ADRENERGIC NATURE OF VAGAL ACCELERATION OF THE FROG HEART

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The study of the mechanisms of the positive chronotropic effect of the vagus nerve on cardiac activity has a very long history, for it was discovered as long ago as in 1846 [10]. However, our ideas about this phenomenon are still extremely contradictory, many hypotheses are without solid grounds, and only two of them have received reliable experimental confirmation: cholinergic and adrenergic.

According to the adrenergic hypothesis, acceleration of the heart arising in response to stimulation of the vagus nerves or their centers is the result of excitation of intracardiac adrenergic neurons, connected synaptically with preganglionic parasympathetic fibers of the vagus nerve [2, 3]. This hypothesis is confirmed by a number of facts and, in particular, by experimental results showing that the β-adrenoblocker inderal completely abolishes the vagal acceleration effect in frogs [1, 2, 5], whereas the muscarinic cholinergic blocker atropine does not abolish it [1, 5].

According to the cholinergic hypothesis, acceleration of the heart in response to stimulation of the vagus nerves or their centers is realized through the same cholinergic neurons and, naturally, the same mediator (acetylcholine): if the action of the vagus nerve leads to secretion of large pulses of mediator inhibition arises, if small pulses, acceleration of the heart is produced [8, 9]. This hypothesis is based on the results of experiments which showed, in particular, that the vagal acceleration effect is blocked by atropine, a muscarinic cholinergic receptor blocker, and is not abolished by surgical or pharmacological desympathization in experimental animals [6, 8, 9]. It is also confirmed by the results of experiments showing that stimulation of the septal nerve in the frog heart causes acceleration of conduction of excitation in the atrioventricular node, which is not abolished by propranolol (obsidan) [4].

Clearly, therefore, in experiments by different workers the same pharmacological agents (atropine and sympatholytics) had diametrically opposite effects on the vagal accelerating effect, although it is not clear why. It is likewise not clear what the effect of surgical desympathization of the heart alone on vagal acceleration might be. The aim of this investigation was to continue the study of the mechanisms of acceleration of the heart arising in response to stimulation of the vagus nerves or their centers.

EXPERIMENTAL METHOD

Experiments, acute and chronic, were carried out on 98 frogs (*Rana temporaria*) during the fall and winter. In the chronic experiments preliminary desympathization of the heart was carried out surgically by the method described previously [5]. After the operation the frogs were maintained at a temperature of 10°C and were used in the experiments 15-30 days after the operation. This time is long enough for degeneration of sympathetic postganglionic nerves [14] but not long enough for their regeneration [13]. The heart preparation for the main part of the experiment was performed under ether anesthesia. The entire central nervous system was destroyed except the medulla. The spinal cord was divided immediately below the medulla and destroyed along its whole length by means of a probe. After craniotomy (through the mouth, for the experimental animal was fixed in the experiment in the supine position) the anterior regions of the brain were removed as far as the medulla. Preparation for the experiment ended with thoracotomy and catheterization of the ventricle of the heart for perfusion with various solutions and for recording cardiac activity. A cannula was introduced through the left arch of

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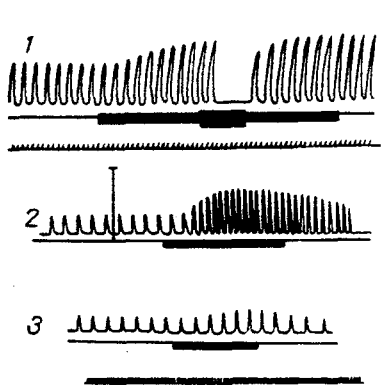

