EFFECT OF PROCAINE AND CALCIUM IONS ON SLOW SODIUM INACTIVATION IN THE FROG RANVIER NODE MEMBRANE

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The effect of procaine and Ca^{++} ions on slow sodium inactivation (SSI) was studied by the voltage clamp method in nodes of Ranvier of the frog. The results obtained with procaine confirm the earlier hypothesis that only those sodium channels which were initially inactivated by a fast mechanism pass into a state of slow inactivation. Under the influence of procaine SSI also develops in a potassium-free solution; this conflicts with the view that SSI is based on the accumulation of potassium in the premembranous space during prolonged depolarization. The addition of Ca^{++} ions to the solution leads to partial abolition of SSI. The effect of Ca^{++} cannot be reduced to a change in the surface charge on the membrane. It is postulated that Ca^{++} ions displace K^+ or procaine ions from those sites on the membrane which play an important role in the mechanism of SSI development.

A previous investigation [2] showed that besides fast sodium inactivation [12, 17] in the node of Ranvier of frog nerve fibers there is also a process of slow sodium inactivation (SSI) with a time constant (τ_s) of the order of hundreds of milliseconds [7, 8]. The curve expressing the proportion of sodium channels free from SSI (S_∞) as a function of potential E is similar in shape to the curve of stationary fast inactivation ($h_\infty - E$). However, whereas h_∞ tends toward zero at high values of E, S_∞ in the same region of values of E becomes stabilized at a constant level (S_∞^{min}) that differs from zero. The value of S_∞^{min} is highly dependent on the external K^+ ion concentration ($[K^+]_0$), for SSI does not develop in potassium-free solution. At $[K]_0$ = 2.5 mM S_∞^{min} is 0.75-0.8, and it falls to 0.35 at $[K]_0$ = 50 mM. Unlike τ_h, the value of τ_s is independent of potential. With an increase in $[K]_0$ from 2.5 to 25 mM the value of τ_s is approximately halved (from 170 to 80 msec).

The effect of procaine and Ca^{++} ions on SSI was studied. This investigation was carried out because of the discovery that action potential generation is restored in the procainized node of Ranvier during hyperpolarization of the membrane and an increase in the external Ca^{++} ion concentration [3-5].

EXPERIMENTAL METHOD

Experiments were carried out by the voltage clamp method on nodes of Ranvier of Rana ridibunda [9, 11, 19]. The potassium current was blocked by tetraethylammonium chloride (10 mM). To abolish the initial SSI, the membrane potential of the node was maintained at high negative values of E (from −90 to −100 mV).

The volt-ampere characteristic curves of the node were measured at each change of the solution (Fig. 1). To investigate SSI a depolarizing step of varied amplitude and duration was applied and short testing

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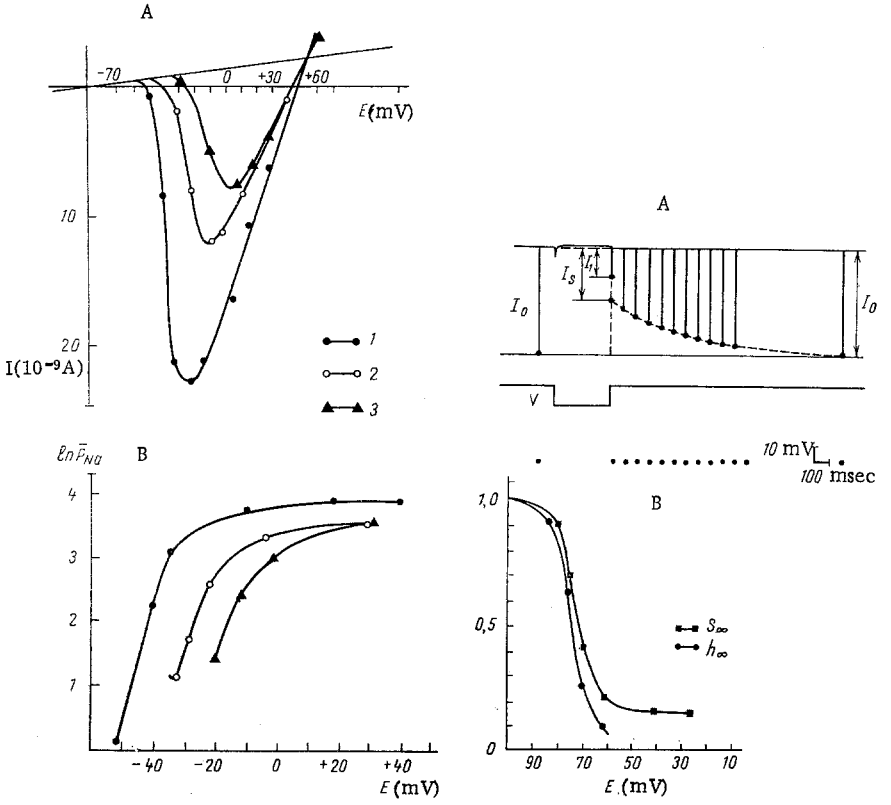

