# THE EFFECT OF ACETYLCHOLINE ON THE RESTING CURRENT OF A NERVE*

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Since acetylcholine formation occurs regularly during the process of excitation in many parts and in particular in myelinated nerve fibers, the question arises as to its possible role in the conduction of impulses along a nerve fiber, and so in turn to the possible electrogenic effects of acetylcholine, i.e. its effect on the electrical properties of the nerve.

According to M.A. Menshikova [11], acetylcholine causes a maintained depolarization of nerves bathed in Ringer's solution. This result does not agree with the findings of Lorente de No [13]. In his experiments, acetylcholine only caused a delay in anoxic depolarization, and a more rapid recovery after it. The polarizing effect of acetylcholine has been observed by E.B. Babsky, A.G. Mershchikov and F.D. Sheikhon [5] in experiments on the circumesophageal commissure of the Anodonta. However, Barnes and Beutner [12] maintain that acetylcholine has no effects on the resting current of a nerve immersed in saline (Ringer), but depolarizes the nerve placed in isotonic glucose. S.D. Kovtun [10] found that acetylcholine had no effect on the resting current. In view of this disagreement we investigated the action of acetylcholine on nerves immersed in Ringer solution and in a solution of glucose.

## EXPERIMENTAL METHOD

The isolated sciatic nerve of the frog (Rana temporaria) was placed lengthwise in an ebonite bath in a special groove. At the bottom of the bath there were holes 1 mm in diameter through which contact with the nerve was made by nonpolarizable Du Bois-Reymond electrodes. The groove of the bath was divided by vaseline partitions into two or three sections. Each section was filled with a solution bathing the part of the nerve investigated. We used Ringer's solution, and isotonic and hypotonic (1.8%) solutions of glucose. The resting current was recorded using a mirror galvanometer (sensitivity 1 mm at 1 m = $0.53 \times 10^{-9}$ A; R = 2065 ohms; $R_{cr}$ = 10,835 ohms; T = 5.9 seconds). The potential difference between two portions of nerve was measured using a compensating arrangement. Acetylcholine was used in concentration of $10^{-3}$ and $10^{-4}$ (in concentrations of $10^{-5}$ it had no effect) and was placed on the nerve by two methods: it was either dissolved before the experiment in the solution, or it was added to it in drops during the experiment.

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## EXPERIMENTAL RESULTS

### I. Effects of Acetylcholine on the Resting Current of a Nerve Immersed in Ringer's Solution

Two of the sections of the bath in which the nerve lay were filled with Ringer's solution. The difference of potential between the two portions of the nerve was measured before and after adding acetylcholine. Acetylcholine in a concentration of $10^{-3}$ was added at first to one, and after a considerable time to the other portion of the nerve. The nerve was treated with acetylcholine either by replacing the Ringer's solution with a solution of acetylcholine in Ringer (10 experiments), or the acetylcholine solution was added in drops to the Ringer solution (10 experiments). Acetylcholine had no effect in any of the experiments.

### II. Effect of Acetylcholine on the Resting Current of a Nerve Immersed in Glucose Solution

The proximal portion of the nerve was immersed in isotonic glucose, and the distal in an isotonic solution of KCL. Acetylcholine was added to the electropositive proximal portion. The change of potential difference was so rapid that we had to abandon introducing acetylcholine together with the solution. Therefore in most experiments of this group, and in subsequent experiments, acetylcholine was added in drops to the solution containing the nerve. In a concentration of $10^{-4}$ it caused a change of potential difference of 1-3 mv which reached a maximum value in 5-10 seconds and fell to zero after 10-15 minutes. In all 8 experiments of this group an increase in positivity was observed. In a concentration of $10^{-3}$ acetylcholine causes a more marked change of potential difference. In some of the experiments both parts of the nerve were placed in the solution and acetylcholine added in turn to each portion. Before the beginning of the experiment the nerve was kept for 60-90 minutes in the glucose solution. Sometimes a positive (Fig. 1) and sometimes a negative (Fig. 2) effect was produced and quite frequently the two effects could be observed on different parts of one and the same nerve (Fig. 3). Out of 100 experiments carried out to investigate this twofold action of acetylcholine, negative changes were found in about 10%, and positive changes in about 90% of the cases. The maximum positive change was 28 mv and the maximum negative – 21.7mv. On the average the change in potential difference was 8-10 mv. The change occurred immediately after adding the acetylcholine and reached a maximum value after 5-30 seconds or more; the return to the initial potential difference was 80-90% complete in approximately the same time; this initial potential difference was completely re-established after 5-10 minutes, or in some cases after 20-30 minutes. In most of the experiments the change in the potential difference ended by reaching approximately the initial value. However in certain cases the change proceeded further in the direction opposite to the initial one. In control experiments a drop of sodium chloride solution was added to the nerve immersed in the glucose solution. An average positive potential change of 3 mv was produced (16 observations).

