ORDER OF DISAPPEARANCE OF VARIOUS TYPES OF POSTSYNAPTIC INHIBITION IN EXPERIMENTAL TETANUS

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In experiments on cats in which a fixed time interval separated intramuscular injection of tetanus toxin and division of the ventral roots, the order of disappearance of various types of postsynaptic inhibition of monosynaptic reflex discharges of motoneurons for the gastrocnemius muscle was studied. Inhibition produced by volleys of impulses in cutaneous afferent fibers disappeared first, followed by inhibition from group II muscle fibers, and last of all, by direct inhibition from group Ia muscle fibers. When direct inhibition of a test reflex produced by single inhibitory volleys appeared to be completely suppressed in the course of development of tetanus poisoning, it could be detected after preliminary tetanization of the inhibitory nerve.

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Tetanus toxin is known to reach the central nervous system by traveling from its point of formation or injection at the periphery along the trunks of muscular nerves and through the ventral roots [2]. The method generally used to investigate the various types of postsynaptic inhibition involves division of the muscular nerves and ventral roots, interrupting the pathway for the toxin. For this reason the action of tetanus toxin on the central nervous system has usually been studied in animals with already developed tetanus [1-3, 4]. To examine the disappearance of various types of postsynaptic inhibition in the course of development of tetanus, Brooks and co-workers [6] injected tetanus toxin directly into the spinal cord or high into the trunk of the sciatic nerve.

We have examined the complete dynamics of disappearance of various types of postsynaptic inhibition after intramuscular injection of tetanus toxin, which most closely approximates to the conditions of natural tetanus infection. This could be done by keeping to a strictly definite time interval between injection of the toxin and division of the ventral roots.

EXPERIMENTAL METHOD

Experiments were carried out on 28 cats. Tetanus toxin in a dose of 2-3 mg/kg body weight was dissolved in 0.5 ml physiological saline and injected into the extensor muscles of the left leg (1 mg dried toxin contained 800 LD_{50} for mice). Under nembutal anesthesia (25 mg/kg) the spinal cord was divided at the level $T_{10}-T_{12}$ 16 h after injection of the toxin. The ventral roots of segments L_6-S_2 were isolated and divided 18-20 h after injection of the toxin. The sural nerve (SUR), the peroneous profundus nerve (PP), and both branches of the nerve to the gastrocnemius muscle (G) were dissected and divided into the lower limbs. After fixation of the animal, the ventral roots of L_7 and S_1 were placed on platinum electrodes for monopolar recording of the action potentials. The peripheral nerves were stimulated electrically with pulses 0.1 msec in duration. The amplitude of afferent volleys entering the spinal cord was determined by means of a silver wire electrode in contact with dorsal surface of segment L_7 at the point of entry of the dorsal roots on the corresponding side. Because of the considerable duration of the experiment, exposed tissues were covered with mineral oil (36-38°) and irrigated with penicillin solution. Penicillin was also injected intramuscularly (100,000-200,000 units). Inhibition was investigated by Lloyd's method [10], i.e., the action of conditioning volleys of impulses was studied on the magnitude of maximal monosynaptic test reflexes arising in the ventral roots in response to stimulation of the nerve to the gastrocnemius muscle.

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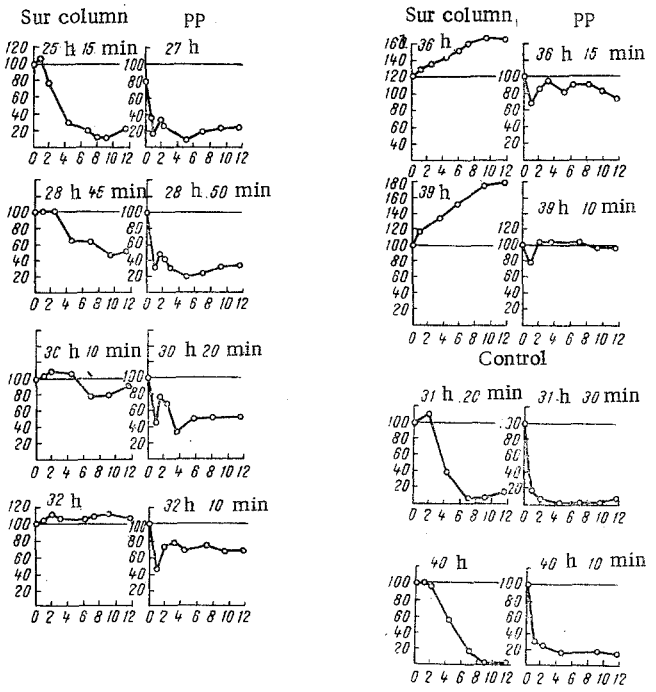

