PRESYNAPTIC CHARACTER OF THE PESSIMUM IN THE MOTOR INNERVATION OF SMOOTH MUSCLE

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Pessimal inhibition, discovered by N. E. Vvedenskii, has been studied extensively in nerve-muscle preparations from cold and warm blooded animals. Only by the use of intracellular methods of investigation of the synaptic potentials, however, has it been possible to bring to light some of the concrete mechanisms of development of pessimal inhibition in the nerve-muscle apparatus.

According to the findings of various workers [9-12], inhibition at nerve-muscle junctions is brought about by a decrease in the amount of mediator liberated at nerve endings. On the other hand, it has been reported that inhibition develops as a result of a decrease in the sensitivity of the end plate under the influence of the secreted mediator [5, 13].

The cell structure of smooth muscle has been determined by electron microscopy, and at the points of contact between the autonomic axons and the smooth muscle cells structures resembling synapses have been found [7]. It may be assumed from these facts that one of the above-mentioned mechanisms of synaptic inhibition is present in the nervous apparatus of smooth muscle. Isolated strips of smooth muscles are unsuitable for the study of this problem for they contain ganglion cells; in such cases access to the postganglionic nerves is impracticable.

A convenient object for investigation of the mechanisms of nervous action on smooth muscle and, in particular, for the study of pessimal inhibition, is the retractor penis muscle of the dog. This muscle has a dual innervation. The sympathetic nerve causes contraction of the muscle, and the parasympathetic relaxation. The postganglionic fibers of each system of innervation extend beyond the limits of the muscle. The postganglionic sympathetic fibers originate from the last sacral sympathetic ganglia and their course lies in the pudendal nerve.

EXPERIMENTAL METHOD

Experiments were conducted on dogs under morphine-urethane anesthesia. The postganglionic sympathetic fibers were isolated and placed in buried platinum electrodes. The distal tendinous end of the muscle was attached to an isometric lever, connected to a mechanical system for recording changes in the tension of the muscle. The nerves were stimulated by means of a generator of rectilinear impulses, the output of which had a cascade with a radiofrequency coupling. The duration of the rectilinear impulse was 3-5 msec. The muscle potentials were registered intracellularly by means of glass microelectrodes filled with 3M potassium chloride solution. The resistance of the microelectrodes varied between 10 and 20 MΩ. The microelectrode was connected to a constant current amplifier through an additional input cascade, assembled in the manner of a cathode repeater [1]. The input cascade was fixed directly to a micromanipulator, by means of which the microelectrode was introduced into the smooth muscle cell. The potentials were registered by means of a beam of light, immobile in a horizontal direction, on a moving film. The deflection of the beam upward corresponded to negativity, and downward to positivity of the microelectrode.

EXPERIMENTAL RESULTS

During rhythmic stimulation of the postganglionic sympathetic nerve a gradual decrease in the membrane potential of the smooth muscle fiber was recorded. The critical value of the depolarization of the smooth muscle cell membrane, at which peak potentials developed, varied from 17 to 20 mv [4]. The time taken for the depolarization of the muscle membrane to rise to the critical value depended on the frequency of stimulation of the sympathe-

tic nerve. The duration of development of depolarization varied from 1.5 second at a frequency of stimulation of 1-2 impulses per second to 0.3-0.5 second at a frequency of 15-25 impulses per second (Fig. 1). A gradual increase in the frequency of stimulation to 30-35 impulses per second led to some increase in the frequency of the resulting peak potentials, which did not correspond to the frequency of stimulation of the nerve. The increase in frequency of the peak potentials was usually associated with some delay in the repolarization of each individual junction. Stimulation applied at a frequency of 50-60 impulses per second was pessimal, and led to the cessation of the peak activity and to some degree of lowering of the level of the membrane potential or, more accurately, to a return to the initial level as it was before stimulation (Fig. 2). The change from the pessimal to a slower—optimal—frequency of stimulation was not accompanied by delay in the appearance of the peak potential. Depolarization first developed, and when this reached the critical level, peak potentials appeared.

