ANALYSIS OF THE HUMORAL BASIS UNDERLYING PERIODIC ALTERNATION OF CARDIAC INHIBITION AND STIMULATION ON PROLONGED STIMULATION OF THE VAGUS

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Present day data on the mechanism of the vagal effect on the heart indicate that the vagus can not only inhibit but also, under certain conditions, stimulate cardiac activity. Detailed studies of such dual influence of the vagus has led many scientists to conclude that the different nature of these different vagal effects is associated with secretion of chemically active substances during its stimulation [5, 6, 7, 8, 9, 10, 11].

In the light of the problem being considered in the present work of great interest are the researches of L. Went, E. Szucs and E. Hetenyi [10], L. Went, E. Szucs, T. Kovacs [11], M. Szentivanyi, A. Kover [12] and others which deal with the interrelations of acetylcholine and adrenaline. It has been shown that prolonged action of acetylcholine can promote liberation of adrenaline in the heart, i.e., acetylcholine stimulates cardiac activity by way of liberation of its antagonist – adrenaline. In the analysis of the humoral basis of vagal action on the heart a study of the Sechenov-Mechnikov phenomenon is especially relevant; this phenomenon consists of the fact that prolonged stimulation of the vagus (up to 40 minutes) elicits periodic changes in cardiac activity manifested by alternating inhibition and restoration of cardiac activity.

The present work is concerned with an attempt to find the answer to the question of whether the periodic alternation of inhibition and stimulation of cardiac activity observed on prolonged vagal stimulation is associated with a periodic appearance in the heart of chemically active substances which act differently on rhythmic cardiac activity during the different phases of vagal effect.

METHODS

All the present experiments were performed in accordance with the following scheme: three Straub-isolated frog (Rana temporaria) hearts were used. One of these – the donor heart – retained its connection with the medulla oblongata which was subjected to prolonged electric stimulation. It must be noted that both cardiac reactions (inhibition and restoration) occurred under identical experimental conditions, viz., with the same strength and frequency of stimulation. The heart was perfused with Ringer's solution; during definite phases of periodic cardiac activity under conditions of prolonged vagal stimulation the perfusate from the donor heart was transferred to the solution maintaining the recipient heart. Two recipient hearts were used in the experiment: one served as a testing agent for the phases of inhibition of the donor heart, the other for the phases of its restoration to full activity.

RESULTS

In the first series of experiments an attempt was made to clarify the question of whether it was possible for the Sechenov-Mechnikov effect to be mediated humorally. The results of these experiments are given in Fig. 1.

As kymogram I shows, prolonged stimulation of the vagus (31 minutes) elicits periodic alternation of inhibition and restoration of cardiac activity. Cessation of stimulation abolishes all forms of periodic activity.

Kymograms II and III show the results of experiments analyzing the possibility of "humoral transmission" of the periodic alternation of inhibition and restoration of cardiac activity upon prolonged stimulation of the vagus. As can be seen from kymogram II, the perfusate taken from the donor heart during a period of inhibition (a, c) causes a negative inotropic reaction in the recipient heart. Thus, perfusate (a), taken at the moment of the first arrest, diminishes the amplitude of cardiac contractions by 20%. Kymogram III shows the changes observed in the recipient heart treated with the perfusate collected from the donor heart during the phases of restoration (b, d, e). This perfusate causes a positive inotropic reaction in the recipient heart. Analogous data were obtained in all the other experiments of this series. The results thus indicate that the periodic vagal effect seen on prolonged stimulation of the nerve is accompanied by a periodic secretion of chemical agents which exert alternate inhibitory and stimulating influences on the heart.

The second series of experiments was devoted to a pharmacologic analysis of the nature of the substances liberated during the different phases of the periodic effect exerted on the heart by prolonged vagal stimulation.

The study of this question was begun by investigating the role of acetylcholine in the periodic appearance of inhibitory phases of cardiac activity on prolonged vagal stimulation. Experiments with proserine* and atropine were therefore carried out.

