CHANGES IN THE DURATION OF THE ELECTRIC RESPONSE OF SINGLE NERVE FIBERS FOLLOWING REPETITIVE STIMULATION

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Following prolonged repetitive stimulation the properties of the nerve trunk are markedly modified. The amplitude of the action potential is lowered (9, 11, 13), conduction velocity decreased (10), rheobase increased (2), refractory period lengthened (12), after-potential increased (1, 5, 8), and oxygen consumption (7) and heat production (6) per impulse are decreased.

The interpretation of such results is often uncertain because differences exist in the size and form of the action potential, in the excitability, and in the conduction rate, of the fibers comprising the nerve trunk. In the present investigation the effects of tetanization on nerve activity were reexamined using toad single nerve fibers. The most striking changes observed involved the duration of the electric response. This report is mainly concerned with these changes.

Methods

Single nerve fiber responses were recorded with three arrangements of the "bridge insulator" method (14). The nerve innervating the semitendinous or sartorius muscle of the toad (*Bufo marinus*) was used. A segment (about 1 mm. long) of the nerve was desheathed and all except one of the fibers in the desheathed region were cut. The isolated fiber preparation was mounted on a bridge insulator (Fig. 1). The Ringer fluid bathing the preparation was divided into two pools. With the methods illustrated in Figs. 1 *A* and 1 *C* the air gap separating the two pools measured 0.1 to 0.2 mm. With the method illustrated in Fig. 1 *B* the air gap measured 0.5 mm. With all three methods an internode of the isolated fiber was suspended across the air gap. With methods 1 *A* and 1 *B* this internode was located in the dissected region. With method 1 *C* this axis cylinder was located in the undissected portion of the nerve.

The response recorded was derived primarily from the activity of the two nodes adjacent to the internode suspended across the gap. With methods 1 *A* and 1 *B* the response recorded represented the *current* flowing through this internodal segment. When both pools were filled with normal Ringer a binodal action current was obtained. A strong diphasicity or a duration (of the phase of strong current flow) longer than 0.1 to 0.2 msec. was taken as an indication of injury and the fiber was dis-

carded. When the fluid in the distal pool (left in Fig. 1) was replaced with 0.05 to 0.1 per cent cocaine-Ringer, the configuration of the response changed into a triangular form (Fig. 2, record at 0 minutes). This was the response of the node on the proximal side of the air gap (mononodal action current). With method 1 *C* the electrical response

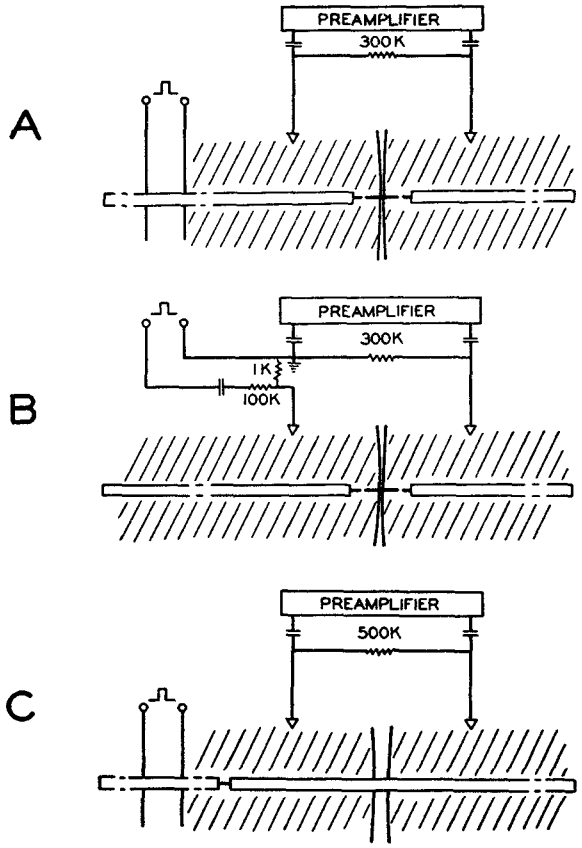

FIG. 1. Three arrangements for recording single nerve fiber responses.

was recorded as *voltage* (15). The configuration of this response was similar to the configuration of the binodal action current.

Tetanic stimuli at 50 to 150 shocks per second were delivered to the fiber for a period of 20 to 140 minutes. Test stimuli were presented at 2 to 5 shocks per second. In most experiments test stimuli were applied continuously following tetanization. In a few experiments a short train of test stimuli was delivered at intervals of 5 minutes to 1 hour. With method 1 *B* the node under study was stimulated directly by passing a current across the two pools. With methods 1 *A* and 1 *C* the node under study was excited indirectly by stimulating the proximal end of the nerve trunk.

In a series of experiments the sciatic plexus of the toad was tetanized *in vivo*. Fol-

lowing tetanization the nerve innervating the sartorius and semitendinosus muscle was excised. Single fiber responses were then studied with methods 1 *A* or 1 *B*.

