EFFECT OF REPETITIVE STIMULATION ON AFTER-POTENTIALS OF A NERVE AFTER REMOVAL OF THE EPINEURIUM AND PERINEURIUM

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The magnitude and duration of after depolarization and hyperpolarization of the isolated frog sciatic nerve with its epineurium and perineurium intact and removed were studied during repetitive stimulation under various conditions. It is concluded from the experimental results that the outer membranes of the nerve play no essential role in the formation of the after-potentials of the nerve trunk. This is confirmed indirectly by the fact that all effects due to the great magnitude and duration of after depolarization are reproduced in nerve trunks after removal of the outer membranes: the after-period of increased excitability (phase of exaltation), the phenomenon of the tetanized single response, and the increase in amplitude of the action potentials during submaximal tetanization.

The duration of after-potentials in the intact frog nerve is measured in tens or hundreds of milliseconds, while after repetitive stimulation it is measured in seconds and minutes [2, 3, 5, 6, 12, 13, 16]. The maximal duration of the after-potentials of the isolated nerve fiber, on the other hand, does not exceed 50 msec while after a single excitation it is only a few milliseconds [17, 18].

It has accordingly been postulated [18] that the relatively long duration of the after-potentials of the isolated nerve trunk is caused by interruption of the circulation of blood in the trunk and by swelling of the Schwann cells, as a result of which during excitation there is an appreciable shift of ion concentration in the small volume of tissue fluid surrounding the nerve fibers, leading to prolonged changes in the permeability and polarization of the excitable membrane. In isolated fibers, on the other hand, according to this hypothesis, ions diffuse freely into the surrounding solution or, in intact animals, into the tissue fluid and blood, so that their concentration near the excitable membrane remains relatively constant.

However, the presence of positive after-potentials in the intact nerve with its blood supply intact was demonstrated originally by Lorente de No [16] and later by the work of Böhm and Straub [7]. The suggestion by Meves [18] that prolonged after-potentials are absent in the fibers of an intact nerve was thus not

TABLE 1. Duration of After-Depolarization and Hyperpolarization of a Nerve at Different Frequencies of Stimulation (M ± m)

Frequency of stimulation per second	Duration (in msec) of after-depolarization of nerve		Duration (in sec) of after-hyperpolarization of nerve	
	with intact membranes	with membranes removed	with intact membranes	with membranes removed
10	144±12,6	138±12,9	—	—
50	84±9,2	80±8,6	1,04±0,05	1,02±0,05
100	47±4,3	48±4,2	1,09±0,05	1,09±0,05
500	66±0,6	7±0,6	1,18±0,07	1,18±0,07

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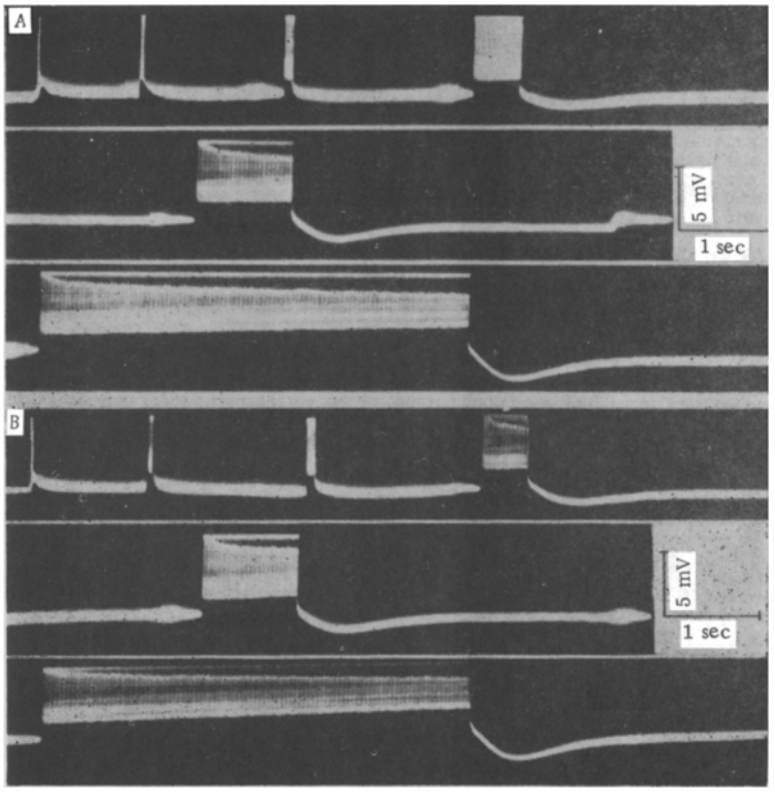

Fig. 1. Effect of repetitive stimulation on after-potentials of nerve before (A) and after (B) removal of the epineurium and perineurium. Duration of stimulation 0.01, 0.05, 0.1, 0.5, 1, and 4 sec respectively. Frequency of stimulation 300/sec.

