MECHANISM OF PARADOXICAL RESISTANCE OF THE DIGASTRIC MUSCLE TO THE ACTION OF TETANUS TOXIN

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In tetanus poisoning as a rule hyperactivity of the sleketal muscles develops, in connection with inhibition of mediator release in the inhibitory synapses of motoneurons [4, 10] or with loss of sensitivity of the postsynaptic membrane to it [7]. The muscles of mastication becoming involved [3, 5, 15] in the pathological process of trismus, it is not yet clear to what extent the function of the muscles, namely opening the mouth, is altered under these circumstances. Under physiological conditions this group of muscles has a weaker force of contraction than the group of mouth-opening muscles [6, 9], and for that reason masking of their injury in tetanus cannot be ruled out. It was accordingly decided to reproduce a local form of rigidity from tetanus in the mouth-opening muscles and to discover whether changes in the parameters of excitability of the muscle fibers typical of tetanus develop in them.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-260 g. Under sterile conditions and pentabarbital anesthesia (4 mg/100 g) the corresponding belly of the digastric muscle (DM) was exposed and 40 mouse MLD of tetanus toxin (TT) was injected into it (1 MLD for mice = 0.0001 mg of dried TT) in a volume of 0.04 ml. In some animals this evoked ipsilateral trismus. After injection of a similar dose into the limb, the rats developed ascending tetanus, leading to death of most animals. In comparative investigations TT was injected under open ether anesthesia into the masseter muscle (MM), by injection of 20 mouse units through the skin. Larger doses caused bilateral trimus. The animals were used in acute experiments at various times — 3-4 and 10-15 days — after poisoning. Under pentabarbital anesthesia (4 mg/100 g) the corresponding belly of DM or MM was dissected and part of the fascia removed. A bath was formed from the skin and adjacent tissues and filled with warm mineral oil at 37°C. The animal was fixed to a frame for microelectrode investigations, and the jaws were fixed by metal clamps to the upper and lower incisors. To investigate DM the corresponding belly was stretched by a glass hook fixed to the intermediate tenson. MM was stretched by selarating the incisors. To record resting membrane potentials (RMP) and, at the same time, to stimulate the cells, glass (Pyrex) microelectrodes filled with 3M KCl solution (resistance 4-10 MΩ) were used. For intracellular testing of the cytoplasmic membrane of the fiber with depolarizing pulses the same microelectrode was used, connected to a bridge circuit [2]. In this case the amplitude of the action potential (AP), the critical level of depolarization (CLD), and the strengths of the rheobase currents were investigated. Microelectrodes were inserted into the muscle to a depth of 2.5 mm in DM and of 3 mm in MM. Considering the laminar organization of the muscles of mastication [1], an equal number of cells was recorded in each layer. In the control series TT which had been boiled for 30-40 min was injected into the muscles. The experimental results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Since DM in the rat has no stretch receptors, but the motoneurons innervating it possess only presynaptic inhibition [8, 11, 13, 14], comparative investigations were carried out on MM, which has the typical afferent and efferent nervous apparatuses for skeletal muscle.

