IONO-HUMORAL INTERRELATIONS IN THE PROCESS OF APPEARANCE AND DEVELOPMENT OF VAGAL INHIBITION OF THE HEART

COMMUNICATION I

THE INFLUENCE OF ATROPINE ON THE NORMAL AND DESYMPATHIZED FROG HEART

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The present series of communications is devoted to descriptions of experimental data indicating certain distinctive features of the ionic, humoral and polarization factors and their interrelations in the process of the appearance and development of vagal inhibition of the heart.

In the course of development of various concepts of the mechanism of vagal inhibition much attention was paid to the fact that the usual effect disappeared when the vagus was stimulated against the background of atropine action. Loewi and Navratil [5] established the possibility of differential elimination of the vagal effect. They showed that under the influence of atropine vagal stimulation was accompanied by liberation of acetylcholine (i.e., from the humoral point of view the nerve endings were not touched) but the inhibitory effect was absent (consequently acetylcholine did not exert the usual effect).

It was later established that under the influence of atropine not only was there a change in the cardiac reaction to acetylcholine but there was also impairment of the potassium ion activity in the process of development of the vagal effect [4]. Thus the action of atropine disturbs the usual interrelation of the ionic and humoral factors and this, evidently, leads to the disappearance of vagal inhibition.

The present author considers that a study of the altered activity of potassium ions and of acetylcholine as well as of the polarization properties of the atropinized heart should lead to proper understanding of the interrelations of these factors in the process of the development of inhibition of the normal organ.

It was essential to check the action of atropine on heart muscle. Special attention was paid to the effect of atropine on the polarization properties of the heart as no data on the subject could be found in the literature.

EXPERIMENTAL

Experiments were performed on isolated frog hearts (Rana temporaria) using the Samoilov method and that of "circular" perfusion. Using the first method the heart, with a Straub cannula inserted into the arch of the aorta, was left attached to the frog's head by means of dissected vago-sympathetic trunks. The second method permitted the direct observation of the effect exerted by the substance being tested on the venous sinus of the heart.

The isolated heart preparation, connected to the frog's head by vago-sympathetic trunks, was set up; a small funnel was inserted into the inferior vena cava, the end of the funnel being drawn out to form a cannula; a second cannula was inserted into the arch of the aorta and connected to a narrow bent tube. The funnel was filled with Ringer's solution or with the test substance in Ringer's solution. The perfusion fluid left by way of the

aortic cannula, passed through the tube and re-entered the venous cannula (funnel). The amount of fluid in the whole system did not exceed 2-3 ml. Ventricular contractions or contractions of both the atria and the ventricle were recorded on a moving smoked drum. The cardiac action was recorded electrographically by means of an Einthoven string galvanometer (large Engelman model) connected to a two-stage balanced direct current amplifier. The input resistance reached $6 \cdot 10^6$ ohms, the output—5000 ohms. The maximal sensitivity (outflow current) was 45 mA/v; amplification (direct current) approximately $3 \cdot 10^8$. The use of directly coupled d.c. amplifier converted the electrographic string galvanometer apparatus into a potentiometric one. Thus, changes in the potential of the heart muscle were recorded and not changes in resistance (an error permitted in some investigations of the polarization properties of the heart muscle). In all the experiments with electrographic recording monophasic potentials were led off by the M. G. Udelnov method. The exploring electrode was placed on an intact area of the active heart. The indifferent electrode, consisting of a loop of a fine cotton-wool wick soaked in Ringer's solution, was tightened round the top of the heart, isolated by a tight ligature and cauterized.

The electrodes used were the nonpolarizable Dubois-Raymond type.

