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REVERSE TRANS-SYNAPTIC REGULATION OF CATECHOLAMINE SYNTHESIS IN ADRENERGIC NEURONES

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Abstract—It was established that the blocking agent of beta-adrenoceptors, propanolol (1×10^{-6}M), activates [^{3}H]catecholamine synthesis from [^{3}H]tyrosine in isolated rat organs (vas deferens and atrium) by 40–80%. The blocking agent of alpha-adrenoceptors, phentolamine (1×10^{-6}M) activates [^{3}H]catecholamine synthesis by 30–60% only in the organ possessing postsynaptic alpha-adrenoceptors (vas deferens). The activator of beta-adrenoceptors, isopropylnoradrenaline (1×10^{-6}M), was shown to produce a decrease in [^{3}H]catecholamine synthesis by 30–40% in both organs investigated. The substance activating alpha-adrenoceptors, phenylephrine (1×10^{-5}M), inhibits [^{3}H]catecholamine synthesis in the organ with postsynaptic alpha-adrenoceptors by 40–50%. Activation and inhibition of [^{3}H]catecholamine synthesis induced by adrenotropic drugs is due to the release of chemical factors from the effector cell and their influence on the adrenergic neurone. The formation of chemical factors changing the intensity of catecholamine synthesis is related to the activation of protein synthesis in the effector cell. The processes which proceed in the adrenergic neurone are not connected with protein synthesis *de novo*.

Existence of the common mechanism for reverse trans-synaptic regulation of noradrenergic synthesis and uptake via the adrenoceptors of the effector cell is discussed.

Noradrenaline synthesis from tyrosine is one of the major processes providing the necessary level of adrenergic mediator in the neurones. Certain relationships were shown to exist between the intensity of catecholamine synthesis and the functional state of adrenoceptors. In the *in vivo* experiments following repeated injections of an alpha-adrenoceptor blocking agent (phenoxybenzamine) for 24 hr, the synthesis of [^{3}H]noradrenaline from [^{3}H]tyrosine is activated in the rat brain and heart [1, 2]. It is suggested that this activation is due to the central feed-back mechanism. A change in catecholamine metabolism is also observed after a short-term action of the substances affecting the adrenoceptor activity. Thirty minutes after the injection of the alpha-adrenoceptor blocking agent, phentolamine, a significant increase of noradrenaline concentration is observed in the rat spleen and vas deferens [3].

Recently the attention of investigators is more and more attracted to the study of the local mechanisms of regulation and self-regulation of mediator processes at the synaptic level. Thus it was shown that cholinergic processes were inhibited by a physiologically active substance, such as uridinepolyphosphate which was released from the effector cell under acetylcholine action [4]. A number of local mechanisms were discovered in the regulation of the adrenergic system [5, 6]. For example, in the experiments with the isolated rat organs, it was shown that blockade or activation of adrenoceptors produced an increase or inhibition of [^{3}H]catecholamine synthesis in the adrenergic neurones [7, 8]. Similar data on the action of adrenotropic drugs were obtained when investigating [^{3}H]noradrenaline uptake by isolated organs, where the humoral way for transmission of the regulatory influence from the effector cell to adrenergic neurones was shown [9, 10]. It seems quite possible that the same course of trans-synaptic regulation is also characteristic of catecholamine synthesis from tyrosine.

The present work was undertaken in order to study the regulatory influence of the effector cell on [^{3}H]catecholamine synthesis in the adrenergic neurone.

MATERIALS AND METHODS

The experiments were performed on male white Wistar rats, weighing 180–200 g. The animals were decapitated, and the vas deferens (80–90 mg) and atrium (60–80 mg) were excised and incubated in Tyrode solution (NaCl, 153.9 mM; KCl, 4.5 mM; $CaCl_2$, 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 a 5 ml chamber at 37° under oxygenation (95% O_2, 5% CO_2). After 30 min of incubation the solution was changed and preparations were incubated with the studied substances (antagonists and agonists of adrenoceptors), then for another 30 min in Tyrode solution with the studied substance and one of labelled noradrenaline precursors: [^{3}H]tyrosine, 8 μCi/ml (5.9×10^{-7} M); [^{3}H]-3,4-dioxyphenylalanine ([^{3}H]DOPA), 1 μCi/ml (2.1×10^{-5} M); or [^{3}H]dopamine, 0.1 μCi/ml (4.2×10^{-8} M).

