EFFECT OF THYROID HORMONES ON THE HISTOTOPOGRAPHY OF LECTIN RECEPTORS IN RAT SALIVARY GLANDS

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The use of lectins in histochemical investigations has made possible the selective demonstration of those carbohydrate-containing biopolymers of cells and tissues that are distinguished by terminal nonreducing carbohydrate residues [6]. Glycoconjugates of cell membrane surfaces, of intracellular secretory inclusions, and of extracellular structures, binding with lectin molecules, have been called lectin receptors. Recent investigations have demonstrated the marked effect of estrogens and progesterone on the histotopography of lectin receptors in organs of the female reproductive system [9, 11, 12], so that the use of lectins can be recommended to predict the effectiveness of hormone therapy of human breast tumors [9] and also for the early detection of pregnancy [11]. Because of their high content and the varied nature of glycoconjugates present in their composition, the salivaty glands are a convenient object for histochemical investigations with lectins [14]. The writers previously studied the histotopography of receptors of eight lectins, differing in their carbohydrate specificity, in the submandibular and sublingual salivary glands of rats at consecutive stages of postnatal development [13]. The aim of the present investigation was to use the submandibular salivary gland of rats as the test object with which to study the effect of hyper- and hypothyroidism on lectin receptor histotopography.

EXPERIMENTAL METHOD

Experiments were carried out 38 noninbred female albino rats weighing 180-220 g. The experimental animals were divided into three groups, with nine animals in each group. Exogenous hyperthyroidism was induced in the rats of group 1 by feeding them daily with a dry thyroid preparation in a dose of 150 mg/kg body weight with the food for 40 days. Animals of group 2 (hypothyroidism) received the thyrostatic agent methimazole in a dose of 3 mg/kg body weight with the food for 30-35 days. Both preparations were products of the Soviet pharmaceutic industry (Thyroidin and Merkazolyl). Experimental group 3 consisted of animals after subtotal thyroidectomy. Changes in thyroid hormone levels were judged by determination of the animals' gas exchange and body weight. The schemes of administration of the preparations indicated above ensured the development of persistent hyper- or hypothyroidism for 15-17 days after the beginning of feeding [2]. The submandibular salivary glands were removed after decapitation of the animals, fixed for 12 h in Bouin's mixture, and embedded in paraffin wax. Since the content of secretory granules in cells of the granular efferent duct may undergo cyclic fluctuations depending on the phase of the estrous cycle [1], material was sampled in the stage of metestrus. Sections were treated with the following lectins: peanut agglutinin (PNA, specific for D-galactose), wheat germ agglutinin (WGA, specific for N-acetyl-D-glucosamine and sialic acid), and *Laburnum anagyroides* lectin (LAL, specific for L-fucose), obtained from Soviet raw materials by the appropriate methods* [4, 5, 8], and also with concanavalin A (con A, specific for D-mannose and D-glucose), obtained from Sigma (USA). After dewaxing, the

*Conjugates of peanut and wheat germ agglutinins and LAL with horseradish peroxidase, and also the purified horseradish peroxidase for indirect demonstration of concanavalin A were obtained from the L'vov branch of the A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR.

