THE EFFECT OF DISTURBANCE OF ACETYLCHOLINE METABOLISM ON CATHODE THRESHOLD PARABIOSIS CURVES AND ON THE FUNCTIONAL STABILITY OF THE NERVE TRUNK

V. I. Alatyrev and L. N. Zefirov

Department of Normal Physiology (Head, Professor I. N. Volkova),
Kazan' Medical Institute
(Presented by Active Member AMN SSSR A. V. Lebedinskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 3,
pp. 6-10, March 1963
Original article submitted April 9, 1962

In our previous researches [4, 5, 6] we showed that if the formation of acetylcholine in the body was disturbed by the preliminary operative removal of the pancreas, in addition to the changes taking place in certain functional indices relating to the nerve trunk the time taken for nonconductivity to develop in the nerve during accommodation is shortened and certain changes arise in the typical parabiotic phases.

In the present research we investigated the development of parabiosis of a nerve during experimental impairment of acetylcholine synthesis brought about in various ways. As criteria of the state of parabiosis we used the curve of cathode threshold parabiosis, as defined by L. L. Vasil'ev [1], characterizing the resistance of both the intact nerve and the nerve during the action of alteration, and also the time taken for parabiosis to develop when a standard strength of direct current was applied (D. G. Kvasov's functional resistance [9]).

EXPERIMENTAL METHOD

Experiments were carried out on the ordinary nerve-muscle preparation of the frog (Rana ridibunda). Alteration of the nerve was induced by a dc cathode, using widely separated nonpolarizing electrodes ($Zn-ZnSO_4$). The anode was placed near the remains of the spine, and the cathode 2.5 cm distal to it. The strength of the direct current was graded by means of a rheochord and checked by means of a microammeter included in the circuit. The nerve was stimulated by means of balanced single induction pulses through silver electrodes, situated at an indifferent point between the nonpolarizing electrodes. For the investigation of the changes in threshold cathodic parabiosis alteration began with a current strength of 22 μA. After the development of nonconductivity the direct current was reduced successively every 2-3 min until the minimal level maintaining the state of nonconductivity was reached. The strength of the stimulating induction current remained constant throughout the experiment, at the level of 3 cm above the threshold of stimulation before alteration. The functional resistance of the nerve was determined from the time of onset of nonconductivity during the action of a direct current of standard strength of 15 μA. Maximal induction pulses were used to stimulate the nerve.

Acetylcholine synthesis was impaired by the preliminary (6-10 days before the experiment) operative removal of the pancreas [10] or by treating the nerve with the cholinesterase inhibitor 2-methylnaphthoquinone [7, 13, 14]. In the latter case experiments were carried out on two preparations from the same frog: one was soaked in a 1:10,000 solution of 2-methylnaphthoquinone in Ringer, and the other (the control) in Ringer's solution. More than 150 experiments were performed, 26 on preliminarily pancreatectomized animals and 52 using 2-methylnaphthoquinone.

EXPERIMENTAL RESULTS

Investigation of the changes in threshold cathodic parabiosis in nerve-muscle preparations from intact frogs showed that nonconductivity usually developed 2-3 min, or more rarely 5-6 min, after making the direct current with a standard strength of 22 μA. Thereafter, the minimal strength of current maintaining nonconductivity of the nerve decreased successively. The curve of threshold cathodic parabiosis, plotted from the magnitudes of the threshold blocking current, was regular in character and approximated to the shape of a rectangular hyperbola (Fig. 1, 1). In only one or two experiments, although the threshold depolarizing current fell regularly, the course of the curve was fluctuating.

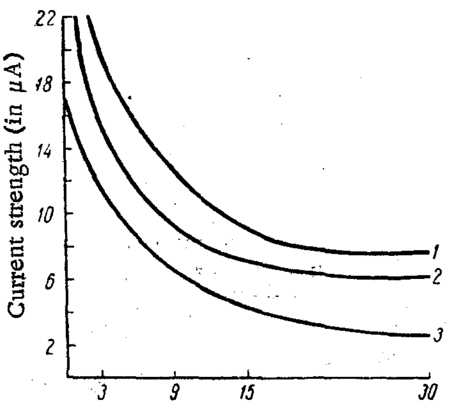

Fig. 1. Curves of threshold cathodic parabiosis of the sciatic nerve of normal frogs (1), on the 6th-10th day after pancreatectomy (2) and after the action of a 1:10,000 solution of 2-methylnaphthoquinone for 60 min (3). Mean values are plotted.

