

## IDENTITY OF THE ACETYLCHOLINE, ADRENALIN AND SEROTONIN RECEPTORS

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Interaction between two antagonistic mediators, acetylcholine and serotonin, was investigated by a kinetic method on the isolated ventricle of Mytilus grayanus and Neptunea sp. When both substances act simultaneously on the myocardium, they behave as antagonists of competitive and mixed types. Similar results were obtained on the isolated frog ventricle during simultaneous action of acetylcholine and adrenalin on the myocardium. Non-specific factors inducing reversible denaturation of proteins (brief exposure to a high temperature or to 15% urea) led to temporary blocking of sensitivity to both mediators. After their action the sensitivity of the frog heart muscle to acetylcholine and adrenalin is gradually restored, and the recovery curves for the two mediators coincide. The identity of the receptor proteins for the antagonistic mediators is discussed.

### INTRODUCTION

Recent investigations have demonstrated the protein nature of cholinergic, adrenergic and serotonergic receptors, namely the specific structures of the chemoreceptive surface membrane of the effector cell through interaction with which the mediators of nerve impulses — acetylcholine (ACh), adrenalin, (A) or noradrenalin, and serotonin (ST) — exert their action. Facts indicating the nucleoprotein nature of cholinergic receptors (1) and of the lipoprotein receptors of serotonin (2) have been obtained.

Studies of the kinetics of the reaction between ACh and cholinergic receptors and between A and adrenergic receptors in experiments on the frog's heart have shown that both these reactions have the same temperature optimum and similar thermodynamic parameters of cold inactivation of both receptors (3, 4). During the action of high temperatures, reversible and irreversible inactivation of both cholinergic (3) and adrenergic receptors of the frog myocardium (5) have been observed. All these observations suggest the identity of the receptor proteins of both mediators (6).

New data are presented which identify properties of the ACh and A, and also of ACh and ST receptors obtained in experiments on the heart muscle of various animals sensitive to these two mediators with antagonistic actions.

### METHOD

The test objects were the frog ventricle made to contract at 30/min by means of an electrical stimulator, and isolated ventricles of Mytilus grayanus and Neptunea sp., contracting spontaneously under the pressure of the column of perfusion fluid (1.5 cm) in the cannula. The frog's heart was perfused with Ringer's solution (g/liter): NaCl 6.5, KCl 0.14, CaCl<sub>2</sub> 0.12, NaHCO<sub>3</sub> 0.20, and the mollusc heart with filtered seawater.

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The experiments on *M. grayanus* and *Neptunea* sp. were performed at the fisheries establishment on Popov Island (Vladivostok). To determine the affinity of the receptors for the corresponding mediators quantitatively, kinetic methods of determining  $K$ , the apparent dissociation constant of the mediator-receptor complex (3, 4) were used. Competitive relationships between the antagonistic mediators during their simultaneous action on the myocardium were assessed from changes in the kinetics of the reaction between one mediator and the receptor under the influence of the other mediator, and the inhibition constant ( $K_i$ ) of the reaction was calculated (7).

The effects of chemical, physicochemical, and physical factors on sensitivity of the myocardium to ACh and A were studied in frogs and results are described below.

### RESULTS

The first series of experiments on the frog myocardium were concerned with the effect of A on interaction between ACh and cholinergic receptors and the effect of ACh on interaction between A and adrenergic receptors. The effect of ACh on interaction between ST and serotonergic receptors and the effect of ST on interaction between ACh and cholinergic receptors were investigated on the heart muscle of the molluscs.

The effects of A in different concentrations on the cholinergic response of the frog's heart are shown graphically in Fig. 1. In the presence of A the gradients of the concentration-action lines for ACh in a system of double reciprocal coordinates are increased. The higher the concentration of A, the greater the increase in gradient of the straight line, indicating an increase in the value of  $K$  for the cholinergic reaction under these conditions. In other words, A reduces the affinity of cholinergic receptors for ACh. Since the point of intersection of the straight lines with the ordinate on the graph (Fig. 1A) is unchanged in the presence of A, this means that the maximal reaction and the number of active cholinergic receptors are unchanged by the action of A, from which it follows that it behaves as a competitive inhibitor of the cholinergic reaction.