Part of the experiments were performed on frogs with chronically desympathized hearts. The frogs were anesthesized with ether. The anesthesized frog was placed on its back, the lower jaw was pulled down with Pean's forceps and a small incision was made in the mucosa of the upper jaw along the midline, posterior to the eyes. The mucosa was drawn apart and by means of blunt dissection with glass hooks the head ganglion was dissected as well as part of the sympathetic trunk leading to it from the stellate ganglion. The head ganglion was completely and bilaterally extirpated. No hemorrhage accompanied the operation; the oral mucosa was sutured and painted with iodine. According to B. I. Lavrentiev 8-10 days after bilateral extirpation of the head sympathetic ganglion regeneration of post-ganglionic sympathetic fibers can be observed in the frog heart. The operated frogs in the present experiments were kept on ice in an aquarium for 25-35 days; control frogs were kept together with the operated ones.

Atropine, varying in concentration from 10^{-3} to 10^{-6}, was prepared with Ringer's solution from standard 0.5% solution of atropine sulphate or from the solid preparation.

The experimental data given in the present communication were obtained in 1947-1949.

RESULTS

In the first series of experiments more than 300 tests of the action of atropine on isolated frog hearts were carried out under different experimental conditions. Figure 1 shows the most typical effects of atropine on the strength and rhythm of cardiac contractions. In 80% of the cases the introduction of atropine into the heart was followed by a positive effect, but the strength of contractions returned to normal within 20-40 minutes. The inotropic effect reached 20-25% on the average, its magnitude being independent of the concentration of atropine used. Thus, Fig. 1, a) shows the effect of atropine in 10^{-5} concentration, while Fig. 1, b that of a 10^{-4} concentration; in the first case the positive inotropic effect is 30%, in the second case 28%, and sometimes the interrelation of the effects is reversed.

A negative inotropic effect during the action of atropine was observed in approximately 14% of cases; Fig. 1, c) shows that a concentration of 10^{-4} evokes such an effect reaching 21%, while a concentration of 10^{-5} (Fig. 1, d) decreases the strength of contractions by 20%. It must be stressed again that the effects obtained are independent of the concentration of atropine used (concentrations of 10^{-3} and 10^{-6} were also used). A positive chronotropic effect was observed in 36% of the cases; it reached, on an average, 10%. A biphasic effect of atropine on the rhythmic activity of the heart was noted in 19% of the cases; the cardiac rhythm, first slowed and then accelerated by atropine, returned to normal within 30 minutes. In the remaining cases no change in the rhythm of cardiac contractions occurred.

The data obtained make it possible to assert that the positive inotropic effect which is seen in 80% of the cases immediately after the introduction of atropine can be one of the characteristic features of its physiologic action on the heart. Haberlandt [3] who observed positive and chronotropic effects in the heart of Ranae esculentae following the introduction of atropine (concentration 10^{-3}) suggested that these effects were the consequence of excitation of sympathetic nerve endings in the heart muscle by atropine. He did not observe any positive atropine effects after regeneration of cardiac nerves following section of vago-sympathetic trunks.