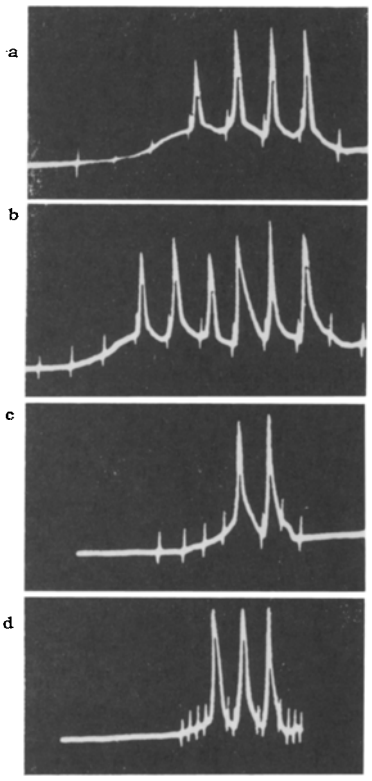

Fig. 1. Rate of development of depolarization of a cell depending on the frequency of stimulation of the sympathetic nerve. a, b) Frequency of stimulation from 3 to 5 impulses per second; c, d) the same from 8 to 20 impulses per second.

Consequently, the changes in the membrane potential of the muscle cell were insignificant during stimulation of the postganglionic sympathetic fibers with the pessimal frequency.

Inhibition of the pulsed activity of the smooth muscle cell during stimulation of the sympathetic nerve with a pessimal frequency differed from the reaction in response to stimulation of the parasympathetic nerve. The inhibitory effect in response to stimulation of the parasympathetic nerve took place through the development of primary hyperpolarization of the membrane of the smooth muscle cell, evidently on account of the direct action of the cholinergic mediator on the excitable membrane of the muscle cell [4].

It might have been assumed that the inhibition of the pulsed activity of the smooth muscle resulted from the fact that the increase in the frequency of stimulation either delayed in some way or other the liberation of the sympathetic mediator or, on the other hand, the excessive liberation of the mediator blocked the excitability of the muscle membrane.

If the second hypothesis is taken to be true, it would be expected that the application of a pessimal frequency of stimulation initially would cause the development of depolarization and the appearance of at least one peak potential. However, the results of the experiments suggest that this is not so. Stimulation with a frequency of 60 impulses per second did not cause depolarization of the membrane or the appearance of peak potentials. Other evidence against the hypothesis that pessimal inhibition is the result of the accumulation of an excess of mediator was provided by the following fact. Application of adrenalin to the surface of the muscle fiber in a dilution of 1×10^{-6} during or immediately after the cessation of stimulation of the postganglionic sympathetic nerve with a pessimal frequency caused the development of depolarization of the muscle membrane and the appearance of a volley of peak potentials, i.e., the same response reaction to the administration of adrenalin was observed as in the absence of stimulation of the sympathetic nerve (see Fig. 2).

These experiments thus showed that pessimal inhibition is not the result of suppression of the excitability of the cell membrane, but rather that it is related to a disturbance of the function of the presynaptic apparatus, leading to a decrease or inhibition of the liberation of the sympathetic mediator.

It was natural to suppose that if the synthesis of the sympathetic mediator was disturbed or its amount in the body reduced, the pessimal reaction in the neuromuscular synapse of smooth muscle must develop in response to the slower rhythms of stimulation. Extirpation of the adrenal medulla is known to lead to a temporary disturbance of synthesis of the sympathetic mediator [2]. The most marked disturbances of the activity of the sympathetic innervation were observed on the 6th, 7th, and 8th days after operation. We therefore carried out experiments on these days after extirpation of the adrenal medulla. The results of the investigations confirmed our hypothesis.

A frequency of stimulation of 10-20 impulses per second caused the appearance of peak potentials, but if the stimulation was continued, after a short time the frequency and amplitude of the peak potentials diminished. The

application of higher frequencies of stimulation—up to 30-40 impulses per second—which were not pessimal in ordinary conditions, led to inhibition of the activity of the cell. The application of adrenalin to the surface of the muscle caused a response reaction of the cell which did not differ from the usual, emphasizing once again the presynaptic character of the reaction observed. Consequently, the disturbance of the synthesis of sympathin is revealed primarily by a worsening of the state of the presynaptic apparatus responsible for liberation of the mediator, as a result of which even low frequencies of stimulation bring about inhibition of the pulsed activity of the cell.

