PHYSIOLOGY

THE ROLE OF ACETYLCHOLINE IN THE FUNCTIONING OF THE AUTONOMIC NERVE TRUNKS

R. S. Orlov

From the Department of Normal Physiology of the 1st I. P. Pavlov Leningrad Medical Institute
(Chairman – Corresponding Member Acad. Med. Sci. USSR A. V. Kibiakov)

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Calabro [10] was able to establish that during excitation of the nerve trunks,physiologically active mediating substances are released. Certain authors, such as Nachmansohn [13], minimize the role of acetylcholine to mere depolarization of the cell membrane which produces the action current, and enables it to travel along the nerve fiber. The concept that acetylcholine plays the role of a mere depolarizer of the cell membrane has encountered a whole series of objections [6, 9, 11, 12].

As far back as 1935, A. A. Ukhtomskii [8] suggested the hypothesis that mediators of the nerve impulses play the role of humoral "conditioners" preparing the system for a more adequate utilization of the action currents.

In subsequent studies,the physiological role of acetylcholine was investigated by demonstrating the direct effect of acetylcholine or eserine upon the functional state of the nerve trunks. E. B. Babskii and his co-workers [1, 2, 3] showed that large concentrations of acetylcholine caused a prolongation of both the relative and absolute refractory phases and a diminution of the sensitivity of the nerve trunks to stimulation, while small doses exerted just the opposite effects, namely, increased their functional sensitivity. However, experiments in which the concentration of the mediating substance within the nerve tissue has been artificially increased are still not such as to permit a complete visualization of their physiological significance.

In the present study we attempted to clarify the role played by acetylcholine in the functioning of the autonomic nerve trunks, by means of the method which utilized a temporary destruction of the acetylcholine-forming tissue within the organism. Under such conditions we studied the excitability, refractory states and lability of the nerve trunks.

EXPERIMENTAL METHODS

The experiments were conducted on the individual nerve trunks of the sympathetic and parasympathetic systems in the neck region of the cat. For the purpose of investigating absolute and relative refractory states, as well as the phase of reinforcement we used a special generator of paired impulses, so constructed as to make possible independent variation of the amplitude, duration, and time interval between the pulses. For rhythmic stimulation we generated direct impulses with a frequency of from 3 to 800 cps. The action currents were recorded on photofilm with the aid of a two-channel cathode oscillograph type OB-2. The oscillograph had a range of 0.1 to 2000 cps.

The exposed portions of the stimulating and discharging electrodes (made of silver or platinum) were insulated and so prevented from making contact with the surrounding tissues. The action currents were recorded at a distance of 8 to 12 cm from the stimulating electrodes. A grounded silver plate was placed between the stimulating and discharging electrodes, the purpose being to prevent a short circuit. Experiments were carried out on normal, unoperated cats as well as on animals which had been previously subjected to removal of some 60% of their pancreatic gland, in order to temporarily disturb the acetylcholine-forming mechanism of the organism. Such an operation was not observed to upset the functions of islet cells in the body. As controls we used operated animals who received after the operation, along with the eserine, 1-2 cc of a 1 :10,000 solution of acetylcholine. This solution was given intramuscularly beginning with the fourth postoperative day, while in many experiments the acetylcholine was given intravenously one hour before beginning the study.

EXPERIMENTAL RESULTS

In unoperated animals the absolute refractory phase in the sympathetic as well as the parasympathetic cervical nerve trunks lasted from 1.5 to 3.8 σ. The relative refractory phase lasted 8 to 18 σ. The phase of reinforcement, determined by the second stimulus having a threshold value, would make itself manifest 12 to 40 σ after the first impulse. When thresholds were being investigated for stimuli of very short duration, namely of the order of 0.5 to 1 σ, it was found that they had to be quite powerful. For this reason we chose stimuli lasting 3-4 σ, this duration being quite adequate for our purposes. The use of direct impulses of such duration

