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Saturation effects and rectifier properties of sodium channels in human skeletal muscle *

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Abstract. Sodium outward currents were measured in human myoballs with the whole-cell recording method. The electro-chemical gradient of the sodium ions across the cell membrane was modified over a wide range by variations of the clamped membrane potential and of the internal and external soidum concentration. Up to 50 mV positive to the sodium equilibrium potential, E_{Na}, the current-voltage relation is linear. At a potential 80 mV positive to E_{Na} the sodium outward current has a maximum and decreases with a further increase in electrochemical gradient. Investigating the instantaneous current change in experiments in which the membrane potential was changed while the channels were already open we could exclude the possibility that the gates of activation or inactivation are responsible for this effect. Therefore we postulate that the sodium channel has a valve-like mechanism producing a negative slope conductance at highly positive membrane potentials, a current saturation with self-inhibition by the intracellular sodium concentration, and a blockade of the channel on reduction of the extracellular sodium concentration.

Key words: Human skeletal muscle, myoballs, sodium channels, rectification, saturation

Introduction

Since Hodgkin and Huxley published the first kinetic analysis of sodium channels in 1952, sodium inward currents have been investigated by many authors and in a great variety of preparations. Saturation of the sodium current has been explored in the node of Ranvier (Hille 1975) and in planar bilayers (Andersen et al. 1986; Hartshore et al. 1986). From single channel experiments it has been concluded that the conductance of the open sodium channel in skeletal muscle is independent of the membrane potential (Weiss and Horn 1986). Depending on the sodium equilibrium potential some of the recordings also showed small sodium outward currents during the largest positive test pulses and a non-linear current-voltage relation in this range (Woodhull 1973). Sodium outward currents were usually not included in the numerical analysis because in the range of highly positive membrane potentials the kinetics of the sodium channels become so fast that the accuracy of the recorded current transients is limited by the speed of the clamp and by the time resolution of the recording system.

When cells having a high internal sodium concentration (like *Xenopus* oocytes injected with cRNA for rat brain sodium channels) are investigated with a fast voltage clamp, the results sometimes suggest a decline of the m_∞ curve at highly positive membrane potentials (Stühmer et al. 1987). This observation led us to the question of whether this decline is indeed an effect of the steady-state activation of the channel (m_∞), or if there is an extremely accelerated inactivation at highly positive potentials, or if this decline is caused by yet another reason.

Methods

Human myoballs were grown from the satellite cells of adult human skeletal muscle sacrificed during hip surgery. The culturing procedure (Yasin et al. 1977) was modified as has been described by Pröbstle et al. (1988).

The myoballs were investigated with the whole-cell recording technique. Low resistance pipettes (200–400 kΩ) were used for the passage of large currents (up to 200 nA) into the cell. To optimize the voltage clamp, the sum of pipette and access resistance (series resistance) was compensated by a fast negative

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impedance in the clamp system, leaving a series resistance of about 50–100 kΩ.

Variation of the intracellular sodium concentration was achieved by means of a 150 μm polyethylene tubing, placed inside the tip of the pipette, through which a sodium solution containing 1 mol/l NaCl, 10 mmol/l EGTA, 10 mmol/l HEPES, and 1.5 mmol/l magnesium was slowly washed into the cell. This enabled us to replace all of the internal monovalent cations with sodium and to reach sodium activities up to 140 mmol/l. Higher concentrations could not be attained for osmotic reasons. The values of the internal sodium concentration were calculated from the given external sodium concentration (the standard external solution contained 135 mmol/l NaCl, 4 mmol/l KCl, 10 mmol/l HEPES, 1.5 mmol/l $CaCl_2$ and 1.4 mmol/l $MgCl_2$) and the measured sodium equilibrium potential. For the sake of simplicity we use the term sodium concentration instead of sodium activity. We no not take into account the external activity coefficient, therefore, we should not designate a value calculated in this way as internal activity because it is only a relative measure (Ruppersberg and Rüdel 1985). The sodium-free internal solution contained 10 mmol/l EGTA, 10 mmol/l HEPES, 1.5 mmol/l magnesium, 110 mmol/l cesium and chloride at a pH of 7.4.

