Biochimica et Biophysica Acta, 943 (1988) 1–12
Elsevier

BBA 74068

The action of arginine-specific reagents on ionic and gating currents in frog myelinated nerve

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(Received 7 March 1988)

Key words: Myelinated nerve fiber; Sodium current; Potassium current; Arginine residue; (Frog)

(1) The effect of arginine-specific reagents on the sodium current (I_{Na}), potassium current (I_K) and gating current (I_{gat}) of myelinated nerve fibres was investigated. (2) Externally applied camphorquinone-10-sulfonic acid (Cqs-OH) had little effect, but 50 mM Cqs-OH applied to the cut ends of the fibre progressively reduced the amplitude of I_{Na} without significantly altering its time course. After 30 min I_{Na} was reduced to 52% (pH 9.0) or 66% (pH 6.75–7.6) of the control value. I_K was decreased to a similar extent without changing its kinetics. I_{gat} was less affected than the ionic currents. (3) Externally applied phenylglyoxal markedly reduced I_{Na} and I_{gat}, but many fibres were lost during or shortly after the treatment. A few min treatment with 5 mM phenylglyoxal at pH 9 reduced I_{Na} to 20% and the on-response of I_{gat} to 69.5%. The effect was to a large extent irreversible. (4) External nitrophenylglyoxal and hydroxyphenylglyoxal significantly reduced I_{Na} and were less damaging than phenylglyoxal. I_{Na} was decreased to 34.5% by 10 mM nitrophenylglyoxal and to 28.3% by 20 mM hydroxyphenylglyoxal. The effect of nitrophenylglyoxal was little reversible, but that of hydroxyphenylglyoxal to a large extent reversible. 20 mM hydroxyphenylglyoxal reduced the on-response of I_{gat} to 62.5% of the control value, i.e. much less than I_{Na}. (5) 5 mM phenylglyoxal, 10 mM nitrophenylglyoxal and 20 mM hydroxyphenylglyoxal shifted the steady-state inactivation curve by 10–15 mV to more negative values of membrane potential but did not affect the descending branch of the $I_{Na}(E)$ curve. (6) 20–30 mM glyoxal, 20 mM 1,2-cyclohexanedione and 10 mM 4-hydroxy-3-nitrophenylglyoxal had no effect on I_{Na}. (7) The results are compatible with the idea that arginine residues are principal components of the sodium channel macromolecule.

Introduction

Experiments on the squid giant axon show that arginine residues are involved in the inactivation process of the sodium channel [1,2], probably together with tyrosine residues (Refs. 3 and 4, see also Ref. 5). Analysis of the primary structure of the sodium channel deduced from cDNA suggests that arginine and lysine residues act as positive gating charges, possibly in conjunction with negative charges [6]. To elucidate the role of arginine residues in sodium channel gating, we have studied the effect of externally applied arginine-specific reagents, especially phenylglyoxal and its derivatives, on the sodium and gating currents of the frog node of Ranvier.

Recently, the arginine-specific reagent camphorquinone-10-sulfonic acid (Cqs-OH) has been reported to reduce the amplitude of I_{Na} of the squid giant axon when added to the internal perfusion fluid; by contrast, its main effect on I_K is a slowing of the kinetics [7]. In the myelinated nerve

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fibre the internal effect of substances can be studied by applying them to the cut ends of the fibre (see, for example, Refs. 8 and 9). With this method we have investigated the effect of internally applied Cqs-OH on the ionic and gating currents of the node of Ranvier. External application of Cqs-OH had little effect.

Our results support the view that arginine residues play a key role in channel gating. Part of the results have been reported in abstract form [10].

Methods

The experiments were done on single motor or sensory nerve fibres from the tibial or peroneal nerve of the frog *Rana esculenta*. A node of Ranvier was voltage clamped at 10 °C [11]. The fibre was cut on both sides of the node. The cut ends were at a distance of about 0.75 mm on one side and (owing to the additional length of the air gap (see Ref. 11)) at about twice that distance on the other side. The potential at which I_{Na} was 70% of the maximum I_{Na} (measured with a prepulse of -50 mV and 40 ms duration) was taken as the normal resting potential ($E = -70$ mV). The holding potential was -90 mV for the measurement of I_{Na} and I_K and -100 mV for the measurement of I_{gat}; the more negative holding potential served to enhance the size of I_{gat}. Membrane currents were filtered at 25 kHz and sampled on-line at 10 μs intervals by a 12 bit A/D converter [12]. To obtain absolute values of membrane current, the recording resistance was assumed to be 10 MΩ; this is justified because for a 14 μm fibre the resistance per unit length of axis cylinder is 140 MΩ/cm [13], corresponding to 10.5 MΩ per 0.75 mm.

