Properties of an Early Outward Current in Single Cells of the Mouse Ventricle

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Abstract. Single ventricular myocytes of adult mice were prepared by enzymatic dissociation for voltage clamp experiments with the one suction pipette dialysis method. After blocking the Na current by 10^{-4} mol/l TTX early outward currents (I_{EO}) with incomplete inactivation could be elicited by clamping from -50 mV to test potentials (V_T) positive to -30 mV. Interfering Ca currents were very small (<0.6 nA at $V_T = 0$ mV). The approximation of I_{EO} by the q^4r-model showed a pronounced decrease in the time constant of activation (τ_q) to more positive potentials. At 50 ms test pulses the time course of the incomplete inactivation could be described by two exponentials and a constant. The time constant of the fast exponential (τ_{r1}) showed a slight decline towards more positive test potentials (8.1 $\pm$ 1.0 ms at -10 mV; 5.8 $\pm$ 1.2 ms at $+50$ mV, mean $\pm$ SD, $n = 5$) whereas the time constant of the slow exponential (τ_{r2}) was voltage independent (41.1 $\pm$ 7.9 ms, mean $\pm$ SD, $n = 5$). The contributions of the fast exponential and the pedestal increased towards positive test potentials. The Q_{10} value for the time constants of activation and fast inactivation was 2.36 $\pm$ 0.19 and 2.51 $\pm$ 0.09 (mean $\pm$ SD, $n = 3$), respectively. After an initial delay the recovery of I_{EO} at a recovery potential of -50 mV could be fitted monoexponentially with a time constant of 16.3 $\pm$ 2.9 ms (mean $\pm$ SD, $n = 3$). The time course of the onset of inactivation determined with the double pulse protocol was slower than the decay at the same potential, and could be described as sum of a fast ($\tau = 18.4 \pm 6.0$ ms) and a slow ($\tau = 62.1 \pm 19.9$ms, mean $\pm$ SD, n = 3) exponential. I_{EO} could be blocked completely by 1 mmol/l 4-aminopyridine at potentials up to $+20$ mV. Stronger depolarizations had an unblocking effect.

Key words: Myocardial mouse cells — Early outward current — Activation — Inactivation — 4-Aminopyridine

Introduction

Transient outward currents have been identified in several molluscan neurons (Hagiwara et al. 1961; Connor and Stevens 1971a; Thompson 1977), in skeletal muscle fibres (Ashcroft and Stanfield 1982), and also in egg cells (Hagiwara et al. 1981; Miledi 1982). In the heart, such currents were first described in Purkinje fibres of several species and have been named dynamic outward current (Peper and Trautwein 1968; Fozzard and Hiraoka 1973), transient outward current (Boyett 1981; Coraboeuf and Carmeliet 1982) or Ca-activated transient outward current (Siegelbaum and Tsien 1980). Later, a transient (early) outward current has been described in rat ventricular cells (Josephson et al. 1984), and similar currents have been found recently in the rabbit crista terminalis cells (Giles and Van Ginneken 1985) and atrioventricular node cells (Nakayama and Irisawa 1985). The ionic nature of these currents had been a subject to controversy for a long time. It were but the measurements of single channel currents that convincingly demonstrated, at least for the transient outward current in atrioventricular node cells (Nakayama and Irisawa 1985), that the main charge carrier is the K^+ ion and that Na^+ ions carry a minor fraction of the current.

In the present experiments we used single ventricular myocytes of the mouse heart. This preparation was chosen since the extremely rapid heart rate of this species (520 – 780/min) made us suggest the existence of a pronounced outward current as would be necessary for a sufficiently fast repolarization of the action potential. This suggestion could soon be sustained by the experiments which revealed that the mouse myocardial cell was very appropriate for the analysis of a transient outward current in mammalian heart, since the currents were large and no application of Ca channel blockers to resolve the outward current was necessary. Conclusively, the transient outward current in the mouse ventricular cell is faster than all those described so far, and it most resembles the corresponding current described in rat ventricular cells (Josephson et al. 1984).

Isolation of single heart cells

Heart cells were obtained by enzymatic dispersion as described in detail by Benndorf et al. (1985). Briefly, the heart of adult mouse was quickly removed and mounted on the cannula of a perfusion system for retrograde perfusion through the aorta at a constant flow of 1 ml/min. At first, the heart was washed for three minutes with Tyrode solution containing 2.5 mmol/l Ca^{2+}. This was followed by a 5 min period of perfusion with Ca^{2+}-free solution. After changing to recirculation the heart was perfused with the enzyme solution for 30 min. A 3 min washing period with Ca^{2+}-free solution followed. All perfusions were performed at 37 °C with oxygen-saturated solutions. Then, the ventricles were

cut away and minced in a Petri dish containing a K^+-rich recovery solution (Taniguchi et al. 1981; Isenberg and Klöckner 1982). The resulting suspension was sieved through a nylon mesh (250 μm) and stored at room temperature for 30 min. The cell sediment was finally washed twice with Tyrode solution and stored at room temperature until used for experiments.

The suction pipette technique

The method was essentially the same as described by Benndorf et al. (1985). In brief: A small aliquot of the cell suspension was given to the experimental chamber (volume 0.1 ml) mounted at the stage of an inverted microscope (Zeiss Jena). The chamber contained Tyrode solution. Then, an appropriate cell was sucked to a fire polished glass pipette (tip opening 5—8 μm). Under steady suction (30 cm H_2O) leakage decreased to a steady state level within 4 min. A short suction pulse destroyed the membrane under the tip opening. Then, a sodium current was elicited and effectively blocked by TTX at a final concentration of 10^{-4} mol/l. All experiments were carried out at 36 ± 0.5 °C. The flow through the interior of the suction pipette was adjusted to 50 μl/min.

