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Mechanism of Heart-Rate Acceleration Caused by Stimulation of Vagal Centers in the Frog

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The mechanism by which heart rate is increased upon stimulation of vagal centers is studied using frog heart preparations perfused with Ringer—Locke solution containing atropine and/or benzohexonium. Atropine stimulates vagus-induced heart-rate acceleration in dilutions of 10^{-6} and 10^{-5} g/ml. In a dilution of 10^{-4} g/ml both atropine and benzohexonium abolish vagal tachycardia. Rausedyl (3-4 injections, 5 mg/kg, at 18-20-h interval) prevents tachycardia. Stimulation of both halves of the medulla oblongata increases heart rate to a greater extent than stimulation of one half.

Key Words: *heart; regulation; vagus*

Stimulation of the vagus not only decreases but also increases heart rate [3,4,8-12]. Although the acceleration phenomenon was discovered in the 1850s, the controversy over its mechanisms still exists. In our view, only adrenergic and cholinergic hypotheses have gained sufficient experimental support. According to the adrenergic hypothesis, acceleration of heart rate is mediated by intracardiac adrenergic neurons forming synapses with preganglionic parasympathetic fibers of the vagus [3,4,11]. This hypothesis is supported by experiments in the vagal accelerating effect was abolished by sympatholytics [1,3] and ganglionic blocking agents [3], but not by atropine [1,12]. The adrenergic hypothesis is also consistent with the tentative presence of adrenergic neurons in the heart of various animal species [2].

The cholinergic hypothesis postulates that acceleration of cardiac rhythm upon vagal stimulation with a series of electric pulses is mediated by the same cholinergic neurons and, consequently, by the same neurotransmitter (acetylcholine): strong stimulation and high concentration of acetylcholine increase heart rate, while weak stimulation and low concentration of the neurotransmitter decrease it [5,6,8,9]. This hypothesis is supported by the absence of inhibitory and acceleratory effects in frogs with atropine-blocked M-cholinergic receptors [5, 6], preservation of vagal acceleratory effect in the presence of sympatholytics [5,6], and tentative absence of cardiac adrenergic neurons in some animal species.

Thus, on the one hand it was demonstrated that vagal acceleration of cardiac activity is prevented by blockade of the sympathetic nervous system and is not influenced by atropine that blocks the autonomic nervous system. On the other hand, there is evidence that atropine, but not sympatholytics, prevents vagal acceleration of heart rate.

Our goal was to analyze these results and to identify the neurotransmitter mediating vagal stimulation of cardiac activity in frogs.

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MATERIALS AND METHODS

The study was conducted during fall and winter using 77 *Rana temporaria* frogs. Heart preparations were obtained as follows: frog was fixed in supine position under ether anesthesia, all structures of the central nervous system except the medulla oblongata (MO) were destroyed, and the spinal cord under MO was cut along the entire length. After opening the cranium from the oral cavity, all brain structures up to the MO were removed, the chest was opened, and a cannula was inserted into cardiac ventricle. The cannula was introduced through the left aortic arch, and the right aortic arch was loosely ligated. The heart was perfused using special apparatus (Fig. 1). Perfusate from a low-pressure (2-3 mm Hg) vessel simulating venous blood flow entered the ventricle and discharged into a high-pressure (20-30 mm Hg) vessel simulating arterial blood flow. The cannula was connected via a polyethylene tube to a highly sensitive sensor, and electric signal from it was amplified and recorded.

The heart was perfused with Ringer—Locke solution containing atropine (10^{-6}, 10^{-5}, and 10^{-4} g/ml) or benzohexonium (10^{-4} g/ml) or both. In order to deplete the sympathetic nervous system of catecholamines, 5 mg/kg rausedyl was injected subcutaneously in the back (into the lymph sac) once or 3-4 times at 18-20-h intervals between the injections and the start of experiment.

After the manipulations had been completed, the main experiment was started *in situ* interconnected by the vagus. Tachycardia was induced by bilateral 20-60-sec stimulation of vagal centers in medulla oblongata [1,3,8,9] with electric pulses (5-20 V, 10-15 Hz, 2-10 msec series) delivered via bipolar platinum electrodes using an ESL-2 device. The chosen experimental conditions were the same as those used by advocates of adrenergic [3,4] and cholinergic [8,9] hypotheses except a different dosage of pharmacological agents, which was done in an attempt to reconcile these hypotheses.

