PHYSIOLOGY

IONO-HUMORAL INTERRELATIONS IN THE ORIGIN AND SPREAD OF VAGAL INHIBITION OF THE HEART

Article II

THE EFFECT OF ACETYLCHOLINE AND K-IONS ON THE NORMAL AND ATROPINIZED FROG'S HEART

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(Received December 29, 1954. Presented by M. S. Vovsi, Active Member of the Academy of Medical Sciences, USSR)

During the development of vagal inhibition of the heart, the tissues secrete acetylcholine [19], and the K-ion concentration in the perfusate is increased [6, 11, 15, 18, 23]. For this reason, physiologists believe that the development of a vagal effect is determined by the ultimate effect of either acetylcholine or K-ions on the cardiac muscle.

Both seem probable, since the acetylcholine on the heart is attended by K-ion secretion (Lehnartz, [17] – turtle heart; Holland, [14] - guinea pig auricles), and acetylcholine appears when the concentration of K-ions increases in a heart perfusate (Beznak [7]). Physiologists base the association of vagal inhibitory development with the ultimate effect of K-ions or acetylcholine on the phenomenological similarity of the effects caused by vagus nerve stimulation and by the action of these substances extraneously introduced. This is probably the reason why there are so many works studying the "inhibitory" effect of acetylcholine and K-ions.

Actually, such an important sign of vagal inhibition as a decreased cardiac contraction amplitude can be observed during the action of both K-ions and acetylcholine. Many authors mention the slowed rhythm and subsequent cessation of the heart under the influence of these agents. The problem of the polarized shifts which are observed in the heart with the action of acetylcholine or of K-ions is widely discussed.

Using a needle and mirror galvanometer, A. S. Mozzhukin [4], V. B. Boldyrev [1], and V. B. Boldyrev and P. A. Kiselev [2] observed the resting current of the cardiac muscle to increase under the influence of acetylcholine.

V. B. Boldyrev [1] recorded an increase in the resting current during the action of surplus K-ions.

However, there are contradictory data. It has been definitely established that K-ions actively depolarize different stimulated tissues [9, 10, 12]. There are indications that acetylcholine cannot increase the polarization of the cardiac muscular structures [3, 5, 21, 22].

We propose that neither acetylcholine nor K-ions alone can produce those changes in the functional properties of the cardiac muscle which are observed during the development of vagal inhibition. Only a mutually caused disturbance of the ionic balance, the release of acetylcholine and the change in the polarization faculty of the cardiac muscle cellular structures can bring about the initiation and development of the complex process of cardiac inhibition.

The purpose of this work was to study the action of acetylcholine and K-ions on a frog's heart.

EXPERIMENTAL METHODS

The experiments were done on the hearts of male and female Rana temporaria over a two-year period from 1947-1949. We used the rotary perfusion method and also a preparation made from a heart isolated according to Shtraub; heart action was electrocardiographically recorded in strictly potentiometric conditions on a string galvanometer connected with a continuous current booster by a direct connection (for details of methods, see Article I). Acetylcholine was prepared in a Ringer's solution from a dry preparation of acetylcholine hydrochloride. The concentration of the potassium solution was usually 10^{-3} (1 g of potassium chlorate to 1,000 ml of Ringer's solution). Therefore, the K-ion content in the active solution was 0.114% instead of the 0.014% in an ordinary Ringer's solution.

Two series of experiments were done.

EXPERIMENTAL RESULTS

The first series of experiments studied the action of acetylcholine and of potassium on a normal frog's heart. About 500 tests were done to determine the effect of acetylcholine in concentrations of 10^{-5} to 10^{-9} on the isolated frog's heart.

