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EVIDENCE FOR EXISTENCE OF TWO ACID GROUPS CONTROLLING THE CONDUCTANCE OF SODIUM CHANNEL

G.N. MOZHAYEVA, A.P. NAUMOV and Yu.A. NEGULYAEV

Institute of Cytology, Academy of Sciences of the U.S.S.R., Leningrad 190121 (U.S.S.R.)

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Summary

The inhibition of the sodium current in nodal membrane at low pH external solutions was studied under voltage clamp conditions. Analysis of the data for membrane potentials from +10 to +150 mV shows that the inhibition of the Na^+ currents at high positive potentials cannot be described by a titration curve of a single acid group. The data can be explained on assumption that the conductance of each sodium channel is controlled by two acid groups: one is located within the pore, the other just near the outer mouth of the pore. The affinity of both groups for H^+ is estimated.

The results of experiments on inhibition of the sodium currents by H^+ [1—6] have led to a conclusion that there exists an acid group within the sodium channel. This group is thought to be a part of the selectivity filter [7], and its properties are essential for conductance and selectivity of the channel. Recent studies of the sodium channels modified by aconitine [8,9] and trimethyloxonium ions [10,11] have suggested that the channel contains some other acid group, which seems to be a part of the tetrodotoxin receptor. In the present work, the inhibition of sodium channels by H^+ was studied with a view to determining how many acid groups are directly involved in the control of the channel conductance.

The work was done on myelinated fibres of the frog, *Rana ridibunda*, using voltage clamp technique [8]. Membrane potential (V_m) was referred to the outside. Holding potential was set at −100 mV. Currents through the sodium channels (I_{Na}) were evoked by depolarizing test pulses. In order to remove steady-

state inactivation, test pulses were preceeded by prepulses to −140 mV (100 ms). Leakage and slow capacitative currents were subtracted automatically with an analogue electronic device.

External solutions contained either 20 mM biphthalate (pH 4.0—6.3) or 10 mM Tris-HCl (pH 7.65—7.70) buffers. The content of physiologically essential ions was the same in all solutions: 80 mM Na^+/30 mM K^+/2 mM Ca^{2+}/10 mM tetraethylammonium ions. The ends of the fibres were cut in solution containing 115 mM KF/5 mM tetraethylammonium chloride/5 mM Tris-HCl, pH 7.7.

Experiments were carried out at 9—10°C.

Ratio of the peak conductance for low pH to that for high pH ($g_{pH}/g_{7.7}$) was used as a measure of H^+ block. The use of conductance ratio instead of current ratio enables us to reduce error due to a drift of reversal potential of I_{Na} during experiment. The measurements at normal pH were repeated following each series of measurements at low pH solutions. $g_{7.7}$ values from measurements before and after low pH solutions were averaged and this averaged value was used for calculating each $g_{pH}/g_{7.7}$ value. Such a procedure enabled to minimize error due to progressive rundown of the sodium conductance in the course of experiment. If the $g_{7.7}$ value turned out to be markedly altered after pH lowering, results of corresponding measurements were discarded.

Fig. 1 shows the family of experimental $g_{pH}/g_{7.7}$ vs. pH curves corresponding to different levels of potential during the test pulse. It can be seen that $g_{pH}/g_{7.7}$ vs. pH curves corresponding to more positive V_m values shift to the right, that is to lower pH values. The shift of the experimental relation to the right is in qualitative accordance with the data on decrease of proton block of the