The value of the inhibition constant of A ( $K_{iA}$ ) during its action on the cholinergic reaction averages  $2.98 \times 10^{-6} M$  (Table 1), and as an inhibitor of the cholinergic reaction A is 20 times less active than ACh is as an activator of this reaction ( $K_{ACh} = 1.2 \times 10^{-7} M$ ). ACh has an inhibitory effect on the adrenergic reaction (Fig. 1B), but its manifested inhibitory action on the adrenergic reaction differs from that on the cholinergic reaction (Fig. 1). ACh not only changes the gradient of the lines but also shifts them along the ordinate which indicates a decrease in affinity of the adrenergic receptors for A, and a decrease in the number of active adrenergic receptors, under the influences of ACh. Hence it follows that ACh is an inhibitor of mixed type of the adrenergic reaction.

It must be emphasized that ACh is much more active as an inhibitor of the adrenergic reaction than as an activator of its specific cholinergic reaction. It is clear from Table 1, for instance, that ACh is about 10 times stronger as an inhibitor of the adrenergic reaction ( $K_{iACh} = 1.5 \times 10^{-8} M$ ) than as an activator of the cholinergic reaction ( $K_{ACh} = 1.2 \times 10^{-7} M$ ). Consequently, the inhibitory effect of ACh on the positive inotropic reaction of the heart to A will be manifested in concentrations giving no negative inotropic effect. As an example, the kymogram of an experiment on the frog ventricle is shown in Fig. 2C; ACh in concentrations not affecting the amplitude of the cardiac contraction completely abolish the positive inotropic reaction to A.

In the next series of experiments, on the work of the mollusc heart, ACh has a negative, and ST a positive inotropic action. Because of a well-defined concentration-effect relationship for ST and ACh, serotonergic and cholinergic reactions can be analyzed by the kinetic method. Kymograms for the action of ST on the heart of *Neptunea* sp. and of ACh on the heart of *M. grayanus* are shown as examples in Fig. 2 A, B. During their simultaneous action on the mollusc heart they behave as antagonists, just as ACh and A in their action on the frog's heart.

The effects of ST in different concentrations on the effect of ACh on the heart of *Neptunea* sp. are shown graphically in Fig. 3A. The change in position of the straight lines under the influence of ST is characteristic of a competitive type of inhibitory action on the cholinergic reaction. Results showing the mutual effect of ACh and ST on

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TABLE 1. Constants (K) of Reaction between Acetylcholine and Cholinergic Receptor ( $K_{ACh}$ ) Adrenalin and Adrenergic Receptor ( $K_A$ ), and Constants of Inhibitory Action ( $K_i$ ) of Adrenalin on Cholinergic Receptor ( $K_{iA}$ ), and Acetylcholine on Adrenergic Receptor ( $K_{iACh}$ ) of Isolated Frog Ventricle

Adrenalin (M)	Acetylcholine (M)	Adrenalin (M)	Acetylcholine (M)
Adrenergic receptor		Cholinergic receptor	
$K_A$ $2.9 \cdot 10^{-9}$	$K_{iACh}$ $1.5 \cdot 10^{-9}$	$K_{iA}$ $8.4 \cdot 10^{-7}$	$K_{ACh}$ $2.2 \cdot 18^{-7}$
$2.7 \cdot 10^{-9}$	$1.0 \cdot 10^{-9}$	$2.3 \cdot 10^{-7}$	$2.7 \cdot 10^{-8}$
$1.8 \cdot 10^{-8}$	$2.2 \cdot 10^{-8}$	$2.6 \cdot 10^{-7}$	$3.5 \cdot 10^{-8}$
$2.0 \cdot 10^{-8}$	$1.4 \cdot 10^{-8}$	$4.7 \cdot 10^{-6}$	$3.7 \cdot 10^{-7}$
$2.2 \cdot 10^{-8}$	$3.8 \cdot 10^{-8}$	$8.4 \cdot 10^{-6}$	$6.2 \cdot 10^{-8}$
		$2.4 \cdot 10^{-6}$	$6.4 \cdot 10^{-8}$
		$4.0 \cdot 10^{-6}$	$6.6 \cdot 10^{-8}$
$\bar{x} = 1.3 \cdot 10^{-8}$	$\bar{x} = 1.5 \cdot 10^{-8}$	$\bar{x} = 2.98 \cdot 10^{-6}$	$\bar{x} = 1.2 \cdot 10^{-7}$

TABLE 2. Constants (K) for Reaction of Acetylcholine with Cholinergic Receptors ( $K_{ACh}$ ), of Serotonin with Serotonergic Receptors ( $K_{ST}$ ) and Constants of Inhibitory Action ( $K_i$ ) of Serotonin on Cholinergic Receptors ( $K_{iST}$ ) and of Acetylcholine on Serotonergic Receptors ( $K_{iACh}$ ) of Isolated Heart of Neptunea sp.