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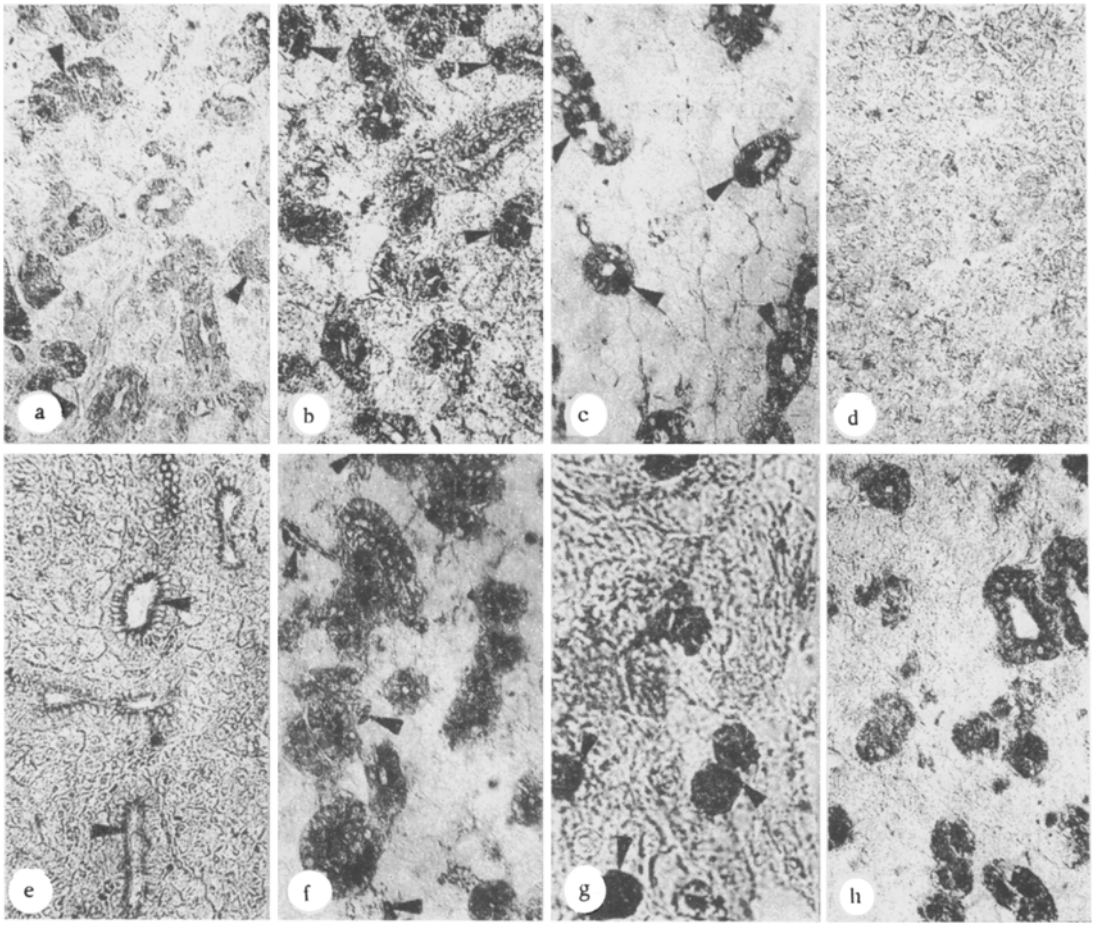

Fig. 1. Histotopography of lectin receptors in submandibular salivary gland of intact rats and of rats with hypo- and hyperthyroidism. a) Gland of intact rat. Moderately positive reaction of epithelium of granular and striated efferent ducts with con A. Cytoplasmic granules visible (arrow) in cells of granular ducts. Treatment with con A and HRP. 180 ×; b) Hypothyroidism due to administration of methimazole. Increased intensity of staining of granular efferent ducts, appearance of numerous cells giving strongly positive reaction with con A (arrows). Treatment with con A and HRP. 180 ×; c) Hypothyroidism induced by subtotal thyroidectomy. Marked intensification of staining of epithelium of granular ducts superposed on complete areactivity of cells of the terminal secretory portions. Individual cells of the granular duct exhibit very high affinity for con A (arrows). Treatment with con A and HRP. 180 ×; d) Hyperthyroidism. Complete areactivity of gland parenchyma. Treatment with con A and HRP. 180 ×; e) Gland of intact rat. Distinct outlines of luminal surface of efferent duct visible (arrows) after treatment with peanut agglutinin, conjugated with HRP. 180 ×; f) Hypothyroidism induced by administration of methimazole. Sharp increase in number of PNA receptors in cells of granular and striated efferent ducts, redistribution of PNA receptors from luminal surface into cytoplasm of cells. Increase in number of tissue basophils (arrows) in connective-tissue stroma of gland. Treatment with PNA conjugated with HRP. 180 ×; g) Detail reaction with PNA. Treatment with PNA, conjugated with HRP. 690 ×; h) Gland of intact rat. Intense staining of epithelium of efferent ducts superposed on complete areactivity of cells of terminal secretory portions. Treatment with LAL, conjugated with HRP. 180 ×.

sections were incubated for 30 min in methanol containing 0.3% H_2O_2 to inactivate endogenous peroxidase, and taken through alcohols of decreasing concentration to buffered physiological saline, pH 7.4. The lectins were labeled with horseradish peroxidase (HRP) and lectin receptors subsequently visualized with the aid of diaminobenzidine tetrahydrochloride as described previously [3].

EXPERIMENTAL RESULTS

On treatment of sections through the submandibular salivary glands of animals of the control group a marked affinity of con A for epitheliocytes of the granular and striated efferent ducts was observed against the background of total areactivity of the remaining structural components of the gland parenchyma (Fig. 1a). The plasmalemma of the epitheliocytes of the efferent ducts was distinctly outlined and the cytoplasm of the cells gave a positive reaction with con A; cytoplasmic granules were found in many cells of the granular efferent ducts. After administrationof dried thyroid total areactivity of the gland parenchyma with con A was observed (Fig. 1d), evidence of disappearance of the receptors of this lectin. In hypothyroidism caused by administration of methimazole, increased reactivity of the cells of the striated and granular efferent ducts with con A was observed (Fig. 1b). Many cells giving a strongly positive reaction with con A appeared in the composition of the granular efferent ducts. Similar changes, but rather more marked, also were observed in hypothyroidism due to thyroidectomy (Fig. 1c).

