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REVERSE TRANS-SYNAPTIC REGULATION OF NEURONAL NORADRENALINE UPTAKE

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Abstract—It was established that the blocking agent of β-adrenoceptors, propranolol (5.10^{-7}–1.10^{-6} M), activates [^{3}H]noradrenaline uptake by isolated rat organs (vas deferens, spleen, small intestine, atrium, uterus) by 30–180 per cent. The blocking agent of α-adrenoceptors, phentolamine (5.10^{-7}–1.10^{-6}M), activates noradrenaline uptake by 30–55 per cent only in the organs possessing postsynaptic α-adrenoceptors (vas deferens, spleen, small intestine). The activator of β-adrenoceptors, isopropylnoradrenaline (5.10^{-7}–1.10^{-6}M), was shown to produce a decrease in [^{3}H]noradrenaline uptake by 15–50 per cent in all the organs investigated. The substances activating α-adrenoceptors—urea (2.10^{-3}–5.10^{-3}M) and mesatone (1.10^{-6}–1.10^{-5}M)—inhibit [^{3}H]noradrenaline uptake in the organs with postsynaptic α-adrenoceptors by 20–45 per cent. Activation of neuronal [^{3}H]noradrenaline uptake induced by phentolamine (1.10^{-6}M) is due to the release of a humoral factor from the effector cell and its influence on adrenergic neurone. Mesatone (1.10^{-5}M) causes the formation and release of a humoral factor inhibiting neuronal [^{3}H]noradrenaline uptake. The formation of humoral factors changing the intensity of amine uptake is related to the activation of protein synthesis in the effector cell. The possible mechanism of the reverse trans-synaptic regulation of neuronal noradrenaline uptake via the adrenoceptors of the effector cell is discussed.

The functional state of adrenoceptors is one of the essential factors determining adrenergic responses [1, 2]. Different factors producing the changes in adrenoceptor sensitivity, such as temperature [3, 4], experimental pathology [5, 6], various types of denervation [7–9] and a number of pharmacological drugs [10–12], affect the specific response of the effector cell. At the same time it was established that regulation of some processes occurring in the presynaptic site can be realized via the adrenoceptors. Thus, the functional state of adrenoceptors affects the tissue content of noradrenaline and its release on the stimulation. In the *in vivo* experiments following repeated injections of an α-adrenoceptor blocking agent, phenoxybenzamine, for 24 hours, the synthesis of [^{3}H]noradrenaline from [^{3}H]tyrosine is activated in the rat brain and heart [13]. A change in catecholamine metabolism is also observed after a short-term action of the substances affecting the adrenoceptor activity. As soon as 30 min after the injection of the α-adrenoblocking agent, phentolamine, a significant increase of noradrenaline concentration is observed in the rat spleen and vas deferens [14]. The electrical stimulation of adrenergic neurones following the administration of phentolamine and phenoxybenzamine produces the increased excretion of noradrenaline from the isolated rat organs, namely, heart [15, 16], spleen [17] and brain [18]. For isolated rat organs, it has also been shown that under the effect of substances blocking or activating adrenoceptors a change in the rate of [^{3}H]noradrenaline uptake takes place [19–21].

The present work was undertaken in order to study the effect of the functional state of postsynaptic adrenoceptors on the intensity of neuronal noradrenaline uptake and elucidating possible ways for realizing this regulatory influence.

MATERIALS AND METHODS

The experiments were performed on white Wistar rats of either sex weighing 180–200 g. The animals were decapitated, and vas deferens (80–90 mg) atrium (60–80 mg), pieces of spleen (65–75 mg), uterus (45–55 mg) and small intestine (ileum, 60–70 mg) were excised and incubated in Tyrode solution (NaCl—153.9 mM, KCl—4.5 mM, CaCl—2.5 mM, $MgCl_2$—1.0 mM, $NaHCO_3$—11.9 mM, NaH_2PO_4—1.0 mM, glucose—5.5 mM, ascorbic acid—2.8 mM) in 5 ml chambers at 37° under oxygenation (95% O_2 + 5% CO_2). After 30 min of incubation the solution was changed and preparations were preincubated with the studied substances (inhibitors of noradrenaline uptake, antagonists and agonists of adrenoceptors), then for another 30 min—in Tyrode solution with the studied substance and [^{3}H]noradrenaline in concentrations of 0.01 or 0.025 μCi/ml (1.2 or 3.0×10^{-9}M). After incubation the preparations were rinsed for 4–5 min with 5-fold replacement of the solution. Good agreement of parallel samples leads us to believe that the rinsing conditions employed provide similar removal of unbound [^{3}H]noradrenaline in the control and treated preparations. Then preparations were placed into scintillation cuvettes and after addition of 1 mM of ethanol left for 16–18 hr. Whereupon 10 ml scintillation liquid were added to the cuvettes (4 g of PPO and 100 mg of POPOP per 1 litre of toluene). In our experiments this method of treatment [22] provided a yield of 90 per cent as compared with the yield of amine after adsorption and elution on the columns with Dowex-50 ion-exchange resin [23].