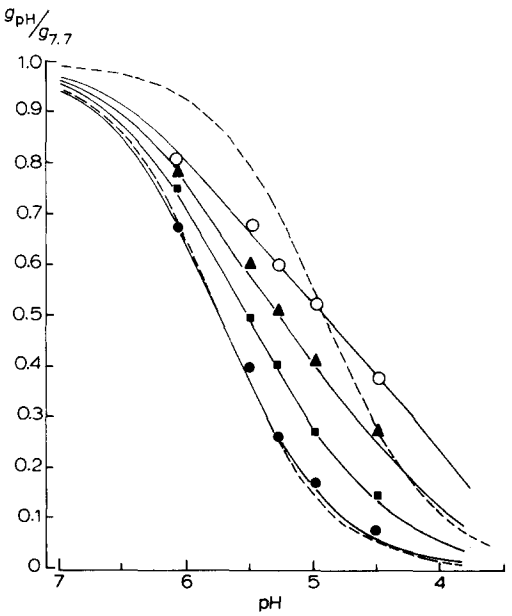

Fig. 1. The block of the sodium conductance by H^+ at different levels of membrane potential. Symbols represent experimental $g_{pH}/g_{7.7}$ values at V_m (mV): ○, 140; △, 100; □, 50 and ●, 10, during test pulse. Dashed lines are theoretical titration curves of a single acid group. Solid lines are calculated by Eqn. 2 with the following values of parameters: $pK_1 = 5.12$, $\delta = 0.40$, $pK_2 = 5.82$.

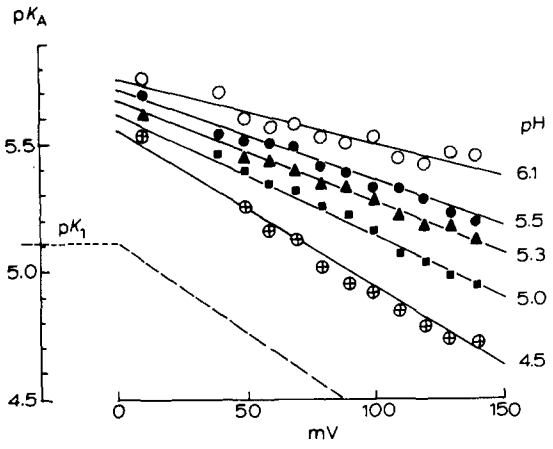

Fig. 2. Apparent pK values of the acid group in the sodium channel as calculated with Eqn. 1. The numbers to the right denote the pH of external solutions. Symbols denote pK_A values calculated by substitution of experimental $g_{pH}/g_{7.7}$ values into Eqn. 1. Solid lines were obtained by substitution of theoretical $g_{pH}/g_{7.7}$ values from Eqn. 2 into Eqn. 1. The same parameter values were used as in Fig. 1. Dashed line represents pK of the inner acid group. See text for details. The same experiment as in Fig. 1.

sodium channels as potential increases [2]. However, if the reduction of the sodium conductance were due to the protonation of one acid group with potential-dependent affinity for H^+, $g_{pH}/g_{7.7}$ vs. pH curves for different V_m values would differ from each other only in position along pH axis, but not in shape. Calculations on the assumption that there is only one titrable group in the channel gives reasonable fit to experimental points for $V_m = +10$ mV, whereas it fails to do it for more positive potentials (Fig. 1).

Another representation of the data also shows that the assumption that the conductance of the sodium channel is controlled by one acid group is inadequate. If this assumption were correct, the apparent pK for the group can be calculated from the following equation [9]:

$$pK_A = \log[(g_{7.7}/g_{pH}) - 1] + pH \quad (1)$$

When deriving this equation, H^+ concentration at normal pH was neglected. Calculated pK_A for any particular V_m should not depend on pH at which measurements were made. However, as shown by experiments in Fig. 2, with the decrease of pH the calculated pK_A decrease and pK_A vs. V_m curves become steeper.

These results can be interpreted as follows. Let us assume that there are two acid groups: the first one deep inside the pore and the second just at the external end of the pore. In this case, the protonation of the first group would depend on V_m [2] and the protonation of the second would not. Let us further assume that the protonation of the first group results in complete block of the channel and the protonation of the second reduces conductance (current) of the channel to the fraction α of its normal value. Then the relative sodium conductance can be expressed by the equation:

$$\frac{g_{pH}}{g_{7.7}} = \frac{\alpha K_2 C_H + 1}{(1 + K_2 C_H)(1 + K_1 C_H)} = \frac{\alpha K_2 C_H + 1}{(1 + K_2 C_H)(1 + S K_2 C_H)} \quad (2)$$

where C_H is activity of H^+ in external solution; K_1 and K_2 are binding constants of H^+ with first (inner) and second (surface) groups, respectively; $S = K_1/K_2$.