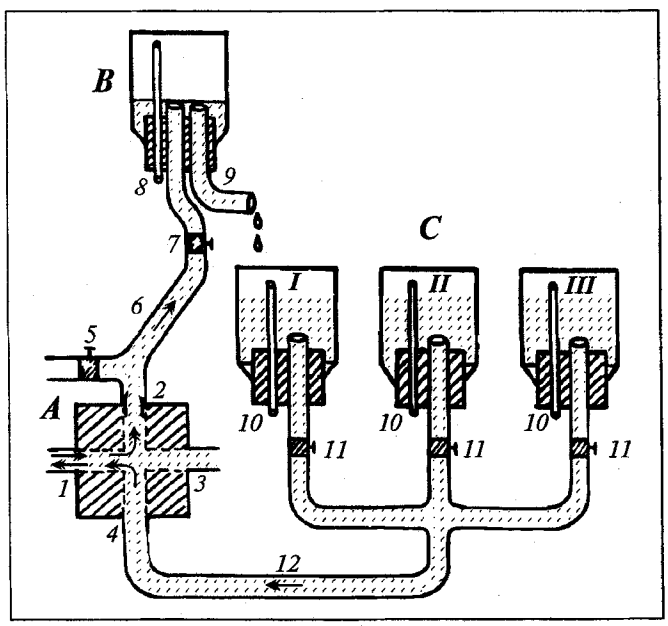

Fig. 1. System used to perfuse frog heart. *A*) Valve arrangement; *B*) high-pressure vessel simulating the arterial system; *C*) low-pressure vessels simulating the venous system; *1*) connection to the heart; *2*) arterial valve; *3*) connection of pressure gauge; *4*) venous valve; *5*, *7*, and *11*) stopcocks; *6*, *12*) elastic tubes; *8*, *10*) air-receiving tubes; *9*) output tube for spent solution and maintaining constant pressure in vessel *B*. The solution enters the heart from one of the *C* vessels via valve *4* during diastole and is discharged to vessel *B* via valve *2* during systole. Some quantity of the solution flows out from the heart through damaged vessels and the loosely ligated right aortic arch, which causes fresh solution to enter the heart. *I*) physiological saline; *II*) reagent No. 1; *III*) reagent No. 2.

RESULTS

Each experiment was started by control stimulation of vagal centers, and, as expected, both inhibitory and acceleratory cardiac responses were observed. Weak stimulation (0.5-5 V) often increased heart rate by 8%, while strong stimulation (5-10 V) decreased it (from 45±3 to 28±4 beats/min), sometimes leading to cardiac standstill. After control stimulations, atropine was added to the perfusate, and vagal centers were stimulated again.

Atropine was used in the following concentrations: 10^{-6} g/ml (12 tests, 12 preparations), 10^{-5} g/ml (18 tests, 18 preparations), and 10^{-4} g/ml (7 tests, 7 preparations). It was suggested that a more complete blockade of M-cholinergic receptors by atropine diminishes or even abolishes the vagal acceleratory effect if its is mediated by cholinergic neurons.

In fact, stimulation of MO in the absence of pharmacological preparations increased heart rate only by 8%, while in the presence of 10^{-6} g/ml, atropine it increased by 24% (from 38±4 to 47±4 beats/min; $p<0.01$) at 10^{-5} g/ml atropine by 39% (from 28±4 to 39±4 beats/min; $p<0.01$) and at 10^{-4} g/ml by 14% (from 21±2 to 24±2 beats/min; $p<0.05$), i.e., the effect of atropine at 10^{-4} g/ml was 2.8 times weaker than at 10^{-5} g/ml (Fig. 2, *3*).

Tachycardia was observed in 50% of the preparations when MO was stimulated in the absence of atropine and in 66%, 100%, and 57% of them, respectively, in the presence at 10^{-6}, 10^{-5}, and 10^{-4} g/ml atropine (Fig. 2, *2*). In further tests we used atropine in the concentration 10^{-5} g/ml, i.e., the concentration at which it produced the greatest acceleratory effect.

