SLOW SODIUM INACTIVATION IN THE RANVIER NODE MEMBRANE

ROLE OF EXTERNAL POTASSIUM

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A process of slow sodium inactivation (SI) with time constant τ_S = 150-300 msec and dependent on external potassium was found by the voltage clamp method in single nodes of Ranvier. Unlike the fast inactivation described by the variable h, SI in the intact fiber does not develop in potassium-free medium. The value of τ_S is independent of potential and falls with a rise of $[K]_0$. The steady-state value of S (the fraction of sodium channels remaining free from SI after depolarization for 1 sec) falls with a rise of potential, but only up to a certain limiting, nonzero level of S_∞^{min} for the given $[K]_0$. With an increase in $[K]_0$ from 2.5 to 25 mM S_∞^{min} falls from 0.75 to 0.4. The fact that S_∞ reaches the level S_∞^{min} in the region of values of E where h_∞ becomes the zero suggests that only those channels which were inactivated by the "fast" mechanisms pass into a state of slow inactivation.

Recent investigations have shown that besides the fast processes described by Hodgkin and Huxley [12] in the membranes of nerve and muscle fibers (cells) there are also slow changes in ionic permeabilities characterized by time constants measured in hundreds of milliseconds, in seconds, or even in minutes. These processes include the slow sodium inactivation [3, 4, 6, 7, 13] which lies at the basis of Vvedenskii's "parabiosis" [1, 2]. So far as the writers know, quantitative studies of slow inactivation under voltage clamp conditions have so far been carried out only in experiments on squid giant axons [3, 4].

However, it is difficult to interpret the results obtained by investigators using that object because allowance must be made for the accumulation of K^+ ions in the space between the surface of the membrane undergoing prolonged depolarization and the layer of Schwann cells [9]. It was therefore decided to investigate myelinated fibers in which the membrane of the nodes is relatively exposed and, consequently, the accumulation of K^+ in the juxtamembranous space is unlikely to take place, especially if the potassium channels are blocked by tetraethylammonium.

EXPERIMENTAL METHOD

Experiments were carried out on isolated nerve fibers of Rana temporaria using the voltage clamp method [5, 8, 11]. The apparatus designed by V. V. Belyi and Z. S. Volovich fixed the membrane potential at an assigned level with a transition process of less than 50 μsec. The arrangement of the fiber in the experimental chamber and the theoretical circuit of the apparatus used are shown in Fig. 1a.

Solutions of the following composition were used in the experiments: NaCl 114 mM, $CaCl_2$ 2 mM, KCl 0 mM, 2.5 mM, and 50 mM; pH = 7.2. Since solutions containing 25 and 50 ml KCl were hypertonic, all measurements were made during the first 10 min after the solution was changed [10]. In every case the initial membrane potential was stabilized at between −90 and −100 mV, so that not only the initial fast, but

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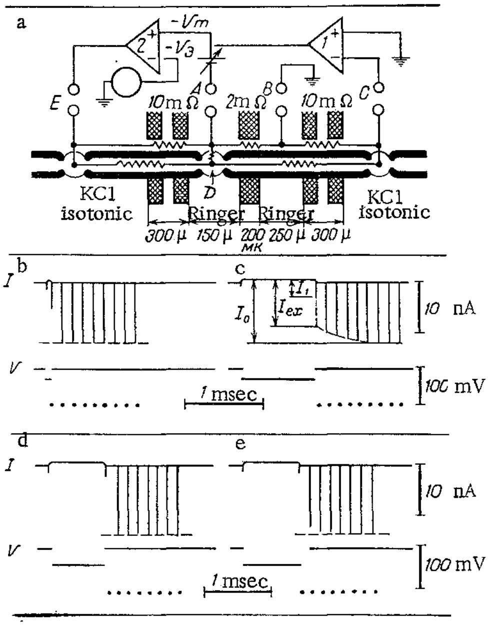

