Substates of Cardiac Sodium Channels Are Due to Both Decrease in Conductance and Changes in Selectivity

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Abstract. Currents through DPI 201-106 modified single cardiac sodium channels in guinea pig ventricular cells were measured using the patch clamp technique in the cell-free configuration to control the sodium concentrations on both sides of the patch membrane. Current-voltage relationships of the single channels were obtained by application of linear voltage ramps from −140 to 100 mV. With 10 mmol/l Na^+ at the inner surface of the patch, openings of sodium channels with conductances of 17 pS (selectivity ratios $P_K/P_{Na} = 0.083$ and $P_K/P_{Na} = 0.58$) and 12 pS (selectivity ratios $P_K/P_{Na} = 0.084$ and $P_K/P_{Na} = 1.832$) were obtained. With 30 mmol/l internal sodium, conductances of 20, 10, and 7 pS and selectivity ratios of 0.084, 0.386, and 0.543, respectively, could be measured. It is concluded that substates of sodium channel currents are due to changes in single channel conductance as well as in selectivity, or to changes of both independently of each other which accounts for the variability of conductance levels of cardiac Na channels.

Key words: Heart muscle — Patch clamp — Sodium channel — Substates — Conductance — Selectivity

Introduction

A number of recent studies have shown that as a rule, ion channels open to different states rather than only to a single open (conducting) state. Substates of single channel currents are known for the inwardly rectifying potassium channel (Matsuda 1988, Matsuda et al. 1989; Kell and DeFelice 1988), the acetylcholine receptor channel (Hamill and Sakmann 1981;

Auerbach and Sachs 1983; Sigworth 1985, 1986; Strecker and Jackson 1989), acetylcholine and other receptor operated channels (reviewed by Gage 1987; Jahr and Stevens 1987; Cull-Candy and Usowicz 1987), the sarcoplasmic Ca^{2+}-release channel (Hymel et al. 1988; Kiu et al. 1989) and also sodium channels (Nagy et al. 1983; Kiss and Nagy 1985; Scanley and Fozzard 1987; Kohlhardt et al. 1987; Nagy 1987a, b; Sigel 1987a, b; Barnes and Hille 1988; Kohlhardt et al. 1988; Nagy 1988a, b; Patlak 1988; Chinn and Narahashi 1989; Meves and Nagy 1989; Schreibmayer et al. 1989; Nilius et al. 1989; and Carmeliet et al. 1989) in native membranes as well as in black lipids (Green et al. 1987).

Substate levels of the inward rectifier potassium channel can be reliably reproduced by application of micromolar concentrations of blocking divalent and monovalent cations (Mg^{2+}, Cs^+, and Rb^+) (Matsuda 1988; Matsuda et al. 1989) and are explained by aggregation to a triple barrel structure of this channel (Matsuda et al. 1989). Substates of the sodium channel, however, are rather inhomogeneous.

Although most of the studies on substates of Na channels were done in the presence of compounds that abolish fast inactivation, evidence has also been given for the existence of substates in nonmodified Na channels (Nagy et al. 1983; Scanley and Fozzard 1987). Substances like DPI 201-106, veratridine, chloramine-T, STX, BTX and others were used to increase the probability of the appearance of different levels of sodium channel currents by increasing the lifetime of the open state of the channel. A wide range of levels has been described. Only few data are available so far on the voltage dependency of microscopic currents through substates (Kohlhardt et al. 1987; Sigel 1987a, b; Nilius et al. 1989; Schreibmayer et al. 1989), the reversal potential and selectivity of substate sodium channels. The question remains open whether sodium channel substates are due to changes in conductance or selectivity, or both. Using the advantage of linear voltage ramps, we present here data that favor the coexistence of all these mechanisms.

Materials and Methods

Single cardiomyocytes from guinea pig ventricular tissue were dissociated by a procedure similar to that used by Mitra and Morad (1985). All experiments were carried out in the inside-out configuration of the patch clamp technique. The solution in the patch pipette contained (in mmol/l): 140 Na^+, 4K^+, 1Ca^{2+}, 1Mg^{2+}, 145Cl^-, 5 HEPES, and the pH was adjusted to 7.4 with NaOH; the bath solution contained 10 or 30Na^+, 130 or 110K^+, respectively, 5 HEPES, 5 EGTA, pH adjusted to 7.2 with KOH. Five mmol/l of the S-enantiomer of the piperazinylindole compound DPI 201-106 (Sandoz Ltd., Basel) were added to the bath solution.

