GATING CURRENTS IN THE RANVIER NODE MEMBRANE STUDIED BY RAMP POTENTIAL CONTROL

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Asymmetrical displacement currents in the Ranvier node membrane of Rana ridibunda, treated with tetrodotoxin and tetraethylammonium, were studied by the use of ramp voltage pulses. In some experiments both ramp- and step-voltage pulses were used. The net displacement current is the sum of two components, one of which can be blocked by the local anesthetic trimecaine or inactivated by a preliminary 10-msec depolarizing pulse of sufficient amplitude to inactivate sodium currents in the control solution. The parameters of distribution of the charge carrying the inactivated part of the displacement current are close to (although not identical with) the parameters of the sodium conductivity versus membrane potential curve. The inactivated charge is 30-50% of the total displaced charge. It is suggested that the inactivation- and trimecaine-sensitive components of the displacement current may be the true gating current.

KEY WORDS: gating current; node of Ranvier; inactivation; trimecaine.

The nerve fiber membrane voltage clamp conditions and after complete blocking of the sodium and potassium channels exhibits asymmetry of its displacement current, which is attributed to a redistribution of charges in the membrane dielectric produced by the electric field [1, 3, 4, 6, 9]. Definite correlation has been found between the characteristics of the sodium permeability system and the properties of mobile charges carrying the displacement current [2, 9]. This suggested that these charges (possibly some sort of constant dipoles) control the activation gates of the sodium channels. The displacement current was therefore called the gating current.

However, full agreement between the displacement currents and the characteristics of the gating currents expected on the basis of the Hodgkin–Huxley theory [2] has not yet been obtained. Meanwhile there are indications that the population of charges contributing to the displacement current is nonhomogeneous and that only part of the displacement current is concerned with the gating function [7, 10]. The problem accordingly arises of distinguishing that component of the displacement current which is the true gating current.

To study this problem, it was decided to use ramp-voltage pulses ($V = kt$) to investigate displacement currents rather than the step pulses usually employed in such experiments. The quantity of charge carried (Q), the integral of the displacement current with time, can be regarded in this case as a function of both potential (V) and time (t). The slope (k) in this case acts as a parameter. In this way it was possible to compare the kinetic characteristics of the displacement current and sodium current (I_{Na}), measured at the same slope (k), directly, and also to compare Q and the coefficient of sodium permeability (P_{Na}) as functions of potential. Because of the continuity of the act of measurement of the curve $Q(V, t)$, all anomalies in it could evidently be regarded as a direct indication of the nonhomogeneity of the charge population. It will be understood that such anomalies, in the case of discrete measurement of $Q(V)$ can easily be taken as the result of the natural scatter of the experimental data.

EXPERIMENTAL METHOD

Experiments were carried out on Ranvier nodes of isolated frog nerve fibers by the voltage clamp method [5]. The ends of the fiber on either side of the test node were divided in isotonic CsCl solution. During measurement of the sodium current the potassium channels were blocked by 10 mM tetraethylammonium chloride and Cs^+ ions, diffusing in the axoplasm through the divided internodes. To investigate the displacement cur-

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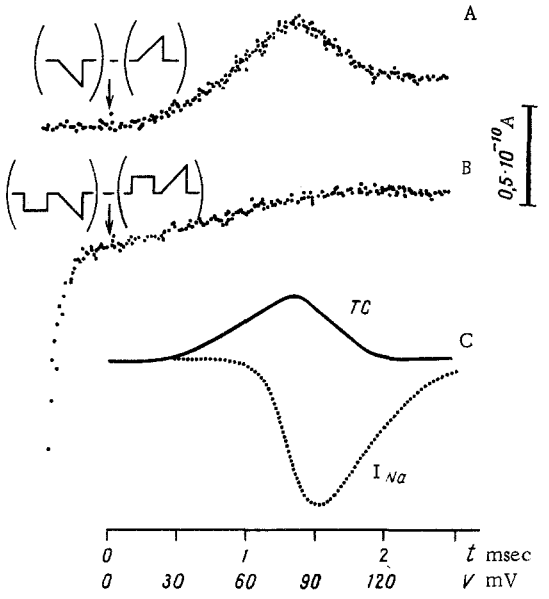

Fig. 1

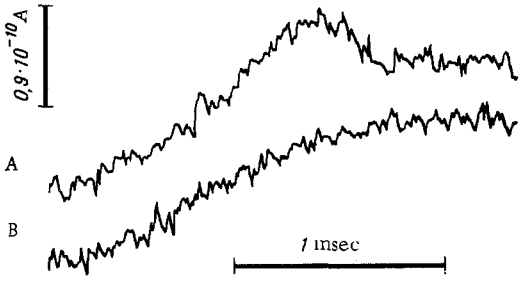

Fig. 2

Fig. 1. Displacement currents in response to ramp-voltage pulse and effect of preliminary depolarization. Ramp-voltage pulse applied from held potential (−100 mV) in one case without prepulse (A), in the other 0.5 msec after end of prepulse (10 msec) with amplitude 80 mV (B). Difference between curves A and B and record of sodium current in response to same pulse, obtained in control solution, shown below (C). Averager triggered by trailing edge of prepulse. Moment of stimulation indicated by arrow. Calibration given for displacement current. Leakage current automatically subtracted when recording ion currents. Experiment 15.2.7.

