EFFECT OF ACONITINE ON SOME PROPERTIES OF THE SODIUM CHANNELS OF THE RANVIER NODE MEMBRANE

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Aconitine causes the appearance of two types of modified channels in the Ranvier node membrane. Channels of the first type are activated at high negative potentials and are inactivated only partly or not at all; their selectivity is sharply reduced: The mean ratio of potassium to sodium permeability is 0.72. The properties of these channels are stable with time. The second type of modified channels lose their conductivity during the action of aconitine, and their kinetic characteristics and region of activation are similar to those of normal sodium channels. They are less selective than normally: the ratio of potassium to sodium permeability is 0.22. The sensitivity of the modified channels to tetrodotoxin is at the same level as that of normal sodium channels.

INTRODUCTION

Recent experimental data on the properties of sodium channels of excitable membranes has led to the development of detailed models of their structure in which parts of the channel regarded as having different functions are distinguished [4, 9, 10, 11]. In the modern view, for instance, outer and inner "mouths" a "selective filter," a "hilum," and a tetrodotoxin receptor can be distinguished in the sodium channel. To increase the accuracy and continue the development of models, fresh investigations are required in order to determine structural connections between the various parts of the channel. In this respect the results obtained by modifying the properties of the sodium channels by means of various active substances could be useful. Several agents modifying the normal function of sodium channels in one way or another are now known: scorpion venom [15, 16], batrachotoxin [3, 13, 20], grayanotoxin [24], veratridine [3, 25, 26], aconitine [22], etc.

In this investigation the effect of the alkaloid aconitine on the sodium currents through Ranvier node membranes was studied. Attention was concentrated chiefly on properties of the sodium channels which are modified by aconitine, such as the relationship between conductance and potential, ability to undergo inactivation, selectivity, and sensitivity to tetrodotoxin.

EXPERIMENTAL METHOD

Experiments were carried out on single nerve fibers of Rana ridibunda by the voltage clamp method.

The basis for the voltage clamp method was Nonner's technique [21] as modified by Lonskii et al. [1]. The surface resistance of the internodal segments was increased with the aid of air bridges. The screened potential electrode (point C in Nonner's scheme) was connected to the amplifier input, not directly, but through a source follower; the screen of the electrode was connected to the output of the follower. In this way the frequency characteristics of the preparation could be improved, with a corresponding decrease in the demands on the frequency characteristics of the amplifier. Furthermore, in these experiments an additional electrode, connected to the internodal segment (point B in Nonner's scheme), was connected to the output of the follower and not to ground. On the one hand, this simplified the preparation and use of the chamber by comparison with the method of Lonskii et al. [1], for the additional electrode also served as part of the screen, and on the other hand, it prevented the current from flowing over the outer surface of the internode [2].

The input cascade of the follower incorporated a KP 305 D transistor with isolated gate. The operational amplifier was mounted on the base of a standard 1 UT 401 B integral circuit. The necessary frequency
corrections were done with the aid of RC-couplings. The time for the voltage to become established on the membrane varied from 15 to 25 μsec for different fibers. The output voltage of the operational amplifier, proportional to the membrane current, was led to the input of an oscilloscope and photographed by means of a camera attachment. During calibration of the membrane current the resistance of the axoplasm on the side of the current electrode (point E in Nonner’s scheme) was taken to be 20 MΩ.

For external perfusion of the node solutions of the following ionic composition (in mM) were used: 120 Na⁺, 2.5 K⁺, 2 Ca²⁺, 5 Tris⁺, 10 TEA⁺, 131.5 Cl⁻, 10 Br⁻ (solution A); 120 K⁺, 2 Ca²⁺, 5 Tris⁺, 10 TEA⁺, 129 Cl⁻, 10 Br⁻ (solution B). The pH was kept at 7.3. TEA⁺ was added to the solutions to suppress potassium currents through the node membrane [6, 14, 19].

The ends of the dissected fiber on either side of the node to be tested were cut off in a solution containing either 124 mM KCl or 100 mM KCl and 20 mM TEA⁻Br⁻. In the latter case, perfusion of TEA inside the fiber compensated for the reduced effectiveness of external TEA⁺, as is usually observed when high positive potentials are present on the membrane [19].

Tetrodotoxin (Sankyo), aconitine nitrate, and aconitine chloride* were used.

Experiments were carried out at room temperature (20-22°C).

**EXPERIMENTAL RESULTS**

The action of aconitine in a concentration of 1·10⁻⁴ g/ml on the sodium currents of the node membrane is illustrated in Fig. 1; here and in the remaining experiments potassium currents were absent because of application of TEA⁺ to both sides of the membrane. The initial membrane potential (holding potential), Eₗ, was maintained at −90 (Fig. 1a, b) and −120 (Fig. 1c, d) mV. The value of the membrane potential (Vₘ) during the testing pulse is shown near the appropriate records.

An inward current, which showed no visible inactivation, appeared 3.5 min after application of aconitine, when Vₘ was −75 and −60 mV (Fig. 1b). With an increase in amplitude of the depolarizing pulse (from −30 to 60 mV) an inactivating current, initially inward, later outward in direction, was added to the steady-state current.

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