Response of Glandular Organs to Experimental Macroglossia

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Response of glandular organs to tongue enlargement was studied in 16 outbred male rats; each of them received injections of hydrophobic polyacrylamide gel (0.05 ml) in midline of the tongue. Changes in the studied glandular organs of external secretion (salivary glands) and internal secretion (thyroid and adrenal glands) were morphometrically detected.

Key Words: *tongue; macroglossia; glands; morphometry*

In recent decades, macroglossia increasingly attracted attention of researchers of different specialties. This is due to several aspects, among which pathophysiological (development of sleep apnea, poor articulation), anatomical, cosmetic, dental ones, *etc*. The polyetiological nature of this problem is also of interest [8,9,12].

True congenital macroglossia usually manifests in similar external features and developmental disorders of internal organs. First of all, these are changes in skull dimensions as well as nose, eyes, ears, lips and other changes in appearance. Developmental disorders of heart, kidneys, altered brain size and impaired endocrine function are also observed. At the same time, we have not found published reports on functional changes of endocrine organs in acquired macroglossia, which may be associated, for example, with type 2 diabetes mellitus, thyrotoxicosis, oncological and other diseases developing during postnatal period.

In light of this, experimental reaction of such rat glandular organs as adrenal glands, salivary and thyroid glands, on the changes in tongue volume is of particular interest.

Here we studied the impact of the experimental macroglossia modelled in laboratory animals on endo- and exocrine glands.

MATERIALS AND METHODS

The study was carried out on 32 outbred male rats weighing 120-160 g. The animals of experimental group (n=16) received single injections of 0.05 ml hydrophobic polyacrylamide gel into the tongue on both sides of the midline in the middle of the tongue length. Control group consisted of 16 rats. Within 4 months, the animals were kept on standard feed with free access to water. The rats were sacrificed in two stages after 2 and 4 months. After decapitation, the studied organs were removed as a whole. Halves of unpaired organs (fragment of the trachea with thyroid and parathyroid glands) and left paired organs (salivary glands, adrenal glands) were routinely fixed in 10% buffered formalin and embedded in paraffin; 5-μ slices were stained with hematoxylin and eosin. Morphometric study was carried out using program SigmaScanPro. After morphometry of the thyroid gland, follicle epithelium index (FEI) was calculated as a ratio of follicle diameter to thyrocyte height.

Second halves of unpaired organs and right paired organs were frozen and then 10 μ thick slices were cut. Activity of alkaline phosphatase (ALP) was detected by the method of Burstone [5], nonspecific esterase (NE), by simultaneous azo coupling with 1-naphthyl acetate and stabilized diazonium salts in the modification of Gomori [5]. Enzyme activity was evaluated by photometry in transmitted light on Micmed-2 microscope with photographic attachment type FMEL-1 and FEU-79 at the output voltage of the amplifier 1200 V and an interference filter with a maximum transmittance at λ=624 nm (for ALP). NE activity was assessed without the latter. Light transmission was recorded using a digital voltmeter Schch-4300 followed by taking the negative decimal logarithm;

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then light transmission was transformed into light absorption, which was expressed in optical density units (OD units) [1]. The content of catecholamines in the salivary gland was determined by gas paraformaldehyde luminescent histochemical method [10]. Catecholamines luminescence was measured spectrophotometrically in luminescence units using scale of voltmeter Schch-4300.

The obtained data were processed by routine statistical methods using the Microsoft Office Excel 2003 software. The arithmetic mean *M* and standard error of the mean *m* were calculated. Significance of intergroup differences was evaluated using Student's t test.

RESULTS

Microscopic structure of glands depended on the type of secretion (Fig. 1). Submandibular glands of rats, unlike human, consisted of two structural units clearly separated by the connective tissue septum, and were represented by different types of salivary glands. In the larger part (about $^{3}/_{4}$), numerous and well-developed intermediate and intralobular ducts lined with cuboidal epithelium, stained homogeneously and eosinophilic were found in contrast to the epithelium of the acini. This part had a structure typical of the complex alveolar-tubular serous gland. The part smaller by volume, in which intralobular ducts occurred in smaller amounts, had the structure typical for mucous glands.

