MECHANISMS OF THE PATHOLOGICALLY INCREASED SCRATCH REFLEX IN ANIMALS WITH EXPERIMENTAL TETANUS

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The mechanisms of the pathologically increased scratch reflex from the receptive fields of the forelimb into which tetanus toxin had previously been injected (the mechanism of the "departure station" phenomenon) were investigated. In rats with a pathologically increased scratch reflex discharges of high amplitude occurred in the ipsilateral peroneal nerve during stimulation of the radial nerve on the side of injection of the toxin. In animals with hemisection of the spinal cord at the level C_2 stimulation of the ipsilateral nerve to the "tetanus" forelimb in a strength of 4 threshold units (4T) induced inhibition followed by facilitation of the lumbar flexor motoneurons on the ipsilateral side; the inhibition was deeper in rats with a pathologically increased scratch reflex than in healthy rats. The state of segmental inhibition of the lumbar extensor motoneurons and the ability of monosynaptic extensor reflexes to reproduce the frequency of afferent stimulation were the same in the rats with a pathologically increased scratch reflex as in healthy animals. It is concluded that the increase in strength of the long spinal reflexes from the receptive fields of the "tetanus" forelimb is connected with de-inhibitory action of tetanus toxin on interneurons in the brachial segments of the spinal cord.

Previous investigations [8-11] showed that in animals with relatively local toxic damage to the rostral portions of the spinal cord following the arrival of tetanus toxin along the regional neural pathway [2] from one of the forelimbs, stimulation of the receptors of that limb induces a pathologically increased scratch reflex of the ipsilateral hind limb. This is a manifestation of the "departure station" phenomenon [2, 3], in which the "station" is located in the rostral portions of the spinal cord. It has been postulated that the pathologically increased scratch reflex is a special form of tetanus hyperreflexia, the production of which is unconnected with the suppression of motoneuronal inhibition [7-11].

The investigation described below was carried out to test this hypothesis experimentally.

EXPERIMENTAL METHOD

Experiments were carried out on 47 albino rats. A pathologically increased scratch reflex of the hind limb was induced by injecting tetanus toxin in a dose of 3-4 MLD into the triceps muscle of the ipsilateral forelimb. To block the spread of the toxin by the blood, antitoxin was injected in a dose of 0.025 a.u. The rats were used in the experiments 3-4 days later. Tracheotomy was performed under ether anesthesia, the cervical and lumbar enlargements of the spinal cord were exposed, and the spinal roots and peripheral nerves were dissected. For the electrophysiological investigations the animals were immobilized with D-tubocurarine (0.3-0.4 mg/kg) or Remyolan (5-7 mg/kg) and maintained on artificial respiration. General anesthesia was not used during the experiments.

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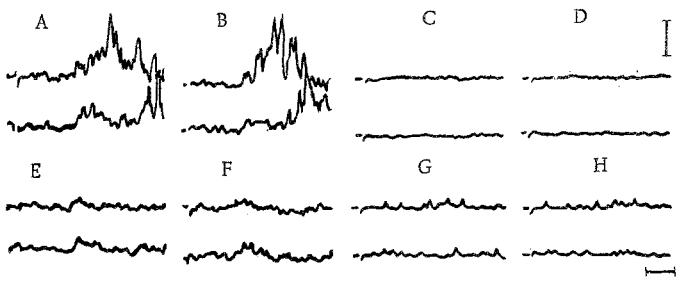

Fig. 1. Reflex activity in nerves of hind limbs evoked in rat with pathologically increased scratch reflex by a single stimulus applied to the radial nerves of the forelimbs. A and B) Responses in peroneal nerves on both sides to stimulation of radial nerve of "tetanus" forelimb at strengths of 4 and 40T; E and F) the same, but to stimulation of nerve on side opposite to injection of toxin; C and D) responses in tibial nerves on both sides to stimulation of nerve to "tetanus" forelimb in strengths of 4 and 40T; G and H) responses in nerves to medial heads of gastrocnemius muscles to stimulation of radial nerve to "tetanus" forelimb in strengths of 4 and 40T. On all records responses in nerves on "tetanus" side are shown above, and responses in nerves on opposite side below. Calibration: signal 500 μV, time 6 msec.

TABLE 1. Amplitudes of Reflex Discharges in Peroneal Nerves Evoked in Rats with a Pathologically Increased Scratch Reflex by Stimulation of Ipsilateral and Contralateral Radial Nerves in a Strength of 4T

Side of recording	Amplitude (in μV)					
	stimulation of ipsilateral radial nerves			stimulation of contralateral radial nerves		
	n	$\bar{x} \pm S_{\bar{x}}$	P	n	$\bar{x} \pm S_{\bar{x}}$	P
"Tetanus"	6	198±49	<0,05	4	64±38	>0,05
Opposite	5	42±26		4	180±52	

Legend. Here and in Table 2: n) number of observations; x) arithmetic mean; $S_{\bar{x}}$) standard error; P) probability of null hypothesis. Level of significance taken as 0.05. Differences significant when $P \leq 0.05$.