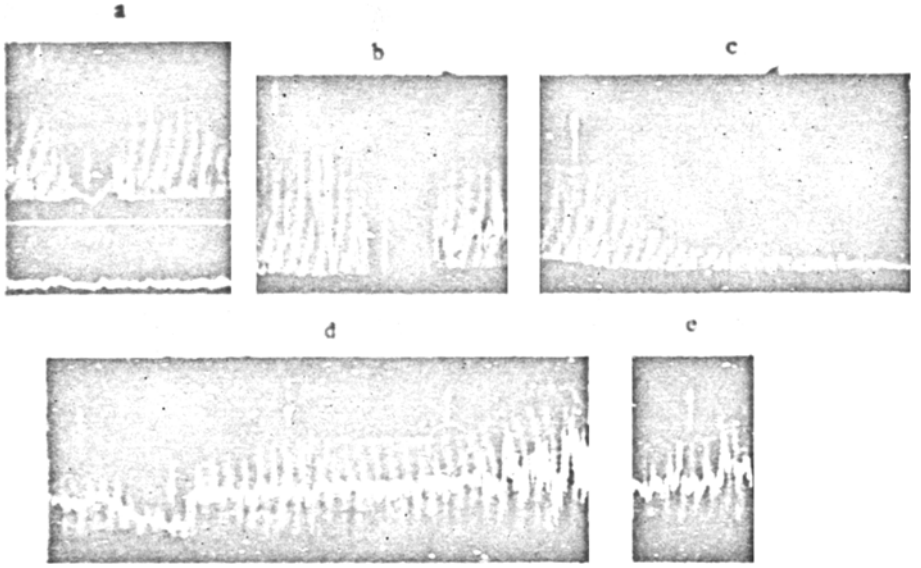

Fig. 1. The action of acetylcholine on an isolated frog's heart in conditions of Shtraub's method (a,e) and of rotary perfusion (b,c,d): a, b, c) reaction of a normal heart; d) reaction of normal and atropinized heart (atropine 10^{-5}); e) reaction of a previously desympathized and atropinized heart. Acetylcholine concentration: a, d, e) 10^{-7}; b) 10^{-6} c) 10^{-5}.*

A negative inotropic effect, which depended on the concentration of the substance administered, was observed in all cases. Figure 1, a,b, and c show the effect of acetylcholine in concentrations of 10^{-7}, 10^{-6} and 10^{-5} respectively. Kymogram a, in Fig. 1, illustrates an acetylcholine injection into a heart isolated according to Shtraub. Figure 1, b and c show the action of acetylcholine when it was administered directly to the sinus venosus by rotary perfusion. In no case were any changes in the inherent rhythm of the heart recorded. The apparent retardation of rhythm in Fig. 1, a and b is the result of blocking atrioventricular conduction 1:2 and

* ...e explanation for arrows given in Russian text – Publisher's note.

1:4 (in these cases, the electrocardiogram showed a normal sinus rhythm). In Fig. 2, one can see the effect of acetylcholine on the form and duration of development of the action potentials, and also on the resting potential level of the cardiac muscle; mechanograms of the auricles and ventricles were recorded simultaneously with the electrocardiogram. Acetylcholine, especially, sharply changed the monophasic action potential tracing, destroying its normal plateau and accelerating the development of the descending limb, i. e., the restorative processes after depolarization.

Reports have recently appeared in the literature which agree with the data we obtained; Hoffman and Suckling, especially, observed while recording monophasic action potentials of a dog's heart with intracellular electrodes that the repolarization processes were accelerated under the influence of acetylcholine.

However, the acceleration of the restorative processes was attended by the development of residual negativity; the tissue could not fully restore the original potential, and the resting potential was observed to decrease (an upward shift of the base line, which occurred as a secondary phenomenon after the development period of the monophasic action potentials had been reduced – see Fig. 2, a).

In cases where an atrioventricular blockade developed and where there were no ventricular monophasic potentials, the sinus rhythm was unchanged (Fig. 2 b), and a small secondary increase in the resting potential was observed.

Therefore, the injection of acetylcholine into the heart (either into the ventricles or directly into the sinus venosus) was attended by a decrease in contraction amplitude, the degree of which depended on the concentration of the acting substance. High concentrations of acetylcholine caused a blockade of the atrioventricular and sino-atrial conductions to develop. The fact that acetylcholine did not affect the level of the resting potential of the cardiac muscle directly must be emphasized. This could only be detected when working with a potentiometric apparatus by which one could record the primary effect of acetylcholine on the restorative processes (repolarization processes).

