Different Blocking Effects of Cd^{++} and Hg^{++} on the Early Outward Current in Myocardial Mouse Cells

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Abstract. The effects of external Cd^{++} and Hg^{++} on the early outward current (I_{EO}) in myocardial mouse cells were studied using voltage clamp with one suction pipette. Both Cd^{++} in the millimolar and Hg^{++} in the micromolar range shifted the half time to peak I_{EO} to positive potentials more than the current amplitude after 50 ms. Incomplete blocking of I_{EO} could be obtained by Cd^{++} concentrations between 10^{-3} and 2×10^{-2} mol/l and by Hg^{++} concentrations between 10^{-6} and 5×10^{-5} mol/l. The current-voltage relationships of I_{EO} at peak time and at 50 ms were mainly shifted to the right by Cd^{++} whereas Hg^{++} mainly decreased the slope. At incomplete blocking concentrations Cd^{++} slowed down I_{EO} activation more strongly than did Hg^{++}. All Cd^{++} effects were completely reversible. The action of Hg^{++} was irreversible. It is concluded that both ions act directly on the gating machinery of the channels rather than simply binding to homogenous surface charges.

Key words: Myocardial mouse cells — Voltage clamp — Early outward current — Cd^{++} — Hg^{++}

Introduction

In the heart muscle, transient (early) outward currents carried mainly by K^+ and not activated by Ca^{++} have been described in several single cell preparations: in crista terminalis cells (Giles and VanGinneken 1985) and in atrioventricular node cells (Nakayama and Irisawa 1985) of the rabbit as well as in ventricular cells of the rat (Josephson et al. 1984) and the mouse (Benndorf and Nilius 1988). In ventricular mouse cells the early outward current which is responsible for the rapid repolarization of the action potential can be activated by depolarizing pulses to potentials positive to −30 mV. This current could be characterized without blocking Ca channels because in this preparation, the Ca current is very small in amplitude. Hence, these cells can be used to investigate the influence on

the early outward current of blockers which may have an additional effect on Ca channels.

The actions of heavy metal ions on ionic currents in excitable tissue have been described in the squid giant axon (Gilly and Armstrong 1982a,b), myelinated nerve fibers (Arhem 1980), the lobster axon (Blaustein and Goldman 1968), and in heart muscle fibers (Takahashi et al. 1958). Here we report different effects of the divalent cations Cd^{++} and Hg^{++} on the early outward current in ventricular mouse cells as described in detail by Benndorf and Nilius (1988).

Materials and Methods

Myocytes were isolated from adult mouse hearts as described previously (Benndorf et al. 1985). The cells were stored at room temperature in Tyrode solution containing (in mmol/l): NaCl 150, KCl 5.4, $MgSO_4$ 0.4, $CaCl_2$ 2.5, glucose 11.1, HEPES 5, pH 7.4. A small amount of the cell suspension (0.1 ml) was given to the experimental chamber containing Tyrode solution plus 10^{-4} mol/l TTX. The cells were sucked to the pore (6–8 μm) of a fire polished glass pipette and voltage clamped (see Benndorf et al. 1985). The solution continuously flowing inside the pipette contained (in mmol/l): KH_2PO_4 52, K_2HPO_4 65, NaH_2PO_4 13, HEPES 10, EGTA 2, pH 7.1. All experiments were performed at 36 ± 0.5 °C. Current traces were either photographed from a storage oscilloscope (OG 31 Messelektronik Berlin) or fed after A/D conversion (8 bit, sampling rate 4 kHz) to a MC 80 microcomputer (Elektronik Gera) and stored on tape. The sampled currents were corrected for linear capacitive and leakage components by subtracting an averaged current trace obtained by five hyperpolarizing pulses of 20 mV.

Results

Fig. 1*A* shows the action of 10 mmol/l Cd^{++} on I_{EO} for test pulses up to +50 mV. The rapidly activating and incompletely inactivating currents under control conditions (top) are slowed down in their activation time course by the heavy metal ion and they loose their transient character. Panel B (Fig. 1) illustrates the current-voltage relations of the peak I_{EO} of the same cell at control conditions and at 2×10^{-3}, 5×10^{-3}, and 10^{-2} mol/l Cd^{++} in the bathing solution. The heavy metal ion alters the curve by (i) shifting towards positive potentials and (ii) slightly flattening it. If the shift to positive potentials is caused by fixed surface charges, the activation kinetics and the potassium conductance at equilibrium should be shifted by equal amounts (Blaustein and Goldman 1968; Hille 1968). Fig. 1*C* illustrates current-voltage characteristic of the currents in A at the end of the test step (50 ms). Although by that time steady state is not fully reached the current amplitudes should also show identical shifts as does activation kinetics if due to surface charge effects. Cd^{++} concentration of

