Interaction of H^+ Ions with Acid Groups in Aconitine-modified Sodium Channels

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Abstract. Ionic currents through aconitine-modified sodium channels of nodal membrane were measured under voltage-clamp conditions at normal and low pH external solutions. The pattern of the dependence of sodium conductance on pH is consistent with supposition that the channel conductance is controlled by two acid groups, one of which (inner) is situated within the pore and the other (surface) localized at the external end of the pore. Calculations according to the model proposed in a previous paper (Mozhayeva et al. 1982) show that affinity of the surface group to hydrogen ions remains essentially unchanged and that of the inner group is lowered when modifying the channel with aconitine. The hydrogen-sodium permeability ratio (P_H/P_{Na}) was estimated from the reversal potential measurements to be equal to about 2000.

Key words: Nodal membrane — Voltage clamp — Sodium channel — Aconitine — Acid groups — Permeability

Introduction

In a previous study (Mozhayeva et al. 1982) evidence was presented for the existence of at least two acid groups controlling sodium channel conductance, accessible for protonation from external solution. The first (inner) is situated within the pore and seems to be a part of selective filter (Hille 1975 a, b), the second (surface) is localized at external end of pore and seems to be part of a tetrodotoxin receptor (Spalding 1978, 1980). Within the simple model proposed in that study, the affinity of each group to hydrogen ions, and the electric distance between the external solution and the inner group were estimated.

From a theoretical viewpoint the properties of a negatively charged acid group within the channel should influence essentially selective properties of the channel. The most plausible hypothesis concerning a relationship between properties of acid group(s) and the selectivity of the ionic channel is based on Eisenman's equilibrium binding theory (Eisenman 1962) and can be briefly formulated by saying that the higher the "field strength" of the acid group, the more selective the channel for small cations and vice versa (Chandler and Meves 1965; Hille 1975 a, b). Until

recently, the only experimental evidence for this idea being applicable to the problem of selectivity of ionic channels in a biological membrane was the fact that the apparent affinity to hydrogen ions, and consequently "field strength" of acid group(s) is higher in sodium channels than in potassium channels (Hille 1973; Hille 1975 a, b). Additional and apparently more certain information concerning the relation between properties of the acid group and the type of selectivity of the ionic channel may be obtained from a comparison of the properties of acid group(s) in normal channels and those with changed selectivity. Changes in the selectivity of sodium channels can be achieved by means of modification with alkaloid aconitine (Mozhayeva et al. 1976; Mozhayeva et al. 1977; Campbell 1978). In the only study dealing with currents through aconitine-modified sodium channels at low pH (Naumov et al. 1979), the sensitivity of these channels to H^+ blockage was shown to be lower than that of normal sodium channels. However, data were obtained at a single pH value (4.77) in that work and therefore are insufficient to evaluate the parameters of acid groups at the channel (Mozhayeva et al. 1982). In the present study H^+ blockage of aconitine-modified sodium channels was investigated over wide range of acid pH values of external solutions. The data obtained demonstrate that the modification of the channel with aconitine results in a lowering of affinity of the inner acid group to hydrogen ions without essential changing in properties of the surface group. In addition, the hydrogen-sodium permeability ratio was evaluated from reversal potential measurements at low pH solutions.

Methods

The work was carried out on single nerve fibres from frogs *Rana ridibunda* under voltage clamp conditions. The methods, procedure of measurements and composition of solutions were essentially the same as described in a previous study (Mozhayeva et al. 1982). Aconitine at saturating concentrations ($2—4 \times 10^{-4}$ g/ml) was only added to the normal pH solution (7.65—7.70) because its effect is practically irreversible. In order to enhance the development of its effect a series of depolarizing pulses (+60 mV) at a frequency of 10 Hz was applied for 1—2 min (Mozhayeva et al. 1977). Under these conditions almost all sodium channels are modified. All external solutions contained (in mmol.l^{-1}): 80 Na^+, 30 K^+, 2 Ca^{2+} and 10 tetraethylammonium ions (TEA^+). TEA-Cl (5 mmol.l^{-1}) was also added to the solution in which the ends of fibres were cut (115 mmol.l^{-1} KF).

In order to minimize any error due to progressive rundown of sodium conductance, measurements at normal pH were repeated after each series of measurements at low pH, and the corresponding conductance values were averaged.

Because aconitine-modified sodium channels are activated at rather high negative potentials (Schmidt and Schmitt 1974; Mozhayeva et al. 1976), the holding potential was set at −120 and −140 mV.

