POSTTETANIC HYPERPOLARIZATION OF THE ISOLATED NERVE FIBER OF Rana ridibunda

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The parameters of posttetanic hyperpolarization (PTH) of a single node of Ranvier are unchanged by an increase of duration of stimulation of over 0.1 sec. PTH was not sensitive to strophanthin but was abolished by tetraethylammonium. The membrane resistance of the Ranvier node, measured by the amplitude of anodic shocks, was reduced by 50% during PTH. It is concluded that PTH of isolated nerve fibers is due to preservation of increased potassium permeability during and after the end of tetanus. An increase in the duration of tetanization does not cause the appearance of PTH connected with active ion transport.

Key words: posttetanic hyperpolarization; single node of Ranvier; isolated nerve fiber.

In 1961, Meves [12] showed that the duration of posttetanic hyperpolarization (PTH) of the isolated nerve fiber does not exceed 50-80 msec. At the same time, in recordings from the whole nerve, PTH is known to last several tens of seconds or even minutes [2, 6, 7, 9, 11]. The duration of PTH of the whole nerve depends very largely on the frequency and, particularly, the duration of preceding stimulation [7, 9, 11]. Böhm and Straub [7], for instance, showed that during wide variation of stimulus duration the duration of PTH of the sciatic nerve in frogs and rabbits varied from tens of milliseconds to 1-1.5 min. On this basis they postulated that the short duration of PTH of the isolated nerve fiber described by Meves [12] could be explained by the fact that he used only very brief stimulation (10-120 msec) in his experiments.

This investigation was carried out in order to test the validity of Böhm and Straub's hypothesis and also to continue the study of PTH of isolated nerve fibers.

EXPERIMENTAL METHOD

Over 30 experiments were carried out on single nodes of Ranvier of myelinated nerve fibers of Rana ridibunda by a somewhat modified Kato–Tasaki technique [5]. A fiber 8-12 μ in diameter and 5-6 mm long, isolated from the sciatic nerve, was placed in a special chamber with two air insulating bridges (Fig. 1A). The node to be studied (N_2) was placed in the middle section, consisting of a plastic gutter 1 mm wide. This gutter was filled with Ringer's solution or with solutions of the test substances. Two other nodes N_1 and N_3, with the adjacent parts of the trunk, were placed in the lateral divisions on rounded and polished slides. The activity of nodes N_1 and N_3 was inhibited by 0.2% procaine solution.

The node was stimulated and action potentials recorded through nonpolarizing $Zn-ZnSO_4$ electrodes. The Verigo–Khodorov scheme [3] was used for simultaneous polarization and stimulation of the node. Action potentials were recorded by means of a cathode follower (R_{input} 20 GΩ) with positive feedback, and a dc amplifier.

EXPERIMENTAL RESULTS AND DISCUSSION

A single spike of the isolated nerve fiber was accompanied usually by slight after-depolarization lasting a few milliseconds (time constant 1.2 msec). During repetitive stimulation of the single fiber the resting potential shifted a little toward hyperpolarization (Fig. 1B). This hyperpolarization increased in

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intensity with an increase in the frequency of stimulation, and at 300 stimuli/sec it reached 1.4±0.1 mV. The time constant of its rise was 30.4±1.4 msec. After the end of repetitive stimulation hyperpolarization of the nerve fiber membrane increased to 3.2±0.4 mV. The duration of PTH of the fiber in ordinary Ringer's solution, after moderately frequent stimulation with a duration of 1 sec, varied within narrow limits (60-120 msec). The time constant of rise of the PTH was 4±0.02 msec, and the decay constant 34±1 msec.

PTH of the isolated nerve fiber increased considerably if potassium ions were removed from the surrounding solution [12]. In that case the after-hyperpolarization was observed even after the single action potential (Fig. 2A: 6), its amplitude was 2.7±0.18 mV, and its time constant of rise and fall was 0.26±0.01 and 2.4±0.04 msec, respectively. If two stimuli separated by a fairly long interval were applied to the nerve fiber (Fig. 2A: 1) the after-hyperpolarization after the second response had a similar time course to that after the first. With shorter intervals (Fig. 2A: 2, 3) the hyperpolarization after the second action potential was a little increased. With even shorter intervals, when the second stimulus became subliminal, the after-hyperpolarization was interrupted by the local response (Fig. 2A: 4, 5), then recovered again, although it did not reach its initial value, but only roughly the level that it would have had at that time in the absence of the second stimulus. Admittedly, the total duration of after-hyperpolarization in that case was a little increased.