Fig. 1. Diagram of voltage clamping apparatus and experimental records showing character of change in ionic currents after end of depolarization: a) myelinated nerve fiber placed in chamber consisting of 4 compartments isolated from each other by petrolatum partitions (shaded). Note for testing kept in bath A. Both extreme partitions consist of two petrolatum columns separated by an air space. Numbers above each partition show their resistance (in MΩ). Numbers below show width of corresponding partitions and baths (in μ). Black dots mark boundary of conventionally shown equivalent electrical circuit of the preparation for a steady current. The chamber is connected by 4 salt bridges with an electronic circuit (shown above the chamber). Amplifier 1 holds the potential at point D inside the fiber at the zero level, amplifier 2 ensures that the membrane potential is equal to the assigned value; b–e) peaks of inward current developing in response to a series of testing pulses applied after removal of the conditioning depolarization. Top record shows ionic current; bottom record shows membrane potential (depolarization downward); b) node in Ringer's solution with 2.5 mM KCl, duration of conditioning pulse (t) 50 msec. Fiber 3.5.72; c) the same as in b, t = 1 sec; d) node in potassium-free solution, fiber 19.4.72; e) parameters of stimuli the same as in d; potassium-free solution replaced by solution containing 2.5 mM KCl.

also the slow inactivation was almost completely abolished. Conditioning and testing changes of potential were carried out from that initial level. In the present investigation all the depolarizing pulses used did not exceed 1 sec in duration. In this way it was hoped to eliminate the contribution of any other slower (time constant 30–200 sec) inactivation process independent of external potassium [3, 4, 7]. In all cases the potassium channels were blocked by the addition of 10 mM tetraethylammonium chloride (TEA) to the Ringer's solution. The experiments were carried out at 20–22°C.

EXPERIMENTAL RESULTS AND DISCUSSION

The order in which the experiment was carried out was as follows: the leak current was measured by means of a hyperpolarizing pulse, after which the relationship between the sodium current I_{Na} and the amplitude of the depolarizing step was studied; the stimulus evoking the greatest peak of inward I_{Na} was used as the testing stimulus. Conditioning depolarization 50–1000 msec in duration was then applied in 1 step, and immediately after it, without any delay, a series of 1-msec testing stimuli was applied at intervals of 100–150 msec. The peak value of the sodium current developing in response to the first testing pulse of the series I_1, divided by the amplitude of the current (I_0) arising in response to the same pulse without preliminary depolarization, is the usual measure of the fraction (h) of the channels not becoming inactivated during the action of the conditioning pulse. With the interval between pulses chosen, the fast processes described

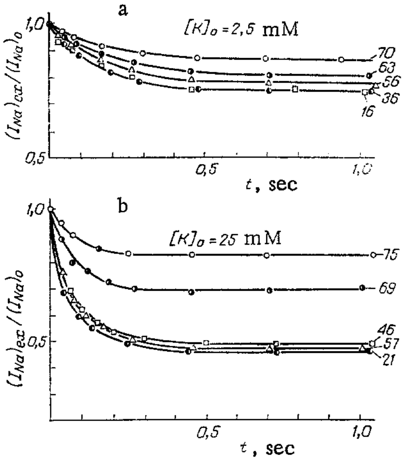

Fig. 2

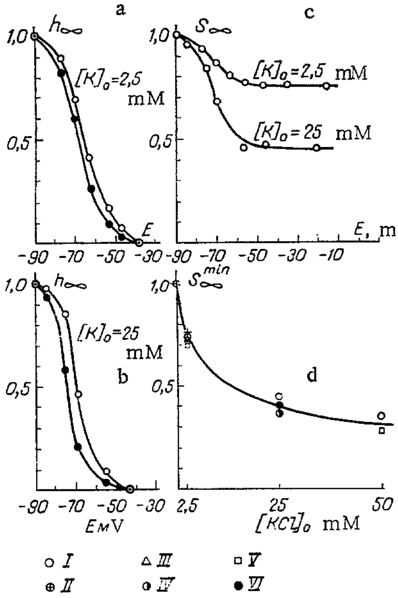

Fig. 3

Fig. 2. S = I_{ex}/I_0 as a function of duration of conditioning depolarizing pulse. Numbers by each curve indicate absolute value of potential (E) of conditioning pulse. Fiber 12.4.72: a) KCl = 2.5 mM; b) KCl = 25 mM.