The effect of surplus K-ions on an isolated frog's heart was tested 120 times. In no case did the recordings show the inherent rhythm of the heart to be retarded. In 40% of the experiments, a positive chronotropic effect was observed, reaching 10% (rhythm accelerated to 6-7 contractions per minute). As a rule, the action of K-ions in a 10^{-3} concentration was attended by a considerable negative inotropic effect, sometimes involving the disappearance of the ventricular contractions. The kymogram in Fig. 3, a was obtained from K-ions acting (in a concentration of 10^{-3}) directly on the ventricles, and the one in Fig. 3,b from the same action on the sinus venosus of a frog's heart. In Fig. 3,a, one can see the effect frequently caused by the action of potassium – a blockade of the atrioventricular conduction developing. Sometimes a sino-atrial or even a sino-sinusal blockade was observed.

Studying the effect of K-ions on the bioelectric tonicity of the cardiac muscle was especially interesting. The experiments were conducted in the usual way. A typical result of the K-ion action is shown in Fig. 2, d. The injection of potassium in a 10^{-3} concentration was attended by a sharp decrease in the resting potential (i.e., negativation) of the cardiac muscle, with a simultaneous decrease in the amplitude and development time of the monophasic action potential. We observed a negative inotropic and a positive chronotropic effect on the mechanogram of this experiment.

In many cases, a sharp alteration of the monophasic action potential was observed, which undoubtedly indicates that the depolarization processes are disturbed by K-ion action.

In the case of the cardiac muscle also, therefore, K-ions are a factor actively depolarizing the cellular structures of the myocardium.

The second series of experiments examined the action of acetylcholine and K-ions on an atropinized frog's heart.

In 1924, O. Loewi and V. Navratil [20] concluded that acetylcholine and atropine in the cardiac muscle were interrelated. The authors believed that atropine reduced the sensitivity of the cardiac muscle to additionally administered acetylcholine or to that secreted by the vagus nerve endings. In spite of the many works treating this problem, there is still no clear understanding of the changes effected by the action of acetylcholine on an atropinized heart.

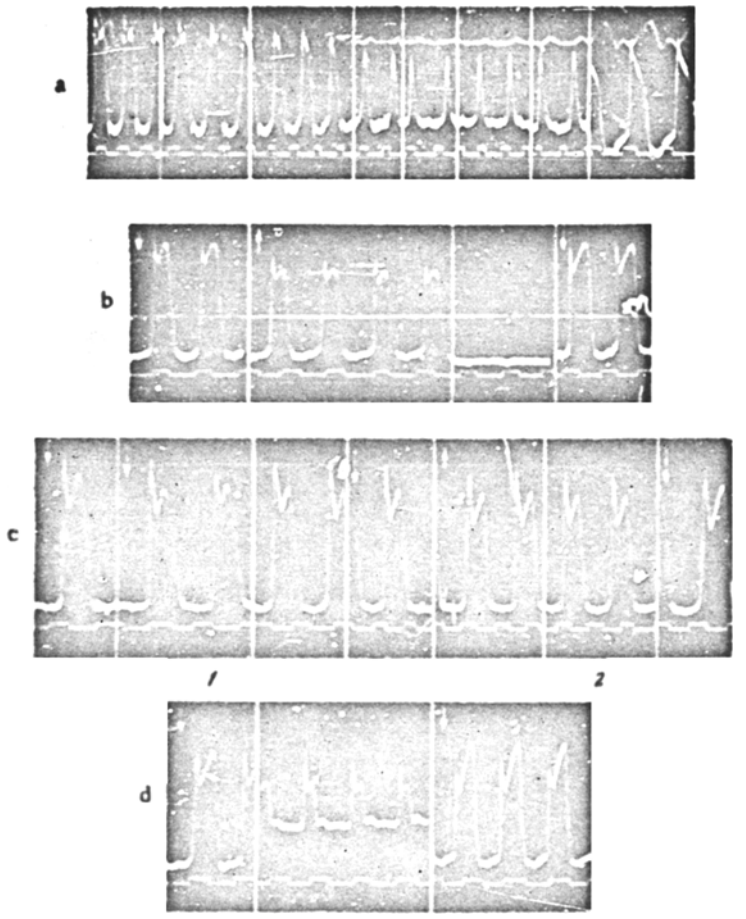

Fig. 2. Change in monophasic action potentials and resting potential of an isolated frog's heart during the action of acetylcholine in a 10^{-7} concentration (a,b,c) and of K-ions in a 10^{-3} concentration (d). The tracing b shows the effect of acetylcholine on a previously atropinized heart. Explanation of tracings: from top to bottom: a) mechanogram, electrogram, time indication; b, c, d) electrogram, time indication. Arrows mean the same as in Fig. 1.

