ANALYSIS OF ADRENERGIC RECEPTORS OF THE FROG AND RAT MYOCARDIUM AT DIFFERENT TEMPERATURES

I. I. Abramets, I. V. Komissarov, and G. I. Reutskaya

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Chelating agents inhibit the positive inotropic action of catecholamines on the isolated frog's heart at 7 and 17°C but not at 27°C. In experiments on the isolated perfused rat's heart, chelating agents did not modify the cardiotonic effects of catecholamines.

It is generally held that the positive inotropic effect of catecholamines on the heart of frogs [8] and warm-blooded animals [5, 6, 11, 14] is mediated through β-adrenergic receptors, which are iron-containing complexes [3]. On the other hand, evidence has been obtained to show that α-adrenergic receptors are also concerned in the mechanism of the inotropic effect of catecholamines on the heart [10].

The object of the present investigation was to determine the degree to which both types of adrenergic receptors participate in the action of catecholamines in strengthening cardiac contractions.

EXPERIMENTAL METHOD

Experiments were carried out on isolated perfused rats' hearts and the hearts of spring frogs isolated by Straube's method.

The isolated rat's heart was placed in an ultrathermostat at 37 and 27°, in modified Ringer's solution aerated with oxygen [12]. The heart was perfused with the same oxygenated solution. Spontaneous contractions of the heart were recorded on the drum of a kymograph. The drugs for testing – noradrenalin (hydrotartrate), adrenalin (hydrochloride), isoprenalin (sulfate) and phenylephrine (hydrochloride) – were injected into the perfusion fluid in a concentration of 10^{-8} g/ml. The inotropic effect of the catecholamines and phenylephrine on the heart was investigated before and after treatment with chelating agents: 8-hydroxyquinoline (5 min), $Na_2CaEDTA$, and sodium diethyldithiocarbamate (10 min) in concentrations of 10^{-3} g/ml. After treatment with the chelating agents the heart was perfused with ordinary Ringer's solution for 15 min.

In experiments on isolated frogs' hearts the influence of the chelating agents on the effects of catecholamines and caffeine was studied at temperatures of 7, 17, and 27°C. Details of the method were described previously [3].

The effects of the catecholamines, phenylephrine, and caffeine were estimated from the increase in amplitude of the cardiac contractions above the initial value, taken as 100%. The mean values of these effects found by averaging the results of 5-10 experiments.

EXPERIMENTAL RESULTS

Adrenalin, noradrenalin, isoprenaline, and phenylephrine at 37° increased the amplitude of contractions of the isolated rat's heart by 3-4.5 times. The chelating agents used in this investigation caused no

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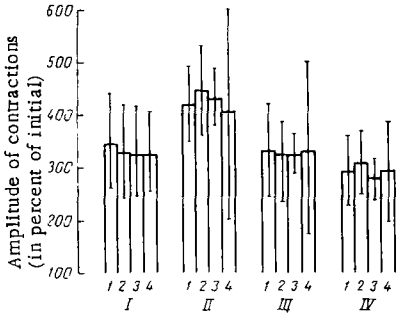

Fig. 1

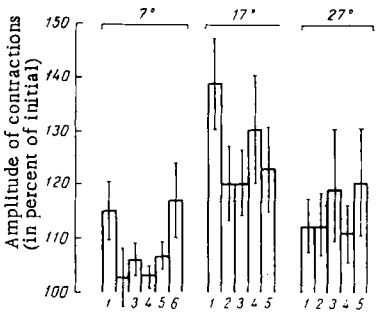

Fig. 2

Fig. 1. Influence of chelating agents on effects of catecholamines and phenylephrine ($1 \cdot 10^{-8}$ g/ml) in experiments on isolated rats' hearts at 38°: I) effects of adrenalin; II) of noradrenalin; III) of isoprenaline; IV) of phenylephrine. 1) Reaction to injection of agonist; 2) the same, after treatment with 8-hydroxyquinoline; 3) reaction to injection of $Na_2CaEDTA$; 4) to sodium diethyldithiocarbamate. Here and in Figs. 2 and 3, mean values and confidence limits ($P < 0.05$) are shown.

