Effect of pH on Fast Sodium Channels in Neurons of the Rat Dorsal Root Ganglion

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Abstract. Ionic currents through fast sodium channels in the neuronal somatic membrane were measured under voltage clamp conditions using external solutions of normal and low pH. Voltage-dependent inhibition of ionic currents through open channels was observed in acidic solutions. The voltage-dependent block of sodium channels may be explained by the presence of two acid groups at the channel. The parameters of the inner and outer acid groups calculated according to this model are similar to those reported for the nodal membrane.

Key words: Neuronal membrane — Voltage clamp — Fast sodium channels — Proton interaction — Acid groups

Introduction

Various reports have shown that the lowering of the external pH resulted in inhibition of currents through sodium channels (I_{Na}). Models were proposed explaining this effect on the assumption that protons interact with one (Woodhull 1973) or two (Mozhayeva et al. 1982) acid groups at the channel preventing the passage of sodium ions. According to an alternative hypothesis, the decrease of I_{Na} is due only to a reduction in the near-membrane ion concentration owing to a change in the membrane surface potential (Mironov 1983). At the same time, no data concerning the effect of increasing external concentration of hydrogen ions on sodium channels in a wide voltage range are available for neuronal membranes. For these reasons, it seems necessary to study the interaction of protons with sodium channels in neuronal membranes at different pH of the external solution. Such studies can be carried out on dialysed neuronal membranes under voltage clamp conditions (Krishtal and Pidoplichko 1975), with an improved experimental setup allowing to measure inward and outward I_{Na} up to large positive potentials.

Materials and Methods

Our experiments were performed on isolated neurons from the dorsal root ganglia of adult rats. The technique of the isolation of single cells was described earlier (Kostyuk et al. 1981). Neurons without

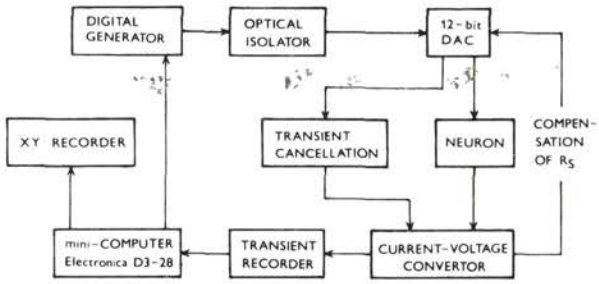

Fig. 1. Block-diagram of the experimental setup.

slow sodium channels (Veselovsky et al. 1980) were used. Calcium currents were inhibited by intracellular perfusion with fluoride ions. Potassium ions were removed from all solutions in order to abolish potassium current components resistant to tetraethylammonium (TEA) blockage. Thus the currents recorded were currents through fast sodium channels (I_{Na}). This was checked in some cases by tetrodotoxin application.

The solution for intracellular perfusion contained (in mmol . l^{-1}): 130 Na^+; 10 TEA^+; 5 $Tris^+$; 130 F^-; 15 Cl^-; pH 7.4. Extracellular solutions contained: 120 Na^+; 2 Ca^{2+}; 10 TEA^+; 134 Cl^-; 10 Tris-buffer, pH 7.4 or 19—20 glutamate (pH 4.4—5.8). pH values of the test solutions were checked both before and after the application.

The voltage clamp technique and intracellular perfusion of the neurons were principally similar to those described earlier (Kostyuk et al. 1981). Fig. 1 shows a block-diagram of the experimental setup. Membrane potential levels were set using a programmable pulse generator with a memory controlled by a computer Electronica D3-28. Communication between the digital rack and the digital-analog converter was by way of optical isolators (Bezanilla and Armstrong 1977).

The membrane current signal from the neurons was amplified in the analog rack and then fed into the input of a transient recorder DL-905, where it was digitalized. The contents of the recorder memory were transferred into the computer. The current signal was reproduced in the analog form by a pen-recorder.

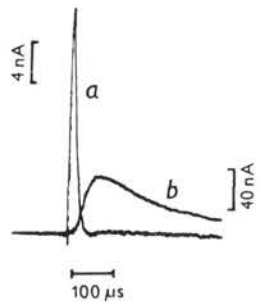

Fig. 2. Current record at a voltage jump from −100 mV to −80 mV (displacement 20 mV) without using the P — (−P/4) programme (a), and current record at a voltage jump from −100 mV to +100 mV (displacement 200 mV) using the P — (−P/4) programme (the current scale increased 10 times). Neuron 36.

