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ALTERATION OF THE CONDUCTANCE OF Na^+ CHANNELS IN THE NODAL MEMBRANE OF FROG NERVE BY HOLDING POTENTIAL AND TETRODOTOXIN

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(1) Na^+ currents and Na^+-current fluctuations were measured in myelinated frog nerve fibres at 15°C during 7.7 ms depolarizations to $V = 40$, 60 and 80 mV. (2) The conductance γ of a single Na^+ channel and the number N_0 of channels per node were calculated from ensemble average values of the mean Na^+ current and the variance of Na^+-current fluctuations. (3) For a hyperpolarizing holding potential of $V_H = -28$ mV the mean values of the channel conductance and number were $\gamma = 9.8$ pS and $N_0 = 74000$. (4) After changing the holding potential to the resting potential ($V_H = 0$) the conductance γ increased by a factor of 1.37 whereas the number N_0 decreased by a factor of 0.60. (5) Addition of 8 nM tetrodotoxin at a holding potential of $V_H = -28$ mV increased γ by a factor of 1.55 and reduced N_0 by a factor of 0.25. (6) The increase of the channel conductance at reduced channel numbers suggests negative cooperativity between Na^+ channels in the nodal membrane.

Introduction

In myelinated nerve Na^+ channels are confined to the nodes of Ranvier [1]. From binding studies with radioactively labelled toxins and from electrophysiological measurements a high Na^+ channel surface density of the order of $1000/\mu m^2$ was determined. The resulting small distances between channels could produce interactions among neighbouring channels similar to the ones suggested to exist between gramicidin A channels in lipid bilayer membranes [2]. In the present investigation we have studied such a possibility for Na^+ channels in frog myelinated nerve by measuring ensemble average values of the mean Na^+ current and the variance of Na^+-current fluctuations. From these values the conductance of a single Na^+ channel and the number of Na^+ channels per node were calculated [3,4]. We found that reducing the number of conducting Na^+ channels either by a more positive holding potential or by the addition of tetrodotoxin increases the conductance of a single Na^+ channel. These results can be interpreted by a hindrance of Na^+ current flow through individual channels by neighbouring channels which means negative cooperativity between Na^+ channels in the nodal membrane.

Methods

Single motor or sensory fibres were dissected from the tibial nerve of the frog, *Rana esculenta* [5], and a node of Ranvier was voltage-clamped at 15°C [6]. The ends of the fibre were cut in a solution containing 113 mM CsCl and 7 mM NaCl. The extracellular solution was composed of 110.5 mM NaCl, 2 mM $CaCl_2$ and 10 mM tetraethylammonium chloride. The solution with 8 nM tetrodotoxin was prepared by adding toxin from a stock solution. Extra- and intracellular solutions

were buffered with 4 mM morpholinopropanesulphonic acid and adjusted to pH 7.2 with NaOH.

At the beginning of an experiment the voltage clamp was balanced to give a resting inactivation of Na^+ channels equivalent to $h_\infty = 0.7$–0.75. Displacements of the membrane potential from this value are denoted by V. First, linear components of leakage and capacity currents were compensated during a hyperpolarizing pulse by an analogue circuit and the reversal potential V_{Na} of the Na^+ currents was determined. In 16 fibres this potential ranged between 118 and 152 mV (mean value $\pm$S.E.: 132 ± 2 mV). The holding potential was then set to $V_H = -28$ mV or kept at the resting value ($V_H = 0$). Membrane currents were measured 4 min after a change of the holding potential when the holding current had reached a stationary value. At the end of each experiment the longitudinal axoplasm resistances from the test node to the cut ends of the fibre and the resistance of the nodal membrane were evaluated from electrical measurements [3].