In the experiments with the inhibitor of protein synthesis cycloheximide [24] the latter was added to

the medium during the first 30 min of incubation. The preparations of isolated organs after being washed 8–10 times with Tyrode solution were incubated with the studied substances and [^{3}H]noradrenaline according to the aforementioned scheme. In the control experiments on the effect of cycloheximide on the protein synthesis [^{14}C]leucine in a concentration of 1 μCi/ml was used as a labelled precursor. The preparations were incubated in Tyrode solution for 30 min, then for 30 min—with cycloheximide and after washing cycloheximide off 8–10 times—with [^{14}C]leucine for an additional 30 min. After being washed 5 times and treated with 5 per cent trichloroacetic acid according to standard procedure the preparations were placed into scintillation cuvettes and 1 ml of hyamine was added. After 16–18 hr, 10 ml of scintillation liquid were added.

The experiments on cycloheximide action on the response of rat vas deferens to exogenous noradrenaline and transmural electric stimulation were carried out. Isolated vas deferens incubated with Tyrode solution was contracted under the effect of exogenous noradrenaline in a concentration of 1.10^{-7}g/ml and by electrical stimulation with rectangular stimuli of 20 V magnitude, 0.3 msec duration, 15 Hz frequency and 10 sec time stimulation through ring electrodes. The isotonic contraction amplitude of the isolated rat vas deferens was recorded mechanographically on a kymograph.

To detect the humoral factor of trans-synaptic regulation in the incubation medium ("donor–recipient" experiments) vas deferens and atrium were incubated together in the same bath in the presence of α-adrenotropic agents. In some "donor–recipient" experiments one of the organs was previously treated with cycloheximide in the separate bath as described.

Radioactivity was measured on a liquid scintillation counter "Intertechnique SL-30" (France) with an external standard and expressed in terms of disintegrations per min per gram of material (d.p.m./g). The results were subject to statistical processing using the Student criteria.

The drugs used were imipramine (melipramine—EGYT, Budapest, Hungary), normetanephrine (Sigma, U.S.A.), cycloheximide (Serva, BRD), noradrenaline (Koch-Light Lab. Ltd., England), cocaine, desmethylimipramine, phentolamine, propranolol, mesatone (phenylephrine), urea, isopropilnoradrenaline (U.S.S.R.), [^{3}H]noradrenaline (Radiochemical Centre Amersham, England, specific activity 8.2 Ci/m-mol) and [^{14}C]leucine (Isotop, U.S.S.R., specific activity 30 mCi/m-mol).

RESULTS AND DISCUSSION

Effect of adrenotropic substances on the neuronal [^{3}H]noradrenaline uptake

Effect of inhibitors of neuronal and extraneuronal uptake. To demonstrate that the observed [^{3}H]noradrenaline binding by isolated organs is due to its uptake by adrenergic neurons, a series of control experiments was run.

The effect of specific inhibitors of neuronal (desmethylimipramine, imipramine, cocaine) [25] and extraneuronal (normetanephrine) [25] uptake on the rate of [^{3}H]noradrenaline binding under the experimental conditions was studied. A marked and significant inhibition of [^{3}H]noradrenaline binding under the effect of the inhibitors of neuronal uptake (Table 1) was established. Desmethylimipramine in concentrations of 5.10^{-6}M and 1.10^{-5}M produced a decrease in the rate of [^{3}H]noradrenaline uptake by 60–70 per cent in vas deferens and atria and by 40–50 per cent in spleen, uterus and small intestine. Other inhibitors of neuronal uptake—imipramine and cocaine (1.10^{-5}M)—also reduced the rate of [^{3}H]noradrenaline uptake: by 60–70 per cent in vas deferens and by 40–45 per cent in atria.

Similar data on the effects of neuronal uptake inhibitors during the incubation of isolated organs are obtained in the studies of the other authors [26–28].

To determine the degree of extraneuronal [^{3}H]noradrenaline uptake in the tissues a study was made of the amine uptake under the effect of normetanephrine (1.10^{-5}M)—the inhibitor of extraneuronal uptake of catecholamines (Table 1). Both in vas deferens and atria the rate of amine uptake was shown to be

Table 1. Effect of inhibitors on [^{3}H]noradrenaline uptake by rat organs

	[^{3}H]Noradrenaline uptake (d.p.m. × 10^3/g ± S.E.)					
		Inhibitors of neuronal uptake				Inhibitor of extraneuronal uptake
		Desmethylimipramine		Imipramine	Cocaine	Normetanephrine
Organ	Control	5×10^{-6}M	1×10^{-5}M	1×10^{-5}M	1×10^{-5}M	1×10^{-5}M
Vas deferens	165.8 ± 6.5	51.4 ± 1.5*	—	62.1 ± 2.9*	59.3 ± 2.1*	144.3 ± 3.9†
Spleen	32.1 ± 1.8	—	18.9 ± 1.0*	—	—	—
Small intestine	35.3 ± 2.6	—	17.3 ± 1.6*	—	—	—
Atrium	117.4 ± 4.5	49.9 ± 1.7*	—	69.7 ± 4.2*	63.7 ± 1.2*	103.3 ± 2.1*
Uterus	42.2 ± 3.5	—	23.5 ± 1.1*	—	—	—

The values for [^{3}H]noradrenaline uptake given in Tables 1–5 in control experiments are the means of 12–24 experiments; figures for treatment, those of 6–12 experiments.

