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MODIFICATION OF SODIUM INACTIVATION IN MYELINATED NERVE BY *ANEMONIA* TOXIN II AND IODATE

ANALYSIS OF CURRENT FLUCTUATIONS AND CURRENT RELAXATIONS

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Summary

(1) Na^+ currents and Na^+ current fluctuations were measured in single myelinated nerve fibres of *Rana esculenta* under voltage-clamp conditions. The process of Na^+ inactivation was modified by external treatment with 7 μM *Anemonia* Toxin II or by internal application of 20 or 40 mM IO_3^-.

(2) At depolarizations of 24 and 32 mV the spectral density of Na^+ current fluctuations could be described as the sum of two contributions, $S_h(f)$ and $S_m(f)$, representing the spectrum from fluctuations of the inactivation (h) and activation (m) gates, respectively. At higher depolarizations of 40 and 48 mV the low frequency (h) fluctuations could be better fitted by the sum, $S_{h1}(f) + S_{h2}(f)$, of two separate Lorentzian functions.

(3) The Na^+ current and the variance of Na^+ current fluctuations between 150 and 450 ms after depolarization are increased by one order of magnitude after application of *Anemonia* Toxin II or IO_3^-.

(4) The kinetics of Na^+ current inactivation were described as $A_1 \cdot \exp(-t/\tau_{h1}) + A_2 \cdot \exp(-t/\tau_{h2}) + B$. The time constant, τ_{h1}, of fast Na^+ inactivation was the same in normal and modified nerve fibres. The slow inactivation time constant, τ_{h2}, increased with increasing depolarizations in modified fibres but decreased under control conditions. In all cases τ_{h2} showed a similar voltage dependence as the time constant found by fitting the low frequency fluctuations of Na^+ current with one Lorentzian function, $S_h(f)$.

(5) It is concluded that *Anemonia* Toxin II and IO_3^- modify a fraction of Na^+ channels in an all-or-none manner. A lower limit of the number of modified Na^+ channels is estimated from the Na^+ current and the variance of Na^+ current fluctuations. 7 μM external *Anemonia* Toxin II modifies more than 17% and

20 or 40 mM internal IO_3^- more than 8% of all Na^+ channels. The inactivation gates in modified channels experience an electric field different from that in normal fibres.

Introduction

Upon depolarization the Na^+ current in nerve quickly rises to a peak value and thereafter decays to a small steady-state level. The two phases of Na^+ permeability changes are denoted as Na^+ activation and inactivation, respectively. In recent years many studies have been undertaken to explore the molecular basis of the gating processes underlying Na^+ activation and inactivation. Useful tools in these investigations are substances modifying the kinetics of gating since they may yield information on the location and chemical composition of receptors connected to the gates and could give indications whether Na^+ inactivation is independent of or coupled to the process of Na^+ activation [1].

This paper describes experiments on myelinated nerve fibres in which the Na^+ channels of the nodal membrane were modified by external treatment with *Anemonia* Toxin II or by internal application of IO_3^-. Both substances are selective in the sense that they significantly modify Na^+ inactivation with only minor effects on Na^+ activation. In a first extensive investigation of the effects of *Anemonia* Toxin II on myelinated nerve fibres the kinetics of Na^+ inactivation were studied at various toxin concentrations, and a value of the equilibrium binding constant was estimated [2]. From the rates of toxin action reported in the same paper and in a recent abstract [3], it was suggested that the toxin receptor would be located at the external membrane surface. IO_3^-, on the other hand, slows the kinetics of Na^+ inactivation in myelinated nerve only if it is applied intra-axonally [4].