### III. Investigation of the Twofold Effects of Acetylcholine

#### a) Dependence of the effect of acetylcholine on the time of immersion of the nerve in the glucose solution.

The nerve was immersed in a glucose solution. The initial potential difference was determined. Immediately after this acetylcholine was added first to one portion, and then after a definite interval of time to the other. From 38 observations made during 21 experiments a definite relation was found between the absolute value and the sign of the effect of acetylcholine (whether positive or negative) and the time of immersion of the nerve in the glucose solution. In 20 out of these 38 observations a time of from 1 minute to 6 minutes elapsed between the immersion of the nerve in the glucose solution and the action of acetylcholine on it. A positive effect was found in 12 cases; the maximal positive effect was 22.5 mv, the minimum – 0.3 mv, the average – 6.2 mv; an electronegative effect was found in 8 cases, the maximum value was 15.5 mv, the minimum – 1.4 mv, and the average – 4.9 mv.

In the remaining 9 of the 38 observations, the time interval between immersing the nerve in the glucose solution and the action of the acetylcholine was 10-34 minutes; a positive effect was found in 8 cases, the

maximum being 17.5 mv, the minimum – 1.0 mv and the average 7.1 mv; a negative effect of 16.5 mv was found in one case.

In the remaining 9 cases, the time between the immersion of the nerve in glucose solution and the addition of acetylcholine was 70-195 minutes; the maximum positive effect was 34 mv, the minimum – 13.8 mv, and the average – 23.3 mv; no negative effect was found, the change being positive in every instance.

From these results we concluded that the longer the nerve remains in glucose, the greater is the positive potential developed, and the fewer are the cases where a negative effect is produced in response to acetylcholine. It seemed probable that the electronegative effect of acetylcholine is associated with the condition of the nerve in the early stages, and the electropositive with the later stages of immersion in glucose solution. Actually, in 13 of the 17 experiments in which the interval for the first portion of the nerve treated with acetylcholine was equal to 1 minute, and that for the second 2-6 minutes, a negative effect was found. In the case of a treatment with acetylcholine repeated after 30-40 minutes, a positive effect only was produced. In a careful study of the action of isotonic glucose on the resting current we found that a short electropositive phase lasting a few minutes preceded a steady electronegative phase; in these experiments one part of the nerve was placed in Ringer and the other in glucose solution. However in subsequent experiments it was found that the sign of the acetylcholine effect did not depend on whether or not a positive effect had occurred in the glucose solution.

In several experiments the nerve was placed in a hypotonic glucose solution. In these cases too, a double acetylcholine effect was found, i.e. both positive and negative potentials were developed.

b) Action of acetylcholine on the dead nerve

Experiments in which it was shown that acetylcholine exerts only an electropositive effect on nerves kept for a long time in glucose solution, suggested that in the glucose solution considerable changes in the functional condition of the nerve take place, and that these are due to the passage of ions out of the nerve. The question then arose as to how a nerve which had suffered irreversible changes, i.e. a nerve which had been killed, would react to acetylcholine. To this end a nerve was placed for 30 seconds in boiling Ringer, and then kept in glucose solution for several minutes. Acetylcholine was placed in succession on both portions of the nerve. No negative effect was found in any of this group of experiments. At the same time the effect of acetylcholine was approximately twice as great (in several experiments about 50 mv, on average – 20 mv), and the positive effect developed and ended more rapidly than in the living nerve. Within the limits of accuracy possible with such large fluctuations, the potential difference returned to its original value after the action of acetylcholine.

## DISCUSSION

We were not able to confirm the results of M.A. Menshikova on the depolarizing action of acetylcholine, and concluded that acetylcholine does not affect the resting current of a nerve immersed in Ringer. The same conclusion was reached previously by Lorente de No and Barnes and Beutner. According to Netter [15], the electrogenic effect of substances is greatly reduced by immersing the nerve in saline. In studying the action of acetylcholine on a nerve immersed in a salt-free medium, Barnes and Beutner found a depolarizing effect. In similar experiments we found that the effect could be either negative or positive.