Under normal conditions PNA receptors are concentrated on the luminal surface of the epithelium of the efferent ducts (Fig. 1e). In hyper- and hypothyroidism concentration of PNA receptors was observed both on the luminal surface of the efferent duct cells and in the composition of their cytoplasm (Fig. 1f). In hypothyroidism caused by thyroidectomy, weak homogeneous staining of cells of the terminal secretory portions also was observed. The increase in the number of PNA receptors was more marked in hyperthyroidism than in hypothyroidism. Against the background of areactive cells of the connective-tissue stroma of the gland, tissue basophils binding PNA intensively were clearly visible (Fig. 1f, g). Characteristically both in hyperthyroidism and in hypothyroidism due to administration of methimasole, the number of tissue basophils was increased four to sixfold.

Under normal conditions WGA binds homogeneously with the tissue components of the gland, with some outlining of the luminal surface of the striated duct and cells of the serous demilunes. In hyper- and hypothyroidism a reduction of the intensity of staining of the sections could be seen, without any appreciable changes in WGA receptor topography.

Normally LAL exhibits marked affinity for epitheliocytes of the efferent ducts of salivary glands (Fig. 1h). Reactivity of the epithelium of the efferent ducts to LAL was sharply depressed in hyperthyroidism and weak homogeneous staining of the gland parenchyma was found. In hypothyroidism the number of LAL receptors in the composition of the epithelium of the efferent ducts also was appreciably reduced, although it still remained higher than in cells of the terminal secretory portions.

The investigations showed that hyperthyroidism is accompanied by a sharp decrease in the number of receptors of all the lectins used except PNA, whose receptors increased appreciably in number. On the basis of the carbohydrate specificity of the lectins it can be postulated that hyperthyroidism causes accumulation of glycoconjugates with terminal residues of D-galactose in the cells, and this is accompanied by a decrease in the content of glycoconjugates containing D-mannose, D-glucose, N-acetyl-D-glucosamine, sialic (N-acetyl-neuraminic) acid, and L-fucose as terminal residues. A similar pattern of lectin receptor distribution is observed in the submandibular gland of newborn rats [13] and has also been observed by many investigators in other embryonic or malignant tissues [10]. This phenomenon is evidently based on the disturbance of the final stages of biosynthesis of carbohydrate-containing biopolymers in the cell, consisting of blocking of the addition of sialic acid residues (of D-mannose, D-glucose, N-acetyl-D-glucosamine, and L-fucose) to D-galactose residues.

Interpretation of the results characterizing the state of hypothyroidism is difficult. In view of the marked affinity of con A for aldehyde-fuchsinophilic granules of the granular efferent duct (whose cells perform an endocrine function [1]), it can be postulated that hypothyroidism is accompanied by delay of elimination of biologically active substances from the cells. Similar inhibition of exocytosis in hypothyroidism has been described for the B-insulinocytes of the pancreas [7]. The increase in the number of PNA receptors, with a simultaneous decrease in the number of WGA and LAL receptors, evidently reflects a process of destabilization of the terminal stages of glycoconjugate biosynthesis, which was observed in hyperthyroidism also. The patterns of redistribution of lectin receptors discovered in the tissues against the background of an overabundance or deficiency of thyroid hormones must be taken into account in subsequent research involving the use of lectins as histochemical reagents.

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EFFECT OF REMOVAL OF THE MATERNAL LUNG ON DEVELOPMENT OF THE FETAL LUNG IN RATS

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Pathology of an organ in a pregnant animal is known to disturb the development mainly of the homonymous organ of the fetus, although at the same time it leads to developmental disturbances of the other organs [1-6, 8]. There have been few experimental investigations of interaction between maternal and fetal lungs [7], and no information has been published concerning the structural changes taking place in the fetal lung in the case of a deficiency of lung tissue in the mother.

The aim of this investigation was accordingly to study the structural features of the lungs in fetuses obtained from unilaterally pneumonectomized rats.

EXPERIMENTAL METHOD

The lungs of Wistar rat fetuses were studied on the 18th and 21st days of intrauterine development. At each time in the experiment lungs were obtained from 10 fetuses — two each from the litter of a pneumonectomized mother The operation was performed on the 9th day of pregnancy. Lungs of the same number of fetuses from five litters of intact rats were used as the control. In all the experiments tissue of the left fetal lung was studied histologically and electron-microscopically. The lungs were fixed in a 10% neutral formalin solution by Lillie's method and embedded in paraffin wax. Sections were stained with hematoxylin and eosin. Under the light microscope, photographs were taken of histological preparations under

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