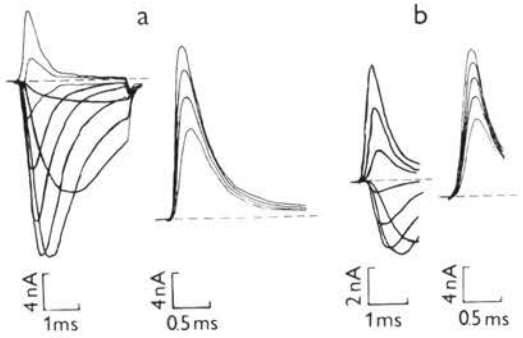

Fig. 3. (*a*) Records of currents through fast sodium channels at voltages from −60 to +60 mV (10 mV steps), pH 7.4, and (*b*) at voltages from −40 to +80 mV (10 mV steps), pH 5.4. Programme P — (−P/4). Holding potential −100 mV. Neuron 26.

Cells characterized by a relatively small series resistance (R_S) were selected for experiments. In addition, a compensation of R_S by introducing positive charging current may lead to some distortion of measured I_{Na} because of the saturation of current amplifier. In order to compensate for the major portion of the capacity transient, current injection into the sum point of the amplifier was produced (Hamill et al. 1981). Leakage currents were subtracted using the P — (−P/4) pulse programme (Mozhayeva and Naumov 1983). Holding potential was set at −100 mV. Experiments were carried out at 22—24 °C.

Fig. 2 shows a current record at a voltage jump from −100 mV to −80 mV (displacement 20 mV) without using the programme P — (−P/4) and a current record at a voltage jump from −100 mV to +100 mV (displacement 200 mV) using the programe P − (−P/4) (the current scale increased 10 times). It can be seen that the current transient did not affect the recording of the peak current at a test membrane potential (E_M) of +100 mV. Records of inward and outward currents I_{Na} under normal conditions and in acidic external solution (pH 5.4) are presented in Fig. 3.

Results and Discussion

Fig. 3 shows that at low pH, I_{Na} decreases and both the activation and inactivation kinetics become slower as compared with those in normal external solution. The current reduction is less prominent at higher test depolarizations. Fig. 4 shows peak current-voltage relations at different pH values of the external solution. It can be seen that, in addition to potential-dependent depression of I_{Na}, in low pH solution also the voltage range of activation shifts to more positive potentials as compared with the normal one.

Results obtained earlier on the node of Ranvier (Woodhull 1973; Mozhayeva et al. 1982) allow us to consider the ratio of peak chord conductance at low and normal pH ($g_{pH}/g_{7.4}$) as the measure of the blockage of open channels. In order to analyse the interaction of protons with open channels only values for positive E_M were used. The value $g_{7.4}$ was determined using the average of two values measured before and after the application of test acidic solution. Fig. 5 shows that the depression of I_{Na} decreased with the increasing positive E_M.

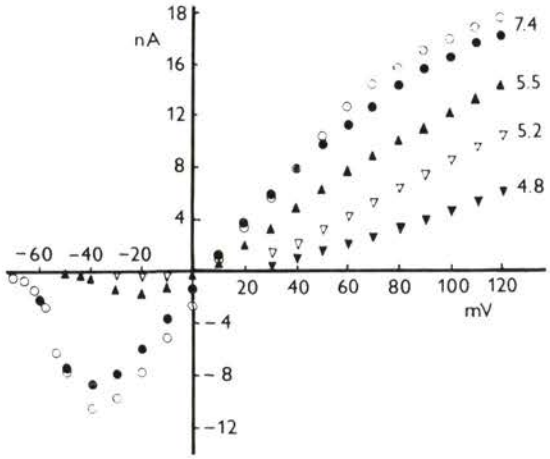

Fig. 4. Peak current-voltage relations at different pH of the external solution: 7.4 (○); 5.2 (▲); 4.8 (▽); 4.4 (▲); 7.4 (●). Holding potential −100 mV. Neuron 20.

The shift of the voltage range of activation (ΔE) is considered by many authors to be due to a decrease of the surface potential ($\Delta\psi$) (see, e. g. Hille et al. 1975). In order to estimate the possible decrease of I_{Na} due to a reduction of the near-membrane ion concentration as a result of a change in the surface potential ($\Delta\psi$) secondary to pH decrease we have chosen an experiment with the largest ΔE values at a given pH (pH 5.2, −18 mV; pH 4.8, −23 mV). This particular experiment is illustrated in Figs. 4. and 5. The values $g_{pH}/g_{7.4}$ were calculated assuming $\Delta\psi = \Delta E$ and using the equation (4) from Mironov's paper (Mironov 1983). The results given in Fig. 5 (lines 1b, pH 4.8; and 2b, pH 5.2) show that the degree of I_{Na} inhibition observed in the experiment was greater and the potential dependence curves were steeper even at maximal ΔE. Consequently, the decrease in I_{Na} at low pH of external solutions cannot be explained only by a reduction of the near-membrane ion concentration.

