KINETICS OF SODIUM CHANNEL INACTIVATION IN THE FROG RANVIER NODE

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The kinetics of inactivation of the sodium channels was studied by the voltage clamp method in experiments on the nodes of Ranvier in frogs. The measure of inactivation chosen was the maximal value of the first derivative of the sodium current developing in response to a test stimulus. In response to a stepwise change of potential of the membrane inactivation was shown to develop after an initial delay, which decreased with an increase in the steepness of rise of the sodium conductance. The results do not accord with the view that inactivation is an independent process.

When they created their formal model to describe changes in sodium conductance, for simplicity Hodgkin and Huxley [3] assumed that the processes of activation and inactivation are independent. It was later shown [4] that inactivation in response to a stepwise change of potential toward depolarization develops without any initial delay and simultaneously with activation. This result gave evidence in support of the above hypothesis and against all those models in which inactivation depended on activation [2, 5, 6, 7]. In this experiment the measure of the proportion of the channels converted into an inactivated state by the end of the first depolarizing (conditioning) pulse, the duration of which is an experimental variable, was the peak value of the inward sodium current developing in response to the second (testing) pulse of the same amplitude. However, the peak value of the sodium current is not reached instantaneously, but after a short time. During this time the membrane remains depolarized, and the level of inactivation at the time of reaching the peak differs from that found at the end of the conditioning pulse. This fact is connected with measurement of the error, and the way in which this error can influence the form of the experimental curve cannot be allowed for without making assumptions regarding the kinetics of the inactivation process which is actually being studied.

It was natural, therefore, to try to find a measure of inactivation which could be obtained experimentally with the introduction of the smallest possible disturbance into the parameter measured. In the writer's opinion one such measure is the maximal value of the first derivative of the sodium current (I_{Na}) developing in response to a testing change of potential. In fact, I_{Na} must be proportional to the rate of activation of of the sodium channels. The latter, in turn, is proportional to the fraction of the channels which can in general be activated, represented by the term h in Hodgkin's definition [3]. At the same time, the maximal value of the derivative is reached much sooner than the peak value of the current (in the node at 22°C after approximately 50 and 200 msec, respectively, when $E = -15$ mV). This paper describes a study of the kinetics of the inactivation process using this measure.

EXPERIMENTAL METHOD

Experiments were carried out on isolated fibers of frogs (Rana temporaria and Rana ridibunda), using the voltage clamp method. The apparatus for voltage clamping was built to the previous design [1] and could maintain the potential at an assigned level with a transition process less than 50 μsec in duration.

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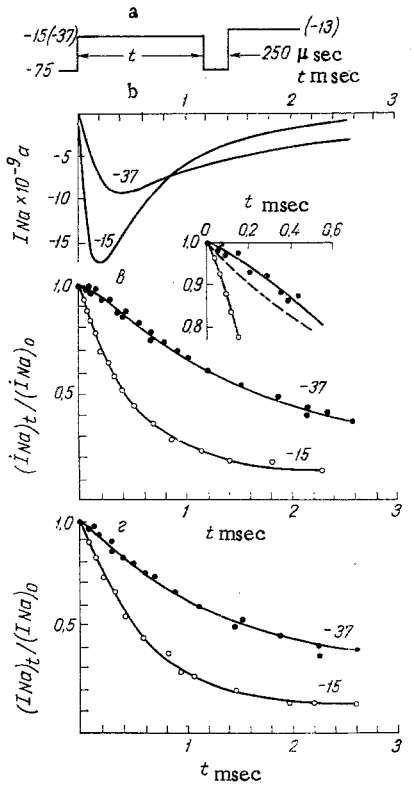

Fig. 1

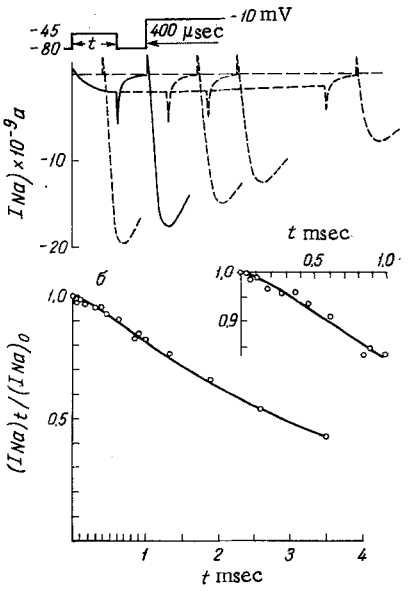

Fig. 2

Fig. 1. Development of inactivation measured by maximal values of derivative and peak value of sodium current: a) scheme of experiment. Conditioning stimulus (duration is an experimental variable t) is followed after 250 μsec by the testing stimulus (numbers show absolute values of potential in millivolts). Peak value (I_{Na}) and maximal value of derivative ($\dot{I}_{Na}$) of sodium current developing in response to testing stimulus were measured; b) sodium current during action of conditioning stimulus; c) curve showing $(\dot{I}_{Na})_t/(\dot{I}_{Na})_o$ as a function of t for two values of conditioning stimulus (−37 and −15 mV). The inset shows the initial portion of the curves on a larger scale (the broken line marks the course of the change in inactivation calculated for the same conditions from the Frankenhaeuser–Huxley equations); d) curves of $(I_{Na})t/(I_{Na})_o$ as a function of t for the same values of the conditioning stimulus. Fiber 18.5.72, temperature 20°C.