In experiments carried out on the 6th-10th day after pancreatectomy, when the same strength of direct current was used (22 μA) to induce alteration, nonconductivity of the nerve developed more rapidly (after 1-3 min) and, in some experiments, almost instantaneously (within a few seconds). Only in one or two experiments performed on the 10th day after operation did nonconductivity take longer to develop. The lowering of the threshold of blocking during polarization of the nerve took place much more quickly than in the control experiments. For instance, 3 min after making the direct current the average blocking threshold was 15.1 μA compared with 18.9 μA in normal frogs (a decrease to 69 and 86% of the initial value, respectively). After 9 min the minimal polarization was 9.35 μA compared with 13.2 μA in the control (a decrease to 42.5 and 60% of the initial value respectively). After 15 min the threshold strength of current to induce alteration fell to 7.26 μA compared with a normal value of 9 μA (a decrease to 33 and 41% of the initial value respectively), and after 30 min its value was 6.5 μA compared with 7.7 μA in the control preparations (a decrease to 29.5 and 35% of the initial value respectively). The time taken for the blocking current to fall to half its initial value was 6.6 min in these experiments, compared with 12.1 min in normal conditions, or a decrease of almost 50%. Hence, the minimal polarization of the nerve at the 6th-10th day after the operation diminished in value at a more rapid rate and reached lower levels than in control preparations. The curve of threshold cathodic parabiosis correspondingly showed a marked shift downward and to the left, and its fall was steeper than in the curves obtained with preparations from intact frogs (Fig. 1, 2). It should also be noted that after pancreatectomy the decrease in the curve of threshold cathodic parabiosis of the nerve was characterized not only by its greater rapidity and depth, but also by the absence of fluctuations and temporary rises.

Since the effects of preliminary pancreatectomy in cold-blooded animals are primarily associated with disturbance of acetylcholine synthesis, we carried out a series of experiments in which pancreatectomized animals received compensatory injections of acetylcholine. These showed that systematic injection of small doses of acetylcholine (0.2-0.3 ml of a 1:10,000 solution daily, starting on the 3rd-4th day after operation) caused a slight delay in the development of parabiosis, as shown by the later development of nonconductivity and the less rapid fall of the curve of threshold cathodic parabiosis in the initial period of alteration. However, the subsequent course of the curve was similar to that observed in the experiments on the pancreatectomized animals not receiving acetylcholine. Only if large doses of acetylcholine were administered (2 ml of a 1:10,000 solution 2 h before the experiment) were a larger decrease in the rate of development and an increase in the level of the curve of threshold cathodic parabiosis observed.

For the investigation of the action of 2-methylnaphthoquinone (2MN) on the changes in threshold cathodic parabiosis, the nerve-muscle preparation or its nerve was kept before the experiment in a solution of this compound for 1 hr. A control preparation was kept for the same length of time in Ringer's solution. After treatment of the preparation with 2MN nonconductivity of the nerve developed very quickly during the action of a direct current with a standard strength of 22 μA, almost instantly (in the control preparations the time required for this was usually 1-6 min). The same effect was also frequently observed after application of much weaker currents (12-15 μA). The minimal strength of direct current maintaining a state of nonconductivity fell much more rapidly than in the control preparations. For instance, 3 min after making the altering current the average blocking threshold was 11 μA (a decrease to 50% of the initial value), after 9 min – 7 μA (32%), after 15 min – 4.4 μA (20%), and after 30 min – 3 μA (14%). The time during which the strength of the current producing alteration fell to half its initial value was only 3 min. The decrease in threshold alteration after treatment of the nerve with 2MN also required a longer time and was more profound in character. At the end of the first hour the value of the blocking direct current often was lowered to zero, whereas in control preparations it remained considerable. The curve of threshold cathodic parabiosis was correspondingly displaced towards the axis of ordinates, as a result of which the curves of the experimental and control preparations became apparently divergent in character (see Fig. 1, 1; Fig. 1, 3). If the duration of exposure of the preparations to the 2MN solution were increased, the displacement of the curve of threshold cathodic parabiosis was increased still further.

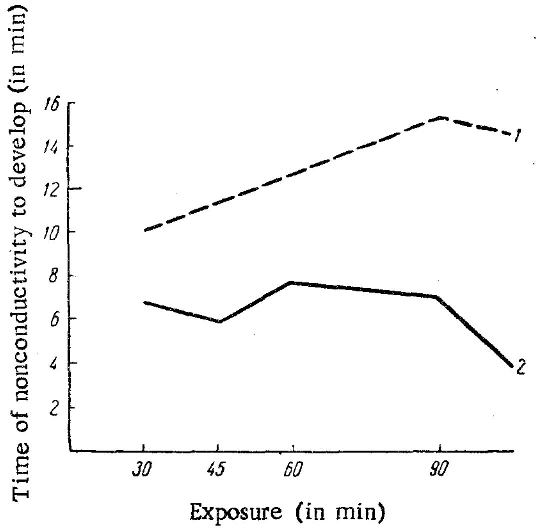

Fig. 2. Rate of development of parabiosis of the sciatic nerve of the frog in control preparations kept in Ringer's solution (1), and after treatment with 2MN in a concentration of 1:10,000 (2). Mean values. Strength of current 15 μA.