I_{Na} and I_K were measured with 14 ms test pulses of varying amplitude. They were corrected for capacitative and leakage currents; in order to do this the current elicited by a -30 mV pulse was suitably scaled and subtracted from the currents produced by the test pulses. The steady-state inactivation curve $h_\infty(E)$ of the sodium permeability was measured with 50 ms prepulses of varying height followed by a 5 ms test pulse to 0 mV. Plotting normalized test pulse current against prepulse potential gave the $h_\infty(E)$ curve. The experimental points were fitted by the equation

$$h_\infty(E) = [(1-C)/(1+\exp((E-E_h)/k_h))] + C \qquad (1)$$

In this equation, E_h is the potential at which $h_\infty = 0.5(1 + C)$, C is a small noninactivating component of the sodium permeability and k_h is the number of mV required to change h_∞ e-fold.

When measuring I_{gat}, an analog circuit was used to compensate most of the linear component of the capacitative and leakage currents. The pulse program for measuring I_{gat} is shown in Fig. 6. It consisted of a depolarizing test pulse of variable amplitude and three hyperpolarizing pulses of fixed size (-30 mV), superimposed on a reference potential of -130 mV. The -30 mV pulses were used to measure residual capacitative and leakage currents; the latter was suitably scaled and subtracted from the current during the test pulse. To improve the signal to noise ratio, 24 records were averaged with a repetition rate of 1 Hz. Gating currents were corrected for the effect of the low-pass filter (25 kHz) and for the additional delay caused by the programmable amplifier. For this purpose, the time axis of the data points was shifted 15 μs with respect to that of the pulses [14]. Integration of the on-response of I_{gat} gave records like those in Fig. 6. Before integrating the on-response the small time-independent current flowing during the pulse was measured (by taking the average of the last 20 current points) and subtracted. The integrated on-responses were fitted with single- or double-exponential functions using the equation

$$Y(t) = A\exp(-t/\tau_1) + B\exp(-t/\tau_2) + C \qquad (2)$$

The relation between steady-state values of charge movement during the on-response, Q_{on} ($= C$ in Eqn. 2) and test pulse potential, E, was fitted by the equation

$$Q_{on}(E) = Q_{on\,max}/[1+\exp((I_{mid}-E)/k)] \qquad (3)$$

where E_{mid} is the potential at which $Q_{on} = 0.5\,Q_{on\,max}$ and k is the number of mV required to change Q_{on} e-fold.

Solutions

For the measurement of ionic currents, the node was superfused with Ringer's solution containing (mM): 110 NaCl, 2.5 KCl, 1.8 $CaCl_2$ and 4-morpholinepropane sulfonic acid (Mops). The pH was adjusted to 7.2 with 1 M NaOH. In some cases alkaline Ringer's solution (pH 8.5 or 9) with 10 mM 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) instead of Mops was used. When measuring I_{Na} the Ringer's solution also contained 12 mM tetraethylammonium chloride to block K^+ channels from the outside. For the measurement of I_{gat}, Na-free Ringer's solution (105 mM tetramethylammonium chloride, 1.8 mM $CaCl_2$, 10 mM Mops, pH 7.2) + 12 mM tetraethylammonium chloride + 300 nM tetrodotoxin was used.

When recording I_{Na} or I_{gat}, the ends of the fibre were bathed in 113 mM CsCl, 7 mM NaCl + 4 mM MOPS of pH 7.2 to block K^+ channels from the inside. When recording I_K, the CsCl in this solution was replaced by KCl. In some experiments an alkaline solution (pH 8.5 or 9) with 10 mM CHES instead of Mops was used.

Arginine-specific reagents

Seven different α dicarbonyl reagents, known for their specificity for arginyl side chains in proteins [15–19], have been tested (Fig. 1). Glyoxal was purchased from Serva (Heidelberg, F.R.G.), phenylglyoxal and 1,2-cyclohexanedione from Sigma, *p*-hydroxyphenylglyoxal and *p*-nitrophenylglyoxal from Pierce, Cqs-OH from Fluka. 4-Hydroxy-3-nitrophenylglyoxal was a gift from Dr. Laila Zaki (Max Planck Institute for Biophysics, Frankfurt (Main)); she also supplied some *p*-nitrophenylglyoxal.

The reagents were either added to the Ringer's solution (external application) or to the axoplasm solution bathing the ends of the fibre (internal application). Most reagents could be dissolved without difficulty. Nitrophenylglyoxal and 1,2-cyclohexanedione had first to be dissolved in methanol or dimethylformamid; once added to the final solution, the concentration of the organic solvent did not exceed 2%. Hydroxyphenylglyoxal was dissolved with the help of ultrasound. Adding the reagents to the Ringer's solution or to the axoplasm solution lowered the pH. It had to be readjusted with tetramethylammonium hydroxide.

