INVESTIGATION OF THE NATURE OF DIFFERENCES BETWEEN β-ADRENERGIC RECEPTORS

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The results of an investigation of the affinity of propranolol, alprenolol, and practolol for β-adrenergic receptors of various organs confirm the division of these receptors into two subtypes. The action of isoprenaline on β_1-adrenergic receptors of the smooth muscles of the gastrointestinal tract and myocardium is accompanied by a change in the inflow of Ca^{++} ions into the cells. Under the influence of isoprenaline on β_2-adrenergic receptors of the vessels and trachea an increase in the outflow of Ca^{++} ions from the cells and a decrease in tone of the smooth muscles are observed.

KEY WORDS: β-adrenergic receptors; classification; conjugation mechanisms.

Many effects of the catecholamines take place with the participation of β-adrenergic receptors [2]. Evidence of the pharmacological nonhomogeneity of β-adrenergic receptors has now been accumulated. For instance, β_1-adrenergic receptors of the myocardium and intestine and β_2-adrenergic receptors of the blood vessels, trachea and, perhaps, uterus are distinguished [5]. However, this classification has aroused certain objections [4, 6, 9].

The object of this investigation was to study the special features of receptors mediating the β-adrenergic effects of catecholamines.

EXPERIMENTAL METHOD

Antagonism between propranolol, alprenolol, and practolol, on the one hand, and isoprenaline, on the other hand, was investigated on the isolated atria and spiral strips of the trachea from guinea pigs, strips of the fundus of the rat stomach, and strips of the rabbit carotid artery. The character of the shift of the logarithm of concentration–effect curves of isoprenaline was determined in the presence of adrenolytics in concentrations of $1 \cdot 10^{-7}$ g/ml ($1 \cdot 10^{5}$ g/ml in the case of practolol), and the difference in the values $pA_2 - pA_{10}$ was determined for all adrenolytics. The values of pA_2 and pA_{10} were determined by Schild's method [8] after contact between the β-adrenolytics and the organs for 14 min.

The effect of changes in the ionic medium on isoprenaline-induced relaxation of the smooth muscles and the positive chronotropic effect also was studied. The intracellular Na^+ ion concentration was altered by replacing half of the NaCl in Krebs's solution by sucrose and inhibiting the K^+-, Na^+-activated membrane ATPase by strophanthin ($5 \cdot 10^{-6}$ g/ml). The dependence of the effects of isoprenaline on the Ca^{++} ion concentration was investigated by increasing (up to 25 mM) and reducing (down to 0.25 mM) their concentrations, and also by exposure to the action of $MnCl_2$ (2 mM) and $LaCl_3$ (1 mM). In each experiment a control curve of logarithm of concentration–effect of isoprenaline was first obtained. The organ was then rinsed for 30 min and then treated with one of the agents described above, after which the curve of logarithm of concentration–effect of isoprenaline was again plotted.

In the last series of experiments the effect of isoprenaline on the membrane potential (MP), action potentials (APs), and tone of a longitudinal strip of the guinea pig large intestine and rat portal vein was investigated by the "sucrose gap" method [1]. Resting and action potentials of the smooth muscles were recorded with the aid of Ag–AgCl electrodes from the sucrose gap chambers on a dc amplifier of the VEKS-4m

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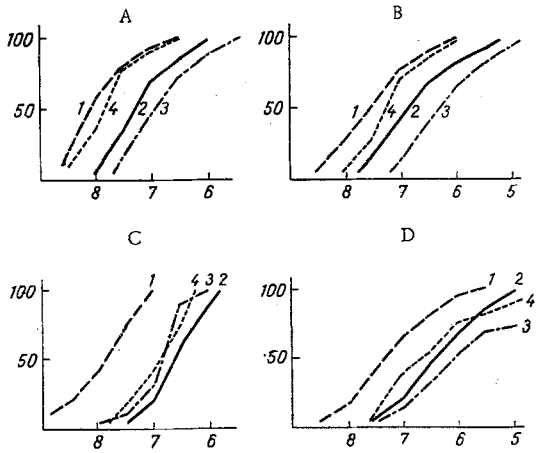

Fig. 1. Effect of propranolol, alprenolol, and practolol on log concentration–effect curves of isoprenaline: A) guinea pig trachea; B) rabbit carotid artery; C) guinea pig atrium; D) strip of rat stomach. 1) Control; 2, 3, and 4) in the presence of propranolol, alprenolol ($1 \cdot 10^{-7}$ g/ml), and practolol ($1 \cdot 10^{-5}$ g/ml) respectively. Abscissa, negative logarithms of isoprenaline concentration (in % of maximal).