Membrane currents were recorded through a 10 kHz low-pass filter during a 45 ms prepulse to $V_R = -28$ mV and during subsequent 7.7 ms positive and negative test pulses. The height of the two test pulses was symmetrical with respect to the reference level V_R (compare Fig. 1). Positive and negative pulses followed alternatingly, the pause between pulses was 1 s. After the onset of each test pulse 245 current values were recorded. The sampling interval was 10.2 μs until 0.5 ms, 18.5 μs until 3.2 ms and 200 μs until the end of the pulse. Ensemble average values of the mean current and of the variance of current fluctuations were calculated from current samples in a group of six subsequent positive or negative pulses (for details compare [7]). The procedure was carried out on 15 groups and the mean currents and the variance values of the last 14 groups were averaged and stored. These measurements were performed in each fibre at two or three test potentials of $V = 40$, 60, 80 mV for $V_H = 0$ and -28 mV or at $V_H = -28$ mV for extracellular solutions containing no and 8 nM tetrodotoxin.

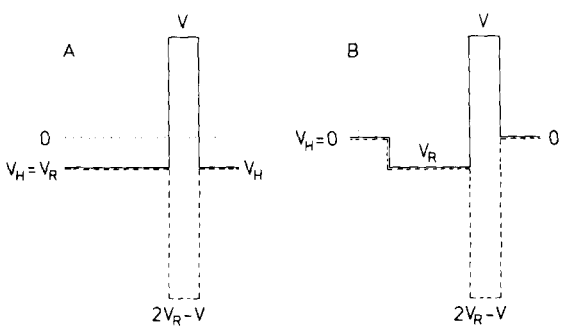

Fig. 1. Pulse programme for a hyperpolarizing holding potential $V_H = -28$ mV (A) and for $V_H = 0$ (B). For positive (full lines) and negative sweeps (interrupted lines) the current baseline was measured during a 45 ms reference potential to $V_R = -28$ mV. The height of the subsequent 7.7 ms test potentials was $V = 40$, 60 or 80 mV and $2 \cdot V_R - V = -96$, -116 or -136 mV.

The mean current during the prepulse to $V_R = -28$ mV was subtracted from all current values obtained during the subsequent test pulses. For each time t after the beginning of the test pulses the current during the negative pulse was then added to the corresponding positive pulse value to yield the Na^+ current $I(t)$. Its magnitude and kinetics were characterized by the peak value, I^{peak}, by the time to peak, t^{peak}, and by the parameters of the expression

$$A_1 \exp(-t/\tau_{h1}) + A_2 \exp(-t/\tau_{h2}) + B$$

which was fitted to the early and late phases of Na^+ inactivation with the least-squares method of Gauss.

The variances, var(t), of nonstationary Na^+-current fluctuations were obtained by subtracting the variances during the negative pulse from the isochronical positive pulse values. The relation

$$\text{var}(t) = i \cdot I(t) - I(t)^2/N + c \qquad (1)$$

was then fitted to the (var, I) data where i is the current through one open Na^+ channel and N the number of channels per node [3]. The variance, c, at $I = 0$ represents small contributions not arising from Na^+ channels. From i the chord conductance

$$\gamma = i/(V - V_{Na}) \qquad (2)$$

of a single Na^+ channel was calculated where V denotes the potential of the positive test pulse and V_{Na} the measured Na^+ reversal potential. At large Na^+ current amplitudes the driving force $V - V_{Na}$ is significantly reduced by the voltage drop $R_s \cdot I$

across the resistance R_s in series with the nodal membrane. Hence the single channel current i varies with I according to

$$i = i_o\left(1 - \frac{R_s \cdot I}{V - V_{Na}}\right) \quad (3)$$

Inserting this expression into Eqn. 1 yields an actual single-channel current i_o which is equal to the fitted value i, whereas the channel number N_o corrected for series-resistance effects is higher than the fitted parameter N and given by

$$N_o = \frac{N}{1 - R_s \cdot N \cdot \gamma} \quad (4)$$

Corrected channel numbers N_o were calculated with $R_s = 220$ kΩ [8] and used to determine the probability

$$p^{peak} = \frac{I^{peak}}{N_o \cdot i} \quad (5)$$

of the open state of Na^+ channels at the time of the peak Na^+ current.

For five fibres the axoplasm resistances could not been measured. In these cases no absolute values of the Na^+ channel conductance γ could be determined, whereas the conductance ratio at different holding potentials or extracellular solutions and the parameter N in Eqn. 1 are independent of the calibration resistance. Corrected channel numbers N_o were then calculated from Eqn. 4 with mean γ values from other experiments.