After incubation the preparations were rinsed for 4–5 min with 5-fold replacement of the solution. Then the tissues under study were homogenised in 6 ml of 0.2 M $HClO_4$ with 0.02 M EDTA and 30 min later centrifuged in the cold (6000 *g*, 15 min). The

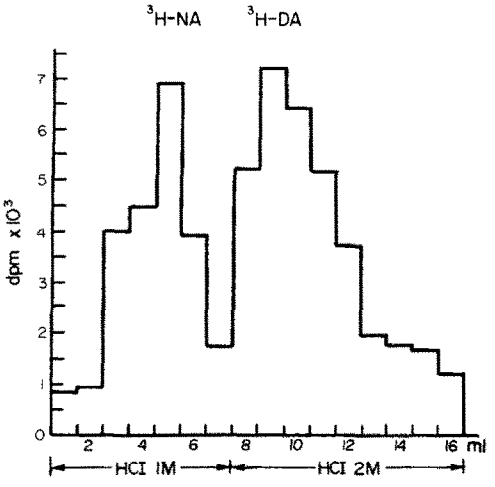

Fig. 1. Chromatographic separation of [^{3}H]-noradrenaline and [^{3}H]dopamine on column with ion-exchange resin Dowex-50. The *x* coordinate represents the fraction number (1 ml of eluate in each fraction). The *y* coordinate represents the amount of [^{3}H]catecholamine in dpm (fractions 1–7, elution with 1 M HCl; fractions 8–16, 2 M HCl).

pH of the centrifugate was brought up to 6.5 with 3.5 M Na_2CO_3.

Chromatographic separation of catecholamine was carried out on the columns with ion-exchange resin Dowex-50 (Serva) in the sodium form: lower part (height, 50 mm)—Dowex-50 wx10 mesh 20/50, upper part (70 mm)—Dowex-50 wx 4 mesh 100/200 [11, 12].

[^{3}H]Noradrenaline was eluted from the columns with 5 ml of 1 M HCl and then [^{3}H]dopamine with 5 ml of 2 M HCl [13]. This method of catecholamine chromatography allows purification of catecholamines from amino acid precursors (tyrosine and DOPA) and will separate noradrenaline and dopamine. In Fig. 1 the diagram of [^{3}H]catecholamine distribution in different eluate fraction is demonstrated. For [^{3}H]noradrenaline determination the eluate was taken from 3 to 7 ml, for [^{3}H]dopamine from 8 to 12 ml. On calculation it was taken into consideration that in the [^{3}H]noradrenaline fraction there was 5% of [^{3}H]dopamine and in the [^{3}H]dopamine fraction there was 15% of [^{3}H]noradrenaline, which corresponds to literature data [14].

The eluates were dried in scintillation cuvettes, then 1 ml of ethanol was added to the residue for [^{3}H]catecholamine extraction and then 10 ml of scintillation liquid (4 g PPO and 100 mg POPOP per 1 litre toluene) were added.

In the experiments with the inhibitors of protein synthesis, puromycin and cycloheximide [15], the latter was added to the medium during the first 30 min of incubation. The preparations of isolated organs, after washing 8–10 times with Tyrode solution, were incubated with the studied substances and [^{3}H]tyrosine according to the aforementioned scheme. In the control experiments on the effect of cycloheximide and puromycin on the protein synthesis, [^{14}C]leucine at concentration 1 μCi/ml (1.3×10^{-5}M) was used as a labelled precursor. It was shown that under these conditions cycloheximide (3.5×10^{-5}M) and puromycin (2.0×10^{-5}M) irreversibly inhibited the protein synthesis in rat vas deferens and atrium by 75–90% [10]. To study the effect of adrenotropic drugs on [^{3}H]tyrosine and [^{14}C]leucine incorporation into protein after routine incubation and washing procedure, the preparations were placed into scintillation cuvettes and 1 ml of hyamine was added. After 16–18 hr 10 ml of scintillation liquid was added.

To detect the chemical factor of trans-synaptic regulation in the incubation medium (donor-recipient experiment), vas deferens and atrium were incubated together in the same bath in the presence of alpha-adrenotropic agents. In some 'donor-recipient' experiments one of the organs was pretreated with cycloheximide in a 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 minute per gram of material (dpm/g). The results were subject to statistical processing using Student's criteria.

