INVESTIGATION OF THE RATES OF INTERACTION BETWEEN ADRENOLYTICS AND α-ADRENERGIC RECEPTORS OF SMOOTH MUSCLES

I. I. Abramets and I. V. Komissarov

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Velocity constants of occupation and liberation of adrenergic receptors of the smooth muscles of the rat vas deferens were determined for phentolamine and dihydroergotoxine. Under anoxic conditions and, in particular, after preliminary exposure for 15 min to the action of noradrenalin (1×10^{-5} g/ml) the constant of the direct reaction, but not of the reverse reaction, was reduced. It is concluded that energy is expended in the formation of the antagonist–receptor complex, but the breakdown of this complex does not require the expenditure of energy.

The interaction of agonists and antagonists with specific receptors is accompanied by conformational changes in the latter [3, 5]. It has been shown, in particular, that interaction between noradrenalin and the α-adrenergic receptors of smooth muscles causes conformational changes which can easily be found because of the reduced affinity of the receptors for dihydroergotoxine. The affinity of receptors for the α-adrenolytic is restored "spontaneously," but restoration is sharply delayed under anoxic conditions and in the presence of dinitrophenol [1]. It is not clear, however, for what the energy of oxidation is required – for the conformational changes arising during interaction between the receptors and noradrenalin or for restoring the original confirmation of the adrenergic receptors.

In the investigation described below an attempt was made to solve this problem by measuring the kinetic velocity constants of interaction between adrenolytics and α-adrenergic receptors.

EXPERIMENTAL METHOD

The principle suggested by Paton [8] was used to determine separately the velocity constants of direct (k_1) and reverse (k_{-1}) interaction between α-adrenolytics and adrenergic receptors. Since the formation of the antagonist–receptor complex obeys the law of mass action

$$R + B \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} RB,$$

where R is the total number of receptors, B the concentration of the antagonist, and RB the concentration of receptors occupied by the antagonist, the change in the occupancy of the receptors with time during the development of the block will be determined by the difference between the velocities of formation and breakdown of the antagonist–receptor complexes, i.e.,

$$\frac{RB}{dt} = k_1 \cdot B(1 - RB) - k_{-1}RB. \quad (1)$$

The velocity of breakdown of the complexes is proportional to the concentration of receptors occupied by the antagonist:

$$\frac{RB}{dt} = k_{-1} \cdot RB. \quad (2)$$

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TABLE 1. Concentrations of Noradrenalin Causing Effects in the Presence of Agonists Equal to Effect of Testing Concentration of Noradrenalin, and Proportion of Receptors Occupied by the Agonist (RB) during Development and Disappearance of Adrenergic Block of the Smooth Muscles of the Rat Vas Deferens