In the experiments on animals with an intact spinal cord reflex responses were recorded in the nerves of both hind limbs to stimulation of the common trunk of the radial nerve on the "tetanus" and contralateral sides at a strength of 4 and 40 threshold units (4 and 40T).

The segmental inhibition of lumbar motoneurons was investigated in spinal rats. Chordotomy was performed at the level T_7 2 h before the beginning of the experiment. Inhibition of extensor monosynaptic reflexes evoked by stimulation of the tibial nerve in the ventral root L_5 and L_6 in response to conditioning stimulation of the afferent fibers of the peroneal nerve was studied. The strength of conditioning stimulation was 3T, and the strength of testing stimulation a little more than that required to induce a monosynaptic reflex of maximal amplitude. In the same experiments the ability of the monosynaptic reflexes to reproduce the rhythm of stimulation (100, 150, and 200/sec) of the tibial nerve with a strength of 3T was studied. The procedures of preliminary preparation, stimulation of the nerves, and recording the responses were similar to those described previously [5, 6, 9].

The effect of conditioning stimulation of the common trunk of the radial nerve in strengths of 4 and 40T on the amplitude of the ipsilateral lumbar flexor monosynaptic reflexes evoked in the ventral roots L_5 and L_6 by stimulation of the peroneal nerve was studied in animals with ipsilateral hemisection of the spinal cord (at the level C_2). Hemisection was carried out to abolish the spino-bulbospinal component of the limb-to-limb reflex [1, 13].

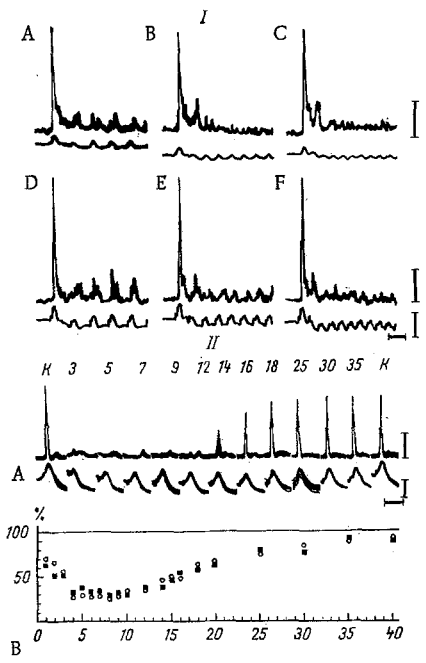

Fig. 2. Inhibition of lumbar extensor monosynaptic reflexes in rats with a pathologically increased scratch reflex. I. Changes in spinal reflexes in the course of repetitive stimulation of afferent nerves. A, B, and C) Monopolysynaptic reflexes (above) and dorsal surface potentials (DSPs) of spinal cord (below) in a healthy rat during stimulation of tibial nerve in a strength of 3T and frequencies of 100, 150, and 200/sec; D, E, and F) analogous curves obtained with a rat with pathologically increased scratch reflex. Calibration of signal for DSP and time the same for all records (2 mV and 10 msec), shown in F. Calibration of signal for efferent discharges for records A, B, and C (0.25 mV) given in C, for records D, E, and F (0.10 mV) given in F. II. Inhibition of lumbar extensor reflexes. Inhibition evoked by stimulation of tibial nerve in a strength of 3T. A) Inhibition of monosynaptic reflex in root L_5; B) in root L_6. Curve of inhibition of monosynaptic reflex in healthy animals (circles) plotted from mean data obtained in 11 experiments, curve of inhibition in animals with pathologically increased scratch reflex (filled squares) plotted from mean data obtained in experiments on 15 animals. Calibration of signal in A: top curve 500 μV, bottom curve 250 μV; time 10 msec.

EXPERIMENTAL RESULTS

In the rats with a pathologically increased scratch reflex stimulation of the radial nerve on the "tetanus" side in a strength of 4T evoked efferent discharges, after a delay of 12-13 msec, in the peroneal nerves on both sides (Fig. 1a). In response to stimulation of the radial nerve to the contralateral forelimb at the same strength or more (40T), responses of lower amplitude and shorter duration were recorded in these same nerves of the hind limbs (Fig. 1E, F). The difference in amplitudes of the responses evoked by stimulation of the radial nerves on the "tetanus" and opposite sides, in the peroneal nerves ipsilateral relative to stimulation, is statistically significant (Table 1). A study of the electrical responses in the nerves to the flexor and extensor muscles of the ankle showed that efferent activity evoked by stimulation of the radial nerve to the "tetanus" forelimb is directed chiefly toward the flexor muscles: it was recorded regularly only in the peroneal nerves (Fig. 1).

To investigate the causes of the increase in efferent activity of the crural portion of the spinal cord in rats with a pathologically increased scratch reflex, inhibition of the extensor and flexor lumbar motoneurons was studied on the "tetanus" side. Curves showing inhibition of motoneurons of the ankle extensors evoked by stimulation of the peroneal nerve in a strength of 3T are shown in Fig. 2, II. It is clear from graphs showing inhibition of the monosynaptic reflexes in root L_6 and from the records showing inhibition of the monosynaptic reflex in the more rostral root L_5 that inhibition of motoneurons in the lumbo-sacral portion of the spinal cord in animals with a pathologically increased scratch reflex was not significantly changed.