Absolute K_2 values affect only the position of $g_{pH}/g_{7.7}$ vs. pH curve on pH axis and can be determined by shifting calculated curve (with arbitrary K_2) along pH axis until it fits to experimental points. The shape of $g_{pH}/g_{7.7}$ vs. pH curve depends on α and S values, which are not known beforehand. As follows from studies by Sigworth and Spalding [11,12], both treatment of the membrane by trimethyloxonium ions and decrease of pH of the external solution result in nearly 3-fold reduction of the single channel conductance at zero potential. It is natural to identify the assumed surface groups with the groups available for esterification by trimethyloxonium ions. Both esterification and protonation of this group remove its negative charge. So let us assume α to be equal to 0.3 at $V_m = 0$ mV. The above mentioned studies as well as our own experiments (not illustrated) with triethyloxonium ions, which exert the same action as trimethyloxonium ions [13], show that removal of the negative charge of the surface group makes the current-voltage curve less convex at positive potentials. In terms of our model, this implies that α would increase as V_m becomes more positive. The comparison of current-voltage relations before and after triethyloxonium treatment enables to evaluate approximately α values over all the positive potential range used. For instance, at potentials +50, +100 and +140 mV (see Fig. 1), α = 0.34, 0.43 and 0.54, respectively.

A number of experimental data (unpublished observations) suggests that interaction of H^+ with the inner group is not at equilibrium because of proton motion through sodium channels. Therefore, K_1 obtained from H^+ block experiments is not, strictly speaking, genuine equilibrium-binding constant and it should depend on potential in a more complicated way than a single exponential (see e.g. Ref. 2). However, data available are not enough to use the model taking into account permeation of H^+ for calculations. Therefore, following the simple Woodhull's model [2], let us assume for the present that K_1 (or S) depends exponentially on potential, at least at positive V_m, with the factor in the exponent proportional to the fraction of the way across the membrane from the outside to the binding site (δ).

Solid curves in Fig. 1 were calculated from Eqn. 2 and in Fig. 2 from Eqns. 1 and 2. A rather good agreement between calculation and experiment was obtained with the following numerical values of parameters of the model: $pK_{1,0}$ = 5.12, δ = 0.40, pK_2 = 5.82. $pK_{1,0}$ is the value of pK_1 at $V_m = 0$ mV, obtained by means of extrapolation. It should be noted that owing to the fact that the theoretical curves had to be fitted to experimental points obtained for a wide range of pH and V_m values, arbitrariness in the choice of parameters is significantly minimized. The calculations for eight experiments gave the following average (±S.D.) values of parameters: $pK_{1,0} = 5.02 \pm 0.11$, $\delta = 0.42 \pm 0.04$, $pK_2 = 5.39 \pm 0.25$.

An attempt was made to take into account coulombic interaction between protons bound to two acid groups, with a result that the same δ values and lower values of inherent binding constants for both groups were obtained.

Thus, the results of the study presented enable us to make the following conclusions. (1) The conductance of the sodium channel is controlled by at least two acid groups with different affinities to H^+ and different localization in the

channel. Properties of the surface group unlike those of the inner group do not seem to be affected by aconitine [9], while the inner group seems to be inaccessible for trimethyloxonium ions [10,11]. (2) It is impossible to estimate the parameters of the inner group without taking into account the existence of the surface group. Thus, calculation based on the assumption that the channel conductance is affected by the inner acid group alone (Fig. 2) gives overestimated pK value and underestimated δ value for the inner group. The higher the pH, the more pronounced this discrepancy. Thus, δ value is estimated to be no less than 0.4 in the present study, while previous studies [2,5,9] gave δ values of approx. 0.25. Hence, taking into account the influence of the surface group on the channel conductance, it can be assumed that the inner acid group and consequently the whole selectivity filter is located deeper within the channel than has been thought previously.

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