Results

Camphorquinone-10-sulfonic acid

As shown in Fig. 2, when Cqs-OH was applied at the relatively high concentration of 50 mM to the cut ends of the fibre, the amplitude of I_{Na} was progressively reduced without significantly altering its time course. The small increase of the time

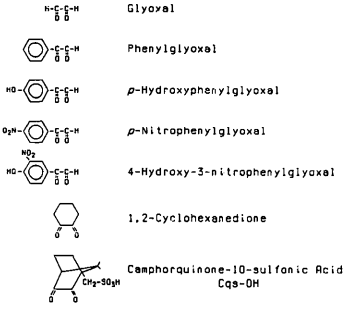

Fig. 1. Arginine-specific reagents.

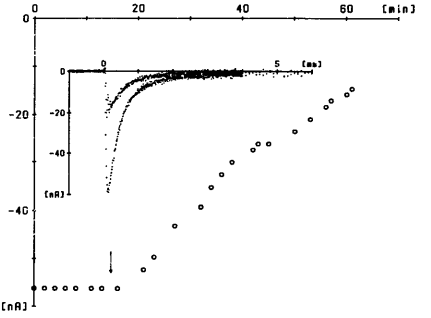

Fig. 2. Effect of 50 mM Cqs-OH (applied at $t = 15$ min, see arrow, to both cut ends) on the peak sodium current I_{Na} which was measured with pulses to -10 mV. The Cqs-OH was applied in axoplasm solution of pH 9.03. Inset shows I_{Na} recorded at $t = 9$ min and $t = 60$ min. The time to peak is 0.135 ms and 0.165 ms, respectively, and the decay time from peak to half peak 0.30 and 0.36 ms, respectively.

to peak and the time from peak to half peak mentioned in the figure legend is probably due to series resistance artifact. I_{Na} was reduced to 72 and 47% of its original value after 30 or 45 min, respectively. In another, similar experiment the reduction was to 78 or 57% after 15 or 30 min, respectively, and in a third experiment to 63% after 15 min, giving an average reduction to 71% at 15 min ($n = 3$) and to 52% at 30 min ($n = 2$).

In the three experiments described the pH of the Cqs-OH containing solution was adjusted to 9.0. This was done because the Cqs-OH effect was expected to be more pronounced at alkaline pH than at neutral pH (see Ref. 15). Further experiments were done at lower pH. In two experiments with 50 mM internal Cqs-OH at pH 6.75 and one experiment with 50 mM internal Cqs-OH at pH 7.6 we observed an average reduction to 82% at 15 min ($n = 3$) and to 66% at 30 min ($n = 3$). The reduction at 30 min is not significantly different from that at pH 9.0 (t-test, $P > 0.1$).

In one experiment we tried to reverse the Cqs-OH effect by applying Cqs-OH-free solution to the cut ends. Even after 18 min I_{Na} showed no sign of recovery.

Internal Cqs-OH also reduced the amplitude of I_K (Fig. 3). Again the time course was not measurably altered as indicated by the approximate constancy of the half time which was 1.22, 1.09, 1.15 ms in control, after 30 min and after 59 min, respectively, at a pulse potential of 70 mV. At the smaller pulse potential of 10 mV, the half time was 2.96, 2.38, 2.55 ms, respectively; the slight decrease is probably due to the decreasing voltage drop of I_K on the resistance in series with the nodal membrane. In the two experiments with 50 mM internal Cqs-OH at pH 6.75 and the one experiment with 50 mM internal Cqs-OH at pH 7.6 we found an average reduction of I_K (measured with 14 ms pulses to 70 mV) to 61% at 30 min ($n = 3$), not very different from the reduction of I_{Na} which was to 65% at 30 min ($n = 3$) (see above) in the same experiments. In the experiment of Fig. 3 the decrease of I_{Na} and I_K was followed for almost an hour. After 59 min I_{Na} was reduced to 58% and I_K (measured with 14 ms pulses to 70 mV) to 51%, demonstrating again that the two types of current are affected to the same extent. Another experiment showed that the inward K tail currents following 50 ms pulses to 60 or 70 mV

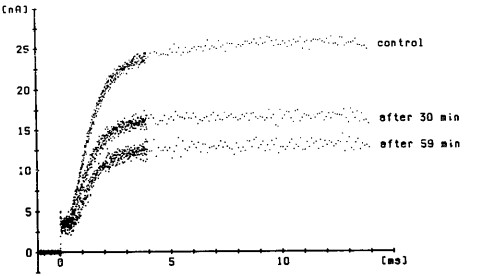

Fig. 3. Effect of 50 mM Cqs-OH (applied to both cut ends in a solution of pH 7.6) on the potassium outward current I_K which was measured with 14 ms pulses to 70 mV. The records show I_K before, 30 min after and 59 min after application of Cqs-OH. I_{Na} reversed sign at 64, 62 and 58 mV, respectively. A small Na outward current is visible at the beginning of the pulse.

were somewhat slowed by internal Cqs-OH; the time for decay to half the initial amplitude was 0.275 ms in control and 0.85 ms after 65 min internal Cqs-OH application. The leakage current (measured with −30 mV pulses) was not altered by internal Cqs-OH.