The drugs used were phentolamine, propranolol, phenylephrine, isopropylnoradrenaline (U.S.S.R.), cycloheximide, puromycin (Serva, West Germany), [^{3}H]tyrosine (sp. act. 13.5 Ci/mmole), [^{3}H]dopamine (sp. act. 2.4 Ci/mmole, Radiochemical Centre,

Table 1. Effect of adrenoceptor blocking agents on [^{3}H]catecholamine synthesis from different precursors in rat vas deferens and atrium

Organ	Precursor	[^{3}H]Catecholamine synthesis (dpm × 10^3/g ± S.E.)			
		Catecholamine	Control	Phentolamine (10^{-6}M)	Propranolol (10^{-6}M)
Vas deferens	[^{3}H]Tyrosine	[^{3}H]Dopamine	42.9 ± 2.3	55.4 ± 3.2*	61.5 ± 1.9*
		[^{3}H]Noradrenaline	25.3 ± 2.4	41.4 ± 4.6*	37.9 ± 1.9*
	[^{3}H]DOPA	[^{3}H]Dopamine	58.9 ± 3.6	64.7 ± 3.4	60.3 ± 2.7
		[^{3}H]Noradrenaline	11.3 ± 0.6	13.5 ± 1.3	9.8 ± 1.3
	[^{3}H]Dopamine	[^{3}H]Noradrenaline	40.9 ± 4.7	41.4 ± 2.7	43.5 ± 4.8
Atrium	[^{3}H]Tyrosine	[^{3}H]Dopamine	48.2 ± 3.5	50.1 ± 6.0	78.0 ± 4.1*
		[^{3}H]Noradrenaline	33.0 ± 3.0	32.9 ± 2.8	59.4 ± 3.2*
	[^{3}H]DOPA	[^{3}H]Dopamine	36.1 ± 3.8	35.4 ± 4.6	37.0 ± 5.6
		[^{3}H]Noradrenaline	19.5 ± 2.8	20.0 ± 3.8	21.6 ± 4.4
	[^{3}H]Dopamine	[^{3}H]Noradrenaline	23.1 ± 2.4	26.2 ± 5.5	25.9 ± 6.3

Values are means of 12–18 experiments.
Difference from the control is statistically significant: * $P < 0.01$.

Amersham, U.K.), [^{3}H]DOPA (sp. act. 47 mCi/mmole), and [^{14}C]leucine (sp. act. 30 mCi/mmole, Isotop, U.S.S.R.).

RESULTS

Effect of adrenotropic substances on [^{3}H]*-catecholamine synthesis.* Catecholamine synthesis involves a number of enzymatic processes, so we studied the action of adrenotropic drugs, all noradrenaline precursors being used as a substrate (tyrosine, DOPA, dopamine). The experiments were carried out on vas deferens—the organ with postsynaptic alpha and beta-adrenoceptors—and on atrium, which had mainly beta-adrenoceptors.

As seen in Table 1 the adrenoblocking agents increase [^{3}H]catecholamine synthesis only from [^{3}H]tyrosine, i.e. a change in the adrenoceptor functional state influences the [^{3}H]catecholamine synthesis only from its main precursor. Here the alpha-adrenoblocking agent, phentolamine, activates [^{3}H]catecholamine synthesis only in vas deferens ([^{3}H]dopamine by 29%, [^{3}H]noradrenaline by 64%), while the beta-adrenoblocking agent, propanolol, increases this process, both in vas deferens (by 43 and 50%) and in atrium (by 62 and 80%, respectively). Similar results were obtained when studying the adrenomimetics action on catecholamine synthesis. Thus the activator of alpha-adrenoceptors, phenylephrine, reduced the rate of [^{3}H]noradrenaline synthesis from [^{3}H]tyrosine in vas deferens only (Table 2), while the activator of beta-adrenoceptors, isopropylnoradrenaline, inhibited this process both in vas deferens (Table 3) and in atrium (Table 4).

It can be concluded from the results obtained that it is through the adrenoceptors that the effect of adrenoblocking agents and agonists on catecholamine synthesis, as well as on noradrenaline uptake [10], is realized. This is indicated by the following facts. All the drugs used produced changes of catecholamine synthesis, when taken in very low physiological concentrations, specifically blocking or activating adrenoceptors and producing no toxic effect on the functional state of the cells. Effects of the drugs known as agonists (phenylephrine) and blocking agents (phentolamine) of alpha-adrenoceptors are manifested only in the organ possessing postsynaptic alpha-adrenoceptors (vas deferens). The effect of the beta-blocking agent, propranolol, and the beta-agonist, isopropylnoradrenaline, is observed in all the organs (vas deferens and atrium) regardless of their functional adrenoception. This might be due to the fact that all the organs with sympathetic innervation have the receptors of beta-type by means of which the main influences of the adrenergic system on metabolic processes are realized. Thus, the effect of all alpha- and beta-agonists and blocking agents used, is precisely correlated with the presence of corresponding alpha- and beta-adrenoceptors in the tissues.

