# PHYSIOLOGY

# Postnatal Changes in Muscarinic Cholinoception, β-Adrenoception, and Monoamine Content in the Cranial Cervical Sympathetic Ganglion of Desympathized Rats

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The content of catecholamines and serotonin in rat cranial cervical ganglion increased during postnatal ontogeny. $^3$H-Quinuclidinyl benzilate binding approached the adult values at the age of one month and then remained at this level, while the binding of $^3$H-dihydroalprenolol increased until maturation and then declined in senescent animals. Early chemical sympathectomy reduced the tissue content of monoamines and had little effect on the binding of labeled ligands.

**Key Words:** *sympathetic ganglion; β-adrenoception; muscarinic cholinoception, chemical sympathectomy*

Physiologically, sympathetic regulation is directed to setting functional activity of organs and systems into accord with demands [3]. However, the contribution of different anatomical substrates (the central nervous system, para-, prevertebral ganglia, intramural ganglia, and paraganglia) to sympathetic regulation remains unclear. Many pathological conditions caused by dysfunction of the sympathetic system manifest themselves at advanced age. Impaired metabolism of biogenic amines is implicated in the pathogenesis of schizophrenia, catatonia, and affective disorders. Abnormal content of monoamines deteriorates the integrative function and stimulates the development of pathology. Early sympathectomy in animals represents a convenient model for studying the changes in the postnatal ontogeny caused by alterations in the functional activity of the sympathetic nervous system.

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## MATERIALS AND METHODS

Male albino rats were studied at the age of 1 day, 2 and 4 weeks, and 6 and 24 months. Within the first two weeks after birth, the rats of the experimental group received subcutaneous injections of guanethidine (Isobarine, Pliva) in a dose of 20 mg/kg. The control group received saline injections at the same time.

Cryostat 15-μ sections were prepared for a fluorescent histochemical analysis by a modified Bjorklund's technique [6]. Cytospectrofluorimetry was performed using a LYUMAM-3 microscope with an FMEL-1A attachment. Serotonin and catecholamine fluorescence was measured at 524 and 481 nm, respectively, and quantified by a digital ShChH-300 device.

Specific receptor binding was assayed autoradiographically. To this end, the animals narcotized with Nembutal were perfused (through the left ventricle) with 0.5% glutaraldehyde in phosphate buffer. Cryostat sections (15 μ thick) were mounted on gelatin-coated slides, dried and incubated in buffer solution containing either $^3$H-quinuclidinyl benzilate ($^3$H-QNB),

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or $^3$H dihydroalprenolol ($^3$H-DHA), highly specific ligands of muscarinic cholinergic receptors and β-adrenoceptors, respectively. Unlabeled atropine and isoproterenol were used as competitors, when measuring the nonspecific binding. After incubation the sections were dried, covered with a tritium-sensitive (LM-1 emulsion) lavsan film, and exposed for 6 months at 4°C. The intensity of labeling was measured by optical densitometry using an Opton cytophotometer.

## RESULTS

In the cranial cervical ganglion of normal rats, single and grouped elements with intense fluorescence can be distinguished against the background neurocyte and neuropil fluorescence. The content of fluorophores varied considerably within the age groups, but tended to increase with age (Fig. 1).

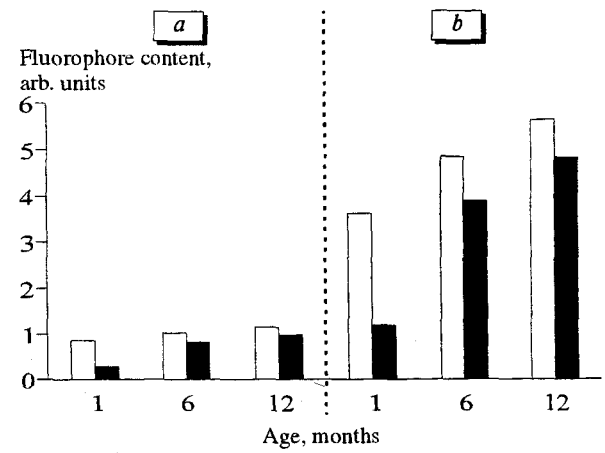


**Fig. 1.** Content of catecholamines (*a*) and serotonin (*b*) in sympathetic ganglion from control (open bars) and sympathectomized (filled bars) rats of different age.