Action of proserine. These experiments were based on the hypothesis that proserine, being anticholinesterase preparation, should enhance the effect of the inhibitory substance appearing in the donor heart during the inhibitory phase if this substance were in fact acetylcholine. The experiments were performed as follows: proserine was introduced into both recipient hearts (concentration 1×10^{-5}). Perfusate taken from the donor heart during inhibitory and restorative phases was then tested on these hearts. The results obtained in this series of experiments are given in Fig. 2.

Kymogram III shows that proserine promotes a considerably more profound negative inotropic effect of perfusate collected from the donor heart during phases of inhibition (a, c, e, f, h). For instance, perfusate (a), taken at the moment of the first arrest of the donor heart, decreases the amplitude of cardiac contractions by 90% with simultaneous slowing of the rhythm by 25% as compared with normal; in the second phase of inhibition of the donor heart the perfusate collected from it (c) again exerts strong inhibition on the recipient heart, decreasing the amplitude of cardiac contractions by 62.5% this time. Analogous data were obtained throughout the duration of the whole experiment (kymogram III, e, f). Analysis of the effect of perfusate collected from the donor heart during the phase of restoration (Fig. 2, kymogram II, b, d, g) revealed that the response reaction of the recipient heart was unaltered; the perfusate in this case elicited the usual positive inotropic effect.

Treatment of recipient hearts with an anticholinesterase preparation – proserine – thus leads to marked deepening of the effect exerted by the "inhibitory" substance liberated by the donor heart during the phases of cardiac arrest. The effect of perfusate taken from the donor heart during the inhibitory phases is increased four-fold as compared with the effect of the same perfusate on the recipient heart which had not been treated with proserine; at the same time the effect of the "restorative" perfusate elicits the usual positive inotropic cardiac reaction. This fact appears to the present authors to indicate that periodic arrest of myocardial activity associated with prolonged vagal stimulation depends on the presence of acetylcholine. During the phases of restoration substances different in nature are probably liberated; this question is to be considered more fully below.

In the next series of experiments an attempt was made to discover the effect of atropine on the response of the recipient heart to the action of perfusate from the donor heart; atropine was chosen as a substance possessing properties abolishing the effect of acetylcholine on the one hand, and not interfering with the action of adrenaline on the other [4].

The effect of atropine. The experimental conditions were the same as in the preceding series except that the recipient hearts were treated preliminarily with atropine (concentration 1×10^{-5}).

Atropine abolishes the negative inotropic effect: perfusate taken from the donor heart at the moment of the first arrest does not alter the amplitude of contractions of the recipient heart (3.7-3.6 cm); using perfusate taken at the moment of the second arrest also leaves the amplitude of recipient heart contractions unchanged (3.5-3.5 cm).

* Russian trade name.