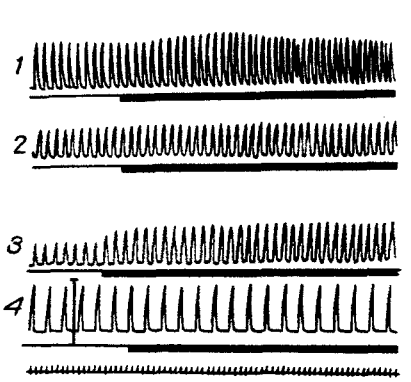

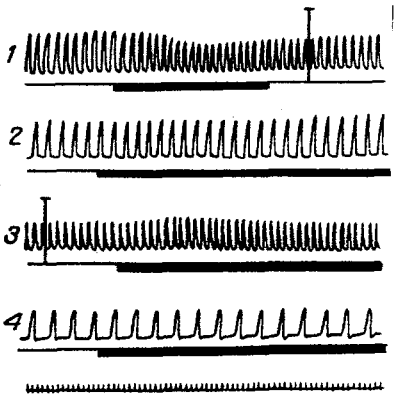

Fig. 1. Fig. 2 Fig. 3

Fig. 1. Changes in work of frog's heart during stimulation of medulla under different conditions: 1) perfusion of heart with Ringer—Locke solution without pharmacological agents: potentiating—accelerating effect to stimulation of 5 V, 20 Hz, 2 msec (marker of stimulation is initial part of bold line) and cardiac arrest to stimulation by 10 V, 20 Hz, 2 msec (marker of stimulation is middle, broader part of the same line); 2) perfusion of the heart by Ringer—Locke solution with atropine in a dilution of 10^{-5} g/ml: a marked increase in frequency and strength of cardiac contractions in response to stimulation by 10 V, 10 Hz, 10 msec; 3) marked decrease in potentiating—accelerating effect with an increase in atropine concentration in perfusion fluid to 10^{-4} g/ml (parameters of stimulation the same: 10 V, 10 Hz, 10 msec). Pressure scale 40 mm Hg, marker of stimulation shown below each fragment (broader part of line, also serves as zero line for counting pressure); time marker 1 sec (above — for fragment 1, below — for fragments 2 and 3).

Fig. 2. Change in heart rate of frogs during stimulation of vagus nerve centers when heart perfused with Ringer—Locke solution containing atropine in a dilution of 10^{-5} g/ml. 1) Acceleration of heart; 2) marked reduction of accelerating effect on addition of benzohexonium (10^{-4} g/ml); 3) acceleration of heart rate in frog desympathized surgically; 4) abolition of accelerating effect by benzohexonium (10^{-4} g/ml) in a desympathized frog. Time marker 1 sec; scale 50 mm Hg.

Fig. 3. Change in heart rate of frogs in response to stimulation of vagus nerve centers with perfusion of heart by Ringer—Locke solution with atropine in a dilution of 10^{-5} g/ml. 1) Acceleration; 2) acceleration blocked by addition of inderal in a solution of $2.5 \cdot 10^{-5}$ g/ml; 3) acceleration in surgically desympathized frog; 4) acceleration in desympathized frog blocked by propranolol ($2.5 \cdot 10^{-5}$ g/ml). Time marker 1 sec. Scale 50 mm Hg.

the aorta, and the right arch of the aorta was ligated. The perfusion solution passed into the atrium through a valve of our own design, from a low-pressure (venous pressure: 2-3 mm Hg) reservoir, and the solution was expelled by the ventricle into a high-pressure reservoir (arterial: 20-30 mm Hg). In the experiment the heart was perfused with Ringer—Locke solution with the addition of atropine in dilutions of 10^{-6}, 10^{-5}, and 10^{-4} g/ml, benzohexonium 10^{-4} g/ml, and propranolol (inderal) (1 or 2.5)·10^{-5} g/ml. To rule out the possibility of excitation of sympathetic nerve fibres of the vagosympathetic trunk, the nerves themselves were not stimulated, as by other investigators, but their centers in the medulla; both halves of the brain, moreover, were stimulated by series of pulses (5-10 V, 10-15 Hz, 2-10 msec) for a period of 20-60 sec through bipolar platinum electrodes.

EXPERIMENTAL RESULTS

In the first stage of the study of the mechanisms of the acceleration phenomenon, atropine in a dilution of 10^{-6} g/ml (12 experiments) and 10^{-5} g/ml (18 experiments, Fig. 1, 2) facilitated, whereas atropine in a dilution of 10^{-4} g/ml

(seven experiments, Fig. 1, 3) prevented the onset of the vagal accelerating effect; in the first case (10^{-6} g/ml) during stimulation of the vagus nerve centers of the frog the heart rate rose from 38 ± 4 to 47 ± 4 beats/min (by 24%, $p < 0.01$), in the 2nd case (10^{-5} g/ml) it rose from 28 ± 4 to 39 ± 4 beats/min (by 39%, $p < 0.01$) but in the 3rd case (10^{-4} g/ml) by only 14%: from 21 ± 2 to 24 ± 4 beats/min ($p < 0.05$). Without the use of atropine, the accelerating effect was only 8% (Fig. 1, 1).

The results of these experiments indicate that vagal acceleration of the heart is noncholinergic in its nature, for the accelerating effect was maximal against the background of the action of atropine in a dilution of 10^{-5} g/ml, when muscarinic cholinergic receptors of the heart were reliably blocked by atropine. These results are also evidence that atropine, in large doses (10^{-4} g/ml) is a ganglion blocker, i.e., it blocks not only muscarinic, but also nicotinic cholinergic receptors. This was the cause of the weakening of the vagal accelerating effect. Other investigators also have indicated a ganglion-blocking action of atropine in large doses (10^{-4} g/ml) [7]. Experiments [5, 8, 9] in which the accelerating effect was blocked by atropine in large doses (10^{-4} g/ml — it is this fact which is the main argument in support of the cholinergic hypothesis) do not prove the muscarinic cholinergic mechanism of vagal acceleration of the heart.