Experimental conditions	No. of experiments	Index	Development of block, time (in min)							Disappearace of block, time (in min)		
			3	6	9	12	15	18	21	10	20	30
Dihydroergotoxine ($1 \cdot 10^{-7}$ g/ml)	20	A*	$1{,}6 \cdot 10^{-6}$	$2{,}1 \cdot 10^{-6}$	$2{,}7 \cdot 10^{-6}$	$\underline{3{,}5 \cdot 10^{-6}}$	$3{,}5 \cdot 10^{-6}$			$3{,}2 \cdot 10^{-6}$	$2{,}8 \cdot 10^{-6}$	$2{,}4 \cdot 10^{-6}$
		n	1,6	2,1	2,7	3,5				3,2	2,8	2,4
		RB	0,37	0,52	0,63	0,71				0,69	0,64	0,58
		$RB_{eq} - RB_t$	0,34	0,19	0,08	0						
Phentolamine ($1 \cdot 10^{-8}$ g/ml)	18	A*	$1{,}2 \cdot 10^{-6}$	$1{,}6 \cdot 10^{-6}$	$1{,}9 \cdot 10^{-6}$	$\underline{2{,}1 \cdot 10^{-6}}$				$1{,}9 \cdot 10^{-6}$	$1{,}5 \cdot 10^{-6}$	$1{,}4 \cdot 10^{-6}$
		RB	0,17	0,38	0,47	0,53				0,47	0,36	0,33
		$RB_{eq} - RB_t$	0,36	0,15	0,07	0						
Dihydroergotoxine ($1 \cdot 10^{-7}$ g/ml) after treatment with noradrenalin ($1 \cdot 10^{-5}$ g/ml)	10	A*	$1{,}1 \cdot 10^{-6}$	$1{,}3 \cdot 10^{-6}$	$1{,}9 \cdot 10^{-6}$	$2{,}5 \cdot 10^{-6}$	$\underline{3{,}5 \cdot 10^{-6}}$			$3{,}2 \cdot 10^{-6}$	$2{,}7 \cdot 10^{-6}$	$2{,}4 \cdot 10^{-6}$
		RB	0,09	0,23	0,47	0,60	0,71			0,69	0,63	0,58
		$RB_{eq} - RB_t$	0,62	0,48	0,24	0,11						
Phentolamine ($1 \cdot 10^{-8}$ g/ml) after treatment with noradrenalin ($1 \cdot 10^{-5}$ g/ml)	10	A*	$1{,}0 \cdot 10^{-6}$	$1{,}1 \cdot 10^{-6}$	$1{,}5 \cdot 10^{-6}$	$1{,}9 \cdot 10^{-6}$	$\underline{2{,}1 \cdot 10^{-6}}$			$1{,}9 \cdot 10^{-6}$	$1{,}6 \cdot 10^{-6}$	$1{,}5 \cdot 10^{-6}$
		RB	0	0,09	0,33	0,47	0,53			0,47	0,36	0,33
		$RB_{eq} - RB_t$	0,53	0,44	0,20	0,06						
Dihydroergotoxine ($1 \cdot 10^{-7}$ g/ml) under anoxic conditions	10	A*	$1{,}2 \cdot 10^{-6}$	$1{,}6 \cdot 10^{-6}$	$2{,}0 \cdot 10^{-6}$	$2{,}5 \cdot 10^{-6}$	$2{,}9 \cdot 10^{-6}$	$3{,}3 \cdot 10^{-6}$	$\underline{3{,}5 \cdot 10^{-6}}$	$3{,}2 \cdot 10^{-6}$	$2{,}7 \cdot 10^{-6}$	$2{,}4 \cdot 10^{-5}$
		RB	0,17	0,37	0,50	0,60	0,65	0,70	0,71	0,69	0,62	0,58
		$RB_{eq} - RB_t$	0,54	0,34	0,21	0,11	0,06	0,01	0			
Phentolamine (1.10^{-8} g/ml) under anoxic conditions	10	A*	$1{,}1 \cdot 10^{-6}$	$1{,}2 \cdot 10^{-6}$	$1{,}3 \cdot 10^{-6}$	$1{,}4 \cdot 10^{-6}$	$1{,}6 \cdot 10^{-6}$	$1{,}8 \cdot 10^{-6}$	$\underline{2{,}1 \cdot 10^{-6}}$	$1{,}9 \cdot 10^{-6}$	$1{,}5 \cdot 10^{-6}$	$1{,}4 \cdot 10^{-6}$
		RB	0,09	0,17	0,23	0,28	0,38	0,44	0,53	0,47	0,36	0,33
		$RB_{eq} - RB_t$	0,44	0,36	0,30	0,25	0,15	0,09	0			

*Concentration of noradrenalin equal in effect to testing concentration (1×10^{-6} g/ml).

Note: Concentrations of noradrenalin found for the moment of equilibrium are underlined.