[^{3}H]Noradrenaline concentration for all organs is 0.01 μCi/ml (1.2×10^{-9}M, 22×10^3d.p.m./ml), except the concentration for vas deferens and atrium in Tables 1, 2, 5, where it is 0.025 μCi/ml (3.0×10^{-9}M, 55×10^3d.p.m./ml);

Difference from the control is statistically significant:

* $P < 0.01$, † $P < 0.02$, ‡ $P < 0.05$.

reduced by 10–13 per cent only, thus indicating that the role of extraneuronal uptake in the whole process of catecholamine uptake must be rather insignificant.

The results presented make it possible to believe that changes in the accumulation of [^{3}H]noradrenaline (observed in the later experiments) reflect some specific influences on the [^{3}H]noradrenaline uptake by adrenergic neurones.

In the subsequent experiment a study was made of the [^{3}H]noradrenaline uptake by isolated rat organs under conditions of the blockade and activation of adrenoceptors.

Effect of the blocking agents of postsynaptic adrenoceptors. Blockade of α-adrenoceptors by phentolamine in concentrations of 5.10^{-7} and 1.10^{-6}M results in the activation of [^{3}H]noradrenaline uptake in the organs with α-adrenoceptors: in the vas deferens by 30 and 40 per cent; in the spleen by 30 and 55 per cent; and in the small intestine by 40 and 55 per cent, respectively. No significant changes were recorded in the organs without α-adrenoceptors (atria, uterus) (Table 2). Consequently, the activating effect of the α-adrenoblocking agent phentolamine shows itself only in the organs possessing α-adrenoceptors.

Next, the effect of β-adrenoblocking agent propranolol on the same organs was studied. It was established that in concentrations of 5.10^{-7} and 1.10^{-6}M propranolol activates [^{3}H]noradrenaline uptake in all the organs under study (Table 2). In the organs with α-adrenoceptors (vas deferens, spleen, small intestine) activation amounted to 30–45 per cent; in those having functional β-adrenoceptors (atria, uterus) activation of noradrenaline uptake was more pronounced. In the uterus it amounted to more than 50 per cent and was especially pronounced in atria where it was 1.8 times above the control level at a concentration of propranolol of 5.10^{-7}M. It should be noted that the activating effect of phentolamine appears at the concentration of 5.10^{-7}M, that of propranolol at 2.10^{-7}M, and both increase with greater concentrations up to 1.10^{-6}M; further increase of concentration causes a well known inhibitory effect of adrenoblocking agents on noradrenaline uptake.

Thus, both adrenoblocking agents at concentrations specifically blocking α- and β-adrenoceptors increase the [^{3}H]noradrenaline uptake by rat organs, and the interesting thing is that while the action of the α-adrenoblocking agent phentolamine is confined to the organs possessing functional α-adrenoceptors, β-adrenoblocking agent propranolol activates the amine uptake in all organs investigated. It seems that the blockade of adrenoceptors results in the increase of noradrenaline uptake, thus providing for the accelerated accumulation of noradrenaline in adrenergic neurones. If this is the case it can be supposed that activation of adrenoceptors must result in the opposite reaction—inhibition of noradrenaline uptake by rat organs. To test the hypothesis a study was made of the effect of the drugs activating adrenoceptors on the [^{3}H]noradrenaline uptake by different organs.

Effect of adrenoceptors agonists. The drugs used as the agonists of α-adrenoceptors were: urea (enhances adrenoceptors sensitivity [29] and mesatone (α-sympathomimetic agent of direct action [30]. Urea in concentrations of 2.10^{-3} and 5.10^{-3}M (such a high concentration is explained by the fact that the physiological concentration of urea in the blood of rats amounts to 3–10.10^{-3}M [31]) reduces the rate of [^{3}H]noradrenaline uptake by the organs with functional α-adrenoceptors: by 40–45 per cent in the vas deferens, 30–40 per cent in the spleen, and 20–25 per cent in the small intestine (Table 3). In the organs without α-adrenoceptors (atria, uterus) no significant changes in the rate of the amine uptake were observed (Table 3). Similar results were obtained for mesatone: in concentrations of 1.10^{-6} and 1.10^{-5}M it reduced the rate of [^{3}H]noradrenaline uptake in vas deferens by 30–40 per cent and showed no effect on the amine uptake in atria (Table 3). As the agonist of β-adrenoceptors, isopropylnoradrenaline was used. It is one of the most active β-sympathomimetic agents whose characteristic feature is that it cannot be taken up by neurones [32]. In all the organs studied, irrespective of the absence or presence of α-adrenoceptors in them, isopropylnoradrenaline produces the inhibitor effect on [^{3}H]noradrenaline uptake (Table 3). Thus, isopropylnoradrenaline in a concentration as low as 5.10^{-7}M reduces the amine uptake by 15–25 per cent in all the organs and with increasing concentrations the inhibitory effect increased; at the concentration of 5.10^{-6}M it reached 40–50 per cent in some organs (atrium, uterus and small intestine).