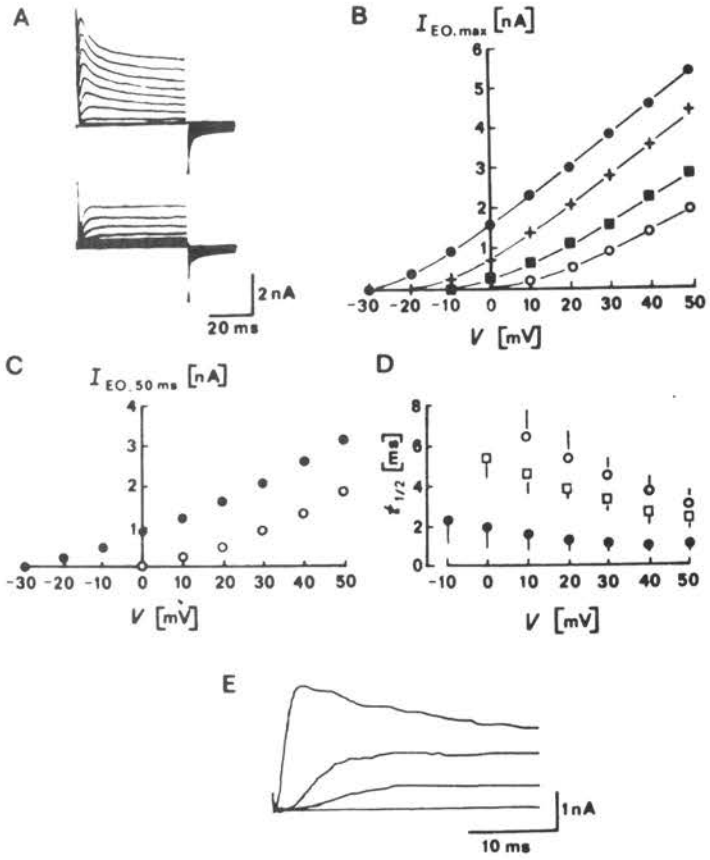

Fig. 1. Action of Cd^{++} on I_{EO}. *A*: A family of I_{EO} under control conditions (*top*) and at 10^{-2} mol/l Cd^{++} (*bottom*); $V_h = -50$ mV; V_T -40 to $+50$ mV step 10 mV; leakage compensated; cell 120285-6. *B*: Current-voltage characteristics of $I_{EO,max}$ under control conditions (filled circles), at 2×10^{-3} (crosses), at 5×10^{-3} (squares), and at 10^{-2} (open circles) mol/l Cd^{++}. At 10^{-2} mol/l Cd^{++} the maxima of currents were evaluated. The curves have been fitted by eye; same cell. *C*: Current-voltage characteristics of I_{EO} 50 ms after the beginning of the test pulse under control conditions (filled circles) and at 10^{-2} mol/l Cd^{++} (open circles); same cell. *D*: Half time ($t_{1/2}$) to peak or to current maximum, respectively, as the function of voltage under control conditions (filled circles), at 5×10^{-3} (squares), and at 10^{-2} mol/l Cd^{++} (open circles, mean of 3 cells, bars indicate SD). *E*: Effects of two Cd^{++}-concentrations on the time course of I_{EO} (top: control; middle: 5×10^{-3} mol/l; bottom: 10^{-2} mol/l), $V_h = -50$ mV; $V_T = +20$ mV. Similar traces corrected for capacitive and leakage components were used to determine $t_{1/2}$ in *D*. Cell 180285-8.