All experiments were carried out at room temperature (20 $\pm$ 1 °C). The patch clamp device used was standard (Hamill et al. 1981): EPC 7 (List electronics), Lab Master for ADC and DAC, Clampex 5.0 on an IBM desktop PC. To evaluate current-voltage relationships of the single channel

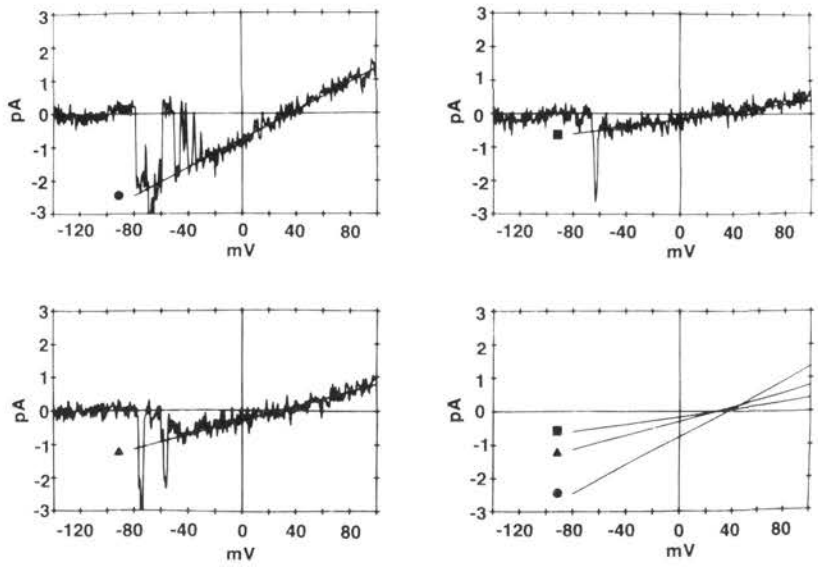

Fig. 1. Single channel current-voltage relationship of a sodium channel obtained by application of linear voltage ramps from −140 to 100 mV to an inside-out patch. The pipette solution was physiological with 140 mmol/l Na^+ and 4 mmol/l K^+ whereas the inner side was in contact with 30 mmol/l Na^+ and 100 mmol/l K^+ (for further compositions see Methods). The different traces represent i-V curves for the sodium channel in distinct conductance states. Linear regressions were fitted to the original data. Filled circle: The channel opens to the main conductance level (g = 20 pS; E = 36.6 mV). Filled triangle: Opening to a medium conductance state (g = 10 pS; E = 19.2 mV). Filled square: Another event of open state with the lowest conductance (g = 7 pS; E = 13.2 mV). The superposed plots of all the three regressions (lower right pannel) illustrates the shift of the reversal potential and single channel conductance during subconductive openings.

sodium current, linear voltage ramps of 50 ms from a holding potential of −140 mV to 100 mV were applied to the patches. Single channel conductances and reversal potentials were obtained from linear regressions fitted to the original data. Selectivity ratios were calculated by use of the Goldman equation

$$P_K/P_{Na} = (Na_o - \exp(EF/(RT)) * Na_i)/(\exp(EF/(RT)) * K_i - K_o \quad (1)$$

where E is the measured reversal potential; F, R, and T have their usual meanings; Na_o, Na_i, K_o, and K_i are the concentrations at both sides of the membrane.

Results

The appearance of multiple current levels in nonmodified single sodium channels is rare. However, in the presence of tools that interfere with fast inactiva-

