Adrenergic Structures, M-Cholino-, and β-Adrenoception in Thyroid Gland of Desympathized Rats during Postnatal Ontogenesis

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The density of adrenergic nerve terminals in thyroid gland decreases with age, and this decrease is more pronounced in parafollicular terminals. Density of ^{3}H-dihydroalprenolol binding sites increases in 1-month-old rats and decreases in senescent rats. In comparison with control rats, in sympathetically denervated rats the density of nerve fibers increases at the age of 1-6 months and decreases at the age of 12 months. Binding of ^{3}H-dihydroalprenolol is considerably higher in immature sympathetically denervated rats.

Key Words: *thyroid gland; adrenergic terminals; adrenoception; chemical desympathization*

The key role in adaptive reactions is played by neuroendocrinal system, which has a wide repertoire of physiological effects and is characterized by pronounced structural and functional lability in responses to exo- and endogenous factors. The integral scheme of relationships between neural agents includes transmitter sources, specific membrane receptors, and coupled modulating systems of cell-cell transmission. In the sympathetic part of the nervous system these roles are predominantly played by monoamines and their receptors. It is important to study the interaction of monoaminergic structures in the nervous system and paraganglionic subdivisions supplying tissues with biological amines at various periods of life. Thyroid gland (TG) is a convenient test object, because the mechanism of its neurohumoral regulation by the hypophyseal system is known in details, and a clear-cut dependence of TG function on sympathetic innervation is established [2,5,15]. Animals desympathized in the early postnatal period are a well known model for studying the postnatal ontogenesis under conditions of altered functional activity of the sympathetic nervous system [6].

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Our aim was to study the age-related dynamics of adrenergic innervation and reception in TG under various modes of sympathetic activity.

MATERIALS AND METHODS

The study was carried out on 1-day, 2 and 4-week, 6, 12, and 24-month-old male albino rats. The test rats were injected subcutaneously with guanethidine (Isobarin, Pliva) in a dose of 20 mg/kg body weight from postnatal day 0 through 14. The control rats were injected with physiological saline in the same period. The fluorescent histochemical analysis was performed on 15-μ TG cryostat slices processed according to Bjorklund's modified method [7].

To evaluate specific binding of transmitters in tissue, the rats narcotized with Nembutal were perfused through the left atrium with 0.5% glutaraldehyde in phosphate buffer. The cryostat TG sections were mounted on gelatinized slides, dried, and incubated in a buffer containing ^{3}H-quinuclidinyl benzilate (^{3}H-QB) and ^{3}H-dihydroalprenolol (^{3}H-DA), highly specific agonists of muscarinic cholinergic- and β-adrenoceptors, respectively. Unlabeled atropine and isoproterenol were used as the antagonists. After incubation, the preparations were dried, covered with a lavsan film

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TABLE 1. Density of Adrenergic Fibers in TG of Control and Desympathized Rats (*M±m*)

Age, month		Density of adrenergic nerve fibers, rel. units	
		parafollicular network	perivascular plexuses
1	Control	15.8±0.9	2.3±0.2
	Test	4.0±0.6*	1.4±0.1*
6	Control	9.8±0.8	2.3±0.3
	Test	4.9±0.7*	1.9±0.2*
12	Control	8.6±0.8	2.4±0.3
	Test	3.9±0.9*	1.6±0.4*
24	Control	6.2±0.7	2.1±0.4

Note. *$p<0.01$ compared with the control group.

with LM-1 emulsion and exposed during 6 months at 4°C. Binding intensity was evaluated by optical density of the autograph measured with an Opton cytophotometer.

RESULTS

The fluorescent-histochemical study of TG from normally developing rats revealed perivascular sympathetic terminals with varicosities, desquamated elements in the colloid, labrocytes arranged along blood vessels, and C cells. The density of adrenergic fibers reaches maximum to the 1-month age and then decreases (Table 1). The changes in density of parafollicular sympathetic terminals began earlier and were more pronounced.

It is difficult to determine the absolute number of tissue receptors by radioligand binding assay, although this method makes it possible to characterize their changes during postnatal ontogeny. Binding of labeled ligands by TG tissue in control rats is somewhat different for agonists of muscarinic receptors and β-adrenoceptors. In neonatal rats the density of labeling on autographs only slightly surpassed the baseline values. To the age of 2 weeks the tissue labeling with ^{3}H-DA and ^{3}H-QB attained the level characteristic of mature rats. However, binding of ^{3}H-QB remained virtually unchanged during postnatal ontogeny, while binding of ^{3}H-DA gradually increased until maturity and decreased in 24-month-old rats (Table 2).