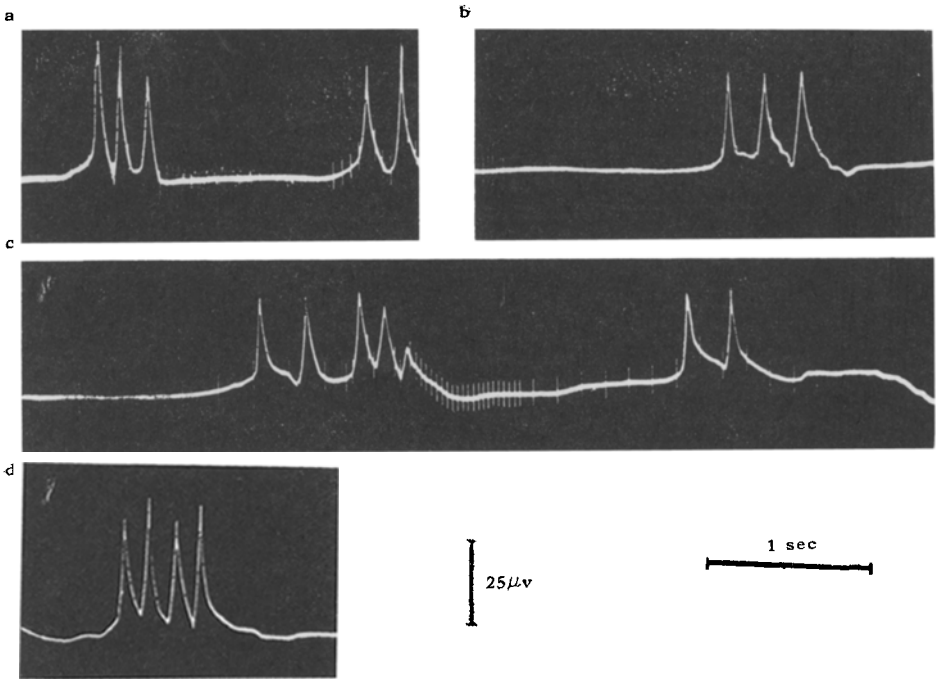

Fig. 2. Response reaction of the cell during stimulation of the sympathetic nerve with a pessimal frequency. a) Development of pessimal inhibition during a change from a slow to a faster frequency; b) pessimal frequency of stimulation initially; c) pessimal response of the cell during disturbance of sympathin synthesis; d) volley of peak potentials of the cell during the action of adrenalin in dilution of 1×10^{-6} immediately after cessation of pessimal stimulation of the sympathetic nerve.

The character of the response reaction of the smooth muscle cell to a pessimal frequency of stimulation of its motor nerve thus suggests that pessimal inhibition is presynaptic in nature. Our results agree with those of other workers [3, 9, 11] maintaining the view that the pessimum in the synapse of skeletal muscle is presynaptic in nature.

It may be assumed that the pessimal frequency of stimulation of the postganglionic sympathetic nerve prevents the liberation of the mediator, which accounts for the absence of depolarization and of peak potentials. Interesting results in this direction were obtained by Robertis and Ferreira [10], who observed under the electron microscope a change in the number of synaptic vesicles in response to difference frequencies of stimulation of the splanchnic nerve. With a frequency of stimulation of 400 impulses per second a decrease in the number of synaptic vesicles was observed. When the frequency of stimulation was 100 impulses per second, the number of vesicles increased. It is probable that at the pessimal frequency of stimulation the delay in liberation of the mediator is associated with its incomplete destruction. According to Brown and Gillespie [6], who studied the output of adrenergic mediator during stimulation of the sympathetic nerves of the spleen, complete destruction of the mediator secreted in response to a single impulse takes 100 milliseconds.

In conclusion, it must be pointed out that in smooth muscle, standing in the scale of evolution below striped muscle, and possessing a dual innervation—excitatory and inhibitory—with its respective chemical transmitters, the presynaptic localization of the pessimum in the motor innervation is evidently a transitional stage to the more perfect form of synaptic function which is found in skeletal muscle.

SUMMARY

Microelectrodes were used to investigate the response of the smooth muscle cells of the m. retractor penis of dog to stimulation of the postganglionic sympathetic nerve. An interruption of the spike activity of the cells in stimulating the nerve by a frequency of 50-60 impulses per second causes no noticeable changes of the membrane potential. Supplication of adrenalin to the cell surface against the background of the sympathetic nerve stimulation with the pessimum frequency provokes a discharge of peak potentials. A suggestion was made on the presynaptic character of the pessimum in the motor innervation of the smooth muscle.

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