After 2 months, the animals of experimental group tended to reduce the weight of the salivary gland in comparison with the control group (0.214±0.007 and 0.234±0.002 g% respectively). After 4 months, the weight of submandibular salivary gland in animals with macroglossia was significantly ($p<0.05$) reduced (0.194±0.004 g% against 0.221±0.006 g% in the control).

The volumes of the epithelial nuclei in different functional units of the salivary glands at different stages of the experiment are presented in Table 1.

Two months after the start of the experiment, epithelial nuclei of serous ducts in experimental animals began to decrease, while nuclei of mucous ducts, on the contrary, significantly increased in volume. By the 4th month of macroglossia, epithelial nuclei in acini and ducts of both parts of the gland were significantly reduced in comparison with the control.

Evaluation of ALP and NE activity in epithelial cells of different parts of salivary gland in macroglossia showed that ALP acticity in acini after 2 months was significantly higher than in the control (0.18±0.01 and 0.12±0.01 OD units respectively, $p<0.05$); after 4 months its activity in epithelial cells of serous part

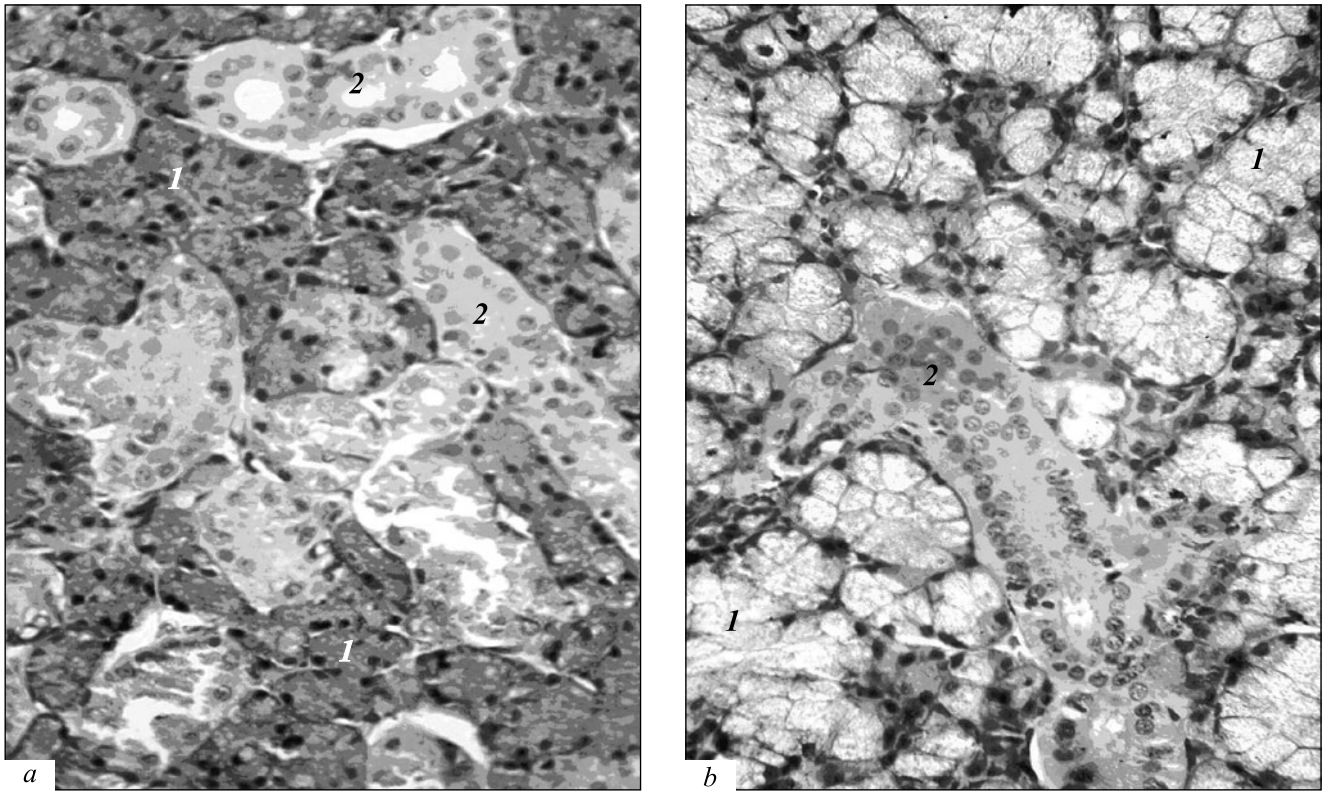

Fig. 1. Structures of submandibular salivary gland, ×100. *a*) serous part; *b*) mucous part. *1*) acinus; *2*) ducts. Here and in Figs. 2 and 3: hematoxylin and eosin staining

TABLE 1. Nucleus Volume (μ³) in Various Structures of Rat Salivary Gland (*M*±*m*)