In accordance with Woodhull's model, I_{Na} inhibition is due to the blockage of channels by protons when interacting with an acid group within the channel (Woodhull 1973). In that case, the apparent pK (pK_a) of this group should be given by the following equation (Naumov et al. 1979):

$$pK_a = \log (g_{7.4}/g_{pH} - 1) + pH \qquad (1)$$

Fig. 6 shows the dependence of pK_a on the potential. $pK_a - E_M$ curves corresponding to different pH do not coincide: the lower the pH the steeper the curve. This implies that proton blockage of sodium channels in neuron membrane cannot be described by the model with a single acid group (Woodhull 1973).

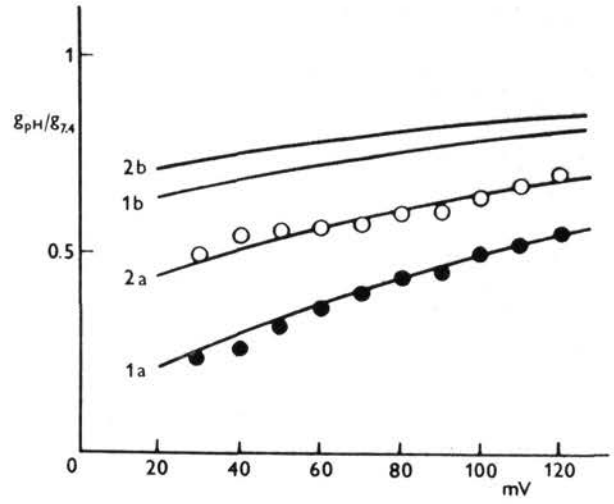

Fig. 5. Dependence of the conductance ratio $g_{pH}/g_{7.4}$ on the potential. Symbols are experimental $g_{pH}/g_{7.4}$ values. Lines 1a and 2a were plotted using equation (2) according to the model of two acid groups at the channel. Lines 1b and 2b were calculated from the possible change in surface potential (for details see text). (1) and (●) pH 4.8; (2) and (○) pH 5.2. Neutron 20.

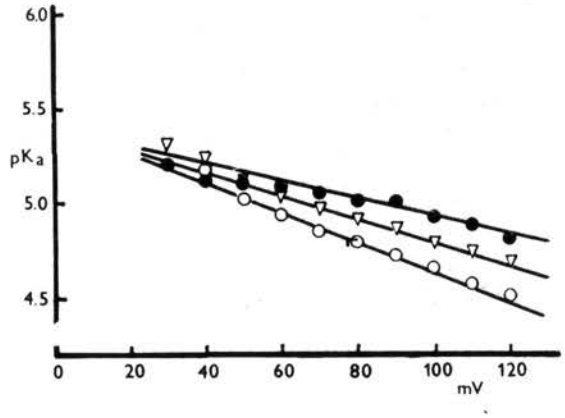

Fig. 6. Dependence of the apparent pK (pK_a) on the potential at different pH: 5.2 (●); 4,8 (▽); 4.4 (○). Solid lines were obtained by fitting parameters in equation (2) to the experimental points; values $g_{pH}/g_{7.4}$ calculated using equation (2) were substituted into equation (1).

Table 1. Parameters of the inner (1) and the surface (2) acid groups.

Neuron	pK_1	pK_2	$\alpha(E_M=0)$	$\alpha(E_M=100)$	δ
12	5.2	5.6	0.5	0.22	0.55
19	4.8	5.4	0.5	0.15	0.60
20	4.9	5.4	0.5	0.25	0.50
23	5.1	5.3	0.5	0.12	0.45
25	5.3	5.6	0.5	0.15	0.35
31	4.8	5.4	0.5	0.30	0.55
33	5.1	5.1	0.4	0.12	0.45
Mean	5.0	5.4	0.49	0.19	0.49
±SD	±0.2	±0.2	±0.04	±0.07	±0.08