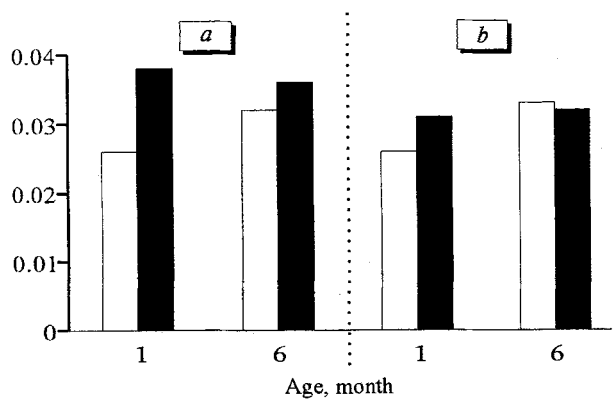

Fig. 1. Binding of ^{3}H-dihydroalprenolol (*a*) and ^{3}H-quinuclidinyl benzilate (*b*) in the thyroid gland of control (light bars) and desympathized (solid bars) rats of different age. Ordinate: autograph optical density, rel. units.

In the 1-month-old rats that were desympathized at the early age, the density of TG adrenergic fibers in the perivascular plexuses and particularly in the parafollicular network was considerably lower than in controls (Table 1). To the age of 6 months, the density of nerve fibers approximates the control value, especially in the perivascular nervous plexuses. In normally developing rats at the age of 6-12 months the density of adrenergic nerve fibers remained practically unchanged, while in desympathized rats it decreased considerably. While the decrease in the density of adrenergic nerve fibers immediately after sympathetic denervation (irrespective of its mechanism) was observed in many tissues of various species, the age-related dynamics of the density of adrenergic terminals is described in a few papers and only for juvenile and early reproductive age [6].

The age-related dynamics of the cytomorphological indices of TG at juvenile and early reproductive age in rats subjected to immune [4] or guanethidine-induced [5] sympathetic denervation can be explained by partial recovery of the adrenergic innervation. The densities of adrenergic fibers in the parafollicular nervous network and perivascular nervous plexuses of 1-month-old desympathized rats were 25 and 61% of the control, respectively, while the corresponding figures in 6-month-old rats were 50 and 83%. Although the density of adrenergic fibers and the content of neurotransmitters remained decreased, the sympathetic influences were enhanced due to increased tissue sensitivity to neurotransmitters resulting from the increase in the receptor number.

In the one-month-old rats desympathized at the early age, the binding of ^{3}H-DA were higher than in

control rats ($p<0.05$, Fig. 1). In 6-month-old experimental and control rats this difference was less pronounced. Differences in ^{3}H-QB binding in control and experimental rats were insignificant.

In most tissues, adrenergic fibers are the major sources of norepinephrine, therefore the total content of catecholamines in the organ depends on innervation density [12]. The neurotransmitters from the sympathetic terminals are more efficient than those released into the bloodstream from the vascular nervous plexuses, or supplied by C-cells [1,13]. In peripheral tissues innervated by sympathetic terminals, the neurotransmitter (norepinephrine) is released not from the nerve endings, but from the axonal varicosities. In this case there are no active zones from where neurotransmitter is released. Neurotransmitter diffuses widely and affects several neighboring cells acting as a local chemical transmitter [2]. Norepinephrine downregulates the number of postsynaptic β-adrenoceptors and in high concentration can disturb their ontogenesis [9]. A pronounced decrease of adrenergic innervation of visceral organs was the reason to outline "postmediator stage" in the ontogenesis of the autonomic nervous system [7,8].

The data on changes of the receptor field are predominantly based on tissue reaction to agonist [6]. Only few papers studied the tissue receptor apparatus in desympathized animals. It was shown that adrenoceptors density in the myocardium [10] and brain [14] increased 7 days after injection of 6-hydroxydopamine.

The density of ligand binding sites corresponds to the amount of mRNA, i.e. depends on the intensity of gene transcription [11]. In desympathized rats the blood level of norepinephrine and chromatin transcription activity in survived neurocytes in sympathetic ganglia are enhanced [6]. Presumably, increased number of receptors is a compensatory reaction of desympathized tissue.

TABLE 2. Ligand Binding in TG in Normal Rats of Different Age ($M\pm m$)

Age	Binding of labeled ligands by TG, rel. units	
	^{3}H-DA	^{3}H-QB
1 day	0.004±0.002	0.007±0.003
15 days	0.024±0.002*	0.028±0.003*
1 month	0.025±0.003	0.026±0.002
6 months	0.032±0.004	0.033±0.004
24 months	0.022±0.004*	0.031±0.006

Note. *$p<0.05$ compared with previous age group.

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