ELECTRONIC DORSAL-FOOT POTENTIALS OF THE FROG SPINAL CORD IN EXPERIMENTAL TETANUS

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In experiments on frogs tetanus toxin, in doses causing generalized tetanus, depressed reciprocal inhibition of spinal reflexes. The depression of inhibition was not connected with blocking of depolarization of the central endings of primary afferent fibers.

KEY WORDS: spinal cord; tetanus toxin; presynaptic inhibition.

It was shown originally in experiments on cats [11, 12] and rats [5-7] that tetanus toxin, in doses completely blocking postsynaptic inhibition of spinal neurons, does not change or strengthen depolarization of central endings of primary afferent fibers, with which presynaptic inhibition of spinal reflexes is associated [19]. Recently, however, Curtis et al. [16] have reported that tetanus toxin, injected into the lumbosacral enlargement of the cat spinal cord, depresses electronic dorsal-root potentials (DRPs), generated by depolarization of the central endings of afferent fibers. The mechanisms of these DRPs are similar in all respects with the mechanisms of DRPs in mammals [9, 14, 19]. Meanwhile, frog DRPs are characterized by particularly high amplitude and duration, which is why the frog spinal cord has been the traditional object for analysis of the mechanisms of depolarization of primary afferents [9, 13, 14, 17, 19, 20].

The object of the present investigation was to study the action of tetanus toxin on DRPs of the frog spinal cord.

EXPERIMENTAL METHOD

Male frogs (Rana temporaria) were used. Tetanus toxin (1000-1500 mouse MLD of dry toxin, dissolved in 0.5 ml physiological saline) was injected into the lymph sac or femoral muscles of the animals. The animals were kept thereafter at a temperature of 18-22° C, but two days later they were placed for 4 h in an incubator at 31-32° C [2]. The first signs of tetanus appeared in most animals on the 7th-8th day after injection of the toxin. Frogs with marked spasm of the trunk and limb muscles 8-14 days after the injection were chosen for the electrophysiological investigations. The spinal cord was divided at the level of the 2nd-3rd segment. Electrical activity of the flexors (tibialis anterior) and extensors (gastrocnemius) of the leg was recorded simultaneously by two pairs of electrodes (copper wire, 0.08 mm in diameter), inserted into the proximal ends of these muscles to a depth of 1-2 mm. The skin of the foot was stimulated by nichrome wire electrodes 0.5 mm in diameter, with interelectrode distance 2 mm. Laminectomy of six caudal vertebrae was carried out either immediately after chordotomy or after the electromyographic investigation. The 9th and 10th dorsal roots were divided distally; the 10th root was placed on platinum electrodes for electrical stimulation, the 11th on Ag–AgCl electrodes for recording the DRPs. The myogram was recorded by means of an amplifier with RC-coupling; DRPs were recorded by a symmetrical amplifier with direct coupling.

EXPERIMENTAL RESULTS

According to the results of experiments on mammals, a characteristic feature of depression of central

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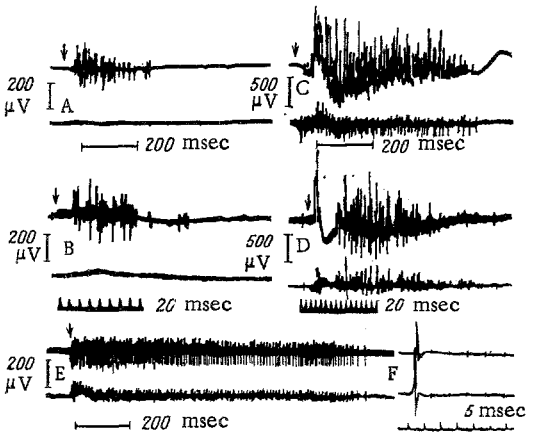

Fig. 1

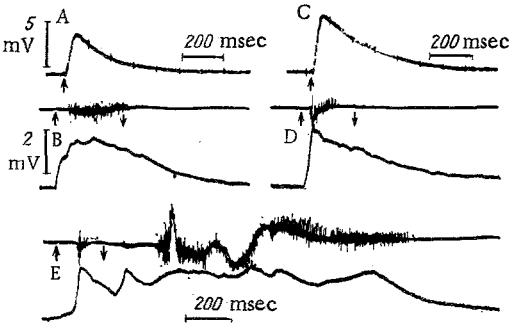

Fig. 2

Fig. 1. Electromyograms of antagonist muscles of leg: A, B) in control frog; C-F) in frogs with generalized tetanus. A, B) reflex responses to touching the skin of the ipsilateral foot with a soft hair brush; B, D. E) the same, to stimulation of skin of foot with four electric pulses, duration 1 msec, frequency 300/sec. Arrows indicate times of stimulation; F) electrical responses of same muscles as in E, but to stimulation of distal end of divided sciatic nerve by single electric pulse. Remainder of legend as in text.