A voltage clamp amplifier was connected with the external and internal solution via Ag/AgCl/3 mol/l-KCl-agar electrodes. Currents were measured with a virtual ground circuit and series resistance errors were reduced by a compensated feedback. No major errors were expected from that source (cf. Benndorf et al. 1985).

Recording and data analysis

Currents were photographed from a storage oscilloscope (OG 31, Messelektronik Berlin). For the analysis of the time course of the outward current the traces were digitized by hand (40 points) and fitted by means of the Levenberg-Marquardt algorithm (Brown and Dennis 1972) on a Commodore 64 computer.

In some experiments currents were stored in the memory of an MC 80 microcomputer (Elektronik Gera). The signals were digitized at 0.28 to 0.32 ms time intervals with an 8-bit A/D converter and filtered at 1 kHz. The current traces were then corrected for their linear capacitive and leakage parts by subtracting an averaged hyperpolarizing 20 mV-pulse multiplied by a corresponding factor. The digitized traces were recorded on a tape for later analysis.

Solutions

Tyrode solution contained (mmol/l): NaCl, 150; KCl, 5.4; $CaCl_2$, 2.5; $MgSO_4$, 0.5; HEPES, 5; glucose, 11.1; pH 7.4. Ca^{2+}-free solution contained: NaCl, 140;

KCl, 5.8; KH_2PO_4, 0.5; Na_2HPO_4, 0.4; $MgSO_4$, 0.9; glucose, 11.1; HEPES, 5; fatty acid free albumin (FFA) 0.1 g/ml; pH 7.4. The enzyme solution was composed of: NaCl, 120; KCl, 5.8; KH_2PO_4, 0.5; Na_2HPO_4, 0.4; $MgSO_4$, 0.9; HEPES, 20; glucose, 11.1; FFA, 0.1 mg/ml; collagenase, 0.4 mg/ml; pH 7.1.

The recovery solution contained: glutamic acid, 70; KCl, 25; taurine, 10; glucose, 11; EGTA, 0.5; oxalic acid, 10; KH_2PO_4, 10; HEPES, 5; pH 7.1 adjusted with KOH.

The suction pipettes were filled with the intracellular solution containing: KOH, 150; EGTA, 2; HEPES, 10; pH 7.1 adjusted with H_3PO_4.

The chemicals used were of analytical grade. FFA was prepared from human albumin for clinical use (Serumwerk Dessau), 4-aminopyridine was obtained from Merck, Collagenase (Type 1) from Sigma.

Results

Separation of the early outward current

In all experiments Na channels were blocked by 10^{-4} mol/l TTX and additionally inactivated by adjusting the holding potential (V_h) to -50 mV. It will be shown later that at this voltage the early outward current (I_{EO}) is in fact not inactivated. The inset in Fig. 1 illustrates a series of current traces when no leakage compensation used. In the vicinity of V_h the input resistance is high (33.6 ± 4.9 MΩ, mean $\pm$ SD, $n = 6$). Hyperpolarizations larger than 30 mV induce a nonlinear current increase that certainly reflects inwardly rectifying channels as described in detail in ventricular cells of the guinea pig (Sakmann and Trube 1984a, b). Depolarizing pulses to potentials positive to -30 mV make a nonlinear increase of currents appear. The activation phase of those currents obviously overlaps with the late capacitive transients. After a maximum there is a decline of the current in the sense of inactivation. The currents maxima are shifted to earlier times at stronger depolarizing pulses.

The points in Fig. 1 reflect the current-voltage (IV)-characteristic of the peak currents. The current between —80 and -20 mV is considered as pure linear leakage current which is mainly the shunt current between pipette tip and cell membrane. In following experiments this linear leakage current was compensated for electronically, and the remaining current at depolarizing pulses will be denoted the early outward current. The arrows point to the amplitude of the maximum early outward current ($I_{EO,\,max}$) shown in the inset.

In all experiments the external solution contained 2.5 mmol/l Ca^{2+}. At this concentration small inward current components that could be attributed to Ca-currents (I_{Ca}) were detectable in only 20 % of the cells, but with a maximum