Fig. 3. Steady-state values of h and S: a, b) h_∞ as a function of E for 2 values of $[K]_0$. Empty circles denote values of h_∞ for a conditioning pulse of 50 msec, filled circles for a pulse of 1 sec in duration; a) KCl = 2.5 mM; b) KCl = 25 mM. Fiber 12.4.72; c) S_∞ as a function of potential for 2 values of $[K]_0$ (shown next to each curve). Fiber 12.4.72; d) S_∞^{min} as a function of external concentration $[KCl]_0$. Different figures correspond to different fibers: I) 12.4.72; II) 19.4.72; III) 13.6.72; IV) 14.6.72; V) 15.6.72; VI) 19.6.72.

by the Hodgkin–Huxley equations [12] would already have ended by the time of application of the second testing pulse. As Fig. 1c shows, after a conditioning pulse of 1 sec (by contrast with that observed after depolarization for 50 msec, Fig. 1b), I_{Na} to the second testing pulse was much less than the initial I_0 and rose to that level on an approximately exponential curve with a time constant of about 300 msec. Presumably during the prolonged depolarization some of the sodium channels passed into a new nonconducting, inactivated state from which they slowly emerged during repolarization. The value of I_{ex}, obtained by extrapolation of the peak values of the currents by the end of the conditioning pulse, divided by I_0 was taken as a measure of the fraction S of the sodium channels which were unable to pass into the "slow" inactivated state during the preliminary depolarization (S = I_{ex}/I_0). In a potassium-free external medium virtually no slow inactivation developed, but it appeared when the potassium-free solution was replaced by a solution containing 2.5 mM KCl (Fig. 1b,c). The relationship between S and the duration and strength of the conditioning depolarization for 2 volumes of $[K]_0$ is shown in Fig. 2. For a given value of $[K]_0$ the process developed exponentially with a time constant τ_S independent of potential. With an increase in $[K]_0$ from 2.5 to 25 mM τ_S was reduced by about half (in this case from 170 to 80 msec). Curves showing h_∞ and S_∞ as functions of potential (E) are given in Figs. 3a-c. For each value of E, h_∞ measured with conditioning depolarization lasting 1 sec was less than that for depolarization for 50 msec; the difference was greater for $[K]_0$ = 2.5 mM than for $[K]_0$ = 2.5 mM. The steady-state level S (see Fig. 3c) fell with an increase in E, but only up to a certain limiting value S_∞^{min}; this limiting level itself fell with an increase in $[K]_0$ (Fig. 3d).

The results described above agree well with those obtained [3, 4] on the squid giant axon. However, the method of investigation used in the present experiments (the initial value of E clamped at the same level for different values of $[K]_0$) made it possible to determine several other facts which indicate that the phenomenon can be interpreted within the framework of a mechanism which differs in principle from that discussed in the investigations cited above [3,4]. The fact that no slow inactivation was detected in a potassium-free medium indicates simultaneously both the need for K^+ ions in order for this process to develop, and also that even if accumulation of K^+ took place it in any event determined neither the kinetics nor the steady-state level of slow inactivation, as the authors cited above claimed [3,4]. The fact that τ_S is independent of E, whereas S_∞ was characteristically dependent on potential indicates a link between slow inactivation and some fast potential-dependent process. It is clear from Fig. 3 that the steady-state level of slow inactivation S_∞ no longer depends on potential in that region of values of E where h_∞ becomes zero. This evidently indicates that those, and only those channels which were inactivated by the fast mechanism described by the variable h pass into the slow inactivated state. Under these circumstances potassium ions are necessary for this change of state to take place. The simplest scheme in qualitative agreement with these facts is that one or more K^+ ions are bound with a certain center in the inactivated channel and give rise to a new state of Na nonconduction. If, however, allowance is made for the fact that the external potassium influences the steady-state level and the time constant of fast inactivation [3,4], the question of whether the effect of potassium on fast and slow inactivation can be explained from identical standpoints must be considered. The answer to this question, however, will be obtained only after further and more detailed investigation of the effect of potassium on fast and slow inactivation simultaneously.

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