TABLE 2. Velocity Constants of Direct (k_1) and Reverse (k_{-1}) Reactions between Adrenolytics and Adrenergic Receptors of the Rat Vas Deferens and Constants of Affinity of Adrenolytics for α-Adrenergic Receptors (K_B)

Experimental conditions	κ_1	κ_{-1}	K_B (in g/ml)
Dihydroergotoxine	$1{,}14 \cdot 10^4$	$6 \cdot 10^{-5}$	$1{,}9 \cdot 10^{-8}$
Phentolamine	$7{,}2 \cdot 10^4$	$1{,}3 \cdot 10^{-4}$	$5{,}5 \cdot 10^{-8}$
Dihydroergotoxine after preliminary treatment with noradrenalin	$0{,}44 \cdot 10^4$	$6 \cdot 10^{-5}$	$0{,}7 \cdot 10^{-8}$
Phentolamine after preliminary treatment with noradrenalin	$1{,}8 \cdot 10^4$	$1{,}3 \cdot 10^{-4}$	$1{,}4 \cdot 10^{-8}$
Dihydroergotoxine under anoxic conditions	$0{,}84 \cdot 10^4$	$6 \cdot 10^{-5}$	$1{,}4 \cdot 10^{-8}$
Phentolamine under anoxic conditions	$3{,}8 \cdot 10^4$	$1{,}3 \cdot 10^{-4}$	$3 \cdot 10^{-8}$

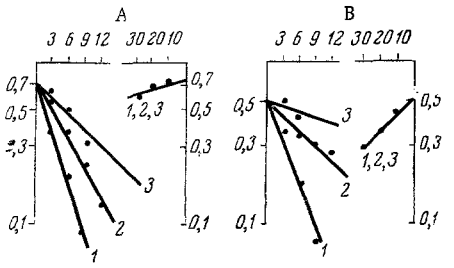

Fig. 1. Graphic analysis of onset (left hand side of figures) and disappearance (right hand side of figures) of adrenolytic action of dihydroergotoxine in a dose of 1×10^{-7} g/ml (A) and phentolamine in a dose of 1×10^{-8} g/ml (B). Abscissa, time (in min); ordinate, on left – difference between proportion of receptors occupied in equilibrium and at time t ($RB_{eq} - RB_t$), on right – proportion of receptors occupied (RB). 1) Control; 2) anoxia; 3) after preliminary exposure to noradrenalin (1×10^{-5} g/ml).

Integrating Eqs. (1) and (2) and making the necessary transformations, then taking logarithms of the expressions obtained, we find that

$$ln\ (RB_{eq} - RB_t) = ln\ RB_{eq} - (k_1[B] + k_{-1}) \cdot t \quad (1a)$$

$$\text{and } ln\ PB_t = ln\ RB_{eq} - k_{-1} \cdot t, \quad (2a)$$

where RB_t is the concentration of receptors occupied by the antagonist before equilibrium is reached, and RB_{eq} is the same at the moment of equilibrium.

Expressions (1a) and (2a) are equations of a straight line of the general form $y = a + bx$, and they can be used to determine the constants k_1 and k_{-1} graphically. If time is plotted along the abscissa and the difference between the concentrations of receptors occupied by the antagonist at the moment of equilibrium and that time t is plotted along the ordinate (on a logarithmic scale), the resulting straight line will form an intercept on the ordinate equal to RB_{eq}, and the tangent of the angle of inclination is equal to $-(k_1 \cdot B + k_{-1})$ (see Eq. 1a). By constructing a similar graph for Eq. (2a) a straight line is obtained which makes an intercept on the ordinate equal to RB_{eq}, with a tangent of the angle of inclination of $-k_{-1}$.

To plot the graphs it is necessary to find the proportion of receptors RB occupied by the antagonist at particular moments of time until the moment of equilibrium. This can be done by finding the ratio between the concentrations (n) of the agonist at which the same effect is produced both in the absence and in the presence of the antagonist [7]. Since