In many experiments designed to reveal the part played by acetylcholine in nervous activity, the double action of acetylcholine occurs. It is well shown in the action of acetylcholine on the reflex function of the nerve centers [3,4,7,8], and on their electrical activity. We found this same double effect of acetylcholine in the present investigation. From our results it appears that the recorded changes in the demarcation potential, due to the action on the nerve of acetylcholine, can at any one moment be considered to be made up of the sum of two components: the electropositive and the electronegative. One or the other of these two components may preponderate according to the time at which the acetylcholine acts on the nerve immersed in the glucose solution. It is abundantly clear that the nerve thus immersed undergoes changes in its functional condition which show up in a study of the demarcation potentials as a predominance of the positive acetylcholine effect. This conclusion is indirectly confirmed by the results of the experiments on the action of acetylcholine on the killed

nerve, in which there was no electronegative effect, and the positive effect was twice as great. We conclude that the electronegative effect of acetylcholine is characteristic of the living nerve only, and that it has a functional significance.

Our results on the double action of acetylcholine not only disagree with those of Barnes and Beutner, but also are opposed to the theory of Nachmansohn [14] on the exclusively depolarizing role of acetylcholine. Here we reach the same conclusion which was stated previously by E.B. Babsky [6] and A.G. Ginetsinsky and Z.I. Barbashova [9].

Further investigation is required of the problem of the nature of the changes which occur in the nerve when immersed in isotonic glucose, and which determine the sign of the acetylcholine effect.

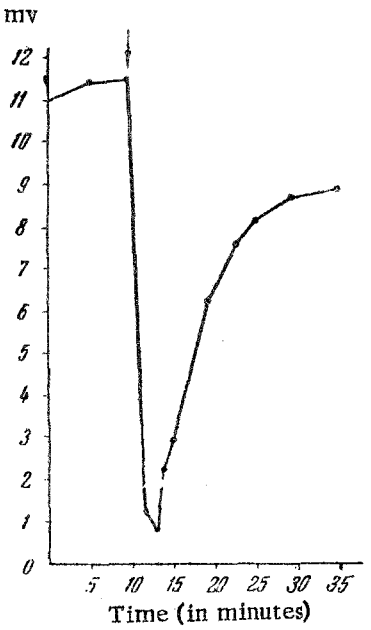


Fig. 1. Positive electrical change caused by acetylcholine (↓).

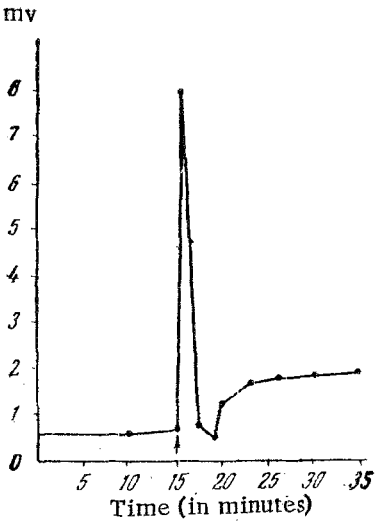


Fig. 2. Negative electrical change caused by acetylcholine (↓).

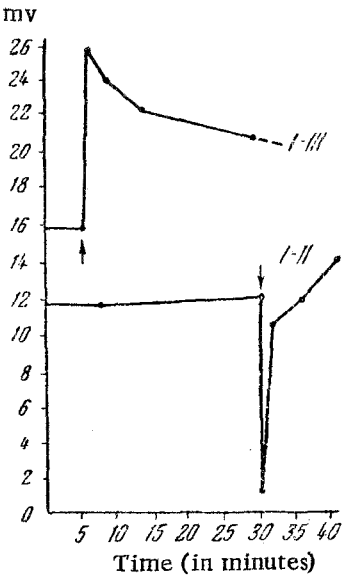


Fig. 3. Electropositive (leads I and II) and electronegative (leads I and III) effects caused by acetylcholine on different parts of the same nerve.

SUMMARY

It was established in experiments on Rana temporaria that acetylcholine has no effect on the demarcational potentials of the nerve, immersed in Ringer solution. Acetylcholine in $10^{-4}$, $10^{-3}$ concentrations acts on the nerve immersed in glucose solution and causes the change of demarcational potentials. Development of either electronegativity or electropositivity takes place in these conditions. The effect of acetylcholine depends on the duration of immersion of the nerve in the glucose solution. The longer it is soaked in this solution –the more the absolute value of electropositivity and the less the number of cases with electronegative effect. Electronegative effect produced by acetylcholine is the inherent property of the live nerve.

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