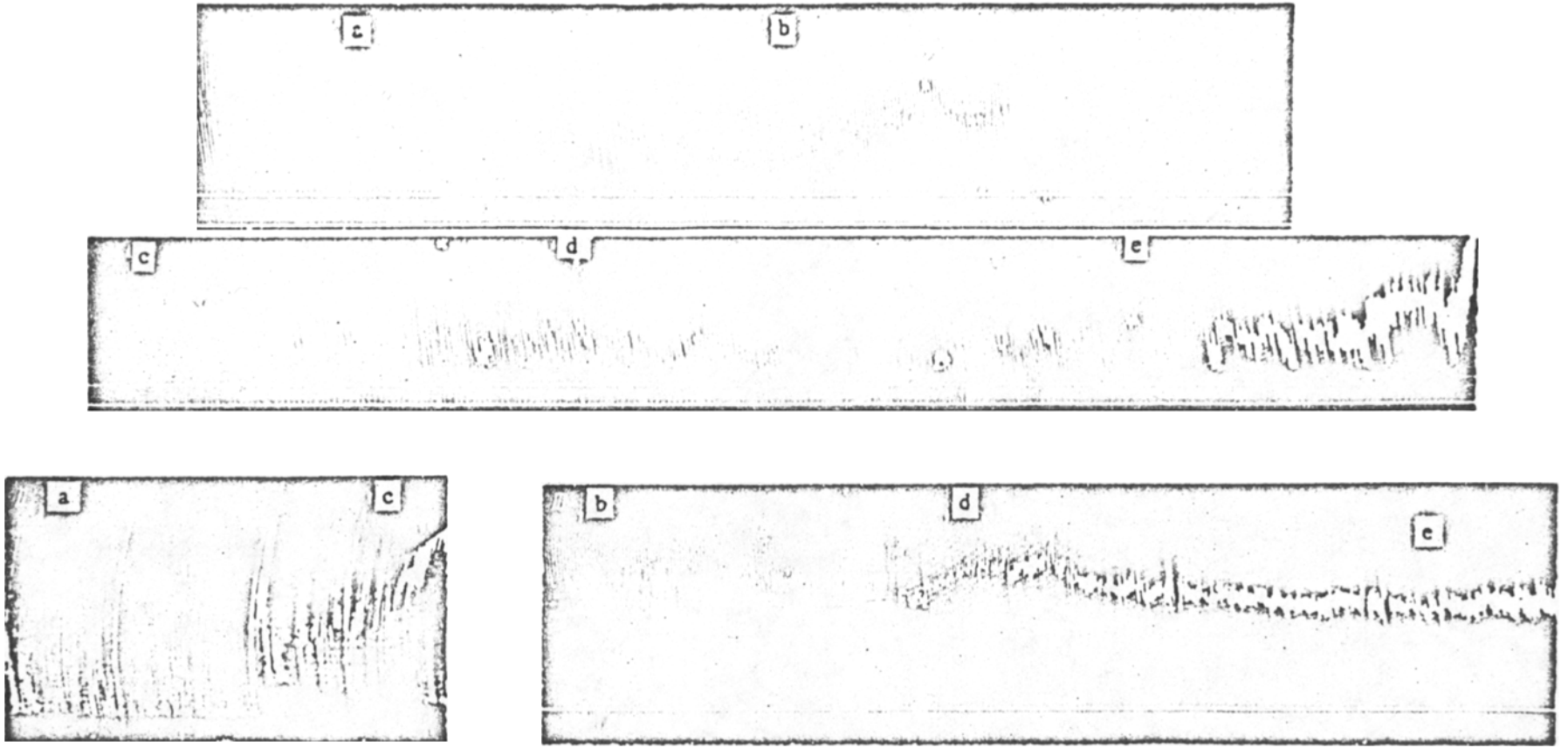

Fig. 1. I) Periodic alternation of inhibition and restoration of activity of the donor heart on prolonged vagal stimulation (duration of stimulation 31 minutes, strength 6 v, frequency 20 cps; II) changes in activity of the recipient heart receiving perfusate from the donor heart during the inhibitory phases; III) changes in activity of the recipient heart receiving perfusate from the donor heart during the restorative phases. The time marker is common for the recipient hearts; letters and arrows in all cases denote the moment of perfusate withdrawal from the donor heart and its transfer to the corresponding recipient hearts.

