Effects of octylguanidine on the electrical activity of *Xenopus* heart

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Summary. The effects of laboratory synthesized octylguanidine are described. There is an early decrease of the maximum rate of rise of the action potential with negligible reduction of the overshoot although the membrane resting potential is unchanged. Subsequently, there is a remarkable reduction of both membrane potential and overshoot, while the plateau is shortened. The former effects resemble those seen with tetrodotoxin, the latter ones, with metabolic poisons.

Alkylguanidines interfere with a number of biological processes. For instance, on isolated mitochondria they depress respiration and Ca^{++}-induced adenosine triphosphatase activity[2], depress oxidative phosphorylation[3], and compete with Na^+ in mitochondria[4]; in barely root cells they inhibit K^+-transport, not as much through a depression of the energy transducing systems as through a direct action on the membrane permease[5]. Since most of the reported effects of alkylguanidines are on metal ion-dependent systems, both in intact cells and isolated organules[3,5-8], we thought it would be interesting to investigate the action of an alkylguanidine, the octylguanidine, on the electrical activity of the cardiac cell where the movement of metal ions is bidirectional and both active and passive. In such a system it ought to be possible to decide if the drug has an action on the surface membrane and/or intracellular sites.

Methods. Atrial muscle of *Xenopus laevis* (Daud.) was perfused in a 5-ml lucite chamber at the rate of about 5 ml/min with a Ringer solution containing 88.1 mM NaCl, 1.8 mM KCl, 1.08 mM $CaCl_2$, 23.8 mM $NaHCO_3$, 2 mM tris-maleate-NaOH buffer of pH 7.4 and 3 mM glucose per l at a temperature of 24 °C. The fluid was oxigenated and carbonated with a mixture of 95% O_2 and 5% CO_2 resulting in a pH near 7.4. The tissue was stimulated at the rate of 1/sec through 2 isolated suction electrodes. Conventional microelectrode recording was used. Octylguanidine, in the salt form of octylguanidinium sulfate, was synthesized by Prof. M. Bellando, Istituto di Fisiologia Vegetale, Orto Botanico, Università di Torino, Italy.

Results. Octylguanidine reduced both excitability and contractility; stimulus intensity had to be increased throughout each experiment, while contraction strength visibly decreased. At a concentration of 4.4×10^{-4} M the electrical and mechanical activity ceased after 40 min, and at a concentration of 2.2×10^{-4} M after about 70 min. At both concentrations, during the initial period of treatment (5 min), the overshoot decreased slightly at unchanged resting potential, while the upstroke of the action potential was greatly modified (figure, trace a). Even in untreated preparations[9] 2 phases of the upstroke were apparent (figure, trace d). There was a 'break' or a 'notch' near zero potential separating the fast phase (dVmax/dt = 37.5 V/sec) and the slower one. The maximum depolarization rate was reduced to 7.2 V/sec and the position of the notch was lowered toward more negative values (−17.0 mV) in trace e. At this initial stage of poisoning the effects were reversible by washing in drug-free solution.

