CHARACTER OF THE INFLUENCE OF THE INHIBITORY NERVE ON THE ADDUCTOR OF THE CLAW IN THE CRAYFISH

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V. G. Ovechkin

Department of Physiology of Animals (Head, Active Member AMN SSSR Professor A. V. Lebedinskii), M. V. Lomonosov Moscow State University
(Presented by Active Member AMN SSSR V. V. Parin)
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The inhibition arising in the skeletal muscles of crustaceans in response to stimulation of specific nerve fibers was discovered long ago [7], and it has not yet been fully explained notwithstanding several investigations [9, 14]. It has been found that the "slow" muscular system is more easily inhibited than the "fast," in which inhibition develops only in response to intensive stimulation of the inhibitory nerve [6, 10, 13].

The "slow" muscle system is represented in its purest form in the abductor muscle of the pincer, whereas, the adductor muscle contains both fast and slow contracting elements. Both pincer muscles are supplied by motor and inhibitory nerve fibers, but most studies of the nature of inhibitory phenomena have been carried out on the abductor muscle. The influence of the inhibitory nerve on the adductor muscle has received less study, even though it was on this muscle that it was first shown that the so-called inhibitory nerve can not only inhibit the contractions of the muscle but also intensify them [1, 2]. These findings were soon confirmed [3], although Western investigators have not yet reported this intensifying influence of the inhibitory nerve.

It has been shown [4, 5] that the intensifying action of the inhibitory nerve on preparations of the Black Sea crab is most clearly revealed in the fast muscular system, but this problem has not been studied in preparations of crayfish.

The object of the present investigation was to study the degree of the intensifying and the inhibitory influences of the "inhibitory" nerve on the "fast" and "slow" systems of the adductor muscle of the crayfish in order to shed further light on the assumed functions of this nerve.

EXPERIMENTAL METHOD

Experiments were conducted on the afferent muscles of the isolated pincers of the crayfish Astacus leptodactilis and Astacus astacus during 1963. The muscle is supplied by two motor nerve axons—one "slow" and the other "fast"—running in a common trunk, and by one inhibitory axon running in a separate trunk [12]. The nerves were carefully dissected in a small bath of physiological saline for fresh-water crustaceans [11], ligated, and placed on two pairs of silver electrodes in a special wet chamber. The nerves were stimulated by rectangular pulses from a type GRAKh-1 stimulator. The recording electrode, made of thin platinum wire, was introduced into the muscle through an opening in the sclerite, and the indifferent electrode was fixed to a stationary gill. A two-channel ac amplifier with a symmetrical input and a special output to a type MPO-2 loop oscillograph was used to record the potentials. The sensitivity of the system was 1 μA/mm. In some experiments, besides recording the potentials on film, the contractions of the muscle were recorded on a revolving kymograph. Depending on the intensity of stimulation of the common motor nerve, monophasic or biphasic potentials were recorded from the muscle fibers, and their amplitude and rhythm changed in response to stimulation of the inhibitory nerve. The duration of stimulation of the inhibitory nerve was 15 sec, and the duration of the testing stimuli applied to the motor nerve was 10-15 sec, at intervals of 5 sec, with a constant frequency and strength within the limits of each experiment.