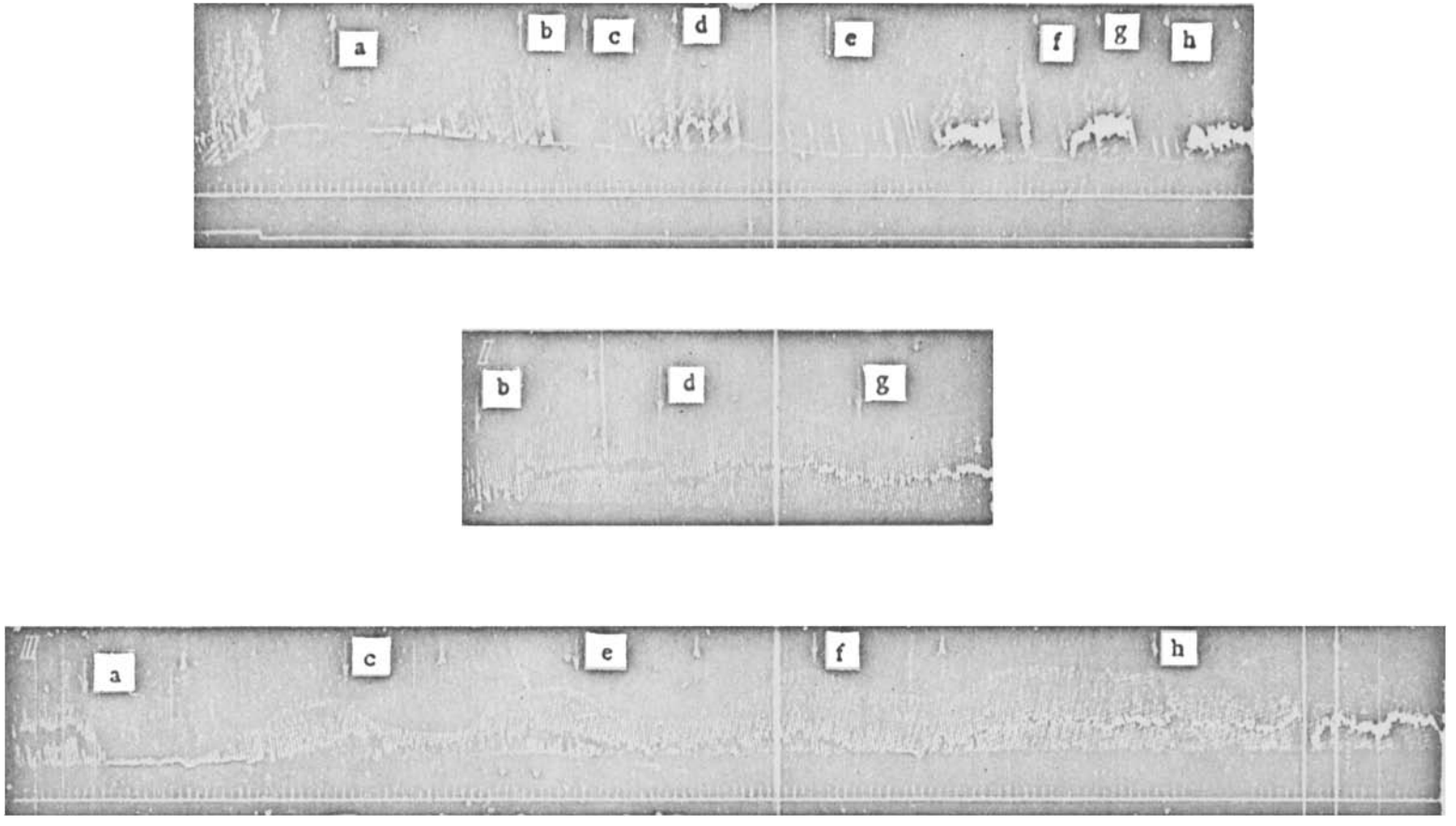

Fig. 2. I) Periodic alternation of inhibition and restoration of the activity of the donor heart on prolonged vagal stimulation (duration — 12 minutes, strength 5 v, frequency 20 cps; II) changes in the recipient heart activity on receiving perfusate from the donor heart during phases of restoration; III) changes in activity of recipient heart receiving perfusate from the donor heart during phases of inhibition. Both recipient hearts treated preliminarily with proserine (1×10^{-5}).

