Current-Dependent Slow Channel Inactivation in Heart Muscle

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Abstract. Slow inward currents were measured under voltage clamp conditions on isolated trabeculae of frog atrial myocardium (*R. esculenta*). A twin-pulse with a gap programme was used: after a 300 s rest a conditioning voltage step was applied followed by a test step; both steps were identical as for their amplitudes and duration. The coupling interval was always 2 s to avoid incomplete recovery from voltage-dependent gating processes. It was found that an increase in the conditioning slow inward current resulted in a decrease of the subsequent current activated by the test step. This decrease was U-shaped and depended on the voltage of the conditioning step. The decrease of the test current was proportional to the amount of the charge transported inwards during the conditioning step. This observation of the current-dependent inactivation is well described quantitavely on the assumption of the screening of negative surface charges as a result of the conditioning inward current.

Key words: Ca channel — Inactivation — Heart — Surface charges

Introduction

As in many other tissues, the predominatly Ca-dependent slow inward current in the heart muscle is activated by depolarization (for a review see Corabeuf 1980). However, it has been suggested that its inactivation may differ from the voltage-dependent inactivation of the sodium channel by being dependent on the Ca entry (Paramecium: Brehm and Eckert 1978, Brehm et al. 1980; Aplysia neurons: Tillotson 1979; Helix neurons: Standen 1981, Plant and Standen 1981; embryonic chick sensory neurons: Dunlap and Fischbach 1981; insect skeletal muscle: Ashcroft and Stanfield 1981; frog heart: Fischmeister et al. 1981).

The results of the experiments bring further evidence for the hypothesis

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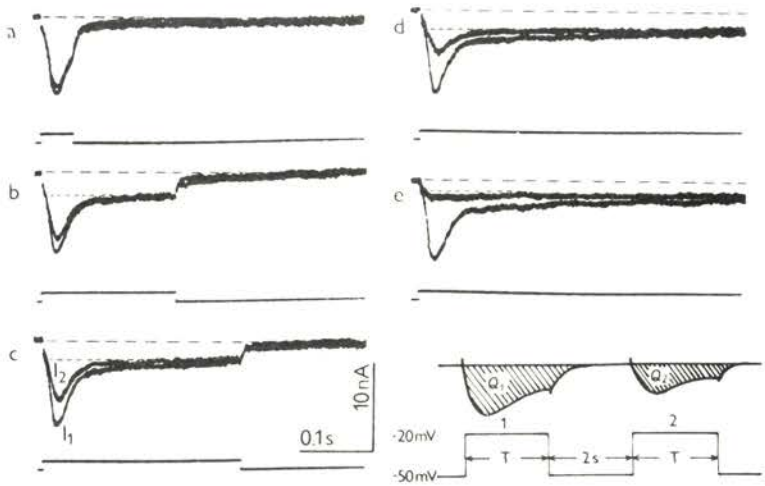

Fig. 1. Slow inward currents recorded using a twin-pulse programme with resting interval interposed between succesive pulses. The pulse duration T was varied. The resting interval was kept constant at 2 s. All currents were activated by depolarizing voltage steps to −20 mV from a holding potential of −50 mV. The smaller currents are test currents. The duration T in ms of the depolarizing pulses is as follows: *a* — 50; *b* — 200; *c* — 300; *d* — 500; *e* — 900. Upper trace: current, lower trace: voltage.

concerning a Ca-dependent slow channel inactivation in heart muscle. It was demonstrated that the Ca conductance is reduced by increasing the conditioning slow inward current. Such a calcium-controlled process could be of physiological relevance in the regulation of the internal Ca concentration.

Material and Methods

Ionic currents were recorded from isolated atrial trabeculae of the frog *R. esculenta* (55 to 165 μm in diameter). The voltage clamp technique was described in detail previously (Nilius and Henček 1982).

In all our experiments the frog Ringer solution was used containing (mmol/l): NaCl 110, KCl 2.5, $CaCl_2$ 4, $NaHCO_3$ 2.38, K_2HPO_4 0.08, glucose 5.5, pH 7.13.

