ANALYSIS OF THE TRANSIENT OUTWARD CURRENT (I_{to}) IN SINGLE DIALYSED RAT VENTRICULAR CELLS

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Single rat ventricular cells were prepared by a modification of the method described by Mitra and Morad (Am. J. Physiol. 1986, vol. 249: H1056–1060). Briefly, rats were anesthetized with pentobarbital and their hearts were rapidly removed to a zero-calcium Tyrode solution. The heart was then mounted in a Langendorff apparatus and perfused for 5 min. at a constant flow with the zero-calcium Tyrodes. After this period, the heart was perfused for 20 min with an enzyme Tyrode solution, containing protease and collagenase. Finally, the tissue was perfused briefly with a 0.2 mM calcium Tyrode solution prior to dispersal of single cells.

The resulting single myocytes (of which approximately 75–80% were viable) were voltage-clamped in 5 mM calcium Tyrode solution (137 mM NaCl, 5.4 mM KCl, 5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.33 mM Na_2HPO_4, 10 mM Hepes, pH 7.4) using the whole cell patch clamp technique, and their internal contents were dialysed with a solution of the following composition: 10 mM NaCl, 120 KCl, 5 mM MgATP, 20 mM Hepes, 14 mM EGTA, 1 mM $CaCl_2$, pH 7.2. EGTA and $CaCl_2$ were added in concentrations designed to buffer the Ca activity at a value lower than the threshold of mechanical activation. When the membrane voltage was held at -100 mV, positive clamp steps in the range of -40 to +40 mV activated increasing amounts of a transient outward current (I_{to}) which displayed outward-going rectification. The amount of this current that could be elicited varied with the holding potential, such that at -40 mV, positive pulses no longer activated any I_{to}. The current also showed a negative relationship with respect to rate of stimulation, at higher rates being much depressed. Addition of 20 mM Cs^+ outside, 20 mM tetraethylammonia or replacement of internal cations with CsCl reduced I_{to}, showing its dependence on potassium. Tetrodotoxin (10^{-5}M) and replacement of sodium by choline also markedly reduced a component of I_{to}. These findings suggest that activation of I_{to} is in part related to the presence of the sodium current. Additionally, it was found that the inorganic calcium channel blockers Co^{2+} (1mM), Cd^+ (200uM–1mM) and Ni^{2+} (2mM) substantially reduced a component of I_{to}. Tetracaine (1mM) completely abolished the current. Whilst 2 mM 4-amino-pyridine blocked nearly all the I_{to}, the remaining current could be abolished by increasing the concentration to 20mM.

Figure 1 illustrates several of these findings. Panel A shows voltage clamp records obtained with a holding potential (HP) of -80 mV. I_{to} was measured as the peak outward current relative to the maintained outward current. When plotted in panel E (open circles) it yielded a curve with a characteristic sigmoid voltage dependency. In panel B the holding potential was changed to -40 mV. This abolished I_{to} as well as the excitatory Na current, leaving mainly the slow inward calcium current (I_{si}) which was plotted in panel F (filled circles). Panels C and D show the effect of Ni, which is known to block I_{si} and shift the activation and inactivation potentials of the excitatory Na current to more positive potentials. With a holding potential of -80 mV (compare panels A and C and the curves based on

open symbols in panel E) it was found that I_{to} was reduced at potentials below 0 mV and enhanced at more positive potentials. If the reduction were related to suppression of I_{si}, the enhancement of higher potentials might then be related to increased Na loading. Panel D is consistent with this idea. A transient outward current was clearly observed even though the holding potential was reduced once again to -40 mV. The separation of I_{to} from I_{si} clearly represents a problem in Fig. 1. Other experiments with a less noticeable I_{si} gave similar changes in I_{to}. Under these conditions, however, I_{si} was measured less reliably.

It appears that while interventions that affect the sodium gradient tend to block I_{to} in the upper voltage range of its activation curve (+20 to +60 mV), the inorganic calcium channel blockers exert the majority of their blocking effects at the lower range of the activation curve (-30 to +20 mV). While such results tend to support the existence of several components to I_{to}, the results are also consonant with the idea that a component of I_{to} is calcium activated, and that reducing the sodium gradient and blocking the calcium influx achieve, by different degrees, a reduction in internal calcium levels.

Figure 1. The effect of Ca^{2+} and holding potential (HP) on the transient outward current (I_{to}) and the slow inward current (I_{si}). Panels A through D show voltage clamp records obtained with and without 2 mM Ni with holding potentials of -80 mV and -40 mV. The upper traces in each panel are membrane current while the lower traces are the voltage clamped membrane potential. The panels are labelled with holding potential, the presence of Ni, if relevant, and the symbol used in the lower graphs. Panel A shows how the I_{to} was measured as the difference between the peak outward current and the maintained outward current. I_{si} was measured only with a holding potential of -40 mV (which presumably inactivates the Na current). The plotted value is the early minimum. Panel E shows the voltage-dependence of I_{to} and panel F the voltage dependence of I_{si}. Details of the figure are described in the text.