Effect of Tetrapeptides Lys-Glu-Asp-Gly and Ala-Glu-Asp-Gly on the Structure and Function of the Thyroid Gland in Neonatally Hypophysectomized Chickens

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Tetrapeptides Lys-Glu-Asp-Gly and Ala-Glu-Asp-Gly were synthesized on the basis of amino acid composition of pituitary cytomedins. Administration of these tetrapeptides to hypophysectomized chickens for 40 days was followed by an increase in the concentrations of thyrotropic hormone and thyroid hormones and recovery of thyroid gland structure.

Key Words: *pituitary gland; thyroid gland; tetrapeptides*

Hypophysectomy (HE) in birds at various stages of ontogeny leads to cellular and humoral immunodeficiency, hypercoagulation, and suppression of fibrinolysis [1-5]. These changes are accompanied by degenerative processes in central organs of cellular and humoral immunity (thymus and bursa of Fabricius). Tetrapeptides Lys-Glu-Asp-Gly (adenohypophyseal peptide, AHP) and Ala-Glu-Asp-Gly (neurohypophyseal peptide, NHP) were synthesized on the basis of amino acid composition of adenohypophyseal and neurohypophyseal cytomedins [7]. Administration of these tetrapeptides to hypophysectomized (HE) birds not only normalizes immune function and hemostasis, but also promotes structural recovery of the thymus and bursa [4,5]. It can be hypothesized that these tetrapeptides partially substitute for pituitary hormones or stimulate their production in cells and organs of HE birds.

The pituitary gland is the major regulator of thyroid function. HE is accompanied by the development of severe hypothyroidism.

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Here we studied the effects of AHP and NHP on the structure and function of the thyroid gland in HE birds.

MATERIALS AND METHODS

Experiments were performed on 60 KROSS-Rodonit 2 chickens over the first hours of life. The chickens were divided into 4 groups (n=12-15 in each group). Group 1 consisted of control chickens with sham-HE. HE chickens of group 2 received physiological saline. HE chickens of group 3 received AHP. HE chickens of group 4 received NHP.

The surgical field was treated with 70% ethanol. The basioccipital bone and sella turcica were punctured through the middle of the third palatine fold in ether-anesthetized birds. The needle was removed. Endoscopic forceps were introduced into the puncture channel to reach the sella turcica. The depth of forceps insertion was determined experimentally. The pituitary gland was cut off and removed. The bone defect was filled with sterile dental wax. Damage to the palate mucosa was treated with BF-6 medicinal glue and brilliant green. The quality of hypophysectomy was verified after sacrifice.

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Sham HE included all surgical manipulations except for removal of the pituitary gland. The birds received 2 mg levomycetin for 5 days after surgery. The living room was daily treated with a bactericidal lamp.

The test peptides in a daily dose of 0.1 mg/kg were injected intraperitoneally for 40 days starting from the 5th day of the study. The blood was sampled on day 45. The concentrations of pituitary thyrotropic hormone (TTH), thyroid triiodothyronine (T_3) and tetraiodothyronine (T_4), and free thyroxin (T-free) were measured by ELISA.

TTH concentration was measured using standard reagents Tiroid IFA-TTG-1 (AlkorBio). Histological study of the thyroid glands was performed routinely. Sections were stained with Ehrlich hematoxylin and eosin.

Samples of the thyroid gland were studied and photographed using an Olympus CX 31 microscope equipped with a JVC Color Video Camera (MEKOS-Ts software) [1]. Macro photos of these organs were obtained with a digital photo system. The width of the connective tissue capsule in the thyroid gland was measured in 10 fields of view. Serial sections were obtained from the histological sample of one animal.

The following morphological and morphometric parameters were studied: shape factor and mean area of follicles, number of follicles in the field of view, percent of large, medium, and small follicles, height of the thyroid epithelium, area of thyrocyte nuclei, nucleus/cytoplasm ratio, and amount of the follicular and interfollicular epithelium. The presence of resorption vacuoles and shape of thyroid epithelium were evaluated to estimate the consistency of colloid. The mean area of follicle cross-sections was calculated by means of MEKOS software.