Results and Analysis

Fig. 1 shows peak currents (I_{Na}) through aconitine-modified sodium channels versus membrane potential (V_m) at different pH values of external solution. It can

be seen that lowering pH results in decrease in I_{Na} values and in a shift of reversal potential (E_r) to more positive values. Both effects are more pronounced with lower pH external solutions. In the experiment presented in Fig. 1 the shift of E_r (ΔE_r) amounted to 20 mV at pH 3.95. In some experiments this value reached up to 35 mV (see Table 2). Low initial values of E_r are due to a higher (in comparison with normal sodium channels) potassium-sodium permeability ratio (Mozhayeva et al. 1976; Mozhayeva et al. 1977). It should be noted that the peak current-voltage relation at positive potentials for aconitine-modified sodium channels is concave towards the I_{Na} axis at all pH values, unlike those for the normal sodium channels which are concave towards the V_m axis at normal pH (see e. g. Mozhayeva et al. 1982). As pH is reduced, the curves become slighly more concave.

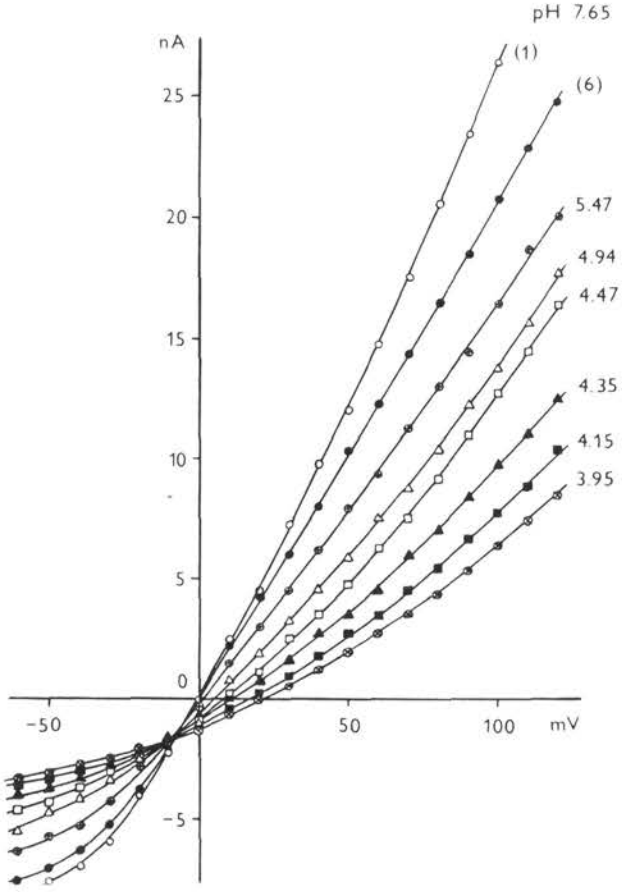

Fig. 1. Peak-current-voltage relations for the node treated with aconitine at different pH values indicated by numbers. For pH 7.65 only first and sixth curves are shown. Curves are visually estimated. Holding potential is -130 mV; conditioning pulse is -140 mV (100 ms in duration). See text for details. Node 47.

reflects the properties of open channels (Dodge and Frankenhaeuser 1959; Woodhull 1973; Mozhayeva et al. 1982).

Fig. 4 shows the typical "instantaneous" current-voltage relations at several pH values. It is evident that at normal pH the current-voltage relation reveals a rectification of the outward current over all potential range tested, that is, at