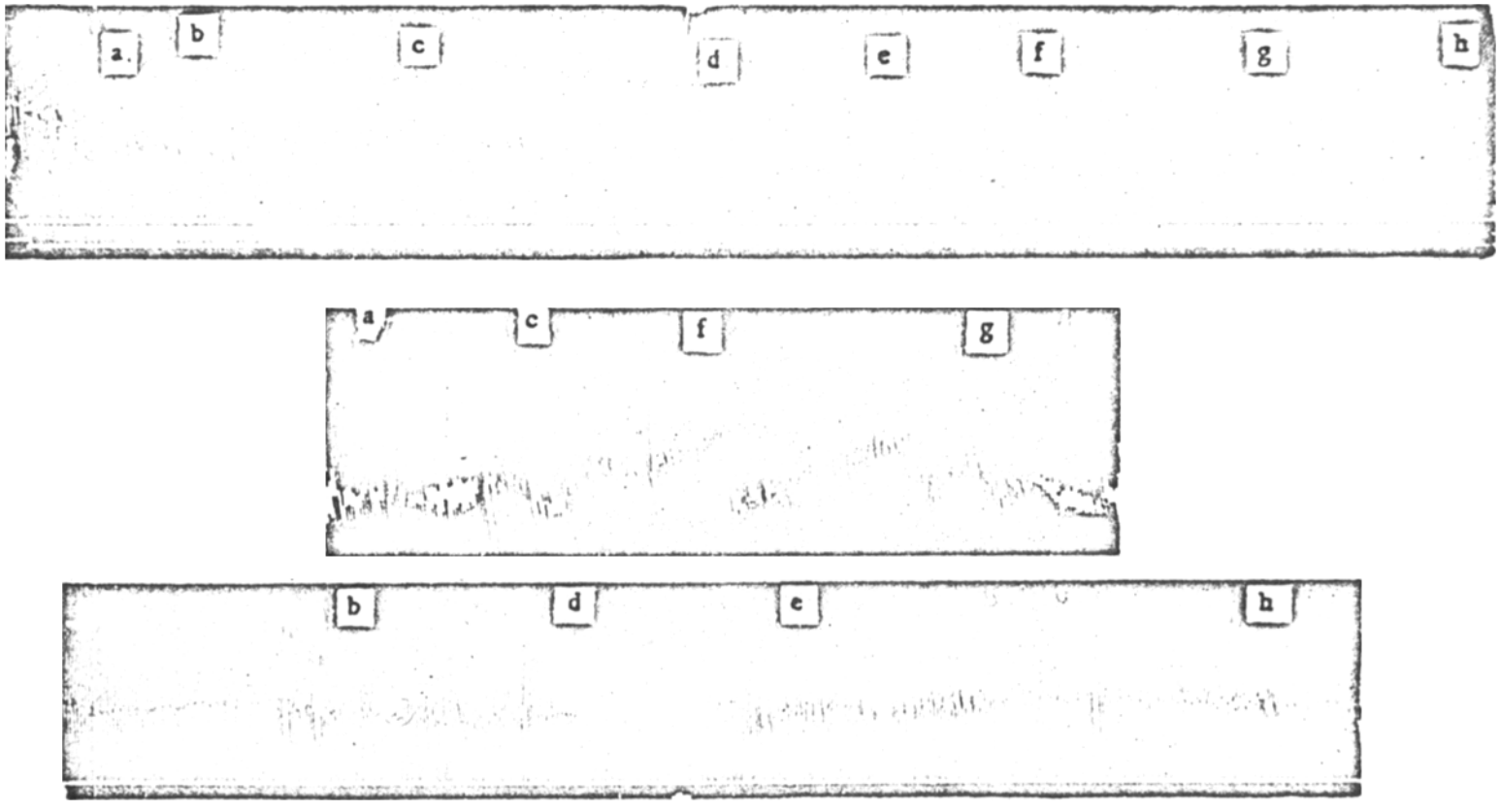

Fig. 3. I) Periodic alternation of phases of inhibition and restoration of the donor heart on prolonged vagal stimulation; II) changes in the activity of recipient heart receiving perfusate from donor heart during restorative phase; III) changes in recipient heart activity upon receiving perfusate from donor heart during inhibitory phases. Both recipient hearts subjected to preliminary treatment with dibenamine (1×10^{-5}).

Examination of the action of perfusate collected from the donor heart during the phase of restoration revealed that the response of the recipient heart was usually positively inotropic in character.

Treatment of recipient hearts with atropine thus leads to abolition of the negative inotropic effect caused by perfusate collected during the phases of inhibition of the donor heart, while the reaction of atropinized recipient hearts to the action of "restorative" perfusate preserves the usual positive inotropic character of the response.

It follows from all the experiments carried out that during the different phases of cardiac activity under the influence of prolonged vagal stimulation substances of different nature are apparently liberated. This conclusion is supported by the fact that both proserine and atropine change the character of the effect exerted by the substance liberated during the inhibitory phase but do not change the effect of the substance secreted during the restorative phase of the donor heart. The data cited above suggest that the agent acting during the inhibitory phases is acetylcholine, while during the restorative phases adrenaline-like substances are most probably liberated. To test this hypothesis a series of experiments with dibenamine – an antisympathetic and anti-adrenaline agent were performed.