Results

Effects of holding potential

The Na^+ currents, I, and variances, var, of Na^+-current fluctuations during a depolarizing pulse to the potential V depend on the holding potential V_H. Fig. 2 shows values of I and var recorded during $V = 60$ mV with the pulse programmes illustrated in Fig. 1. For $V_H = -28$ mV the currents reach higher values than for $V_H = 0$. Their kinetics are hardly affected by the holding potential (compare the values of t^{peak}, τ_{h1} and τ_{h2} in the legend to Fig. 2), only the mean values of the time constants from all experiments reveal a small decrease with hyperpolarization (see Table I, A and B).

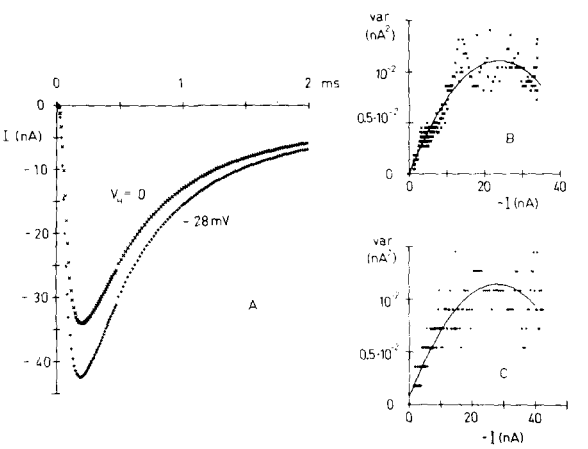

Fig. 2. Na^+ currents, I, and variances, var, of Na^+-current fluctuations during a depolarization to $V = 60$ mV. Holding potentials $V_H = 0$ (×) and -28 mV (+). (A) Kinetics of I during the first 2 ms of depolarization (current values between 2 and 7.7 ms are not shown). $t^{peak} = 0.20$ ms (×) and 0.19 ms (+), $\tau_{h1} = 0.42$ ms (×) and 0.48 ms (+), $\tau_{h2} = 1.23$ ms (×) and 1.23 ms (+). (B) var as function of I for $V_H = 0$. (C) var as function of I for $V_H = -28$ mV. The curves in (B), (C) represent fits by Eqn. 1, the fitted parameters are: $i = -0.933$ pA, $N = 51.2 \cdot 10^3$, $c = -0.29 \cdot 10^{-4}$ nA² (B) and $i = -0.768$ pA, $N = 72.9 \cdot 10^3$, $c = 7.29 \cdot 10^{-4}$ nA² (C). Single-channel conductances $\gamma = 13.7$ pS (B) and 11.3 pS (C). The channel numbers N_o corrected for series-resistance effects are $60.5 \cdot 10^3$ (B) and $89.0 \cdot 10^3$ (C). Experiment 8/81, motor fibre. Na^+ reversal potential $V_{Na} = 128$ mV. Tempeature 15°C.

The parabolas in Figs. 2B and 2C represent fits of Eqn. 1 to isochronical (I, var) pairs. From the fitted parameters i, N the single-channel conductances γ and the channel numbers N_o were calculated as described in Methods. The results are listed in the legend to Fig. 2. Table II contains the mean values of γ, N_o from all experiments and at various depolarizations. It is obvious that at a holding potential of -28 mV the conductances γ are smaller and the channel numbers N_o higher than at the resting potential $V_H = 0$. The ratios of γ and N_o in Table II with respect to standard conditions (no tetrodotoxin in the extracellular solution, holding potential $V_H = -28$ mV) were determined in the same fibres and at the same test potentials. Hence the means of these ratios are not identical to the ratios of the mean values of γ, N_o obtained from different fibres and at various test potentials.