Duration of the experiment	Group	Serous		Mucous	
		acinus	duct	acinus	duct
2 months	Control	46.53±2.70	52.07±3.27	35.84±1.63	43.86±1.46
	Experiment	46.36±2.00	47.49±2.00	39.69±3.16	59.65±3.05*
4 months	Control	45.61±3.19	52.91±2.45	36.48±1.47	46.17±2.98
	Experiment	34.22±4.53*	38.95±3.25*	26.79±2.15*	27.36±1.71*

Note. Here and in Tables 2, 3: *$p<0.05$ compared with control.

was significantly reduced 0.21±0.06 to 0.11±0.02 OD units ($p<0.05$).

NE activity in experimental animals was significantly increased in all structures of the salivary gland (except mucous acini) throughout the experiment. The intensity of catecholamine luminescence was also insignificantly increased in macroglossia.

According to the literature [6], morphofunctional changes observed in the gland at the end of the experiment can be regarded as signs of the reduction of secretory function and impaired passage of substances through cell membranes. Increased level of neurotransmitters of the sympathetic nervous system against the background of intensified destruction of acetylcholine could affect the quantity and viscosity of the submandibular gland secretion [4].

Microscopic examination of the thyroid gland showed that the tissue was divided into segments, consisting of rounded follicles of different sizes; colloid was located in their lumens (Fig. 2).

In compared groups small and medium-sized follicles lined with cuboidal or columnar epithelium predominated. Experimental animals more often showed large follicles located mainly at the periphery