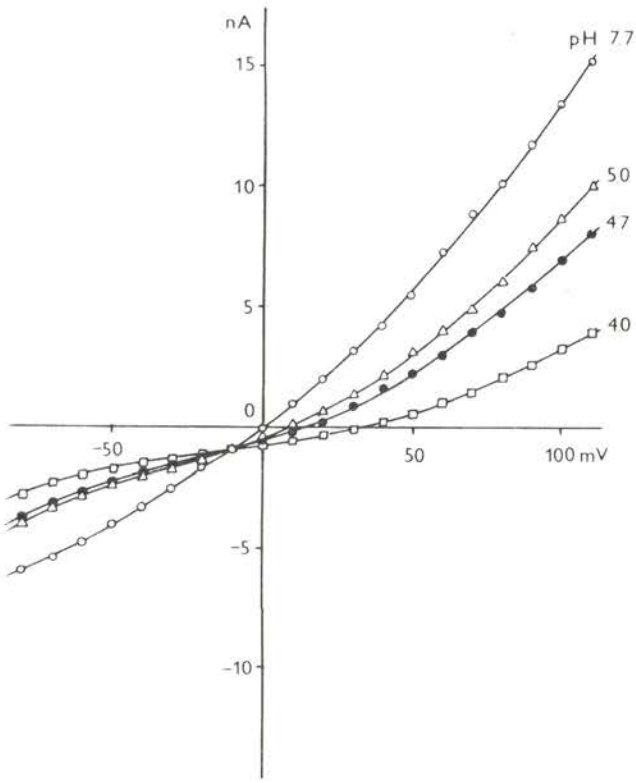

Fig. 4. "Instantaneous" current-voltage relations for the aconitine-modified channels at different pH values of external solution. Curves are visually estimated. The same node as in Fig. 3.

positive potentials it is concave towards the I_{Na} axis and at negative potentials towards the V_m axis. Such a peculiarity of current-voltage relation of aconitine-modified sodium channels was observed earlier (Mozhayeva et al. 1977). As the pH of the external solution is reduced, the "instantaneous" curves also become concave towards the I_{Na} axis at negative potentials. In other words, they become more or less symmetrical on both sides of the reversal potential.

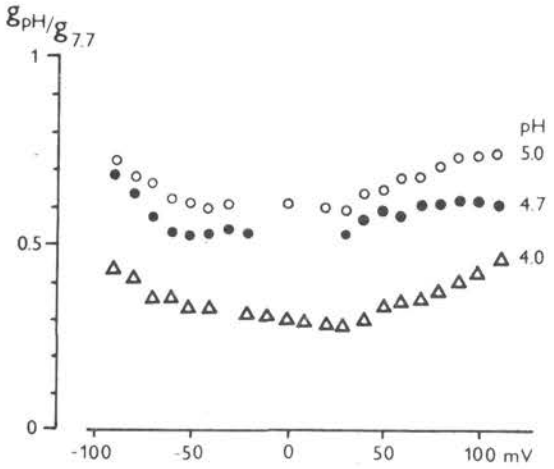

Fig. 5. The dependence of relative conductance of aconitine-modified sodium channels on membrane potential at different pH values of external solution. Symbols indicate $g_{pH}/g_{7.7}$. The same node as in Fig. 3 and 4.

Fig. 5 shows the relative conductance ($g_{pH}/g_{7.7}$) versus membrane potential for three pH values calculated from the data of the same experiment that is presented in Fig. 3 and 4. It can be seen that hydrogen blockage is decreased both at high positive and high negative potentials, with this effect being more pronounced at lower pH. The $g_{pH}/g_{7.7} - V_m$ curves for modified channels are qualitatively similar to those for the normal channel (Mozhayeva et al. 1982), however they are less steep at positive potentials and consequently more symmetrical relative to the zero potential value.

As noted in a previous study (Mozhayeva et al. 1982), the decrease in hydrogen blockage at negative potentials may be due to a high rate of H^+ ions passing from the inner binding site to the internal solution at zero potential and its enhancement at increasing negative potential values. If so, high proton permeability may be expected for aconitine-modified sodium channels. Indeed, observed shifts of reversal potentials (ΔE_r) when reducing pH support this expectation. According to constant field theory (Hodkin and Katz 1949) ΔE_r is related to relative permeability (P_H/P_{Na}) by the following equation:

$$\frac{P_H}{P_{Na}} = \left(\frac{P_K}{P_{Na}} \cdot \frac{C_K}{C_H} + \frac{C_{Na}}{C_H}\right) [\exp(\Delta E_r/24.4) - 1] \quad (2)$$

where C_{Na}, C_K and C_H are activities of Na^+, K^+ and H^+ ions in the external solutions. C_H in the solution with normal pH is neglected when deriving the equation. C_{Na} and C_K are the same in all solutions. The activity coefficient is

the open channels but also to a decrease in the number of open channels. That is why the pK value of the acid group(s) at the batrachotoxin-modified channel derived from such data seems to be an overestimate. In order to settle the issue conclusively, voltage-clamp experiments on neuroblastoma membrane treated with batrachotoxin are needed.