The slow development of the Cqs-OH effect made measurements over a fairly long period of time necessary. This complicated the gating current measurements considerably. In one successful gating current experiment we were able to demonstrate convincingly that 50 mM internal Cqs-OH reduced Q_{on} to 82% of the control value within 35–40 min. Q_{on} (measured with 0.6 ms pulses to 0 mV) was 54.7 ± 0.8 fC under control condition (average ± S.E. from eight measurements over a period of 22 min). After the last control measurement, axoplasm solution with 50 mM Cqs-OH (pH 9.0) was applied to the cut ends. 31–42 min after application five further measurements were done, giving Q_{on} = 44.7 ± 0.6 fC which is 82% of the control value. A second experiment which was done in exactly the same way failed to show any significant change of Q_{on} after Cqs-OH application. The conclusion from these two experiments is that Cqs-OH affects the charge movement less than the ionic currents.

Externally applied Cqs-OH (pH 7.2 or 9.0–9.3) had little or no effect on I_{Na} even at the high concentration of 50 mM. It was difficult to rule out that the small effects which were seen were due to fibre run down. However, one experiment showed convincingly that the small reduction of I_{Na} during external 50 mM Cqs-OH application is genuine. In this experiment, I_{Na} was reduced to 76% of control value during application and recovered to 89% after a 10 min wash.

The sulfonic acid probably prevents externally applied Cqs-OH from reaching the site of action. Likewise, externally applied isethionyl acetimidate which contains a negatively charged sulfonic acid group has no effect on I_{Na} whereas ethyl acetimidate which lacks the sulfonic acid irreversibly reduces I_{Na} [20].

Phenylglyoxal and its derivatives

Alkaline Ringer's solution with 2.5 or 5 mM phenyiglyoxal markedly reduced I_{Na} and I_{gat}, but many fibres were lost during or shortly after the treatment. Fig. 4 shows the reduction of I_{Na} following a few min treatment with 2.5 or 5 mM phenylglyoxal at pH 9.0 and a subsequent wash with neutral reagent-free Ringer's solution. The reduction was to, respectively, 44% and 28% of the control value and was not accompanied by a change in kinetics. The average reduction during treatment with 2.5 or 5 mM phenylglyoxal was to 40.3 ± 6.2% (mean ± S.E., n = 3) and 20.0 ± 2.2%

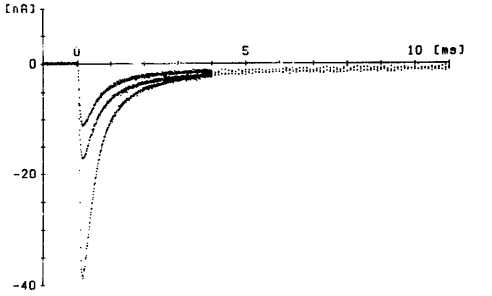

Fig. 4. Effect of externally applied phenylglyoxal on I_{Na} measured with pulses to −10 mV. Largest current: Control record. Medium sized current: after 10 min treatment with 2.5 mM phenylglyoxal and 4 min wash. Smallest current: after 4 min treatment with 5 mM phenylglyoxal and 9 min wash. The phenylglyoxal was applied in Ringer's solution of pH 9.0. The three records were taken in neutral, reagent-free Ringer's solution. The time to peak is 0.165 ms in control, 0.175 ms in 2.5 mM phenylglyoxal and 0.165 ms in 5 mM phenylglyoxal. The decay time from peak to half peak is 0.41 ms in the control record and 0.58 ms in the two other records.

($n = 5$), respectively. During wash with neutral reagent-free Ringer's solution recovery to 62.2 ± 8.6% ($n = 3$) and 27.0 ± 1.3% ($n = 4$), respectively, took place. Phenylglyoxal applied at neutral pH was less effective: a 14 min treatment with 5 mM phenylglyoxal at pH 7.0 reduced I_{Na} to 45.8% ($n = 2$) of the control value; recovery during wash with reagent-free Ringer's solution was to 66.7% ($n = 2$). From the seven fibres treated with 5 mM phenylglyoxal, two collapsed a few min after washing and taking the records. Others were lost even before records could be taken. The leakage current (measured with −30 mV pulses) increased 2 min before the collapse; in those fibers which survived the phenylglyoxal treatment no change of leakage current was observed.