Fig. 2. Development of inactivation measured by peak values of sodium current (I_{Na}): a) parameters of stimuli (absolute values of membrane potential in millivolts and duration of intertrial interval in microseconds are shown) and ionic current ($I_{Na}+I_L$) developing under the influence of the conditioning stimulus, in the intertrial interval and during the action of the testing stimulus. Broken line shows currents for several other values of t. (Time scale the same as in b); b) curve of $(I_{Na})t/(I_{Na})_o$ as a function of t. Inset shows initial portion of curve on a larger scale. Fiber 21.1.72, temperature 14°C.

Ringer's solution of ordinary composition, pH 7.2, containing 10 mM tetraethylammonium (TEA) to block the potassium channels, was used in the experiments. All experiments were carried out at room temperature. The experimental scheme was exactly the same as in [4], except that in addition to I_{Na} the maximal value of $\dot{I}_{Na}$ was measured. The potential of the testing stimulus was chosen to be a little higher than that corresponding to the maximal amplitude of the sodium current, while the duration of the interval between the testing and conditioning stimuli was so calculated that during this time the level of activation (the variable m in Hodgkin's description) could return to its initial value while the level of inactivation changed

negligibly. In the present experiment this interval was 250-400 sec, or approximately equivalent to $4\tau_m$ and less than 0.1 τ_r for the resting potential (−75 mV). The derivative of the current was obtained by means of a differentiating circuit with a time constant of 10 μsec (100 kΩ, 100 pF). Before amplification, the $\dot{I}_{Na}$ signal was freed from high-frequency noise by means of a filter with a transmission band of 10 kHz. No corrections for the change in h during the intertrial interval were introduced.

EXPERIMENTAL RESULTS AND DISCUSSION

The relationship obtained experimentally between $\dot{I}_{Na}$ ($\dot{I}_{Na}$ before the beginning of conditioning depolarization was taken as unity) and the duration of the conditioning pulse at two of its values (E = −37 mV and E = −15 mV) is shown in Fig. 1. At E = −37 mV there was some delay in the fall of $\dot{I}_{Na}$, as is clearly seen when the experimental curve is compared with that calculated for the same conditions by the Frankenhaeuser–Huxley equations. This statement must be qualified by saying that no delay in the complete sense of this term existed. It will be remembered here that the steepness of the curve increased initially and then gradually decreased. By delay is meant the portion of the curve before the point of maximal steepness. For high values of the depolarizing stimulus in response to which the sodium current increased more steeply, delay in the development of inactivation was significantly reduced or even became almost indistinguishable (see the curves for E = −15 mV). It must be noted that during weak conditioning depolarization (from −60 to −45 mV) delay in the development of inactivation also was discovered from the peak values of $\dot{I}_{Na}$ (Fig. 2). Let us assess the possible experimental errors. The error connected with deviation of the recorded potential from a rectangular step during the transition period can be allowed for by discarding an initial portion of the experimental curve equal in duration to the transition process τ. In the experiments illustrated in Figs. 1 and 2, the values of τ were 35 and 40 μsec, respectively. The error ΔV – the deviation of the actual potential difference on the membrane from that recorded, arising because of the presence of stray capacitance between the points E and C (the output and input of the system) – can be estimated on grounds that the time constant of the capacitance coupling $\tau_{ED} = CR_{ED}$ in the present circumstances was below 0.4 μsec (C < 0.01 pF, by direct measurements; the resistance of the axoplasm in the internodal portion R_{ED} was taken to be 40 mΩ). In that case, in the experiment shown in Fig. 1, for E = −37 mV, when the maximal value $\dot{V}$ at point E was 2×10^3 V/sec, $\Delta V = V\tau_{EC}$ will be smaller than 1 mV. Correspondingly, the possible relative change in the time constant of inactivation $\Delta\tau_h/\tau_h$ will be less than 5%. In the experiment, however, the steepness of the curve in the initial portion (approximately equal to the reciprocal of the effective τ_h) changed by 1.5 times. In the experiment shown in Fig. 2, ΔV was of the order of 0.1 mV ($\dot{V} = 3 \times 10^2$ V/sec), which is small enough to be disregarded.

This investigation showed that during membrane depolarization the inactivation process develops after an initial delay, which is reduced with an increase in the depolarization shift and, consequently, with an increase in the steepness of rise of the sodium conductance. This conclusion agrees with the results described in [5],* the authors of which found delay in the development of inactivation in the giant axon of the worm Myxicola from the peak values of I_{Na}. They cite unpublished data of Armstrong, who detected the same delay in giant axons of the Chilean squid. If the possibility of a formal explanation of the delay by raising the variable h to a certain power is rejected, it must evidently be assumed that either the inactivation process itself is complex and must be described by a second-order differential equation, or it is not independent. If the second explanation is correct, correlation between the duration and steepness of rise of the sodium conductants suggests that a connection exists between the process of inactivation and the process of activation: the higher the activation, the more rapidly inactivation takes place.

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*This paper [5] was published after the present investigation was completed.