The accommodation constant (Hill's λ) of single nerve fibers was determined using Tasaki's method (17)

With all three methods the recording electrodes were of the Ag–AgCl–Ringer (agar)

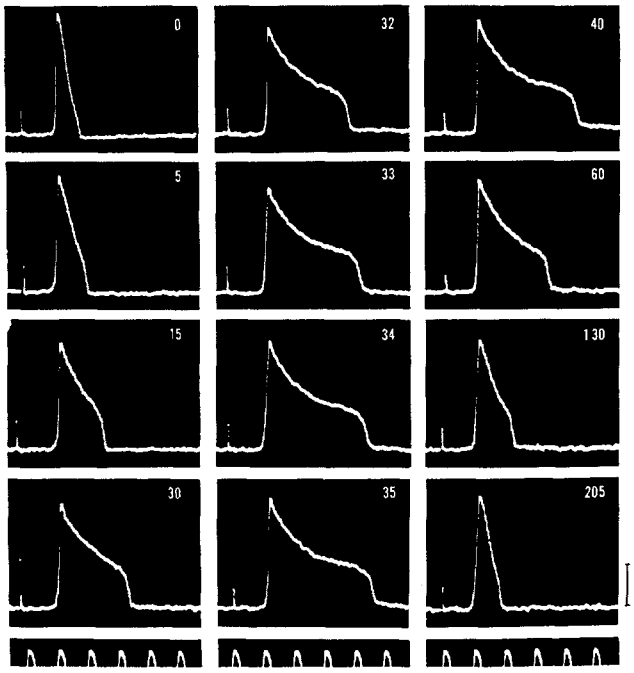

FIG. 2. Action current prolongation during and following 30 minutes' tetanization at 150 shocks per second. The time in minutes from the beginning of tetanization is indicated on the upper right hand corner of each frame. Tetanization was interrupted for a few seconds when tracings were photographed. Test stimuli were delivered at 2 shocks per second. Toad motor nerve fiber. Vertical bar subtends 1×10^{-9} amperes. Time marking, 1 K. C. Temperature, 22°C.

type. With methods 1 *A* and 1 *C* the stimulating electrodes were a pair of platinum wires. With method 1 *B* the stimulating electrodes were of the Ag–AgCl–Ringer (agar) type. The instruments employed included a Grass SA4 stimulator, a Tektronix 122 preamplifier, a Dumont 322A dual channel oscilloscope, and a Grass or Beattie-portronic camera. Observations were made on 112 large (10 to 15 μ) motor nerve fibers. The Ringer's fluid contained 0.2 per cent $NaHCO_3$, 0.014 per cent KCl, 0.65 per cent NaCl, and 0.012 per cent $CaCl_2 2H_2O$. In some experiments it contained in addition 0.001 per cent NaH_2PO_4 and/or 0.2 per cent glucose. Experiments were carried out either at room temperature (22–25°C.) or in a refrigerated room (10–13°C.).

RESULTS

The Properties of Untetanized Fibers

At room temperature (22–25°C.) the duration of the mononodal action current was 0.7 to 0.9 msec., the amplitude 2 to 3 $\times$ 10^{-9} amperes, the rheobase (for direct node stimulation) 16 to 25 mv., and the accommodation constant (Hill's λ) 6 to 13 msec. At low temperatures (10–13°C.) the duration was 2 to

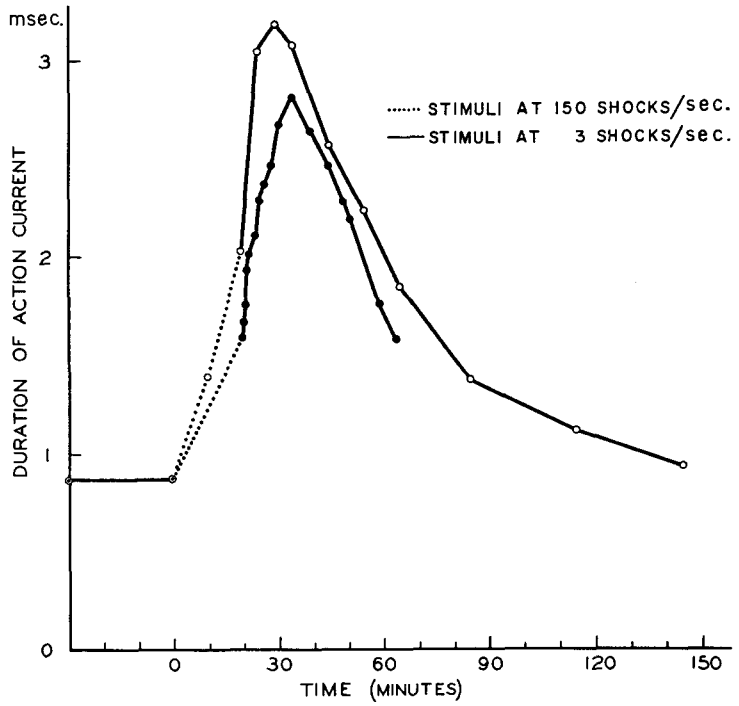

FIG. 3. Action current prolongation during and following tetanization of two toad motor nerve fibers. Temperature, 22°C.