Serotonin (M)	Acetylcholine (M)	Serotonin (M)	Acetylcholine (M)
Serotonergic receptor		Cholinergic receptor	
$K_{ST} (\cdot 10^{-9})$ 0.23	$K_{iACh} (\cdot 10^{-8})$ 3.67	$K_{iST} (\cdot 10^{-8})$ 0.67	$K_{ACh} (\cdot 10^{-7})$ 1.30
1.45	5.31	1.06	0.37
1.42	2.04	4.25	0.65
2.16	2.08	3.45	0.37
1.21	2.96	2.10	1.29
2.63	3.78	4.25	0.95
1.39	—	0.57	0.73
0.79	—	3.74	1.18
4.32	—	—	0.39
0.44	—		3.20
1.03	—		3.54
		$\bar{x} \pm S_{\bar{x}} = 2.48 \pm 0.48$	0.99
$\bar{x} \pm S_{\bar{x}} = 1.54 \pm 0.39$	$\bar{x} \pm S_{\bar{x}} = 3.31 \pm 0.52$		0.23
			0.10
			0.33
			2.03
			$\bar{x} \pm S_{\bar{x}} = 1.10 \pm 0.25$

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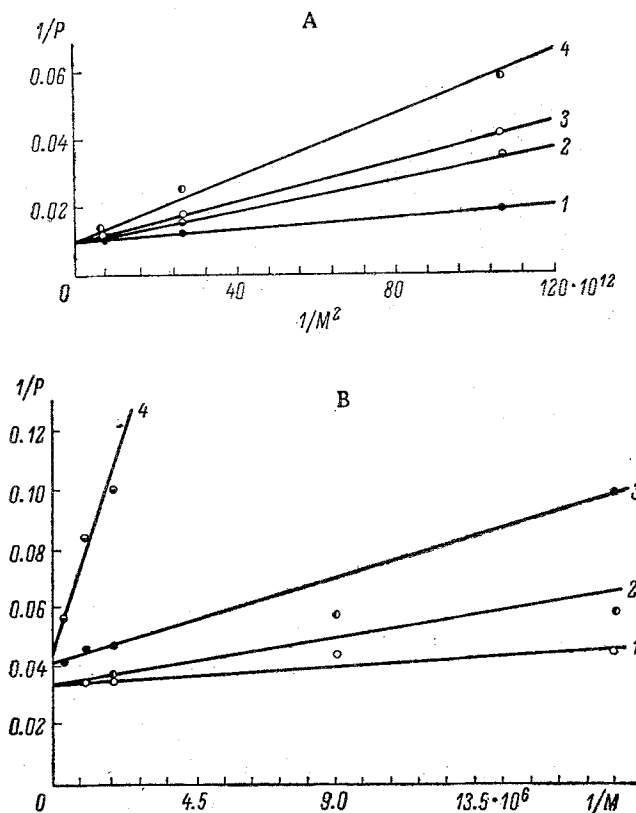


Fig. 1. Interaction between ACh and A on adrenergic and cholinergic receptors. A) Inhibition by adrenalin of reaction between ACh and cholinergic receptors.  $1/P$  — reciprocals of cholinergic response (% of maximum);  $1/M^2$  — reciprocals of square of molar concentration of ACh. 1) Magnitude of cholinergic reaction as a function of ACh concentration under normal conditions ( $K_{ACh} = 6.4 \cdot 10^{-8} M$ ), 2-4) Same in presence of A in concentration (M) of  $5.55 \cdot 10^{-7}$ ,  $1.11 \cdot 10^{-6}$ , and  $5.55 \cdot 10^{-5}$  respectively. Mean value of  $K_{iA}$  for curves 2-4 is  $4.9 \cdot 10^{-6} M$ . B) Inhibition by ACh of reaction between A and adrenergic receptors.  $1/P$  — reciprocals of magnitude of adrenergic response (amplitude of contractions of heart muscle in mm);  $1/M$  — reciprocals of molar concentration. 1) Magnitude of adrenergic response as a function of A concentration under normal conditions; 2-4) Same in presence of ACh in concentrations (M) of  $3.40 \cdot 10^{-8}$ ,  $6.80 \cdot 10^{-8}$ , and  $1.35 \cdot 10^{-7}$  respectively. Mean value of  $K_{iACh} = 2.5 \cdot 10^{-8} M$ .