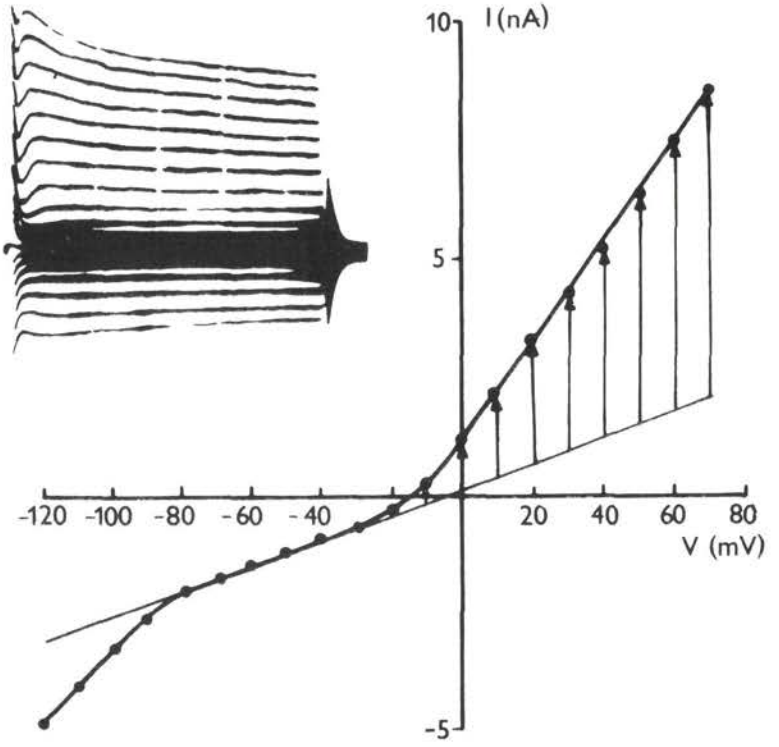

Fig. 1. IV-characteristic of maximum transient outward currents without leakage compensation. The inset shows current traces elicited by 7 hyperpolarizing and 12 depolarizing test pulses in 10 mV steps; $V_h = -50$ mV. The points between -30 and -70 mV have been fitted by a regression line; an input resistance in this voltage range of 31.6 MΩ has been calculated. In successful experiments this linear current was compensated for electronically and the remaining outward currents at depolarizing pulses are termed I_{EO}. The $I_{EO,max}$ values of the currents in the inset are represented by the arrows. The current at -50 mV was defined as zero because linear leakage was compensated for at this potential; cell 200186-3.

at $V_T = 0$ mV they never exeeded 0.6 nA. Fig. 2 gives an example of the overlap between one of the largest I_{Ca} observed and I_{EO}. Between -20 and 0 mV the still small amplitude of I_{EO} is decreased due to the interference of I_{Ca} but at stronger depolarizations the diminishing amplitude of I_{Ca} becomes small compared with the enormously uprising I_{EO}. In most of the cells investigated (80 %) the Ca currents were too small to be detected as could definitely be concluded from the missing peak of I_{Ca} after 3 to 4 ms at potentials around -10 mV at which I_{EO} activation is far slowlier. For evaluation of the early outward currents any data for cells were used in which I_{Ca} could interfere with the properties of I_{EO}. Because of the only tiny magnitude of I_{Ca}, if separable at all, we decided not to use any

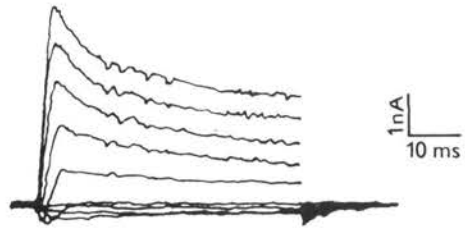

Fig. 2. Interference of I_{Ca} with I_{EO}. $V_h = -50$ mV. $V_T = -40$ to $+40$ mV in 10 mV steps. The inward current, if present (only 20 % of the cells investigated), has its maximum between -10 and 0 mV but is small compared with I_{EO} at positive test potentials. This offers the possibility to investigate I_{EO} without preceding block of I_{Ca}, and makes the myocardial mouse cell extraordinary suitable for an analysis of the transient outward current in heart; cell 160585-8.

Ca-channel blockers for our analysis of I_{EO} in the ventricular mouse cell; we thus had no interfering nonspecific effects of these substances. After clamping back to $V_h = -80$ mV the I_{EO} traces are followed by inward tails which have been proved to be the larger the longer and the stronger the preceding depolarizations (cf. Fig. 3). Since there is some evidence that the magnitude of the leakage current which was compensated for in our protocol decisively influences the tail currents the latter are considered to provide no information about I_{EO}.

Peak current voltage characteristic and steady state inactivation

When compensating for leakage, a series of early outward currents as shown in Fig. 3*A* could be elicited. The currents appear at potentials positive to -30 mV, they are activated faster at stronger depolarizations, and are of a more pronounced transient nature. The IV-relation (Fig. 3*B*) of the peak currents reveals a linear dependence at positive potentials.

Steady state inactivation (r_∞) was determined by the conventional double pulse protocol. The holding potential was set to -80 mV because at -50 mV some cells showed slight inactivation. Prepulses were chosen of 500 ms duration. This prepulse duration (t_p) was sufficient for reaching a steady state at all potentials. Testpulse potentials were set between 0 and $+30$ mV. No dependence of the r_∞-curve on V_T could be found. Fig. 3*C* shows a series of currents at $V_T = +10$ mV. At less negative prepulse potentials the currents become steadily more inactivated and reach a steady state value at 0 mV different from 0 nA. This time-independent current component behaved linearly at positive test potentials, and test current amplitudes were related to this current level. The corresponding steady state inactivation is plotted in Fig. 3 panel D. The smallest depolarizing prepulses produce no inactivation. Full inactivation is reached at