TABLE 1. Values of pA_2 and pA_{10} and their Difference for β-Adrenolytics Tested in Experiments on Smooth Muscles and Myocardium (M ± tm for P = 0.05)

β-Adrenolytic	Guinea pig trachea	Rabbit carotid artery	Guinea pig atrium	Strip of rat stomach
Propranolol				
pA_2	7,59±0,19	7,68±0,18	8,64±0,21	7,18±0,36
pA_{10}	6,54±0,26	6,62±0,22	7,60±0,23	6,15±0,10
pA_2—pA_{10}	1,05*	1,06*	1,04*	1,03*
Alprenolol				
pA_2	8,54±0,21	8,55±0,47	7,21±0,36	7,46±0,33
pA_{10}	7,41±0,39	7,55±0,09	6,56±0,30	6,57±0,30
pA_2—pA_{10}	1,13*	1,00*	0,65	0,89
Practolol				
pA_2	<4	<4	6,47±0,27	6,61±0,18
pA_{10}	—	—	5,50±0,19	5,51±0,24
pA_2—pA_{10}	—	—	0,97*	1,10*

*Absence of statistically significant difference with theoretical value of difference $pA_2 - pA_{10} = 0.95$.

vector-electrocardioscope. The changes observed were recorded by means of a camera attachment on photographic paper. Changes in tone of the smooth muscles were recorded by a variable-capacitance transducer with spring-steel membrane with initial stretching of the taenia coli by 1 g and the portal vein by 0.5 g.

EXPERIMENTAL RESULTS AND DISCUSSION

It will be clear from Fig. 1 that β-adrenolytics caused a shift of the logarithm of concentration–effect curves of isoprenaline to the right along the concentration scale.

Whereas propranolol, judging from the values of pA_2 and pA_{10}, had about equal affinity for the β-adrenergic receptors of the organs studied, practolol had greater affinity for the β-adrenergic receptors of the myocardium and stomach, and alprenolol had greater affinity for the β-adrenergic receptors of the smooth muscles of the blood vessels and trachea (Table 1). The difference between the values of pA_2 and pA_{10} for the β-adrenolytics was 0.95, excluding the affinity of alprenolol in experiments on the strip of stomach and myocardium and practolol on the carotid artery and trachea; in those cases a parallel initial shift of the log concentration–effect curves of isoprenaline to the right was observed. These facts point to the presence of competitive antagonism between the β-adrenolytics and isoprenaline. The stronger affinity of alprenolol for β-adrenergic receptors of the trachea and blood vessels and of practolol for the β-adrenergic

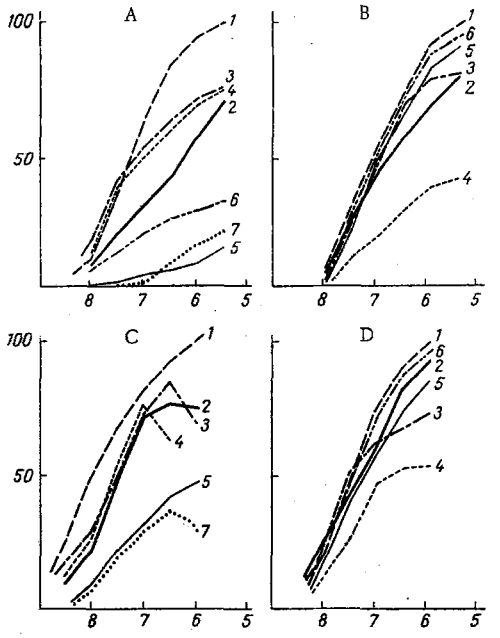

Fig. 2. Action of isoprenaline in experiments on smooth-muscle organs and myocardium after modification of ionic medium: A) strip of stomach; B) guinea pig trachea; C) guinea pig atrium; D) rabbit carotid artery. 1) Control; 2) replacement of half of NaCl in Krebs's solution by sucrose; 3) treatment with strophanthin ($5 \cdot 10^{-6}$ g/ml); 4) Ca^{++} concentration in Krebs's solution 25 mM; 5) Ca^{++} concentration 0.25 mM; 6) treatment with 2 mM $MnCl_2$; 7) treatment with 1 mM $LaCl_3$. Remainder of legend as in Fig. 1.