The actions of *Anemonia* Toxin II and IO_3^- on Na^+ channels were also studied by analyzing the fluctuations of the steady-state Na^+ current [5,6]. From the high-frequency fluctuations associated with the activation process it was concluded that the conductance of single Na^+ channels remained unaffected though the inactivation process was modified [6]. However, a direct analysis of fluctuations of inactivation gates was not possible at that time because the measured spectral densities below 300 Hz were disturbed by a large amount of low-frequency excess noise [5]. With an improved technique (Conti, F., Neumcke, B., Nonner, W. and Stämpfli, R., unpublished results) we are now able to extend the reliable frequency range down to 3 Hz and to study components attributable to Na^+ inactivation processes. The present investigation was devoted to an analysis of such low-frequency fluctuations recorded at various voltages in normal and modified myelinated nerve fibres. The characteristic time constants extrapolated from Na^+ current fluctuations at a given test potential are compared with the kinetics of Na^+ current relaxations after a step to the same potential. The results suggest that *Anemonia* Toxin II and IO_3^- modify only a fraction of the Na^+ channels and that the inactivation gates of modified channels experience an electric field different from that in normal fibres.

Methods

Single motor and sensory fibres were dissected from the tibial nerve of the frog, *Rana esculenta* [7], and a node of Ranvier was voltage-clamped at 18°C [8]. Displacements of the membrane potential from its resting value (where 30% of the Na^+ channels were inactivated) are denoted by V. Membrane currents were calibrated using a longitudinal axoplasm resistance determined from electrical measurements [9]. This method yielded resistance values in agreement with those calculated from the dimensions of the fibre and a specific axoplasm resistance of 110 $\Omega \cdot$ cm [10].

The ends of the fibre were cut in isotonic CsCl solution to block K^+ channels from inside by Cs^+ diffusing through the axoplasm to the node. In addition all extracellular solutions contained 10 mM tetraethylammonium ions to block K^+ channels also from outside.

Control values of Na^+ currents and Na^+ current fluctuations were recorded in K^+-free Ringer's solution (test solution) containing 110.5 mM NaCl, 2 mM $CaCl_2$, 10 mM tetraethylammonium chloride and 4 mM morpholinepropanesulphonic acid/NaOH buffer at pH 7.1. At the end of the experiment current fluctuation measurements were repeated in test solution with 300 nM tetrodotoxin to block Na^+ channels.

Anemonia Toxin II is a polypeptide of molecular weight 4197 extracted from tentacles of *Anemonia sulcata.* It was a kind gift of Dr. L. Beress, Kiel [11], and kept frozen in stock solution (35 μM added to test solution). Before the experiment some millilitres of 7 μM *Anemonia* Toxin II in test solution were prepared. The toxin-containing solution was perfused into the fluid pool surrounding the test node and the flow across this compartment was turned off. After a few minutes measurements were begun.

Fibres were treated with internal IO_3^- by cutting the fibres in side pools containing 20 mM $CsIO_3$ and 100 mM CsCl or 40 mM $CsIO_3$ and 80 mM CsCl. The lengths of the cut internodes were smaller than 1.5 mm, thus IO_3^- had reached the node by intra-axonal diffusion within 10 min [12]. Usually after 10 min the solutions in the side pools were replaced by isotonic CsCl. Nevertheless, the IO_3^- effects were steadily increasing during the subsequent measurements, and the fibre sometimes broke before the end of the experiment.

Between test pulses the fibre was kept at a holding potential of V_H = −20 mV. Relaxations of membrane currents after step depolarizations were photographed on Polaroid film and corrected for leakage to obtain Na^+ currents. The decline of the Na^+ current after its peak value was then digitized for further analysis. Measurements and analysis of currents and current fluctuations at the end of a depolarizing voltage step were performed as described recently [13]. In brief, current samples were recorded between 145 and 460 ms after depolarization. 60 test pulses at 5-s intervals were applied for averaging. The pulse sequence was first performed in test solution and, again, in test solution with 300 nM tetrodotoxin. The differences between the currents, variances or spectral densities in both solutions were considered as the respective quantities arising from Na^+ channels. Except for small depolarizations of 24 mV, the subtraction of the tetrodotoxin reference values gave a negligible correction. Therefore, if in IO_3^- experiments the fibre broke during measure-

ments in the tetrodotoxin-containing solution, the data from the previous runs in test solution were taken as Na^+ currents and Na^+ current fluctuations, respectively.

