PRESENCE AND CHARACTER OF THE METAL IN THE ACTIVE CENTER OF β-ADRENERGIC RECEPTORS OF SMOOTH MUSCLES

I. V. Komissarov and G. I. Reutskaya

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Experiments on the tracheal rings of guinea pigs have shown that preliminary treatment with the chelating agents 8-hydroxyquinoline, diethyldithiocarbamate, thiourea, and $Na_2CaEDTA$ does not affect the sensitivity of the tracheal smooth muscles to adrenalin, isoprenaline, and papaverine. The chelating agents reduce sensitivity of the smooth muscles of the guinea pig ileum to adrenalin but not to papaverine. If the α-adrenergic receptors of the ileum are blocked with dibenamine the effect of the chelating agents does not appear, but propanolol blocks adrenalin effects.

It is concluded that the β-adrenergic receptors of smooth muscles, unlike those of the heart and the α-adrenergic receptors of the intestine, are not iron-containing complexes, but probably have magnesium or calcium ions in the structure of their active centers.

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Experiments on the vas deferens of rats [1] and the frog's heart have shown that preliminary administration of chelating compounds reduces the sensitivity of the myocardium and smooth muscles of the vas to catecholamines, but not to other substances causing contraction of the vas (serotonin) or exerting a positive inotropic action on the heart (serotonin, caffeine, calcium). Lost sensitivity to catecholamines is completely restored by the subsequent action of ferrous chloride, but not by ions of other metals. It has been suggested that the α-adrenergic receptors of the smooth muscles of the vas and β-adrenergic receptors of the myocardium are iron-containing macromolecular complexes.

In this investigation the β-adrenergic receptors of smooth muscles are analyzed as possible metal-containing macromolecules.

EXPERIMENTAL METHOD

The concentration–effect relationship of adrenalin, noradrenalin, papaverine, and theophylline was studied in experiments on the isolated ileum and tracheal rings of guinea pigs kept in Krebs' solution at 37° with constant oxygenation. The details of the method were described previously [1]. Logarithms of concentration–effect curves were compared with the analogous curves obtained after treatment of the smooth-muscle preparations with various chelating compounds: 8-hydroxyquinoline, sodium diethyldithiocarbamate, $Na_2CaEDTA$ and thiourea (10^{-3} g/ml).

The chelating compounds were added to the bath for 7 min, after which the smooth-muscle preparation was repeatedly washed with Krebs' solution. The effect of the chelating compounds or propanolol was studied in experiments on the ileum after preliminary exposure of the intestine to dibenamine. The smooth-muscle preparation was treated with dibenamine for 10 min, for the next 7 min with thiourea or $Na_2CaEDTA$ and was then washed three times (15 min). In experiments in which propanolol was used instead of chelating agents, the drug was added to the bath after washing to remove dibenamine (17 min) in a concentration of 10^{-6} g/ml for 5 min.

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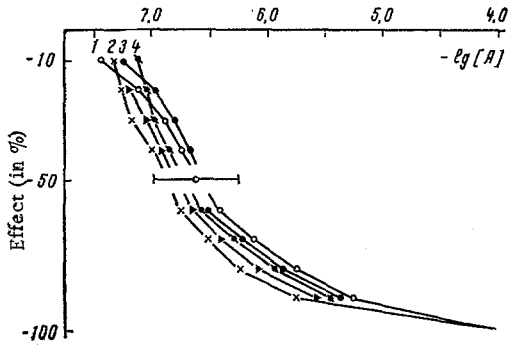

Fig. 1

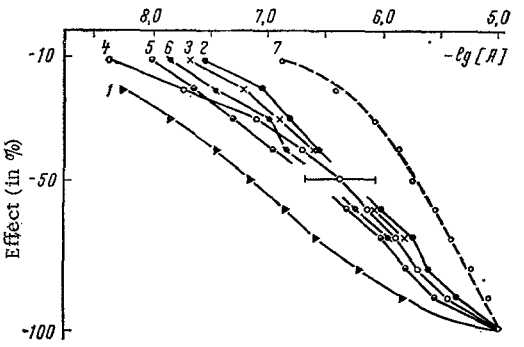

Fig. 2

Fig. 1. Influence of chelating agents on adrenalin effect in experiments on tracheal rings of guinea pigs. Log of concentration–effect curves obtained before (1) and after treatment with thiourea (2), 8-hydroxyquinoline (3), $Na_2CaEDTA$ (4), and sodium diethyldithiocarbamate (5).