Fig. 2. DRPs in frogs with generalized tetanus. Arrows in A and C show times of stimulation, arrows in B, D, and E show beginning and end of stimulation. Remainder of legend in text.

inhibition by tetanus toxin is a disturbance of reciprocal innervation of antagonistic muscles [1, 3, 10, 15, 18]. The results of the present experiments showed that evident disorders of the mechanisms of reciprocal innervation of the skeletal muscles were present also in frogs poisoned with tetanus toxin. The records in Fig. 1A, B show typical reflex electrical responses of antagonist muscles of the leg (tibialis anterior above, gastrocnemius below) in control frogs to stimulation of the skin of the ipsilateral foot, fixed in a position of extension. Clearly both tactile (Fig. 1A) and electrical (Fig. 1B) stimuli evoke volleys of spikes in the flexor muscles, accompanied by almost total "silence" of the extensor muscles. The records in Fig. 1C, D, E show reflex electrical responses of the same leg muscles to stimulation of analogous areas of skin of the ipsilateral foot in frogs with generalized tetanus. Clearly both tactile (Fig. 1C, E) and electrical (Fig. 1D) stimulation evoked prolonged bursts of spikes in both muscles simultaneously.

Reflex electrical activity of the skeletal muscles in mammals poisoned with tetanus toxin is characterized by long after-discharges [1, 3, 18]. Long after-discharges are also characteristic of reflex responses of skeletal muscles of "tetanized" frogs (Fig. 1C, D, E). These discharges were not the result of damage to neuromuscular transmission, for in the "tetanized" animals, just as in the control frogs, single pulses in the motor nerves produced single-action potentials in the muscles supplied by them (Fig. 1F). Typical examples of electrotonic dorsal-root potentials (DRPs) in frogs with generalized tetanus are given in Fig. 2. Figure 2A shows a submaximal, Fig. 2B a maximal electrotonic potential of the 9th dorsal root evoked by stimulation of the ipsilateral 10th root with single pulses. As in normal animals, these DRPs reached a maximum comparatively quickly (after about 40 msec), and then slowly decreased exponentially. The half-decay time of the maximal DRP (240 msec) and its amplitude (about 6 mV) correspond to the maximal values of the same parameters for DRPs evoked similarly in normal frogs (personal observation and data in the literature [9, 14]).

Records (Fig. 2B, D, E) from an experiment on another frog give electronic potentials of the 9th dorsal root (bottom beam) and electrical reflex activity of the tibialis anterior muscle (top beam), arising as a result of mechanical stimulation of the ipsilateral foot. Potentials in Fig. 2B were evoked by stroking the skin of the infero-lateral surface of the foot with a soft hair brush; potentials in Fig. 2D were evoked by squeezing the base of the 3rd toe with forceps. In both cases stimulation led to the appearance of considerable DRPs. Squeezing the toe in Fig. 2E evoked a long burst of paroxysmal activity of the muscles (top beam). Throughout the paroxysmal discharge, negativity of the central regions of the primary afferents (bottom beam) was recorded. DRPs similar to those in Fig. 2 appeared in all the frogs with generalized tetanus in response to electrical stimulation of afferent nerves and to adequate cutaneous stimulation.

The character of the disturbances of reciprocal innervation of the antagonistic muscles in frogs with generalized tetanus suggests that tetanus toxin damages the inhibitory mechanisms of spinal neurons in amphibians in the same way as in mammals. Recordings of DRPs show that the depression of inhibition is not connected with blocking of the depolarization of central endings of primary afferent fibers. This is in agreement with the original data showing the resistance of depolarization of the primary afferents to tetanus toxin [5-7, 11, 12], but it contradicts the later results according to which tetanus toxin injected into the spinal cord depresses DRPs [16]. The results of injection of the toxin into the spinal cord were explained by blocking of the synaptic liberation of γ-aminobutyric acid (GABA) [16]. In that case, the authors cited based their conclusions on personal data showing that toxin depresses liberation of GABA from inhibitory neurons of the cerebellar cortex and, second, on the fact that GABA is a possible chemical mediator of membrane depolarization of the endings of afferent fibers [19]. Fullest evidence of the role of GABA in depolarization of primary afferents was obtained in experiments on the frog spinal cord [13, 17, 19, 20]. Information that the toxin does not damage DRPs in these animals thus seems to be particularly important. The discrepancy between the results described in this paper and previously [5-7, 11, 12] and the results obtained by Curtis et al. [16] can be explained by differences in the methods of injection of the toxin. The DRPs were preserved whenever the tetanus was produced in a way similar to the natural infection – by intramuscular injection of the toxin. The toxin then entered the spinal cord, migrating centripetally along the trunks of the motor nerves [3]. Depression of DRPs evoked by direct injection of the toxin into the spinal cord [16] could be the result of its nonspecific action, mainfested both on account of its high local concentration, and also as a result of the unusual way of its penetration into the nerve centers. It is worth noting that high local concentrations of tetanus toxin block the liberation of acetylcholine from endings of the axons of motor neurons in skeletal muscles [4, 8]. Meanwhile under ordinary conditions of infection, the toxin in doses completely blocking liberation of the chemical mediator from the axons of spinal inhibitory neurons, does not affect the liberation of acetylcholine from axon endings of motor neurons in skeletal muscles [1, 3], nor likewise from recurrent collaterals of these axons terminating on Renshaw cells [10, 15].

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