The variation of the external sodium concentration was made by the use of a second pipette filled with 1 mol/l NaCl, 10 mmol/l HEPES, 1.5 mmol/l $CaCl_2$ and 1.4 mmol/l $MgCl_2$. When a gentle pressure was applied to the back of this pipette a sodium concentration gradient developed in the surroundings of the tip. The culture dish contained a sodium-free solution in which sodium was replaced by either cesium or glucamine. Attached to the clamp pipette and perfused with 150 mmol/l sodium solution, the cell was then moved in this gradient. This shift could be made very quickly. We found that even bigger osmotic differences between intra- and extracellular fluid were tolerated by the cell for a short time.

Changes of the temperature could be effected by having the myoball surrounded by a mini-coil perfused with hot or cold water.

The quantities of TTX-sensitive and TTX-insensitive sodium channels were estimated with the methode described in Ruppersberg et al. (1987).

Results

Sodium currents in the inward and outward directions were recorded over wide ranges of membrane voltage, internal and external sodium concentrations, and temperatures.

The most obvious finding was that under nearly all conditions at test-pulse potentials beyond +90 mV the current amplitude of the outward current was the smaller the higher the voltage (Fig. 1 A). A further increase, rather than a decrease, of the outward current would be expected with increasing electrical force. This negative slope conductance was equal in both TTX-sensitive and TTX-insensitive sodium channels (the percentage of TTX-sensitive channels varied between 40% and 60%). To test this, recordings were made with and without 1 μmol/l TTX in the bathing solution (Ruppersberg et al. 1987).

To test the possibility that a very fast component of inactivation was responsible for the negative slope conductance we applied a double step pulse protocol. First a full activation was provoked by a prestep from −100 mV to 0 mV and then the electric driving force was increased by a test step from 0 mV to either +140 mV or +90 mV. The resulting fast increase of the current reflects the clamp speed as discussed below. It therefore underestimates the instantaneous current change in both cases with a similar amount of error. However in the test step to +140 mV the fast increase of the outward current was only half as large as in the test step to +90 mV (Fig. 1 B) whereas the inactivation of the current elicited at +140 mV was not markedly faster than at +90 mV.

To test the possibility that the steady-state activation (m_∞) is decreased at highly positive potentials we used another double step protocol. Here a prestep to +140 mV, a potential at which the sodium outward current is already reduced, was followed by a test step to +90 mV, the membrane potential at which the sodium outward current is maximal. The sudden increase of the current provoked by the test step at arrow (b) in Fig. 1 C needs 0.15 ms to hit the trace of the current elicited by a direct step to +90 mV whereas the activation of the current at +90 mV (arrow (a) Fig. 1 C) needs 0.3 ms. Therefore it is unlikely that both increases of the current depend on the activation time constant of the channels at +90 mV. On the other hand the sudden increase needs exactly the same time (0.15 ms) as the increase of the inward current provoked by the backstep from −30 to −100 mV (Fig. 1 C). In both cases the speed of this fast change in current is assumed to reflect the clamp speed.

These results show that the negative slope conductance at highly positive membrane potentials is not a consequence of changes in the kinetics of the activation or inactivation mechanism that would produce a reduction in the number of channels in the open state. Therefore we have to assume that the non-linear current-voltage relation is a property of every single channel in the open state.