One more conclusion can be drawn from this fact. It seems likely that the receptors (the changes of functional state of which result in the changes of the rate of noradrenaline synthesis in the rat organs) are located on the postsynaptic membrane of the effector

Table 2. Effect of alpha-adrenotropic agents on [^{3}H]catecholamine synthesis by isolated vas deferens and atrium under conditions of separate and simultaneous incubation

Drug	Concentration (M)	Incubation	Catecholamine synthesis (dpm × 10^3/g ± S.E.)			
			Vas deferens		Atrium	
			[^{3}H]Dopamine	[^{3}H]Noradrenaline	[^{3}H]Dopamine	[^{3}H]Noradrenaline
Control		Separate	81.7 ± 3.5	36.1 ± 1.9	48.2 ± 3.5	33.0 ± 2.9
Control		Simultaneous	74.7 ± 2.5	37.6 ± 1.8	46.7 ± 2.4	31.2 ± 2.1
Phentolamine	1×10^{-6}	Separate	118.6 ± 6.1*	59.6 ± 5.2*	50.2 ± 6.0	32.9 ± 2.8
Phentolamine	1×10^{-6}	Simultaneous	96.3 ± 4.2†	61.2 ± 3.8*	69.3 ± 3.2‡	47.4 ± 2.0*
Phenylephrine	1×10^{-5}	Separate	42.5 ± 3.0*	19.1 ± 2.1*	45.8 ± 2.9	34.7 ± 2.1
Phenylephrine	1×10^{-5}	Simultaneous	50.6 ± 1.1*	20.9 ± 1.9*	30.9 ± 2.6*	22.1 ± 1.2*

Values are means of 12–18 experiments.
Difference from control is statistically significant:* $P < 0.01$; †$P < 0.02$; ‡$P < 0.05$.

cell, since the effect of alpha-adrenotropic drugs is precisely correlated with the presence or absence of alpha-adrenoceptors on the postsynaptic membrane. The possiblity of the action of adrenotropic drugs 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 distribution of alpha- and beta-receptors on the presynaptic membrane corresponding to that of the receptors on the postsynaptic membrane. At present we have no evidence in favour of such an assumption.

Thus, the results obtained suggest the existence of the regulatory influence from the effector cell on the processes of catecholamine synthesis in adrenergic neurones. It might be supposed that this regulatory influence on presynaptic processes is realized by means of chemical factors, since first, the existence of other ways in isolated organs seems unlikely and, second, the humoral way of the reverse transsynaptic regulation was earlier shown for neuronal noradrenaline uptake [10].

Mechanism of regulatory influence of the effector cell on the [^{3}H]*catecholamine synthesis in adrenergic neurones.* For studying the possibility of the humoral way in transmitting the influence from the effector cell to adrenergic neurones, vas deferens and atrium were incubated together in the presence of alpha-adrenoblocking agent, phentolamine, in a concentration of 1×10^{-6}M. As a control the effect of phentolamine on [^{3}H]catecholamine synthesis in these organs at their separate incubation was studied. As seen in Table 2, the combined incubation of the organs in itself produces no marked changes in the rate of [^{3}H]catecholamine synthesis. With separate incubation phentolamine produces a significant increase in the rate of [^{3}H]noradrenaline (65%) and [^{3}H]dopamine (45%) synthesis in vas deferens, but does not affect that in atrium. With the combined incubation of the organs in the presence of phentolamine besides the activation of [^{3}H]catecholamine synthesis in vas deferens (by 63 and 29%, respectively) an increase is also observed in the rate of amine synthesis in atrium which is rather great (40 and 23%) and significant. This could be considered as an evidence that under the effect of phentolamine a chemical factor activating [^{3}H]catecholamine synthesis both in vas deferens and atrium is released from vas deferens.

Similar experiments were carried out using the activator of alpha-adrenoceptors, phenylephrine (1×10^{-5}M), which produces the inhibitory effect of [^{3}H]catecholamine synthesis only in the organ with postsynaptic alpha-adrenoceptors (Table 2). When the organs were incubated separately phenylephrine showed the inhibitory effect on the [^{3}H]catecholamine synthesis in vas deferens ([^{3}H]dopamine by 48%, [^{3}H]noradrenaline by 47%) and produced no effect on the rate of amine synthesis in atrium. The results obtained in the experiment with the combined incubation of the organs were similar to those using the adrenoblocking agent: phenylephrine inhibited the [^{3}H]catecholamine synthesis both in the vas deferens (38 and 42%) and atrium (33 and 38%), respectively.