$$n = \frac{1}{1 - RB}, \text{ it follows that } RB = \frac{n-1}{n}. \quad (3)$$

Experiments were carried out on isolated vasa deferentia of rats. To reduce the effect of diffusion, ducts with the thinnest walls were used. They were placed in oxygenated Krebs' solution at 37°C. Contractions under isotonic conditions were recorded by a lever with a ratio of 1 : 20 between the arms and with a load on the vas of 0.5 g. Contractions from the testing concentration of noradrenalin (1×10^{-6} g/ml) were determined every 3rd minute. Between exposures to the testing concentrations the vas was twice rinsed. When a constant amplitude of contractions was obtained from the testing concentration of noradrenalin, dihydroergotoxine (1×10^{-7} g/ml) or phentolamine (1×10^{-8} g/ml) was added to the bath, and the concentration of noradrenalin which, in the presence of the antagonist, gave rise to an effect equal (±10%) to that of the testing concentration of noradrenalin, was found at the 3rd min. During the next 2 min the vas was rinsed to remove noradrenalin with Krebs' solution containing dihydroergotoxine or phentolamine (10^{-7} and 10^{-8} g/ml, respectively). This procedure was repeated at the 6th and 9th min, and so on, until equilibrium blocking of the receptors took place, at which, in order to obtain an effect equal to that of the testing concentration of noradrenalin in the presence of the antagonist, no further increase in noradrenalin concen-

tration was required. The ratio between the noradrenalin concentrations found and the testing concentration of noradrenalin gave the value of n for the chosen moments of time.

The experiment was continued after rinsing the smooth muscle preparation with Krebs' solution. The dynamics of disappearance of the blocking of the receptors by the adrenolytics was studied under these conditions. Using the method described above the ratios between the noradrenalin concentrations (n) were found at the 10th, 20th, and 30th min.

Similar experiments were carried out on vasa deferentia after preliminary exposure for 15 min to noradrenalin (1×10^{-5} g/ml) and under anoxic conditions.

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results are given in Table 1. Values of n and the proportion of adrenergic receptors occupied by the antagonist are given in this table for each moment. On the basis of the results given in Table 1 graphs were plotted (Fig. 1), from which the velocity constants of the direct and reverse reactions of dihydroergotoxine and phentolamine with the adrenergic receptors of the vas were determined as the tangents of the angle of inclination of the straight lines obtained. The values of these constants and also the constants of affinity ($K_B = k_1/k_{-1}$) of the adrenolytics for the adrenergic receptors calculated from them are given in Table 2.

The affinity (K_B) of dihydroergotoxine and phentolamine for α-adrenergic receptors was substantially reduced if the smooth muscles of the vas deferens were first exposed to the action of noradrenalin. This reduction in affinity was due to a decrease in the velocity of interaction of the adrenolytics with the adrenergic receptors: the velocity constant of the reaction of dihydroergotoxine and phentolamine was reduced by 2.6 and 4 times, respectively, whereas the rate of the reverse process, as reflected in the value of k_{-1}, was unchanged. Similar although less marked changes in the affinity of the adrenolytics for adrenergic receptors were observed if the vas deferens was under anoxic conditions.

It was shown previously [1] that the decrease in affinity of adrenolytics for receptors is due to a change in the conformation of the adrenergic receptors under the influence of noradrenalin, and that restoration of affinity requires the expenditure of energy which is provided by oxidative phosphorylation. Since the decrease in affinity, as the results of the present experiments show, depends on a decrease in the rate of the direct, but not of the reverse, reaction it must be assumed that the interaction between adrenolytics and adrenergic receptors is an energy-dependent process whereas the breakdown of their complexes is not. The development of conformational changes thus requires the expenditure of energy whereas the process of breakdown of adrenolytic–adrenergic receptor complexes takes place without the expenditure of energy.

The necessity for and possibility of the provision of energy for interaction of agonists and antagonists with adrenergic receptors must be closely bound up with the structure of α-adrenergic receptors. Belleau [6] postulated previously that in the presence of Ca^{++} noradrenalin stimulates the hydrolysis of ATP by membrane ATPase, which must be a component part of the α-adrenergic receptor. On the other hand, these receptors are oligomeric macromolecules containing Fe^{++} in the active center [2]. Like certain other nonhemin iron-containing proteins [4, 9], the ferroprotein adrenergic receptors, forming complexes capable of transporting electrons with catecholamines, may participate in the coupling of oxidation and phosphorylation.

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