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SLOW ACTIONS OF HYPERPOLARIZATION ON SODIUM CHANNELS IN THE MEMBRANE OF MYELINATED NERVE

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Summary

The mean sodium current, I, and the variance of sodium current fluctuations, var, were measured in myelinated nerve during a depolarization to $V = 40$ mV applied from the resting potential ($V_H = 0$) or from a hyperpolarizing holding potential $V_H = -28$ mV. From I and var the relative variations in the number N and the conductance γ of sodium channels following changes of the holding potential were calculated. Hyperpolarizing the membrane from $V_H = 0$ to -28 mV increased N by a factor of 3.7, whereas γ decreased by a factor of 0.53. These actions of holding potential on sodium channels develop slowly since 500 ms prepulses to 0 or -28 mV do not alter the values of N and γ.

Introduction

Hyperpolarization removes the inactivation of sodium channels in nerve. According to Hodgkin and Huxley [1] and Frankenhaeuser [2] this behaviour can be described by a voltage- and time-dependent sodium inactivation function h. In their formalism the kinetics of h follow an exponential time course with time constants of some milliseconds. In addition to this 'classical' h inactivation slower sodium inactivation processes with time constants in the s and min range have been found in squid giant axons [3—5], in myelinated nerve [6—10] and in *Myxicola* axons [11].

In this paper we are concerned with a slow inactivation process in myelinated nerve occurring within 2—3 min after changes of the holding potential. It appears to be independent of the h inactivation process [7,9,10], and it is controlled by the electric field strength in the membrane since the effects of holding potential can be compensated or even reversed by simultaneous changes of the external surface potential [8]. The removal of slow sodium inac-

tivation upon hyperpolarization only increases the magnitude of the available sodium current without significant effects on the kinetics of sodium activation (m) and normal sodium inactivation (h) [7,10]. Thus hyperpolarization slowly changes the number and (or) the conductance of sodium channels in the nodal membrane without affecting the driving force for sodium currents or gating properties of individual sodium channels. The present study was undertaken to quantify the relative changes of the number and conductance of sodium channels upon hyperpolarization from an analysis of sodium current fluctuations. Part of our results has been reported previously [12].

Methods

Single motor and sensory fibres were dissected from the tibial nerve of the frog *Rana esculenta* [13]. A node of Ranvier was voltage-clamped at 15°C using the method described by Nonner [14]. The ends of the fibre were cut in isotonic CsCl solution. The potassium channels of the nodal membrane were thus blocked by Cs^+ having reached the node by diffusion through the axoplasm and by 10 mM tetraethylammonium ions applied externally. Displacements of the membrane potential from its resting value are denoted by V. Membrane currents were calibrated using the dimensions of the fibre and a specific axoplasm resistance of 110 Ω/cm [15].

The peak sodium currents of Fig. 1 were measured in an external solution (solution I) containing 110.5 mM NaCl, 2 mM $CaCl_2$, 10 mM tetraethylammonium chloride and 4 mM morpholinopropanesulphonic acid/NaOH buffer at pH 7.1.

Measurement and analysis of currents and current fluctuations at the end of a depolarizing voltage step were performed as follows: Currents were measured through a 100 Hz low-pass filter. Linear components of leakage and capacity currents were subtracted by an analogue circuit. 32 current values at about 10 ms intervals were recorded at approximately steady-state conditions between 145 and 460 ms after a depolarizing voltage step to $V \approx 40$ mV. Current fluctuations were measured during the same period at 80 μs intervals through a separate channel at higher gain and through a 5 kHz low-pass filter [15]. Two procedures were applied to avoid artifacts from slow systematic variations of the mean current: The drifts were compensated by subtracting an exponential waveform produced by an analog trend simulator, and current samples from each two successive test pulses were subtracted. From these differences of current fluctuation samples the spectral density and the variance of current fluctuations were calculated. These procedures to be described in detail elsewhere (Conti, F., Neumcke, B., Nonner, W. and Stämpfli, R., unpublished) removed almost all contributions of $1/f$ noise at low frequencies. 60 test pulses at 5-s intervals were applied and currents, variances and spectral densities averaged. The pulse sequence was first performed in solution I and, again, in solution II containing 300 nM tetrodotoxin or 50 nM saxitoxin in addition to block sodium channels. The differences between the currents, variances or spectral densities of current fluctuations in solutions I and II were considered as sodium current and variance or spectral density of sodium current fluctuations, respectively.