The results were analyzed by Student's *t* test.

RESULTS

The concentrations of TTH and thyroid hormones in chickens sharply decreased on day 45 after neonatal HE: the level of TTH and T_3 decreased by 7 times, T_4 by 3.5 times, and T-free by more than 4 times (Table 1).

Administration of AHP was followed by an increase in the concentrations of TTH, T_3, T_4, and T-free (by 2.2, 2.5, 2.3, and 1.9 times, respectively). The concentrations of these hormones approached the control level.

After injection of NHP, the concentrations of TTH, T_3, and T_4 increased by 2 times on the 45th day of life; the concentration of T-free increased by 1.8 times.

The weight of the thyroid gland changed insignificantly after HE. However, this surgery led to significant morphological changes in the thyroid gland.

Under normal conditions, the thyroid parenchyma is of a mixed follicular type. It consists of follicles, interfollicular epithelial islets, and parafollicular cells (C cells). These cells are pituitary-independent. Therefore, C cells were not identified in the present work.

The shape factor of follicles approached 0.9. Hence, these follicles had round shape. Follicles were lined with a single-layer cubical epithelium. The height of this epithelium varied. The mean height was 6.72 μ. Polymorphic thyrocytes contained a large round nucleus with light nucleoplasm and nucleoli. The cell cytoplasm was oxyphilic and heterogeneous. The follicles were filled with oxyphilic colloid. It included a small number of resorption vacuoles that were localized above the apical pole of thyrocytes.

AHP considerably changed morphological characteristics of the thyroid gland in HE birds. The mean area of follicles decreased by 14 times compared to the control. This parameter in treated birds was 2.5-fold lower than in intact chickens (Table 2, Fig. 1). Microfollicles contained a small amount of oxyphilic colloid. Resorption vacuoles were identified at the colloid-epithelium boundary. Strong proliferation of interfollicular epithelial islets occurred

TABLE 1. Effects of AHP and NHP on Thyroid Function in HE Chickens ($M \pm m$)

Parameter	Group			
	1 (n=15)	2 (n=12)	3 (n=13)	4 (n=14)
Weight of thyroid gland, mg	79.5±1.5	60.5±1.9**	66.5±0.45^{+}	68.98±0.5^{+}
TTH, μU/liter	0.135±0.01	0.023±0.007**	0.05±0.01**$^{+}$	0.046±0.01**$^{+}$
T_3, nmol/liter	4.36±0.17	0.81±0.15**	2.00±0.13**$^{+++}$	1.65±0.27**$^{+++}$
T_4, nmol/liter	4.98±0.13	1.45±0.16**	3.33±0.21*$^{+++}$	2.90±0.35**$^{+++}$
T-free, pmol/liter	3.21±0.19	0.76±0.12**	1.51±0.17**$^{+++}$	1.4±0.13**$^{+++}$

Note. Here and in Table 2: *p<0.01 and **p<0.001 compared to group 1; ^{+}p<0.05, ^{++}p<0.01, and ^{+++}p<0.001 compared to group 2.

TABLE 2. Effects of AHP and NHP on Morphological Characteristics of Thyroid Gland in HE Chickens (*M*±*m*)