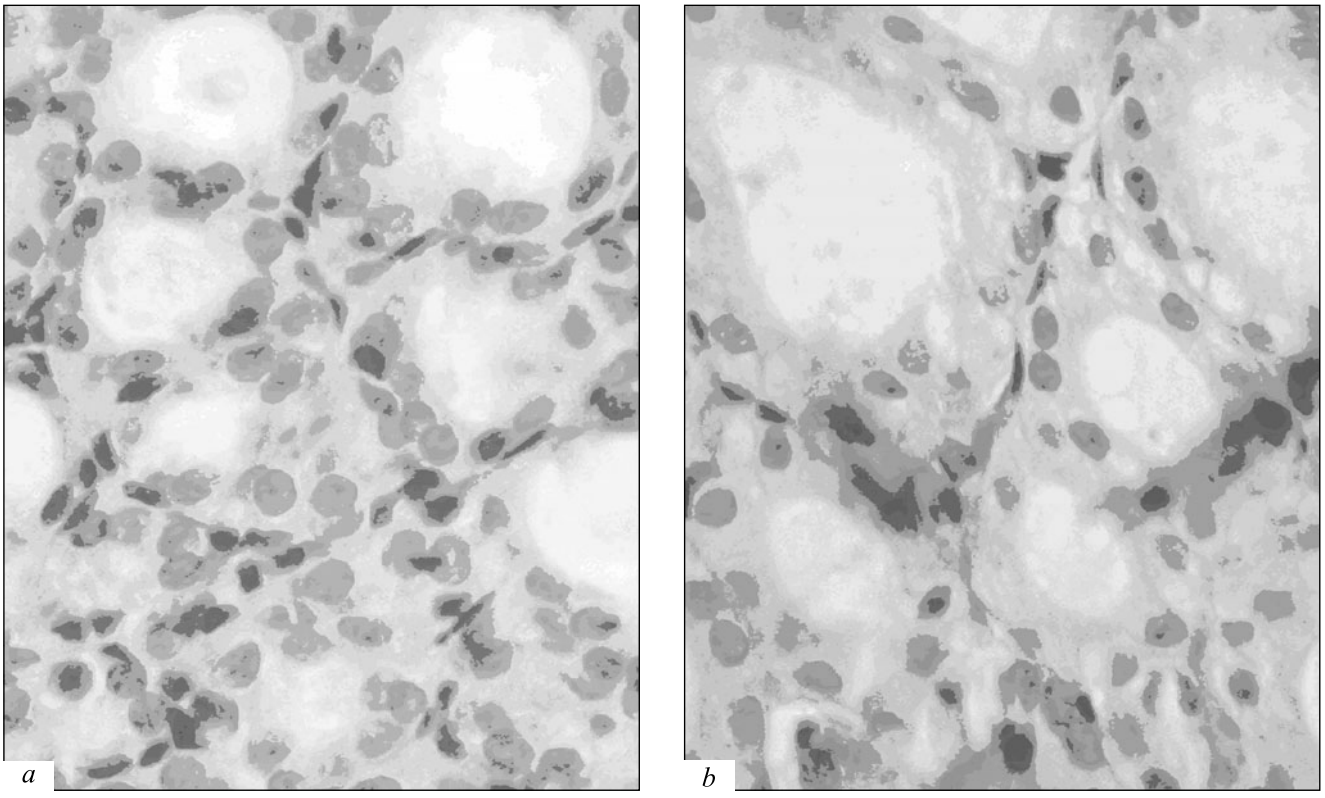

Fig. 2. Epithelial cell nuclear diameter in thyroid gland, ×40. *a*) control; *b*) experiment.

TABLE 2. Morphometric Parameters of the Thyroid Gland (μ)

Duration of the experiment	Group	Height of epithelium	Colloid diameter	Follicule diameter	FEI
2 months	Control	8.56±0.37	26.98±2.32	44.91±2.20	5.29±0.34
	Experiment	11.58±0.36*	35.47±2.71*	58.40±2.38*	5.07±0.28
4 months	Control	11.93±2.99	30.09±3.85	53.95±4.14	5.18±1.03
	Experiment	17.34±1.44	28.16±6.64	62.83±5.00	2.99±3.73

of the gland. Moreover, the colloid in the follicles, as a rule, was homogeneous and intensively stained with eosin.

The data on morphofunctional state of the gland are presented in Table 2.

In animals with macroglossia, epithelium height and diameter of colloid significantly increased after 2 months, with simultaneous enlargement of the follicular diameter. At the same time, FEI did not differ significantly from the controls. These results can indicate a trend toward increased functional activity of the thyroid gland, which at the time was determined by the balance of cumulative and endocrine processes. After 4 months, experimental animals demonstrated significant increase in the height of glandular epithelium, which resulted in significantly reduced FEI values. According to some authors [2,3], this suggests a functional stress of the thyroid gland.

Typical histological picture was observed on the transverse central sections of adrenal glands of control and experimental rats (Fig. 3).

The parameters of adrenal structures are shown in Table 3.

After 2 months, volumes of nuclei in widened zona reticularis decreased in experimental group ($p<0.05$) and in other areas tended to decrease. After 4 months, the volume of the nuclei decreased in all cortical areas, and the zona glomerulosa simultaneously expanded ($p<0.05$).

Thus, experimental tongue enlargement was accompanied by specific morphological changes in all the studied endo- and exocrine organs depending on