Due to the absence of significant $1/f$ noise the sodium current fluctuations originate mainly from the statistical open-close kinetics of sodium channels (conductance fluctuations). If all sodium channels are identical, are non-interacting and have only one conducting state, the variance (var) of sodium current fluctuations is given by [16,17]:

$$\text{var} = N\, i^2\, p\, (1 - p) \tag{1}$$

where N is the number of sodium channels, i the current through one open channel and p the probability of the open channel state. Introducing the mean sodium current $I = N\, i\, p$ yields the relation

$$\text{var}/I = i\, (1 - p) \tag{2}$$

which reduces to $\text{var}/I = i$ at the end of a depolarizing voltage step at which the sodium current is almost completely inactivated ($p << 1$). Hence the ratio of the expressions var/I determined at a fixed test potential and two different holding potentials is equal to the ratio of the single channel currents i. Since the sodium reversal potential does not depend on holding potential (compare Fig. 1), i is also proportional to the channel conductance γ. The ratio of I^2/var finally gives the ratio of the channel numbers N at different holding potentials (see Eqn. 1).

Results

Fig. 1 shows peak sodium currents I_{Na} during various depolarizations V recorded first by holding the fibre at the resting potential ($V_H = 0$), then at a hyperpolarizing holding potential of $V_H = -28$ mV and, again, at $V_H = 0$. To remove fast sodium inactivation ($h_\infty = 1$) a hyperpolarizing prepulse (—40 mV, 50 ms) was applied between the holding and test potential. The data demonstrate that the sodium reversal potential (intercept of the $I_{Na} - V$ curve with

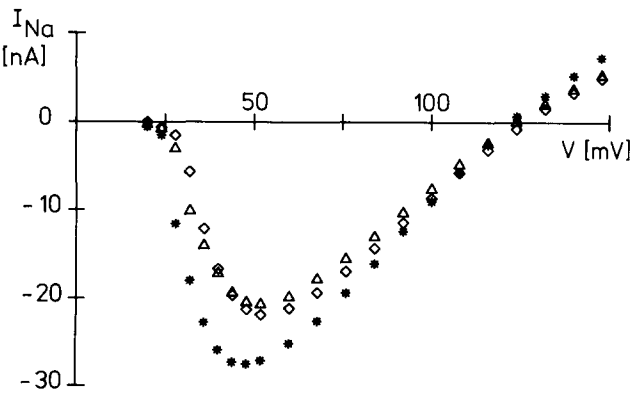

Fig. 1. Peak sodium current I_{Na} as a function of depolarization V. The fibre was first held at the holding potential $V_H = 0$ (◇), then at $V_H = -28$ mV (*) and, again, at $V_H = 0$ (△). Between V_H and V a hyperpolarizing prepulse (—40 mV, 50 ms) was applied to remove fast sodium inactivation ($h_\infty = 1$). I_{Na} values were recorded 4 min after changes of the holding potential. Exp. 5/79, motor fibre.

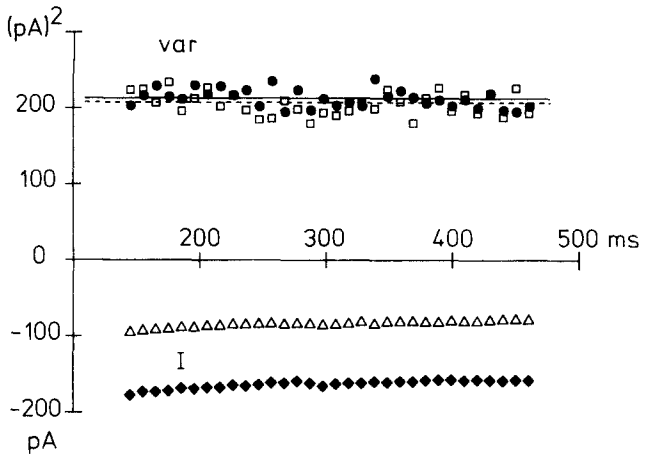

Fig. 2. Sodium current, I, and variance of sodium current fluctuations, var, between 145 and 460 ms after a depolarization to V = 40 mV from two different holding potentials V_H = 0 (△, □) and V_H = −28 mV (♦, ●). ------, mean of variance at V_H = 0; ———, mean of variance at V_H = −28 mV. Current values were recorded 4 min after change of holding potential. Exp. 10/78, motor fibre.

the V-axis) is not affected by the holding potential and that the increase of sodium currents upon hyperpolarization is reversible.