Plotting peak I_{Na} against pulse potential E showed that phenylglyoxal does not change the reversal potential and does not shift the descending branch of the $I_{Na}(E)$ curve; it simply reduces the size of I_{Na}. For instance in the experiment of Fig. 4, the potential at which peak I_{Na} reached half its maximum was −44 mV in control, −40 mV during treatment with 5 mM phenylglyoxal and −48 mV after treatment. There was, however, a clear negative shift of the steady-state inactivation curve during treatment with 5 mM phenylglyoxal. As shown in Fig. 5, E_{mid} changed from −65.6 mV in control to −81.9 mV during treatment and back to −70.9 mV after treatment. In the experiment of Fig. 5, I_{Na} measured with a 50 ms prepulse to −150 mV was reduced to 73% of the control value by 5 mM phenylglyoxal. I_{Na} measured without a prepulse was more strongly reduced, namely to 58%, the stronger reduction being due to the negative shift of the $h_\infty(E)$ curve. I_{Na} measured without a prepulse partly recovered (to 70% of the control value) after the treatment, most likely because the $h_\infty(E)$ curve shifted back towards its normal position. By contrast, I_{Na} measured with a prepulse to −150 mV showed very little recovery (from 73 to 74% of the control value). In conclusion, phenylglyoxal appears to have two effects: it irreversibly reduces the maximum sodium permeability $\bar{P}_{Na}$ and reversibly shifts the $h_\infty(E)$ curve to more negative values of membrane potential.

Because phenylglyoxal tended to damage the fibres, gating current measurements were difficult. Nevertheless a few results could be obtained. As shown in Fig. 6, an 8 min treatment with 5 mM phenylglyoxal reduced the on-response (measured

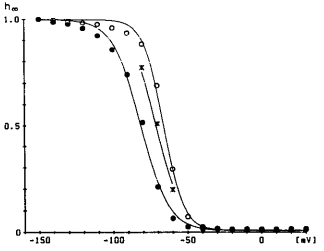

Fig. 5. Effect of externally applied phenylglyoxal on the steady-state inactivation curve $h_\infty(E)$ of the sodium permeability. ○, control. ●, after 13 min treatment with 5 mM phenylglyoxal in neutral Ringer's solution; ★, after 11 min wash with reagent-free Ringer's solution. The experimental points were fitted by Eqn. 1 with the following parameters:

	E_h	k_h	C
control	−65.6 mV	6.5 mV	0.010
in phenylglyoxal	−81.0 mV	9.1 mV	0.008
after phenylglyoxal	−70.9 mV	8.3 mV	0.015

with 0.6 ms pulses to 0 mV) from 95 to 66 fC, i.e. to 69%, without significantly altering the time constant, τ_1, of the fast component; in addition, it almost abolished the slow component of the charge movement. Fig. 7 shows complete $Q_{on}(E)$ curves before and after treatment with 5 mM phenylglyoxal. The treatment reduced $Q_{on\ max}$, the maximum charge displaced at strong depolarizations, from 92.5 to 65.1 fC, i.e. to 70%. The midpoint potential, E_{mid}, and the slope factor, k, were not significantly altered, i.e. the $Q_{on}(E)$ curve was simply scaled down by a factor 0.7. Q_{off}, the reverse charge movement at the pulse end, was determined for the pulses to 0 mV (8 measurements before and 6 measurements after phenylglyoxal treatment); it was reduced to 78% by the treatment, i.e. slightly less than Q_{on}. As in the experiment of Fig. 6, the time constant of the on-response changed little; for the pulses to 0 mV it was 74 μs before and 80 μs after treatment (mean values from six and five measurements, respectively).

Comparing the effects of phenylglyoxal on I_{gat} and I_{Na}, the two experiments in Figs. 6 and 7 show a reduction of Q_{on} to 69 or 70% of the control value by 5 mM phenylglyoxal at pH 9 whereas the same treatment reduced the sodium current to 20% (see above). Part of the difference is certainly due to the difference in holding potential which was −90 mV for the measurement of I_{Na} and −100 mV for the measurement of I_{gat} (see Methods). At a more negative holding potential the negative shift of the inactivation curve will have less effect on the size of the current.

In search for more mildly acting arginine reagents, we did further experiments with the phenylglyoxal derivatives *p*-hydroxyphenylglyoxal, *p*-nitrophenylglyoxal and 4-hydroxy-3-nitrophenylglyoxal (see Fig. 1). 4-Hydroxy-3-nitrophenylglyoxal, externally applied in a concentration of 10