Results

Low frequency fluctuations of Na^+ current

Fig. 1 shows one-sided spectral densities of Na^+ current fluctuations at test pulses of $V = 24$ and 40 mV under control conditions (A) and after application of *Anemonia* Toxin II (ATX-II) (B) or IO_3^- (C). The most prominent effect of the substances is an about 10-fold increase in the spectral densities with respect to the control values. It is also obvious that in all cases the spectral densities exhibit two humps, one at low and one at high frequencies. These humps can be regarded as the contributions of fluctuations of the inactivation (h) and activation (m) gating subunits in Na^+ channels [5]. The solid curves through the points are fits to the spectral density, $S(f) = S_h(f) + S_m(f)$, predicted by the Hodgkin-Huxley m^3h formulation of Na^+ currents (Eqn. 18 of Ref. 5). In a previous investigation [6] the high frequency part $S_m(f)$ was studied in fibres with modified Na^+ inactivation. Due to the presence of excess $1/f$ noise below 300 Hz, the low frequency $S_h(f)$ contribution from inactivation processes could not be revealed. With an improved technique (Conti, F., Neumcke, B., Nonner, W. and Stämpfli, R., unpublished results) we can now extend the reliable frequency range down to 3 Hz, hence it includes frequencies corresponding to inactivation time constants τ_h. The corresponding frequency components, $S_h(f)$, will now be analyzed in detail.

If the process of Na^+ inactivation can be formulated as a first-order reaction,

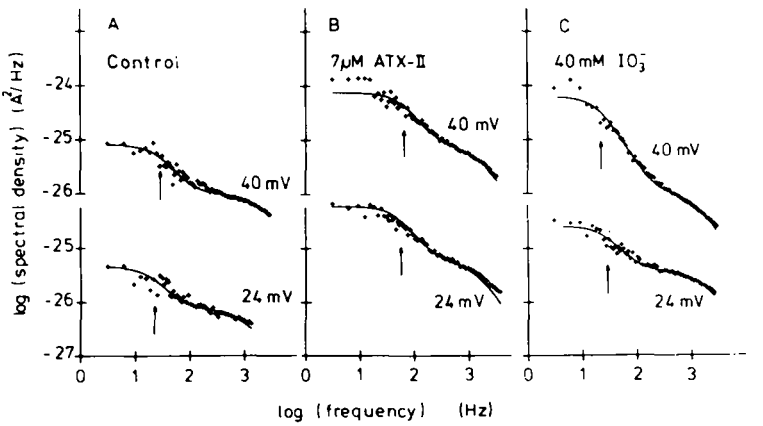

Fig. 1. Spectral density of Na^+ current fluctuations at 24 and 40 mV. Symbols (+) represent data calculated from current fluctuations recorded between 145 and 460 ms after the onset of the test pulse. Solid lines are fits corresponding to an m^3h process. Arrows indicate corner frequencies, f_c, of the h Lorentzian spectrum, $S_h(f)$ (Eqn. 1). (A) With test solution: f_c (24 mV) = 21.9 Hz, f_c (40 mV) = 29.5 Hz (Exp. A5). (B) With 7 μM *Anemonia* Toxin II (ATX-II) added to the test solution: f_c (24 mV) = 56.0 Hz, f_c (40 mV) = 67.4 Hz (Exp. A5). (C) With 40 mM IO_3^- applied to the cut ends of the fibre for 14 min: f_c (24 mV) = 29.1 Hz, f_c (40 mV) = 22.3 Hz (Exp. I1). Temperature 18°C.