Fig. 1

Fig. 2

Fig. 1. Peak values of I_{Na} (A) and $\bar{P}_{Na}$ (B) as functions of potential E in node of Ranvier in normal Ringer's solution (1), after addition of $1.5 \cdot 10^{-5}$ g/ml procaine to the solution (2), and after combined addition of procaine and 20 mM Ca (3). Values of $\bar{P}_{Na}$ in conventional units. Procaine clearly diminishes the maximal sodium permeability and shifts the $P_{Na} - E$ curve. An increase in $[Ca]_0$ causes a further shift of the curve but does not change the maximum of P_{Na}.

Fig. 2. Slow sodium inactivation in procainized node of Ranvier: A) method of measurement (for explanation see text); B) curves of fast ($h_\infty - E$) and slow ($S_\infty - E$) sodium inactivation in node of Ranvier treated with procaine ($2.5 \cdot 10^{-5}$ g/ml).

stimuli were applied at various time intervals (from 0 to 1-2 sec) after its end. The peak value of the inward sodium current (I^p_{Na}) to a testing stimulus applied at the moment of the discontinuation of the conditioning depolarization (I_1), expressed as a ratio of the original value I_0 obtained in response to the same stimulus but without preliminary depolarization, was used as the ordinary measure of h.

EXPERIMENTAL RESULTS

In potassium-free Ringer's solution 50 msec after the end of the depolarization lasting 1 sec the values of I^p_{Na} to the testing stimuli were completely restored to the I_0 level. On the addition of procaine ($2.5 \cdot 10^{-5}$ g/ml) to the solution, however, the picture illustrated in Fig. 2A was observed. Clearly I^p_{Na} to the second testing stimulus was considerably less than I_0, and it increased to that level approximately exponentially with a time constant of about 700 msec. This increase in I^p_{Na} was connected with the gradual abolition of SSI developing during the prolonged depolarization. In other experiments the time constant of emergence from SSI varied from 400 to 890 msec. The values of I_s, obtained by extrapolation of the peak I_{Na} values to the end of the conditioning depolarization (Fig. 2A), expressed as a ratio of I_0 was taken as the measure of the proportion (S) of sodium channels free from SSI. The variable S decreases with an increase in length of the conditioning step. This "entry" into SSI took place with two time constants: an initial fast (τ'_s of the order of 55-80 msec) and a subsequent slow constant (τ''_s 200-300 msec). Both time constants

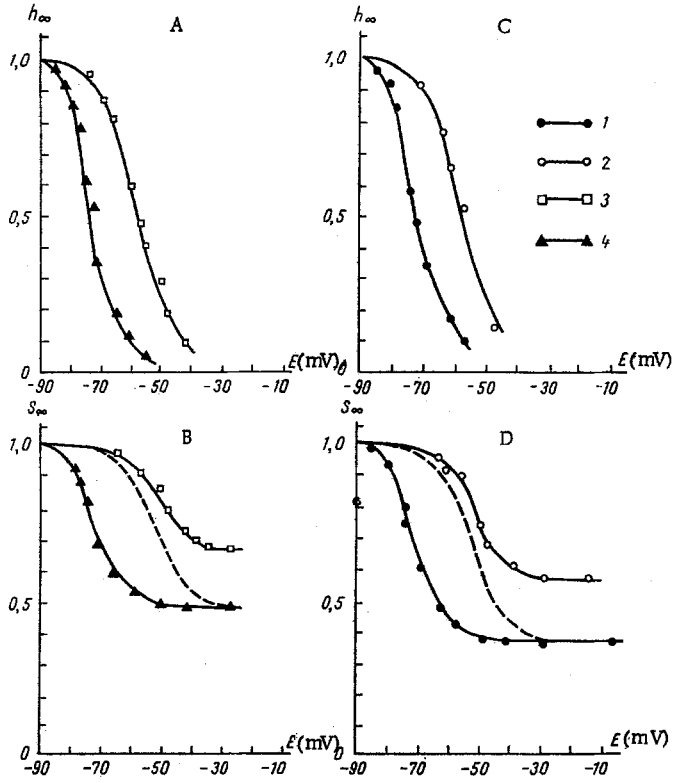

Fig. 3. Effect of Ca^{++} ions on curves of fast and slow inactivation in node of Ranvier: A, B) Ringer's solution with K concentration increased to 10 mM; C, D) Ringer's solution with $1.5 \cdot 10^{-5}$ g/ml procaine. Value of $[Ca]_0$ increased from 2 to 20 mM. 1) Procaine $1.5 \cdot 10^{-5}$ g/ml; 2) procaine + 20 mM Ca^{++}; 3) 20 mM Ca^{++}; 4) 10 mM K^+.

were independent of potential. Curves of $h_\infty - E$ and $S_\infty - E$ plots in a node treated with $2.5 \cdot 10^{-5}$ g/ml procaine are shown in Fig. 2B. In the different experiments S_∞^{min} varied from 0.02 to 0.38. Even in 50 mM K, S_∞^{min} varied from 0.02 to 0.38. The value of S_∞^{min} at the resting potential (E = −75 mV) varied in different experiments from 0.15 to 0.76. This means that in the presence of procaine ($1.5\text{-}5 \cdot 10^{-5}$ g/ml), from 85 to 24% of the sodium channels may be in a state of SSI. The maximal sodium permeability $\bar{P}_{Na}$ fell under these conditions by 30-61% of its initial value.