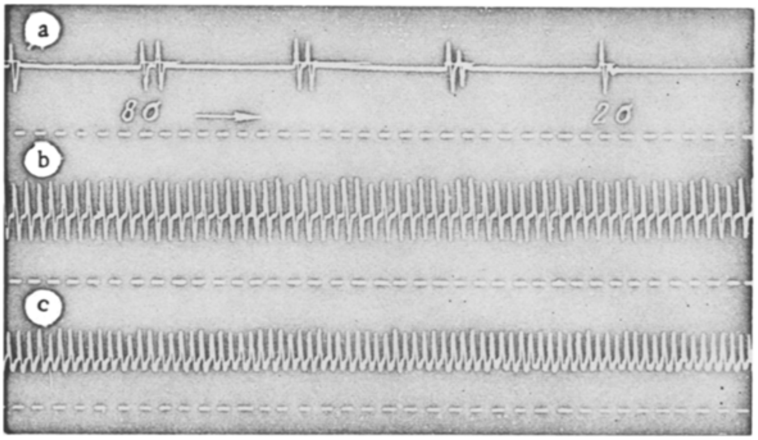

Fig. 1. Action currents in the cervical sympathetic nerve of the cat. Interval between stimuli: a) from 8 to 2 σ. Frequency of stimuli: b) 100 cps; c) 200 cps. Time marker 0.01 second.

produced no alterations in the length of the relative refractory phase. Only when the impulses were lengthened to 5-6 σ could there be noted a change in the time of the relative refractory phase. The threshold of stimulation comprised 4.5-5.5 scale divisions. The maximum stimulatory rhythm which could be carried by the nerve trunks without a labile transformation equaled 200-250 cps. When the frequency of stimulations rose to 300 cps and higher, the rhythm of the action currents became transformed, being accompanied by lower amplitudes of the action currents.

Figure 1,a shows that with intervals between stimuli of 8 σ or less there is observed a lower amplitude from the second stimulus, i.e., the relative refractory phase, which becomes absolute as the interval between the two stimuli is decreased to below 2 σ, there then being no action current produced by the second stimulus. Figure 1,b shows that a frequency of 100 cps gives the highest amplitude of the action currents, while Fig. 1,c demonstrates that increase of the frequency to 200 cps depresses somewhat the amplitude without upsetting the rhythm of stimulation.

The second series of experiments was conducted on 20 cats 6 to 14 days after the major portion of their pancreas had been removed. In previous investigations [4, 5] it had been shown that the greatest disturbances in the acetylcholine balance of the organism occur at this time.

In the operated animals it was found that the absolute refractory phase lengthened to 2.6-6.4 microseconds, compared with the normal 1.5-3.8 microseconds. Also, the relative refractory phase grew from a normal of 8-18 microseconds to 10-25 microseconds.