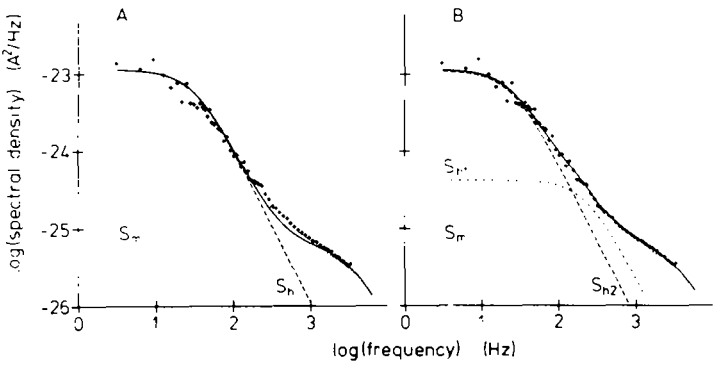

Fig. 2. Spectral density of Na^+ current fluctuations at 48 mV with 7 μM *Anemonia* Toxin II in the test solution. Symbols (+) represent data calculated from current fluctuations. (A) Fit corresponding to an m^3h process with one inactivation time constant: $S(f) = S_h(f) + S_m(f)$, τ_h = 5.16 ms. (B) Fit with two inactivation time constants: $S(f) = S_{h1}(f) + S_{h2}(f) + S_m(f)$, τ_{h1} = 0.68 ms, τ_{h2} = 6.43 ms, and $S_m(f)$ taken from A. The fitted spectral densities $S(f)$ are represented by solid curves, the spectral components S_m, S_h, S_{h1} and S_{h2} by dashed lines (Exp. A4). Temperature 18°C.

$S_h(f)$ is described as a Lorentzian spectrum [14]:

$$S_h(f) = \frac{S_h(0)}{1 + (2\pi f \tau_h)^2} \tag{1}$$

where $S_h(f)$ is constant at low frequencies, $f \ll 1/(2\pi\tau_h)$, and decays proportionally to $1/f^2$ at high frequencies, $f \gg 1/(2\pi\tau_h)$. The transition between both frequency dependencies occurs around the corner frequency, $f_c = 1/(2\pi\tau_h)$. In Fig. 1A—C the locations of the corner frequencies are indicated by arrows. The mean values of the corresponding time constants, $\tau_h = 1/(2\pi f_c)$, at various voltages are plotted as symbols in Fig. 5 and will be discussed below.

At depolarizations above 40 mV the spectral density points between 100 Hz and 1 kHz lie systematically above the fitted curve, $S(f) = S_h(f) + S_m(f)$. This is already visible at 40 mV in Fig. 1A and C, but becomes more pronounced at 48 mV as shown in Fig. 2A in the presence of *Anemonia* Toxin II.

Obviously, the low-frequency part of the spectral density can only poorly be described by one single Lorentzian function at high depolarizations. Fig. 2B illustrates that the same data can be perfectly fitted by the sum of two Lorentzian functions, $S_{h1}(f) + S_{h2}(f)$. Here the fast time constant (τ_{h1}) was taken from the decline of the Na^+ current at the same depolarization and in the same fibre (compare Fig. 4B), whereas the slow time constant (τ_{h2}) and the amplitudes of both Lorentzian functions were obtained from fits to the spectral points. Also, under control conditions and with IO_3^-, two inactivation time constants give better fits to the spectral density. Even though, for convenience, only one time constant was used for further analysis of the spectral densities. The better fits with two Lorentzian functions for perfect description of the low-frequency fluctuations of Na^+ currents indicate that Na^+ inactivation is a multi-step process rather than a first-order reaction. This conclusion is in agreement with the analysis of the kinetics of Na^+ current inactivation described below.

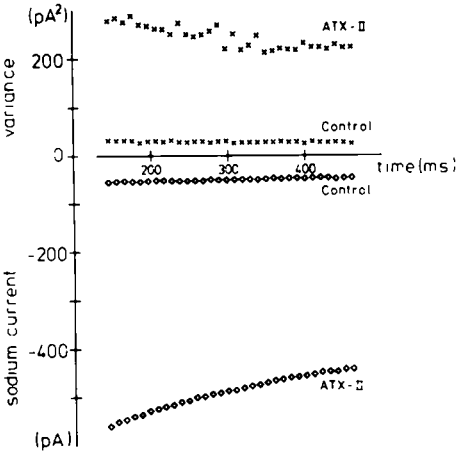

Fig. 3. Variance (X) of Na^+ current fluctuations and Na^+ current (◇) at 40 mV. Samples were taken between 145 and 460 ms after the onset of the test pulse under control conditions and 7 μM *Anemonia* Toxin II (ATX-II) in the test solution. (Exp. A5). Temperature 18°C.

