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EFFECT OF PARASYMPATHETIC ACCELERATION ON BIOELECTRICAL ACTIVITY OF PACEMAKER CELLS OF THE DESYMPATHIZED AND RESERPINIZED FROG HEART

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The mechanisms of parasympathetic acceleration was studied in experiments on the heart of *Rana temporaria* after preliminary exhaustion of the catecholamine reserves by desympathization and reserpinization of the animals. Electrical activity of cells of the isolated pacemaker was recorded. During parasympathetic acceleration the rate of rise of slow diastolic depolarization was found to increase (evidence of the active mechanism of this acceleration), and this was accompanied by slight hyperpolarization and by shortening of the duration of the action potential. After treatment of the preparation with atropine the accelerating effect and the changes in shape of the action potential disappeared, confirming the cholinergic nature of the parasympathetic acceleration. It is suggested that acetylcholine, the mediator of the parasympathetic system, may reduce potassium or increase sodium permeability of the pacemaker cell membrane, thus increasing the rate of rise of slow diastolic depolarization and causing acceleration of discharges.

KEY WORDS: *desympathization; reserpinization; parasympathetic acceleration; atropine; action potential.*

Much experimental evidence has now been obtained of the ability of the parasympathetic system both to inhibit and to accelerate the heart beat [2, 3, 7, 8, 10]. It has been shown by pharmacological methods that both types of parasympathetic influences travel along cholinergic nerve pathways [1, 4].

In this investigation the mechanism of parasympathetic acceleration was studied on frogs' hearts after preliminary exhaustion of the catecholamine reserves by desympathization and treatment with reserpine.

EXPERIMENTAL METHOD

Experiments were carried out on a preparation of the isolated sinus of the heart of *Rana temporaria* with the extracardiac nerves running to it. The frogs were desympathized by bilateral extirpation of the sympathetic chain at the level of the second and third ganglia, after which the animals were kept at 11-14°C for 27-30 days. Reserpine (or Rausedil) was injected subcutaneously 2 days before the experiments in a dose of 50 μg/g. The catecholamine fluorescence of the heart is known to disappear almost completely under these circumstances [9]. The results of control experiments showed that stimulation of the sympathetic chain in frogs after reserpinization does not accelerate the cardiac rhythm.

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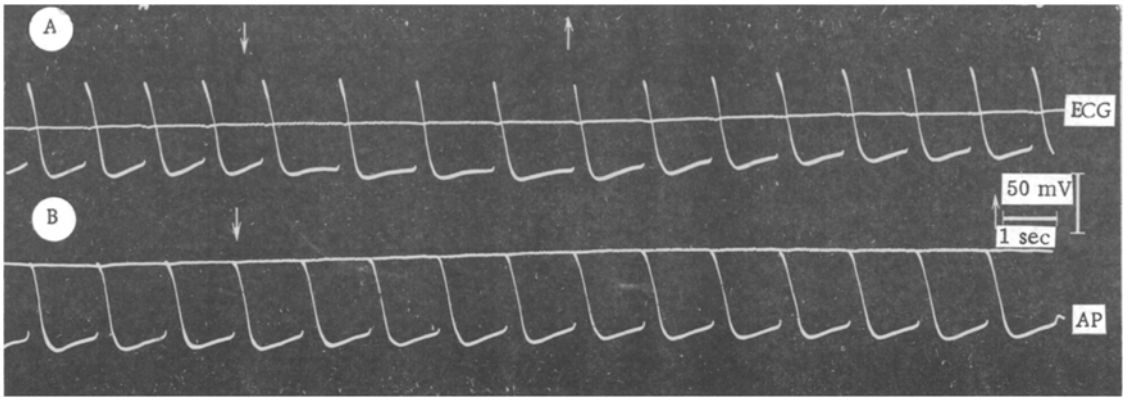

Fig. 1. Changes in AP of sinus venosus of heart of desympathized and reserpinized frogs during inhibition of rhythm by stimulation of vagosympathetic trunks (20 Hz, 3.5 V) before (A) and after atropinization (B). A) Inhibition of rhythm from 57 to 40 beats/min against the background of hyperpolarization, slowing of SDD, and shortening of duration of AP; B) abolition of inhibitory response after addition of atropine ($1 \cdot 10^{-4}$ g/ml). Here and in Fig. 2, beginning and end of stimulation of nerves marked by arrows.

