ADRENERGIC RECEPTORS OF THE FROG MYOCARDIUM AS IRON-CONTAINING COMPLEXES

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Chelating agents reduce the positive inotropic effect of catecholamines. Addition of ions of bivalent iron restores this effect.

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Since many enzymes concerned in the synthesis and conversion of catecholamines are metalloenzymes [1, 4, 7], it is also assumed [8, 9, 10] that metals (ions of magnesium or calcium) are present in the structure of adrenergic receptors, which have definite enzymic properties.

Previous investigations [2, 3] have shown that ions of bivalent iron participate in organization of the active center of the α-adrenergic receptors of smooth muscles of the vas deferens, and adrenergic receptors of smooth muscles of the guinea pig ileum contain iron or manganese.

The object of the present investigation was to study the character of the metal incorporated into the active center of adrenergic receptors of the frog heart, belonging to Ahlquist's category of typical β-adrenergic receptors [4, 5, 8].

EXPERIMENTAL METHOD

The hearts of winter frogs (Rana esculenta), isolated by Straub's method and perfused with Ringer's solution, were used in the experiments. The positive inotropic effect of the substances used was assessed from the increase in amplitude of cardiac contractions above the initial value, taken as 100%. The mean value of the effect was determined by averaging the results of 6-8 series of experiments. Confidence limits of the means were determined at $P = 0.05$.

The effect of the following substances was studied on the heart: adrenalin hydrochloride, 1×10^{-8} and 1×10^{-7} g/ml; noradrenalin bitartrate, 1×10^{-8} and 1×10^{-7} g/ml; isopropyl-noradrenalin, 1×10^{-9} and 1×10^{-8} g/ml; serotonin creatinine-sulfate, 1×10^{-8} g/ml; caffeine sodium benzoate, 1×10^{-8} g/ml; and potassium chloride, 1×10^{-3} g/ml. The effects of these substances were also studied on hearts preliminarily treated with chelating agents. These agents—8-hydroxyquinoline (8-HQ), sodium diethyldithiocarbamate, $Na_2CaEDTA$, and thiourea—were added to the perfusion fluid in concentrations of 2×10^{-3} g/ml for 7 min, after which the heart was washed out 4 times in the course of 12 min.

In the next series of experiments the positive inotropic effect of adrenalin was studied on hearts treated with 8-HQ and subsequently by ferrous or ferric chlorides of cobalt, copper, manganese, or zinc. These salts were added in concentrations of 1×10^{-6}, 2×10^{-5}, and 1×10^{-4} g/ml. They were allowed to act on the heart for 3 min, after which the heart was washed out with Ringer's solution.

EXPERIMENTAL RESULTS

Treatment with solutions of thiourea, sodium diethyldithiocarbamate, and $Na_2CaEDTA$ in concentrations of 1×10^{-4}-2×10^{-3} g/ml changed neither the frequency nor the amplitude of the cardiac contractions. 8-HQ, when used in a concentration of 2×10^{-3} g/ml, rapidly caused cardiac arrest; the activity of the heart was restored completely to normal after 3 rinses with Ringer's solution containing calcium.

The positive inotropic effect of adrenalin on the heart was not exhibited or was substantially reduced if the heart was preliminarily treated with chelating agents (Fig. 1). Only thiourea was almost without effect on the action of adrenalin.

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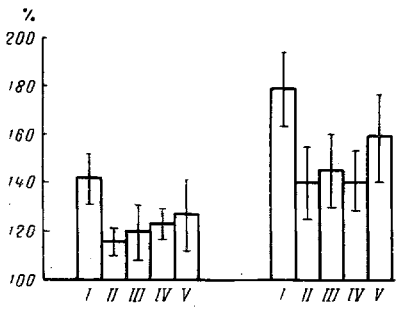

Fig. 1. Effect of chelating agents on positive inotropic effect of adrenalin in concentrations of 1×10^{-8} g/ml (left) and 1×10^{-7} g/ml (right). Horizontally: effect of adrenalin before (I) and after treatment with 8-HQ (II), sodium diethyldithiocarbamate (III), $Na_2CaEDTA$ (IV), and thiourea (V); vertically: increase in amplitude of contractions of isolated heart (in percent of initial value).

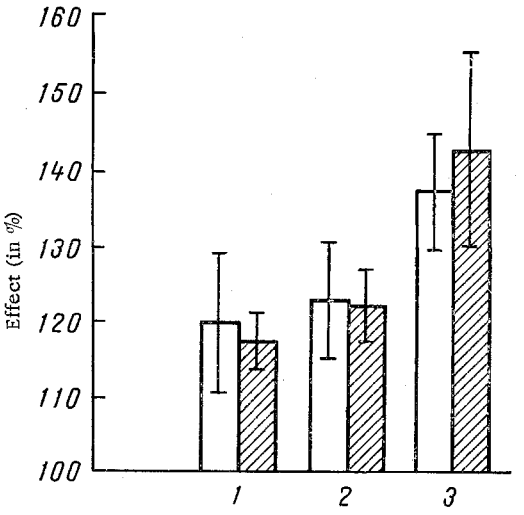

Fig. 2. Effect of 8-HQ on positive inotropic effect of serotonin (1), caffeine (2), and calcium ions (3). Unshaded columns: control; shaded columns: after treatment with chelating agents.