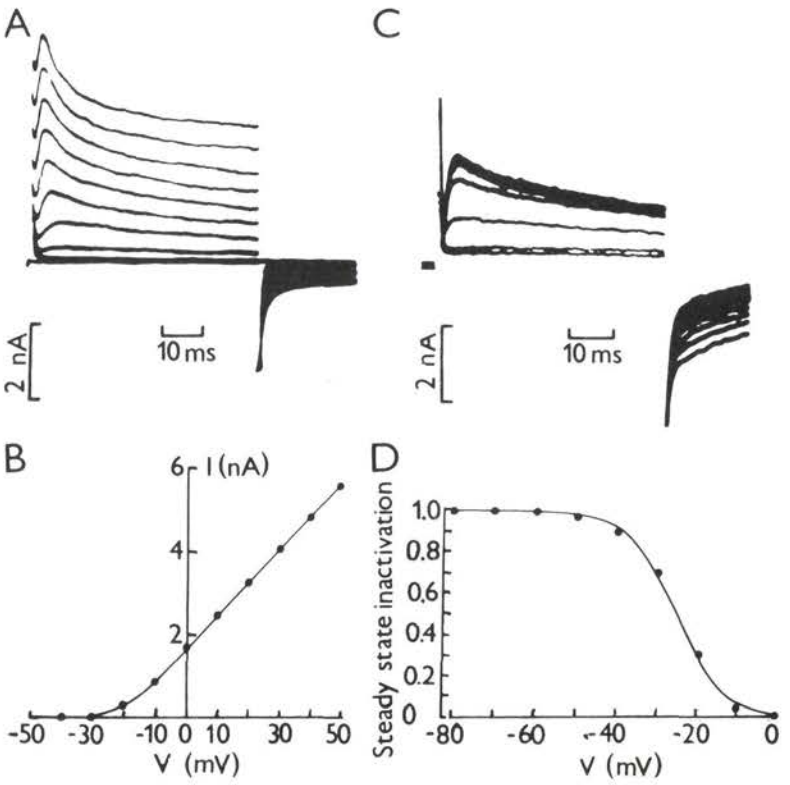

Fig. 3. IV-characteristic of $I_{EO,max}$ and steady state inactivation. *A*. Series of currents for V_T between -40 and $+50$ mV in 10 mV steps. $V_h = -50$ mV. The transient nature of the current becomes more pronounced at stronger depolarizations. *B*. IV-relationship of peak currents in *A*. The first I_{EO} is elicited at -20 mV, at positive potentials the values can be fitted by a straight line. The curve has been fitted by eye. *C*. Series of currents to obtain steady state inactivation, $V_h = -80$ mV, $V_T = +10$ mV, $t_p = 500$ ms, $V_p = -80 \ldots 0$ mV in 10 mV steps. *D*. Plot of the inactivating part of the currents in *C* as amplitude normalized to the steady state current level. The data points could be described by (1) with $V_H = -25.5$ mV and $s = -5.9$ mV; cell 240185-17.

0 mV. The data points were fitted by

$$r_\infty = [1 + \exp(V_H - V)/s]^{-1} \quad (1)$$

yielding the potential of half maximum inactivation (V_H) and the slope parameter (s). From 7 cells we obtained $V_H = -23.7 \pm 3.9$ mV and $s = -5.8 \pm 0.4$ mV (mean $\pm$ SD, $n = 6$).

Identification of the ionic nature of the early outward current

Since elevated extracellular potassium concentrations increase conductance of

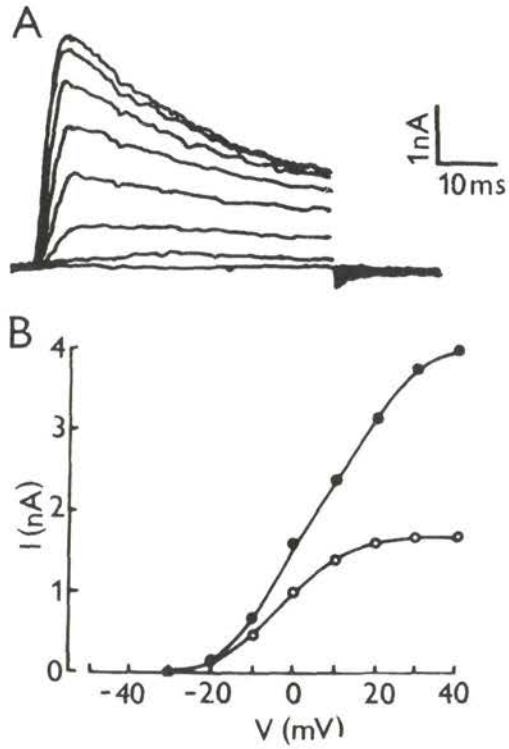

Fig. 4. 4-Aminopyridine block of I_{EO}, cell 160585-8. *A*. 4-Aminopyridine-sensitive currents as difference between control and test currents at $V_T = -30, -20, -10, 0, 10, 20, 30, 40$ mV; *B*. Plot of the amplitudes of the currents in A at the peak (filled circles) and at the end of the 50 ms test pulse (open circles) as a function of test potential. The curves have been fitted by eye. Note the saturation characteristic of both curves suggesting a mechanism of unblocking at large depolarizations.

the inward rectifier channels (Sakmann and Trube 1984a) in the range of the holding potential of -50 mV used in our experiments, the linear leakage could not be compensated for as simply as it would be at normal extracellular potassium. Thus, our protocol to obtain I_{EO} is not appropriate to identify this current as potassium current by checking the influence of an altered driving force. Here we present arguments for the early outward current being a K current which is not activated by Ca.

1. The incubation of an aliquot of the cells in an altered recovery solution containing Cs^+ ions instead of K^+ ions and additionally 40 mg/l of the ionophore Nystatin which is able to exchange both ions (Russel et al. 1977; Tillotson and Horn 1978; Tillotson 1979; Marban and Tsien 1982) completely abolished I_{EO} in those cells whereas a control group of cells of the same preparation

showed the typical outward currents. In experiments with the Cs^+-treated cells all K^+ ions in the solutions of both the pipette and the bath were replaced by Cs^+ ions.