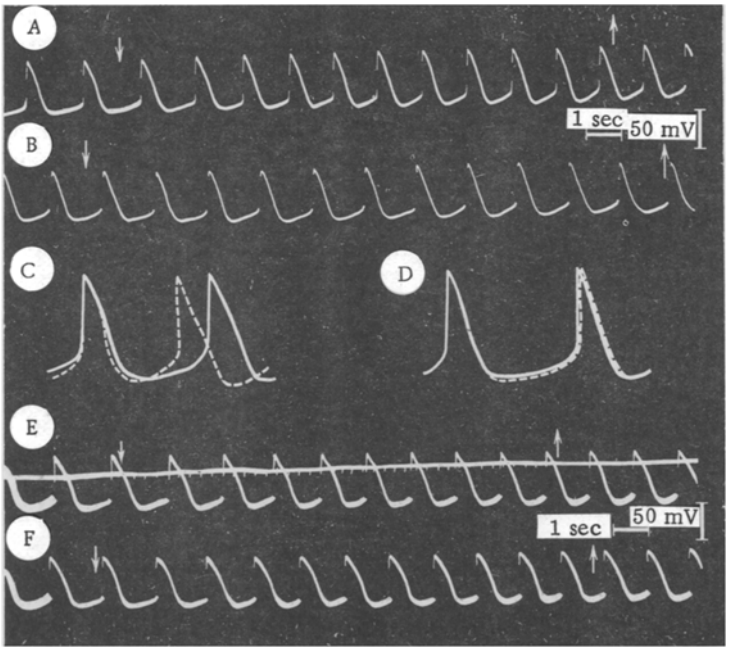

Fig. 2. Comparison of changes in AP of pacemaker structures during parasympathetic (A, B, C, D) and sympathetic (E, F) acceleration of rhythm. A) Acceleration of rhythm from 38 to 48 beats/min during stimulation of vagosympathetic trunk (10 Hz, 3.5 V) of desympathized and reserpinized frog's heart (C — superposition of AP before and during stimulation of nerves); B) abolition of acceleration by atropine ($1 \cdot 10^{-4}$ g/ml) (D — superposition of AP before and during stimulation of nerves). E) Acceleration of rhythm from 38 to 48 beats/min during stimulation of sympathetic chain of intact frog; F) preservation of accelerating response after addition of atropine.

The vagosympathetic trunks were stimulated by bipolar silver electrodes (0.5 msec, 7-15 Hz) for 10 sec. Electrical activity of the pacemaker cells was recorded by means of glass microelectrodes with a tip under 0.5 μ in diameter, filled with 3 M KCl solution. Action potentials (AP) were recorded on a loop oscillograph. Atropine sulfate ($1 \cdot 10^{-4}$ g/ml), which was applied to the surface of the sinus, was used to test the mediator nature of the accelerating effects.

TABLE 1. Changes in Bioelectrical Indices during Parasympathetic Acceleration of Cardiac Rhythm

Expt. No.	Heart rate, beats/min			Rate of SDD, mV/sec			Amplitude of AP, mV			Duration of AP, msec			Hyperpolarization, mV
	initial	maximal acceleration	change, %	initial	during maximal acceleration	change, %	initial	during maximal acceleration	change, %	initial	during maximal acceleration	change, %	
1	38	50	+31,5	13,6	23,9	+75,9	74,4	75,6	+1,6	512	487	—4,9	1,2
2	48	57	+21	16	16,6	+3,8	65,2	68,9	+5,6	439	410	—6,5	1,5
3	57	67	+17,5	29,7	40	+35	75	76,2	+1,6	426	409	—4	0
4	44	50	+14	25	26,2	+4,8	70,1	72,6	+3,5	512	512	0	2,5
5	43	53	+23	16	36,2	+12,6	72,2	77,7	+7,7	560	531	—5,2	0
6	48	60	+25	17,5	24,9	+42	76,3	79	+3,5	463	414	—11	0
7	41	48	+17	11,6	14,7	+27	75,6	78,3	+3,6	365	365	0	2,7
8	58	71	+22	—	—	—	—	—	—	—	—	—	—
P			0,01			0,1			0,01				0,05

EXPERIMENTAL RESULTS AND DISCUSSION

In response to stimulation of the vagosympathetic trunks positive chronotropic effects were observed in 10 of the 20 experiments, negative in seven, and in two experiments the sign of the response depended on the parameters of stimulation: Weak stimuli of low frequency caused acceleration, strong or high-frequency stimuli inhibition; in one experiment biphasic effects were found.