In the same way the chelating compounds suppressed the positive inotropic effect of noradrenalin and isoprenaline on the heart. However, this effect of the chelating compounds was specific only for catecholamines, for the positive inotropic effect of serotonin, caffeine, and calcium ions was not affected by these substances (Fig. 2).

Metallic chlorides added to the perfusion fluid in a concentration of 1×10^{-6} g/ml had no effect on the amplitude of contractions of the intact, isolated frog's heart. In concentrations of 2×10^{-5} g/ml, zinc chloride stopped the heart while ions of the other investigated metals either had no effect on its function or slightly (by 5-7%) increased the amplitude of the contractions. An effect similar to that described above was produced by metallic ions in a concentration of 1×10^{-4} g/ml, but the chlorides of ions and manganese ($FeCl_2$, $MnCl_2$) in the same concentration usually reduced the amplitude of cardiac contractions by 10-15% of the initial value.

Metallic ions in concentrations of 2×10^{-5} g/ml had no effect on the positive inotropic effect of adrenaline on the intact heart, except for zinc chloride which, as usual, stopped the heart.

The effect of metallic ions was different in experiments on hearts preliminarily treated with chelating agents. In experiments on hearts preliminarily treated with 8-HQ and then with ferrous chloride in a concentration of 2×10^{-5} g/ml, adrenalin produced the same positive inotropic effect as in experiments on the intact hearts (Fig. 3b). Ions of bivalent iron thus increased the sensitivity of the frog's myocardium to adrenalin if this sensitivity had previously been reduced by treatment with a chelating compound. Ions of other metals, including trivalent iron, had no such action, but copper chloride under these conditions actually reversed the positive inotropic effect of adrenalin.

The ability of ions of bivalent iron to increase myocardial sensitivity to adrenalin when previously reduced by treatment with chelating agents was seen to an even greater degree when ferrous chloride was given in a concentration of 1×10^{-4} g/ml (Fig. 3c). Cobalt chloride in the same concentration, however, reversed the positive inotropic effect of adrenalin, and manganese chloride had no significant effect.

The results of experiments with adrenalin given in a concentration of 1×10^{-8} g/ml are given in Fig. 3. Identical results were obtained in experiments in which adrenalin was given in a concentration of 1×10^{-7} g/ml.

The ability of chelating compounds to depress the positive inotropic effect of catecholamines is evidently due to a deficiency of ions of the metal incorporated into the structure of the active center of the

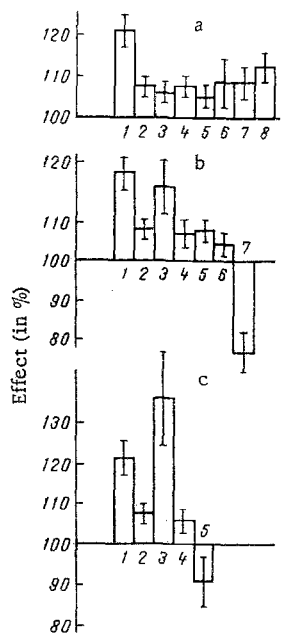

Fig. 3. Positive inotropic effect of adrenalin (1×10^{-8} g/ml) before (1) and after treatment of heart with 8-HQ (2) and subsequent treatment with solutions of chlorides of bivalent metals: iron (3), manganese (4), cobalt (5), ferric iron (6), copper (7), zinc (8). a) Salts of metals in concentration of 1×10^{-6} g/ml; b) in concentration of 2×10^{-5} g/ml; c) in concentration of 1×10^{-4} g/ml.

adrenergic receptors under the influence of chelating agents, the presence of the metal being essential for exhibition of the inotropic effect of the catecholamines. This metal cannot be either Ca^{++} or Mg^{++} ions [8, 10], for repeated washing out of the chelating compounds with Ringer's solution containing calcium and magnesium completely abolishes the deficiency of these ions in the heart tissues if present (experiments to study restoration of cardiac contractions stopped by 8-HQ). Since the sensitivity of the heart to adrenalin, if depressed by 8-HQ, is completely restored by subsequent treatment of the heart with Fe^{++}, it may be considered that ions of the metal incorporated into the structure of the active center of adrenergic myocardial receptors are in fact ions of bivalent iron. This conclusion is confirmed by the fact that ions of the other investigated metals, including trivalent iron, had no such action.

LITERATURE CITED

1. V. Z. Gorkin, in: The Biogenic Amines [in Russian], Moscow (1967), p. 146.
2. I. V. Komissarov, Abstracts of Proceedings of a Plenary Session of the All-Union Pharmacological Society [in Russian], Riga (1957).
3. I. V. Kormissarov and G. I. Reutskaya, Byull. Éksperim. Biol. i Med., No. 11, 61 (1968).
4. Ya. V. Peive, Trace Elements and Enzymes [in Russian], Riga (1960).
5. R. P. Ahlquist, Am. J. Physiol., 153, 586 (1948).
6. R. P. Ahlquist, Arch. Internat. Pharmacodyn., 139, 38 (1962).
7. Z. Axelrod, Pharmacol. Rev., 18, 95 (1966).
8. B. Belleau and J. Burba, J. Med. Pharm. Chem., 6, 755 (1963).
9. B. Belleau, Proc. 1st Internat. Pharmacol. Meeting, Vol. 7, Oxford (1963), p. 75.
10. B. Belleau, Ann. N. Y. Acad. Sci., 139, 586 (1967).
11. D. Erlij, R. Centrangolo, and R. Valadez, J. Pharmacol. Exp. Ther., 149, 65 (1965).