Thus, the inhibitory effect of the activator of

Table 3. Effect of cycloheximide and puromycin on [^{3}H]catecholamine synthesis in vas deferens under the action of adrenotropic agents

Drug	Concentration (M)	[^{3}H]Catecholamine synthesis (dpm × 10^3/g ± S.E.)					
		Control		Cycloheximide		Puromycin	
		[^{3}H]Dopamine	[^{3}H]Noradrenaline	[^{3}H]Dopamine	[^{3}H]Noradrenaline	[^{3}H]Dopamine	[^{3}H]Noradrenaline
Control		42.9 ± 2.3	25.3 ± 2.4	43.7 ± 5.3	28.8 ± 3.2	41.6 ± 4.9	33.9 ± 3.8*
Phentolamine	1×10^{-6}	55.4 ± 3.2*	41.4 ± 4.6*	40.8 ± 2.2	26.9 ± 2.7	38.2 ± 3.9	31.0 ± 4.1
Propranolol	1×10^{-6}	61.5 ± 1.9*	37.9 ± 1.9*	46.8 ± 2.0	30.3 ± 2.7		
Phenylephrine	1×10^{-5}	26.6 ± 2.1*	14.4 ± 2.0*	40.2 ± 3.1	25.9 ± 2.2	39.7 ± 3.0	30.1 ± 3.9
Isopropylnoradrenaline	1×10^{-6}	29.8 ± 2.0*	15.8 ± 1.9*	50.4 ± 3.8	31.6 ± 6.1		

Values are means of 12–18 experiments.
Difference from control is statistically significant: * $P < 0.01$.

Table 4. Effect of cycloheximide on [^{3}H]catecholamine synthesis in atrium under the action of adrenotropic agents

Drug	Concentration (M)	[^{3}H]Catecholamine synthesis (dpm × 10^3/g ± S.E.)			
		Control		Cycloheximide	
		[^{3}H]Dopamine	[^{3}H]Noradrenaline	[^{3}H]Dopamine	[^{3}H]Noradrenaline
Control		26.1 ± 2.2	18.4 ± 2.0	29.8 ± 1.6	22.7 ± 2.9
Phentolamine	1×10^{-6}	27.2 ± 3.7	18.2 ± 2.8		
Propranolol	1×10^{-6}	42.9 ± 1.7*	33.2 ± 2.2*	28.8 ± 1.3	26.7 ± 6.5
Phenylephrine	1×10^{-5}	25.1 ± 2.4	19.1 ± 2.1		
Isopropylnoradrenaline	1×10^{-6}	18.5 ± 2.9*	12.5 ± 1.8†	27.9 ± 5.3	24.5 ± 2.4

Values are means of 12–18 experiments.
Difference from control is statistically significant: * $P < 0.01$; † $P < 0.02$.

alpha-adrenoceptor phenylephrine is also realized through a chemical factor. This result can be interpreted as evidence of the fact that the action of the alpha-adrenotropic drugs, phentolamine and phenylephrine, produces the release of some chemical factors in vas deferens and these factors can influence catecholamine synthesis both in vas deferens and atrium.

Studying the adrenotropic drugs action on [^{3}H]noradrenaline uptake, the necessity of protein synthesis activation was established for the effects of these substances to be realized [9, 10]. Therefore, in this work adrenotropic drugs action on [^{3}H]catecholamine synthesis was investigated in the presence of the protein synthesis inhibitors cycloheximide (3.5×10^{-5}M) and puromycin (2.0×10^{-5}M). Under the conditions employed these drugs showed no appreciable effect on the rate of [^{3}H]catecholamine synthesis in rat organs (Tables 3 and 4). Some activation of [^{3}H]catecholamine (especially [^{3}H]noradrenaline) synthesis in the presence of cycloheximide and puromycin can be noted. Apparently, protein synthesis blockade increases the pool of free amino acid [^{3}H]tyrosine in the cell available for catecholamine synthesis. It is known that an increase of the precursor concentration can lead to fast activation of noradrenaline synthesis [2].

In the next experimental series the action of adrenotropic agents on [^{3}H]catecholamine synthesis in vas deferens and atrium in the presence of protein synthesis inhibitors was studied. It was found that in the presence of cycloheximide and puromycin the activating effect of phentolamine and propranolol and the inhibitory effect of phenylephrine and isopropylnoradrenaline on the amine synthesis in vas deferens and atrium are completely abolished (Tables 3 and 4).