10^{-2} mol/l shifts this current-voltage relation to the right by about 30 mV leaving the slope unchanged. The same was observed in four other cells. The slowing down of the activation phase was evaluated from current traces correc-

ted digitally for capacitive and leakage components as described in Methods. Fig. 1*E* shows such I_{EO} traces obtained from a single cell under control conditions and at 5×10^{-3} and 10^{-2} mol/l Cd^{++}, respectively. At the same test potential (+20 mV) the enormous slow down of activation is quite obvious. The speed of activation was quantitated simply by the half time ($t_{1/2}$) to peak or to maximum current, respectively. Fig. 1*D* shows the dependence of the $t_{1/2}$ values on voltage under control conditions and at both concentrations of the heavy metal ion. I_{EO} activation is slowed at increased Cd^{++} at all potentials and the shift along the voltage axis at 10^{-2} mol/l Cd^{++} considerably exceeds 30 mV observed for $I_{EO,\,50\,ms}$. This suggests that external Cd^{++} acts not only by binding to uniform surface charges but also by modifying the gating apparatus of the channel as supposed for the effects of heavy metal ions on the K current in the squid giant axon (Gilly and Armstrong 1982b). All the Cd^{++} effects were found to be completely reversible.

The effect of Hg^{++} on I_{EO} was tested at concentrations between 10^{-6} and 10^{-4} mol/l. Fig. 2*A* shows the influence of 5×10^{-6} mol/l Hg^{++} on the current traces. At all potentials the amplitude is diminished but the slowing is not very pronounced. The current-voltage relation of the peak current (panel B) reveals only a decrease in slope and nearly no shift along the voltage axis. Concentrations higher than 5×10^{-6} mol/l Hg^{++} were not considered because the corresponding currents kept increasing after 50 ms (cf. panel E) and the peak amplitude could not be measured. 10^{-4} mol/l Hg^{++} abolished all I_{EO} up to +50 mV. Panel C (Fig. 2) illustrates the current-voltage characteristics of I_{EO} at 50 ms for control and for 10^{-5} mol/l Hg^{++}. In contrast to Cd^{++} the effect of Hg^{++} is dominated by a decrease in the slope which is accompanied only by a slight shift. Similar effects were observed in three other cells. Fig. 2*E* shows corrected current traces under control conditions, and at 10^{-6}, and 10^{-5} mol/l Hg^{++}, respectively. All the currents shown were obtained from the same cell. The higher concentration was added to the lower one because all Hg^{++}-effects proved to be irreversible. The current at 10^{-5} mol/l Hg^{++} lost its transient nature and its amplitude even increased after 30 ms. Though Hg^{++} at these micromolar concentrations considerably suppressed I_{EO}, the activation seemed to be influenced to a lesser extent. Pantel D illustrates the dependence of $t_{1/2}$ on voltage (mean values of three cells). A shift to the right by about 30 mV can be seen at 10^{-5} mol/l Hg^{++}. This is more than the only small shift of the current-voltage relation seen after 50 ms (panel C); this also suggests a more intricate action of Hg^{++} with the channel than simple binding to surface charges. At 10^{-6} mol/l Hg^{++}, $t_{1/2}$ remained unaltered.