Thus, activation of adrenoceptors of both types results in the inhibition of noradrenaline uptake. In the organs with functional α-adrenoceptors the inhibitor effect is observed under the action of both α- and β-adrenoactivators, while in the organs having no α-adrenoceptors, only under the action of the agonists of β-adrenoceptors.

From the results obtained it can be concluded that it is through the adrenoceptors that the effects of adrenoblocking agents and agonists observed in our experi-

Table 2. Effect of adrenoceptors blocking agents on [^{3}H]noradrenaline uptake by rat organs

Organ	[^{3}H]Noradrenaline uptake (d.p.m. × 10^3/g ± S.E.)						
		Phentolamine			Propranolol		
	Control	2×10^{-7}M	5×10^{-7}M	1×10^{-6}M	2×10^{-7}M	5×10^{-7}M	1×10^{-6}M
Vas deferens	165.8 ± 6.5	159.8 ± 8.1	218.5 ± 7.4*	226.8 ± 16.9*	164.1 ± 7.2	226.5 ± 6.9*	238.9 ± 19.3*
Spleen	32.1 ± 1.8	31.8 ± 1.0	42.6 ± 2.6*	49.8 ± 2.3*	30.3 ± 1.8	41.4 ± 2.9*	42.8 ± 2.7*
Small intestine	35.3 ± 2.6	38.6 ± 2.3	49.6 ± 2.5*	53.9 ± 2.9*	36.6 ± 2.6	49.9 ± 2.9*	50.6 ± 4.6*
Atrium	117.4 ± 4.5	128.1 ± 6.9	129.4 ± 8.4	137.4 ± 9.0	205.4 ± 6.3*	323.9 ± 10.2*	231.6 ± 8.2
Uterus	42.2 ± 3.5	39.6 ± 4.3	41.7 ± 2.8	41.9 ± 2.3	42.8 ± 2.4	62.7 ± 2.5*	63.2 ± 2.6*

* See footnote to Table 1.

Table 3. Effect of adrenoceptors agonists on [^{3}H]noradrenaline uptake by rat organs

Organ	[^{3}H]Noradrenaline uptake (d.p.m. × 10^3/g ± S.E.)							
		Urea		Mesatone		Isopropylnoradrenaline		
	Control	2.10^{-3}M	5.10^{-3}M	1.10^{-6}M	1.10^{-5}M	5.10^{-7}M	1.10^{-6}M	5.10^{-6}M
Vas deferens	65.4 ± 6.3	40.1 ± 2.6*	36.6 ± 1.3*	46.8 ± 1.4*	41.7 ± 2.5*	47.5 ± 2.2*	47.6 ± 1.9*	45.4 ± 1.8*
Spleen	32.1 ± 1.8	23.3 ± 1.0*	1(9.7) ± 1.9*	—	—	27.4 ± 1.9‡	25.9 ± 1.8†	—
Small intestine	35.3 ± 2.5	28.9 ± 2.4	26.8 ± 3.1‡	—	—	30.2 ± 2.4	23.8 ± 2.2*	18.9 ± 1.4*
Atrium	39.9 ± 1.9	38.7 ± 1.6	34.9 ± 1.9	39.0 ± 2.2	42.1 ± 2.5	32.0 ± 2.3†	24.0 ± 1.3*	24.4 ± 1.2*
Uterus	42.2 ± 3.4	40.7 ± 2.0	41.3 ± 2.2	—	—	35.1 ± 3.6	24.7 ± 1.7*	—

*†‡ See footnote to Table 1.

ments are realized. This is indicated by the following facts. All the drugs used produce their effect when taken in very low physiological concentrations, specifically blocking or activating adrenoceptors and producing no toxic effect on the function of the cells. Effect of the drugs known as agonists (mesatone, urea) and blocking agents (phentolamine) of adrenoceptors is manifested only in the organs possessing postsynaptic α-adrenoceptors: vas deferens, spleen and small intestine. Effect of a β-adrenoblocking agent (propranolol) and agonist (isopropylnoradrenaline) of β-adrenoceptors is observed in all the organs studied regardless of their functional adrenoception. This might be due to the fact that all the organs with sympathetic innervation have the receptors of β-type, via which the main influences of the adrenergic system on metabolic processes are realized. Thus, the effect of all α- and β-agonists and blocking agents used is precisely correlated with the presence in the tissues of corresponding α- and β-adrenoceptors.