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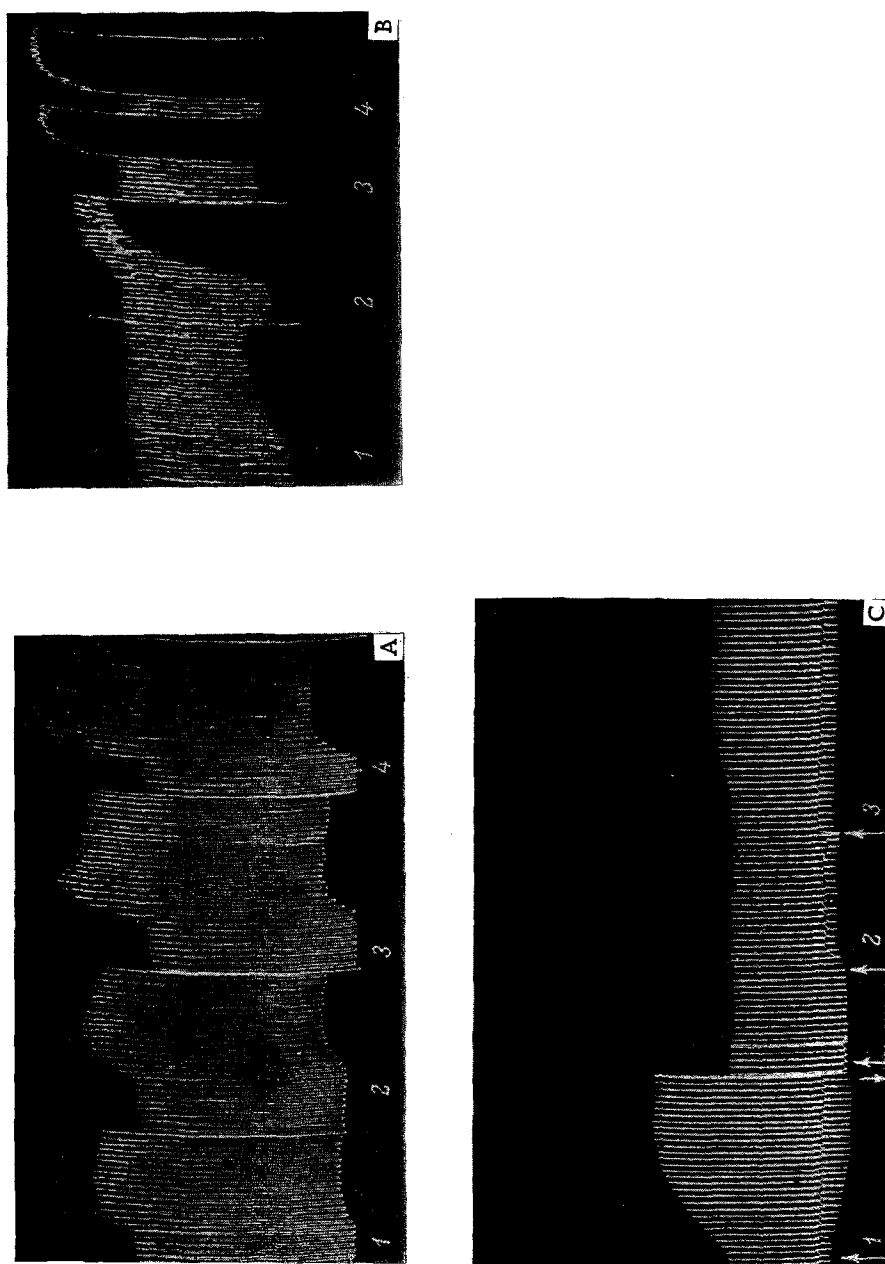