Parameter	Group			
	1 (*n*=15)	2 (*n*=12)	3 (*n*=13)	4 (*n*=14)
Weight of thyroid gland, mg	79.5±1.5	60.51±1.9*	68.98±0.5**	66.56±1.4**
%	100	75.9±2.3	86.1±2.4	83.5±3.1
Relative weight of the gland	0.028±0.0005	0.044±0.0014**	0.028±0.0002^{+++}	0.031±0.0002^{++}
%	100	157.4±5.2	100.2±2.8	110.7±1.9
Width of capsule, μ	94.32±3.1	75.45±2.6**	89.57±4.26**	86.52±3.6
%	100	79.7±2.3	94.6±2.5	91.4±2.1
Mean area of follicles, μ^2	932.2±43.2	5081.0±145.5**	410.70±22.2**$^{+++}$	360.6±34.1**$^{+++}$^{o}
%	100	590.1±2.8	43.9±3.1	38.6±1.8
Shape factor of follicles	0.83±0.054	0.67±0.043**	0.84±0.026^{++}	0.78±0.045^{++}
%	100	80.7±5.2	101.2±3.5	93.9±2.9
Height of epithelium, μ	6.70±0.08	1.25±0.008**	5.74±0.06*$^{+++}$	5.43±0.04*$^{+++}$
%	100	18.6±2.7	85.6±1.5	81.1±2.2
Nucleus/cytoplasm ratio	0.53±0.01	0.67±0.03*	0.56±0.03^{++}	0.61±0.02
%	100	126.4±3.1	105.6±2.1	108.9±1.8

Note. $^{o}p<0.01$ compared to group 3.