In order to attain cholinergic acceleratory effect on cardiac activity, it is necessary to stimulate the

vagus with weak pulses at a low frequency or to stimulate a small number of its fibers after the nerve had been cut [8,9]. Adrenergic acceleratory effect and its enhancement by stimulation of a sympathetic nerve can be achieved by application of stronger stimuli to the vagus or a large number of the vagus fibers should be stimulated. Therefore, one half and both halves of the MO were alternately stimulated with bipolar electrodes in subsequent 15 preparations in the presence of atropine (10^{-5} g/ml), i.e., under excluding the putative cholinergic mechanism, which led to additional increase in heart rate in 14 out of 15 tests: upon stimulation of one half heart rate increased from 36±3 to 40±3 beats/min (11%, $p<$ 0.01, Fig. 3, *1*) and from 32±3 to 41±3 beats/min (28%, $p<$ 0.001) upon stimulation of both halves (Fig. 3, *2*), indicating an adrenergic nature of the acceleration phenomenon.

The third series of experiments (13 frogs) proved the adrenergic nature of vagal acceleration of cardiac activity. These frogs were injected with rausedyl which "switched off" their sympathetic nervous system by depleting it of catecholamines. As expected, stimulation of vagal centers before the addition of atropine decreased heart rate (22±5 beats/min *vs.* 45±5 before stimulation) and caused no changes in cardiac activity in the presence of atropine (Fig. 3, *3*) except two frogs in which some acceleration was noted. However, repeated stimulation induced no acceleratory effect because norepinephrine stores in the sympathetic endings were exhausted.

The fourth test series included 12 frogs whose hearts were perfused with atropine and benzohexonium, an ganglioblocker. Control stimulation of vagal centers in the presence of atropine (10^{-5} g/ml) increased heart rate from 34±2 to 43±2 beats/min (by 30%, $p<0.001$), while similar stimulation in the presence of both atropine (10^{-5} g/ml) and benzohexonium (10^{-4} g/cm) led to a much smaller mean increase in heart rate, from 35±3 to 39±3 beats/min (11%, $p<0.05$), and in 4 frogs the ganglioblocker abolished the acceleratory effect.

These finding indicate that the nerve fibers whose stimulation accelerates heart rate are preganglionic: sympathetic or parasympathetic [3,4,11,12] that anastomize with intracardiac adrenergic neurons. However, some fibers stimulating cardiac activity are postganglionic sympathetic fibers excited by the loops of stimulating current. In our view, preganglionic fibers are also sympathetic. In frogs they are located in close proximity to the MO and could be excited by current loops when its vagal centers were stimulated. This may account for the results obtained after surgical interruption of sympathetic nerve flows to the heart [8,9] since the possibility that some

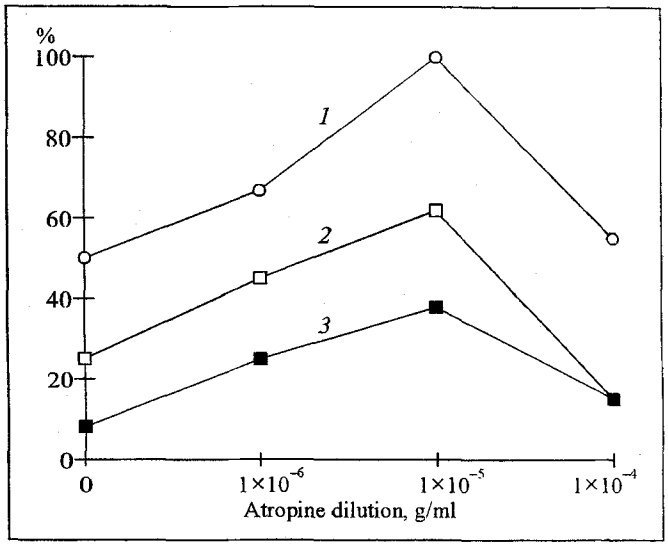

Fig. 2. Heart-rate acceleration in frogs upon stimulation of vagal centers in the presence of 10^{-6}, 10^{-5}, and 10^{-4} g/ml atropine and in its absence. *1*) Hearts with heart rate acceleration; *2*) percentage of stimulations with acceleration relative to the total number of stimulations; *3*) heart rate.

vagosympathetic fibers remaining intact after the surgical sympathectomy cannot be excluded.

Thus, the controversy over the mechanisms of vagal accelerating effects on heart action in frogs results from the use of pharmacological agents in different concentrations by different investigators. Specifically, it was found that atropine (10^{-4}-10^{-5} g/ml) abolishes vagal acceleration of heart rate by blocking M-cholinergic receptors in various structures of the heart [5,6,8,9] .