Fig. 2. Effect of mediators on isolated ventricle of different animals: A) Action of ST on isolated ventricle of *Neptunea* sp.: 1-4) injection of ST into cannula in concentrations (M) of  $0.69 \times 10^{-8}$ ,  $1.38 \times 10^{-8}$ ,  $2.76 \times 10^{-8}$ , and  $5.52 \times 10^{-8}$ . B) Action of ACh on isolated ventricle of *Mytilus grayanus*: 1-4) injection of ACh into cannula in concentrations (M) of:  $2.68 \times 10^{-7}$ ,  $5.36 \times 10^{-7}$ ,  $1.07 \times 10^{-6}$  and  $2.14 \times 10^{-6}$ . C) Effect of ACh in subthreshold concentration (M) on action of A on frog's heart: 1) A ( $2.78 \times 10^{-8}$ ), 2) ACh ( $1.39 \times 10^{-8}$ ), 3) A ( $2.78 \times 10^{-8}$ ) + ACh ( $1.39 \times 10^{-8}$ ). Arrow above - injection of substances into cannula; two arrows - rinsing out of substances.

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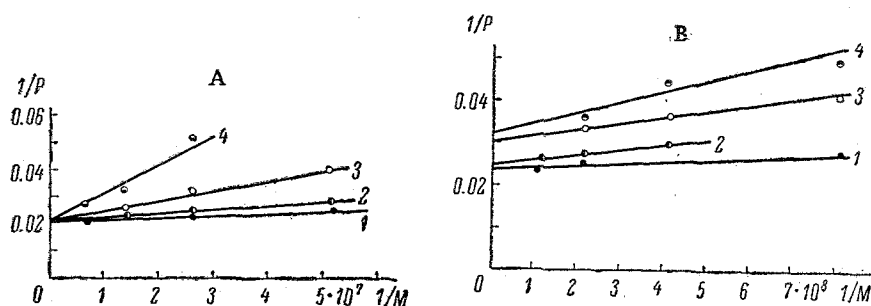


Fig. 3. Interaction between ST and ACh on cholinergic and serotonergic receptors of the heart of *Neptunea* sp. A) Inhibition by ST of reaction between ACh and cholinergic receptors. 1/P— reciprocals of cholinergic reaction (in mm); 1/M— reciprocals of molar concentration of ACh. 1) Magnitude of cholinergic reaction as a function of ACh concentration under normal conditions ( $K_{ACh} = 4.6 \times 10^{-8} M$ ); 2-4) same in presence ST in concentrations (M) of  $0.69 \times 10^{-8}$ ,  $1.38 \times 10^{-8}$ , and  $2.76 \times 10^{-8}$  respectively. Mean value of  $K_{IST} = 1.79 \times 10^{-8} M$ . B) Inhibition by ACh of reaction between ST and serotonergic receptors. 1/P— reciprocals of magnitude of serotonergic reaction (MM); 1/M— reciprocals of molar concentration of ST. 1) Magnitude of reaction of heart as a function of ST concentration (M) under normal conditions ( $K_{ST} = 1.24 \times 10^{-9}$ ); 2-4) same in presence of ACh in concentrations (M) of  $6.66 \times 10^{-8}$ ,  $1.33 \times 10^{-7}$ , and  $2.66 \times 10^{-7}$  respectively. Mean value of  $K_{iACh} = 5.8 \times 10^{-8} M$ .

TABLE 3. Constant (K) of Reaction between Acetylcholine and Cholinergic Receptors ( $K_{ACh}$ ), and Serotonin and Serotonergic Receptors ( $K_{ST}$ ) and Constants of Inhibitory Action of Serotonin on Cholinergic Receptors ( $K_{IST}$ ) of Isolated Heart of *Mytilus grayanus*.