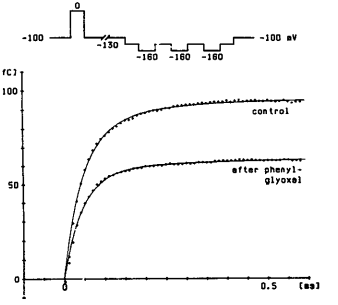

Fig. 6. Effect of externally applied phenylglyoxal on the on-response of the gating current measured with 0.6 ms pulses to 0 mV. Pulse program on top. Ringer's solution with 5 mM phenylglyoxal (pH 8.45) was applied for 8 min; afterwards the fibre was washed with reagent-free solution for 5 min. Records 'control' and 'after phenylglyoxal' were taken in neutral Na^+-free Ringer's solution + 12 mM TEA + 300 nM tetrodotoxin. Integrated on-responses were fitted by Eqn. 2 with the following parameters:

	A	B	C	τ_1	τ_2
control	−72 fC	−23 fC	95 fC	44 μs	148 μs
after phenylglyoxal	−59 fC	−7 fC	66 fC	48 μs	519 μs

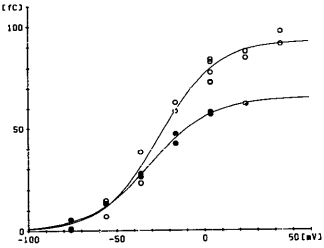

Fig. 7. Effect of externally applied phenylglyoxal on the $Q_{on}(E)$ curve. Q_{on} measured with 0.6 ms pulses before (○) and after (●) a 4 min treatment with 5 mM phenylglyoxal (in Ringer's solution of pH 9.1) followed by a 4 min wash. Points were fitted by Eqn. 3 with the following parameters:

	$Q_{on\,max}$	E_{mid}	k
control (○)	92.5 fC	−21 mV	16.1 mV
after phenylglyoxal (●)	65.1 fC	−26 mV	17.4 mV

mM at pH 9.0–9.1, had no effect on I_{Na} (three experiments) whereas 5–10 mM nitrophenylglyoxal and 10–20 mM hydroxyphenylglyoxal significantly reduced I_{Na} and were indeed somewhat less damaging than phenylglyoxal although by no means harmless. Like phenylglyoxal, nitrophenylglyoxal and hydroxyphenylglyoxal shifted the $h_\infty(E)$ curve to more negative values of membrane potential, the average shift being −10.25 mV in 10 mM nitrophenylglyoxal ($n = 2$) and −14.0 mV in 20 mM hydroxyphenylglyoxal ($n = 2$); the shift was only partly reversible by washing with reagent-free solution.

As shown in Fig. 8, 10 mM nitrophenylglyoxal markedly reduced I_{Na}, but did not alter the reversal potential and did not shift the descending branch of the $I_{Na}(E)$ curve. In a total of seven experiments (four at pH 9.0 and three at pH 7.2) 10 mM nitrophenylglyoxal reduced peak I_{Na} (measured with 80 mV pulses) to 34.5 ± 5.0% (mean ± S.E.). The reduction by 5 mM nitrophenylglyoxal was slightly less, namely to 44.9 ± 10.8% in five experiments (three at pH 7.2–7.9, two at pH 9.0). The nitrophenylglyoxal effect was little or not at all reversible. For instance in the experiment of Fig. 8, I_{Na} (which had been decreased to 22% of the control value by a 10 min treatment with 10 mM nitrophenylglyoxal) increased to 23% after a 9 min wash with reagent-free solution. In another experiment, I_{Na} was decreased to 30% of the control value by a 12 min treatment with 10 mM nitrophenylglyoxal and recovered to 42% during a 10 min wash. In five of the 12 experiments with 5 or 10 mM nitrophenylglyoxal, the fibre was lost during the washing period before records of I_{Na} could be taken.

Fig. 8B shows an interesting side effect, namely a marked increase (factor 2.15) of the decay time

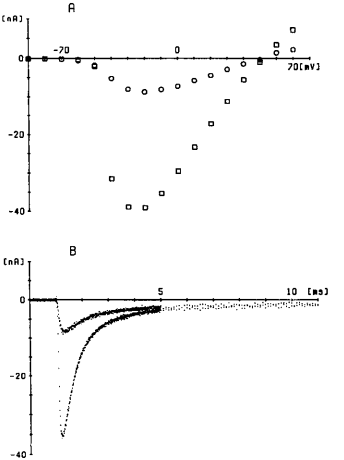

Fig. 8. Effect of externally applied nitrophenylglyoxal on I_{Na}. (A) current-voltage curve in Ringer's solution (pH 9.0) without (□) and with (○) 10 mM nitrophenylglyoxal; the nitrophenylglyoxal containing solution was applied for 10 min. (B) I_{Na} elicited by a pulse to −10 mV in Ringer's solution without and with 10 mM nitrophenylglyoxal (large and small current, respectively). The time to peak is 0.205 and 0.295 ms, respectively, and the decay time from peak to half peak 0.54 and 1.16 ms, respectively.