Since the realization of the action of adrenotropic substances on catecholamine synthesis involves participation of both pre- and postsynaptic sites of adrenergic synapse, we attempted to find out what changes and in what site are connected with the observed effect of the studied adrenotropic substances. For this purpose a study was made of humoral transmission of the regulatory influence of adrenotropic substances during combined incubation of the organs, one of them being pretreated with cycloheximide (Fig. 2). In vas deferens treated with cycloheximide, 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 chemical factors released by vas deferens, while the postsynaptic site of the atrium—the organ possessing beta-adrenoceptors—was unaffected by the alpha-adrenotropic drugs phentolamine and phenylephrine. In vas deferens we were interested precisely in the postsynaptic site in which, under the effect of alpha-adrenotropic substances, the formation and release of chemical agents affecting the rate of amine synthesis by adrenergic neurone took place.

In this experiment phentolamine did not produce a significant change in the rate of [^{3}H]catecholamine synthesis in vas deferens treated with cycloheximide or in the intact atrium (Table 5). These results suggest that in vas deferens cycloheximide might prevent the release and/or formation of the chemical agent activating [^{3}H]catecholamine synthesis 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 amine synthesis.

Consequently, in the following series of experiments we used intact vas deferens as donor and atrium treated with cycloheximide as recipient (Fig. 2). It was shown (Table 5) that under these conditions phentolamine activated [^{3}H]catecholamine synthesis both in vas deferens ([^{3}H]dopamine by 20%,

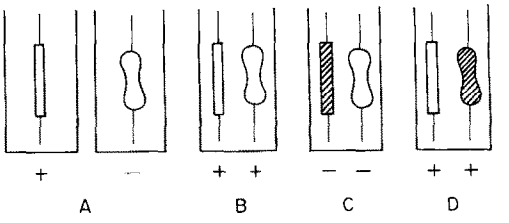

Fig. 2. Experimental scheme for studying the effect of adrenotropic substances on [^{3}H]catecholamine synthesis. A, effect of alpha-adrenotropic substances at separate incubation of vas deferens and atrium; B, effect of alpha-adrenotropic substances at combined incubation of the organs; C, the same 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.

Table 5. Effect of cycloheximide on humoral transmission of activating influence of phentolamine and inhibitory influence of isopropylnoradrenaline on [^{3}H]catecholamine synthesis

Drug	Concentration (M)	Organ treated with cycloheximide	[^{3}H]Catecholamine synthesis (dpm × 10^3/g ± S.E.)			
			Vas deferens		Atrium	
			[^{3}H]Dopamine	[^{3}H]Noradrenaline	[^{3}H]Dopamine	[^{3}H]Noradrenaline
Control			74.7 ± 2.5	37.5 ± 1.8	46.7 ± 2.4	31.2 ± 2.0
Cycloheximide	$3.5.10^{-5}$	Vas deferens and atrium	76.0 ± 5.9	42.8 ± 3.8	52.3 ± 2.8	37.6 ± 3.5
Phentolamine	1.10^{-6}		96.3 ± 4.2†	61.2 ± 3.8*	59.3 ± 3.2*	43.4 ± 3.0*
Phentolamine	1.10^{-6}	Vas deferens	69.6 ± 4.1	36.1 ± 2.0	43.5 ± 2.1	29.3 ± 1.9
Phentolamine	1.10^{-6}	Atrium	89.5 ± 4.1*	54.6 ± 2.8*	57.9 ± 3.2†	41.5 ± 3.1†
Phenylephrine	1.10^{-5}		41.4 ± 1.1*	23.7 ± 1.9*	28.9 ± 2.6*	21.2 ± 1.2*
Phenylephrine	1.10^{-5}	Vas deferens	75.8 ± 3.1	35.1 ± 2.7	48.8 ± 3.1	33.6 ± 2.9
Phenylephrine	1.10^{-5}	Atrium	40.4 ± 2.5*	26.0 ± 4.0†	30.3 ± 2.9*	22.4 ± 2.1*

Values are means of 12–18 experiments.
Difference from control is statistically significant: *$P < 0.01$; †$P < 0.02$.

[^{3}H]noradrenaline by 45%) and atrium (23 and 28%, respectively). This effect corresponds to the usual action of phentolamine on [^{3}H]catecholamine synthesis by vas deferens; and it is transmitted to atrium.