Serotonin (M)	Acetylcholine (M)	Serotonin (M)	Acetylcholine (M)
Serotonergic receptor		Cholinergic receptor	
$K_{ST} (\cdot 10^{-8})$	—	$K_{IST} (\cdot 10^{-7})$	$K_{ACh} (\cdot 10^{-6})$
0.15	—	1.94	0.16
0.49	—	0.91	1.23
2.20	—	1.31	0.31
0.86	—	0.95	0.59
1.99	—	1.08	0.69
1.44	—	2.03	0.79
0.53	—	3.51	0.64
	—	3.12	1.23
$\bar{x} \pm S_{\bar{x}} = 1.10 \pm 0.29$	—	—	1.40
			1.48
			1.41
		$\bar{x} \pm S_{\bar{x}} = 1.86 \pm 0.32$	
			$\bar{x} \pm S_{\bar{x}} = 0.90 \pm 0.13$

serotonergic and cholinergic receptors respectively are given in Table 2. The value of the inhibition constant of ST ( $K_{IST}$ ) is  $2.48 \times 10^{-8} M$ . ACh inhibits the serotonergic reaction (Fig. 3B), and it acts as an inhibitor of mixed (competitive and noncompetitive) type with an inhibition constant ( $K_{IACh}$ ) of  $3.31 \times 10^{-8} M$ . The inhibitory action of ACh on stimulation of cardiac function is thus stronger (three times) on the heart of *Neptunea* sp., just as it is on the frog's heart, than its activating effect on the cholinergic reaction.

Experiments on the heart of *M. grayanus* gave similar results — ST acts on the cholinergic reaction as a competitive inhibitor, with inhibition constant ( $K_{IST}$ ) of  $1.86 \times 10^{-7} M$ . ACh is a mixed inhibitor of the serotonergic reaction (Table 3). No reliable values of the inhibition constant of ACh during its action on the serotonergic reaction could be obtained, because of the rapidly developing and strong desensitization of the heart muscle to serotonin which did not allow satisfactory graphs of the kinetics of the serotonergic reaction to be plotted.

Experiments on the kinetics of the inotropic reaction of the frog and mollusc heart during simultaneous action on the myocardium of two mediators on opposite effects on cardiac contractions (acetylcholine — adrenalin, acetylcholine — serotonin) thus showed that these substances behave as antagonists of competitive or mixed types toward each other.

Further experiments were carried out to study the effect of different factors causing protein denaturation on the activity of cholinergic and adrenergic receptors of frog muscle. It was pointed out above that heating the isolated frog's ventricle to 40°C for 3–4 min leads to temporary loss of sensitivity of the myocardium to both ACh and A. After thermal inactivation, sensitivity returns to normal at room temperature gradually, over a period of about thirty minutes (3); similar results have been obtained during thermal inactivation of adrenergic receptors (5). Because of this fact, it was possible to study recovery of activity of adrenergic and cholinergic receptors in the same heart after their reversible thermal inactivation. Recovery of sensitivity of the myocardium to A and ACh after heating the isolated frog's ventricle in Ringer's solution for 3.5 min at 40°C takes place in complete synchronization (Fig. 4).

Urea is another agent, frequently studied, which produces nonspecific denaturation of the protein molecule. Treatment of the frog's ventricle with 15% urea for 4 min produced reversible inactivation of cholinergic and adrenergic receptors. After rinsing off the urea, activity of the receptors is gradually restored. Although recovery takes place much more rapidly than after thermal inactivation, in this case also the adrenergic and cholinergic receptors recovered their activity simultaneously (Fig. 4).

Reversible thermal or urea inactivation of cholinergic and serotonergic receptors of the mollusc myocardium could not be obtained because it proved impossible to select conditions under which the action of the various denaturing factors did not affect the ability of the myocardium to contract.

## DISCUSSION

In the modern view mediator action consists of a chain of successive biochemical and biophysical reactions. In the effector cell this process begins on the postsynaptic membrane with interaction between mediator and receptor. If the cell is sensitive to two mediators with an opposite final effect, interaction between the systems is antagonistic.