The effect of acetylcholine in different concentrations on a previously atropinized frog's heart was tested 270 times. After the effect of acetylcholine in a Ringer's solution had been recorded, atropine was introduced into a cannula (usually in a concentration of 10^{-5}), and, after a fixed time interval, the effect caused by the same solution of acetylcholine was examined. After the introduction of acetylcholine into the atropinized heart, a positive inotropic effect developed in 256 cases.

Immediately after the Ringer's solution was replaced with the atropine, the usual negative inotropic result effected by acetylcholine was observed to decrease. One to two minutes after the administration of atropine, acetylcholine had no effect on the force of the heart contractions. However, after 2-5 minutes, a second test showed a positive inotropic effect from the action of acetylcholine in the same concentration. The supposed reduced sensitivity of the atropinized heart to acetylcholine should have been evidenced by different reactions to different concentrations of acetylcholine, i. e., in the course of atropinization, a heart which did not react to a 10^{-7} concentration of acetylcholine should have reacted to a 10^{-6} or 10^{-5} concentration, even if only by a weak negative inotropic effect. We did not observe in any case such a gradation in atropinized heart reactions.

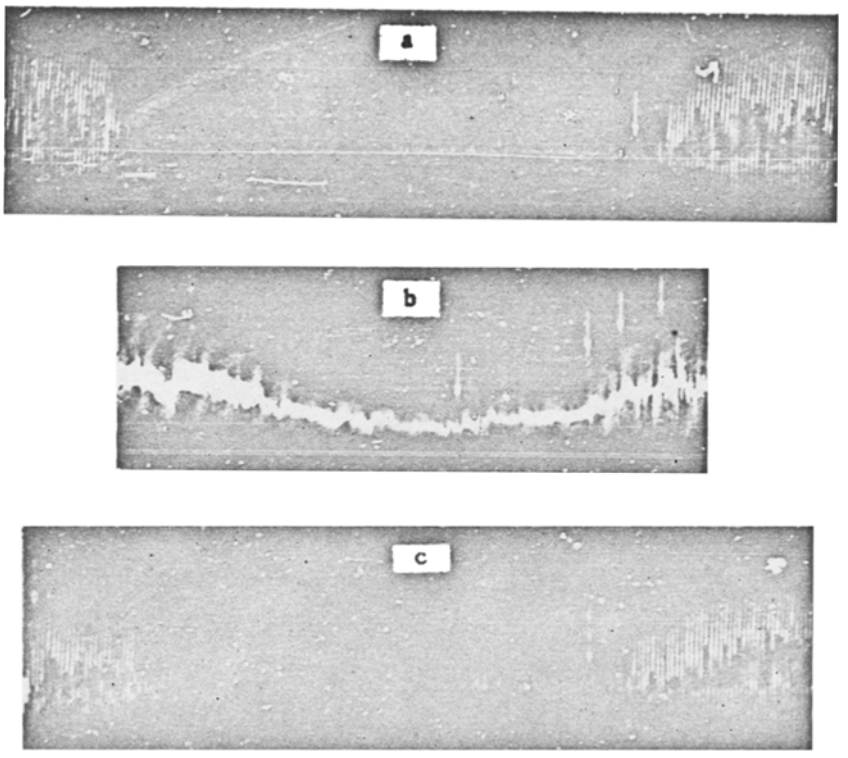

Fig. 3. Effect of surplus K-ions on frog's heart isolated according to Shtraub (a) and under conditions of rotary perfusion (b); c) effect of K-ions on a previously atropinized heart (atropine in a 10^{-5} concentration). The arrows mean the same as in Fig. 1.

Any concentration produced the sequence described above in the development of a positive inotropic effect. There was no change in the inherent rhythm of the heart in any case.