One more conclusion can be drawn from this fact. It seems likely that the receptors (the changes in functional state of which result in the changes in the rate of noradrenaline uptake in rat organs) are located on the post-synaptic membrane of the effector cell, since the effect of α-adrenotropic substances is precisely correlated with the presence or absence of α-adrenoceptors on postsynaptic membrane. The possibility of the action of adrenotropic substances on the adrenoceptors of the neurone itself (the so called presynaptic receptors) seems highly improbable, since in this case it would be necessary to assume the presence of the receptors of both α- and β-type on the presynaptic membrane, with their distribution corresponding to that of the receptors on the postsynaptic membrane. At present we have no evidence in favour of such an assumption.

Of special interest is the response of some organs to the β-adrenotropic substances. The data presented in Tables 3 and 5 indicate that the effect of the substances affecting β-adrenoceptors (propranolol, isopropylnoradrenaline) is more pronounced in the organs having no α-adrenoceptors (atria, uterus). In these organs both metabolic and functional regulatory influences are realized through the adrenoceptors of β-type. It seems likely that in this case summation of the effects caused by the stimulation of functional and metabolic β-adrenoceptors takes place. Similar results—summation of the effects—were obtained for the vas deferens in the study of the simultaneous action of α- and β-adrenoblocking agents phentolamine and propranolol on the rate of [^{3}H]noradrenaline uptake [19].

Thus, the results obtained suggest the existence of the regulatory influences from the effector cell on the processes of noradrenaline uptake by adrenergic neurones. It might be supposed that this regulatory influence on the presynaptic processes is realized by means of humoral (chemical) factors, since the existence of other ways in isolated organs seems unlikely.

Mechanism of regulatory influence of the effector cell on the neuronal [^{3}H]noradrenaline uptake

Humoral transmission of the effects of adrenotropic substances. Assuming the humoral way of transmission of the influences from the effector cell to adrenergic neurone it would be natural to expect the appearance of a humoral factor in the incubation medium. To detect this factor, vas deferens and atria were incubated together in the presence of α-adrenoblocking agent phen-

Table 4. Effect of alpha-adrenotropic agents on [^{3}H]noradrenaline uptake by isolated vas deferens and atrium under conditions of separate and simultaneous incubation

Drug	Concentration (M)	Incubation	[^{3}H]Noradrenaline uptake (d.p.m. × 10^3/g ± S.E.) Vas deferens	Atrium
Control	—	separate	58.2 ± 1.2	45.5 ± 1.8
Control	—	simultaneous	57.8 ± 1.6	44.9 ± 2.7
Phentolamine	1×10^{-6}	separate	84.2 ± 2.8*	44.6 ± 1.2
Phentolamine	1×10^{-6}	simultaneous	83.4 ± 2.6*	56.3 ± 2.1*
Mesatone	1×10^{-5}	separate	36.2 ± 2.3*	45.3 ± 1.9
Mesatone	1×10^{-5}	simultaneous	35.1 ± 1.9*	30.4 ± 2.1*

* See footnote to Table 1.

Table 5. Effect of cycloheximide on humoral transmission of activating influence of phentolamine and inhibitory influence of mesatone on [^{3}H]noradrenaline uptake from vas deferens to atrium

Drug	Concentration (M)	Organ treated with cycloheximide (3.5×10^{-5}M)	[^{3}H]Noradrenaline uptake (d.p.m. $\times 10^3$/g ± S.E.) Vas deferens	Atrium
Control	—	—	185.2 ± 5.4	128.8 ± 4.7
Control	—	vas deferens and atrium	176.7 ± 10.1	135.1 ± 6.2
Phentolamine	1×10^{-6}	—	298.5 ± 9.2*	182.0 ± 6.8*
Phentolamine	1×10^{-6}	vas deferens	167.6 ± 7.9	114.1 ± 7.6
Phentolamine	1×10^{-6}	atrium	313.4 ± 9.8*	207.2 ± 7.1*
Mesatone	1×10^{-5}	—	107.3 + 1.9*	70.7 ± 3.8*
Mesatone	1×10^{-5}	vas deferens	169.4 ± 6.8	117.8 ± 4.8
Mesatone	1×10^{-5}	atrium	110.3 ± 5.3*	88.5 ± 8.9*

* See footnote to Table 1.

tolamine in a concentration of 1.10^{-6}M. As a control the effect of phentolamine on [^{3}H]noradrenaline uptake by these organs at their separate incubation was studied. As seen in Table 4, the combined incubation of the organs in itself produces no marked changes in the rate of [^{3}H]noradrenaline uptake. With separate incubation, phentolamine produces a significant (45 per cent) increase in the rate of noradrenaline uptake in vas deferens, but does not affect that in atria. With the combined incubation of the organs in the presence of phentolamine, besides the activation of [^{3}H]noradrenaline uptake in vas deferens, an increase is also observed in the rate of amino uptake in atria which is rather large (25 per cent) and significant. This could be considered as evidence that under the effect of phentolamine the release from the vas deferens of a humoral factor activating [^{3}H]noradrenaline uptake both in the vas deferens and atria takes place.