In most experiments voltage steps from a holding potential of −50 mV to between −20 and 0 mV were used. In this range the steady state activation and inactivation overlap with long-lasting incompletely inactivated slow inward currents being observed, which are suitable for the induction of current-dependent inactivation. The following activation pattern was used: after a 300 s rest a conditioning pre-pulse was administered followed by a test pulse. Conditioning and test pulses were both identical in amplitudes and duration. A 2 s resting interval was inserted between the pulses to avoid incomplete recovery from voltage-dependent gating processes. Current-time courses were integrated numerically to estimate the charge transferred inwards during the test or conditioning pulses. The least-square two-parameter approximation was used for modelling.

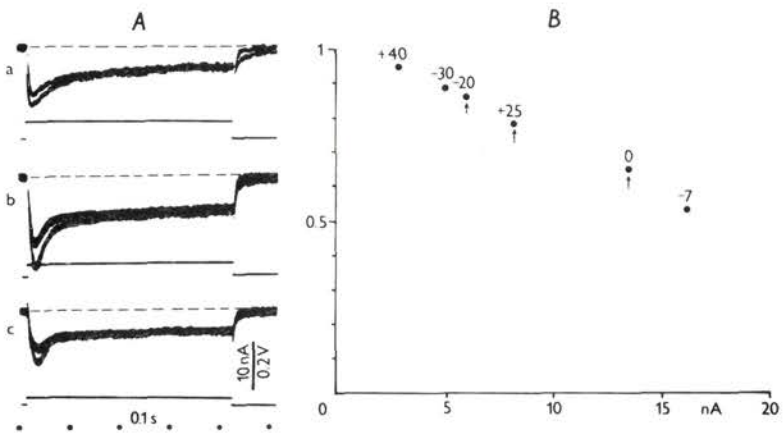

Fig. 2. Dependence of the test current on the depolarizing voltage step. *A*: Slow inward currents recorded by a twin-pulse sequence. The duration of the twin pulses was 400 ms. Currents were activated by clamping the membrane potential to various depolarizing voltages U from a holding potential of -50 mV. These were in mV for *a*:25; *b*:0; *c*:-20 mV. The duration of the interpulse interval was chosen to 2 s. *B*: Dependence of the inactivation of the test current (expressed as test current relative to the conditioning inward current, ordinate) on the absolute value of the conditioning peak inward current. The depolarizing voltage U is indicated at each point in the plot. The arrows mark the values taken from the examples on the left hand side.

Results

Inactivation studied by a double-pulse-with-gap-method

Twin-pulses were used to study the dependence of the test slow inward current on the conditioning current. If the duration of the conditioning pulse was prolonged the test current decreased. Figure 1 illustrates the effect of the duration of the conditioning pulse on the slow inward current elicited by the test pulse. After a 50 ms conditioning pulse the test current remained almost unchanged; however, after a 900 ms prepulse the test peak current disappeared. The prepulse affected the transient time course of the slow inward current only. The remaining slowly inactivating or leakage current was unchanged. If the amplitude of the conditioning prepulse was raised the test current decreased to about zero; it, however, increased when the conditioning pulse was further raised. Figure 2 shows the normalized test pulse currents in dependence on the absolute values of the conditioning currents. The test current was inactivated if the conditioning current was raised independently on the potential. This observation is reflected by the U-shaped inactivation curve (Figure 3). This finding represents the main evidence for the existence of a Ca-dependent inactivation in other types of preparations (Brehm and Eckert

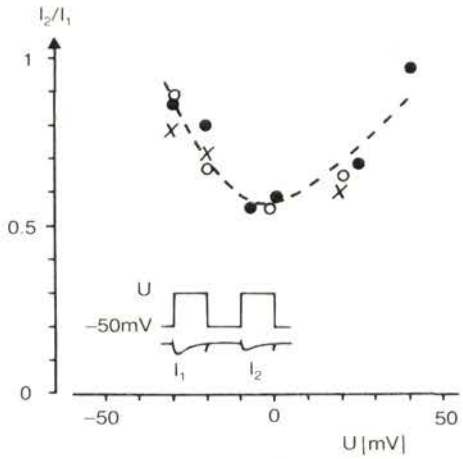

Fig. 3. Effect of the conditioning pulse on the inactivation. Ordinate: peak current of the test pulse relative to the peak current of the conditioning pulse. Abscissa: potential of the depolarizing twin-pulses. Interval between the pulses was 2 s. The different symbols denote different experiments. The interrupted curve was drawn by hand.

1978; Tillotson 1979; Ashcroft and Stanfield 1981; Plant and Standen 1981).