To analyse the mechanism underlying this property we varied the sodium concentrations on either side of the membrane. The maximum and the negative slope of the current-voltage relation were strongly

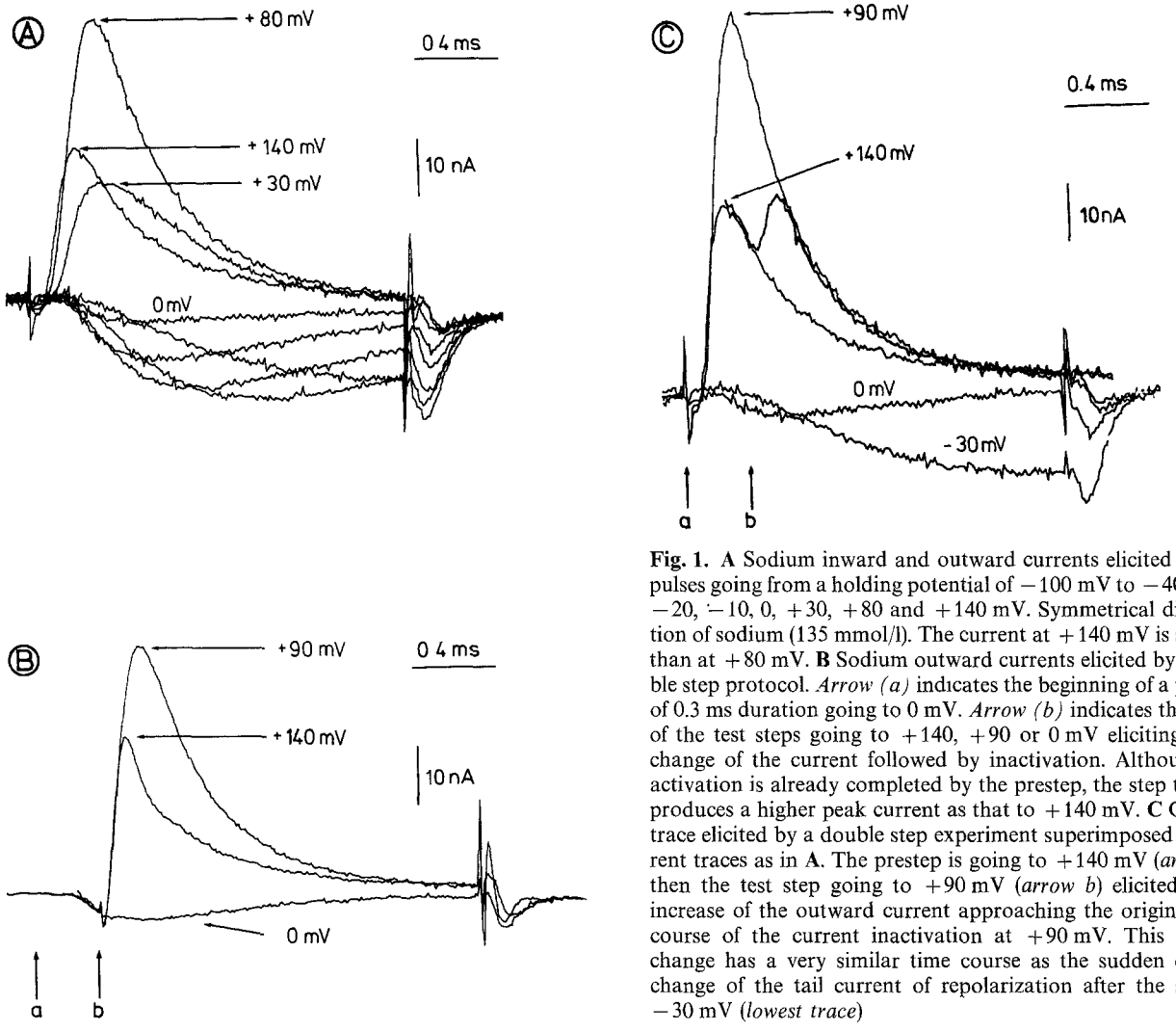

Fig. 1. **A** Sodium inward and outward currents elicited by test pulses going from a holding potential of −100 mV to −40, −30, −20, −10, 0, +30, +80 and +140 mV. Symmetrical distribution of sodium (135 mmol/l). The current at +140 mV is smaller than at +80 mV. **B** Sodium outward currents elicited by a double step protocol. *Arrow (a)* indicates the beginning of a prestep of 0.3 ms duration going to 0 mV. *Arrow (b)* indicates the onset of the test steps going to +140, +90 or 0 mV eliciting a fast change of the current followed by inactivation. Although the activation is already completed by the prestep, the step to +90 produces a higher peak current as that to +140 mV. **C** Current trace elicited by a double step experiment superimposed to current traces as in **A**. The prestep is going to +140 mV (*arrow a*), then the test step going to +90 mV (*arrow b*) elicited a fast increase of the outward current approaching the original time course of the current inactivation at +90 mV. This sudden change has a very similar time course as the sudden current change of the tail current of repolarization after the step to −30 mV (*lowest trace*)

concentration-dependent (Fig. 2). An increase of the internal as well as a decrease of the external sodium concentration reduced the outward current. This result was independent of the kind of positive ion replacing sodium (glucamine or cesium). An osmotic gradient across the cell membrane did not affect this result.

Thus we found by three independent means (variation of voltage, internal and external sodium concentration) that an increase of the outward-going electrochemical gradient for sodium ions reduces the sodium outward current. By contrast, any increase, in the inward-going gradient certainly has an increasing effect on the sodium inward current. The sodium channel therefore acts as an inward-going rectifier.