Experiments to investigate the changes in threshold cathodic parabiosis showed that when the acetylcholine metabolism was disturbed, nonconductivity of the nerve developed more quickly during the action of a direct current than in the control preparations. To study this effect in greater detail we investigated the time taken for nonconductivity to develop after application of a weaker direct current (15 μA) to produce alteration, and after treatment for a different period with 2MN. It was found that the time for nonconductivity to develop in preparations kept in Ringer's solution was increased, and in preparations treated with 2MN it was decreased. For instance, after treatment with 2MN for 60 min, and using a maximal strength of stimulation, nonconductivity developed after 3-12 min (average 7.7 min), compared with 9-16 (average 12) min in the control preparations, and after treatment with 2 MN for 90 min the corresponding figures were 7.2 min compared with 15.3 min in the control preparations (average values). Subsequently, the rate of development of parabiosis increased sharply and the time taken for nonconductivity to develop fell to a matter of minutes, or even seconds (Fig. 2).

The results showed that preliminary pancreatectomy and treatment of the nerve with 2 MN caused basically similar changes in the rate of development and in the pattern of threshold cathodic parabiosis, the changes caused by 2MN being more marked and depending in degree on the concentration and duration of action of the compound. These changes demonstrate that when acetylcholine synthesis is disturbed the functional resistance of the nerve in relation to the action of factors producing alteration is lowered. Not only the initial resistance of the nerve, but also its resistance in the course of alterations, is lowered, as a result of which the curves of threshold cathodic parabiosis show a more rapid and deeper fall. These findings suggest that the capacity of the nerve for resisting the action of parabiotic factors and for compensating their effect and maintaining its functional state during alteration is considerably diminished, i.e., the adaptation power of the nerve towards alteration (to use L. L. Vasil'ev's terminology [1]) is lowered. This is also demonstrated by the absence of fluctuations in the curve of threshold cathodic parabiosis in the experiments on the pancreatectomized animals, resulting from a temporary increase in the functional state of the altered nerve.

A diminished resistance to the action of parabiotic factors and a lowering of adaptation towards alteration evidently affect all the motor fibers composing the nerve trunk. An increase in the rate of development of parabiosis is observed both in experiments to determine the time of onset of nonconductivity during application of maximal test stimuli, exciting all the nerve fibers, and also when relatively weak test stimuli are used to investigate the changes in threshold cathodic parabiosis, acting on only the most excitable motor elements of the common nerve trunk.

These experimental findings obviously do not support the view that acetylcholine is a factor bringing about alteration [2, 12] or, still more, is responsible for the development of parabiosis of the nerve trunk [3, 8], for a disturbance of acetylcholine metabolism and a decrease in its synthesis cause a more rapid development and a deeper degree of parabiotic changes. The results demonstrate that acetylcholine, formed in the nerve trunk, increases the resistance of the nerve to the action of alteration and, by stimulating restorative, compensatory processes, increases the functional state of the nerve conductor. This effect apparently lies at the root of the action of acetylcholine in abolishing parabiosis of the altered nerve [1, 4, 11].

SUMMARY

In disturbance of acetylcholine synthesis caused by preliminary excision of the pancreas or by treating the nerve with 2-methylnaphthoquinone (a cholinesterase inhibitor) there is an accelerated development of nerve parabiosis under the effect of direct current cathode. The minimal intensity of direct current, maintaining a nonconductivity state, decreases in these conditions more rapidly and reaches lower values than in cases of control preparations. As a result of this the curve of threshold cathodic parabiosis proves to be displaced to the axis of ordinates and to the axis of abscissae. The occurring changes testify to a reduction of the nerve resistance to the action of alteration and decrease of restorative, compensatory processes.

LITERATURE CITED

1. L. L. Vasil'ev. The Importance of N. E. Vvedenskii's Teaching in Neuropathology [in Russian]. Leningrad, 1953.
2. Z. S. Dontsova, Nauchn. dokl. vyssh. shkoly. Biol. nauki, 3, 81 (1960).
3. O. V. Zeidlits, Byull. éksper. biol. 7, 6, 514 (1939); 9, 6, 405 (1940).
4. L. N. Zefirov and A. V. Kibyakov, Fiziol. zh. SSSR, 2, 183 (1954).
5. L. N. Zefirov and O. S. Kochnev, Byull. éksper. biol., 4, 3 (1958).
6. L. N. Zefirov and O. S. Kochnev. In the book: The Physiological Role of Mediators [in Russian], p. 19, Kazan', 1959.
7. L. N. Zefirov and L. V. Tukhvatullina, Byull. éksper. biol., 4, 71 (1960).
8. A. A. Zubkov, Byull. éksper. biol. 6, 5, 595 (1938).
9. D. G. Kvasov, Fiziol. zh. SSSR 34, 4, 471 (1948).
10. A. V. Kibyakov and A. A. Uzbekov, Byull. éksper. biol., 3, 202 (1950).
11. D. A. Lapitskii. The Functional Analysis of Certain Pathological Processes [in Russian]. Leningrad, 1948.
12. P. Nordquist, Acta pharmacol. (Kbh.), 1954, v. 10, p. 46.
13. C. Torda and H. G. Wolff, Proc. Soc. exp. Biol. (N. Y.), 1944, v. 57, p. 236.
14. Idem, Science, 1946, v. 103, p. 645.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.