The dependence of the test current on the duration of the conditioning voltage step implies that the inactivation may be favoured by the increase of the conditioning current (Figure 1). Figure 4 shows that the normalized charge transferred inwards and the inward current during the test pulse decrease monotonously.

Modelling of current-dependent inactivation

In this section the experimental findings are described quantitatively using a model for interaction of slow channel conductance and a negatively charged membrane component (see also Hall and Cahalan 1982). The slow channel conductance may depend exponentially on the applied voltage U

$$G_0 = \bar{G}\exp(U/U_0)\,, \tag{1}$$

where $\bar{G}$ is the resting conductance; U_0 is the voltage change that results in an e-fold change in conductance.

It is further assumed that the outer negative charges are completely screened. An internal surface potential, Φ_0, exists resulting from negative charges at the inner side of the membrane. Therefore

$$G_0 = \bar{G}\exp((U-\Phi_0)/U_0)\,. \tag{2}$$

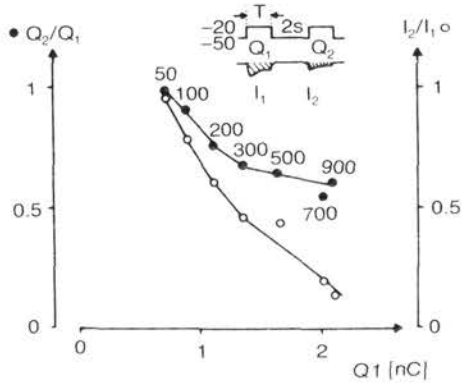

Fig. 4. Dependence of the relative inward transferred charge and the peak inward current respectively during the test pulse on the inward transferred charge during the conditioning prepulse. ○: test peak inward current relative to the conditioning inward current, ●: relative charge during the test pulse (note different ordinates). The numbers denote the duration T of the conditioning pulses. Depolarizing pulses (from −50 to −20 mV) were of different durations (see inset). Abscissa: inward transferred charge during the conditioning pulse in nC.

Using an unstirred layer approximation Hall and Cahalan (1982) a change in the surface potential, $\Delta\Phi$, due to chaning of the Ca concentration within a membrane near unstirred layer can be expressed by

$$\Delta\Phi = (4RT/F)\tanh(F\Phi_0/RT)Q/(Q_M + Q)\,, \tag{3}$$

where Q_M means the inward transported charge necessary for a half screening effect. The relation between the conductance before and after Ca has been accumulated at the inner side of the membrane is, using formula (2) and (3)

$$G_{Ca} = G_0\exp(-\Delta\Phi/U_0)\,, \tag{4}$$

and therefore

$$\Delta\Phi = U_0\ln(G_0/G_{Ca})\,. \tag{5}$$

Equation (4) expresses that the higher the Ca concentration available to screen the surface charges, the lower the membrane conductance. An inward transport of Ca would result in screening of surface charges and effects a potential drop, $\Delta\Phi$. Surface charge clearing means increase, surface charge screening means decrease of the membrane conductance. According to this model G_0 has been identified with the maximum conductance during the conditioning step, G_{Ca} with the maximum conductance during the test pulse. Equation (3) can be rewritten using equation (5) in

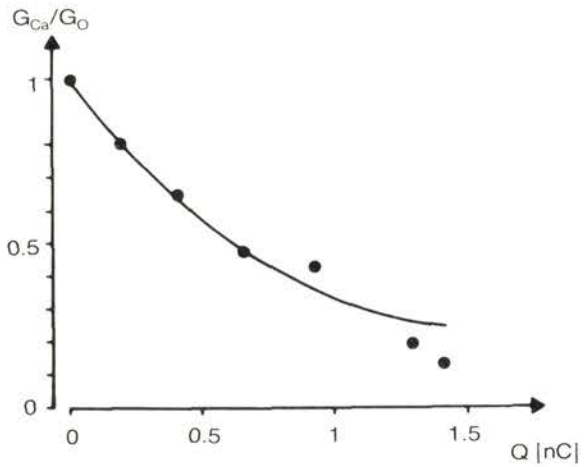

Fig. 5. Approximation of the measured relative conductances obtained by means of the twin-pulse programme. Q denotes the inward transferred Ca^{2+} charge during the conditioning pulse. G_{Ca} is the maximum conductance during the test pulse, G_0 during the conditioning pulse. The results were obtained from an experiment with variable duration of the test pulses. The smooth line was calculated according to eq 6. Using $U_0 = 8$ mV the following parameters were obtained from the best fit approximation: $\Phi_o = -36.8$ mV, $Q_M = 9.51 \times 10^{-9}$ C.