An increase in the concentration of Ca^{++} ions from 2 to 20 mM in a solution containing procaine or an excess of K shifted the $h_\infty - E$ and $S_\infty - E$ curves toward E_{Na} and at the same time significantly increased the level of S_∞^{min} (Fig. 3).

Some important results can be deduced from these observations.

The results of the experiments with procaine agree with the earlier hypothesis [2] that only those sodium channels which were initially inactivated by the fast mechanism pass into a state of SSI. This conclusion is supported by the independence of the time constants of SSI of the potential E and the fact that S_∞ stops falling in the region of values of E in which h_∞ tends toward zero.

The onset of SSI in the potassium-free solution under the influence of procaine conflicts with the view that SSI is based on the accumulation of K^+ ions in the premembranous space during prolonged depolarization [7].

The effect of Ca^{++} ions cannot be reduced entirely to their influence on the surface potential of the membrane [1, 15, 16]; the increase in the level of S_∞^{min} evidently indicates that Ca^{++} ions displace K^+ ions or procaine from sites on the membrane which play the key role in the mechanism of development of SSI. It is tempting to postulate that in the resting state of the membrane these sites are occupied by Ca^{++} ions and that closing the "inactivated h gates" creates the conditions under which Ca can be displaced from those

sites by certain other cations in solution, such as K^+ or procaine. If such an ion-exchange reaction develops, the "slow inactivating S gates" will also be closed. The existence of competitive relationships between Ca and procaine for some structural elements of the membrane has been pointed out elsewhere [6, 10, 18].

The results do not support the view that the blocking action of procaine is due entirely to a decrease in $\bar{P}_{Na}$ [13, 14, 21]. A significant contribution to the effect of procaine is made by sodium inactivation. However, for a correct assessment of this contribution it is not sufficient to use 50-msec hyperpolarizing prepulses only, as is usually done to abolish inactivation – the duration of these prepulses should be not less than 1 sec. There are serious grounds for considering that many observations on changes in $\bar{P}_{Na}$ (or $\bar{g}_{Na}$) described in the literature in various altered states of the nerve fiber require verification because no allowance was made for SSI in those investigations [13, 14].

LITERATURE CITED

1. G. N. Mozhaeva and A. P. Naumov, Biofizika, 17, 801 (1972).
2. É. M. Peganov, B. I. Khodorov, and L. D. Shishkova, Byull. Éksperim. Biol. i Med., No. 9, 15 (1973).
3. B. I. Khodorov, Uspekhi Sovr. Biol., 54, 333 (1962).
4. B. I. Khodorov and V. I. Belyaev, Biofizika, 10, 625 (1965).
5. B. I. Khodorov and V. I. Belyaev, Biofizika, 12, 855 (1967).
6. Yu. D. Kholodova and Z. A. Sorokina, in: The Biophysics of Membranes [in Russian], Part 1, Kaunas (1971), p. 768.
7. W. Adelman and Y. Palti, J. Gen. Physiol., 54, 589 (1969); 53, 685 (1969).
8. K. Cole, cited by Adelman and Palti.
9. F. Dodge and B. Frankenhaeuser, J. Physiol. (London), 143, 76 (1958).
10. M. Feinstein, J. Gen. Physiol., 48, 357 (1964).
11. B. Frankenhaeuser and A. Hodgkin, J. Physiol. (London), 137, 218 (1957).
12. B. Frankenhaeuser and A. Huxley, J. Physiol. (London), 171, 302 (1964).
13. B. Hille, Nature, 210, 1220 (1966).
14. B. Hille, Pharmacological Analysis of the Ionic Channels of Nerve, New York (1967), p. 177.
15. B. Hille, J. Gen. Physiol., 51, 221 (1968).
16. B. Hille, J. Gen. Physiol., 58, 599 (1971).
17. A. Hodgkin and A. Huxley, J. Physiol. (London), 117, 500 (1952).
18. N. Kwant and P. Seeman, Biochim. Biophys. Acta, 193, 338 (1969).
19. T. Narahashi, J. Cell. Physiol., 64, 73 (1964).
20. W. Nonner, Pflug. Arch. Ges. Physiol., 309, 176 (1969).
21. T. Taylor, Am. J. Physiol., 196, 1071 (1959).