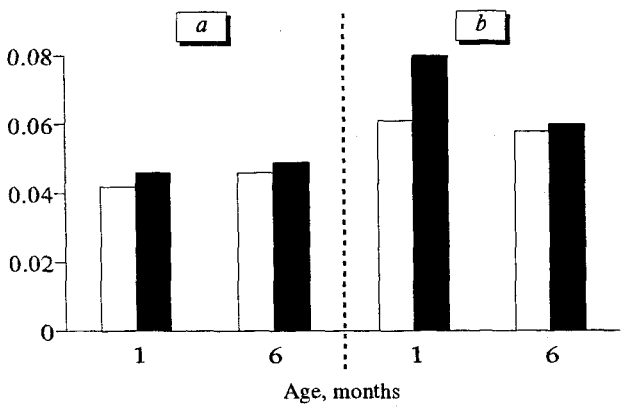


**Fig. 2.** $^3$H-Dihydroalprenolol (*a*) and $^3$H-quinuclidinyl benzilate (*b*) binding in sympathetic ganglion from control (open bars) and sympathectomized (filled bars) rats of different age. Ordinate: optical density of autograph, units.

Although the assessment of the absolute number of binding sites is a problem when using the autoradiography method, it makes it possible to assess the developmental changes in the receptor binding. The autoradiographic images of newborn rats showed low labeling, their density only slightly surpassed the background intensity. The developmental dynamics of binding was different for muscarinic cholinergic and β-adrenoceptors. At the age of 2 weeks the intensity of labeling with both ligands increased to the adult values: $^3$H-DHA binding increased to 0.039±0.002 *vs.* 0.010±0.003 on day 1 ($p<0.01$) and $^3$H-QNB binding to 0.056±0.003 *vs.* 0.009±0.002 in 1-day-old rats ($p<0.01$). Then $^3$H-QNB binding remained at the same level, while $^3$H-DHA binding continued to increase with further development and decreased only at the age of 24 months.

Early sympathectomy significantly decreased the content of fluorophores in the ganglionic tissue of 1-month-old rats ($p<0.01$). This index increased in the 6-month-old rats, but remained below the corresponding control. The difference between sympathectomized and control rats became insignificant only at the age of 12 months (Fig. 1).

In 1- and 6-month-old sympathectomized rats, $^3$H-DHA binding in the cranial cervical sympathetic ganglion was higher than in control animals, while $^3$H-QNB binding was similar in the two groups (Fig. 2).

The data indicate that the receptor structures for muscarinic cholinergic and β-adrenotransmission in the cranial cervical sympathetic ganglion are completely developed by the age of 1 month. This dynamics of the formation of these major receptor systems is also typical of other parts of the autonomic nervous system [14].

The decrease in noradrenaline content in the sympathetic ganglia of adult rats treated with guanetidine depends on the dose of drug and duration of treatment: daily injections for 63 days decreased the content of noradrenaline by more than 80%, while those for 14 days by approximately 25% [5]. In the cranial cervical sympathetic ganglion, noradrenaline is known to suppress the presynaptic release of acetylcholine [8]. It has been reported that noradrenaline activates both retrograde and anterograde axoplasmic transport via activation of $\beta_2$-adrenoceptors and lowering of the cAMP level [9]. Administration of α-methyl-paratyrosine, an inhibitor of catecholamine synthesis, on postnatal days 4 to 6 reduced the content of noradrenaline in 7-day-old rats by 21% and increased $^3$H-DHA binding by 19%; administration of corticosterone induced more dramatic changes (45% and 92%, respectively) [2]. The density of binding sites correlates with the content of mRNA, i. e. depends on gene transcription [7]. Sympathectomized animals showed an increased blood concentration of noradrenaline and

increased chromatin transcription activity in preserved neurocytes of sympathetic ganglia [5]. The increased number of receptors is likely to be a nonspecific compensatory reaction of desympathized tissue. In diabetic patients with severe pathology of the autonomic nervous system, the binding of β-adrenoceptor antagonists markedly increased due to high density of adipocyte β-adrenoceptors [10].

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