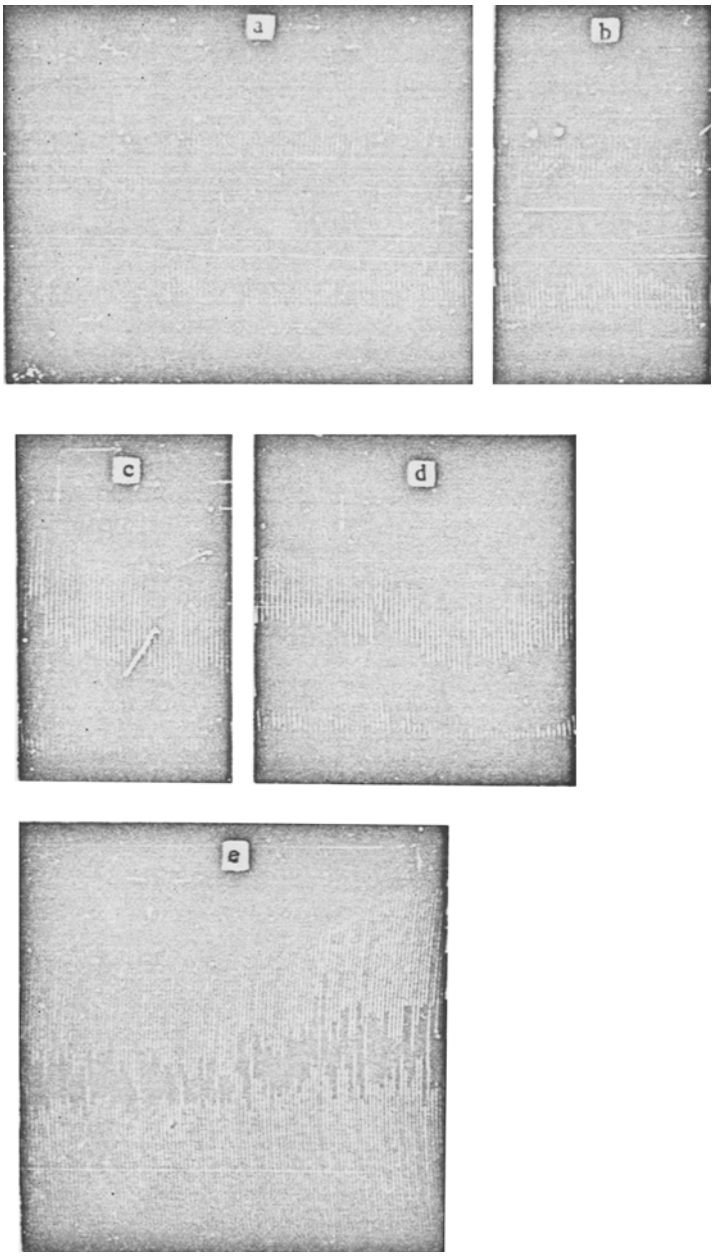

Fig. 1. Mechanograms of isolated frog heart perfused with Ringer's solution. The arrow indicates introduction of atropine, replacing Ringer's solution. e) effect of atropine on desympathized heart (frog operated on February 20th, experiment performed March 26, 1948).

The work of Haberlandt is difficult to assess since he does not give illustrations of his experiments. However, taking his findings into account, investigation of the action of atropine on desympathized frog hearts was undertaken. Forty-one of fifty-two desympathized hearts gave a definite positive inotropic effect with atropine concentrations of 10^{-4} and 10^{-5}. The effect reached 25% on the average. Figure 1, e) gives the kymogram of an experiment which shows that the strength of contraction increased by 23% (the operated frog was kept from February 20 to March 25). Under these conditions a positive chronotropic effect was demonstrated in approximately 30% of the cases. Therefore, sympathetic nerve endings do not participate in the increased contractions of the heart observed under the influence of atropine. The present author considers that this series of experiments suggests that the magnitude of the positive effects evidently depend on the initial functional state of the heart. Moreover, the positive effects of atropine action are most marked during the spring and early summer and in the autumn.

In the second series of experiments 56 tests of the action of atropine on the resting potential and monophasic action potentials of the isolated heart were carried out. In all cases some phenomena were observed which indicated certain distinctive features of the physiologic action of atropine. M. G. Udelnov [1, 2], investigating polarization phenomena occurring in the heart under various conditions (in particular during vagal stimulation) established that the positivity displayed by the heart muscle was accompanied by a growth of the amplitude of the monophasic action potential; negativity produced a decrease in the amplitude of the monophasic action potential. Electrograms recorded from the heart (Fig. 2) show that the introduction of atropine is accompanied by a small increase in resting potential (9% in Fig. 2,a) and 11% in Fig. 2,b); the same records show the increase in the amplitude of the monophasic action potential immediately after the introduction of the active substance. However, 1 minute (Fig. 2,a) or $1\frac{1}{2}$ minutes (Fig. 2, b) later the amplitude of the monophasic action potential returned to normal, despite the persistently raised resting potential. In 7 cases the resting potential was decreased immediately after the introduction of atropine with a parallel decrease in the amplitude of the monophasic action potential, but in the course of the experiment the resting potential increased and the amplitude returned to the original value.