Figure 1, d shows the positive inotropic effect which occurred when acetylcholine was administered in a concentration of 10^{-7} after the 2-minute action of atropine.

Many authors, having observed a positive inotropic effect from acetylcholine under conditions of atropinization, propose that it develops from the action of acetylcholine on the endings of the cardiac muscle sympathetic fibers.

The experiments we conducted on previously desympathized hearts showed that, in 44 out of 52 cases, a clearly expressed, positive inotropic effect was observed from acetylcholine in any concentration (see Fig. 1,e).

Then we studied the action of acetylcholine on the bioelectric tonicity of an atropinized heart. Identical results were obtained in all 56 tests. The electrogram in Fig. 2, c shows that the atropine administration caused the resting potential to slightly increase (downward shift of the base line). The action of acetylcholine in a 10^{-7} concentration on an atropinized heart was attended by a decrease in the resting potential (tracing II — upward shift of base line); in this case, the mechanogram showed a positive inotropic effect.

Therefore, when the heart is atropinized, acetylcholine can no longer accelerate the restorative processes (repolarization) which occur after depolarization; it only lowers the resting potential of the atropinized muscle to the normal level.

In 1936, Lehnartz [17] established that the heart, after atropinization, cannot release K-ions in response to vagus nerve stimulation and to the action of acetylcholine. This fact is extremely important in determining the

role of iono-humoral interrelations in the development of vagal inhibition of the heart. In connection with this, we studied the action of K-ions on a mechanogram and electrogram of the atropinized heart.

When Fig. 3, a is compared with Fig. 3,c, one will observe that there is no difference in the reactions of normal and atropinized hearts to the action of surplus K-ions in a 10^{-3} concentration. Analogous results were obtained in the electrogram recording of the heart; the negativation of the atropinized cardiac muscle was the same as under normal conditions.

There are many works in the literature studying the effects of acetylcholine and K-ions on the normal and atropinized heart. All these works start with a comparison and description of the "inhibitory" effect of acetylcholine, K-ions and vagus nerve stimulation. From this, the conclusion seems logical that "atropinization of the heart, which relieves vagal and acetylcholine inhibition, does not prevent the development of inhibition due to the influence of potassium" [1]. The same author speaks of "the extremely fine differentiation of acetylcholine and potassium inhibitory mechanisms" when the heart is atropinized. However, it seems to us that there is no basis for these conclusions. Vagal inhibition is attended by an increase in the polarization of the cardiac muscle's cellular structures and by the cessation of activity in the sino-atrial node. No similar occurrences were observed under conditions of acetylcholine or K-ion action. Acetylcholine and potassium decrease the force of the heart contractions and aid the development of conduction blockade. Acetylcholine, which accelerates the restoration of the initial potential, decreases its level, i. e., indirectly lowers the polarization of the cardiac muscle. Potassium actively depolarizes the cellular structures of the myocardium, but does not affect the original rate of rhythm. We suggest that neither of these agents are inhibitory, i.e., neither can cause all the signs of vagal inhibition of the heart. Therefore, it is difficult to propose that atropine can "differentiate acetylcholine and potassium inhibition mechanisms".

When the action of acetylcholine on a normal heart is compared with that on an atropinized heart, it appears that atropine does not eliminate the effect of this substance, but only changes the reaction of the heart to the acetylcholine. Atropine, which stabilizes the original polarization of the cellular structures of the heart (see Article II), prevents the acceleration of repolarization under the influence of acetylcholine.

When Lehmartz's data is considered, one can suppose that, under conditions of atropinization, the correlation of the different ions in the cardiac muscle is disturbed, and vagus nerve stimulation is not attended by the evolution of K-ions from the tissues. We observed an intensification of the positive effects from acetylcholine on atropinized hearts of spring and fall frogs. In the spring and fall, a positive inotropic effect could be obtained, even on a normal heart, from the action of acetylcholine. In these periods, the correlation of the K-ions and calcium in the tissues is known to change greatly [8, 16, 24].

All these facts only prove the necessity of normal ionic relations to the realization of the usual effect of acetylcholine, but do not solve the problem of how this substance or K-ions "inhibit".