Thus, as these results indicate, in the presynaptic site the processes that lead directly to the activation of amine synthesis under the effect of adrenoblocking agents are not connected with 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 phenylephrine on [^{3}H]catecholamine synthesis from vas deferens to atrium (Table 5). In the experiment with cycloheximide-treated vas deferens the inhibition of amine synthesis was observed neither in vas deferens nor in atrium, 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 phenylephrine on the amine synthesis from vas deferens to atrium. In this experiment phenylephrine inhibited the amine synthesis by 46% for [^{3}H]dopamine and 31% for [^{3}H]noradrenaline in vas deferens and by 36 and 29% respectively in atrium.

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

It is interesting to note that not only the rate of [^{3}H]catecholamine synthesis changes in the presence of adrenotropic agents but also the intensity of [^{3}H]tyrosine incorporation into protein (Table 6). In this case the rate of protein synthesis is apparently not changed, which is evidenced by the experiments on adrenotropic drugs action on the incorporation of another amino acid—[^{14}C]leucine into protein (Table 6).

DISCUSSION

The results obtained in the present study have shown that the blocking agents and agonists of postsynaptic adrenoceptors produced changes in the rate of amine synthesis in adrenergic neurones. Experiments on the effect of adrenotropic drugs on [^{3}H]catecholamine synthesis during 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 the adrenotropic drugs induce the release of chemical factors from rat vas deferens into incubation medium, which changes the rate of [^{3}H]catecholamines synthesis by adrenergic neurones in both vas deferens itself and atrium. Consequently, these substances are not organ-specific. It is of interest that the chemical factors induced by alpha-adrenotropic drugs also affect the amine synthesis in the organ possessing no alpha-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 alpha- and beta-adrenoceptors produce the same

Table 6. Effect of adrenotropic agents on the incorporation of [^{3}H]tyrosine and [^{14}C]leucine into protein in rat vas deferens and atrium

Drug	Concentration (M)	Activity (dpm × 10^3/g ± S.E.)			
		Vas deferens		Atrium	
		[^{3}H]Tyrosine	[^{14}C]Leucine	[^{3}H]Tyrosine	[^{14}C]Leucine
Control		1130.8 ± 61.2	1525.9 ± 94.1	1008.2 ± 43.1	1443.1 ± 82.1
Phentolamine	1×10^{-6}	1423.8 ± 72.2*	1560.1 ± 96.2	1026.4 ± 51.7	1485.3 ± 71.9
Propranolol	1×10^{-6}	1399.4 ± 89.2†	1477.9 ± 84.4	1298.1 ± 60.2†	1422.8 ± 60.5
Phenylephrine	1×10^{-5}	915.9 ± 64.1‡	1483.4 ± 82.1	994.8 ± 39.2	1410.2 ± 62.4
Isopropylnoradrenaline	1×10^{-6}	882.0 ± 71.9*	1495.9 ± 94.4	821.5 ± 41.9*	1463.1 ± 78.9

Values are means for 12–18 experiments.
Difference from control is statistically significant: *$P < 0.01$; †$P < 0.02$; ‡ $P < 0.05$.

activating effect on [^{3}H]catecholamine synthesis. In turn the factors formed at the activation of the receptors of both types inhibit [^{3}H]catecholamine synthesis.

To answer the question in which of two sites, pre- or postsynaptic one, the mentioned protein synthesis activation takes place we used the model of 'adrenergic synaps' 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]catecholamine synthesis 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.

No significant changes of the rate of [^{3}H]catecholamines synthesis were observed when protein synthesis was blocked in atrium under these conditions, thus providing evidence that the reverse trans-synaptic regulation of catecholamine synthesis is not accompanied by the activation of the protein synthesis in the adrenergic neurone and consequently by the formation *de novo* of the enzymes which take part in amine synthesis.

It is possible that the changes in catecholamine synthesis are related to the changes of the rate of tyrosine transport into the neurone. It follows from the results obtained that the adrenotropic drugs alter the incorporation of [^{3}H]tyrosine into protein. But under the same conditions no changes in the rate of incorporation of [^{14}C]leucine (an amino acid which is not connected with catecholamine synthesis) into protein are observed (Table 6). This fact demonstrates high specificity of this regulatory process. Supporting the evidence of possible selective regulation a certain amino acid transport comes from literature data about the existence of the special system for the transport of aromatic amino acids to which tyrosine belongs [16]. Additionally it was demonstrated that adrenotropic drugs did not change the rate of [^{3}H]noradrenaline synthesis from [^{3}H]DOPA and [^{3}H]dopamine (Table 1) and neuronal uptake of [^{3}H]dopamine [8].

