Alterations in carbohydrate determinants in rat adrenal gland following experimental hypothyroidism

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SUMMARY

Thyroid disorders are currently among the most widespread endocrine pathologies, affecting about 3% of the world's population. Although the thyroid gland interacts with other endocrine organs, including the pituitary and adrenals, the many details of these feedback mechanisms remain obscure. In the relevant literature, no data concerning hypothyroidism-induced remodelling of adrenal gland glycoconjugates were found. Therefore, the aim of the present investigation was to study the effects of experimental hypothyroidism on exposure of glycoepitopes in rat adrenal glands by means of lectin histochemistry. Hypothyroidism was induced by daily diet supplementation of experimental animals with 5 mg/kg mercazolil (1-methyl-2-mercapto-imidazole) for 30 days. Formalin-fixed, paraffin-embedded adrenal glands were labelled by lectin-peroxidase conjugates, with subsequent visualization by diaminobenzidine-tetrahydrochloride. The lectin panel included 12 lectins with different carbohydrate affinities (Con A, PSA, LCA, GNA, PFA, LABA, SNA, RCA, WGA, PNA, SBA, HPA).

The most significant effects of hypothyroidism were detected in blood vessels. They included dilation of the adrenal medulla vascular bed, perivascular oedema, and increased LABA reactivity of the vascular endothelium of both the cortex and medulla. Hypothyroidism induced decreased exposure of αDMan/αLFuc with simultaneous accumulation of βDGal/ DGalNAc sugar determinants within the cells of the adrenal parenchyma; this phenomenon apparently was dependent on incomplete glycosylation patterns – i.e. impairments in the processing of oligomannosidic type N-glycans and of fucose-containing glycoconjugates. There was also an increased count of spider-like cells with strongly lectin-reactive cytoplasmic granularity in the cortical region of the adrenal glands, presumably due to hypothyroidism-induced uncoupling of biosynthesis and secretion, with subsequent retention of bioactive compounds within these cells. It can be concluded that hypothyroidism has significant effects on adrenal gland glycoconjugates, inducing decreased αDMan/αLFuc and enhanced βDGal/ DGalNAc determinant exposure, accompanied by an imbalance in the synthesis and secretion of physiologically active substances.

Key words: Adrenal gland – Rat – Experimental hypothyroidism – Lectin histochemistry

INTRODUCTION

Thyroid disorders are currently among the most widespread endocrine pathologies, affecting about 3% of the world's population (Braverman, 1997;

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Pankiv, 2011). In Ukraine over the past 20 years, the incidence of thyroid-related pathologies, predominantly due to Chornobyl accident contaminants, has increased three-fold. In addition, the population of the Carpathian region, living in an area where iodine deficiency is endemic, requires special care (Pankiv, 2006, 2011).

Clinically obvious hypothyroidism affects 1.5-6% of the adult population, while its sub-clinical forms can affect as many as 4-12% of the population, predominantly affecting women and the elderly (Stechenko et al., 2008; Pankiv, 2011). Hypothyroidism is correlated with significant metabolic disorders, including decreased rates of protein synthesis and degradation, impairments of erythropoiesis, water and electrolyte balance, and glycosaminoglycan turnover, accumulation of mucin-type glycoproteins, hyaluronic acid, and chondroitinsulphates, which, due to an extremely high hydration ratio, cause "mucinous" oedema of organ tissues, as well as ascites, pericardial effusions, and pleural effusions (Stechenko et al., 2008; Prystupjuk, 2011).

The thyroid gland functions are intimately related with those of other endocrine organs, primarily the pituitary and adrenal glands (Braverman, 1997; Balabolkin et al., 2007; Pankiv, 2011). In particular, L-tyrosine, being involved in thyroxin synthesis, simultaneously serves as a precursor in the biosynthesis of noradrenaline and adrenaline (Levite, 2012). Despite the well-documented correlation between hypothyroidism and hypocorticism, as well as its inhibitory effect on catecholamine production (Stechenko et al., 2008; Prystupjuk, 2011), many details of the mechanisms of effects of the thyroid on the adrenal gland remain obscure.

Glycans, by virtue of their mass, shape, charge, or other physical properties, can mediate a wide variety of specific physiological or pathological processes (Gabius, 2009; Varki et al., 2009). Lectins, which are carbohydrate binding proteins, are among the most sensitive probes for testing histopathological changes of organ tissues (Sharon, 2007; Bilyy et al., 2009; Roth, 2011). Since the relevant literature lacks information reflecting the effect of thyroid hormone imbalance on the glycome of the adrenal gland, the aim of the present investigation was to use a set of lectins with different carbohydrate affinities for the histochemical investigation of the effects of experimental hypothyroidism on tissue glycoconjugates of the rat adrenal gland.

