI was an acceleration in the repolarization process. The slope of phase 2 increased and

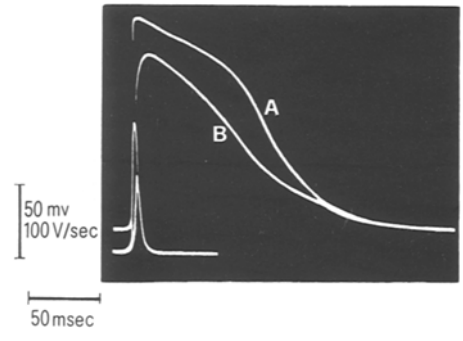


Fig. 1. Influence of high concentrations of CMI ($7\times^{-5}$ M, 30 min) on the action potential characteristics of ventricular fibres. Action potentials in control conditions (A) and in the presence of CMI (B) are superposed. The rate of rise (dv/dt) is shown below the action potentials.