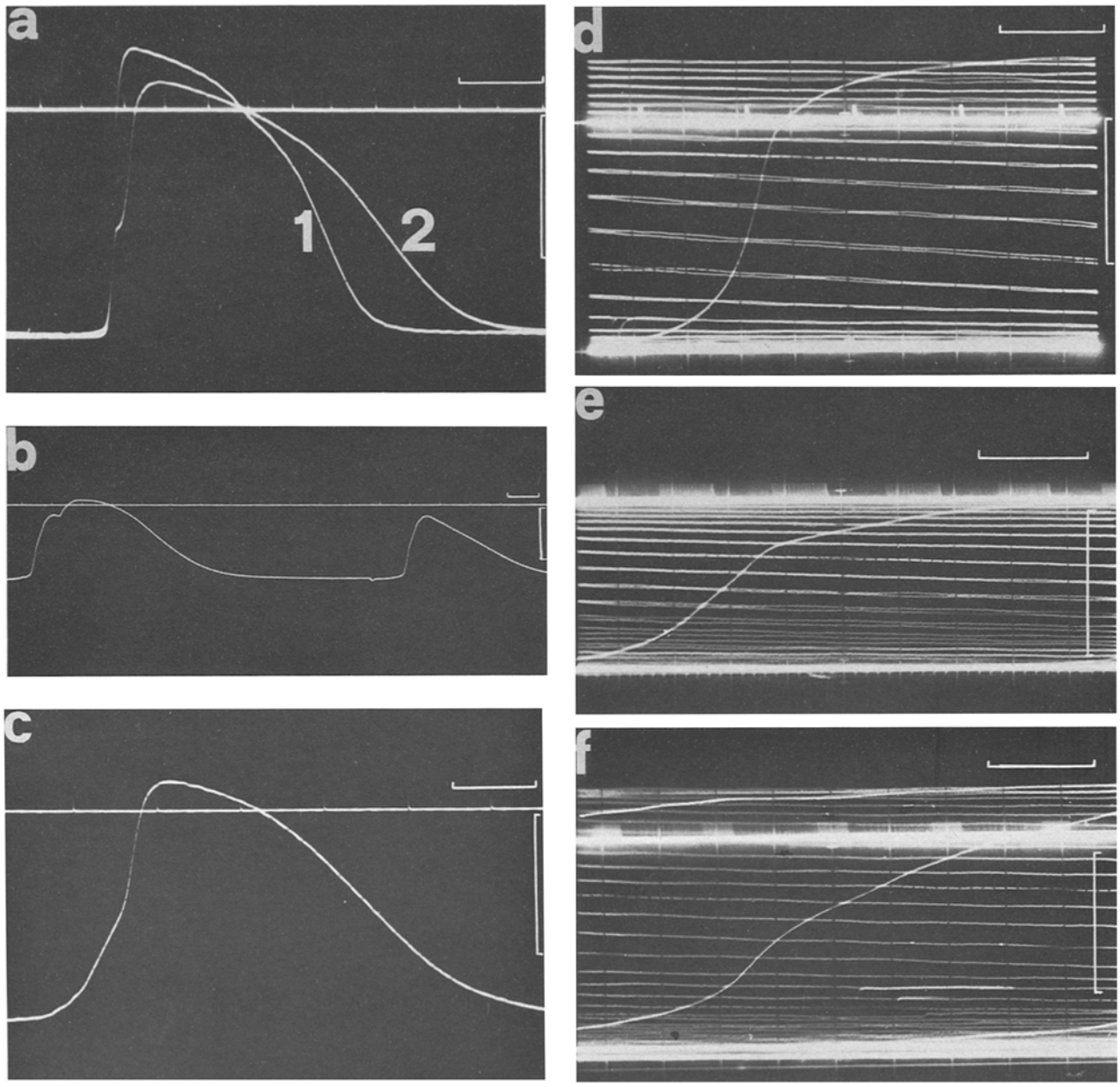
Upon prolonged application of octylguanidine membrane and action potentials progressively diminished until the muscle became unexcitable. During this transition, progressively smaller action potentials could be observed. Occasionally, a sequence of small action potentials accompanied by longer-lasting ones with a large notch in their upstroke could be recorded (trace b). In certain aspects, the action of the octylguanidine resembled that of metabolic inhibitors[10]. However, the administration of isoprenaline 4.5×10^{-5} M for 3 min after the arrest of the preparation, always restored the mechanical activity and produced action potentials with normal overshoots and resting potentials. This suggests the prompt availability of metabolic energy for contraction and excludes an irreversible impairment of the cellular energy transducing systems. The early action potentials, recorded upon the recovery of activity were transitorily preceded by a slow prepotential-like depolarization, visible even at slow recording speed (trace c). The high speed recording provided better evidence for this finding and showed an initial slow depolarization (dVmax/dt = 2.0 V/sec), followed by a faster one (dVmax/dt = 13.3 V/sec) (trace f), producing a picture exactly opposite to that of the normal upstroke, where the maximum depolarization rate is reached in the initial phase (trace d).

Discussion. The decrease of upstroke velocity without a concomitant fall of resting potential is reminiscent of the action of tetrodotoxin, the latter being also a guanidine derivate[12]. The shift of the notch level to more negative potentials and the decrease of fast inward current might be due to several processes: a) partial blockade of Na^+ channels without alteration of the kinetics, b) slowed activation and c) more rapid inactivation of Na^+ channels. Conduction block would be expected to occur when the fast depolarization becomes too small to activate the slow current, whose threshold is reached at about −40 mV[13,14]. It is thought[9,15] that the notch represents the transition between the fast and the slow component of inward current. Action potentials as in the figure b, showing a pronounced 'notch' or even a 'dip' would be typical for the case in which depolarization by the fast inward current can barely activate the slow current.

The late effects of octylguanidine show all characteristics of metabolic poisons: decrease of resting potential and overshoot, shortening of the action potential and a further decrease of upstroke velocity, most likely due to a lowered resting potential[11]. Thus, octylguanidine would appear to have a double biological action: an initial action on the electrical properties of the cell membrane and a late action on cellular metabolism. This latter action is in agreement with results obtained by other methods[2,3,6]. The fact that the substance takes a relatively long time to develop this latter action may mean either that it has to cross the cell membrane and act on an intracellular site or that the late effect does not become apparent until intracellular concentrations of ions have had time to change.

While isoprenaline almost instantaneously restores electrical activity it does not completely reverse the octylguanidine effects on the action potential. Typically, the first propagated action potentials show a step-like prepotential, then a relatively long-lasting 'foot' in their upstrokes. In amphibian atria catecholamines increase the slow inward current and lower its activation threshold[16]. Thus, it may be argued that the immediate effects of isoprenaline are 2-fold: a) to increase slow inward current to a level sufficient for the action potential to propagate and b) to increase the resting membrane potential.

To account for the negative inotropic effect of octylguanidine 2 different mechanisms may be invoked: a) a shortening of the action potential and b) an impairment of metabolism. The simultaneous recovery of electrical and mechanical activity brought about by isoprenaline does not



Action potentials of *Xenopus* atrial muscle. *a* Ringer solution (record 1) and 8 min octylguanidine (record 2); *b* 25 min octylguanidine; *d* and *e* are records taken at higher speed; *d* is control and *e* is obtained after 27 min in octylguanidine; *c* 5 min isoprenaline recovery, *f* same at higher speed. Voltage calibration: vertical bar = 50 mV. Time calibration: horizontal bar = 100 msec for *a, b* and *c;* 5 msec for *d, e* and *f.*

clarify the relative importance of the 2 mechanisms but indicates that cardiac preparations poisoned by octylguanidine still have a metabolic reserve readily available to furnish energy for the contractile process.

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