2. 4-aminopyridine is a well established blocker of transient outward currents in several heart preparations (Isenberg 1978; Kenyon and Gibbons 1979; Coraboeuf and Carmeliet 1982; Josephson et al. 1984; Giles and Van Ginneken 1985). The effect of the drug on I_{EO} of the ventricular mouse cell is summarized in Fig. 4. Testpulses from $V_h = -50$ mV to $V_T = -40 \ldots +40$ mV were elicited first under control conditions and then 10 s after the administration of 1 mmol/l 4-aminopyridine. Fig. 4*A* shows a family of the corresponding 4-aminopyridine-sensitive currents as difference between control and test currents at every voltage. 4-Aminopyridine blocks I_{EO} but an unblock mechanism is present at stronger depolarizations as can be seen in panel B on the saturating curves of the amplitudes of peak currents and currents at the end of the test pulse (50 ms).

3. The replacement of all chloride in the bathing solution by phosphate did not alter the configuration of the early outward currents. Thus, the chloride ion cannot play an important role as charge carrier of I_{EO}.

4. If the early outward current described herein were a Ca-activated current one would expect major alterations of its amplitude when blocking or enlarging the Ca-current. Both mechanisms were checked by adding Cd^{2+} (0.5 mmol/l) or BAY-K 8644 (10^{-6} mol/l) to the bathing solution. Although with Cd^{2+} I_{EO} activation was slightly slowed down no change in I_{EO} amplitude could be observed with both agents.

5. The IV-characteristic of Ca currents nearby the reversal potential is flat (Hagiwara and Byerly 1981). If I_{EO} was Ca-activated at least a slight deviation of the IV-relation of I_{EO} from linearity should be evident around +60 mV. However, a perfect linearity (cf. Fig. 1) was found.

Time course of I_{EO}

For a sufficient time resolution of the activation process only the first 14 ms of I_{EO} were approximated by

$$I_{EO} = \bar{P}_{EO} f(V) q_\infty^4 [1 - \exp(-t/\tau_q)]^4 r_o [\exp(-t/\tau_r) + C]/(C + 1). \quad (2)$$

$\bar{P}_{EO} f(V)$ stands for the maximum conductance and the driving force of I_{EO}, and q_∞ and r_o are activation and inactivation at $t \to \infty$ and $t = 0$, respectively. C is a constant defining the relationship between the transient part and the non-inactivating part of the current. τ_q and τ_r are the time constants for the activation and inactivation process, respectively. $\bar{P}_{EO} f(V) q_\infty^4 r_o$, τ_q, τ_r, and C were determined as parameters by the fitting procedure. The current at the beginning of

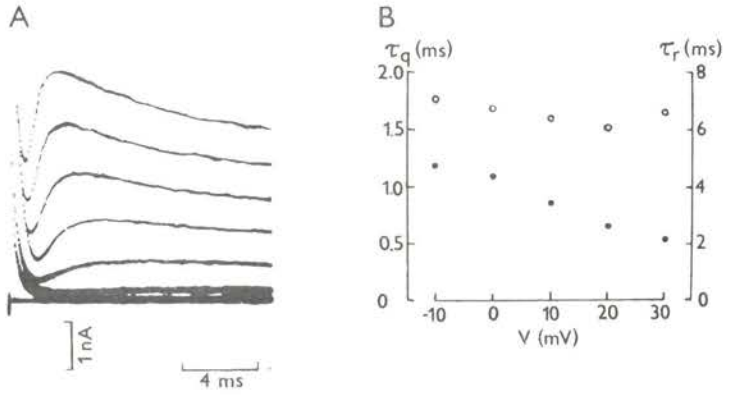

Fig. 5. Time course of activation and early inactivation. *A*. Series of currents, $V_h = -50$ mV, $V_T = -40, -30, -20, -10, 0, 10, 20, 30$ mV. The sampling procedure excluded the time of the capacitive artifacts. *B*. Voltage dependence of τ_q (filled circles) and τ_r (open circles) calculated by (2). τ_q at $V_T = 30$ mV is less than 50 % of the value at $V_T = -10$ mV whereas τ_r varies only less than 20 % in this voltage range.

the clamp pulse was taken for zero (cf. corrected traces, Fig. 2) and the next sampling point for the approximation was taken at 3/4 $I_{EO,\,max}$. Thus, the remaining small interference of a late capacitive component with the activation phase of I_{EO} could only lead to an underestimation of the increase in τ_q at the more depolarizing voltages. From the integer powers between 2 and 5 tested best fits were obtained with the power 4 (correlation coefficient = 0.995). For the other powers, significantly lower correlation coefficients were obtained (2 : = 0.961; 3 : = 0.971; 5 : 0.962). Fig. 5 shows an example of a series of currents used for the approximation, and the voltage dependence of τ_q and τ_r. The time course of activation is far more voltage dependent than the time course of the early inactivation during the first 14 ms.