MATERIALS AND METHODS

Animals

Hypothyroidism was induced in 10 male Wistar rats weighting 150-180 g (12-15 months of age) by supplementation of their daily food allowance with 5 mg/kg of the antithyroid drug mercazolil (1-methyl-2-mercapto-imidazole, Zdorovja, Kharkiv, Ukraine) for 30 days. Control rats (n=10) were maintained in the same conditions on a standard diet. The investigation was carried out according to the ethical criteria for the use and handling of laboratory animals established by Lviv National Medical University in accordance with the "General ethical principles on experiments with animals" of the 1st National Congress on Bioethics (Kyiv, 2001), and in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (1996).

Tissue specimens and lectin histochemistry

Animals were sacrificed by diethyl ether narcosis overdose with subsequent decapitation. The thyroid and adrenal glands were removed immediately, fixed in 4% neutral formalin, and embedded in paraffin wax according to the standard protocol. For general morphology studies, 5- to 7-µm-thick sections were stained with haematoxylin and eo-

Table 1. Lectins and their respective carbohydrate specificities

№	Lectin designation, abbreviation	Specific monosaccharide	Complementary oligosaccharide/ polysaccharide
1.	*Canavalia ensiformis agglutinin*, Con A	αDMan>αDGlc	Man (α1-2)Man(α1-2)Man in N-glycans, non-fucosylated
2.	*Pisum sativum agglutinin*, PSA	αDMan/ αDGlc	Man(α1-3)Man in N-glycans, fucosylated
3.	*Lens culinaris agglutinin*, LCA	αDMan/ αDGlc	GlcNAc-Oligomannose core of N-glycans
4.	*Galanthus nivalis agglutinin*, GNA	αDMan	Man(α1-3)Man, oligomannoside N-glycans
5.	*Perca fluviatilis agglutinin*, PFA	αLFuc	Fuc(β1-2)Gal(β1-3)GlcNAc
6.	*Laburnum anagyroides bark agglutinin*, LABA*	LFuc	Gal(β1-4)Fuc(β1-3)Glc
7.	*Sambucus nigra agglutinin*, SNA	NeuNAc(α2-6)DGal	NeuNAc(α2-6)Gal(β1-4)GlcNAc(β1-2)
8.	*Ricinus communis agglutinin*, RCA	βDGal>NeuNAc	NeuNAc(2-6)Gal(β1-4)GlcNAc
9.	*Wheat germ agglutinin*, WGA	DGlcNAc>NeuNAc	NeuNAc(α2-6)Gal(β1-4)GlcNAc, Man(β1-4) GlcNAc(β1-4)GlcNAc
10.	*Peanut agglutinin*, PNA	DGal	DGal(β1-3)GalNAc
11.	*Soybean agglutinin*, SBA	αDGalNAc> βDGalNAc	GalNAc(α1-3)Gal(β1-3)GalNAc
12.	*Helix pomatia agglutinin*, HPA	αDGalNAc	GalNAc(α1-3)GalNAc

*The preparation and characteristics of the original fucose-specific lectin from Laburnum anagyroides bark was described by Lutsyk and Antonyuk (1982).

sin. Lectin histochemistry investigations were conducted using the peroxidase-diaminobenzidine visualization protocol as described elsewhere (Lutsyk and Sogomonian, 2012; Yashchenko et al., 2012). The lectin panel is presented in Table 1. All lectins used and their peroxidase conjugates were prepared by V. Antonyuk (Lectinotest, Lviv, Ukraine).

Densitometric analysis

Microscopic examinations were performed using a Delta Optical microscope equipped with a Delta Optical (Pro-Micro Scan 5822) digital camera. Microphotographs taken at objective magnification 40× were subjected to densitometric analysis. For this purpose, 20 fields of 1000 square μm each, located subcapsularly at a depth of 75-500 μm from the organ surface, were selected. Integrated optical density (IOD) was estimated using Image-Pro Plus and ImageJ software. For each lectin, 60 fields from three different microphotographs were analysed; background staining was subtracted from the calculated values. Statistical significance was assessed using Student's t-test. Three levels of significance are depicted with asterisks (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

RESULTS

Thyroid specimens were examined morphologically to confirm the induction of hypothyroidism. In particular, mercazolil treatment was accompanied with a so-called "strumogenic reaction", i.e. the thyroid gland was enlarged 2-3 times in comparison with control rats; microscopically, the thyroid follicles of experimental animals developed an irregular shape with prominent microfolds and had a decreased amount of or completely lacked colloid; and the thyroid epithelium, which was cuboidal in control rats, became columnar with certain signs of hyperplasia (Fig. 1).