Variance of Na^+ current fluctuations and Na^+ current

The variance of Na^+ current fluctuations and the Na^+ current at the end of a depolarization to $V = 40$ mV are shown in Fig. 3. In the same nerve fibre the quantities were first measured under control conditions and, again, after application of *Anemonia* Toxin II. The figure illustrates that modification of Na^+ inactivation by *Anemonia* Toxin II drastically increases the variance and the late Na^+ current. Similar effects were observed after treatment with IO_3^-. In Discussion, the mean values of the variance and of the Na^+ currents will be used to estimate the current through single Na^+ channels and the fraction of channels modified by *Anemonia* Toxin II and IO_3^-.

Time constants of decline of Na^+ current

The inactivation of Na^+ currents cannot be described by a single exponential function. Fig. 4A—C demonstrates this for normal fibres and for fibres treated

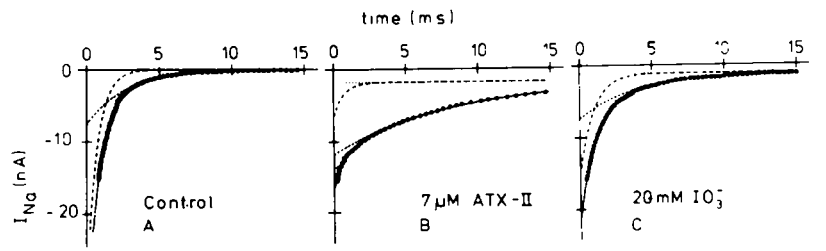

Fig. 4. Inactivation of Na^+ current during 48 mV test pulses applied at $t = 0$. Symbols (○) are measured Na^+ currents after the peak value, solid lines are fits of Eqn. 2 to the data. Dashed lines represent the three components: $A_1 \cdot \exp(-t/\tau_{h1})$, $A_2 \cdot \exp(-t/\tau_{h2})$ and B. (A) Under control conditions (Exp. V3): A_1 = −31.3 nA, A_2 = −7.6 nA, B = 0, τ_{h1} = 0.71 ms, τ_{h2} = 2.65 ms. (B) With 7 μM *Anemonia* Toxin II (ATX-II) added to the test solution (Exp. A4): A_1 = −4.7 nA, A_2 = −10.0 nA, B = −1.8 nA, τ_{h1} = 0.68 ms, τ_{h2} = 7.91 ms. (C) With 20 mM IO_3^- applied to one cut fibre end (Exp. I23): A_1 = −14.1 nA, A_2 = −6.3 nA, B = −1.2 nA, τ_{h1} = 0.96 ms, τ_{h2} = 3.75 ms. Temperature 18°C.

with *Anemonia* Toxin II or IO_3^-. In all cases the relaxation of the Na^+ current after its peak value first occurs rapidly and later with a slower time constant. This behaviour was formulated by the expression:

$$I_{Na}(t) = A_1 \cdot \exp(-t/\tau_{h1}) + A_2 \cdot \exp(-t/\tau_{h2}) + B \quad (2)$$

The dashed lines in Fig. 4A—C represent the single components of Eqn. 2, the values of the parameters are listed in the legend. The steady-state Na^+ current, B, assumes significant values only for fibres with modified Na^+ inactivation, the fits under control condition were normally performed with $B = 0$.

Whilst the amplitudes, A_1 and A_2, of the exponential functions scatter considerably between different test potentials and between different fibres, the time constants, τ_{h1} and τ_{h2}, are reproducible and exhibit a clear voltage dependence. The shaded areas in Figs. 5A—C represent standard deviations of the mean values at various test potentials. In all three cases the fast inactivation time constant (τ_{h1}) becomes smaller with increasing potentials. On the other hand, the slow time constant (τ_{h2}) decreases with depolarization under control conditions, whereas after treatment with *Anemonia* Toxin II or IO_3^- its voltage dependence is reversed.