The decay of the early outward current ($I_{EO,\,dec}$) following 50 ms-testpulses could not be approximated by a single exponential. To keep the number of parameters as low as possible, the inactivation process was quantitated by fitting the currents between peak time +1 ms with the simple model

$$I_{EO,\,dec} = A_1 \exp(-t/\tau_{r1}) + A_2 \exp(-t/\tau_{r2}) + A_3 \qquad (3)$$

τ_{r1} and τ_{r2} are time constants; A_1 and A_2 are the corresponding contributions of both exponentials. A_3 represents the non-inactivated part. Under the assumption that for short test pulses the slow exponential in equation (3) can be taken as a constant, the term $[A_2 \exp(-t/\tau_{r2}) + A_3]$ corresponds to $[\bar{P}_{EO} f(V) q_\infty^4 r_o C/(C+1)]$ in equation (2). Fig. 6 illustrates the calculated voltage dependence of

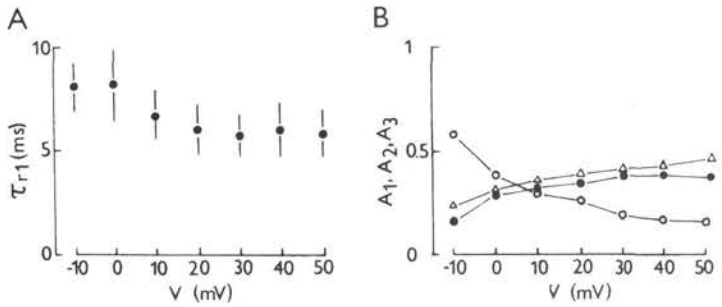

Fig. 6. Biexponential time course of inactivation. Five series of currents as shown in Fig. 2*A* were elicited by test-pulses of 50 ms duration to $V_T = -10 \ldots 50$ mV in 10 mV steps. The decay phases have been approximated by (3). *A*. Voltage dependence of the fast time constant τ_{r1}; bars indicate SD. In the tested voltage range there is only a decline of τ_{r1} at moderate depolarizations. *B*. Voltage dependence of normalized contributions of both exponentials and the pedestal. A_1 (filled circles) and A_3 (triangles) show a similar increase toward positive potentials whereas A_2 (open circles) decreases. Error bars have been omitted.

τ_{r1} and that of the normalized values A_1, A_2, and A_3 as the mean of five cells. τ_{r2} remained voltage independent (41.1 ± 7.9 ms, mean ± SD) between −10 and +50 mV. Since a comparison of the results of the fitting procedure with time constants of the order of the test pulse duration revealed some dependence on the estimates, τ_{r2} was no further considered. On the other hand, a qualitatively different voltage dependence of A_2 and A_3 could be developed independently of the fitting procedure starting conditions. The values for A_2 and A_3 were only accepted if A_2/A_3 varied less than 30% at three different sets of estimated parameters. Fig. 6*B* illustrates similar increases in A_1 and A_3 toward more positive potentials, whereas A_2 declines. The coincidence of A_1 and A_3 suggests that the fast component and the pedestal are generated by the same type of channels which become more activated toward positive potentials. An inverse voltage dependence of A_2 makes it intriguing to speculate that the slow exponential is generated by another type of channel which is already completely activated at −10 mV. However, from the data described so far it has not been possible to decide to what extent the decay is caused by true inactivation or by accumulation of K^+ ions outside the channel. An algorithm for at least the more precisely measurable fast component will be shown in the next section.

Temperature dependence of activation and inactivation

If the fast inactivation component is due to a true inactivation of the channel one should expect a Q_{10} value exceeding at least 2. On the other hand, if ion

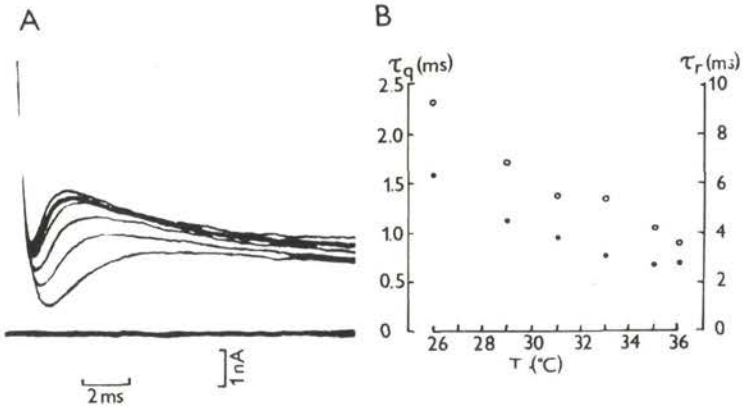

Fig. 7. τ_q and τ_r as function of temperature. *A*. Series of early outward currents during a temperature ramp from 26.0 to 36.0 °C. Currents (from below) were recorded at 26.0, 29.0, 31.0, 33.0, 35.0, and 36.0 °C; ramp duration 4 min; $V_h = -50$ mV; $V_T = +20$ mV; cell 160884-17. *B*. Temperature dependence of τ_q and τ_r. Data were calculated from the approximation of the traces in *A* by (2). Both τ_q (filled circles) and τ_r (open circles) diminish at increased temperature. For τ_q and τ_r Q_{10} of 2.29 and 2.58, respectively, was calculated.

accumulation and related simple diffusion processes determine the fast inactivation Q_{10} below 2 can be expected. A fast overlapping reuptake of accumulated K^+ ions by Na-K-ATPase with a large Q_{10} value would only lead to an underestimation of the temperature dependence of the fast inactivation component. We determined the temperature dependence of the fast inactivation by approximating a series of current traces elicited during a temperature ramp by equation (2) obeying the same sampling protocol as described in the previous paragraph. Figure 7 shows a representative example of early outward current recorded during a temperature increase from 26.0 °C to 36.0 °C within four minutes. Activation and inactivation are accelerated and the current amplitude increased. The time constants decrease at higher temperatures, and a Q_{10} of 2.36 ± 0.19 and 2.51 ± 0.09 (mean $\pm$ SD), respectively, was calculated from data of 3 cells at 26.0 and 36.0 °C for τ_q and τ_r. The value of τ_r seems to strongly argue against diffusion processes as the cause of the fast decline in I_{EO}.