In a further experiment we investigated the temperature dependence of the current-voltage relation, as shown in Fig. 3. With rising temperature the maximum of the current shifted towards more positive potentials. Thus, the mechanism reducing the outward sodium current at highly positive membrane potentials seemed to become less effective the higher the temperature.

Instead of using the terms rectification and negative conductance one could also describe the behaviour of the soidum channels in terms of saturation and self-inhibition of the sodium net outflux. To do it we plotted the amplitude of the sodium outward current against the external and the internal sodium concentrations (Fig. 4). At highly positive potentials (+130 mV to +140 mV) the current is completely saturated, and it is self-inhibited by the internal sodium concentration (Fig. 4 A). The external concentration has a paradoxical enhancing effect on the sodium outward current (Fig. 4 B).

Discussion

Saturation of the soidum current has been studied in planar bilayers, in the frog node of Ranvier and in

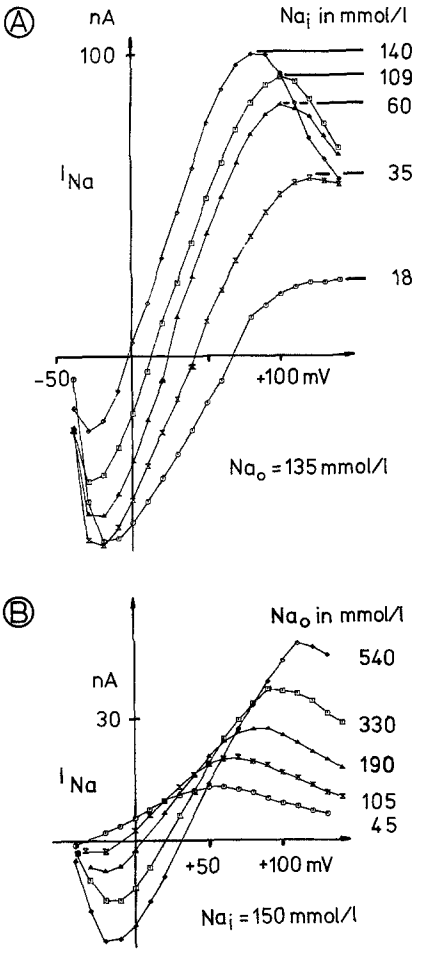

Fig. 2 A and B. Voltage dependence of the sodium peak current at various internal and external sodium concentrations. The peak inward current increases between −50 and −20 mV because of the increase of the steady state activation of the channels. At more positive potentials it decreases as the membrane potential approaches the sodium equilibrium potential. Further on the current changes polarity. The outward current first increases linearly with voltage up to 80 mV positive to the sodium equilibrium potential, reaches a maximum, and then decreases again. **A** Variation of internal sodium concentration at constant external concentration. Internal sodium replaced with cesium. **B** Variation of external sodium concentration at constant internal concentration. External sodium replaced with glucamine. The outward current is the greater the higher the external sodium concentration