The effects of the holding potential on sodium currents and the variance of sodium current fluctuations at the end of a 40 mV depolarization are illustrated in Fig. 2. Measurements started 145 ms after depolarization. Hence there are no aftereffects from a previous holding potential on fast sodium inactivation (h) since τ_h = 3.8 ms at V = 40 mV and 10°C [18]. Nevertheless, the sodium currents for V_H = −28 mV are approximately twice as large as those recorded for the resting potential V_H = 0. On the other hand, the mean of the variance increased only by 4% upon hyperpolarization.

In the experiment illustrated in Fig. 2 the sequence of holding potentials was V_H = 0, −28 mV (solution I), −28 mV, 0 (solution II with tetrodotoxin). In other experiments the sequence of holding potentials was reversed with no influence on the results. Also, no systematic differences were found when sodium channels were blocked by tetrodotoxin or saxitoxin in solution II or when motor or sensory fibres were investigated.

As described in the Methods section the single channel conductance γ is proportional to var/I, and the number N of sodium channels proportional to I^2/var. Taking I as the sodium current at 145 ms after depolarization and var as the mean of the variance of sodium current fluctuations, we obtained from 7 experiments:

$$\gamma(V_H = -28\text{ mV})/\gamma(V_H = 0) = 0.53 \pm 0.06$$

$$N(V_H = -28\text{ mV})/N(V_H = 0) = 3.71 \pm 0.67$$

Thus hyperpolarization increases the number of sodium channels but reduces the single channel conductance.

In 5 additional experiments we applied 500 ms prepulses to −28 mV or 0 between holding potentials V_H = 0 or −28 mV and the test potential V =

40 mV. The prepulses had no significant effect on the sodium current and the variance of sodium current fluctuations measured between 145 and 460 ms after depolarization. Hence the actions of holding potential on number and conductance of sodium channels develop with time constants larger than 0.5 s.

Discussion

The objective of the present investigation was the determination of slow variations in the number and conductance of sodium channels in myelinated nerve following changes of the holding potential. The study is based on an analysis of the mean sodium current and the variance of sodium current fluctuations, a method originally introduced by Sigworth [17]. It has the advantage that it can be applied to stationary or non-stationary processes and that no kinetic details on gating properties of sodium channels are needed. The only necessary assumptions are that the observed fluctuations originate from channel gating only, that all sodium channels are identical, non-interacting and have only one conducting state. Support for these assumptions was provided by measurements of the single channel conductance at various depolarizations starting from a fixed holding potential [17].

In this study we have measured the mean sodium current and the variance of sodium current fluctuations at a fixed depolarization of $V = 40$ mV, holding the fibre at two different potentials $V_H = 0$ (resting potential) or -28 mV. Previous investigations [7,9] had revealed an increase of the available sodium current several minutes after applying a hyperpolarizing holding potential. Hence we expected an increase of the number and (or) of the conductance of sodium channels upon hyperpolarization. Surprisingly, we detected an increase of the number N but a decrease of the conductance γ of sodium channels. The ratio of these quantities at $V_H = -28$ mV and 0 was 0.53 for γ and 3.71 for N resulting in an increase of the mean sodium current by a factor of $0.53 \cdot 3.71 \approx 2$.

To obtain absolute values of the conductance and number of sodium channels in a node of myelinated nerve at different holding potentials, we may use the results $\gamma = 6.8$ pS, $N = 10^5$ which were derived previously for a holding potential of -20 mV [16]. Since the available sodium permeability at decreasing holding potentials has almost reached saturation at -20 mV [9], these numbers are close to those valid for $V_H = -28$ mV. Hence the corresponding values for the resting potential $V_H = 0$ are $\gamma = 6.8/0.53 \approx 13$ pS, $N = 10^5/3.71 \approx 0.3 \cdot 10^5$.

The variation of the conductance and number of sodium channels with the holding potential is a slow process since 500 ms prepulses to a potential different from the holding potential did not change the values of γ or N. Hence the effects of holding potential on sodium channels develop in the same time range as slow changes of the peak sodium current [7,8]. This suggests that the variations of peak sodium current are indeed caused by concomitant alterations of the conductance and number of sodium channels in myelinated nerve.

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