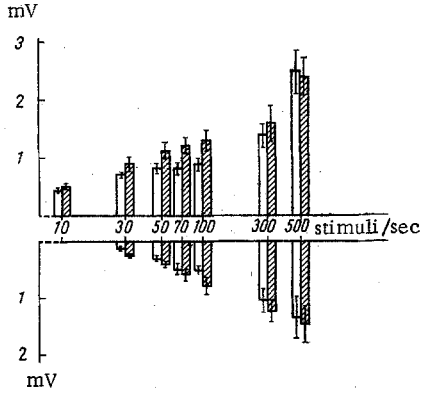

Fig. 2. Changes in maximal value of after-depolarization and hyperpolarization after stimulation of a nerve with intact (shaded columns) and removed (unshaded columns) epineurium and perineurium after stimulation at different frequencies. Abscissa, frequency of stimulation; ordinate, amplitude of after-potentials. After-depolarization shown above the abscissa, after-hyperpolarization shown below.

confirmed experimentally. In order to continue the study of after-potentials it was therefore extremely important to assess the role of the outer membranes of the nerve, which perform a barrier function and, because of their capacity and their high resistance, may affect the character of the recorded electrical response of the nerve.

The object of the present investigation was to study the effect of removal of the epineurium and perineurium on after-potentials of a nerve during repetitive stimulation at different frequencies.

EXPERIMENTAL METHOD

Experiments (more than 60) were carried out on the isolated sciatic nerves of pond frogs (Rana ridibunda) in the period from December, 1970 to March, 1971 and from November, 1971 to March, 1972, at a temperature of 18-20°C. Before the experiment the isolated nerve preparations were kept for 1-1.5 h in Ringer's solution. The membranes were removed under the MBS-2 binocular microscope: initially a longitudinal incision was made in the epineurium and perineurium by means of a sharp-pointed triangular blade, after which they were removed with relatively blunt dissection needles. The nerve was then kept for a further 20 min in Ringer's solution, the pH of which was periodically checked and maintained at 7.3.

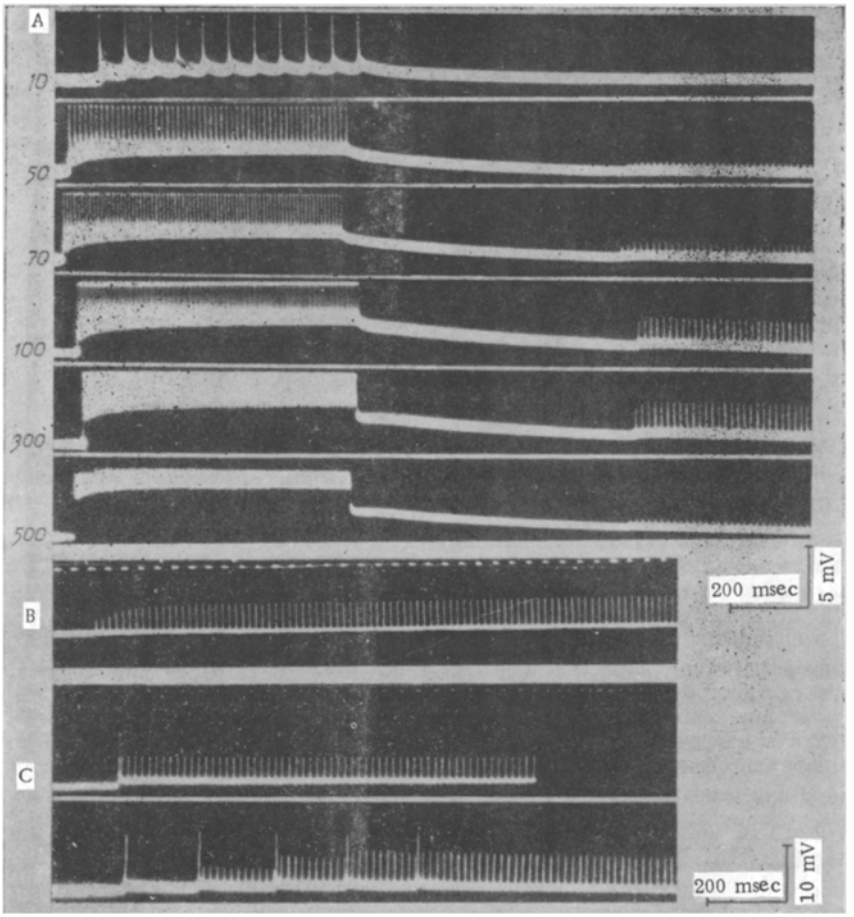

Fig. 3. Effect of frequency (numbers on left) of stimulation on magnitude and duration of after-depolarization of denuded nerve in medium with increased concentration of calcium chloride (A) and effects on nerve trunk caused by high and prolonged after-depolarization: increase in amplitude of action potentials during repetitive stimulation at submaximal strength (B) and increase in above-threshold responses after application of one or several maximal stimuli (tetanized single responses [1]) (C).