Let us assume (according to Mozhayeva et al. 1981) that the inhibition of sodium conductance at low pH is due to the protonation of at least two acid groups at the channel. In case there is no coulombic interaction between protons bound to both groups the conductance ratio may be given by the following equation:

$$\frac{g_{pH}}{g_{7.4}}=\frac{\alpha K_2 a_H+1}{(1+K_2 a_H)(1+a_H K_1 \exp(-\delta E_M F/RT)} \quad (2)$$

where K_1 and K_2 are the binding constants of H^+ with the inner and the outer acid group, respectively; δ is the fraction of the total membrane potential drop from the outer side to the inner group; a_H is the activity of H^+ ions; $\frac{RT}{F}=25.53$ at 23 °C; and the parameter α showing the degree of reduction of the single channel conductance as the surface group becomes protonated. By varying the values of parameters in equation (2) we managed to fit the model predictions to experimental data. When fitting, pK_1 and pK_2 values were changed in steps of 0.1, δ in steps of 0.1, α in steps of 0.01. We have to assume that α increases lineary with the potential. A rather good agreement between the calculated and experimental values is presented in Fig. 6 can be reached with following parameter values: $pK_1=4.9$; $pK_2=5.4$; $\delta=0.5$; $\alpha\ (E_M=0)=0.25$; $\alpha\ (E_M=100)=0.60$. Solid lines in Fig. 6 were obtained by substituting theoretical $g_{pH}/g_{7.4}$ values from equation (2) into equation (1). It should be noted that no reasonable agreement could be obtained with any other combinations of parameters. Table 1 shows the results of fitting for all neurons. Lines 1a and 2a in Fig. 5 show the values of $g_{pH}/g_{7.4}$ calculated using equation (2) in comparison with experimental points and other predictions of the decrease of sodium conductance (lines 1b and 2b).

As shown above, the inhibition of I_{Na} by protons can be described adequately by a model with two acid groups at the sodium channel (Mozhayeva et al. 1981) rather than on other assumptions (Woodhull 1973; Mironov 1983). It should be

concluded that the conductance of fast sodium channels in neuronal somatic membrane is controlled by at least two acid groups with different localization in the pore. Mean values pK_1, pK_2 and δ obtained in our work are similar to those obtained on the node of Ranvier (Mozhayeva et al. 1982). Our results suggest a principal structural similarity between fast tetrodotoxin-sensitive sodium channels in neuronal and nodal membranes.

References

Bezanilla F., Armstrong C. M. (1977): Inactivation of the sodium channel. 1. Sodium channel experiments. J. Gen. Physiol. **70**, 549—566

Hamill O. P., Marty A., Neher E., Sakmann B., Sigworth F. J. (1981): Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. **391**, 85—100

Hille B., Woodhull A. M., Shapiro B. I. (1975): Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions and pH. Phil. Trans. Roy. Soc. London B **270**, 301—318

Katz G. M., Schwartz T. G. (1974): Temporal control of voltage-clamped membranes: an examination of principles. J. Membrane Biol. **17**, 275—291

Kostyuk P. G., Veselovsky N. S., Tsyndrenko A. Y. (1981): Ionic currents in the somatic membrane of rat dorsal root ganglion neurons — 1. Sodium currents. Neuroscience **6**, 2423—2430

Krishtal O. A., Pidoplichko V. I. (1975): Intracellular perfusion of Helix neurons. Neirofiziologiya **7**, 327—329 (in Russian)

Mironov S. L. (1983): Comparison of the structure of selectivity filters of calcium and sodium channels in excitable membranes. Dokl. Akad. Nauk SSSR **268**, 731—734 (in Russian)

Mozhayeva G. N., Naumov A. P., Negulyaev Yu. A. (1981): Evidence for existence of two acid groups controlling the conductance of sodium channel. Biochim. Biophys. Acta **643**, 251—255

Mozhayeva G. N., Naumov A. P., Negulyaev Yu. A. (1982): Interaction of H^+ ions with acid groups in normal sodium channels. Gen. Physiol. Biophys. **1**, 5—19

Mozhayeva G. N., Naumov A. P. (1983): The permeability of sodium channels to hydrogen ions in nerve fibres. Pflügers Arch. **396**, 163—173

Naumov A. P., Negulyaev Yu. A., Nosyreva E. D. (1979): Change in affinity of acid group in sodium channel for hydrogen ions under effect of aconitine. Dokl. Akad. Nauk SSSR **244**, 229—232 (in Russian)

Veselovsky N. S., Kostyuk P. G., Tsyndrenko A. Y. (1980): Slow sodium channels in somatic membrane of spinal ganglia neurons of newborn rats. Dokl. Akad. Nauk SSSR **250**, 216—218 (in Russian)

Woodhull A. M. (1973): Ionic blockage of sodium channels in nerve. J. Gen. Physiol. **61**, 678—708

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