During repetitive stimulation of the isolated nerve fiber kept in potassium-free solution, the hyperpolarization developing after a single action potential subsequently increased. The time constant of rise of after-hyperpolarization during repetitive stimulation was approximately equal in value to the time constant of rise of hyperpolarization developing during repetitive stimulation of the nerve fiber in normal Ringer's solution. In potassium-free medium, after the end of stimulation (50-500 stimuli/sec) PTH of the fiber always developed, but it exceeded only by 0.5-1.5 mV the corresponding hyperpolarization of the nerve fiber kept in Ringer's solution with a normal potassium concentration (2.5 mM).

Within certain limits PTH depended on stimulus duration (Fig. 2B). However, an increase in stimulus duration beyond 0.1 sec had no further effect on either the magnitude or the duration of PTH. After stimulation for 10 sec PTH remained about the same as after stimulation for 0.1 sec.

The hypothesis of Böhm and Straub that PTH of the isolated nerve fiber may have the same duration after prolonged stimulation as when recorded from the whole nerve was thus not confirmed. Under all conditions of stimulation PTH of the isolated nerve fiber remained 3-4 orders of magnitude shorter than PTH of the whole nerve.

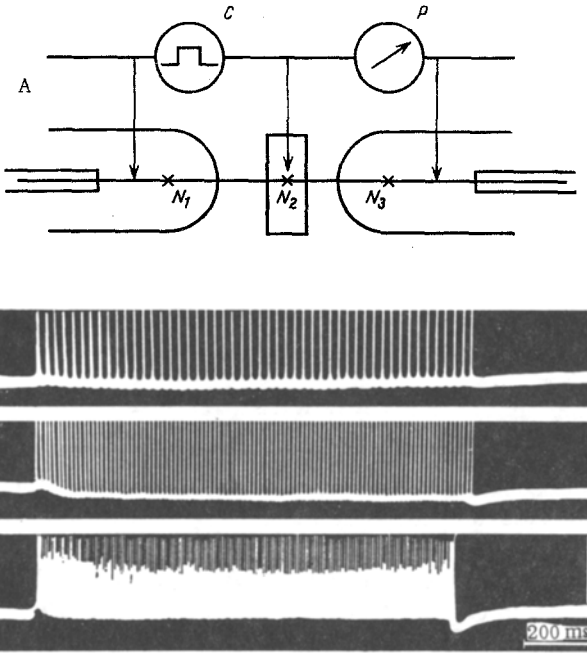

Fig. 1. PTH of nerve fiber: A) scheme of stimulation and recording action potentials; B) combined and after-potentials of isolated nerve fiber arising in response to repetitive stimulation at frequencies of 50, 100, and 300 stimuli/sec.

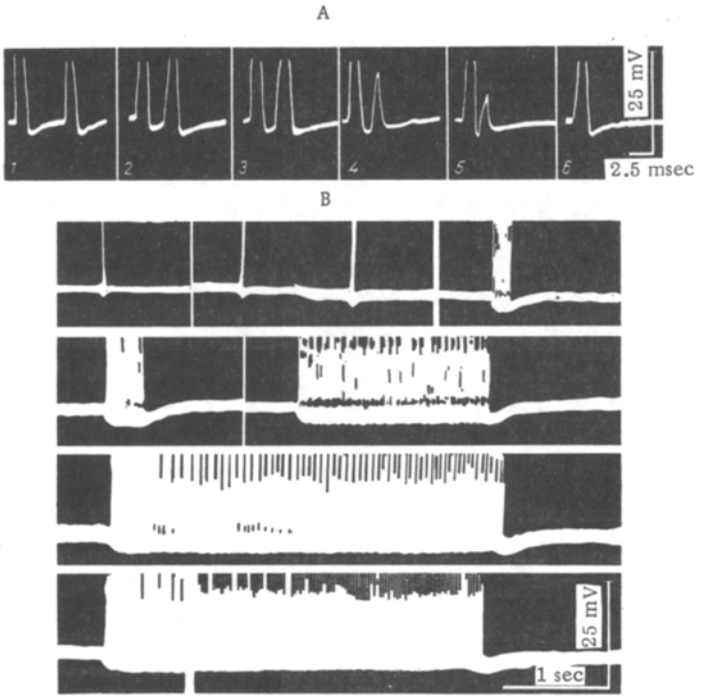

Fig. 2. Potentials of single nerve fiber in potassium-free Ringer's solution: A) after-hyperpolarization after single (6) and paired (1-5) stimulation; B) posttetanic hyperpolarization of isolated nerve fiber arising in response to repetitive (300 stimuli/sec) stimulation of varied duration. Only beginning and end of bottom record shown (duration of stimulation 10 sec), middle omitted.

The resistance of the nerve fiber membrane, measured during PTH from the change in amplitude of electrotonic shock (Fig. 3A), was reduced by approximately 50%. This indicates that during PTH a considerable ionic current flows through the nerve fiber membrane. In magnitude it was much greater than the leak current, for despite the great decrease in membrane resistance, it produced not only complete repolarization of the membrane but even hyperpolarization.