Fig. 2. Effect of trimecaine on displacement current. A) Before application of trimecaine; B) after addition of $5 \cdot 10^{-4}$ M trimecaine to external solution. Held potential −120 mV, slope 30 mV/sec. Integral of difference between curves A and B is $26 \cdot 10^{-15}$ K. Experiment 22.12.6.

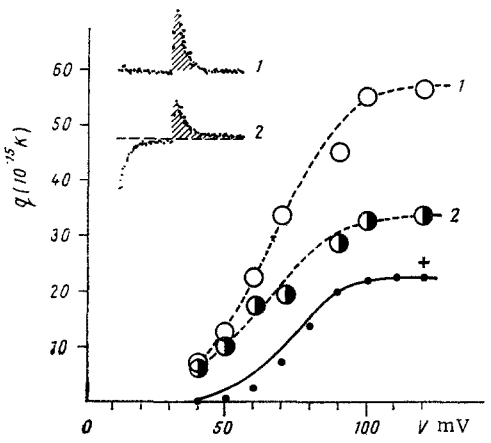

Fig. 3. Carried charge as a function of potential measured as displacement currents to step-voltage pulses. Original recordings of displacement currents for V = 120 mV shown; top record without prepulse (1), bottom 0.5 msec after end of prepulse (10 msec) with amplitude of 80 mV (2). Ordinate, area beneath curves of on responses, in units of $1 \cdot 10^{-15}$ K (shaded). Continuous line indicates difference between broken curves. Filled circles show peak values of P_{Na} as a function of potential. Parameters of distributions: inactivated components: $V_{1/2}$ = 72 mV, gradient of curve 9 mV for an e-fold change in charge; inactivated component: $V_{1/2}$ = 63 mV, gradient of curve 15 mV for an e-fold change in charge; curve of P_{Na} : $V_{1/2}$ = 77 mV, gradient of curve 8 mV for an e-fold change in charge. Experiment 15.2.7.

rents, Na^+ ions in the Ringer's solution were replaced by $Tris^+$; 3×10^{-7} M tetrodotoxin also was added to the solution (for all solutions pH was 7.2 and temperature 8-12°C). The asymmetrical displacement current was obtained by algebraic summation of current arising in response to ramp- or step-voltage pulses, strictly

identical in amplitude and duration, but opposite in sign. The total number of summations was 256. The displacement current was averaged on the ATAS-250 digital averager (256 points per path of 2 or 5 msec, depending on the value of k chosen).

Before averaging, the signal was freed from noise by means of a passive filter with transmission band of up to 50 kHz. The results were displayed either on the oscilloscope screen or by automatic X–Y recorder. The potential on the inner side of the membrane (relative to the external potential, taken as zero) is designated E and the shift of potential from the original held E (E_h) as V.

EXPERIMENTAL RESULTS

A record of the displacement current obtained by algebraic summation of ramp-voltage pulses with k (60 mV/msec) is illustrated in Fig. 1A.

The next record of the displacement current was obtained with the aid of the same ramp pulses (Fig. 1B), the only difference being, however, that they were used 0.5 msec after a 10-msec prepulse with an amplitude of 80 mV (the stimulation procedure is illustrated in Fig. 1). Clearly the prepulse completely removed the "hump" from the displacement current curve. Since the stimulation procedure used leads to inactivation of the inward I_{Na} if sodium ions are present in the medium, it can be concluded that removal of the "hump" of the displacement current is the result of inactivation of one component of the displacement current under the influence of the depolarizing prepulse. Precisely the same effect on the "hump" of the displacement current was produced by the local anesthetic trimecaine in concentrations strong enough to completely suppress I_{Na} in normal salt medium (Fig. 2).