Fig. 1. Disappearance of different types of postsynaptic inhibition of monosynaptic reflexes during development of tetanus. Numbers above graph show time after injection of tetanus toxin. SUR column shows changes in amplitude of test reflexes during the action of conditioning volleys caused by stimulation of SUR by single stimuli three times above threshold strength. PP column shows the same during the action of conditioning volleys caused by stimulation of PP with single stimuli 1.9 times above threshold strength. Abscissa, time between arrival of conditioning and test volleys in spinal cord (in msec), ordinate, amplitude of maximal monosynaptic test G reflexes in ventral root S_1 (in percent of control).

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results given in Fig. 1 show the characteristic order of disappearance of the different types of postsynaptic inhibition during the development of local tetanus. The inhibitory action of volleys of afferent impulses evoked by stimulation of the cutaneous nerve SUR and the muscular nerve PP on monosynaptic reflex discharges in the ventral roots of segment S_1 in response to stimulation of nerve G was investigated. A single conditioning volley in fibers of SUR on the side of injection of toxin and 26 h 15 min after its injection had a marked inhibitory action on the monosynaptic test G reflex. In subsequent tests 28 h 45 min and 30 h 10 min after injection of the toxin, this inhibition progressively diminished. By 32 h the inhibition had completely disappeared and was replaced by slight facilitation of the test response, and this had increased 39 h after injection of the toxin. On the control side (Fig. 1, below) inhibition of the test G reflex by volleys of impulses in afferent fibers of SUR remained deep throughout the experiment.

Parallel with the type of postsynaptic inhibition mentioned above, inhibition of maximal monosynaptic G reflexes by single volleys of afferent impulses in fibers of the PP nerve supplying antagonist muscles of the same joint was investigated. The strength of the conditioning stimulus was maximal for group I afferent fibers and, consequently, it was above threshold for group II muscle afferent fibers [9, 10]. As Fig. 1 shows, 27 h after injection of the toxin, two waves of inhibition of the test G reflex were present on the tetanus side: the first,a wave of rapidly increasing inhibition reaching a maximum at an interval of 1 msec between arrival of the conditioning and test volleys in the spinal cord, and a second wave of inhibition with a maximum in the interval of 4-6 msec. In the generally accepted view the first wave is characteristic of direct inhibition from group Ia afferent fibers of nerves supplying antagonist muscles, while the second wave is due to the action of volleys of impulses in thinner afferent fibers of muscular nerves, group II fibers [5, 10]. On the tetanus side (Fig. 1), starting 27 h after injection of the toxin, inhibition of the test G reflex progressively decreased at all intervals between conditioning and test volleys. By 32 h 10 min after injection of the toxin, when inhibition from the cutaneous fibers had completely disappeared, marked inhibition was still present from the group I and II afferent fibers of PP. These last two types of inhibition were reduced still further 36 h 15 min after injection of the toxin, at a time when impulses from the cutaneous nerves were producing facilitation of the monosynaptic G reflex. Finally, 39 h 10 min after injection of the toxin, inhibition from the group II fibers was completely abolished and replaced by slight facilitation in the interval of 2-8 msec between arrival of the conditioning and test volleys in the spinal cord. Direct inhibition at this time was less than 36 h 15 min after injection, although not completely abolished. On the control side (Fig. 1, below) the inhibitory action of afferent volleys in PP on monosynaptic G reflexes was substantially unchanged throughout the experiment.