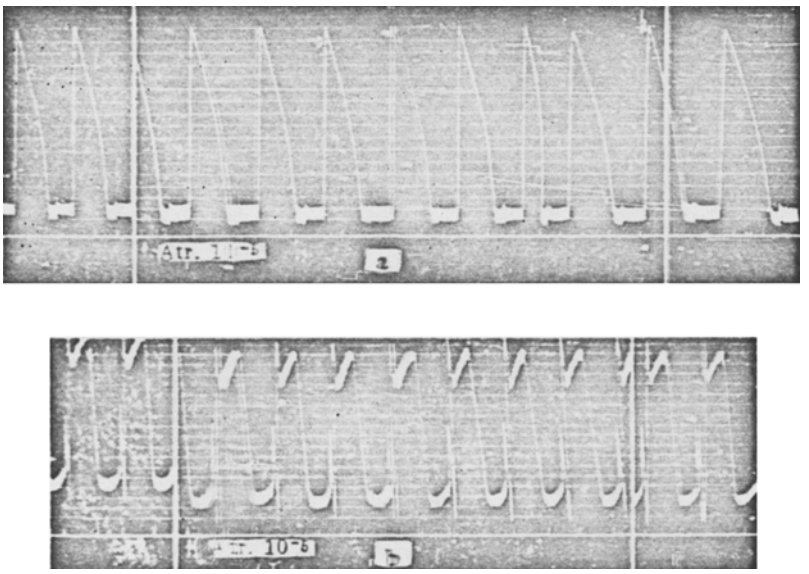

Fig. 2. Electrograms of isolated frog heart. Arrow indicates moment of replacement of Ringer's solution by atropine. Shift of the base line downwards indicates increase of resting potential.

The action of atropine on the heart was accompanied by an increase in the resting potential, which could take place phasically, the end result being expressed in the development of positivity in the heart muscle without a parallel change in the amplitude of the monophasic action potential.

The latter fact makes it possible to assume the existence of a stabilizing effect of atropine. The present author considers that atropine, by inducing positivity in the heart muscle and in some cases producing a phasic effect, creates conditions under which a change of potential exceeding its active level is impossible.

There are some indirect data in the literature which would support such an inference. Thus, Wedd and Blair [8] who investigated the action of atropine on the electrogram of turtle heart, showed that this substance lowered the conductivity of the heart muscle when the heart rate was very high. No changes occur when the heart rate is normal. Rice and Ross [7] note that the normal potential of the intestine reaches 40-100 mv, the surface of the mucosa being negative with respect to the serous layer. If this potential changes under the influence of various factors atropine restores it quickly to the normal level. Rehm and Hokin [6] also showed that when the rate of secretion and the magnitude of the potential of the intestinal wall underwent a change the action of atropine was to reduce secretion to zero and restore the normal value of the potential.

Differences in the action of atropine on the strength and rhythm of contraction of individual hearts at different times of the year, the return of positive changes of strength and rhythm to normal in the course of experiment, as well as the fact that the resting potential of the cardiac muscle increases without a parallel increase in the amplitude of the action potential, all suggest the possibility that atropine can stabilize the bio-electric tonus of the cardiac muscle. The results of experiments performed on desympathized hearts demonstrate the direct action of atropine on heart muscle; the observed positive effects do not result from excitation of sympathetic nerve endings.

SUMMARY

The effect of atropine on the strength and frequency of contractions, rest and action potentials of the normal and desympathized hearts of frogs was studied. Atropine acts directly upon the cardiac muscle, causing a slight increase in the frequency and strength of the contractions. At the same time the resting potential of the heart muscle is increased with no parallel rise in the amplitude of monophasic action potentials. The data obtained suggest the possibility that atropine stabilizes the bioelectrical tonus of the cardiac muscle.

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