# ROLE OF THE METAL IN THE ACTIVE CENTER OF ADRENERGIC RECEPTORS

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Experiments on the isolated vas deferens of rats and ileum of guinea pigs showed that preliminary administration of chelating agents (8-hydroxyquinoline, diethyldithiocarbamate, thiourea, $Na_2Ca$-EDTA) lowers the sensitivity of smooth-muscle preparations to noradrenalin and isoprenaline, but not to serotonin. The lost sensitivity of the smooth muscles of the vas to catecholamines is completely restored by subsequent action of ferrous iron and partly restored by $CuCl_2$ solution, but not by salts of $Fe^{+++}$, $Mn^{++}$, $Zn^{++}$, or $Co^{++}$. Sensitivity of the ileum to catecholamines is restored only by $Fe^{++}$ and $Mn^{++}$ ions.

It is postulated that α-adrenergic receptors and intestinal adrenergic receptors are metallo-(ferro)-proteins. An essential factor in the reaction between catecholamines and adrenergic receptors is the formation of a chelate with the metal contained in the active center of the adrenergic receptors.

* * *

The structure of the active centers of adrenergic receptors has not yet been explained [2].

The suggestion has been made that a phosphate [3] or carboxyl [8] group is located at the anionic point of the α-receptor, and that its role in the interaction between catecholamines and β-adrenergic receptors is unimportant [5]. Interaction with β-receptors takes place mainly on account of phenolic hydroxyl groups reacting with the iron of the metal included in the structure of the β-adrenergic receptor [6]. Experimental confirmation of this hypothesis is given by the ability of catecholamines to act as ligands [9], and also by the property of tropolones, with high affinity for bivalent metals, of inhibiting the metalloenzyme catechol-O-methyl-transferase and to react with β-adrenergic receptors [4].

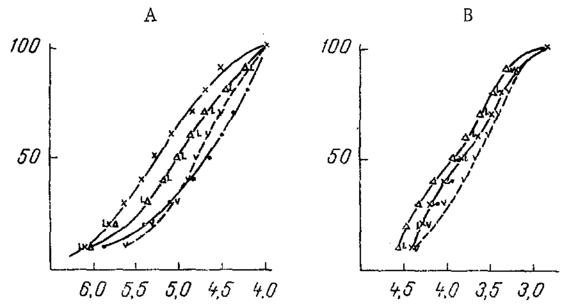


Fig. 1. Action of chelating agents on effects of noradrenalin (A) and serotonin (B) in experiments on vas deferens of rats. Logarithm of concentration-effect curves obtained before treatment (x) and after treatment with 8-HQ (·), DTC(Δ), thiourea (v) and $Na_2Ca$-EDTA (L). Abscissa, log. of concentration; ordinate, effect in percent of maximal contraction of preparation.

The object of the present investigation was to study the presence and character of the metal in active centers of adrenergic receptors.

## EXPERIMENTAL METHOD

In experiments on the isolated vas deferens of rats and ileum of guinea pigs, kept in Krebs or Tyrode solution respectively at 37°, with constant aeration, the relationship between the effects of noradrenalin, isoprenaline, and serotonin and their concentrations was studied by the method of cumulative curves [11]. The effect was calculated as a percentage of the maximal contraction. Each curve was obtained by taking the average of results of experiments on 4-6 smooth-muscle preparations.

Curves of logarithm of concentration versus effect were compared with analogous curves obtained after treatment of the smooth-muscle organs for 10 min with a series of chelating agents: 8-hydroxyquinoline (8-HQ), sodium diethyldithiocarbamate (DTC),

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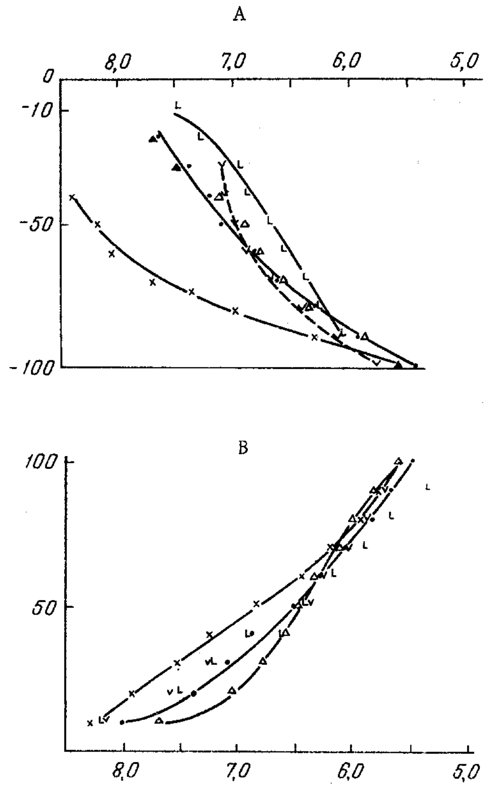


Fig. 2

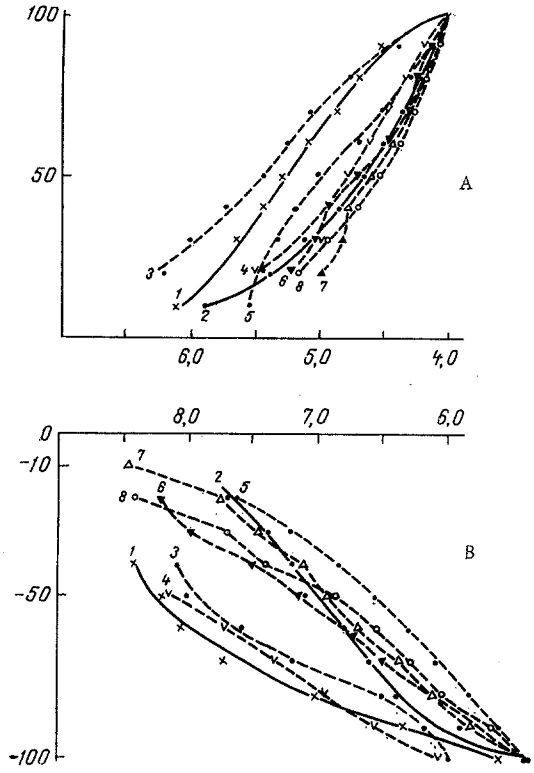


Fig. 3

Fig. 2. Action of chelating agents on effects of noradrenalin (A) and serotonin (B) in experiments on ileum of guinea pigs. Legend as in Fig. 1.