Similar experiments were carried out using the activator of α-adrenoceptors, mesatone (1.10^{-5}M), which produces the inhibitor effect on [^{3}H]noradrenaline uptake only in the organs with postsynaptic α-adrenoceptors (Table 4). When the organs were incubated separately mesatone showed the inhibitory effect on the [^{3}H]noradrenaline uptake in vas deferens (by 38 per cent) and produced no effect on the rate of amine uptake in atria. The results obtained in the experiment with the combined incubation of the organs were similar to those using adrenoblocking agent: mesatone inhibited the [^{3}H]noradrenaline uptake both in the vas deferens (by 40 per cent) and atria (by 32 per cent).

Thus, the inhibitory effect of the activator of α-adrenoceptors, mesatone, is also realized through a humoral factor.

Protein synthesis in the realization of the effects of adrenotropic substances. To reveal possible mechanisms of the formation of humoral factors, a study was undertaken to find out whether the process is connected with the activation of the protein synthesis. For this purpose the effect of adrenotropic substances on the neuronal [^{3}H]noradrenaline uptake in the presence of the inhibitor of protein synthesis, cycloheximide, was

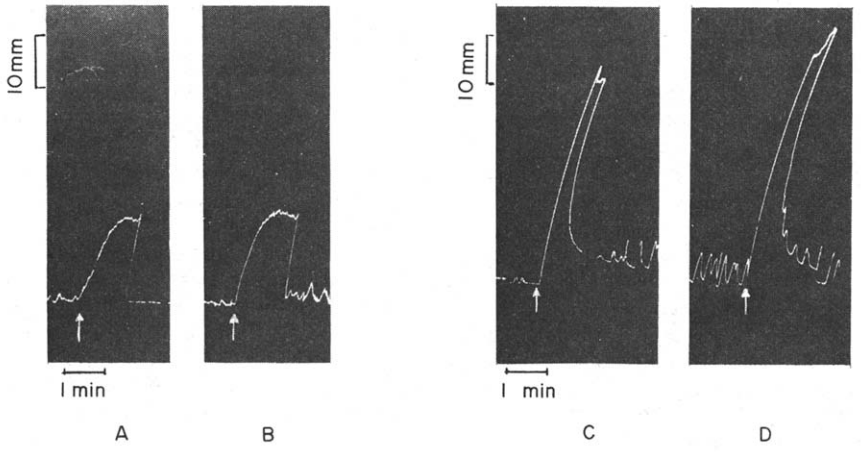

Fig. 1. Effect of cycloheximide on the responses of rat vas deferens to noradrenaline injection and transmural electric stimulation.
A. Injection of noradrenaline (1×10^{-7}g/ml);
B. The same after 30-min cycloheximide treatment (3.5×10^{-5}M);
C. Transmural stimulation 10 sec (20 V, 15 Hz, 0.3 msec);
D. The same after 30-min cycloheximide treatment;
↑. A,B:noradrenaline; C,D:stimulation.

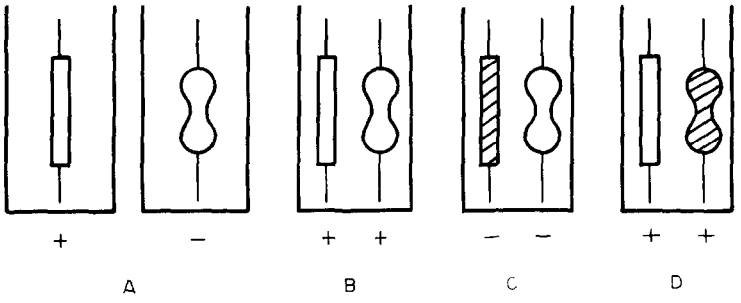

Fig. 2. Experimental scheme for studying the effect of adrenotropic substances on [^{3}H]noradrenaline uptake.
A. Effect of alpha-adrenotropic substances at separate incubation of vas deferens and atrium;
B. Effect of alpha-adrenotropic substances at combined incubation of the organs;
C. The same as (B); vas deferens was treated with cycloheximide;
D. The same as (B); atrium was treated with cycloheximide.
+. Effect
−. No Effect
⟦. Vas deferens: alpha- and beta-postsynaptic adrenoceptors;
⟦. Atrium: beta-postsynaptic adrenoceptors. Hatching: treatment with cycloheximide.

studied. A preliminary test was performed to determine the effect of cycloheximide on the protein synthesis in the rat vas deferens and atria using [^{14}C]leucine as a labelled precursor. Under the conditions employed, cycloheximide in a concentration of $3.5 \cdot 10^{-5}$M irreversibly inhibited the protein synthesis in vas deferens and atria by 75–90 per cent.