The symbols in Fig. 5 are mean values of time constants, τ_h, obtained by fitting the spectral density of Na^+ current fluctuations at low frequencies with one Lorentzian function, $S_h(f)$ (see Figs. 1 and 2A). The time constants derived from the spectral densities approximately follow the voltage dependence of the slow inactivation time constant, τ_{h2}.

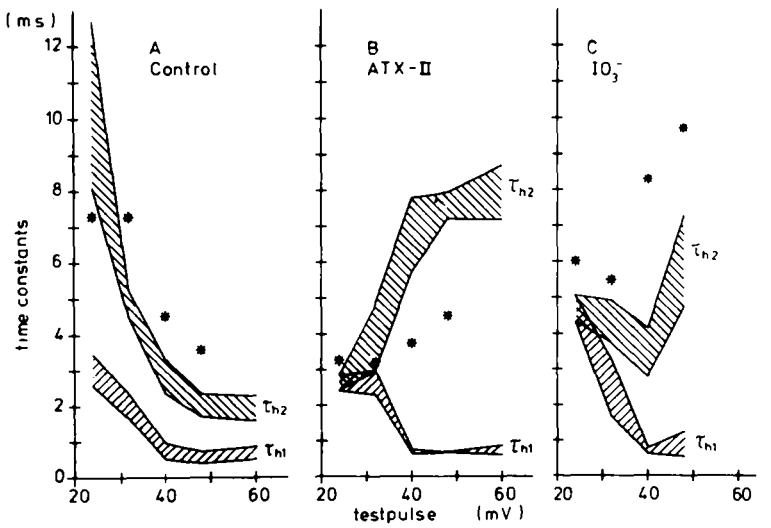

Fig. 5. Voltage dependence of inactivation time constants. Symbols (*) represent τ_h values determined from fits of one inactivation time constant to spectra as shown in Fig. 1, data are mean values of two experiments in A, of five to seven in B, and of five in C. Shaded areas represent standard deviations of mean values (n = 3—5) determined at 24, 32, 40, 48 and 60 mV from Na^+ current inactivation as shown in Fig. 4. (A) Under control conditions; (B) with 7 μM *Anemonia* Toxin II (ATX-II) added to the test solution; (C) with 20 or 40 mM IO_3^- applied to one or both cut fibre ends. Temperature 18°C.

Discussion

At least two time constants are necessary to describe the kinetics of Na^+ current inactivation and the spectral density of low-frequency Na^+ current fluctuations. Hence, Na^+ inactivation must be a multi-step process [15]. In our investigation we have demonstrated this behaviour not only for normal fibres but also for fibres in which Na^+ inactivation was modified by *Anemonia* Toxin II or IO_3^-.

The simplest formulation of a multi-step Na^+ inactivation is the following linear sequence between the open channel state (0) and two closed inactivated states (1) and (2) as proposed recently [15]:

$$(0) \rightleftharpoons (1) \rightleftharpoons (2)$$

This scheme predicts two time constants, τ_{h1} and τ_{h2}, of Na^+ current inactivation and correspondingly two Lorentzian functions, $S_{h1}(f)$ and $S_{h2}(f)$, for low-frequency Na^+ current fluctuations. Whilst the existence of two separate Lorentzian functions is only visible at high depolarizations (compare Fig. 2), the biphasic kinetics of Na^+ current inactivation are clearly seen at all potentials (for 40 mV see Fig. 4). The voltage dependencies of both time constants, τ_{h1} and τ_{h2}, in normal fibres (Fig. 5A) are in agreement with published values [16]. In modified fibres the slow inactivation time constant, τ_{h2}, surprisingly exhibits a reversed voltage dependence. Also, the time constants (τ_h) from the fit of the spectral densities with one Lorentzian function show this peculiarity (symbols in Fig. 5B and C). This indicates that the amplitude of the second Lorentzian function, $S_{h2}(f)$, dominates over that of $S_{h1}(f)$, a prediction which can be directly verified in cases where both spectral components can be resolved (compare Fig. 2B).