The effect of dibenamine. Experiments with dibenamine were carried out as follows: dibenamine (concentration 1×10^{-6})* was introduced into both recipient hearts and the action of perfusate from the donor heart collected during the phases of inhibition and restoration was then tested on these.

As can be seen from the kymogram (Fig. 3. kymogram III, b, d, e, h) dibenamine abolishes the usual positive inotropic effect of perfusate collected from the donor heart during the inhibitory phase. Thus, perfusate (b), taken at the moment of the first period of restoration of the donor heart does not exert the usual positive inotropic effect; on the background of dibenamine action a similar effect is produced by perfusate taken from the donor heart during subsequent phases of restoration – b, d, etc. Examination of the action of perfusate collected from the donor heart during inhibitory phases (Fig. 3, kymogram II, a, c, f, g) showed that the response of dibenamine-treated recipient heart was the same as that of a normal recipient heart: the perfusate in this case evoked the usual negative inotropic effect.

Treatment of recipient hearts with an anti-adrenaline agent – dibenamine – thus leads to the abolition of the positive inotropic reaction occurring in response to the introduction of perfusate collected from the donor heart during restorative phases. The slight diminution in the amplitude of cardiac contractions as compared with the normal, observed in these experiments can be explained either by the presence of a certain amount of acetylcholine in admixture with the adrenaline-like substances in the "restorative" perfusate and the inactivation of these substances by dibenamine with consequent "unmasking" of the action of admixed acetylcholine (Baker, 1954), or by a distorting effect exerted by dibenamine on the action of adrenaline (Zakusov, 1953).

As a control, all the experiments mentioned above were performed on hearts which had been freed from the influence of the sympathetic nervous system. Experiments with such hearts (on the 15th day after denervation) showed that prolonged vagal stimulation, in the absence of sympathetic nervous system influences, was associated with periodic alternation of inhibitory and restorative phases, with "humoral transmission" of this effect.

These experiments, therefore, lead to the following conclusion: functional, periodic changes in the heart muscle which accompany prolonged vagal stimulation (uniform in strength and frequency) are determined by the periodic appearance of chemical substances of different nature: acetylcholine during the phases of cardiac inhibition and adrenaline-like substances during the phases of restoration.

We cannot as yet, on the basis of our data, determine the mechanism of the appearance of these substances during definite phases of cardiac activity. In accordance with the data found in literature [10, 11] the most likely hypothesis is that acetylcholine, which is liberated during stimulation of the vagus, upon reaching a certain degree of accumulation and a certain stage of interaction with the physico-chemical receptor systems promotes the liberation of substances with a stimulating action similar to that of adrenaline-like agents. Acetylcholine, in the opinion of the Oxford school of pharmacologists [5] plays the role of regulator of the rhythmic activity of the heart; it probably plays this part during prolonged vagal stimulation also when periodic alternation of phases of inhibition and restoration of rhythmic cardiac activity are observed.

* This value contradicts the one given in Fig. 3 caption. – Publisher.

SUMMARY

Prolonged stimulation of the vagus nerves (up to 40 minutes) evoked periodic changes in the activity of the heart muscle. It is manifested by the interchange of phases of inhibition and recovery of heart activity (Sechenov-Mechnikov phenomenon).

Experiments were performed on hearts of frogs (Rana temporaria). It was established that the functional periodicity in the activity of the heart, which was observed under conditions of prolonged stimulation of the vagus nerves is associated with periodic appearance of chemically active substances of different origin in the heart. Thus, the periodic appearance of phases of inhibition is connected with periodic secretion of acetylcholine, while the periodic apperance of phases of recovery — with periodical secretion of adrenaline-like substances.

It is probable that acetylcholine plays a regulating role in cardiac activity, promoting liberation of adrenaline-like substances under conditions of prolonged stimulation of the vagus nerve.

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