At the same time cycloheximide showed no appreciable effect on the rate of [^{3}H]noradrenaline uptake by rat organs (Table 5). However, in the presence of cycloheximide the activating effect of phentolamine and the inhibitory effect of mesatone and isopropylnoradrenaline on the amine uptake by vas deferens are completely abolished: no significant changes in the rate of amine uptake were recorded in the presence of cycloheximide, while in its absence phenotolamine activated [^{3}H]noradrenaline uptake by 86 per cent and mesatone and isopropylnoradrenaline inhibited the amine uptake by 31 and 36 per cent respectively (Table 5). A similar effect of cycloheximide was observed in the experiment on the effect of adrenotropic substances on atria. Thus, the rate of [^{3}H]noradrenaline uptake by atria which was shown to be increased by 130 per cent under the effect of propranolol and inhibited by 44 per cent under that of isopropylnoradrenaline showed no significant difference from that of the control in the presence of cycloheximide.

Since the effect of the substances known as specific blocking agents and agonists of adrenoceptors was shown to be completely removed, it seemed logical to study the direct effect of cycloheximide on adrenoceptors. With this goal, a series of control experiments was run on the effect of cycloheximide on the response of rat vas deferens to exogenous noradrenaline and transmural electrical stimulation [18]. Cycloheximide ($3.5 \cdot 10^{-5}$M) produces no decrease in the response of vas deferens to exogenous noradrenaline in a concentration of 1.10^{-7} g/ml (Fig. 1a, 1b). No changes were observed in the response of vas deferens to electrical stimulation (20 V; 15 Hz; 0.3 msec; Fig. 1c, 1d). From these results it can be concluded that the effect of cycloheximide observed in the preceding experiments might be due to its specific influence on the protein synthesis rather than the direct action on adrenoceptors.

Blockade of protein synthesis in pre- and postsynaptic sites. Since the realization of the action of adrenotropic substances on the neuronal uptake involves participation of both pre- and postsynaptic sites of adrenergic synapse we attempted to find out which changes in these sites are connected with the observed effects of studied adrenotropic substances. With this goal, a study was made of the humoral transmission of the regulatory influence of adrenotropic substances during combined incubation of the organs where one was pretreated with cycloheximide (Fig. 2). In the vas deferens treated with cycloheximide, the protein synthesis is inhibited in both pre- and postsynaptic sites, so we used atrium as an organ with an intact presynaptic site. The fact is that the atrial presynaptic site proved to be sensitive to the action of humoral factors released by vas deferens, while the postsynaptic site of the atrium—the organ possessing β-adrenoceptors—is unaffected by α-adrenotropic drugs phentolamine and mesatone. In the vas deferens we are interested precisely in the postsynaptic site in which, under the effect of α-adrenotropic substances, the formation and release of humoral agents affecting the rate of amine uptake by adrenergic neurone takes place.

In this experiment phentolamine did not produce a significant change in the rate of [^{3}H]noradrenaline uptake in the vas deferens treated with cycloheximide or in the intact atrium (Table 5). These results suggest that in the vas deferens cycloheximide might prevent the release and (or) formation of the humoral agent activating [^{3}H]noradrenaline uptake by adrenergic neurone. One cannot, however, exclude the possibility that cycloheximide may also affect the new synthesis of protein in the presynaptic site which is directly related to the neuronal amine uptake.

Consequently, in the following series of experiments we used intact vas deferens as a donor and atrium treated with cycloheximide as a recipient (Fig. 2). It was shown (Table 5) that under these conditions phen-

tolamine produces the activation of [^{3}H]noradrenaline uptake both in vas deferens (by 69 per cent) and atrium (by 61 per cent). This effect corresponds to the usual effect of phentolamine on [^{3}H]noradrenaline uptake by vas deferens (61 per cent activation) which is transmitted to atria (41 per cent activation).

Thus, as these results indicate, in the presynaptic site the processes that lead directly to the activation of amine uptake under the effect of adrenoblocking agents are not connected to a change in the rate of protein synthesis.

Similar results were obtained in the experiments on the effect of cycloheximide on the transmission of the inhibitory effect of mesatone on [^{3}H]noradrenaline uptake from vas deferens to atria (Table 5). In the experiment with cycloheximide-treated vas deferens inhibition of amino uptake was observed neither in the vas deferens nor in the atria, while in that with cycloheximide-treated atrium the results obtained corresponded to those observed in the control experiments on the transmission of the inhibitory effect of mesatone on the amine uptake from vas deferens to atria. In the control experiments mesatone inhibited the amine uptake by 42 per cent in vas deferens and by 45 per cent—in atria, and in the experiment with cycloheximide-treated atria—by 40 and 31 per cent respectively.

Thus, the experiments with cycloheximide suggest that it is in the effector cell that the humoral factors are formed, which are involved in the activation or inhibition of [^{3}H]noradrenaline uptake by adrenergic neurone under the effect of adrenotropic drugs on postsynaptic adrenoceptors.

