SHAPE AND GRADIENT OF CONCENTRATION-EFFECT CURVES OF CATECHOLAMINES IN EXPERIMENTS ON SMOOTH MUSCLE PREPARATIONS

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Experiments on isolated preparations of the guinea pig trachea and on strips of the stomach and spleen of rats showed that concentration-effect curves of catecholamines have the shape of a rectangular hyperbola and a near-theoretical gradient (0.57). Concentration-effect curves of noradrenalin on a strip of rabbit aorta and on the vas deferens of rats are S-shaped and their gradient is greater than theoretical. In experiments on strips of the stomach and spleen of rats and on rats with recording of the blood pressure, the gradients of the curves are increased against the background of the action of propranolol and become significantly greater than the theoretical gradient. It is postulated that α-adrenergic receptors of the blood vessels of rabbits and rats, and of the stomach, spleen, and vas deferens of rats are oligomeric in structure, while the β-adrenergic receptors of the guinea pig trachea and rat stomach are monomeric receptors.

The suggestion has been made that α-adrenergic receptors containing a ferrous ion in their active center are oligomeric in structure [3]. One way of verifying this hypothesis experimentally is to study the shape and gradient of concentration-effect curves of the catecholamines.

It follows from the equation for the reaction between agonists and specific receptors,

$$Y = \frac{[A]}{K + [A]},$$

where Y is the effect, [A] the molar concentration of the agonist, and K the dissociation constant of the agonist–receptor complex, that the relationship between effect and agonist concentration will be described by a rectangular hyperbola, with a gradient of 0.57. The S-shaped curves frequently observed, and a higher than theoretical gradient may be proof of the cooperativeness of interaction between agonist and receptor [6, 9] provided that the tissues do not contain a reserve of receptors and that the possibility of independence or noncompetitive autointeraction is ruled out [2]. S-shaped curves are typical for interaction between substrates with oligomeric allosteric enzymes [1] and for the saturation of the tetrameric hemoglobin of vertebrates with oxygen [4].

EXPERIMENTAL METHOD

Experiments were carried out on isolated strips of the stomach, the spleen, and the vas deferens of rats, strips of rabbit aorta, tracheal rings of guinea pigs, and on rats anesthetized with urethane (1.2 g/kg) in which the arterial pressure was recorded for a short period.

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TABLE 1. Hill's Index and Gradient of Concentration-Effect Curves of Catecholamines on Smooth-Muscle Organs (theoretical gradient 0.57)

Experimental object and conditions	No. of experiments	Hill's index	Gradient (mean)	Difference between experimental and theoretical gradients	3 × Standard error of mean
Rat vas deferens (NA)	10	2.5	0.80	0.23	0.18
Strip of rabbit aorta (NA)	10	1.65	0.69	0.12	0.12
Arterial pressure of rats (NA)	5	1.15	0.59	0.02	0.015
The same against background of propranolol (5 mg/kg)	4	1.55	0.65	0.08	0.06
Rat spleen (NA)	6	1.25	0.56	0.01	0.09[1]
The same against background of propranolol ($5 \cdot 10^{-6}$ g/ml)	5	1.5	0.65	0.08	0.03
Guinea pig's trachea (ISA)	6	1.0	0.52	0.05	0.15[1]
Strip of rat stomach (NA)	10	1.25	0.56	0.01	0.06[1]
The same against background of phentolamine ($5 \cdot 10^{-6}$ g/ml)	5	1.25	0.50	0.07	0.075[1]
The same against background of propranolol ($5 \cdot 10^{-6}$ g/ml)	5	2.25	0.74	0.17	0.06

Note: NA signifies noradrenalin; ISA isoprenaline

[1]Differences between gradients not significant.

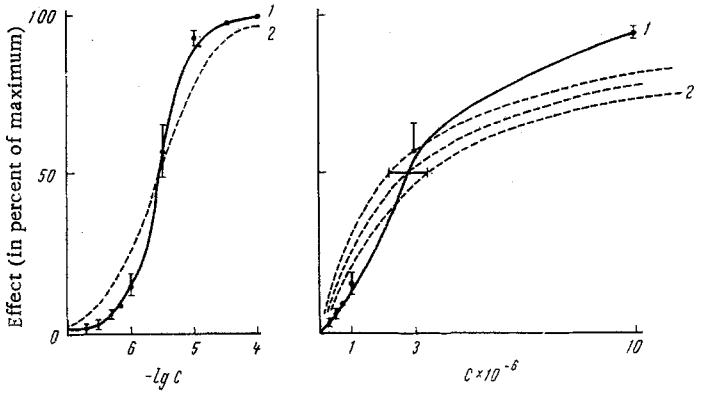

Fig. 1. Experimental (1) and theoretical (2) concentration–effect curves of noradrenalin obtained in experiments on the rat vas deferens. Scale of concentrations on left logarithmic, on right arithmetical. Theoretical curves obtained from equation of mass action counting from the point EC_{50} (± confidence interval at P = 0.05).