Haematoxylin and eosin staining of the adrenal gland showed that hypothyroidism was associated with vasodilatation, predominantly within the medullary region, as well as with perivascular oedema and increased parenchymal basophilia. Many cells showed signs of karyopyknosis and an enhanced number of cytoplasmic vacuoles (Fig. 2).

Lectin histochemistry

In general, there was relatively faint reactivity of the adrenal parenchyma of both control and hypothyroid rats with all lectins used; we refer to this phenomenon as partial loss of lectin receptor sites during formalin fixation and tissue processing. The only exception concerned substantial labelling of the capsule and the superficial layer of the zona glomerulosa, which can be treated as a non-specific, post-fixative artefact.

Of all of the lectins used, the greatest hypothyroidism-related changes in glycoconjugates were detected with LABA, PNA, and SBA. In particular, LABA reactivity in control rats was restricted to the vascular endothelium of the adrenal medulla, while under experimental hypothyroidism, this same lectin also strongly labelled the vascular endothelium of the adrenal cortex (Fig. 3).

It is noteworthy that PFA, another fucose-specific lectin used in this study, did not react with the vascular endothelium; hypothyroidism induced a sig-
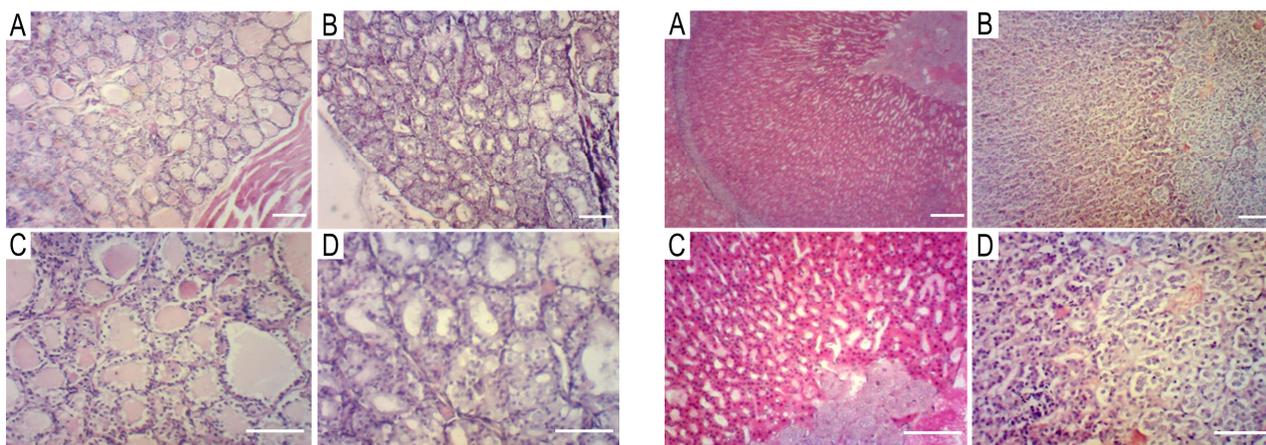

Fig. 1 (left). Thyroid gland of control (A,C), and mercazolil-treated rats (B,D). Thyroid follicles of experimental animals of irregular shape with prominent microfolds, consist of decreased amount of colloid; thyroid epithelium, cuboidal in control rats, has become columnar. Haematoxylin-eosin, ×60 (A,B), ×150 (C,D). Bar, 30 μm.