Heart muscle experiments described here showed that nonspecific physical and physicochemical factors block the sensitivity of the myocardium to both antagonistic mediators—(ACh and A). If the action of these factors was reversible, sensitivity to both mediators was gradually restored, and the process took place simultaneously indicating that structures responsible for the actions of ACh and A are very similar. This similarity is also supported by the identity

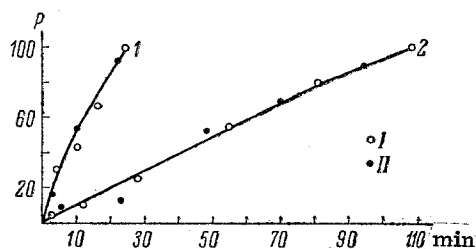


Fig. 4. Recovery of sensitivity of frog's myocardium to ACh (I) and A (II) after treatment with 15% urea (1) and exposure to 40°C (2). P—sensitivity of myocardium to ACh and A (% of initial); min—time after action of denaturing factors.

of the kinetic equations for the reaction between ACh and the cholinergic receptor and between A and the adrenergic receptor, the identical relationship between the sensitivity of the frog's myocardium to ACh and A within 0–40°C, and the identity of the thermodynamic parameters of cold inactivation of sensitivity to both mediators, described previously (3, 4).

On the other hand, experiments to study the effect of mediators on receptors of antagonistic mediators showed that competitive relationships exist between ACh and A (experiments on the frog's heart) and between ACh and ST (experiments on the mollusc heart).

These results, indicating the identity of properties of the antagonistic receptors, on the one hand, and the existence of competitive relationships between mediators on the other hand, suggest the following explanations.

1. Cholinergic and adrenergic mediator processes are two independent chain reactions which converge only on the final common path of both processes (the contractile act). It must be accepted that the cholinergic and adrenergic receptor proteins are extremely similar in their properties. The mutual effect of the mediators on the antagonistic mediator system observed in these experiments is the result of interaction either at the receptor level or on the final common path of the two mediator processes: the contractile act.

2. Although the cholinergic and adrenergic mediator processes have different initial components, they converge on some intermediate component of protein nature which is affected by both changes in temperature and urea. Interaction between the two opposite influences from cholinergic and adrenergic receptors takes place on this same protein.

3. The cholinergic and adrenergic receptors are a single protein with two active centers. One reacts with ACh, the other with catecholamines. During exposure to nonspecific factors (temperature, urea) the activity of both active centers is inhibited equally through a change in the structure of the protein molecule itself. Changes in the conformation of the protein molecule produced by the action of the mediator on the specific active center also have some effect on the activity of the other active center.

The results described here do not provide the final answer to the question of which of these three suggested explanations is the correct one. However, it seems improbable that two different proteins — the cholinergic receptor and the adrenergic receptor — could possess such identical properties: identical rate of recovery after the action of non-specific denaturing agents (urea, high temperature), a common temperature optimum for the reaction between mediator and receptor, simultaneous blocking of activity at high and low temperatures, similar thermodynamic parameters of cold inactivation, etc. In addition, the two receptor proteins must have not only specific active centers, complementary to the structure of the mediators themselves, but also contact areas, interacting with the mediator with antagonistic action. It could be postulated that when interaction between the two mediators acting simultaneously takes place, it is not at the receptor level, but on the contractile substrate. In other words, the competitive relationships observed are the results of events taking place in the final link in the chain of reactions common to both mediator processes. This hypothesis cannot be completely ruled out although experiments to study the effect of a subthreshold concentration of ACh on the adrenergic effect (Fig. 2C) make it improbable. In these experiments, ACh does not inhibit the contractile substrate, but at the same time it considerably reduces the magnitude of the adrenergic effect. The results of these experiments can be readily explained on the basis of the results in Table 1, which show that  $K_{iACh}$  is higher than  $K_{ACh}$ , i.e., the inhibitory action of ACh on adrenergic receptors is almost one order of magnitude greater than the activating action of ACh on cholinergic receptors. This leads to inhibition of the adrenergic reaction by ACh under conditions in which no cholinergic effect is present. Concurrently, the effect of ACh on the adrenergic receptors is mediated only through specific cholinergic receptors. This follows from the fact that atropine completely abolishes the inhibitory action of ACh on the adrenergic receptor.

The second and third explanations seem more likely, for the hypothesis that there exists a single receptor protein or intermediate protein, on which cholinergic and adrenergic mediator processes converge, agrees more with the experimental findings. Changes in the sensitivity of the myocardium to ACh and catecholamines which takes place during the action of different nonspecific factors (temperature, urea) are the result of changes taking place with this single protein. The action of the specific mediator induces changes of conformation in this single protein, leading to a decrease in the effectiveness of action of the antagonistic mediator.



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