Fig. 2. Log of concentration–effect curves of adrenalin obtained in experiments on isolated guinea pigs ileum before (1) and after treatment with thiourea (2), $Na_2CaEDTA$ (3), dibenamine (4), dibenamine and thiourea (5), dibenamine and $Na_2CaEDTA$ (6), and dibenamine and propanolol (7).

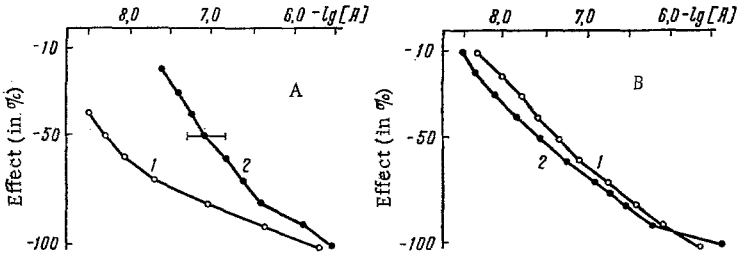

Fig. 3. Effect of 8-hydroxyquinoline (10^{-3} g/ml) on effects of noradrenalin (A) and papaverine (B) in experiments on the guinea pig ileum. Log of concentration–effect curves before (1) and after (2) treatment with chelating compound.

EXPERIMENTAL RESULTS AND DISCUSSION

Preliminary treatment of the smooth-muscle preparation of the guinea pig trachea with chelating compounds caused no significant change in the shape or position of the log of concentration–effect curves of adrenalin (Fig. 1). The chelating compounds likewise did not modify the effects of isoprenaline, theophylline, or papaverine.

The adrenergic receptors of smooth muscles of the guinea pig trachea belong entirely to the β-type [4]. The fact that chelating compounds did not modify the effects of catecholamines on this object is evidence of absence of metal or of the presence of magnesium or calcium ions in the structure of the active centers of the β-adrenergic receptors of the tracheal smooth muscles. In the latter case, treatment with chelating compounds would not necessarily damage the structure of the adrenergic receptors, because immediately after addition of the chelating compounds the tracheal preparation was repeatedly washed with Kreb's solution containing Ca^{++} and Mg^{++}.

In experiments on the ileum, preliminary treatment with thiourea and $Na_2CaEDTA$, despite subsequent removal with Krebs' solution, lowered the sensitivity of the intestinal smooth muscles to adrenalin: the logs of concentration–effect curves were shifted to the right along the concentration scale (Fig. 2). Under the same experimental conditions papaverine did not change the effects (Fig. 3).

After treatment with dibenamine, the sensitivity of the intestinal smooth muscles to adrenalin was lowered. Segments of ileum treated successively with dibenamine and chelating compounds were just as

sensitive to adrenalin as segments treated with dibenamine only or with chelating compounds only. Meanwhile, after treatment with propanolol, the sensitivityof the smooth muscles to adrenalin, when lowered by dibenamine, was lowered further still (Fig. 2).

The lowering of intestinal muscle tone by catecholamines is due to their action on α- and β-adrenergic receptors [5, 7]. Since after blocking of the α-adrenergic receptors of the ileum by dibenamine, chelating compounds did not lower the sensitivity of the smooth muscles to adrenalin, but the adrenergic blocking effect of propanolol persisted, it must be concluded that the lowering of the sensitivity of the intestinal muscles by chelating compounds was due to their effect on the α-adrenergic receptors of the intestine. This suggests that iron or manganese ions, which as was previously shown [1] are present in the structure of the active centers of adrenergic stretch receptors of the guinea pig ileum, belong to the α-adrenergic receptors of the intestine.

The intestinal β-adrenergic receptors, like those of the trachea, are either without metal or they contain magnesium or calcium ions in the structure of their active centers. This hypothesis compels acceptance of differences in the structure of active centers of the iron-containing β-adrenergic receptors of the myocardium and the ion-free β-adrenergic receptors of smooth muscles. The β-adrenergic receptors of the myocardium and smooth muscles of the bronchi can be differentiated pharmacologically by comparison of the relative activity of a series of sympathomimetics [3, 6]. Since the effect of catecholamines in relaxing the intestine is potentiated by theophylline, but is absent in a solution not containig glucose, and it is preceded by an increase in the concentrations of ATP and CP [2], it can be assumed that the β-adrenergic receptors of smooth muscles are identical with adenyl cyclase.

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