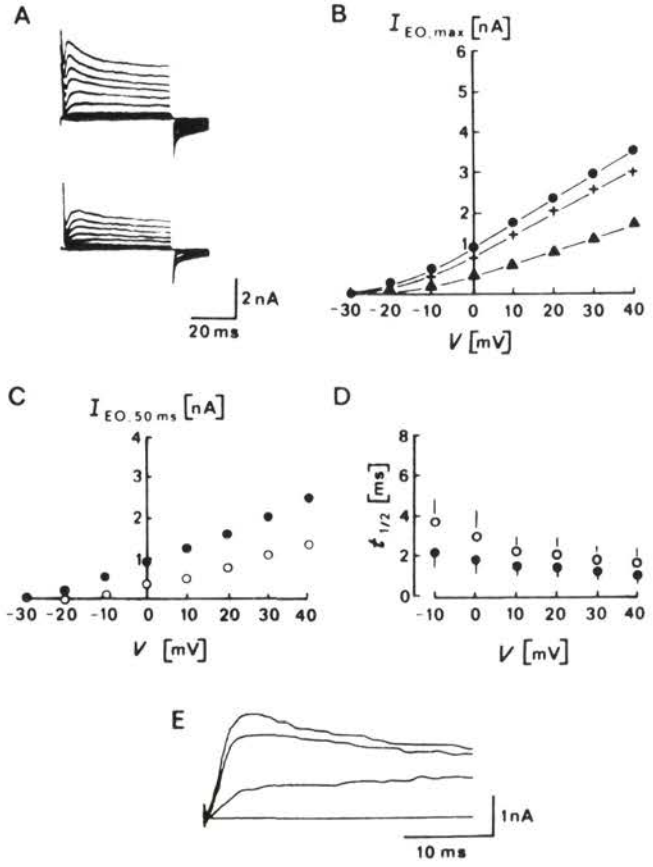

Fig. 2. Action of Hg^{++} on I_{EO}. *A*: A family of I_{EO} under control conditions (*top*) and at 5×10^{-6} mol/l Hg^{++} (*bottom*); $V_h = -50$ mV; $V_T = -40$ to $+40$ mV step 10 mV; cell 250285-3. Current-voltage characteristics of $I_{EO,max}$ under control conditions (filled circles), at 10^{-6} (crosses), and at 5×10^{-6} (triangles) mol/l Hg^{++}. The curves have been fitted by eye; same cell. *C*: Current-voltage relations of I_{EO} 50 ms after the beginning of the test pulse under control conditions (filled circles) and at 10^{-5} mol/l Hg^{++}; cell 180385-6. Half time to peak ($t_{1/2}$) in dependence on potential under control conditions (filled circles) and at 10^{-5} mol/l Hg^{++} (open circles, mean of 4 cells, bars denote SD), *E*: Effects of two Hg^{++}-concentrations on the time course of I_{EO} (*top*: control; *middle*: 10^{-6} mol/l; *bottom*: 10^{-5} mol/l). $V_h = -50$ mV; $V_T = +20$ mV. Cell 250285-9.

Discussion

The major findings of this report are the following.

(a) Cd^{++} and Hg^{++} block I_{EO} in isolated ventricular mouse cells in concentrations similar to those affecting potassium current in squid axons (Gilly and Armstrong 1982b).

(b) In accordance with the findings in squid, both Cd^{++} and Hg^{++} shift the half time of activation to positive potentials more strongly than they shift $I_{EO,\,50\,ms}$; this can be interpreted as a direct action of the ions on the channel gating apparatus.

(c) The blocking mechanisms of Cd^{++} and Hg^{++} differ considerably in many respects. (i) Incomplete blockage occurs between 10^{-3} and 2×10^{-2} mol/l Cd^{++} and between 10^{-6} and 5×10^{-5} mol/l Hg^{++}. (ii) In contrast to Hg^{++}, Cd^{++} does not abolish the transient nature of I_{EO}. (iii) Cd^{++} shifts the current-voltage characteristics of $I_{EO,\,max}$ and $I_{EO,\,50\,ms}$ to the right in a concentration dependent manner, whereas Hg^{++} induces only a flattening of the relation. (iv) In the concentration range inducing incomplete blocking, Cd^{++} considerably slows down the activation kinetics whereas this effect is not as pronounced with Hg^{++}. (v) Cd^{++}-effects are reversible while those of Hg^{++} are not.

The effects of IIB metal ions on K channels in the squid giant axon have been explained on the basis of a model of gating charge movement. This model assumes direct interaction of the blocking ions with a negatively charged element of the gating apparatus that, during activation, moves inward from the membrane outer surface (Gilly and Armstrong 1982b). Since the effects described in this paper are very similar to those reported for the squid giant axon, our results further strengthen the idea of this model.

The substantial differences in the action of both cations may be explained by their binding to different sites. This is supported by the finding of Gilly and Armstrong (1982b) who reported that after treatment of the squid axon with Hg^{++} (which has irreversible effects), Zn^{++} still slowed the opening kinetics of the potassium current. Furthermore, it is known from studies considering the interaction of amino acids with transition metal ions that these ions bind readily to histidine residues; however Hg^{++} binds much more specifically to sulfhydryl groups of cysteine (Eichhorn 1973; Means and Feeney 1971).

On the other hand, the differences in the action of Hg^{++} and Cd^{++} might also be due to the different ionic size: The larger Hg^{++} with a lower charge density could bind to two negatively charged sites of the protein at a time. Assuming some cooperativity for binding, it is the irreversibility of the Hg^{++} action that could be explained in particular in this way. The smaller Cd^{++} then could not bind to two sites at a time.

After the completion of this report the transient outward current of the mouse myocardium has been described to be composed of unitary currents through three types of single K channels (Benndorf et al. 1987, Benndorf 1988). From this point of view it is intriguing to speculate that the differences in the action of Hg^{++} and Cd^{++} can be explained by their blocking different channels. Cd^{++} in millimolar concentrations would then affect more the rapidly activating

channels (27 pS, 5 pS) whereas Hg^{++} in micromolar concentrations would block all types of channels (27 pS, 12 pS, 5 pS) to a similar degree.

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