from peak to half peak after nitrophenylglyoxal treatment (see figure legend). The effect was also seen in other experiments with 10 mM nitrophenylglyoxal and in experiments with other arginine reagents, but much less pronounced. In six experiments with 10 mM nitrophenylglyoxal the decay time from peak to half peak increased on average by a factor of 1.38 ± 0.18 (mean ± S.E.), a value similar to the factors 1.20, 1.41 and 1.54 observed after treatment with internal Cqs-OH, external phenylglyoxal or external hydroxyphenylglyoxal (see legends of Figs. 2, 4 and 9). The phenomenon

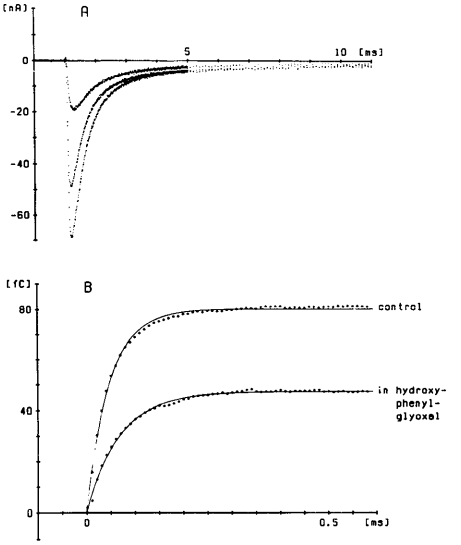

Fig. 9. Effect of externally applied hydroxyphenylglyoxal on I_{Na} (A) and I_{gat} (B). (A) I_{Na} elicited by a pulse to −10 mV before (large current), during (small current) and after (medium size current) application of 20 mM hydroxyphenylglyoxal (pH 7.05). The time to peak is 0.185, 0.285 and 0.165 ms, respectively, and the decay time from peak to half peak 0.48, 0.74 and 0.48 ms, respectively. Hydroxyphenylglyoxal was applied for 10 min and washed out for 21 min. (B) On-response of I_{gat} measured with 0.6 ms pulses to 0 mV before and during application of 20 mM hydroxyphenylglyoxal (pH 7.16). Hydroxyphenylglyoxal was applied for 6 min. Integrated on-responses were fitted by one exponential (i.e. Eqn. 2 with $-A = C$ and $B = 0$) with the following parameters:

	$-A = C$	τ_1
control	80 fC	48 μs
in hydroxyphenylglyoxal	49 fC	69 μs

is probably caused by the voltage drop of I_{Na} on the series resistance; the latter may have been relatively large in the experiment of Fig. 8.

Hydroxyphenylglyoxal was different from the other arginine-specific reagents in that its effect was to a large degree reversible. This is seen in Fig. 9A which shows a reduction of I_{Na} to 28% of the control value by 20 mM hydroxyphenylglyoxal and a recovery to 71% after a 21 min wash with reagent-free Ringer's solution. (Substantial recovery, namely to 68% of the control value, was already observed after 11 min wash). In four experiments with 20 mM hydroxyphenylglyoxal (two at pH 7.1 and two at pH 9.2) the average reduction was to 28.3 ± 4.9% (mean ± S.E.). In three of these four experiments the current recovered to 75.7 ± 0.9% (mean ± S.E.) after washing for 10–21 min; one fibre was lost during washing.

Like Cqs-OH and phenylglyoxal, hydroxyphenylglyoxal reduced the charge movement much less than I_{Na}. As can be seen in Fig. 9B, the on-response of I_{gat} (measured with 0.6 ms pulses to 0 mV) is reduced to 61% of the control value in the presence of 20 mM hydroxyphenylglyoxal. In another experiment Q_{on} was reduced to 64% by a 6 min treatment with 20 mM. In both experiments the reduction of Q_{off} was nearly equal to that of Q_{on}. The reduction of the charge movement was accompanied by a slowing of its kinetics; the time constant τ_1 of the on-response increased by a factor of 1.44 in the experiment of Fig. 9B and by a factor of 1.52 in the second experiment. In both experiments the charge movement failed to recover when washing with reagent-free solution. Instead it became progressively smaller, probably because hydroxyphenylglyoxal–together with the repetitive pulsing required for gating current measurements–had caused some damage to the fibre.

Glyoxal and 1,2-cyclohexanedione

From the reagents shown in Fig. 1 glyoxal and 1,2-cyclohexanedione were without effect. We tested 20 mM glyoxal in the Ringer's solution (pH 9.0) and 30 mM glyoxal in the axoplasm solution (pH 9.0) applied for 21 min; the lack of effect confirms previous observations [21]. Ringer's solution with 20 mM 1,2-cyclohexanedione (pH 8.45) had also no significant effect on I_{Na}.