The temporal course of the inactivated components of the displacement current and the inward I_{Na} of pain in response to the same ramp-voltage pulse in the control solution is compared in Fig. 1C. Clearly the displacement current developed slightly ahead of I_{Na} and ended close to V = 120 mV. This, incidentally, corresponds to the region of values of potential where sodium activation reaches its maximum. The component of the displacement current tested thus showed good agreement with the changes in sodium permeability. The integral of the inactivated component of the displacement current in this experiment was $25 \cdot 10^{-15}$ K. The net carried charge (Q_{max}), measured in this experiment with the aid of step-voltage pulses (Fig. 3), was $58 \cdot 10^{-15}$ K. The inactivated charge was thus rather more than 40% of the total charge displaced by strong depolarization from E_h = 100 mV. In other experiments this value varied between 30 and 50%.

It is natural to suggest that the inactivated and uninactivated components of the displacement current are carried by different charges of different nature. This hypothesis is supported by the results of the following experiments. Curves characterizing the quantity of carried charge in response to a step-voltage pulse as a function of V, in one case without a prepulse and in the other case 0.5 msec after the end of the prepulse almost completely inactivating I_{Na} in the control solution, are shown in Fig. 3. Clearly the curve obtained in the measurements with the prepulse represents the distribution of the "uninactivated charge" (Q) and the difference between the two curves the distribution of the "inactivated charge" (q). The position of the middle points of the curves ($V_{1/2}$) and the gradient of the curves can be seen to differ substantially at these points. This is in full agreement with the hypothesis that the charges are different in nature. The maximal value of q can be seen to coincide, within the limits of accuracy of measurement, with the value of q of the inactivated component of the displacement current measured in response to application of a ramp-voltage pulse to the membrane (marked by a plus sign).

The fact will be noted that the characteristics of distribution of the "inactivated charge" were close to the parameters of the curve of P_{Na} (filled circles), whereas the gradient of distribution of the uninactivated charge was just over half the gradient of P_{Na}, and the $V_{1/2}$ point lay 14 mV to the left of the middle point of the P_{Na} curve. It must be emphasized that the mutual arrangement of the P_{Na} curves and the distribution of the inactivated charge were preserved in those cases when the displacement current was obtained by means of a ramp-voltage pulse with a small slope (30 mV/msec), i.e., when the distribution of the charge was close to stationary (Fig. 3).

To sum up the facts described above, it seems that the inactivated component of the displacement current is evidently the true gating current. The experimental evidence does not yet provide an answer to the question of whether the uninactivated component of the asymmetrical displacement current is related in any way to the gating function. The answer to this question must largely depend on whether a satisfactory quantitative description of the kinetics of P_{Na} can be given by the use of the inactivated component of the displacement current only.

In conclusion, it should be noted that the asymmetrical displacement current in response to a ramp-vol-

tage pulse does not reach zero in the region of values of E of about + 20 mV, whereas the curves of distribution of both types of charges reach saturation in this region. In the writers' view, this is because 10 mM tetraethylammonium chloride did not completely block the potassium channels, which were able to open by the time that these values of E were reached.

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DOPAMINE SENSITIVITY OF HYPOTHALAMIC ARCUATE NEURONS AND THEIR ROLE IN THE REGULATION OF PITUITARY GONATROPIC FUNCTION

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The effect of microiontophoretic injection of dopamine (DA) into the arcuate region of the hypothalamus on single unit activity and the plasma and pituitary levels of luteinizing hormone (LH) was investigated in rats in various stages of the estrous cycle. No significant differences in the number of neurons responding by activation or inhibition or not responding to DA could be observed in the course of the estrous cycle. However, in the first half of the day of proestrus (P) a significant increase in the number of neurons responding by activation to microiontophoretic injection of DA was observed. In all stages of the estrous cycle except the second half of P the activation response of the neurons was coupled with elevation of the plasma LH level. A significant rise in the LH level in the pituitary in response to microiontophoretic injection of DA into the hypothalamic arcuate region was observed only in stage diestrus 2.

KEY WORDS: Hypothalamus; neurons; dopamine; microiontophoresis; luteinizing hormone.

The arcuate region of the hypothalamus (ARC), the tonic center of regulation of pituitary gonadotropic function, must be regarded as a component of the neuroendocrine system in which the action of neuromediators is switched to the corresponding hypothalamic releasing factors. It has been shown that many dopamine terminals originating from higher levels of the CNS are represented in this region [2]. Dopamine (DA), as a mediator, occupies a special place in the regulation of gonadotropin releasing factors and, in particular, of luteinizing hormone releasing factor (LH-RF). Most data in the literature indicate its activating action on the liberation of pituitary luteinizing hormone (LH), mediated through LH-RF [7, 12].

This paper describes a combined study of the sensitivity of the neurons to microiontophoretic injections of DA and subsequent changes in the plasma and pituitary LH levels in rats at different stages of the estrous cycle.

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