The onset of and the recovery from inactivation

Mice have very high heart rates (between 520 and 780 min^{-1}) compared with other mammals. If the early outward current is responsible for the sufficiently fast repolarization the onset of inactivation should be appropriately related to recovery from inactivation: the time course of recovery should be fast enough

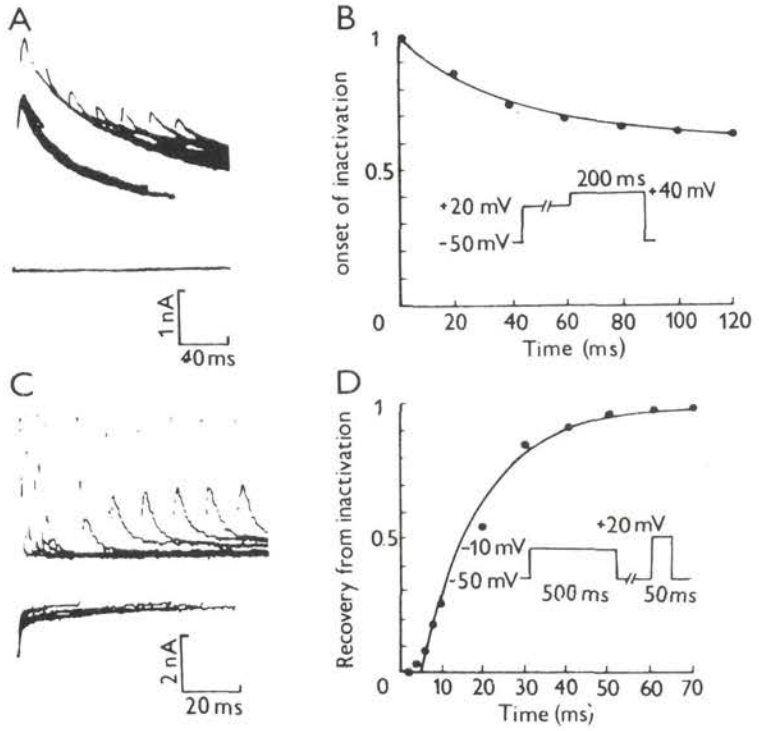

Fig. 8. Time courses of the onset of and the recovery from inactivation of I_{EO}. *A*. Series of currents to determine onset of inactivation with the double pulse protocol as shown in the inset of *B*; prepulse duration 0, 20, 40, 60, 80, 100, 120 ms. The onset of inactivation as decay of the peak currents declines slower as the decay at prepulse potential; cell 121184-9. *B*. Plot of the normalized peak currents in *A*. The onset of inactivation could be described by two exponentials with a fast time constant of 20.1 ms and a slow time constant of 59.6 ms. *C*. Current traces to quantitate the recovery process from inactivation. After about 40 ms an obviously complete recovery of the fast component above the time independent part can be observed. The recovery of the slow component is pointed out; recovery intervals: 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70 ms; voltage protocol, see inset in *D*; cell 240485-3. *D*. Plot of the normalized peak currents in *C* as function of the recovery interval. After an initial delay the recovery time course can be approximated monoexponentially with a time constant of 14.7 ms.

or the time course of the onset of inactivation must not be faster. Fig. 8 gives representative examples of both time courses. The onset of inactivation was determined by the conventional double step technique. The special protocol used is illustrated in inset (Fig. 8). The decline of the peak currents reflecting this onset of inactivation appears to be even slower than the decay at the prepulse potential. The time course of the onset of inactivation could be described by two exponentials with a fast time constant of 18.4 ± 6.0 ms and a slow time constant

of 62.1 ± 19.9 ms (mean ± SD, $n = 3$). The prepulse potential of 20 mV was chosen since it has a fast onset of inactivation within the physiological voltages.

The current recovery from inactivation is shown in Fig. 8*C* and *D*. The protocol is shown in the inset of panel D. The inactivating prepulses were 500 ms long to yield complete inactivation. They were followed by variable recovery intervals at $V_h = -50$ mV and the subsequent test pulses to $V_T = +20$ mV then induced currents as shown in panel C. At first the fast component recovers and later a recovery of the slow component can also be observed. However, 70 ms as the maximum time interval did not permit evaluation of the slow component. Panel D shows a plot of the peak current values. After an initial delay the recovery showed an approximately monoexponential time course with a time constant of 16.3 ± 2.9 ms (mean ± SD, $n = 3$). The recovery potential of −50 mV was chosen with respect to r_∞ (cf. Fig. 3*D*) to avoid disturbing interferences with the Na-current despite the high TTX-concentrations, and to have a slow recovery at the worst. Conclusively, the time courses of the onset of and the recovery from inactivation make the early outward current a favorite candidate dominating a rapid repolarization of the action potential in the mouse ventricle.

Discussion

This paper describes the existence of a transient outward current in single myocardial mouse cells that could be activated by depolarizing pulses from $V_h = -50$ mV to test potentials positive to −20 mV. There is a lot of evidence that this current is mainly carried by K^+ ions as could be demonstrated by the block with Cs^+ ions and 4-aminopyridine and by ruling out the participation of Cl^- ions. However, the very important experiment to study the dependence of I_{EO} on changes in K^+-gradient is hard to evaluate because the shunt currents inherent in the suction pipette method employed make an exact determination of the zero current level of the membrane impossible. Thus, leakage compensation was necessary to define I_{EO}. However, at elevated extracellular potassium concentrations inward rectifier channels sophisticate the amount of leakage at —50 mV (cf. Sakmann and Trube 1984a) and conclusively, I_{EO} cannot be measured under comparable conditions. The inwardly rectifying current has been described in many heart preparations (Hall et al. 1863; Mascher and Peper 1969; Beeler and Reuter 1977; Sakmann and Trube 1984a, b) but it is obviously not present in primary (Noma et al. 1984; Shibata nad Giles 1984) and subsidiary (Giles and Van Ginneken 1985) pacemaker tissue.