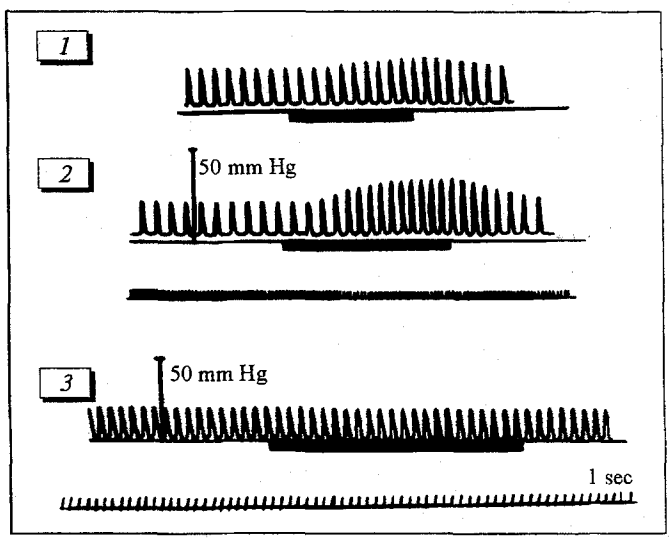

Fig. 3. Heart-rate acceleration observed for frog hearts perfused with Ringer—Locke solution containing 10^{-5} g/ml atropine. *1*) Slight acceleration when one half of medulla oblongata is stimulated; *2*) higher acceleration when both halves of medulla oblongata are stimulated; *3*) no acceleration when both halves of medulla oblongata are stimulated in rausedyl-treated frog. The bold segment of the zero line under each fragment indicates stimulation.

Our findings show that the pronounced decrease or complete disappearance of vagal acceleration occurring in the presence 10^{-4} g/ml atropine is due to the fact that in high concentrations atropine acts as a ganglioblocker, i.e., as a blocker of N-cholinergic receptors in vegetative ganglia, which is an established fact. Electrophysiological experiments on frog heart preparations showed that 10^{-4} g/ml atropine interrupts excitation transmission from preganglionic fibers of the vagus to intracardiac neurons, i.e., blocks not only M-cholinergic but also N-cholinergic receptors. In our experiments the inhibitory effect of the vagus was abolished by 10^{-5} g/ml atropine with the emergence of a stimulatory effect that was eliminated by 10^{-4} g/ml atropine. The same effect was exerted by benzohexonium (10^{-4} g/ml) in the presence of atropine (10^{-5} g/ml).

The conflicting results obtained with 10^{-5} g/ml atropine can be explained by the differences in experimental conditions: we used stronger stimuli (5-10 V, 10-15 Hz, 2-10 msec) than other investigators [5,6,8,9] who used threshold (1 V) or slightly higher stimuli. Therefore, partial blockade of N-cholinergic receptors by atropine did not modulate the acceleratory effect in our experiments tests and abolished or drastically reduced it their investigation [5,6].

So far, it remains unclear why rausedyl abolished vagal acceleratory effect only in our experiments. The explanations is probably as follows: other researchers [5,6] administered rausedyl once in a dose of 50 μg/g and started the experiment after 48 h, when the effect of rausedyl had already disappeared. In our experiments, a single rausedyl dose of 5 mg/kg was injected 20-24 h before experiments, which did not abolish the vagal acceleratory effect. This effect was prevented only after 3-4 rausedyl injections in this dose for 3-4 days at 18-20-h intervals between the injections and the start of the main experiment.

Our results show that acceleration of heart rate observed in frogs upon stimulation of the vagal nerves or their centers is of adrenergic rather than cholinergic nature, being mediated partly by postganglionic sympathetic fibers and partly by preganglionic parasympathetic fibers or, which is more likely, by the sympathetic fibers activating adrenergic neurons. In frogs, like in guinea pigs and rats, vagal acceleration of heart rate probably results from the excitation of only sympathetic fibers of the vagosympathicus [7] rather than of parasympathetic fibers, as it is believed by some researchers [5,6,8,9]. This has been supported by the observation that no acceleration of heart rate in response to vagal stimulation occurs in animal species (turtle, pigeon, and rabbit) whose vagus contains no sympathetic neural elements [7].

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