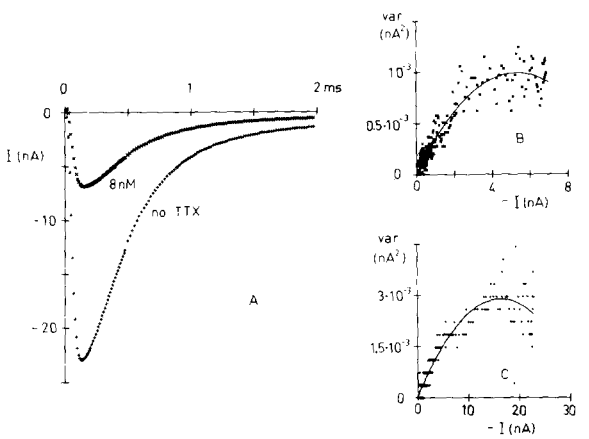

Fig. 3. Na^+ currents, I, and variances, var, of Na^+-current fluctuations during a depolarization to $V = 80$ mV. Concentrations of tetrodotoxin (TTX) in the extracellular solution 8 nM (×) and 0 (+). Holding potential $V_H = -28$ mV. (A) Kinetics of I during the first 2 ms of depolarization (current values between 2 and 7.7 ms are not shown). $t^{peak} = 0.16$ ms (×) and 0.13 ms (+), $\tau_{h1} = 0.40$ ms (×) and 0.42 ms (+), $\tau_{h2} = 0.67$ ms (×) and 0.46 ms (+). (B) var as function of I with 8 nM tetrodotoxin. (C) var as function of I without tetrodotoxin. The curves in (B), (C) represent fits by Eqn. 1, the fitted parameters are: $i = -0.365$ pA, $N = 29.2 \cdot 10^3$, $c = 0.19 \cdot 10^{-4}$ nA² (B) and $i = -0.343$ pA, $N = 96.0 \cdot 10^3$, $c = 0.60 \cdot 10^{-4}$ nA² (C). Single-channel conductances $\gamma = 7.4$ pS (B) and 7.0 pS (C). The channel numbers N_o corrected for series-resistance effects are $30.7 \cdot 10^3$ (B) and $112.7 \cdot 10^3$ (C). Experiment 11/81, sensory fibre. Na^+ reversal potential $V_{Na} = 129$ mV. Temperature 15°C.

Effects of tetrodotoxin

Tetrodotoxin blocks Na^+ channels and thus decreases the amplitude of the Na^+ currents. Also, the process of Na^+ activation is slower in the presence of tetrodotoxin (compare values of t^{peak} in the legend to Fig. 3 and in Table I, A and C). On the other hand, the time constants τ_{h1} and τ_{h2} of Na^+ inactivation are not clearly affected by the toxin.

Figs. 3B and 3C illustrate that 8 nM tetrodotoxin also reduces the variances of Na^+-current

TABLE I

PARAMETERS OF Na^+ CURRENT WITHOUT AND IN THE PRESENCE OF TETRODOTOXIN AND AT VARIOUS HOLDING POTENTIALS (V_H) AND TEST POTENTIALS (V)

Values are mean ± S.E., number of averaged values in parenthesis. n, number of fibres.

A. No tetrodotoxin, $V_H = -28$ mV, $n = 16$.

V (mV)	t^{peak} (ms)	P^{peak}	τ_{h1} (ms)	τ_{h2} (ms)	A_2/A_1
40	0.301 ± 0.012 (11)	0.61 ± 0.02 (11)	0.58 ± 0.05 (11)	2.13 ± 0.17 (11)	0.46 ± 0.06 (11)
60	0.174 ± 0.005 (11)	0.65 ± 0.04 (11)	0.46 ± 0.03 (11)	1.27 ± 0.18 (11)	0.45 ± 0.09 (11)
80	0.132 ± 0.003 (14)	0.62 ± 0.05 (13)	0.33 ± 0.02 (14)	0.86 ± 0.11 (14)	0.30 ± 0.04 (14)

B. No tetrodotoxin, $V_H = 0$, $n = 8$.

V (mV)	t^{peak} (ms)	P^{peak}	τ_{h1} (ms)	τ_{h2} (ms)	A_2/A_1
40	0.351 ± 0.015 (6)	0.49 ± 0.05 (7)	0.70 ± 0.09 (7)	1.91 ± 0.18 (7)	0.66 ± 0.14 (7)
60	0.203 ± 0.006 (5)	0.60 ± 0.04 (5)	0.53 ± 0.04 (5)	1.13 ± 0.17 (5)	0.50 ± 0.12 (5)
80	0.143 ± 0.004 (7)	0.53 ± 0.04 (7)	0.33 ± 0.03 (7)	0.75 ± 0.10 (7)	0.37 ± 0.06 (7)