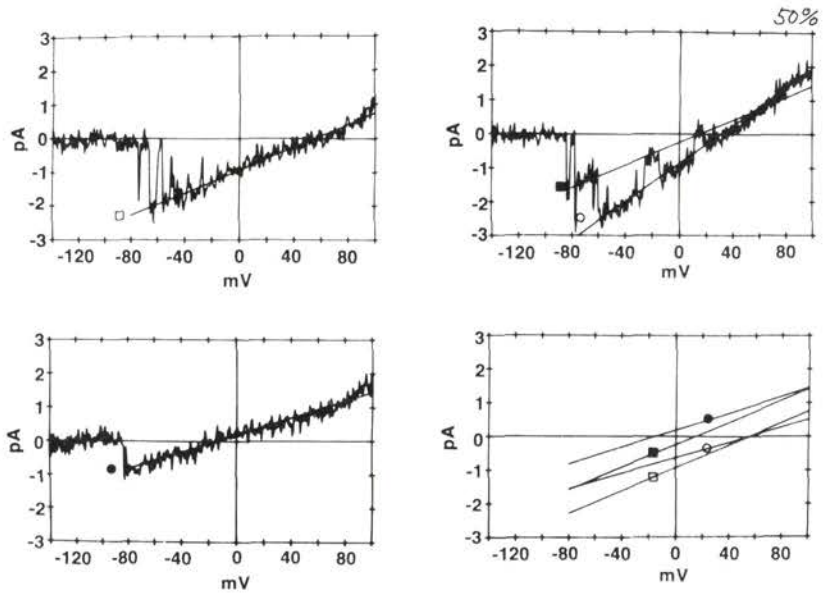

Fig. 2. Single channel current-voltage relationship of sodium channels obtained by application of linear voltage ramps from −140 to 100 mV to an inside-out patch that contained at least two channels. Pipette solution, see legend to Fig. 1; bath solution: 10 mmol/l Na^+ and 130 K^+. Open circle: i-V curve for a sodium channel in the main open state (g = 17 pS; E = 55 mV). Filled square: A second channel with lower conductance and no selectivity between Na^+ and K^+ (g = 12 pS; E = −15 mV). Simultanous opening of two channels (*upper right pannel*). Filled circle: the 17 pS channel, now with a lower selectivity (E = 14.7 mV) superimposed by the 12 pS channel with a high sodium selectivity (E = 55 mV). For permeation ratios, see Results.

tion, detection of substate levels is strikingly improved. We used the cardiotonic compound DPI 201-206 which does not change the main single channel conductance of the sodium channel (Nilius 1988). Because of the long open times of Na channels in the presence of inactivation blockers, instantaneous single channel current-voltage (i-V) relationships including the reversal potential can be obtained from linear voltage ramps. Multiple levels of the sodium channel could be measured in 3 out of 31 patches only.

Data shown in Fig. 1 were obtained from an inside-out patch with 140 mmol/l Na^+ and 4 mmol/l K^+ at the outer side, and 30 mmol/l Na^+ and 110 K^+ at the inner side. The first curve (marked by the filled circle) represents the i-V relationship of the main open state of sodium channel. The single channel conductance was 20 pS. A short opening (partially out of range) of a second channel can be seen on top of a long lasting channel opening. The consecutive trace (recorded immediately after the first one, marked by the filled

triangle) shows normal openings to the full open state interrupted by a short closure; but suddenly it switches to a subconducting state with a medium single channel conductance of 10 pS. After a sweep with no opening (not shown) a further short opening to the full level with a consecutive switch to a low conductance of 7 pS could be observed. Considering the intersections of the linear regressions with the abscissa a shift of the reversal potential of about −20 mV during the substate currents should be noticed suggesting that the decrease of conductance is accommpanied by a loss of selectivity. Considering only sodium and potassium as the main cations at both sides of the membrane, permeation ratios P_K/P_{Na} of 0.084 (full), 0.386 (medium), and 0.543 (low conductance) could be calculated by equation 1 (see Methods).

The situation in Fig. 2 is somewhat more complicated than that in Fig. 1. The patch contained at least two channels. The first trace (open square) represents the i-V curve for the main open state of sodium channel with the same pipette solution as in Figure 1 but with 10 mmol/l Na^+ and 130 K^+ at the inner side of the patch. The reversal potential of 55 mV fits a highly selective sodium channel ($P_K/P_{Na} = 0.083$). Single channel conductance is 17 pS. In other traces of the same patch, a channel with a lower (12 pS) conductance appeared. This channel (filled square) was not able to discriminate between Na^+ and K^+. A permeation ratio $P_K/P_{Na} = 1.832$ was calculated from a reversal potential of −15 mV. In the next sweep simultaneous openings of two channels could be observed. The current-voltage relationship of these two channels together could be considered as the sum of an i-V curve of a 17 pS channel (empty circle) and that of a 12 pS channel (filled circle). Surprisingly, now the 12 pS channel has a reversal potential in the range of 55 mV and the same high selectivy of 0.084 like the ordinary sodium channel. But in contrast, the reversal potential of the 17 pS channel was measured at 15 mV. This again points to a loss of selectivity with a permeation ratio of 0.58. Due to a spontaneous closure of the 12 pS channel the reversal potential could be read directly from the original trace.

Discussion

Our data provide some evidence that multiple current levels of the DPI 201-106 modified cardiac sodium channel are due to changes in single channel conductance, changes in selectivity (Figs. 1 and 2), or both changes in conductance and selectivity (Fig. 1). These changes are probably independent of each other and one alteration does not preclude the other one. Additionally, the coexistence of different populations of sodium channels is possible as a further explanation (Fig. 2). Oiki et al. (1988) have shown that a synthetic 22-mer peptide from sodium channel primary structure can form a channel when incorporated in

lipid bilayers. Probably four of these alpha-helices are necessary to build up a pore which exhibits a single channel conductance of 20 pS in symmetrical 500 mmol/l Na^+ solution. This synthetic channel is nonselective for Na^+ and K^+ but it has subconductance levels. The behaviour of this artificial channel emphasizes the possibility that the conductance determining region of the channel could be responsible for real subconductance states of the channel independently of selectivity. Additionally, alterations of channel regions different from the previous one could evoke changes in selectivity at the same conductance. The result in both cases are current levels that are different from the usually main level of the open channel.

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