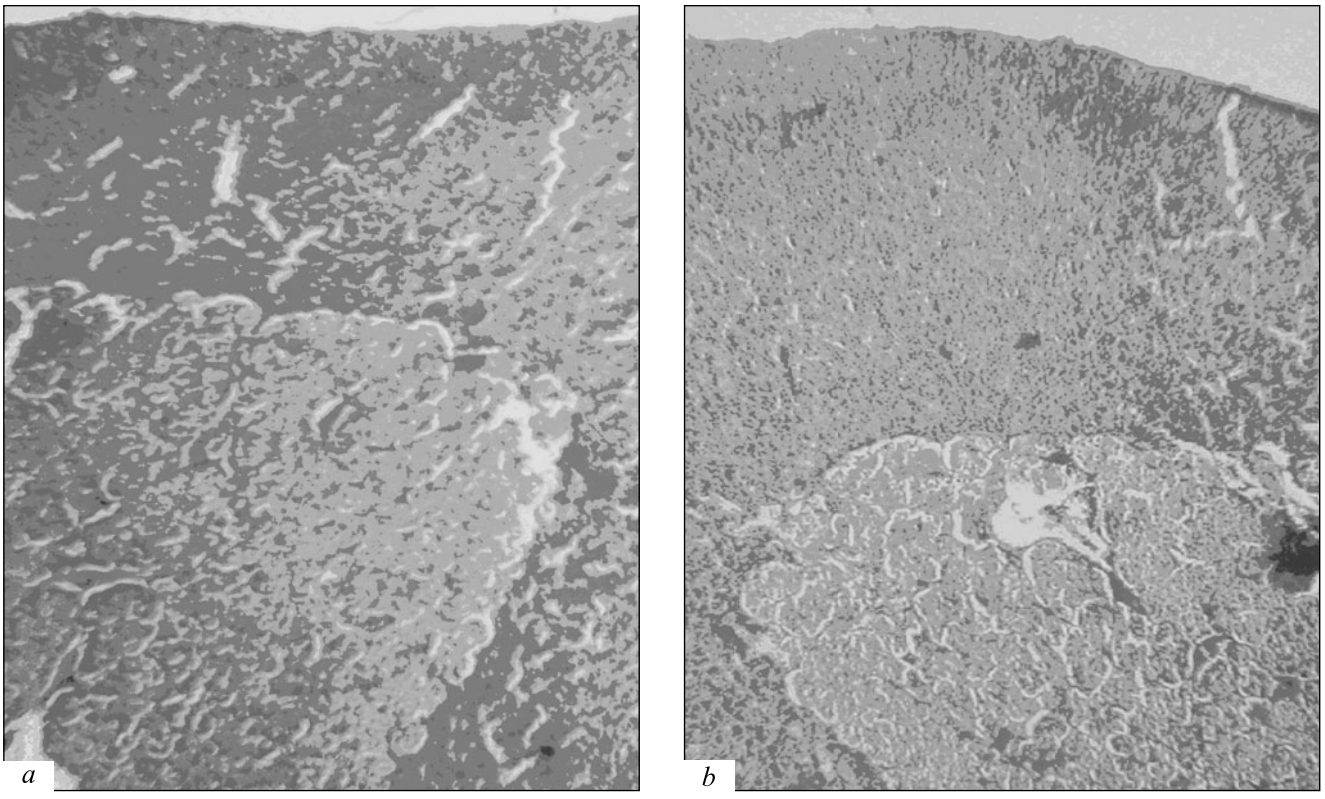

Fig. 3. Total section of adrenal glands, ×40. *a*) control; *b*) macroglossia.

TABLE 3. Morphometric Parameters of the Adrenal Cortex

Time of experiment	Group	Volume of nuclei (μ^3)			Width of the zones (μ)		
		zona glomerulosa	zona fasciculata	zona reticularis	zona glomerulosa	zona fasciculata	zona reticularis
2 months	Control	37.45±2.83	54.10±4.38	48.89±3.38	70.58±2.65	511.59±62.4	181.56±22.65
	Experiment	29.26±3.73	50.18±2.47	32.83±2.79*	75.51±8.57	555.06±14.2	244.89±6.55*
4 months	Control	59.11±3.28	97.37±6.73	84.67±6.84	133.61±4.45	616.68±47.87	337.31±18.09
	Experiment	36.05±2.72*	56.91±4.29*	53.59±4.04*	185.28±16.19*	621.62±90.74	325.33±38.60

the stage of the experiment. Given this and published data confirming the morphofunctional relationship of thyroid, salivary, and adrenal glands with the tongue size [7-9,11], we can assume that any surgical intervention in the tongue entails certain disorders of endo- and exocrine organs.

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