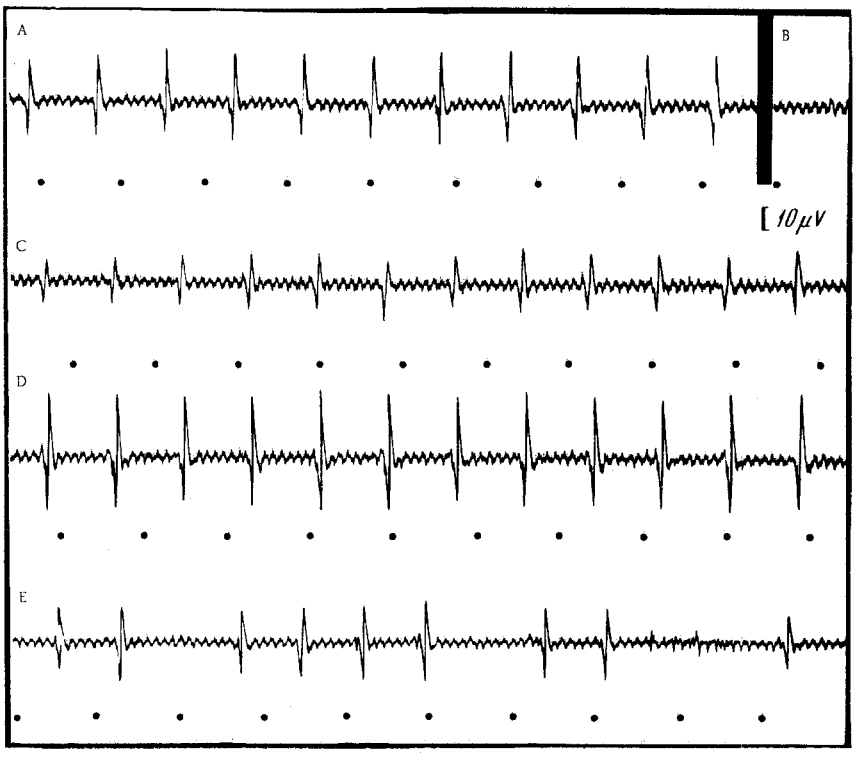

Fig. 1. Influence of the inhibitory nerve on the "fast" system of the adductor muscle. A) Action potentials of muscle fibers during stimulation of the motor nerve (6 cps, 1.5 V); B) stimulation of the inhibitory nerve (10 cps, 1.5 V); C) action potentials 5 sec after stimulation of the inhibitory nerve; D) 15 sec after stimulation; E) incomplete reproduction of frequency of stimulating impulses by muscle fibers after stimulation of the inhibitory nerve by a current of frequency 20 cps and voltage 1.5 V. Dots—time marker (0.2 sec).

EXPERIMENTAL RESULTS AND DISCUSSION

The functional differences between the "fast" and "slow" systems were clearly revealed when the common motor nerve of the adductor muscle was stimulated with different frequencies and voltages. Fast single contractions appeared at a frequency of 2-6 cps and voltage of 1.5-3.0 V, and slow contractions at 6-40 cps and 0.5-2.0 V, depending on the season of the year. In the first case biphasic potentials were recorded from the muscle fibers characteristic of the "fast" systems of crustaceans, and in the second case monophasic potentials characteristic of the "slow" systems.

The results of the study of the influence of the inhibitory nerve on the "fast" system showed that stimulation of the nerve caused distinct changes in the functional state of the muscle fibers. These changes were determined by the frequencies at which the inhibitory nerve was stimulated. At low frequencies (5-10 cps), the influence of the nerve was manifested in the form of the intensification of the bioelectrical activity of the fast contractile elements, as shown by an increase in the amplitude of the potentials by 20-30% (Fig. 1D) by comparison with their amplitude in the background tracing (Fig. 1A). Increased potentials were recorded for 5-10 min, after which they fell to the initial level. Sometimes a slight lowering of the potentials was observed immediately after stimulation of the inhibitory nerve (Fig. 1C), but within 10-15 sec their amplitude returned to the initial level, after which it increased appreciably.

Stimulation of the inhibitory nerve at higher frequencies (10-40 cps) led to depression of the "fast" system, shown initially by a decrease in the amplitude of the potentials, followed by an incomplete reproduction of the frequency of the stimulating impulses (Fig. 1E), and finally, by the absence of the potentials. This last state was defined as profound inhibition, although the duration of the inhibitory state of the "fast" system did not exceed 10 min even with high frequencies of stimulation of the inhibitory nerve.