In the presence of negative chronotropic effects characteristic inhibitory changes occurred in AP of the pacemaker structures (a decrease in the steepness of rise of slow diastolic depolarization (SDD), hyperpolarization of the membrane potential, acceleration of repolarization, and shortening of the duration of AP)(Fig. 1).

A typical example of acceleration of the heart beat caused by stimulation of the vagus nerve of the desympathized and reserpinized frogs is shown in Fig. 2A. The accelerating effect in these cases usually occurred in the first to the third cardiac cycle after the beginning of stimulation. In every case of parasympathetic acceleration an increase in the rate of rise of SDD, measured by ratio of the amplitude of SDD to the duration of the predischarge process (Table 1), was observed. Some shortening of the duration of AP and slight hyperpolarization also were frequently observed. After addition of atropine to the preparation the positive chronotropic effect was completely abolished in 8 of 10 experiments (Fig. 2B).

Changes in the pacemaker potentials observed during parasympathetic acceleration should be compared with changes in AP to stimulation of the sympathetic chain in intact animals. Sympathetic acceleration (Fig. 2E) developed against the background of increased steepness of rise of SDD and a small increase in the duration of AP. After addition of atropine to the preparation the acceleration effect continued and developed against the background of the same changes in AP as in the absence of atropine.

Experiments on catecholamine-deprived hearts thus showed that both inhibitory and accelerating effects can develop in response to stimulation of parasympathetic pathways. They also showed that both effects are abolished by atropine. Consequently, parasympathetic acceleration, like parasympathetic inhibition, is due to activation of cholinergic structures. The hypothesis that adrenergic neurons exist in the parasympathetic innervation structure cannot be evoked to explain these results for the further reason that adrenergic ganglion cells and chromaffin tissue are not found in the hearts of *Rana temporaria* [5, 6, 9, 13], and catecholamine reserves contained in the sympathetic nerve endings were exhausted by preliminary desympathization and reserpinization of the animals. Comparison with sympathetic acceleration, which is not changed by atropinization, confirms the different nature of sympathetic and parasympathetic acceleration. The increase in the rate of rise of SDD observed during the development of parasympathetic acceleration is evidence of an active mechanism for this process.

According to the currently held view, acetylcholine increases the permeability of the cell membrane to potassium ions, and this is responsible for the inhibitory effect of this

mediator. More recently, however, the possibility of an atypical action of acetylcholine has been reported. Pappano [12], who observed accelerating effects of acetylcholine on the chick embryonic hearts, postulates an increase in sodium permeability by this mediator. Other workers [11], on the basis of experiments with radioactive potassium, concluded that in low concentrations both endogenous and exogenous acetylcholine can reduce potassium permeability. The increase in the rate of SDD during parasympathetic acceleration may perhaps be due to a similar "atypical" effect of acetylcholine on the permeability of the cell membrane, i.e., to its ability to increase sodium and reduce potassium permeability. This problem is a matter for future research.

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RESTORATION OF VITAL FUNCTIONS OF ANIMALS REVIVED WITH A DONOR CIRCULATION AFTER PROLONGED CIRCULATORY ARREST

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Anesthetized dogs were revived by means of an artificial donor circulation after circulatory arrest lasting 15-20 min. In group 1 the donor's blood was injected toward the heart of the resuscitated dog, whereas in group 2 it was injected toward the heart and brain. In donor-aided resuscitation (especially in group 2) the vital functions were restored more quickly and the number of surviving animals was greater than when other methods of resuscitation were used. Despite the outwardly full recovery of the animals after prolonged circulatory arrest, various degrees of injury took place to their brains, depending both on the duration of clinical death and on the methods of resuscitation.

KEY WORDS: *resuscitation; donor circulation.*

The question of the longest possible period of anoxia tolerated by the brain and the possibility of resuscitation after long periods of clinical death is not a new one [2, 4-9,

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