HYDROGEN CURRENTS THROUGH ACONITINE-MODIFIED SODIUM CHANNELS IN THE NERVE FIBER MEMBRANE

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Ionic currents through aconitine-modified sodium channels of the Ranvier node membrane were measured by a voltage clamp method in an external medium free from sodium ions. A shift of pH of the solution below 4.6 led to the appearance of inward ionic currents, whose kinetics and activation region were characteristic of aconitine-modified sodium channels at low pH. These currents were blocked by the local anesthetic benzocaine in a concentration of 2 mM. Experiments with variation of the concentration of Ca++, Tris+, TEA+, and choline+ in acid sodium-free solutions showed that these cations make no appreciable contribution to the inward current. It is concluded that the inward currents observed under these conditions are carried by H+ (or H2O+) through aconitine-modified sodium channels. From the shifts of reversal potentials of the ionic currents the relative permeability (PH/PNa) for H+ was determined: 1059 ± 88. The results agree with the view that the aconitine-modified sodium channel is a relatively wide water pore, and that movement of H+ through it is limited by its binding with an acid group.

INTRODUCTION

It was shown in the previous paper [2] that with a shift of pH of a sodium-free solution below 4.0, small (under 0.1 nA) inward currents with a kinetics similar to that of sodium currents can be recorded. Analysis showed they are carried through sodium channels by H+. Under these circumstances permeability of the sodium channels for H+ was approximately 200 times greater than the permeability for Na+, determined from shifts of the reversal potentials of ionic currents (ER). It was concluded from these results that energy barriers for H+ in the sodium channel are small and the velocity of its passage through the channel is limited by binding with an acid group [18]. In this connection it would be interesting to have similar data for channels in which the size of the pore and the properties of the acid group are modified. The alkaloid aconitine is known to have a considerable effect on the selective properties of sodium channels. The diameter of the pore of the channel is evidently increased by modification with aconitine, whereas the affinity of the acid group inside the channel for H+ is reduced [4, 19]. In the present investigation hydrogen currents were investigated through sodium channels of the nerve fiber membrane modified by aconitine.

METHOD

Experiments were carried out on single nerve fibers of the frog Rana ridibunda by the voltage clamp method [18]. Full details of the method of the measurements and preparation of the solutions were described previously [2].

The control solution contained (in mM): Na+ 100, Ca++ 2, tetraethylammonium+ (TEA+) 10, Tris+ 10, Cl− 129; pH 7.4-7.6. To prepare sodium-free solutions with low pH, buffered mixtures of Tris-base and succinic or propionic acid were used. The tonicity of the solutions was maintained constant by addition of sucrose. The concentration of free Ca++ was calculated from the binding of Ca++ and H+ with these acids.

A control solution containing 0.3-0.6 mM aconitine was used to modify the channels. Since the effect of aconitine is virtually irreversible, its presence in the test solutions was unnecessary.

The ends of the fibers were cut off in a solution containing (in mM): KF 90, CsF 20, and Tris-HCl 5; pH 7.6.
The membrane potential ($E_M$) was determined as the potential of the internal solution. When the membrane current was calculated the resistance of the axoplasm measured from the current electrode was taken to be 20 MR

$E_M$ held on the membrane ($E_H$) was established at between -120 and -130 mV after modification of the sodium channels by aconitine. Before replacement of the sodium-free solution with normal pH by acid sodium-free solutions $E_H$ was reduced to between -80 and -70 mV in order to protect the membrane against electrical failure.

In cases when the currents to be measured were large enough they were recorded in response to a single testing depolarization shift of potential. Leakage current and capacitive current were deducted automatically. When the currents were small, measurements were made by summation of responses to four depolarizing and 16 hyperpolarizing shifts, of one-quarter of their amplitude, by summation on a digital averager. The signal of the current amplifier was filtered through a low-frequency filter (2 kHz).

Experiments were carried out at a temperature of 9-11°C.

RESULTS

Traces of the currents in the Ranvier node membrane after treatment with aconitine in the control solution are shown in Fig. 1a. As earlier studies showed [3, 18, 20], currents through aconitine-modified sodium channels are activated at more negative $E_M$ values and are only partially inactivated. Traces of currents in sodium-free solutions, cumulated on an averager, are given in Fig. 1b-d. This figure shows that in the case of a normal pH currents appeared at approximately the same $E_M$ values as in the control solution, but all were outward in direction, even at $E_M$ of -88 mV. With a decrease in pH of the solution to 4.08 and even lower, to 3.80, depolarizing shifts induced inward currents. In this case they reached approximately 0.5 nA, but in some experiments they were much greater and could be observed without cumulation. The increase in current was preceded by a rapidly falling outward current, evidently a gating current [7, 15].

Several features allow the inward ionic currents at low pH values of the sodium-free solution to be identified as currents through aconitine-modified sodium channels. Inward currents appeared at the same $E_M$ values as gating currents. Their kinetics was similar to that of the sodium current through modified channels, i.e., they developed more slowly than currents through normal channels under analogous conditions [2], and after reaching a peak they fell, not to zero but to a certain steady level.

The $E_M$ region where these currents appeared was shifted relative to the activation regions of the currents through normal sodium channels toward hyperpolarization by about 50 mV. This applies also to the $E_M$ region where gating currents were observed. A similar shift in the activation region of sodium channels during modification with aconitine has also been described for ionic [3, 18, 20] and for gating currents [1].

At low pH values currents appeared in the case of more positive $E_M$ values than at normal pH (Fig. 1). Such a shift of the activation region of the sodium channels could be expected as a result of lowering the pH of the solution on account of a change in the sort of charge of the membrane [9, 12, 19].

The local anesthetic benzocaine, in a concentration of 2 mM, completely blocked the inward ionic currents and left only the gating currents (Fig. 2). Benzocaine is known to block aconitine-modified sodium channels almost as effectively as normal channels [5].

It can thus be stated reasonably confidently that the rise and fall of currents recorded in sodium-free acid solutions after treatment of the membrane with aconitine are the result of processes of activation and inactivation of aconitine-modified sodium channels.

In many cases at potentials more positive than -10 mV the current not only fell, but also changed direction to outward. This phenomenon was particularly marked in the experiment whose results are given in Fig. 4. The outward part of the current could not be attributed to channels of any other type, such as potassium, for the latter are blocked by TEA$^+$ from outside and by Cs$^+$ from inside, and before treatment with aconitine there were no signs of a potassium current. The complex, multicomponeut character of the current likewise cannot be explained by the presence of two populations of sodium channels, normal and modified, for currents through normal channels under analogous conditions have a much smaller value. It will be noted that the change in $E_T$ in the course of the depolarizing shift also took place in normal sodium solution (Fig. 1a), although to a much lesser degree. Both the inward and the outward parts of the currents were completely suppressed by benzocaine. It can be concluded from all these findings that the whole current was transported through modified