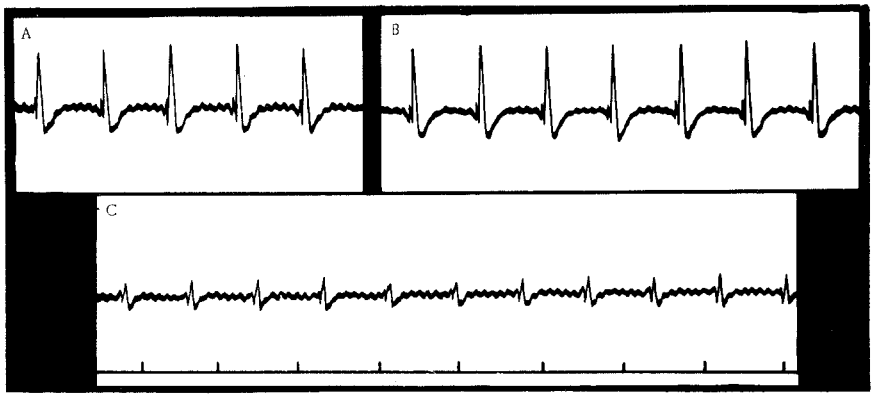

Fig. 2. Influence of the inhibitory nerve on the "slow" system. A) Potentials of muscle fibers in response to stimulation of the motor nerve with a current of frequency 10 cps and voltage 1 V; B) potentials of muscle fibers in response to simultaneous stimulation of the motor and inhibitory nerves at 10 cps, 1 V; C) potentials of muscle fibers in response to simultaneous stimulation of the motor (10 cps) and inhibitory (20 cps) nerves. Below —time marker (0.2 sec).

Some of the oscillograms of experiments in which the influence of the inhibitory nerve on the "slow" system of the adductor muscle was studied are given in Fig. 2. During stimulation of the motor nerve with a current of frequency 10 cps and voltage 1 V, monophasic potentials were recorded from the muscle fibers (Fig. 2A), stable in amplitude and rhythm. If, however, against the background of continuous stimulation of the motor nerve, the inhibitory nerve was stimulated, the character of the responses of the muscle fibers changed. Brief stimulation of the inhibitory nerve (10-15 sec) at a low frequency either had no effect on the amplitude of the potentials or caused it to increase slightly (Fig. 2B), whereas at higher frequencies (20-50 cps), the depressant action of the nerve was observed. At 20 cps and 1.5 V (Fig. 2C), the depression took the form of a reduction of the amplitude of the potentials, while at 40-50 cps total inhibition took place, and lasted for 30 min in some cases. The muscle fibers then again responded with normal excitation potentials to testing stimulation of the motor nerve.

Hence, the "slow" system responded readily to the depressant influence of the inhibitory nerve but hardly responded at all to its intensifying influence, whereas the "fast" system, on the contrary, responded most to the intensifying influence. These results were obtained equally with A. leptodactilis and A. astacus.

The influence of the inhibitory nerve could be demonstrated by the ordinary method of recording the contractions of the muscle on a kymograph Typical results of experiments carried out on the adductor muscles of the pincers of A. leptodactilis (Fig. 3A) and A. astacus (Fig.3B, C) are given in Fig. 3. The slow contractions caused by stimulation of the motor nerve were increased appreciably after stimulation of the inhibitory nerve with a frequency of 20 cps. At 25 cps, however, the depressant action of the inhibitory nerve began to appear, as shown by the fact that the muscle was totally inhibited for 2-3 min. At 40-50 cps the inhibition could last for 30 min, after which the preparation returned to its original functional state.

The intensifying influence of the inhibitory nerve was clearly revealed in preparations from A. astacus, which is less characterized by inhibition, especially in the autumn and winter. In the experiment illustrated in Fig. 3B, the motor nerve was stimulated against the background of continuous stimulation of the inhibitory nerve by a current of moderate frequency and strength. In these conditions the amplitude of the contractions of the muscle often increased considerably, twice or three times greater than the level of the original contractions. Stimulation of the motor nerve at a frequency of 10-15 cps against the background of continuous stimulation of the inhibitory nerve at a frequency of 25-40 cps, on the other hand, led to a decrease in the amplitude of the contractions (Fig. 3C). This slight depressant action of the nerve was often replaced by an intensifying action, but after it had ceased to be stimulated. Characteristically, stimulation of the inhibitory nerve alone did not cause contractions of the muscles (Fig. 3A, B).