C. 8 nM tetrodotoxin, $V_H = -28$ mV, $n = 8$.

V (mV)	t^{peak} (ms)	P^{peak}	τ_{h1} (ms)	τ_{h2} (ms)	A_2/A_1
40	0.402 ± 0.022 (3)	0.42 ± 0.05 (3)	1.09 ± 0.22 (3)	2.79 ± 0.20 (3)	0.85 ± 0.37 (3)
60	0.226 ± 0.016 (7)	0.69 ± 0.05 (7)	0.45 ± 0.03 (7)	1.15 ± 0.04 (7)	0.63 ± 0.16 (7)
80	0.157 ± 0.009 (7)	0.67 ± 0.06 (7)	0.34 ± 0.04 (7)	0.97 ± 0.22 (7)	0.48 ± 0.14 (7)

TABLE II

MEAN VALUES ±S.E. OF THE CONDUCTANCE γ AND NUMBER N_o OF Na^+ CHANNELS WITHOUT AND IN THE PRESENCE OF TETRODOTOXIN AND AT TWO HOLDING POTENTIALS V_H

γ and N_o values were obtained from all fibres and at all test potentials. The ratios of γ and N_o with respect to 0 nM tetrodotoxin and $V_H = -28$ mV were determined in the same fibres and at the same test potentials. Number of averaged values in parenthesis. N, number of fibres. TTX, tetrodotoxin.

TTX (nM)	V_H (mV)	n	γ (pS)	Ratio γ	N_o ($\times 10^3$)	Ratio N_o
0	−28	16	9.8 ± 0.7 (28)	–	73.9 ± 6.1 (32)	–
0	0	8	13.4 ± 1.1 (13)	1.37 ± 0.08 (17)	53.8 ± 9.0 (17)	0.60 ± 0.05 (17)
8	−28	8	12.1 ± 1.6 (13)	1.55 ± 0.10 (14)	16.7 ± 2.0 (17)	0.25 ± 0.02 (13)

fluctuations. The fit of the data by Eqn. 1 yields a drastic reduction of the number of Na^+ channels but reveals also a small increase of the channel conductance after the addition of tetrodotoxin (compare values of N_o and γ in the legend to Fig. 3 and in Table II).

The alterations of the parameters of Na^+ currents and of the conductance and number of Na^+ channels by a change in the holding potential and by tetrodotoxin application were not different for motor and sensory fibres. Therefore, the results from both types of fibres have been averaged in Tables I and II.

Discussion

The amplitude of Na^+ currents during a given depolarization can be reduced by holding the fibre at less negative potentials [9,10] (Fig. 2) or by adding tetrodotoxin to the extracellular solution (Fig. 3). In both cases the time to the peak of the Na^+ current is increased (Table I). The slower kinetics at lower Na^+ current amplitudes I can be explained by the presence of a resistance R_s in series with the nodal membrane. The membrane potential is then no longer equal to the clamp potential V but more positive by the amount $R_s \cdot I$ and all voltage-dependent gating time constants at $V = 40$, 60 and 80 mV become shorter with increasing Na^+ inward currents. This current-dependent change of the kinetics of Na^+ currents seems to account for the observed alterations of the time constants and it is thus not necessary to postulate a direct action of the holding potential or tetrodotoxin on the gating processes in Na^+ channels.

