ACTION OF PILOCARPINE ON THE FROG HEART UNDER NORMAL AND PATHOLOGICAL CONDITIONS

N. N. Alipov, G. I. Kositskii, and T. E. Kuznetsova

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According to the classical view, acetylcholine (ACh) has a negative chronotropic action on the heart. However, there is experimental evidence that this substance may also have a positive chronotropic effect [1, 4, 6, 9-11, 14]. To explain this phenomenon widely different hypotheses have been put forward, but they can all be reduced to two basic concepts. According to the first of these, the positive effect of ACh on the heart is linked with its action on nicotinic acetylcholine receptors (AChR) of intracardiac catecholamine-containing structures: intramural adrenergic neurons [1], chromaffin cells [11], or sympathetic nerve endings [9]. According to the second concept, the positive effect of ACh, like its negative effect, can be explained by its action directly on muscarinic AChR of the myocardium, and the character of the effect is determined by the degree of their excitation [6, 10, 12]. To solve this problem, drugs used for pharmacologic analysis are used, and in particular, the widely used muscarinic cholinolytic atropine. However, experiments with atropine often give contradictory results: some workers observed depression of the positive effect of ACh by atropine [6, 10, 12] whereas others found opposite results [1, 11]. The possibility cannot be ruled out that these contradictory data are attributable to side effects of atropine [5, 6]. For the reasons given above, the problem whether excitation of myocardial muscarinic AChR can lead to a positive effect remains unsolved. The aim of the present investigation was to study this possibility with the aid of the muscarinic cholinomimetic pilocarpine, which does not act on nicotinic AChR [3].

EXPERIMENTAL METHOD

Experiments were carried out in January-March on male frogs (*Rana temporaria*). In the experiments of series I isolated frogs' hearts were used after destruction of the brain spinal cord. The heart was perfused through a cannula in the posterior vena cava with Ringer's solution (winter) of the following composition (in mM): NaCl 103, KCl 1, $CaCl_2$ 0.9, $NaHCO_3$ 1.2. The perfusion pressure, recorded by means of a Y-shaped water manometer, was 0-2 cm water. The perfusion fluid drained through the arch of the aorta into a cannula connected with an EMT-35 pressure transducer, from which it entered a tube the free end of which was located at the level of the preparation. The resistance to outflow was controlled by means of a clamp. Pressure was recorded on the N-327 automatic writer. Ringer's solution was applied constantly drop by drop to the heart. Every 15 min application of Ringer's solution was stopped and 2 ml of pilocarpine solution was applied (exposure 30-60 sec). Immediately after the end of pilocarpine application, application of Ringer's solution was resumed. Solutions of pilocarpine made up in Ringer's solution, in concentration from 10^{-15} to 10^{-4} g/ml, were used. If several applications were made in the experiment, at each successive application pilocarpine was given in a concentration 10^6 times higher than the previous application (for example, 10^{-15}, 10^{-9} g/ml, and so on). In intervals between applications of pilocarpine, control applications of 2 ml of Ringer's solution were made.

In series II and III the hearts were studied in situ without perfusion. The electrogram (EG) of the thymus were recorded by means of a copper wire about 50 μ in diameter and 7 cm long. The free end of the wire touched the sinus and moved during its contractions, while the other end was connected to a cathode follower. EG of the ventricles also was recorded by means of tantalum hook electrodes. A UBP 1-02 amplifier and N-327 automatic

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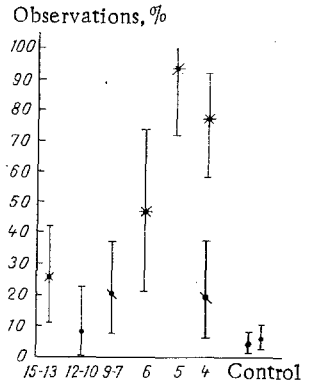

Fig. 1

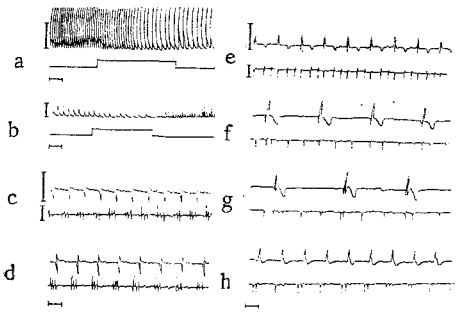

Fig. 2

Fig. 1. Effects of pilocarpine on isolated perfused frog heart. Abscissa, concentration (-log C) of pilocarpine (in g/ml); ordinate, number of observations in which effect was found (in % of total number). Thin lines represent negative chronotropic effects, bold lines — positive chronotropic effect. Confidence intervals at the P = 0.05 level are given. A dot indicates that difference from control is not significant; an oblique stroke indicates that difference from control is significant (P = 0.05); a cross indicates the same (P = 0.01); an asterisk the same (P = 0.001).

Fig. 2. Effect of pilocarpine on the heart. a) Negative chronotropic effect on isolated perfused heart: top curve — pressure in aorta (calibration 1.47 kPa), bottom curve — marker of application (pilocarpine concentration 10^{-6} g/ml). Time marker 10 sec; b) positive chronotropic effect on isolated perfused heart: top curve — pressure in aorta (calibration 1.32 kPa), bottom curve, marker of application (pilocarpine concentration 10^{-4} g/ml). Time marker 10 sec; c, d) negative chronotropic effect on heart *in situ* with normal conduction of excitation; c) EG of ventricle (top curve, calibration 1 mV) and sinus (bottom curve, calibration 50 μV), background; d) EG of ventricle and sinus after application of pilocarpine (10^{-5} g/ml). Time marker 1 sec; e-h) action of pilocarpine on heart *in situ* under conditions of sinoartial block; e) EG of ventricle (top curve, calibration 10 mV) and sinus (bottom curve, calibration 250 μV), background; f) EG of ventricle and sinus, sinoatrial block; g) EG of ventricle and sinus, sinoatrial block, beginning of pilocarpine application (10^{-4} g/ml). Slowing of sinus activity; h) EG of ventricle and sinus 1 min after beginning of pilocarpine application. Slowing of sinus activity, removal of block, quickening of ventricular rhythm. Time marker 1 sec.

writer were used for amplification and recording respectively. Solutions of pilocarpine in concentrations of 10^{-8}-10^{-4} g/ml were applied. In series II tests were carried out under conditions of normal cardiac activity, in series III during sinoatrial block induced by the action of a direct current (amplitude 10-15 V, duration 5-15 sec) to the region of the sinoatrial boundary. Applications of Ringer's solution served as the control in all cases.