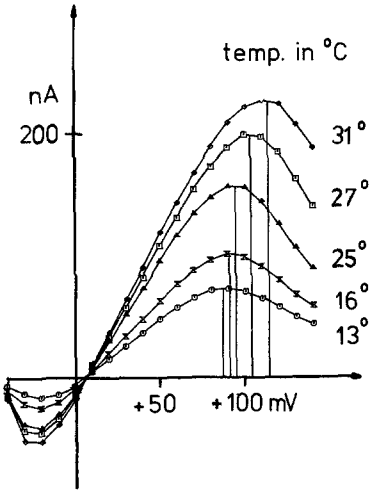

Fig. 3. Temperature dependence of the sodium peak current. The maximum of the peak current is shifted to the left by lowering the temperature. (Symmetrical sodium distribution)

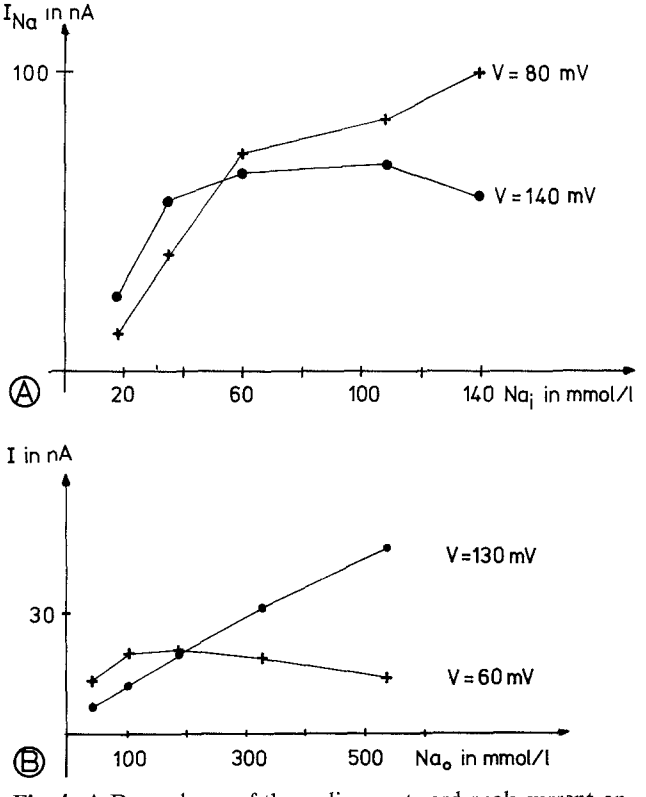

Fig. 4. **A** Dependence of the sodium outward peak current on the internal sodium concentration. Around +140 mV membrane potential, the current is self-inhibited at concentrations above 100 mmol/l although both the concentration gradient and the electric driving force (difference between equilibrium potential and clamp potential) are increased. At +80 mV no self-inhibition is present. **B** Paradoxical enhancement of the outward current by extracellular sodium (intracellular sodium 140 mmol/l). At a membrane potential of +130 mV, the sodium outward current increases linearely with the external sodium concentration although the electro-chemical gradient decreases. At +60 mV the sodium outward current is only little dependent on the external sodium concentration because paradoxical enhancement and decrease of the electro-chemical gradient cancel each other