The most reliable values of Na^+ channel parameters are then those obtained in the presence of 8 nM tetrodotoxin which reduces the Na^+ currents and series-resistance artifacts to small amounts. Comparison of the mean values in Table I shows that the decline of all time constants t^{peak}, τ_{h1}, τ_{h2} and of the amplitude ratio A_2/A_1 of slow and fast Na^+ inactivation with depolarization becomes more pronounced at lower Na^+ current amplitudes. On the other hand, the probability P^{peak} exhibits a steeper increase with depolarization and reaches higher values at $V = 60$, 80 mV in the presence of tetrodotoxin. As explained in Methods the calculated chord conductance γ of a single Na^+ channel is not affected by the membrane series resistance. Nevertheless, the results listed in Table II exhibit a clear dependence of γ on the Na^+ current amplitude: With respect to standard conditions (no tetrodotoxin in the extracellular solution, holding potential $V_H = -28$ mV) the conductances are higher when Na^+ currents are reduced by the addition of tetrodotoxin or by a positive change of the holding potential. Thus the decrease of the number N_o of conducting Na^+ channels by tetrodotoxin or by a more positive holding potential is more pronounced than expected from a comparison of the total Na^+ conductance γN_o per node. For example, 8 nM tetrodotoxin reduces the mean value of γN_o from 9.8 pS · 73.9 · 10^3 = 0.72 μS down to 12.1 pS · 16.7 · 10^3 = 0.20 μS, thus to 28%, whereas the mean value of N_o declines further to 23% from 73.9 · 10^3 to 16.7 · 10^3. Hence, the equilibrium dissociation constant of tetrodotoxin binding is 3.1 nM when calculated from the Na^+ conductance γN_o, but

only 2.3 nM if the number N_o of conducting Na^+ channels is considered. This illustrates that the determination of binding constants of Na^+ channel blockers from measurements of macroscopic Na^+ currents through all channels can yield inaccurate values.

Recently a similar change of γ with holding potential was reported from this laboratory [11]. In these experiments stationary Na^+ currents and variances of Na^+ current fluctuations were measured at the end of a fixed depolarization to $V = 40$ mV applied from holding potentials $V_H = -28$ and 0 mV. The ratio of Na^+ channel conductances at both holding potentials was found to be 0.53, thus γ at $V_H = 0$ would be by a factor of 1.89 higher than at -28 mV. The present investigation based on an analysis of non-stationary Na^+ current fluctuations confirms the increase of γ after a positive change of the holding potential. The different ratios of the conductances γ at $V_H = -28$ and 0 mV found previously and in the present experiments (see Table II) could be due to a small stationary Na^+ inward current of the order of 20 pA at $V_H = 0$. This current had been subtracted from the current values at $V = 40$ mV thus leading to an underestimation of the stationary Na^+ current during the test potential. Therefore, we have recorded in the present experiments the current baseline during a prepulse to $V_R = -28$ mV (see Fig. 1B) at which no stationary Na^+ currents could be detected.

In the present investigation the conductance and number of Na^+ channels were calculated from the parameters i and N of Eqn. 1. This equation can be derived by eliminating the probability P of the open-channel state from the relations

$$I = N \cdot i \cdot P \tag{6}$$

$$\text{var} = N \cdot i^2 \cdot P(1 - P) \tag{7}$$

which describe the mean current, I, and the variance, var, of current fluctuations for ionic channels with only one conducting state [3]. Additional requirements for the validity of Eqns. 6 and 7 are (i) all channels are identical and (ii) neighbouring channels do not interact with each other [3,4,12]. If both assumptions would be fulfilled, the current i through one open channel and its conductance γ should be independent of the channel number. However, the conductance of a single channel increases when the number of conducting channels is reduced either by a more positive holding potential or by addition of tetrodotoxin (see Table II). This alteration of the channel conductance implies that one or more assumptions in the derivation of Eqns. 6 and 7 are not fulfilled and that the fit of our data by Eqn. 1 only yields effective values of the channel number and conductance. Possible reasons for the conductance increase at reduced channel numbers could be:

(i) A non-uniformity of Na^+ channels with a broad distribution of single-channel conductances or different Na^+ channel populations with distinct conductances. A change of the holding potential would then have to affect primarily less conducting channels and tetrodotoxin would have to bind preferentially to those channels. However, it seems unlikely that part of the Na^+ channels has different electrical and pharmacological properties but is otherwise kinetically indistinguishable from the rest of the channels. In addition, recent single-channel records of Na^+ channels in excitable membranes [13–15] do not reveal a broad distribution of γ values between various Na^+ channels.