In the second stage of the investigation the role of sympathetic fibers of the vago-sympathetic trunk in the development of the accelerating effect was determined. For this purpose experiments were carried out on eight surgically desympathized frogs and on 12 intact (control) frogs, using the ganglion-blocker benzohexonium in both cases. The results showed that in intact frogs of this series the heart rate rose during stimulation of the vagus nerve centers from 34 ± 2 to 43 ± 2 beats/min (30%, $p < 0.001$, Fig. 2, 1), whereas in desympathized frogs it rose from 32 ± 3 to 39 ± 4 beats/min (by 20%, $p < 0.05$, Fig. 2, 3). The same stimulation but against the background of atropine and benzohexonium caused acceleration of the heart in intact frogs but only from 35 ± 3 to 39 ± 3 beats/min, i.e., by 11% ($p < 0.05$, Fig. 2, 2), whereas in desympathized frogs the heart rate was in general unchanged under these circumstances at 36 ± 5 beats/min (Fig. 2, 4). In four animals with an intact sympathetic nervous system the accelerating effect was completely blocked by benzohexonium.

Vagal acceleration of the heart in frogs thus is realized partly with the aid of postganglionic sympathetic fibers of the vagosympathetic trunk, and partly with the aid of intracardiac catecholamine-containing cells, evidently connected synaptically with the preganglionic parasympathetic fibers of the vago-sympathetic trunk. Excitation of the sympathetic fibers of the vago-sympathetic trunk, however, arises as a result of the spread of loops of current during stimulation of the vagus nerve centers.

At the third stage of the investigation the precise nature of receptors by means of which vagal acceleration of the heart takes place was identified, and the reason why propranolol, in experiments by some workers abolished, whereas in those by others did not abolish the accelerating effect, was studied. For this purpose a series of experiments were carried out with propranolol (inderal) on frogs with intact [15] and surgically desympathized [8] hearts (chronic experiments). The accelerating effect was obtained as a rule by simultaneous stimulation of both halves of the frog medulla against the background of the action of atropine in a dilution of 10^{-5} g/ml, which increased the heart rate in intact animals of this series from 35 ± 2 to 46 ± 3 beats/min (31%, $p < 0.001$, Fig. 3, 1), but in desympathized frogs from 36 ± 3 to 41 ± 3 beats/min (14%, $p < 0.01$, Fig. 3, 3). The same stimulation, against the background of the action of atropine and propranolol (2.5×10^{-5} g/ml) gave no accelerating effect in intact (Fig. 3, 2) or desympathized animals (Fig. 3, 4), whose heart rate was 23 ± 2 and 19 ± 2 beats/min, respectively. It is important to note that although a smaller dose of propranolol (10^{-5} g/ml) weakened the vagal accelerating effect, it did not abolish it completely.

The results of the acute and chronic experiments with inderal confirm our conclusion regarding the adrenergic nature of vagal acceleration of the heart in frogs and they demonstrate that it is realized through the β-adrenoreceptors of the heart. Preservation of the sympathetic effect in experiments of other investigators with propranolol [4], however, was the result of the use of an insufficient dose of obsidan (10^{-5} g/ml), which they adopted in their experiments. To confirm this conclusion, it must also be noted that, according to our observations, obsidan is a less effective blocker of the β-adrenoreceptors of the heart than inderal, although both are alternative names for propranolol.

Since the surgically desympathectomized frogs in our experiments were kept in a refrigerator before the main part of the experiment (which increased their survival rate), whereas the intact frogs were kept in the general animal house bath, it was necessary to test the effect of different temperatures on the experimental results.

The results of a special series of experiments conducted on eight cooled (taken from a refrigerator) and 10 "warm" (kept in a warm room for 3-5 days) frogs were as follows. The heart rate during medullary stimulation preceded by administration of atropine (10^{-5} g/ml) in cooled frogs increased from 35 ± 3 to 44 ± 4 beats/min (by 25%, $p < 0.02$), whereas in "warm" frogs it increased from 34 ± 4 to 49 ± 4 beats/min (by 44%, $p < 0.01$). The intensity of the accelerating effect in warm and cooled frogs at different times after the beginning of medullary stimulation also differed. For instance, after 5 sec of stimulation the increase in the heart rate in the warm and cooled frogs was 23 and 17%, respectively, whereas after 15 sec it was 29 and 9%, respectively, i.e., the accelerating effect increased in the course of time in the warm frogs, but was quickly exhausted in the cooled frogs.

Thus, allowing for a temperature correction, it is clear that about half the total vagal accelerating effect is realized by sympathetic fibers of the vagosympathetic trunk, while the other half is realized by intracardiac catecholamine-containing cells.

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