Fig. 2. Influence of chelating agents on effects of adrenalin ($1 \cdot 10^{-7}$ g/ml) in experiments on isolated frogs' hearts at different temperatures: 1) effect of adrenalin; 2) the same, after preliminary treatment with 8-hydroxyquinoline; 3) after treatment with $Na_2CaEDTA$; 4) with thiourea; 5) with sodium diethyldithiocarbamate; 6) effect of adrenalin after treatment with 8-hydroxyquinoline and ferrous chloride ($2 \cdot 10^{-5}$ g/ml).

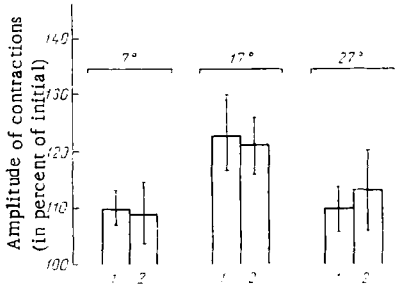

Fig. 3. Influence of 8-hydroxyquinoline on effects of caffeine in experiments on isolated frogs' hearts at different temperatures: 1) effects of caffeine sodium benzoate ($1 \cdot 10^{-7}$ g/ml); 2) the same, after preliminary treatment with 8-hydroxyquinoline.

appreciable change in these effects. Chelating agents are known to remove the ions of metals from the active centers of adrenergic receptors [1]. The results obtained indicate that either the metal is not incorporated into the structure of the active center of adrenergic receptors in the rat myocardium or the active center of these receptors is formed with the participation of Ca^{++} or Mg^{++} ions, which were incorporated into the active center of the adrenergic receptors while the heart was perfused with Ringer's solution containing calcium and magnesium ions after treatment with the chelating agents.

At 27° the positive inotropic action of the catecholamines of the rat's heart was weaker than at 37°; a further reduction of the temperature to 22-23° caused cessation of the spontaneous cardiac contractions.

Preliminary treatment of the isolated frog's heart with chelating agents reduced the positive inotropic effect of the catecholamines but not of the other cardiotonic drugs [3]. This specific action of the chelating agents was clearly apparent at 17°, but at 7° a similar effect was shown even by such a weak chelating agent as thiourea, which gave no visible effect 17°. At 27° the chelating agents did not modify the effects of adrenalin (Fig. 2). The cardiotonic effect of caffeine was not affected by 8-hydroxyquinoline throughout the temperature range: 7, 17, or 27°C (Fig. 3). At 7° the positive inotropic action of adrenalin is evidently mediated through adrenergic receptors whose function is completely blocked at 27°.

An essential discovery was that the sensitivity of the myocardium to adrenalin, when depressed by 8-hydroxyquinoline, could be restored if, after treatment with chelating agent, the heart was perfused with a solution containing ferrous chloride, but not other metals (Fig. 2). At 7°, for example, the increase in

amplitude of the contractions of the frog's heart produced by catecholamines was mediated through adrenergic receptors whose function is depressed by chelating agents and specifically restored by bivalent ferrous ions. These same properties are possessed by α-adrenergic receptors which mediate the adrenergic contraction of the vas deferens of rats and the aorta of rabbits [1, 2], and relaxation of the ileum in guinea pigs [4]. It thus follows that the adrenergic receptors of the frog myocardium, which function at 7°, are α-adrenergic receptors.

Although it has long been known that natural and dihydro derivatives of ergot alkaloids depress the stimulation of the frog's heart produced by stimulation of the sympathetic nerve or by treatment with catecholamines [8, 9, 13], the existence of both α- and β-adrenergic receptors in the frog's heart has been established only recently [7]. At 5-7°, the positive inotropic action of the catecholamines is blocked only by α-adrenolytics, and at 27-28° only by β-adrenolytics; at temperatures of the order of 15-18°, however, both types of adrenergic receptors still function.

The results of these experiments do not only confirm the existence of two types of adrenergic receptors in the frog's heart, but they also show that the α-adrenergic receptors are macromolecular iron-containing complexes functioning at 7-17°. At 27° the positive inotropic action of the catecholamines on the frog's heart is effected mainly through β-adrenergic receptors, the function of which is not disturbed by chelating agents. The positive inotropic effect of catecholamines on the heart of warm-blooded animals is mediated entirely by β-adrenergic receptors, as a result of which chelating agents do not modify the action of catecholamines on the heart of these animals. Isolated reports which have appeared of the presence of α-adrenergic receptors in the heart of warm-blooded animals [10] were not confirmed either by the present investigation or by the findings of other workers [11].

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