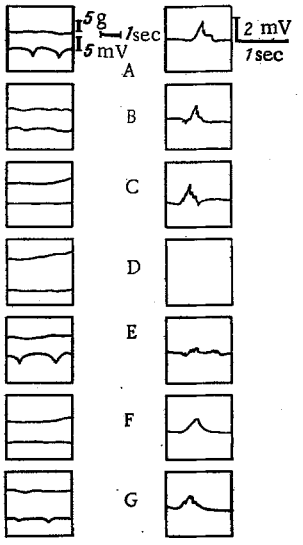

Fig. 3. Effect of isoprenaline on electrical and mechanical activity of guinea pig taenia coli and rat portal vein: A) control; B, C, and D) treatment with isoprenaline in concentrations of $1 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, and $1 \cdot 10^{-5}$ g/ml, respectively; E) treatment with procaine ($1 \cdot 10^{-3}$ g/ml) F) treatment with $LaCl_3$ (1 mM); G) treatment with $LaCl_3$ (1 mM) after isoprenaline ($1 \cdot 10^{-6}$ g/ml). Top curve in each frame on left shows tone; bottom curve, electrical activity of guinea pig taenia coli; curve in each frame on right shows electrical activity of rat portal vein.

receptors of the myocardium and stomach can therefore be understood on the basis of the postulated existence of two subtypes of β-adrenergic receptors [5].

During interaction between isoprenaline and each of these types of adrenergic receptors, different mechanisms evidently link the adrenergic stimulus with the final effect of action of isoprenaline. As Fig. 2 shows, the effect of isoprenaline on the guinea pig atrium and on the strip of rat stomach was significantly reduced with a decrease in the Ca^{++} ion concentration in the external solution and in the presence of Mn^{++} and La^{++} ions, reducing the permeability of the cell membranes to Ca^{++} ions [3, 4], whereas the effect of isoprenaline on smooth muscles of the guinea pig trachea and rabbit carotid artery was greatly reduced only if Ca^{++} ions were present in excess in the Krebs's solution. The explanation of this fact may be that stimulation of β-adrenergic receptors by isoprenaline is accompanied by an increased outflow of Ca^{++} ions from the smooth muscles; as a result their intracellular concentration decreases and the muscle tone is reduced.

This hypothesis was partly confirmed by the fact that isoprenaline did not change MP and did not abolish the spontaneous APs in a β_2-adrenergic preparation (the rat portal vein; Fig. 3), although their frequency was reduced a little. APs in the smooth muscles of the vein were inhibited by treatment with procaine but were not changed in the presence of lanthanum chloride, i.e., they appeared as a result of increased permeability of the cell membranes to Na^+ ions but not to Ca^{++} ions.

Conversely, in experiments on a β_1-adrenergic preparation (guinea pig taenia coli) APs were unrelated in the presence of procaine but were inhibited by the addition of lanthanum chloride. Isoprenaline, exactly like La^{++} ions, abolished spontaneous APs in the smooth muscles of the intestine without changing MP. La^{++} ions and isoprenaline evidently compete for structures of the cell membranes that control calcium permeability and weaken each other's effects. These facts suggest that the action of isoprenaline on β_1-adrenergic receptors modifies the functions of the effector organ cells by changing the rate at which Ca^{++} ions enter the cells.

Hence the β_1- and β_2-adrenergic receptors of the myocardium and smooth muscles differ not only in their affinity for β-adrenomimetics [5] and β-adrenolytics, but also in their mechanisms of conjugation, by means of which isoprenaline exerts its effect on smooth muscles and myocardium.

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