The nerve was stimulated by means of a type ES-10 electronic stimulator with radiofrequency output. The duration of each pulse was 0.1 msec. Action potentials of the nerve were recorded with calomel electrodes, with an interelectrode distance of 2.5 cm. The distal end of the nerve was killed by application of a thermocautery. Action potentials were amplified and recorded on a five-channel UFUPT-5 physiological apparatus operating under dc conditions.

EXPERIMENTAL RESULTS AND DISCUSSION

The combined action potential of a nerve kept for 1-1.5 h in Ringer's solution was accompanied by a well-marked after-depolarization, lasting 125.5 ± 9.2 msec in these experiments.

During repetitive stimulation, summation of the after-depolarization took place. Its duration after short-term stimulation at high frequency showed a slight increase, but after prolonged stimulation (0.5 sec or more) it fell almost to its minimum (Fig. 1A). With an increase in the duration of tetanization (starting with 0.5 sec) the after-hyperpolarization was exhibited more clearly. The longer the preceding tetanization, the greater its amplitude, but its duration remained approximately constant.

Records from the same nerve obtained after removal of the epineurium and perineurium are given in Fig. 1B. The character and magnitude of the after-potentials clearly remained substantially unchanged.

Since there is reason to suppose that the outer membranes play an important role in the genesis of the long after-potentials, it seemed very probable that the after-potentials of a nerve with its outer membranes intact and removed would differ significantly when the frequency of stimulation was varied.

After removal of the membranes the maximal value of the after-potentials fell significantly (Fig. 2). However, this decrease was not directly connected with the absence of the epineurium and perineurium. Actually in these experiments the amplitude of the action potentials of the nerve trunk fell after removal of the membranes by 12 ± 0.92%. This suggests that during the dissection some of the fibers were injured, increasing the shunting not only of the spike potential, but also of the following after-potentials. The duration of the after-potentials remained practically unchanged, however, after removal of the nerve sheaths.

To judge from the data given in Table 1, the after-hyperpolarization observed in the present experiments was identical with the phases P_1 [11] and P_2 [15] described in the isolated and intact nerve. During brief stimulation (less than 10 sec) the phase of slow hyperpolarization is absent [7, 12, 16] and only the phase of fast hyperpolarization is observed. Böhm and Straub [7] attempted to explain the shortness of the after-hyperpolarization of single nerve fibers by postulating that in isolated fibers the phase of fast after-hyperpolarization is present but the corresponding slow phase is absent. However, to judge from data in the literature [12, 16] and from the results of the present investigation the phase of fast hyperpolarization lasts for more than 1 sec, significantly longer than the maximal (50 msec) duration of after-hyperpolarization of single nerve fibers.

After the preparations had been kept for 7-9 h in Ringer's solution or for 10-30 min in a solution with high calcium concentration [5, 6] there was a marked increase in the after-depolarization, which under these conditions could last for 1.5 sec. As the frequency of stimulation of this nerve increased, the duration of the after-depolarization also increased, and this was reflected in an after-period of increased excitability and increased amplitude of the testing action potentials (Fig. 3A). These coupled changes in excitability emphasize the true nature of the recorded after-potentials and they rule out any assumption that they could be artifacts caused by the passive properties of the nerve trunk. This conclusion is confirmed indirectly also by the fact that all the effects caused by the great magnitude and duration of the after-depolarization were reproduced in nerve trunks after removal of the outer membranes: the after-period of increased excitability (or phase of exaltation), the increase in amplitude of the action potentials during submaximal tetanization [5, 6] (Fig. 3B), and the phenomenon of the tetanized single response [1] (Fig. 3C). These effects also took place in response to stimulation at or slightly below threshold strength. This suggests that the after-potentials recorded in the present experiments and the effects produced by them in the nerve trunk are characteristic of the most excitable thick axons which are usually used for making preparations of single nerve fibers.

It can be concluded from the foregoing account that the epineurium and perineurium do not play an essential role in the formation of the after-potentials of the nerve trunk. Prolonged durations of the after-potentials of the nerve trunk are possibly linked with the presence of a surface barrier on each separate fiber [10, 11, 19], leading to the accumulation of ions in the immediate proximity of the excitable membrane, with consequent marked after-changes in polarization and excitability. This barrier on single nerve fibers may, however, be damaged during dissection.

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