EXPERIMENTAL RESULTS

The results of the experiments of series I (70 experiments, 142 applications of pilocarpine) are given in Fig. 1. In concentrations of 10^{-15} to 10^{-7} g/ml pilocarpine gave only a weak negative chronotropic effect, the intensity of which did not depend on dose. In concentrations of 10^{-6}-10^{-4} g/ml pilocarpine gave a stronger negative chronotropic effect, which depended on dose (Fig. 2a). If a concentration of 10^{-4} g/ml was used, a significant positive chronotropic effect was found. The fact that this effect arose only in response to high concentrations of pilocarpine contradicts the view that positive reactions of the heart are ob-

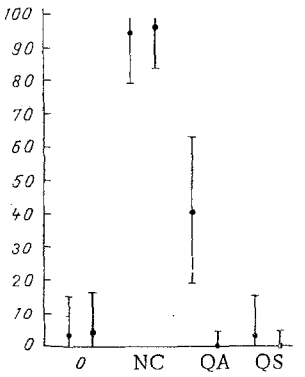

Fig. 3. Chronotropic effects of pilocarpine on heart *in situ* with normal conduction of excitation and with sinoatrial block. Vertically (number of observations in which effect was observed (in percent of total number). O) Absence of effect; NC) negative chronotropic effect; QA) quickening of atrial rhythm; QS) quickening of sinus activity. Thin lines indicate sinoatrial block; bold lines normal conduction of excitation. Confidence intervals given for P = 0.05.

served during weak stimulation of muscarinic AChR [6]. A positive chronotropic effect appeared against the background of a slow initial rhythm, and it took place in a "jump" (without a gradual rise in heart rate). Sometimes the effect was biphasic, i.e., acceleration was preceded by some degree of slowing (Fig. 2b). In experiments in which these effects took place arrhythmias were observed, as shown by the fact that the venae cavae and sinus contracted with a faster rhythm than the atria and ventricles (under the technical conditions used, visual observation of activity of the venae cavae and sinus was by no means always possible). In these cases sinoatrial dissociation evidently took place, possibly due to a conduction block between the venae cavae and atria. It therefore became necessary to determine whether pilocarpine may have a positive action on the heart under conditions of normal conduction. For this purpose an investigation was carried out in which electrical activity was recorded from different parts of the heart.

In the experiments of series II pilocarpine was applied to the heart in concentrations of 10^{-8}-10^{-4} g/ml (the previous series showed that if lower concentrations are used positive chronotropic effects do not arise). Altogether 91 applications of pilocarpine were made in 45 experiments. Comparison of the sinus EG and EG for lower portions of the heart showed that in all experiments the order of conduction of excitation in the heart was normal: activation of the atria was preceded by excitation of the sinus (Fig. 2c, d). In series III (31 experiments, 35 applications of pilocarpine in concentrations of 10^{-6} to 10^{-4} g/ml) applications of pilocarpine were made against the background of sinoatrial block. The results of series II and III are given in Fig. 3.

Under conditions of normal rhythms and during sinoatrial block pilocarpine has a negative chronotropic effect on the sinus. If the rhythm was normal, slowing of activity was observed in lower parts of the heart (Fig. 2c, d). Under conditions of sinoatrial block, however, slowing of activity of the sinus node in 40% of cases was accompanied by reduction or removal of this block and by quickening of atrial and ventricular contractions; and in series I, moreover, this quickening occured in a "jump" against the background of a slow original rhythm, and the effect was biphasic (Fig. 2, e-h). Why did pilocarpine reduce the degree of block? To answer this question, we must examine the functional properties of the region of myocardium causing the block. The resting potential of cells in such an altered area is known to be lowered [2]. Under these conditions inactivation of the fast sodium channels takes place, which leads to an increase in the threshold potential, a decrease in steepness of the rising phase of the action potential and, as a result of this, to a decrease in the reliability factor and in the conduction velocity [7]. Under these circumstances, impulses following with high frequency are delayed in the altered area and a block arises. In this case slowing of the pacemaker by pilocarpine may have the result that more impulses can pass through the altered area, i.e., the block is abolished. This hypothesis is supported by the fact that in the present experiments a reduction in the degree of sino-

atrial block occurred only when activity of the sinus node was slowed. However, there is another possible explanation: Excitation of muscarinic AChR is known to be accompanied by an increase in potassium conductance and by hyperpolarization of the membrane [8]. This may lead to restoration of the lowered resting potential of the altered area and to restoration of its functional properties [13, 15].

This investigation thus showed that under conditions of the normal cardiac rhythm excitation of muscarinic AChR does not lead to a positive chronotropic effect. During heart block, excitation of muscarinic AChR may lead to a "false" positive chronotropic effect, associated with reduction or removal of the block. In experiments on frogs chronotropic effects can be judged only in cases when a conduction block between the venae cavae and lower portions of the heart is ruled out, or activity of the venae cavae and sinus is recorded.

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