3 msec., the amplitude 1.6 to 2.5 $\times$ 10^{-9} amperes, the rheobase 20 to 25 mv., and the accommodation constant 20 to 25 msec.

The Properties of Tetanized Fibers

During tetanization the spike duration was progressively increased (Figs. 2 and 3). Following tetanization, in 98 out of 112 preparations, the spike duration continued to lengthen at a more rapid rate. Within 5 to 60 minutes the post-tetanic prolongation stopped, and within 1 to 10 hours the spike duration was normal. The duration of the response of tetanized fibers was from 2.5 to more than 10 times the spike duration of untetanized fibers. The dura-

tion of the response of 14 out of 112 fibers was doubled or tripled immediately after tetanization but little or no post-tetanic lengthening occurred.

Post-tetanic spike prolongation was observed with all three methods, 1 *A*, 1 *B*, and 1 *C*, at room temperature and in the cold room, in mononodal and binodal preparations, with continuous or intermittent test stimulation, and with all tetanizing frequencies between 50 and 150 shocks per second. Frequent replacement of the fluid bathing the stimulated node with fresh Ringer during or after tetanic stimulation did not prevent prolongation. Prolongation was observed in fibers isolated from nerves tetanized *in vivo*. Injured fibers (fibers with abnormal binodal spike shape) showed little or no post-tetanic lengthening. With longer periods of tetanization the increase in duration was greater and the time required for the duration to return to normal was longer. The exact dependence of the time course of changes in spike duration upon the frequency and duration of tetanization, the temperature, and other experimental conditions is still under investigation. Large differences among fiber preparations have made such a study difficult.

The rate of fall of the shoulder (the sudden sigmoid ending of the falling phase) of the mononodal action current remained relatively unchanged during and after tetanization (Fig. 2).

In the fibers tetanized after isolation, during tetanization the amplitude of the response was progressively decreased, the rheobase increased, and the shock-response interval prolonged. Tetanized fibers showed a prolonged accommodation constant. In response to D. C. stimulation many tetanized fibers fired repetitively.

After a period of tetanization the fiber failed to respond to every stimulus. This failure occurred more readily with higher frequencies of tetanization. When the frequency of tetanization was decreased the fiber responded again to every stimulus.

DISCUSSION

The changes in amplitude, latency, and rheobase obtained as a result of tetanization with whole nerve trunks (1, 2, 5, 13) are similar to those obtained in the present study with toad single nerve fibers. No evidence, however, exists in the literature for a post-tetanic prolongation of the responses of individual nerve fibers.

Since spike prolongation was obtained *in vivo*, with binodal preparations and with method 1 *C*, prolongation could not have been a result of injury during isolation of the fiber, or (in mononodal preparations) a result of the unidirectional current flowing through the node under study. With method 1 *A* and in the *in vivo* experiments most (if not all) the fibers in the nerve trunk were active during tetanization. This could not have been associated with the prolongation, since prolongation was obtained with method 1 *B*

when the fiber under study was the only fiber in the nerve trunk active during tetanization.

The effects of chemicals on the prolongation following tetanization have recently been reported (16). Certain compounds (*e.g.*, tubocurarine) prevented this prolongation or shortened the prolonged spike. Other chemicals (*e.g.*, versene) further lengthened the duration of the prolonged spike. Versene or tubocurarine had no comparable effects on the untetanized fiber. Reversible post-tetanic prolongation occurred in nitrogen (containing less than 1 per cent O_2), 95 per cent O_2 and 5 per cent CO_2, or in the presence of certain glycolytic or TCA cycle inhibitors.

The time course of the response of tetanized nerve fibers is similar to the time course of the response of cardiac muscle fibers (18), of Purkinje fibers (3), of crustacean muscle fibers treated with TEAC (4), and of nerve fibers treated with NaCl (hypertonic), sinomenine, heroine, brucine, emetine (14), or azide (unpublished experiments).

Prolongation was obtained with stimulating frequencies known to occur in the nervous system. The prolongation during and especially following tetanization cannot easily be explained with any of the existing theories of nerve excitation.

SUMMARY

Single nerve fibers were isolated from the nerve innervating the sartorius or semitendinosus muscle of the toad (*Bufo marinus*). Single nerve fiber responses were recorded with three arrangements of the "bridge insulator" method. During stimulation at 50 to 150 pulses per second for 20 to 140 minutes the spike duration was progressively increased. After tetanization the spike duration usually continued to increase at a more rapid rate. Within 5 to 60 minutes further prolongation stopped and within 1 to 10 hours the spike duration was normal. The duration of the response of tetanized fibers was from 2.5 to more than 10 times the spike duration of untetanized fibers. Prolongation was observed in nerve fibers isolated from nerves tetanized *in vivo*.

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