squid axons. In planar bilayers Andersen et al. (1986) found linear current-voltage relations at any, including the highly positive, potentials. In the squid axon Landowne and Scruggs (1981) as well as Chandler and Meves (1965) also found that the sodium currents behave in accordance to Ohm's and Fick's Laws but having a small bend or rectification near +100 mV. Also in frog nerve Hille (1975) did not detect a negative slope in the dependence of the sodium current on voltage or concentration. A marked voltage-dependent block as described by us exists perhaps only in mam-

malian sodium channels. Concentration-dependent block of sodium channels has been described for potassium and hydrogen ions (Cohen et al. 1975; Begenesich and Danko 1983). In our experiments all solutions had a pH of 7.4 and the same results were obtained when sodium was replaced by cesium or glucamine. Thus we cannot explain our results with a blockage of the sodium channels by cations being different from sodium.

There are two explanations for a negative slope in the current-voltage relation of an ionic channel. First, the number of open channels might be inversely related to the electrical driving force because of an adverse potential dependence of the rate constants for opening and closing. In this case the reduction of the current is a time-dependent relaxation process like the potential-dependent inactivation in the potassium inward rectifier (Ohmori et al. 1981). Second, the conducting zone of the channel might be subject to a potential-dependent conformational change influencing the conductance.

A channel showing only rectification is much easier to explain than a channel that has, in addition, a negative slope conductance. Mathematical models of the channel such as the rate reaction models of Eyring (Zwolinksi et al. 1949) or Läuger (1973), as well as a continuous model that takes into account space charge effects (Ruppersberg and Rüdel 1988), permit the generation of any kind of rectifying current-voltage relationships but they do not yield a negative slope conductance.

For the following reasons we think that only the second alternative, a potential-dependent conformational change in the channel's conducting zone can explain our results: with the double pulse experiments we showed that the negative slope conductance is an instantaneous property of the channels and therefore cannot be attributed to the much slower processes of activation or inactivation. Moreover, the negative slope conductance was strongly dependent on the concentration gradient. By contrast, the rate constants for activation and inactivation of the sodium channel are little dependent on the sodium concentration (unpublished observation).

Therefore we have to assume that every single sodium channel shows rectification and on that account it must have a valve-like mechanism sensing the electric field and the sodium ion gradient. Such a mechanism could consist of sodium-binding sites near the channel openings on both sides of the membrane and/or of a sensor inside the ion-selective zone of the channel. The internal binding site then has a blocking while the external binding site has an opening effect on the valve inside the channel.

The negative slope conductance occurred at potentials that were the more positive the higher the temperature. That fits well with the assumption of a conformational change in the channel protein induced by both the electric field strength and the concentration gradient. With increasing temperature the kinetic energy of the moving ions rises almost proportionally whereas macromolecular conformational changes often are less temperature-dependent. On the other hand, one should not imagine this valve in a too mechanical way since even minor movements of charged groups in the channel protein may strongly influence the conductance (Ruppersberg and Rüdel 1988).

The inward rectification, the saturation, and the negative slope conductance of sodium channels occur under conditions that are far from physiological for a living cell. Under these extreme conditions, like low extracellular sodium concentrations, it may be useful for the cell to save sodium ions to maintain the activity of the electrogenic Na/K-ATPase. But one should not overestimate the physiological importance of these effects. In our view, they are most relevant to our understanding of ionic conductance in channels. The described effects provide a simple experimental system for the study of the interaction of the sodium channel with the ions passing through it. For example, the channel seems to "know" the actual sodium equilibrium potential, since the current maximum is exactly 80 mV positive to the sodium equilibrium potential at all internal and external concentrations (at 24° C). Further the channel seems to require minimal extracellular sodium concentrations in order to conduct at all. We found that even high intracellular sodium concentrations yield only a very small sodium outward current when extracellular sodium is absent. These effects must now be studied in other preparations and under further physical and chemical conditions, perhaps even with genetically modified sodium channels.

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