Discussion

The experimental findings (which are summarized in Table I) show that arginine-specific reagents reduce the sodium current and the gating current of the node of Ranvier. The most effective reagents are Cqs-OH for internal application and

TABLE I

EFFECT OF ARGININE-SPECIFIC REAGENTS ON SODIUM CURRENT (I_{Na}) AND ON-RESPONSE OF THE GATING CURRENT (Q_{on})

I_{Na} and Q_{on} were measured during the application of the reagent with pulses to −10 and 0 mV, respectively, and are given in % of the control value.

Application	Reagent	Concn. (mM)	I_{Na} % of control	Q_{on} % of control	Reversibility
Internal	camphorquinone-10-sulfonic acid (Cqs-OH)	50	52–66 [a]	82 [b]	none
	glyoxal	30	no effect		
External	phenylglyoxal	5	20.0	69.5	little
	p-nitrophenylglyoxal	10	34.5		little
	p-hydroxyphenylglyoxal	20	28.3	62.5	> half
	camphorquinone-10-sulfonic acid (Cqs-OH)	50	76		half
	glyoxal	20	no effect		
	1,2-cyclohexanedione	20	no effect		
	4-hydroxy-3-nitrophenylglyoxal	10	no effect		

[a] At pH 9.0 and pH 6.75–7.6, respectively; applied for 30 min.
[b] Applied for 35–40 min.

phenylglyoxal with his derivatives nitrophenylglyoxal and hydroxyphenylglyoxal for external application. The different effectiveness of the different reagents reflects probably mainly the different ability to reach the arginine residues inside the channel macromolecule. It is surprising that the lipophilic 1,2-cyclohexanedione in a concentration of 20 mM has no effect. An about 5 times smaller effectiveness of 1,2-cyclohexanedione compared with that of the phenylglyoxal derivatives has been reported for inhibition of the mitochondrial tricarboxylate carrier and has been explained by assuming that the vicinity of the essential arginine(s) has hydrophilic character [22]. Alternatively, the ineffectiveness of 1,2-cyclohexanedione might be explained by the absence of borate; the latter is reported to accelerate the reaction between 1,2-cyclohexanedione and arginine residues and to stabilize the reaction product [15,16].

The reagents affect mainly the size of I_{Na}. The small increase of the time to peak and of the time from peak to half peak is probably a series resistance artifact. Apart from that the kinetics are not altered. The decrease of I_{Na} is partly due to a shift of the $h_\infty(E)$ curve to more negative values of membrane potential and partly to a decrease of the maximum sodium permeability $\bar{P}_{Na}$; consequently, a strong negative prepulse makes the decrease of I_{Na} smaller but by no means abolishes it. The shift of the inactivation curve is not accompanied by a shift of the activation curve (as judged from the descending branch of the $I_{Na}(E)$ curve); the same has been observed on fibres treated with different amino group reagents or periodate [20,21].

I_{gat} is clearly less reduced than I_{Na}. As explained on p. 7, a difference is expected because of the different holding potential which was -90 mV for the measurement of I_{Na} and -100 mV for the measurement of I_{gat}. It seems, however, likely that the smaller effect on I_{gat} is in part genuine. Procaine [23] and ultraviolet light [24] also decrease I_{gat} much less than I_{Na}. In order to prevent the opening of a channel it seems sufficient to block or immobilize only some of the gating charges of the channel.

The results are consistent with the idea that arginine residues are principal components of the sodium channel macromolecule (see Introduction). Surprisingly, our findings differ in several respects from those obtained in the squid axon. The major discrepancy is that in our experiments internal Cqs-OH affects I_K in the same way as I_{Na}: it reduces the size without significantly altering the time course. By contrast, in the squid axon the essential effect of internal Cqs-OH on I_K is a slowing of its kinetics [7]. One is forced to conclude that the gating mechanisms of the two channels are similar in the frog node but significantly different in the squid axon. In the squid axon, 10 mM Cqs-OH applied internally eliminate I_{Na} [7] whereas we achieve only partial inhibition of I_{Na} with 50 mM Cqs-OH. The difference may be simply due to the fact that Cqs-OH reaches the inner side of the excitable membrane more easily from the perfusion fluid of the squid axon than from the cut ends of the myelinated nerve fibre. It is also surprising that 5 mM glyoxal remove inactivation of I_{Na} completely when applied internally to the perfused squid axon [2], but 30 mM glyoxal applied to the cut ends of the frog nerve fibre are without any effect; it seems unlikely that glyoxal should be unable to diffuse through the axoplasm of the frog nerve fibre while Cqs-OH can.

Acknowledgements

This investigation was supported by the Deutsche Forschungsgemeinschaft. We thank Dr. M. Rack for many helpful suggestions and Professor B. Neumcke for reading the manuscript. The authors are indebted to Dr. L. Zaki (Frankfurt/Main) for a gift of phenylglyoxal derivatives and for advice on arginine-specific reagents.

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