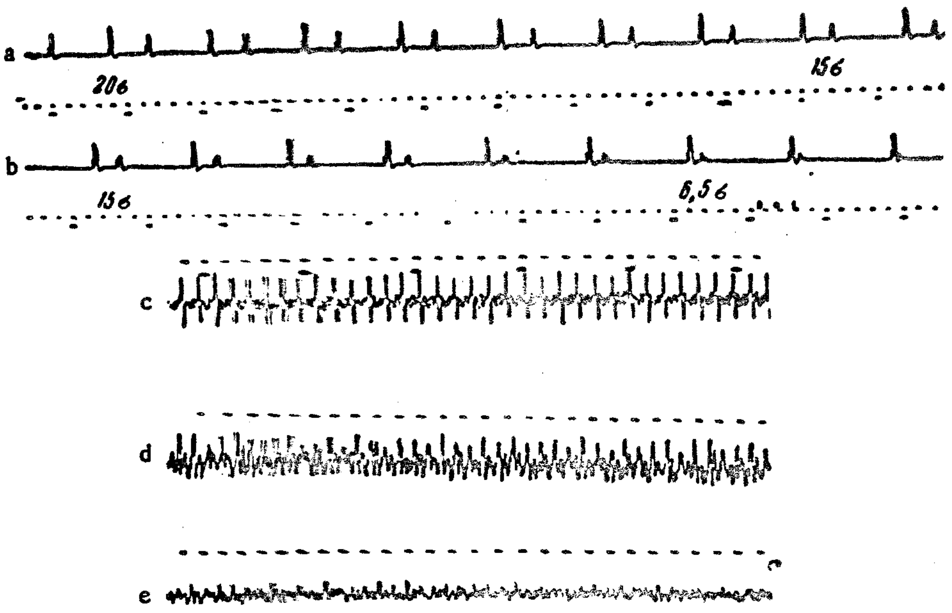

Fig. 2. Action currents in the cervical sympathetic nerve of a cat 8 days following a partial pancreatectomy.
Interval between stimuli: a) from 20 to 15 microseconds; b) from 15 to 6.5 microseconds. Frequency of stimuli: c) 100 cps; d) 125 cps. Time markers (0.01 and 0.05 seconds).

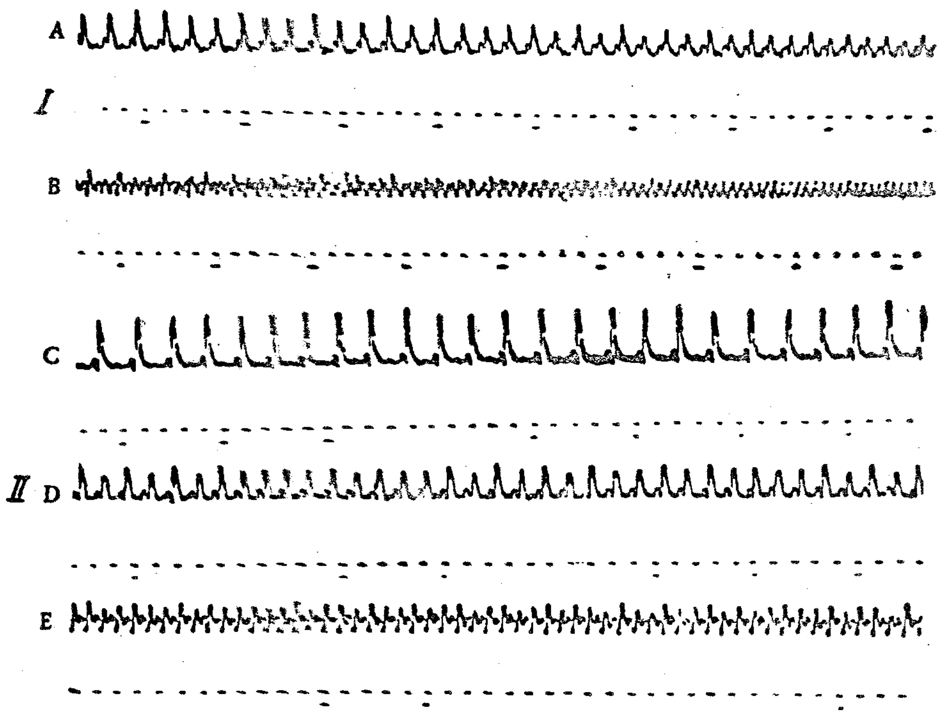

Fig. 3. Action currents in the cervical parasympathetic nerve of the cat 8 days following a partial pancreatectomy.
I) Before introduction of acetylcholine; II) after introduction of acetylcholine.
Frequency of stimulations: B) from 100 to 125 cps; C) 100 cps; D) 125 cps; E) 106 cps.
Time markers 0.01 and 0.05 seconds.

The phase of reinforcement could not be demonstrated in the majority of the experiments. The threshold of stimulation rose by 3-6 scale divisions, while at the same time the stimuli had to be prolonged. The lability of the rhythm fell to 100-110 oscillations, compared to the normal of 200-250 oscillations per second. Stimulation with a frequency of 150 cps would upset the regular rhythm of the action currents and depress the amplitudes up to the point of complete inhibition of the electrical activity of the nerve trunk.