The decrease of affinity of the internal acid group to hydrogen ions may be accounted for by the positive charge of aconitine molecule being located not far from the acid group. Then proton binding to the group should be weakened due to Coulombic repulsion (Naumov et al. 1979). A similar effect is known to occur in a number of aminoacids in which the pK of a carboxylic group is sometimes lowered due to the presence of positive charged amino group (Martin 1964).

Decrease in pK corresponds, in terms of selective binding theory (Eisenman 1962), to a decrease in "field strength" of this anionic group. The lower the "field stregth" of the group is, the more energetically favorable is the binding of large cations from aqueous solutions as compared with small ones. At the same time, a change in selectivity due to modification of the channel may be characterised by an increase in channel-permeability to large cations, such as NH_4^+, K^+ (Mozhayeva et al. 1977; Campbell 1978). Thus, changes both in the properties of the acid group and in selectivity resulted from modification of the sodium channels with aconitine are qualitatively consistent with prediction of Eisenman's theory. Therefore, one can suggest that changes in the channel selectivity induced by aconitine are due not only to an increase in pore diameter (Mozhayeva et al. 1977) but also to decrease in the "field strength" of the anionic inner group.

A decrease in δ value due to modification of the channels with aconitine suggest changes in potential distribution along the pore. If one assumes, as usual, that the electric field is constant across the membrane, the decrease in δ should imply a displacement of the inner acid group towards the external surface of the membrane. This assumption does not seem to be plausible as the inner group should become closer to the surface one and therefore Coulombic interaction between groups and the influence of the surface group on channel conductance, should be more strong in the modified channel, i. e. F^2 value should increase and α value should decrease. However, according to the calculations F^2 remains the same and α becomes somewhat higher due to the modification of the channel. Additional experiments and more detailed theoretical considerations are needed to elucidate observed changes in the properties of open channels due to modification with aconitine.

The use of aconitine enables to discriminate pharmacologically between two functionally significant acid groups at the sodium channels. The fact that aconitine affects neither the properties of the surface group or sensitivity of the channel to tetrodotoxin (Mozhayeva et al. 1976) is consistent with the assumption that this

group is part of a tetrodotoxin receptor (Spalding 1978, 1980; Mozhayeva et al. 1982).

As noted in the preceding paper (Mozhayeva et al. 1982) K_1 values obtained from the fit of the model to experimental data are not, strictly speaking, equilibrium binding constants because the barriers the H^+ ions have to overcome in the channel are low. An increase in the rate of H^+ ions moving from the binding site to the internal solution is equivalent to an increase in the rate of dissociation without a change in the rate of proton binding. If one assumes that rates of H^+ ions moving from binding site to external and to internal solutions are the same, the *inherent equilibrium* pK of the inner group of aconitine-modified sodium channels may be obtained by adding $\lg 2 = 0.3$ to the inherent values given in the "Results and Analysis" section. Thus the inherent equilibrium pK of the inner group of aconitine-modified sodium channels may be estimated to be 4.3—4.5. These values seem to give the upper limit for pK of the inner group of aconitine-modified sodium channels, because the rate of H^+ ions moving to the internal solution is hardly higher than the rate of H^+ ions moving to external solution. The same calculation for normal channels gives analogous quantities equal to 5.1—5.3.

A high hydrogen to sodium permeability ratio of aconitine-modified sodium channels indicates that the height of the energetic barrier relative to some mean level in solutions for H^+ ions in the channel is much lower than that for Na^+ ions (Chizmadzhev et al. 1974; Hille 1975 a). The higher P_H/P_{Na} value, by almost an order for modified as compared with normal channels, seems to be due mainly to a higher absolute permeability to H^+ ions and perhaps to some extent to a lower sodium permeability (Mozhayeva et al. 1977) of the modified channels. The possibility of an enhancement of absolute proton permeability indicates that the structure of the pore imposes some restrictions on the motion of hydrogen (or possibly, hydronium) ions, and that these restrictions are less in the aconitine-modified channel.

It is conceivable that the energetic profile for H^+ ions in the aconitine-modified channel is much the same as in water solution except for a deep energetic well due to the presence of the inner acid group. In spite of the high proton permeability of modified sodium channels, H^+ ions remain blocking ions. In other words, our assumption used in the model that protonation of the inner group results in a complete blockage of the channel seems to hold in this first approximation. A few experiments at pH lower than 4.0 (3.65 – 3.35) show that the conductance of modified channels falls under these conditions to lower than 10% of the initial level. The blocking effect of H^+ ions is accounted for by strong binding of H^+ ions with the acid groups (Chizmadzhev et al. 1974).

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