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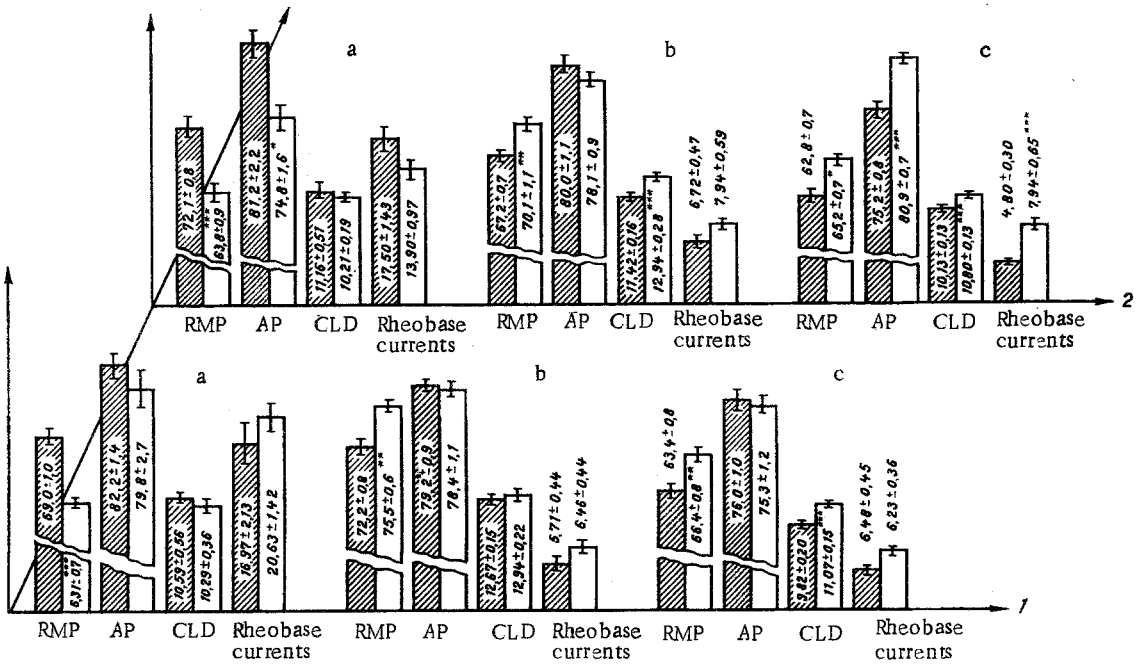

Fig. 1. Changes in parameters of excitability of cytoplasmic membrane of the muscles of mastication during tetanus. a) Masseter; b) anterior belly of digastric; c) posterior belly of digastric muscle. 1, 2) Early (3rd-4th day) and late (10th-15th day) stages after injection of toxin. Values of RMP, AP, and CLD given in millivolts; rheobase currents in nanoamperes. Unshaded columns — experiment, shaded — control. *p < 0.05, **p < 0.01, ***p < 0.001.

The first step was to determine what changes take place in the electrophysiological parameters of the cytoplasmic membrane of the muscle fibers of the rat MM in the course of the local form of tetanus rigidity. It can be seen in Fig. 1a that at the early stages of tetanus poisoning only RMP was reduced, and the remaining parameters were virtually unchanged. At the late stages, however, a significant fall of RMP and of AP was observed, i.e., the typical picture of local tetanus.

During the investigation of the local form of tetanus in DM changes in the electrophysiological parameters in the opposite direction were recorded (Fig. 1b, c), i.e., in the early stages in the anterior belly RMP increased significantly, whereas in the posterior belly both RMP and CLD increased. In the later stages an increase in RMP and CLD was observed in the anterior belly and a significant increase in all parameters (RMP, AP, CLD, and the rheobase currents) in the posterior belly.

These differences in the fibers of the mouth-opening and mouth-closing muscles may evidently arise through strengthening of presynaptic inhibition in motoneurons innervating DM, for it has been found that during artificial stimulation of afferents of the mouth-closing muscles the level of presynaptic inhibition of motoneurons of the mouth-opening muscles always rises and hyperpolarization processes develop in them [11, 12, 14]. The trismus which develops in tetanus is characterized by the appearance of the same processes, but because prolonged hyperpolarization of the motoneurons leads to a prolonged decrease in synaptic bombardment of the fibers of DM, hyperpolarization of the cytoplasmic membrane also develops in them.

The results are thus evidence that injection of TT into the anterior or posterior belly of DM in a dose causing the development of a generalized form of the disease ending in death, if injected into other parts of the skeletal musculature does not cause the appearance of rigidity in the mouth-opening muscles and is not accompanied by the development of disturbances of the parameters of muscle fiber excitability typical of tetanus. This last fact may be evidence that motoneurons innervating the fibers of DM do not lose their neurotrophic effect, evidently because they have not been damaged by tetanus toxin. Since these same motoneurons are characterized by the possession of only excitatory synapses, it can be postulated that the paradoxical resistance of DM to TT is due to the absence of the specific substrate for its action.

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