The results obtained in the present study have shown that the blocking agents and agonists of postsynaptic adrenoceptors produce changes in the rate of amine uptake by adrenergic nerve endings. Experiments on the effect of adrenotropic drugs on [^{3}H]noradrenaline uptake in conditions of combined incubation of vas deferens and atrium suggest the humoral way of transmission of regulatory influences from postsynaptic adrenoceptors to adrenergic terminals. It seems that under the conditions employed, adrenotropic drugs induce the release of humoral factors from rat vas deferens into incubation medium that change the rate of [^{3}H]noradrenaline uptake by adrenergic neurone in both vas deferens itself and atrium. Consequently, these substances are not organ-specific. It is of interest that the humoral factors formed under the effect of α-adrenotropic drugs also affect the neuronal amine uptake in the organ possessing no α-adrenoceptors. Moreover, as it is seen from the data on the action of different adrenotropic drugs on different organs, the factors released at the blockade of both α- and β-adrenoceptors produce the same activating effect on [^{3}H]noradrenaline uptake. In turn, the factors formed at the activation of the receptors of both types inhibit [^{3}H]noradrenaline uptake.

In the course of experiments it was recorded that if the effect of adrenotropic drugs did not exceed 15–20 per cent in the organ-donor (vas deferens), no reliable changes were observed in the rate of [^{3}H]noradrenaline uptake in the recipient (atrium). From this it follows that a minimum concentration of humoral factors must exist which is necessary in order for their effect on the neuronal uptake to be expressed.

It can be concluded that the activation and inhibition of noradrenaline uptake caused by adrenotropic drugs are related to the protein synthesis. Here it should be noted that not only excitation, but the blockade of the adrenoceptors of the effector cell, is an active process resulting in the increase of the protein synthesis and the formation of corresponding humoral agents. This is indicated by the experiments with the inhibitor of protein synthesis cycloheximide in the presence of which the effect of both agonists and blocking agents of α- and β-adrenoceptors on noradrenaline uptake by rat vas deferens and atrium is not manifested.

It can be supposed that either the humoral factors themselves are the protein substances or that their formation demands the activation of some enzyme synthesis or—the last possibility—that the realization of the effects of these humoral factors is related to the activation of protein synthesis. Therefore, after the participation of the protein link in the process of the reverse trans-synaptic regulation had been established, a question arose: in which of the two sites—pre- or postsynaptic—the above mentioned protein synthesis activation takes place. To answer this question we used the model of "adrenergic synapse" where atrium was taken as a presynaptic site and vas deferens as a postsynaptic one. This model made it possible to block the protein synthesis separately either in the presynaptic (atrium treated with cycloheximide) or postsynaptic (cycloheximide-treated vas deferens) site. In these experiments the effect of adrenotropic substances on [^{3}H]noradrenaline uptake was manifested only if the protein synthesis in vas deferens remained intact. These results suggest that the regulatory effect of adrenotropic substances is realized through the activation of the protein synthesis precisely in the postsynaptic site in the effector cell. However, it still remains unclear what events take place in the effector cell; whether the humoral factors of the protein nature are formed or the enzyme systems connected with the formation of these factors are activated.

No significant changes of the rate of [^{3}H]noradrenaline uptake were observed during protein synthesis blockade in atria under these conditions, thus providing evidence that the reverse trans-synaptic regulation of noradrenaline uptake is not accompanied by the activation of the protein synthesis in the adrenergic neurone.

Summing up the results obtained one can infer the following sequence of the processes taking place under the effect of adrenotropic drugs. These substances activating or blocking adrenoceptors produce changes in the intensity of certain enzymatic processes in the effector cell by activating the protein synthesis. This in turn causes the formation and the release from the effector cell of specific humoral factors that reach the presynaptic sites through the gap in the synapse and produce respectively the activation or inhibition of the rate of noradrenaline uptake by adrenergic neurone. It should be noted that the processes that take place in the presynaptic site during these events are not connected with the synthesis of new protein molecules but, presumably are due to the effect of humoral factors on already present enzymes (or their cofactors) which control the uptake of noradrenaline by adrenergic neurone.

The considered humoral mechanism of transmission from the postsynaptic site is not unique in the regula-

tion of adrenergic process. Thus, it has been shown that under the effect of catecholamines a humoral factor is released from the effector cell which increases the adrenoceptor's sensitivity [33]. There is also some evidence suggesting that the effector cell may control the amount of noradrenaline released on the stimulation by acting on the presynaptic membrane of adrenergic neurone through the mediation of prostaglandin E [34–37].

It can be supposed that the reverse trans-synaptic regulation of the rate of neuronal noradrenaline uptake is a local physiological mechanism adapting the rate of mediator uptake to the changing functional state of the postsynaptic adrenoceptors, which is due to the amount of mediator acting on adrenoceptor as well as to the receptor's sensitivity to the mediator.

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