Two possible explanations of the origin of this current could be suggested. It might be either the current of active ion transport, which, as has been suggested [1, 3, 6-8, 10], lies at the basis of the long PTH of fibers of the whole nerve and also of bodies of nerve cells [1, 14], or the passive outward potassium current, connected with the after-increase in potassium conductance of the membrane. If the first suggestion is correct, PTH of the isolated nerve fiber ought to be abolished by strophanthin, a specific inhibitor of active transport. Experiments showed, however, that PTH of the isolated fiber was completely resistant to strophanthin (Fig. 3B).

Small changes in polarization of nerve fibers are known to have virtually no effect on the intensity of active ion transport through their membrane. Only a small increase in PTH of the nerve fibers ought to be expected during hyperpolarization of the membrane [6] as a result of the increase in its resistance and the increase in the quantity of sodium entering during each nervous impulse. The records given in Fig. 3C, parts 1-3, indicate the contrary. In a single node after-hyperpolarization both after a single spike and after a spike train was partly or completely abolished in the case of an anelectrotonic shift of the initial membrane potential level and slightly increased in intensity in the case of catelectrotonic membrane depolarization. These results evidently contradict the attempt to explain PTH of the isolated nerve fibers by an increase in electrogenic ion transport. However, they agree well with the hypothesis that PTH could be the result of the preservation of increased potassium permeability at the end of tetanus. That this explanation is correct was confirmed by experiments with tetraethylammonium, which specifically blocks the membrane potassium current [15, 16]. As Fig. 3D shows, tetraethylammonium completely abolished both the after-hyperpolarization of the single action potentials and also the PTH. Taken as a whole, therefore, the facts

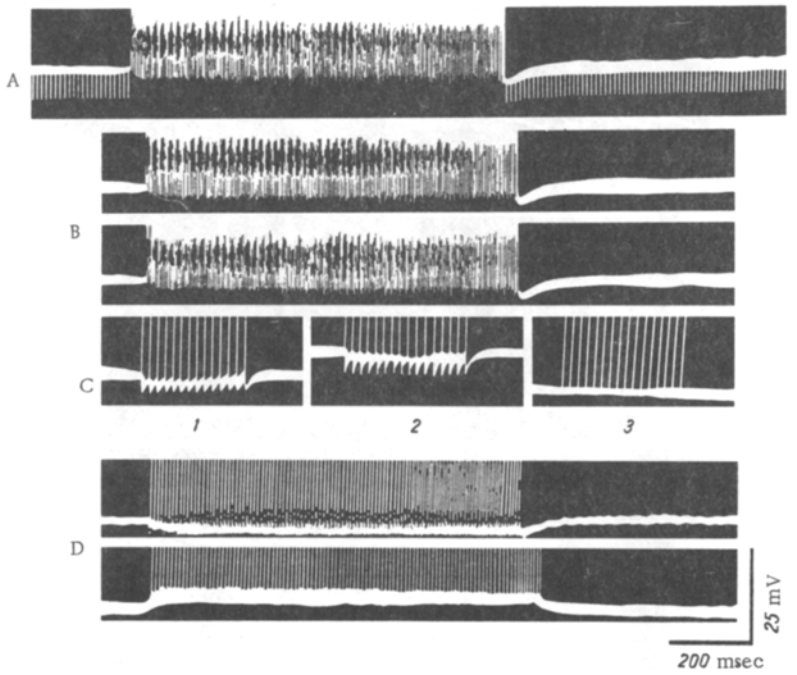

Fig. 3. PTH of single nerve fiber during the action of various factors on its membrane: A) change in amplitude of anodic shocks during posttetanic hyperpolarization of isolated nerve fiber. Frequency of stimulation 300/sec. Frequency of application of anodic shocks 50/sec; B) PTH of isolated nerve fiber before and after addition of 0.5 mM strophanthin to surrounding solution; C) after-hyperpolarization of single action potentials and PTH of fiber: 1) without polarization, 2) against background of depolarization, 3) of hyperpolarization. Frequency of stimulation 50/sec; D) abolition of after-hyperpolarization of action potentials and of PTH by addition of 10 mM tetraethylammonium to solution surrounding single nerve fiber. In all cases preparations kept in potassium-free Ringer's solution.

examined in this paper explain the origin of PTH of isolated frog nerve fibers by the preservation of increased potassium conductance for some time after the end of tetanus. This explanation agrees with the opinions of Meves [12] on this problem but is opposed to the hypothesis of Böhm and Straub [7] that PTH of isolated nerve fibers is identical in nature and similar in its time course with the PTH of the whole nerve.

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