Fig. 3. Curves of logarithm of concentration versus effect of noradrenalin before (1) and after treatment of vas deferens (A) and intestine (B) with 8-HQ (2) and subsequent treatment with solutions of salts of bivalent metals: iron (3), manganese (4), copper (5), cobalt (6), and zinc (7) and of ferric chloride (8). Remainder of legend as in Fig. 1.

$Na_2Ca$-EDTA, and thiourea. These compounds were used in concentrations of $1 \cdot 10^{-3}$ g/ml in experiments on the ileum and $2 \cdot 10^{-3}$ g/ml in experiments on the vas deferens.

In the next series of experiments the relationship between the effect of the amines and their concentration was studied after treatment with chelating agents and subsequent exposure to solutions of $FeCl_2$, $FeCl_3$, $CuCl_2$, $ZnCl_2$, $CoCl_2$, and $MnCl_2$. The concentration of the salts in experiments on the ileum was $1 \cdot 10^{-5}$ and in experiments on the vas $2 \cdot 10^{-5}$ g/ml. The smooth-muscle organs were treated with solutions of these salts 8 times, for 30 sec each time, followed in every case by rinsing for 1 min.

## EXPERIMENTAL RESULTS

Curves of logarithm of concentration versus effect of noradrenalin obtained in experiments on the rat vas deferens are illustrated in Fig. 1, A. After treatment with chelating agents for 10 min, despite five subsequent washings with Krebs solution in the course of 15 min to remove these compounds, the sensitivity of the smooth muscles of the vas deferens to noradrenalin fell significantly: the logarithm of concentration-effect curves shifted to the right on the scale of concentrations, especially after treatment with 8-HQ.

Diminution of the noradrenalin effects by chelating agents was specific and not connected with their action on the mechanisms of muscular contraction, for under the same experimental conditions chelating agents had no effect on contractions of the smooth muscles of the vas if produced by administration of serotonin (Fig. 1, B).

Similar results in principle were obtained in experiments on the ileum when chelating agents very considerably reduced the effects of noradrenalin and also of isoprenaline, shifting the log concentration-effect curves by 1-1.5 orders and only slightly reducing the sensitivity of the smooth muscles to serotonin (Fig. 2).

The specific action of structurally different chelating agents on the effects of noradrenalin and isoprenaline, but not of serotonin, can be interpreted as evidence of the presence of a metallic ion in the active centers both of $\alpha$-adrenergic receptors and of "relaxation adrenergic receptors" of the intestine (of the $\beta$-adrenergic receptor type).

The fact that after treatment with chelating agents the smooth-muscle preparations were rinsed for 15 min with Krebs or Tyrode solution containing high concentrations of $Ca^{++}$ and $Mg^{++}$ ions, yet despite this the smooth muscles lost their sensitivity to catecholamines, makes the supposition that $Ca^{++}$ and $Mg^{++}$ ions are present in the active center of adrenergic receptors improbable. This conclusion is confirmed by the observations showing that smooth-muscle preparations deprived of calcium by treatment with $Na_2$-EDTA readily lose their sensitivity to serotonin [7, 10].

Special analysis showed that $Fe^{++}$ may be incorporated into the structure of the active center of $\alpha$-adrenergic receptors, and probably $Fe^{++}$ or $Mn^{++}$ into the active center of "relaxation adrenergic receptors" of the intestine.

As Fig. 3, A shows, sensitivity of the smooth muscles of the vas deferens to noradrenalin, when lowered by the action of 8-HQ, can be completely restored if the preparation is treated with ferrous chloride immediately after exposure to the chelating agent. $Cu^{++}$ has a weak restorative action, but not salts of $Fe^{+++}$, $Mn^{++}$, $Zn^{++}$, and $Co^{++}$. In experiments on the guinea pig ileum (Fig. 3, B), restoration of sensitivity to noradrenalin lost after treatment with 8-HQ can only be obtained by subsequent treatment with $FeCl_2$ or $MnCl_2$.

The results are evidence of the metalloprotein nature of adrenergic receptors. The $Fe^{++}$ ion (in $\beta$-receptors possibly the $Mn^{++}$ ion) plays the role of central ion capable of forming a chelate with catecholamines. The formation of a chelate compound by the $Fe^{++}$ ion takes place at the expense of the phenolic m-hydroxyl group belonging to the catecholamines, assuming an ionized form when reacting with $\alpha$-adrenergic receptors [1], and also of the phenolic p-hydroxyl group or $\beta$-alcohol hydroxyl group.

Assuming that in chelate compounds the coordination number of $Fe^{++}$ is usually 6, it can be assumed that two molecules of catecholamine can land on the two subunits combined into the dimer structure of the $\alpha$-adrenergic receptor by the $Fe^{++}$ ion. The possibility of oligomerism of the $\alpha$-adrenergic receptors is indirectly confirmed by the gradient of the logarithm of concentration-effect curves of noradrenalin and adrenalin, which is usually higher than the theoretical value if the curves are obtained for smooth muscles possessing $\alpha$-adrenergic receptors.

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