However, we hope that the data we have obtained will help approach an understanding of how the ionic, humoral and polarizing factors are interrelated in the origin and spread of vagal inhibition of the heart.

SUMMARY

The effect of acetylcholine and K-ions upon the rate and force as well as upon the resting and action potentials of normal and atropinized frogs' hearts was studied (over 600 experiments). Acetylcholine and K-ions diminished the force of contraction of the normal heart and promoted the blocking of conductivity, the rate of sinus rhythm being unaltered. Acetylcholine accelerated repolarization leading to a somewhat diminished polarization of the heart muscle. Potassium suppressed the contractions of the heart muscle diminishing at the same time the amplitude and slowing the development of monophasic action potentials. Preliminary atropinization did not alter the effect of surplus potassium on the heart. Acetylcholine however did not accelerate repolarization under these conditions, it only lowered the resting potential of the atropinized muscle down to its normal level. Acetylcholine enhanced the force of contraction of the atropinized normal and permanently desympathized heart.

LITERATURE CITED

[1] V. B. Boldyrev, in the book: Works of the Leningrad Medical Institute of Sanitary Hygiene,* 1950, part 7, pp. 199-215.

* In Russian.

[2] V. B. Boldyrev and P. A. Kiselev, Works of the Leningrad Medical Institute of Sanitary Hygiene,* 1950, part 7, pp. 216-222.

[3] I. A. Keder-Stepanova and M. G. Udelnov, Fiziol. Zhur. SSSR, 1951, Vol. 37, pp. 180-185.

[4] A. S. Mozzhukin, in the book: Collection of Works Dedicated to the 65th Birthday of Academician L. A. Orbeli,* Leningrad, 1948, pp. 233-244.

[5] M. G. Udelnov, in the book: Summaries of the Proceedings of the VIII All-Union Conference of Physiologists,* Moscow 1947, pp. 620-621.

[6] L. Asher, Ztschr. f. Biol., 1923, Bd. 78, S. 297-314.

[7] A. B. Beznak, J. Physiol., 1934, Vol. 82, pp. 129-153.

[8] N. Brock, Arch. f. exp. Path. u. Pharm., 1941, Bd. 198, S. 609-643.

[9] K. Cole, in the book: Cold Spring Harbor Symposia on Quantitative Biology, 1940, Vol. 8, pp. 110-122.

[10] K. Cole and H. J. Curtis, J. Gen. Physiol., 1941, v. 24, pp. 551-563.

[11] N. Gavrilescu and N. Jonescu, Compt. rend. soc. biol., Vol. 124, pp. 971-972.

[12] A. L. Hodgkin and A. F. Huxley, J. Physiol., 1945, v. 104, pp. 176-195.

[13] B. F. Hoffman and E. E. Suckling, Am. J. Physiol., 1953, v. 173, pp. 312-320.

[14] W. C. Holland, C. E. Dunn and M. E. Greig, Am. J. Physiol., 1952, v. 168, p. 546.

[15] W. Howell and W. Duke, Am. J. Physiol., 1908, v. 21, pp. 51-63.

[16] B. Kisch, Arch. f. exp. Path. u. Pharm., 1929, Bd. 116, S. 227-233.

[17] E. Lehnartz, J. Physiol., 1936, 86, pp. 37-38.

[18] E. G. Lipow, S. J. Reed, and W. K. Weaver, Proc. Soc. Exp. Biol. a. Med. 1931, 28, pp. 531-533.

[19] O. Loewi, Arch. ges. Physiol., 1921, Bd. 189, S. 239-242.

[20] O. Loewi and E. Navratil, Arch. ges. Physiol., 1924, Bd. 206, S. 123-134.

[21] W. Rössel, Arch. f. ges. Physiol., Bd. 250, S. 200-227.

[22] K. E. Rotschuch, Arch. f. ges. Physiol., 1952, Bd. 255, S. 367-378.

[23] N. Scheinfinkel, Ztschr. f. Biol., 1925, Bd. 82, S. 285-290.

[24] K. Wachholder and F. Nothmann, Arch. f. ges. Physiol., 1932, Bd. 229, S. 130-132.

* In Russian.