Figure 2 demonstrates the action currents present in the cervical sympathetic nerve of a cat 8 days following the removal of the major portion of its pancreas. The oscillogram in line a shows a diminution of the action current after the second stimulus when the latter follows the first stimulus by 20 microseconds (relative refractory phase), while when the two stimuli are approximate to within 6.5 microseconds of each other, as shown in line b, the action current from the second stimulus disappears altogether (absolute refractory phase). If stimuli have a rhythm of 100 cps, the action currents maintain their regularity as can be seen in line c, but increasing the frequency to 125 cps upsets the rhythm, as can be seen in d, while a further increase to 150 cps leads to infrequent action currents of low amplitude (e).

It can be seen, then, that removal of the major portion of the pancreas affects adversely the functional state of the nerve trunks of the autonomic system. Concretely, these changes consist in a lengthening of the absolute and relative refractory phases, a rise in the threshold of sensitivity, and a depression of the level of lability.

In the succeeding series of experiments the operated animals received daily intramuscular injections of acetylcholine solution with eserine. Under such conditions these experimental animals failed to register any material changes in the state of irritability, length of the refractory phases, or level or lability of the nerve trunks being studied. The absolute refractory phase varied within 2.6 to 5.2 σ. The relative refractory phase was 7.5-14 σ. The reinforcement phase after the first stimulus appeared within 30-40 σ. The threshold of irritability equaled 5-6 divisions of the scale. The maximal rhythm of stimulation, synchronized with the action currents, gave values of 150-200 oscillations per second.

In a series of experiments the operated animals were first tested for the functional state of their nerve trunks and then given a single injection of acetylcholine. The nerve trunks were tested after this injection. In these tests we were able to show a temporary but definite improvement in the functional state of these same nerves. Lability grew and the amplitude of the action currents increased. Figure 3 represents the oscillogram of the biocurrents registered from the parasympathetic cervical nerves of a cat which, 8 days previously, had been subjected to a partial pancreatectomy. When the stimulation frequencies rose from 100 to 125 cps, there could be observed a sharp decrease in the amplitude of the action currents (A). Further increase in the frequency to 150 cps resulted in inhibition of the electrical activity of the nerve (B) (the right side of the film shows only eddy currents). One hour after injecting the acetylcholine (C) the amplitude of the action currents grew with stimulation at a frequency of 100 cps. Increasing the frequencies to 125 (D) and 150 cps (E), while decreasing the amplitude of the action currents, did so to a much lesser degree than seen before the introduction of the acetylcholine.

The conclusion can then be drawn that removal of the major portion of the pancreas leads to definite functional alterations within the nerve trunks. There is observed an increase in the length of both the absolute and relative refractory phases and a shortening (in the majority of instances an absence) of the period of reinforcement. The threshold of reactivity rises while basic lability levels decrease. All this is consistent with a situation in which action currents leave residual effects for a longer time, which is another way of saying that restorative processes have become prolonged. Systematic introduction of acetylcholine into the operated animals compensates the indicated abnormalities. It is instructive to record the fact that introduction of the acetylcholine in to the operated animals improves the functional state of the nerve trunks only as long as they maintain their anatomical ties with the nerve cells. This question has been explored more thoroughly by Kh. S. Khamitov [7] who showed that in the operated animals nerve irritability could be restored to normal either by injecting the acetylcholine intraveneously as long as the nerve trunks maintained their connections with their nerve cells or by injecting the chemical mediator directly into the nerve trunk itself.

Our experiments permit us the conclusion that acetylcholine participates in the functional regulation of the processes determining nerve fiber activities. It seems probable that acetylcholine is one of the components of the current of excitation and that it facilitates the functional transformations of the nervous tissue in such a fashion that conditions for better transmission of the nerve impulses are created, the excitability and level of lability of the nerve conductor being raised.

SUMMARY

The most important indices of the functional condition of the autonomic nerve trunks, i.e., excitability, lability and refractivity, were investigated in normal and in operated animals. Removal of a large part of the pancreas in cats brings about a fall in the level of lability and increased refractivity of the nerve trunks. Acetylcholine abolishes these disturbances.

It is suggested that acetylcholine plays a significant role as a factor promoting change of the functional condition of the nerve tissue, as well as in preparation of the best conditions for transmission of nerve impulses by way of the nerve trunk.

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