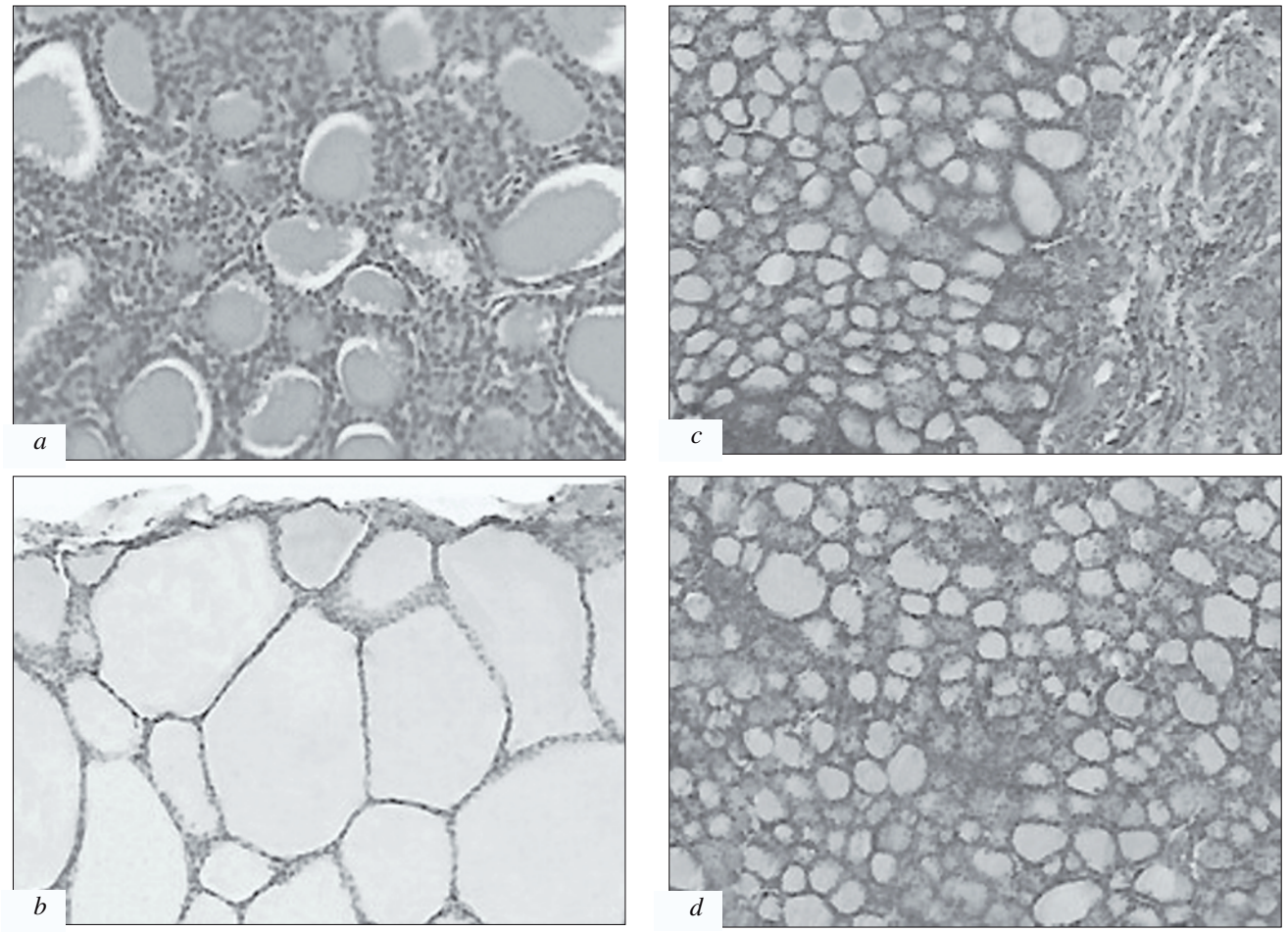

Fig. 1. Effect of tetrapeptides AHP and NHP on morphological characteristics of the thyroid gland in neonatally hypophysectomized chickens. Groups 1 (*a*), 2 (*b*), 3 (*c*), and 4 (*d*). Staining with Ehrlich hematoxylin and eosin, ×100.

between follicles. Large follicles were localized at the peripheral region of the thyroid lobules.

Proliferation of thyrocytes was followed by the formation of folds, protrusions and papillae. They were extended into the cavity of follicles. Besides this, the basal membrane was replaced in the interfollicular space by forming epithelial buds. These buds were separated from maternal follicles, which resulted in the formation of microfollicles. Thyroglobulin synthesis should occur in microfollicles. However, the concentration of thyroglobulin was low. Therefore, the size of microfollicles in these chickens was lower than in intact birds. The intensity of proliferation was maximum in the central area of lobules. Single medium and large follicles were found in the peripheral area. The follicular epithelium was cubical. The height of thyrocytes increased by 4.3 times. The nucleus/cytoplasm ratio decreased. The shape factor in treated specimens was similar to that in intact birds. The width of the thyroid capsule increased (Table 2, Fig. 1).

Similar morphological signs were observed after administration of NHP. Intrafollicular and interfollicular proliferation was accompanied by the formation of microfollicles and increase in interfollicular epithelial islets. The diameter of follicles decreased by 12 times compared to the control. The height of thyrocytes in NHP-treated birds approached that in intact specimens. The nucleus/cytoplasm ratio decreased. The capsule was thickened (Table 2, Fig. 1).

Our results indicate that administration of AHP and NHP was followed by recovery of the absolute weight of the thyroid gland and increase in intrafollicular and interfollicular proliferation. The size of follicles decreased, while the number of follicles in the field of view increased. Tetrapeptides increased the height of thyrocytes and decreased the nuclearus/cytoplasm ratio. These changes attest increased functional activity of the thyroid gland.

Pituitary hormones are produced in various cells and tissues. For example, activated lymphocytes and other immune cells secrete growth factors and various hormones (adrenocorticotropic hormone, somatotropic hormone, thyrotropic hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, chorionic gonadotropin, somatostatin, oxytocin, vasopressin, met-enkephalin, corticotropin-releasing hormone, somatoliberin, substance P, *etc.*) [6,8-10]. It can be hypothesized that the test peptides partially normalize the production of pituitary hormones by lymphocytes, thymocytes, macrophages, APUD cells, etc. This assumption is supported by our results that AHP and NHP increase the concentration of TTH in HE chickens. Moreover, AHP and NHP practically normalize body weight in birds. These changes are probably associated with restoration of the concentration of somatotropic hormone or its analogue.

AHP and NHP produced similar effect on morphological and functional characteristics of the thyroid gland, which was probably related to the same sequence of amino acid residues (Glu-Asp-Gly) in these tetrapeptides.

Our results are of great practical importance. Tetrapeptides AHP and NHP hold much promise for the therapy of diseases associated with hypofunction of the pituitary gland, thyroid gland, and other endocrine glands.

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