(ii) Interactions among Na^+ channels. This possibility has already been suggested but rejected because no alterations of the conductance γ of Na^+ channels were detected at reduced Na^+ currents [4]. The results of the present investigation, however, reveal an increase of γ at lowered numbers of conducting Na^+ channels. A possible explanation for this deviation is that the previous study was performed at the resting holding potential with a low initial number $20 \cdot 10^3$ to $46 \cdot 10^3$ of conducting Na^+ channels per node [3]. Hence, the increase of γ induced by a further decrease of the channel number could have been too small to be detectable. In our experiments the mean number N_o of Na^+ channels declined from $74 \cdot 10^3$ to $54 \cdot 10^3$ after changing the holding potential from -28 mV to the resting potential, and N_o was reduced from $74 \cdot 10^3$ to $17 \cdot 10^3$ after the addition of 8 nM tetrodotoxin. For this strong decrease of the absolute number of conducting Na^+ channels the single-channel conductance increased by 37 and 55%, respectively (see Table II). The significant alteration of γ warrants to revive the idea of

interactions among Na^+ channels. The type of interaction would be a negative cooperativity because the flux of sodium ions through one channel decreases with increasing channel densities. Such an effect of neighbouring channels could occur either by channel-channel interactions in the membrane matrix or by interferences between ion fluxes in the unstirred solution layers around the channels. In the first case the conformational changes of individual channels are also expected to be influenced by the distance and gating states of neighbouring channels. However, no alterations of Na^+ gating time constants could be detected by us which could be attributed to a direct interaction among Na^+ channels. In the latter case a depletion or accumulation of permeant ions at the channel entrances occurs without direct channel-channel interactions due to overlapping ion fluxes outside the channels in the unstirred solution layers [16,17]. To estimate the range ρ of interactions for such diffusion effects we assume a mean lifetime $\tau = 1$ ms of the order of the inactivation time constant for the open state of Na^+ channels and an aqueous diffusion coefficient $D = 10^{-5}$ cm$^2 \cdot$ s^{-1}. This yields $\rho = \sqrt{\pi D \tau} \simeq 1800$ nm. The average distance between Na^+ channels in the nodal membrane is much smaller: If it is assumed that $74 \cdot 10^3$ Na^+ channels are equally distributed in the nodal membrane of area 35–60 μm^2 [18], the distance between channels would be approx. 25 nm. Hence the ion flux through open Na^+ channels can affect the local Na^+ concentration at the entrance of many neighbouring channels.

The following calculations illustrate that the resulting concentration changes may be of the order of 10 mM: A single conducting channel acts as a sink and thus produces a gradient of the concentration c of permeant ions in the aqueous phase. For spherical symmetry the concentration $c(r)$ at a distance r from the channel entrance assumes the stationary value

$$c(r) = c_o - \frac{i}{2\pi FDr} \tag{8}$$

where c_o is the bulk ion concentration, i the current through the channel and F the Faraday constant [16,19]. Neighbouring conducting channels will disturb this spherical concentration profile and cause a further depletion of ions near the channel entrances. To estimate this additional concentration shift Δc we assume that conducting channels are distributed with a uniform surface density δ around an arbitrarily selected reference channel [17]. Channels within the radii r and $r + dr$ will then reduce the concentration at the reference channel by the amount $2\pi\delta r dr i/(2\pi FDr) = i\delta dr/(FD)$. Hence all channels within the range ρ of diffusion interaction produce the concentration change

$$\Delta c = \frac{i\delta\rho}{FD} \tag{9}$$

Inserting the values $i = 1$ pA, $\delta = 74 \cdot 10^3/45$ μm^2, $\rho = 1800$ nm and $D = 10^{-5}$ cm$^2 \cdot$ s^{-1} yields $\Delta c = 31$ mM. Due to random opening and closing of Na^+ channels the actual concentration change will be smaller, but it could be still large enough to cause a measurable decrease of the single-channel conductance. Our interpretation that depletion of sodium ions outside the channel mouth is causing the negative cooperativity between Na^+ channels could be tested by investigating the effects of changes in the external Na^+ concentration on the single-channel conductance.

In conclusion, this study presents evidence that the Na^+ channel conductance in a frog nerve fibre can be reduced by neighbouring channels. The negative cooperativity between channels yields an effective single-channel conductance which is smaller than the value derived in patch-clamp experiments from current recordings of single isolated ionic channels.

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