The isolated organs were placed in Krebs' solution (37°C) strongly aerated with oxygen. Contraction or relaxation of the smooth-muscle preparations was recorded under isotonic conditions, with a minimal load, on Engelmann's levers. The relationship between effects and concentration of catecholamines (isoprenaline, noradrenalin) was studied by the method of cumulative curves [15]. Mean values were calculated from the results obtained with 4-10 preparations, and the shape of the concentration-effect curves judged from the cumulative curve. Hill's index, a qualification of its sigmoid character [9], was found for this same curve. Gradients were determined for each individual curve; their mean value was obtained, together with the standard error of the mean. Differences between the experimental and theoretical gradients were regarded as significant if they did not exceed three times the standard error of the mean [5].

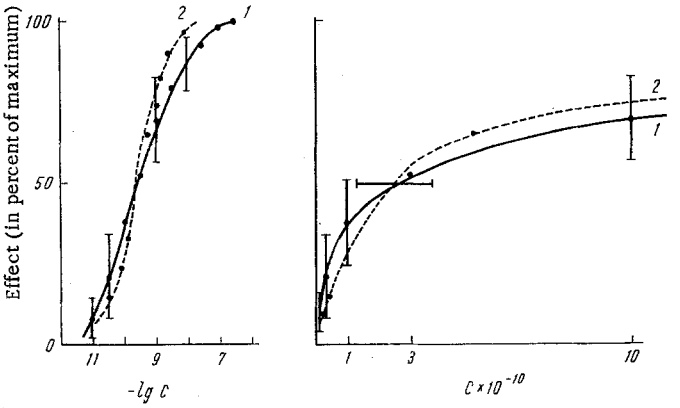

Fig. 2. Experimental (1) and theoretical (2) concentration-effect curves of isoprenaline obtained in experiments on the guinea pig trachea.

In experiments on objects where the effects could be determined by independent interaction between catecholamines and the two types of adrenergic receptors (stomach, spleen [8, 14] of rats), α-adrenolytics (phentolamine, $5 \cdot 10^{-6}$ g/ml) or β-adrenolytics (propranolol, $5 \cdot 10^{-6}$ g/ml) were used.

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental curve of amplitude of contractions of the rat vas deferens versus noradrenalin concentration does not coincide with the theoretical curve (Fig. 1). Unlike the theoretical hyperbola, the experimental curve is S-shaped. Its sigmoid character is confirmed by the value of Hill's index [2, 5] which in this case is much greater than unity (Table 1). The gradient of this curve is also greater than the theoretical gradient.

Similar relationships, as Table 1 shows, were obtained in the experiments on strips of rabbit aorta.

Since the smooth muscles of the rabbit aorta and rat vas deferens contain principally α-adrenergic receptors [7], and reserves of these receptors are virtually absent [11, 13], the increase in gradient and the S-shape of the concentration-effect curves are evidence of the oligomeric character of the α-adrenergic receptors, i.e., that they contain 2 or more subunits of their quaternary structure.

Dose (concentration)- effect curves for which Hill's index was close to unity and whose gradients almost exactly coincide with the theoretical gradient were obtained in experiments to record the arterial pressure and in experiments on the isolated spleen of rats. However, the use of propranolol to abolish the effect of noradrenalin on the β-adrenergic receptors increased both Hill's index and the gradient of the curves above the theoretical value (Table 1). The relationship between the degree of relaxation of the guinea pig trachea and isoprenaline concentration is described by a rectangular hyperbola (Fig. 2). The important feature in this case is that Hill's index is 1, and the gradient of the curve coincides with the theoretical (Fig. 2; Table 1). Similar curves were obtained in experiments on strips of rat stomach. Whereas adrenergic receptors in the smooth muscles of the guinea pig trachea are entirely of the β-type [10], relaxation of the stomach by catecholamines can be achieved by action on both β- and α-adrenergic receptors [12, 14]. Blocking the α-adrenergic receptors with phentolamine produces little change in the gradient of the concentration-effect curves, whereas blocking the β-adrenergic receptors with propranolol increases it substantially; Hill's index also rises significantly (Table 1).

Hence, where the effects of catecholamines are realized entirely (guinea pig trachea) or predominantly (rat stomach) through the β-adrenergic receptors, the shape and gradient of the concentration-effect curves coincide with these predicted theoretically. When catecholamines produce their characteristic effect through the α-adrenergic receptors, the curves are S-shaped and the gradient is higher than theoretical. These results can be interpreted on the assumption that the structure of the α-adrenergic receptors is oligomeric while that of the β-adrenergic receptors in the smooth muscles is monomeric.

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