It has been shown earlier that the adrenotropic drugs changed neuronal uptake of [^{3}H]noradrenaline as well [9, 10]. It is known from literature that transport of noradrenaline and of some amino acids into the cell has some common properties. Thus both processes are enzymatic reactions and carriers are involved in them [17], they are sodium-dependent [16] and are inhibited by tricyclic anti-depressants [18]. All these facts suggest the existence of a very similar regulatory mechanism of transport into adrenergic neurone amino acid tyrosine and noradrenaline which are the main source for maintaining the necessary level of sympathetic mediator in the nervous system. Tyrosine concentration in the plasma is known to be 10^{-4}–10^{-5}M [19, 20]. In our work we used a much lower concentration (6×10^{-7}M), but a series of other concentrations (up to 1.6×10^{-4}M) were tested in our tentative experiments. With all the concentrations studied the observed effects fully coincided with those obtained in this work, i.e. we observed the phenomenon of the reverse trans-synaptic regulation of catecholamine synthesis.

The role of tyrosine concentration in the tissue for the regulation of the rate of catecholamine synthesis still remains unclear. According to the data of Dairman [20] a change in the tyrosine content in the rat blood plasma produced by administration of exogenous tyrosine or by the blockade of the protein synthesis does not lead to a reliable increase of noradrenaline content in the tissue. At the same time, as reported by Sedvall and Kopin [2], a change of the intracellular pool of precursor resulted in a rapid activation of noradrenaline synthesis, and according to Molinoff and Axelrod [21] at the level of tyrosine hydroxylase the rate of catecholamine synthesis can be regulated, among the other factors, also by the rate of tyrosine transport into neurone. So it seems that a change in the intensity of the major precursor transport into neurone could be one of the factors regulating the rate of noradrenaline synthesis.

It was noted before that formation of the chemical factors was connected with the activation of the protein synthesis in the effector cell. It should be emphasized that not only excitation, but the blockade of adrenoceptors of the effector cell is an active process resulting in an increase of protein synthesis and the formation of corresponding chemical agents. Thus not only the presence but also absence of

adrenergic information serves as a trigger for the activation of some biochemical processes in the effector cell.

As regards the effect adrenoblocking agents have on the adrenoceptors of the isolated organs, here one can think of two alternatives: (1) adrenoblocking agents (phentolamine and propranolol) by blocking adrenoceptors prevent the effect on these receptors of spontaneously releasing noradrenaline; (2) the very binding of adrenoceptors with substances inhibiting their functional activity triggers the mechanism of reverse trans-synaptic regulation.

The direct effect of adrenomimetics, phenylephrine and isopropylnoradrenaline on the synthesis of catecholamines in our experimental conditions can be ruled out, since (1) phenylephrine and isopropylnoradrenaline do not inhibit the catecholamine synthesis at the blockade of the protein synthesis, even though the activity of tyrosine hydroxylase remains unchanged (the rate of catecholamine synthesis is unchanged), (2) it is through the postsynaptic level that the effect of these substances is brought about: it is in this link of the adrenergic synapse that interference with the protein synthesis prevents the development of phenylephrine and isopropylnoradrenaline effect on the catecholamine synthesis.

Summing up the results obtained one can infer the following sequence of the processes taking place in the course of reverse trans-synaptic regulation of the catecholamine synthesis from tyrosine. Changes in the functional state of postsynaptic adrenoceptors produce changes in the intensity of certain enzymatic processes in the effector cell by activating protein synthesis. This in turn causes the formation and release of specific chemical factors from the effector cell. These factors reach the presynaptic site through the gap in the synapse and probably produce, respectively, activation or inhibition of the rate of tyrosine transport into adrenergic neurone which leads to corresponding alteration of catecholamine synthesis.

It can be supposed that reverse trans-synaptic regulation of the rate of noradrenaline synthesis and neuronal uptake [10] is a local physiological mechanism adapting the rate of mediator accumulation to the changing functional state of the postsynaptic adrenoceptors. A decrease of adrenoceptor activity brings about quick recovery of its functional pool and, probably, an increase of the quantity of the mediator release under nerve impulses. The adrenoceptor activation, on the contrary, inhibits the rate of noradrenaline accumulation which leads to a decrease of the neurone adrenergic activity. So the feed-back is settled between the innervated cell and adrenergic neurone by means of which the neurone is informed about the adrenergic provision of the affector organ. It allows an exact regulation of the neurone functional activity in accordance with the varying state of the innervated system.

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