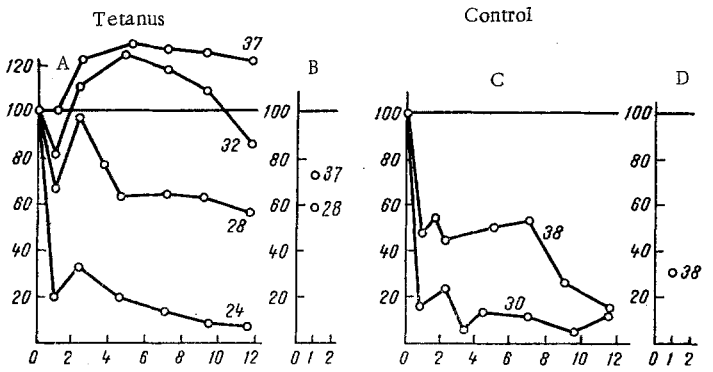

Fig. 2. Posttetanic potentiation of direct inhibition during development of tetanus. A, B) Direct inhibition tested without preliminary tetanization of conditioning nerve PP on tetanus and control sides respectively; C, D) magnitude of test reflexes with an interval of 1 msec between arrival of conditioning and test volleys in spinal cord, 10 sec after preliminary tetanization of PP. Numbers on curves in A and C and by points in B and D show times after injection of toxin (in h). Abscissa, time between moments of arrival of conditioning and test volleys in spinal cord (in msec); ordinate, amplitude of maximal test monosynaptic G reflexes in ventral root S_1 (in percent of control). Remainder of explanation in text.

The direct inhibitory action of volleys of impulses in group Ia afferent fibers can be strengthened by preliminary tetanization of these fibers [11]. One of the 8 experiments in which posttetanic potentiation of direct inhibition was investigated during development of tetanus poisoning is illustrated in Fig. 2. On the tetanus side (Fig. 2A), starting from 24 h after injection of the toxin, both the direct inhibition and inhibition produced by a volley of impulses in the group II afferent fibers of PP were progressively reduced. Inhibition from group II fibers was replaced 32 h after injection of the toxin by marked facilitation, whereas direct inhibition still persisted, although very weak. Direct inhibition of the monosynaptic G reflex was completely abolished 37 h after injection of the toxin, while facilitation of the test response by volleys of impulses in the group II afferent fibers was increased. Evolution of these types of inhibition on the control side (Fig. 2C) could not be compared with that on the tetanus side. The magnitude of direct inhibition of maximal monosynaptic G reflexes produced by single volleys of impulses in afferent PP fibers 10 sec after tetanization of PP, given for 15 sec with stimuli 3 times over the threshold strength and at a frequency of 300/sec, is illustrated in Fig. 2D (control side). If inhibition of the test G reflexes in Fig. 2C (without preliminary tetanization of PP) is compared with inhibition in Fig. 2D, it will be clear that the amplitude of the test reflex for an interval of 1 msec between arrival of the conditioning and test volleys in the spinal cord in Fig. 2C (38 h after injection of the toxin) was reduced by 53% below the control level, whereas in Fig. 2D, when the interval was the same, it was reduced by 68%. In the same way, posttetanic potentiation of direct inhibition on the tetanus side is illustrated in Fig. 2A and B. When, 37 h after injection of the toxin, direct inhibition tested without preliminary tetanization of PP had already completely disappeared on the tetanus side (Fig. 2A), it was still found after preliminary tetanization of PP and amounted to approximately 25-27% (Fig. 2B).

In conjunction with the results of previous investigations [2, 3, 6, 7], the results of these experiments show that tetanus toxin effectively suppresses different types of postsynaptic inhibition of monosynaptic reflexes. It also follows from these experiments that the disappearance of all the investigated types of postsynaptic inhibition starts at about the same time after injection of the toxin and increases along a parallel course for a relatively long time interval. The first to disappear is inhibition of monosynaptic reflexes caused by volleys of impulses in afferent fibers of the cutaneous nerve SUR, followed by inhibition from group II afferent fibers of the PP nerve, and finally, direct inhibition. In the course of development of tetanus poisoning, the inhibitory action of volleys of impulses in cutaneous afferent fibers and group II

muscle afferent fibers is replaced by facilitation. The regular order of disappearance of the inhibitory action of volleys of impulses in SUR and PP on monosynaptic G reflexes described above and its transformation into a facilitatory action during the development of tetanus may be due to differences in the number of inhibitory and excitatory fibers in these nerves or to differences in the anatomical distribution of endings of inhibitory neurons.

These results show that direct inhibition, after its complete abolition during development of tetanus poisoning, can be largely restored by preliminary tetanization of the conditioning nerve. In accordance with modern views, rhythmic activity of presynaptic endings leads to an increase in the probability of liberation of mediator contained in them [8]. Such a mobilization of mediator in inhibitory synapses evidently takes place against the background of action of tetanus toxin which, it is considered [6], blocks the liberation of inhibitory mediator from axon endings of inhibitory neurons.

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