The predominantly intensifying influence of the so-called inhibitory nerve on the "fast" system and its depressant influence on the "slow" has not been satisfactorily explained. The intensifying influence has been attributed to its adrenergic action, by analogy with the sympathetic nerves in vertebrates [1]. Although sympatheticomimetic

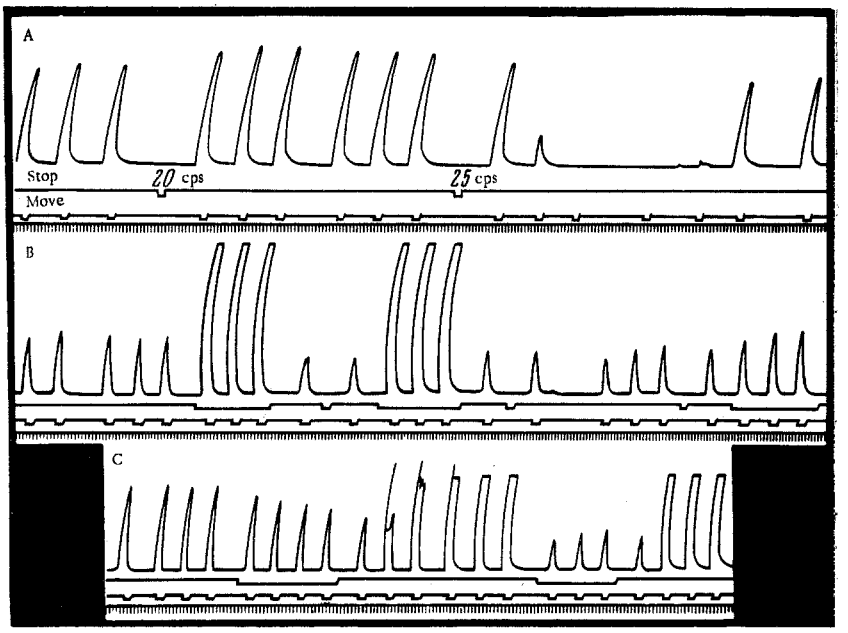

Fig. 3. Influence of inhibitory nerve on contractions of the adductor muscle. A) Stimulation of inhibitory nerve at a frequency of 20 cps and voltage 1 V (increase in strength of contractions) and at a frequency of 25 cps (inhibition); B) increase in strength of muscular contractions in response to stimulation of motor nerve (10 cps, 1.5 V) against the background of continuous stimulation of the inhibitory nerve (10 cps, 1.5 V); C) increase in strength of contractions after depressant influence of the inhibitory nerve stimulated with a frequency of 20 cps, 1.5 V. From top to bottom: contractions of muscle; marker of stimulation of inhibitory nerve; marker of stimulation of motor nerve; time marker (3 sec).

drugs do not fully imitate the action of the nerve on muscles [3], it is possible that the hypothetical mediator of this nerve also is a product of the conversion of one of the amino acids, with an action like that of the adrenergic substances. Otherwise, it is difficult to explain the prolonged intensifying action of the nerve after its single stimulation. Since the intensifying influence of the nerve was clearly exhibited at low frequencies and the depressant at higher, the opposite effects of stimulation of the "inhibitory" nerve may be attributed to the different amount of mediator secreted in these circumstances. In small amounts, the mediator improves the functional state of the neuromuscular apparatus, but in large doses it has a depressant action. Some investigators consider that the mediator increases the permeability of the membranes of the muscle fibers for chloride ions, and in their opinion this is responsible for producing the inhibition [8].

However, the many different influences of the nerve can hardly be reduced simply to manifestations of a change in permeability. The fact that the intensifying influences are most clearly seen in autumn and winter, i.e., after the animals have molted, and the depressant are most marked in spring and summer when ecdysis of the crayfish takes place, indicates that the functions of the nerve are closely related to the animal's functional state, which varies with the season of the year. It is, therefore, possible that the actions of the nerve are not limited to the simple regulation of the rate of movement of certain ions through the membrane of the muscle fiber, but that the whole neuromuscular apparatus is involved and its functional state is changed in accordance with the animals's life cycle. The inhibitory influences on the muscle during ecdysis and the trophic influences between ecdyses are biologically desirable, and the "inhibitory" nerve may therefore be regarded as an auxiliary regulator of the functional state of the peripheral neuromuscular system. This is the more probable because the adductor muscle studied in this investigation consists of a small number of large (up to 1 mm in diameter) muscle fibers which, according to Aleksandrovich (cited in [14]) contain bundles of myofibrils with different functional properties (fast and slow), and also because the organization of the neuromuscular apparatus is based on the presence of a special mechanism of regulation of the contractions.

To reveal the fine mechanism of the influence of the "inhibitory" nerve, further investigations are necessary, more especially because certain authors [10] see an analogy between the myo-neural junctions in crustaceans and the inhibitory synapses of the central nervous system in higher animals.

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