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, 1×10^{-5}M to 7×10^{-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 (1×10^{-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 1×10^{-7} M (0.04 µg/ml) and 7×10^{-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 1×10^{-5} M were omitted from this table, because they were not significantly different from the controls. At concentrations between 1×10^{-5} M and 7×10^{-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

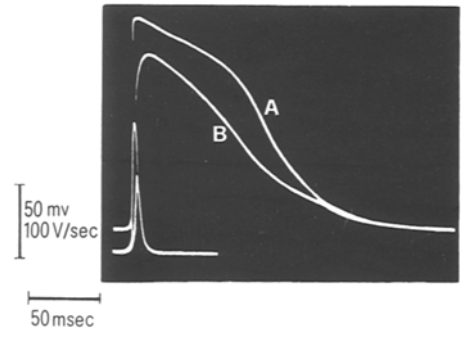

Fig. 1. Influence of high concentrations of CMI ($7\times^{-5}$ M, 30 min) on the action potential characteristics of ventricular fibres. Action potentials in control conditions (A) and in the presence of CMI (B) are superposed. The rate of rise (dv/dt) is shown below the action potentials.

Electrophysiological effects of chlorimipramine)mean ± SEM)

	Resting potential (mv)	Overshoot (mv)	Amplitude (mv)	dv/dt (V/sec)	APD_{50} (msec)	APD_{90} (msec)
Control (8)	88.2 ± 1.3	40.0 ± 3.2	128.2 ± 3.1	198.4 ± 6.0	116.9 ± 3.7	157.3 ± 6.0
CMI, 1×10^{-5} M	87.6 ± 1.6	32.2 ± 1.5*	119.1 ± 2.6*	180.8 ± 5.3*	93.0 ± 7.3*	130.3 ± 5.5*
Control (10)	87.6 ± 2.5	39.5 ± 2.4	126.6 ± 1.6	184.0 ± 7.6	114.5 ± 8.5	149.7 ± 6.2
CMI, $3{,}5 \times 10^{-5}$ M	87.7 ± 2.3	23.5 ± 1.6***	110.3 ± 3.9***	141.5 ± 8.0**	74.2 ± 8.4**	122.5 ± 8.6*
Control (10)	89.5 ± 1.4	37.5 ± 1.2	127.0 ± 2.0	197.5 ± 6.7	111.5 ± 6.7	150.5 ± 5.5
CMI, 7×10^{-5} M	89.8 ± 3.0	8.5 ± 4.5***	98.6 ± 2.5***	115.2 ± 8.6***	70.0 ± 3.1***	146.0 ± 8.6

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The numbers in parentheses represent the number of experiments.

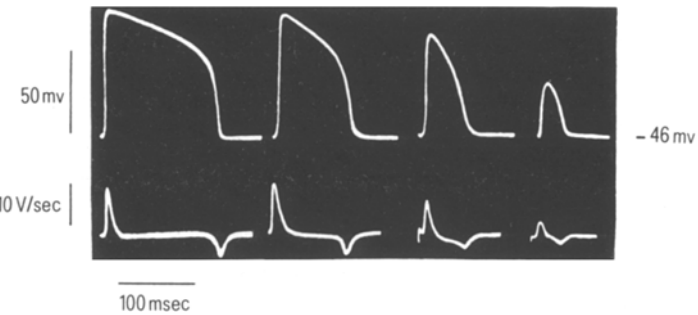

Fig. 2. Effect of CMI on Ca-mediated action potentials elicited by adding isoproterenol (1×10^{-6} M) to high potassium (27 mM) Tyrode solution. A: control. B and C: after 10 and 30 min, respectively, in CMI, 1×10^{-5} M. D: after 30 min in CMI, 3.5×10^{-5} M.

the onset of phase 3 occurred earlier which led to a decrease in the duration of the measured action potential at 50% level of repolarization (APD_{50}). The action potential duration measured at the 90% of repolarization (APD_{90}) decreased at concentrations between 1×10^{-5} M and 3.5×10^{-5} M. A similar shortening was observed during the first 10 min of exposure to higher concentrations of the drug (7×10^{-5} M); exposure to CMI beyond 12–15 min resulted in a lengthening of the final portion of phase 3 so that even though there was proportionately faster repolarization during earlier phases, the (APD_{90}) did not shorten significantly (figure 1).

The electrophysiological changes produced by concentrations up to 1×10^{-5} M were completely reversible within 30–60 min of perfusion with drug-free Tyrode solution. At higher concentrations the changes were only partly reversible.

To obtain information on the change in Ca-conductance during the cardiac action potential, the effects of CMI were studied on the so-called Ca-mediated action potentials. Figure 2 shows one of these experiments. In A the resting membrane potential fell to −46 mv (−45.1 ± 2.5 mv, n = 6) after increasing the external potassium concentration to 27 mM and Ca-action potentials were induced by adding isoproterenol (1×10^{-6} M). CMI, 1×10^{-5} M, did not alter the resting potential but reduced the amplitude, duration and rate of rise of the Ca-action potentials (figure 2, B and C). Higher concentrations 3.5×10^{-5} M, nearly abolished the Ca-action potentials (figure 2, D). These changes were reversible after washing out for 40–60 min.

Discussion. Transmembrane potentials of ventricular muscle fibres were studied during perfusion with CMI. The effects produced by concentrations up to 1×10^{-5} M (4 µg/ml) comprised the observed therapeutic plasma levels (30–300 ng/ml)[7,8] whereas the higher concentrations used in this study could reproduce the effects of toxic plasma levels on heart muscle. However, it is very difficult to relate in vivo plasma levels to the concentration of CMI perfusing isolated preparations of cardiac tissue, because it is known that certain effects of tricyclic compounds appear only after repeated doses administered over days and weeks.

CMI decreased the amplitude, overshoot and rate of rise of the ventricular action potentials in the absence of any change in the membrane resting potential. These effects are similar to those produced by drugs having local anesthetic properties and have been attributed to a drug-induced interference with the increase in the sodium conductance during the phase 0 of the action potential[9].

The effects of CMI on the duration of the action potential are not easy to explain, since the ionic mechanisms responsible for the different phases of repolarization are still uncertain[10]. The plateau of the action potential in muscular fibres is mainly determined by the inward flow of Ca-ions[10–12]. Therefore, the loss of the plateau in the presence of CMI is suggestive of a decrease on the Ca-inward movement. In order to test this hypothesis, the effect of CMI was studied on Ca-action potentials. The dv/dt of these action potentials was reduced by CMI, indicating that it inhibits the rise of the Ca-conductance during the ventricular action potential.

The prolongation of the late phase of depolarization induced by the highest concentration of the drug suggests that CMI might have a depressant effect on the K-conductance responsible for the late outward current in guinea-pig ventricular fibres[13].

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