$$\ln(G_0/G_{Ca}) = 4RT/U_0F \tanh(F\Phi_0/RT)\ Q/(Q_M + Q)\ . \qquad (6)$$

which defines a two-parameter problem ($Y = a_1 Q/(a_2 + Q)$) for aproximation. Y and Q were obtained from measurements similar to those shown in Figure 1 and Fugure 3. Figure 5 demonstrates an example obtained from the measurements shown in Figure 1 and 4. Supposing an U_0 value of 8 mV (Almers 1978 and in accordance to the measured steady state activation) in three cases an unscreened surface potential between -10.5 and -36.7 mV and a maximal shift $\Delta\Phi$ due to the conditioning Ca influx (equation 5) between 4.3 and 15.7 mV were calculated. A half screening was estimated to occur due to an inward transported charge between 1.5×10^{-9} and 2.7×10^{-8} C. The estimated surface potentials obtained from the parameter a_1 agree with those in other tissues (Brown 1974) and supposed for the heart muscle (Coraboeuf 1980).

Discussion

The presented results can be discussed in terms of a number of possible mechanisms which could explain the inactivation of the slow (Ca) inward current: (i) Ca

accumulation or Ca depletion in membrane-near compartments (Almers et al. 1981), (ii) voltage dependent inactivation, (iii) activation of outward currents, (iv) a Ca or current dependent inactivation. Using the twin pulse with gap method a decrease of the test current to about 10 or 20% of the conditioning current could be observed. It seems unlikely that the reduction of the inward current can be explained in terms of a decrease of the driving force. Using a constant field model (Sten-Knudsen 1978) a Ca depletion from 4 to about 10^{-4} mmol/l or an internal Ca accumulation from about 100 nmol/l to 0.1 mmol/l would be necessary. From the measured inward transported charges (see Fig. 4) it seems unlikely that such a distinct alternation in the Ca concentrations on either side of the membrane can be realized by the conditioning step.

The voltage dependence of the observed inactivation was found to be U-shaped in contrast to the monotonous voltage dependent inactivation of other types of pure voltage dependent Ca channels (Fox 1981). Such U-shaped inactivation-potential relationship is one of the main arguments for the existence of a Ca-dependent Ca channel inactivation (Brehm and Eckert 1978; Tillotson 1979; Ashcroft and Stanfield 1980; Plant and Standen 1981; Standen 1981; Fox 1981; Fischmeister et al. 1981). The U-shaped inactivation-potential characteristics (Fig. 3) and a good correlation between conditioning current or charge respectively and inactivation (Fig. 2 and 4) would be inconsistent with a voltage-dependent inactivation hypothesis.

In the present experiments the contribution of the delayed activated or a possibly Ca-dependent outward current to the inactivation was not studied. However, if a delayed outward current would be responsible for the decline of the inward current only this decline had to be increased due to increasing voltage steps in contrast to the demonstrated findings (Fig. 2).

The most probable explanation of the observed phenomenon seems to be the assumption of a calcium current-induced inactivation of the slow (Ca) channel in the heart muscle. The quantitative model derived by Hall and Cahalan (1982) was shown applicable also to inactivation of the slow inward current after the conditioning prepulse current. The calculated surface potentials obtained from best approximation of the measured conductances and currents were quite reasonable with regard to the supposed uncertainties. According to the supposed model the calcium current-induced inactivation is generated by screening of fixed negative charges. The results are also in agreement with the finding of a pacing-induced inactivation. The increase in the pacing rate induced an acceleration of the onset of inactivation and a shift of the steady-state inactivation curve to more negative potentials (Nilius and Henček 1982).

The time constant of the decay of the test current was estimated to be about 800 ms (Fig. 1 and; see also Nilius and Henček 1982); it is of the same order of magnitude as described for the slow inactivation process (Kohlhardt 1981). The

slow inactivation was also found to be accelerated at increased Ca concentrations, what supports the assumption of a relationship between the observed current-dependent inactivation and the slow Ca channel inactivation.

The physiological relevance of such a Ca-dependent inactivation of the slow channel in the heart muscle is obvious; it represent an efficient control of the transported Ca ions into the cell by Ca ions themselves.

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