The early outward current as reported herein has been defined as difference to the current caused by a linear leakage conductance between −30 and

−70 mV. Other transient or early outward currents have been described in isolated ventricular cells of the rat (Josephson et al. 1984) and the guinea pig (Lee and Tsien 1984) and recently in atrioventricular node cells (Nakayama and Irisawa 1985) and crista terminalis cells (Giles and Van Ginneken 1985) of the rabbit. In spite of several differences our findings agree best with the properties of the corresponding outward current in rat ventricular cells. We therefore have termed this current I_{EO}. Particularly in its inactivation time course it differs from the results in atrioventricular node and the crista terminalis cells as well as from transient outward currents recorded in Purkinje fibres (Deck et al. 1964; Deck and Trautwein 1964; Peper and Trautwein 1968; Reuter 1968; Fozzard and Hiraoka 1973; Isenberg 1978; Kenyon and Gibbons 1979; Coraboeuf and Carmeliet 1982).

The activation phase of I_{EO} in ventricular mouse cells lasts only several milliseconds, is voltage dependent, and can be described by the Hodgkin-Huxley like model by power 4 (cf. Connor and Stevens 1971b). The incomplete inactivation phase consists of several components as could be demonstrated by the existence of two time constants and a pedestal. The fast time constant τ_{r1} is less than half the corresponding value reported for other single heart cells (Nakayama and Irisawa 1985; Giles nad Van Ginneken 1985) but might be similar to that for ventricular rat cells (Josephson et al. 1984) if the time courses for the latter are estimated and extrapolated to 36 °C by the determined Q_{10}. In spite of the only moderate voltage dependence of τ_{r1} the Q_{10} value of 2.58 for this time constant holds for the assumption of a true gating mechanism at the very beginning of the inactivation process. From the equal dependence of the contributions of both the fast component of inactivation and the pedestal on voltage and the block by 4-aminopyridine and Cs^+ we conclude that either the same K channel or different types of K channels with a similar selectivity are responsible for these two components. The time constant of the recovery of the fast component from inactivation at the recovery voltage of −50 mV was 16.3 ± 2.9 ms (mean ± SD, $n = 3$). This suggests similarly rapid kinetics of this mechanism as that of the fast decay component, and agrees fairly well with 25.3 ms in rat at room temperature (Josephson et al. 1984). We did not check the voltage dependence of the recovery in detail but there has been evidence for a far faster recovery at more negative recovery potentials indicating a similar bell shaped voltage dependence of the fast inactivating component as has been described for the considerably slower inactivation time course of the transient outward current in crista terminalis cells (Giles and Van Ginneken 1985).

4-Aminopyridine is widely used as blocker of K channels in various preparations of nerve (Pelhate and Pichon 1974; Yeh et al. 1976; Ulbricht and Wagner 1976; Meves and Pichon 1977; Thompson 1977), skeletal muscle (Gillespie 1977; Fink and Wettwer 1978; Molgo 1978), and also heart muscle

(Isenberg 1978; Kenyon and Gibbons 1979). In Purkinje fibres Coraboeuf and Carmeliet (1982) could show that the blocker only affects a slowly inactivating part of the transient outward current. All transient outward currents recorded from single heart cells could also be blocked by 4-aminopyridine, (Josephson et al. 1984; Giles and Van Ginneken 1985; Nakayama and Irisawa 1985) but no voltage dependence in the sense as reported herein has so far been described in heart. A similar reduction of the 4-aminopyridine block of K channels toward more positive potentials has been reported by Ulbricht and Wagner (1976) in the myelinated nerve of frog and in the squid giant axon where it has been partially explained by a voltage dependent binding of the drug to the potassium channel (Meves and Pichon 1977; Yeh et al. 1976). One significant difference in the blocking mechanisms described herein and by Josephson et al. (1984) has been found between the periods since application to full effect of the drug. Whereas several minutes were necessary in Josephson's et al. measurements in rat ventriculocytes, 10 s were the upper limit in our preparation.

An advantage of the myocardial mouse cell for studying I_{EO} is the very small Ca current in this preparation at physiological Ca concentrations in the bath solution. This enabled us to work without any Ca channel blocker and avoided all nonspecific effects of those substances on outward currents. This advantage will be especially useful for analysis of effects of substances that alter in the same concentration range both Ca and K channels of the myocardial cell membrane.

To speculate about the physiological meaning of the early outward current described herein, it is obvious that this current promotes a very rapid repolarization as can be seen in the mouse myocardium. However, similar currents have been detected also in other mammalian heart preparations with far longer action potentials such as guinea pig (Lee and Tsien 1984). The significant variability in the duration of action potentials in heart of different mammalian species may find an explanation only in different channel densities and not in different channel properties.

After the submission of the manuscript, the transient outward current in the myocardial mouse cell was described to be composed of three types of unitary currents with slope conductances of 27 pS, 12 pS, and 5 pS, respectively (Benndorf et al. 1987; Benndorf 1988).

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