There are two extreme possibilities as to how *Anemonia* Toxin II and IO_3^- could modify the process of Na^+ inactivation in myelinated nerve. The substances could affect all Na^+ channels equally (uniform population of channels) by a gradual change of the rate constants between states (0), (1) and (2) of the above reaction scheme. The other alternative is to postulate two distinct populations of Na^+ channels, one normal and one fully affected. In this case the change of the rate constants would be an all-or-none process. In the following we present two arguments against the concept of a uniform population of Na^+ channels after the application of *Anemonia* Toxin II or IO_3^-.

(i) The time constants, τ_{h1} and τ_{h2}, depend on all rate constants of the sequence, $(0) \rightleftharpoons (1) \rightleftharpoons (2)$ (Eqns. 7 and 8 of Ref. 15). Thus, modification of one or more rate constants should affect both inactivation time constants. However, τ_{h1} remains practically unaffected after *Anemonia* Toxin II or IO_3^- treatment (compare Fig. 5A—C).

(ii) With increasing *Anemonia* Toxin II concentrations the amplitude, A_2, of slow Na^+ current inactivation and the steady-state value, B, increase at the expense of the amplitude, A_1, of fast inactivation. But both time constants, τ_{h1} and τ_{h2}, remain unchanged (Table I of Ref. 2). Again, this cannot be explained by a gradual change of the rate constants in all Na^+ channels.

These experimental results suggest that *Anemonia* Toxin II and IO_3^- modify only a fraction of Na^+ channels and that this fraction increases with increasing

concentrations of inactivation modifying substances. A distinction between both populations of Na^+ channels is possible because of the different voltage dependencies of the time constants τ_{h2}. Thus, Na^+ current components decaying with time constants above 5 ms at depolarizations, $V \geqslant 40$ mV, unequivocally can be attributed to the response of modified Na^+ channels, since the Na^+ current from normal channels inactivates with shorter time constants.

This criterium will now be used to estimate the single channel current and a lower limit of the number of modified Na^+ channels. We assume that normal and modified channels have only one conducting state and that Na^+ channels do not interact with each other. The Na^+ current, I, and the variance, *var*, of Na^+ current fluctuations are then given by the sum of the contributions of both populations of Na^+ channels:

$$I = Nip + N^*i^*p^* \tag{3}$$

$$var = Ni^2p(1-p) + N^*i^{*2}p^*(1-p^*) \tag{4}$$

In these equations, i denotes the single channel current and N and p the number and the open state probability of normal Na^+ channels, respectively. The corresponding quantities of modified channels are indicated by the asterisk.

Recently, the corresponding expressions of I and *var* for a uniform population of channels were employed to determine the number and conductance of K^+ and Na^+ channels in nerve [17,18]. But Eqns. 3 and 4 may also be used in our case of two populations of Na^+ channels, because at the end of a depolarizing test pulse the late Na^+ current and the variance mainly originate from modified channels (compare Fig. 3). Hence, the relationship:

$$\frac{var}{I} = i^*(1-p^*) \tag{5}$$

holds even for a mixture of normal and modified Na^+ channels. An upper limit for the open state probability, p^*, of modified channels can be derived from the criterium that at depolarizations of 40 and 48 mV the current component, $A_2 \cdot \exp(-t/\tau_{h2})$, originates from modified channels. Since these channels could also exhibit faster and slower, but unresolved inactivation components, it must be: $p^* < I/A_2$. Inserting this inequality into Eqn. 5 yields a lower and an upper limit for the single channel current, i^*:

$$\frac{var}{I} < i^* < \frac{var}{I(1-I/A_2)} \tag{6}$$

Finally, a lower limit for the number, N^*, of modified Na^+ channels follows from the relationships, $N^* = I/(i^*p^*)$ and $i^*p^* < var/(A_2 - I)$:

$$N^* > \frac{I(A_2-I)}{var} \tag{7}$$

Table I contains values of p^*, i^* and N^* determined at test potentials of V = 40 and 48 mV from fibres treated with *Anemonia* Toxin II or IO_3^-. From i^* = 0.65 pA at V = 40 mV (mean of *Anemonia* Toxin II values) one obtains a conductance of 8.1 pS for a modified Na^+ channel assuming the reversal potential V_{Na} = 120 mV and a linear current-voltage characteristic. This result is in agree-

TABLE I

ESTIMATION OF SINGLE CHANNEL CURRENT i^* AND LOWER LIMIT OF THE NUMBER N^* OF MODIFIED Na^+ CHANNELS

A_2, amplitude of slow Na^+ current inactivation. I and *var*, mean of Na^+ current and variance of Na^+ current fluctuations, respectively, between 145 and 460 ms after the onset of the test pulse. ATX-II, *Anemonia* Toxin II.

Expt.	Treatment	V (mV)	A_2 (nA)	I (pA)	*var* (pA^2)	p^*	i^* (pA)		N ($\times 10^4$)
							Lower	Upper limit	
B 22	ATX-II	40	4.22	535	277	<0.127	0.519	0.595	>0.71
B 23		40	14.03	1225	742	<0.087	0.605	0.663	>2.12
B 24		40	14.41	1950	1211	<0.135	0.621	0.718	>2.01
B 25		40	5.74	1320	1117	<0.230	0.846	1.099	>0.52
A 5		40	9.50	570	244	<0.060	0.428	0.455	>2.09
A 3		48	7.89	1120	349	<0.142	0.312	0.364	>2.17
A 4		48	11.26	1730	848	<0.154	0.490	0.579	>1.94
								mean ± S.E.	>1.65 ± 0.27
I 3	IO_3^-	40	3.85	1630	1610	<0.423	0.99	1.716	>0.22
I 4		40	23.00	230	1473	<0.010	6.40	6.46	>0.36
I 5		40	12.65	2100	1884	<0.166	0.90	1.079	>1.17
I 10		40	9.17	2200	2827	<0.240	1.29	1.697	>0.54
I 15		48	7.36	1450	1072	<0.197	0.74	0.922	>0.80
I 23		48	6.03	2190	1021	<0.363	0.47	0.738	>0.82
I 24		48	5.77	1270	344	<0.220	0.27	0.346	>1.67
I 25		48	3.68	1540	961	<0.418	0.62	1.065	>0.35
								mean ± S.E.	>0.74 ± 0.17

ment with the value of about 7 pS recently determined for the conductance of normal and modified Na^+ channels [6].

In normal fibres the number, N, of sodium channels per node is approx. 10^5 at a holding potential of $V_H = -20$ mV [6,13] which was also applied in our present experiments. *Anemonia* Toxin II and IO_3^- probably do not change the total number of Na^+ channels, since the peak Na^+ current upon depolarization is hardly affected by these substances [2,6]. With the lower limits of N^* listed in Table I we then conclude that more than 17% of Na^+ channels are modified by *Anemonia* Toxin II and more than 8% by IO_3^-. Hence, these substances alter a significant fraction of all Na^+ channels. Whilst the conductance and the process of Na^+ activation seems not to be affected [6], *Anemonia* Toxin II and IO_3^- preferentially act on the inactivation gating subunits. The action is not simply a destruction of inactivation gates, since the Na^+ current through modified channels still inactivates with characteristic time constants. Interestingly, the time constant of slow Na^+ current inactivation increases with increasing depolarizations for modified channels, whereas in normal channels a reversed voltage dependence is observed. This indicates that the gating processes related to the slow inactivation time constant are subject to an electric field different from that in normal fibres. The difference could arise from a different location of the inactivation gates in modified channels or from a change of the dielectric properties around the gating subunits. At present we cannot distinguish between these possibilities.

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