Besides inhibition, in these experiments the ability of the monosynaptic reflexes to reproduce the frequency of afferent nerve stimulation (the carrying capacity of the efferent output) was investigated in these experiments [4, 10]. During suppression of motoneuronal inhibition by the toxin the monosynaptic reflexes reproduced higher frequencies of afferent stimulation if the afferent volley evoked a mixed mono-polysynaptic response in the ventral root [4, 10]. It follows from Fig. 2, I that the ability of the monosynaptic reflexes at the lumbar level to reproduce the rhythm of repetitive afferent stimulation was at the same low level in the animals with a pathologically increased scratch reflex as in the healthy rat.

Stimulation of the common trunk of the radial nerve in a healthy, unanesthetized animal with hemisection of the spinal cord at the level C_2 was accompanied by biphasic changes in excitability of the nuclei of the ipsilateral flexor motoneurons (Fig. 3, curve 1). The first changes in excitability – a decrease in amplitude of the testing monosynaptic reflex – were observed as early as 4 msec after application of the conditioning stimulus. With an increase in the interval between the conditioning and testing stimulation, inhibition of the testing monosynaptic reflex was increased, the maximum corresponding to an interval of 10-15 msec, after which it decreased

TABLE 2. Intensity of Inhibition of Lumbar Flexor Motoneurons during Stimulation of Radial Nerve of Ipsilateral Forelimb in a Strength of 4T

Animals investigated	Amplitude of testing monosynaptic reflex (in % of control) for a time interval between conditioning and testing					
	7 msec			8 msec		
	n	$\bar{x} \pm s_{\bar{x}}$	P	n	$\bar{x} \pm s_{\bar{x}}$	P
Healthy rats	6	79±5	<0,05	6	76±4	<0,05
Rats with a pathologically increased scratch reflex	5	59±9		5	52±12	

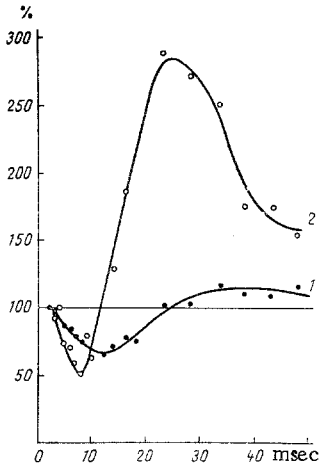

Fig. 3. Changes in excitability in lumbar flexor motoneurons induced by stimulation of radial nerve to ipsilateral forelimb in a strength of 4T in healthy rats (1) and in rats with a pathologically increased scratch reflex (2). Animals with ipsilateral chordotomy at the level C_2. Curves plotted from mean data of 7 experiments on healthy and 8 experiments on "tetanus" animals. Testing stimulation applied to peroneal nerve, monosynaptic testing reflex recorded in ipsilateral root L_6. Abscissa, time interval between conditioning and testing stimulation (in msec); ordinate, amplitude of testing monosynaptic reflex (in percent of control).

steadily. Inhibition was replaced by a very slight increase in the excitability of the flexor motoneurons. An increase in the strength of the conditioning stimulation to 40T did not lead to any further increase in the changes in excitability of the lumbar motoneurons.

In the animals with a pathologically increased scratch reflex the inhibitory effects of the rostral portions of the spinal cord on the lumbar motoneurons remained intact. Moreover, descending inhibition of the testing monosynaptic reflex in the rats with a pathologically increased scratch reflex was deeper than in the healthy rats (Fig. 3). Statistical analysis of the measurements of the depth of inhibition in the animals with a pathologically increased scratch reflex and in the healthy rats for an interval of 7-8 msec (the maximum distance apart of curves 1 and 2 in Fig. 3) led to a significant increase in inhibition in the animals with the phenomenon under investigation (Table 2). Descending facilitation of flexor reflexes in the animals with a pathologically increased scratch reflex also grew in intensity, whereas the latent period of appearance of the facilitatory effects was shortened (Fig. 3).

The facts described above clearly prove that inhibition of the extensor and flexor lumbar motoneurons in animals with a pathologically increased scratch reflex is not suppressed by tetanus toxin. Consequently, the strengthening of inhibitory and excitatory influences of the rostral portions of the spinal cord on the lumbar motoneurons can only be explained by the de-inhibiting action of tetanus toxin on interneurons of the corresponding reflex arcs in the cervical and upper thoracic segments of the spinal cord. The rostral portions of the spinal cord thus exert qualitatively similar effects on reflex activity of the lumbar motoneurons in animals with a pathologically increased scratch reflex and in healthy rats, and the differences in curves 1 and 2 in Fig. 3 resemble the differences in character and shape of the curves obtained in experiments with preparations of the spinal cord, with low and high excitability, respectively, of the healthy spinal cat [12].

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