Fig. 2 (right). Adrenal gland of control (A,C), and hypothyroid (B,D) rats. Hypothyroidism has induced vasodilatation within the medullary region, as well as perivascular oedema and increased parenchymal basophilia. Haematoxylin-eosin, ×60 (A,B), ×150 (C,D). Bar, 30 μm.
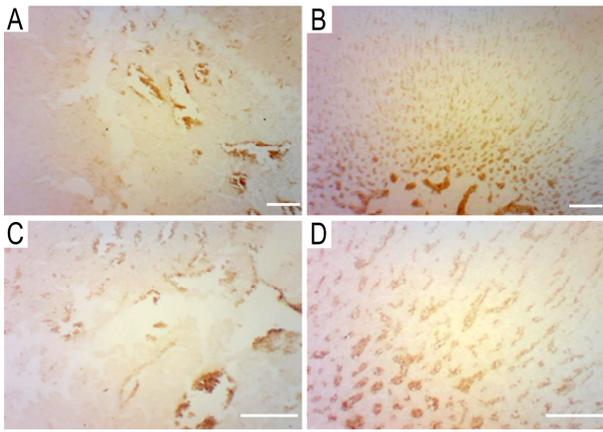
Fig. 3. Adrenal gland vascular bed labelled with fucose-specific Laburnum anagyroides bark lectin (LABA). Enhanced reactivity of vascular endothelium of hypothyroid rats (B,D) in comparison to control rats (A,C). ×60 (A,B), ×150 (C,D). Bar, 30 μm.
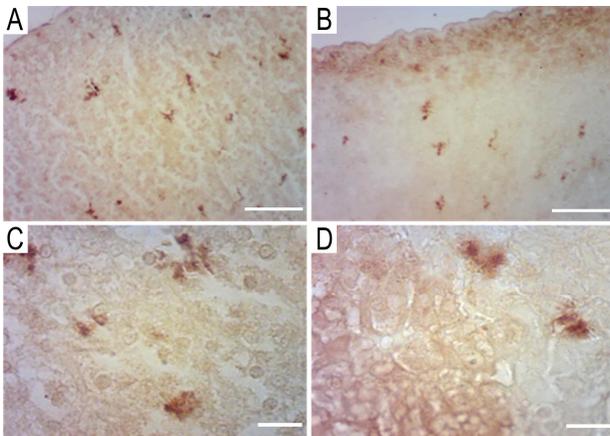

Fig. 4. Binding of a galactose-specific PNA with parenchyma of normal (A,C), and hypothyroid (B,D) adrenal gland. Evidence of spider-like structures within the cortex (A,B), enhanced reactivity of hypothyroid rat zona glomerulosa and medullary cells (C,D). ×150 (A,B), ×600 (C,D). Bar, 30 μm (A,B), 10 μm (C,D).
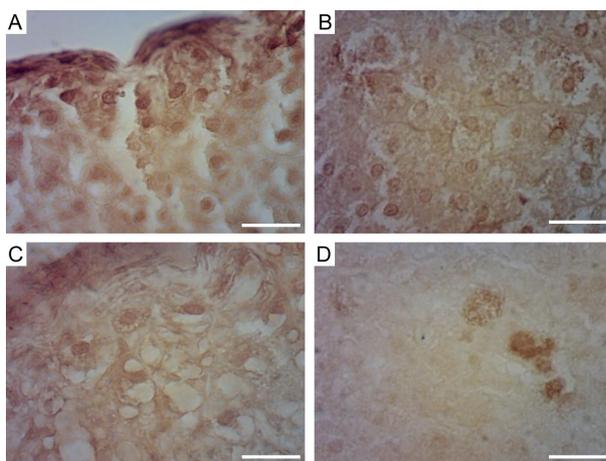

Fig. 5. Reactivity of the mannose-specific lectins GNA (A,B) and PSA (C,D) with adrenals of control (A,C), and hypothyroid (B,D) rats. Decreased exposure of DMan determinants in hypothyroidism, with the appearance of isolated cells with strong PSA-reactive cytoplasmic granularity (D). ×600. Bar, 10 μm.

nificant reduction of this lectin's labelling of the adrenal parenchyma. On the other hand, PNA and SBA reactivity of zona glomerulosa cells was enhanced in hypothyroidism (Fig. 4).

By means of PNA, SBA, and PFA, as well as of the mannose-specific lectins, within the adrenal cortex were found specific spider-like cells with prominent cytoplasmic granularity, the number of which was increased in hypothyroidism. We refer to these cells as hormone-producing cells overloaded with secretive granules, apparently indicating hypothyroidism-induced uncoupling of biosynthesis and secretion, with subsequent retention of bioactive compounds within these cells (Figs. 4, 5). Under experimental conditions, decreased exposure of αDMan determinants, accompanied by the accumulation of isolated cells with prominent cytoplasmic granularity, was also seen (Fig. 5).

Comparison of four mannose-specific lectins (GNA, PSA, LCA, Con A), despite the existence of certain differences in their fine carbohydrate specificities, revealed no differences in the histotopography of these lectin receptor sites within adrenal gland components. However, densitometric analysis demonstrated that the hypothyroidism-induced level of LCA receptors was completely different from that of GNA, PSA, and Con A receptors (Fig. 6), being greatly increased at hypothyroid state.

While measuring the optical densities of the histological slides, the most intensive signal was detected after PFA, HPA, and GNA labelling of tissue specimens in both control and experimental animals, and in hypothyroid rats it was detected after LCA, WGA, HPA, PFA, GNA, and SBA labelling; minimal optical density was detected with Con A and PNA. Hypothyroid rats showed significantly decreased PFA, GNA, PSA, MAA, PNA, and Con A labelling, while LCA and WGA reactivity was significantly higher in experimental animals. This phenomenon needs further investigation. LABA and HPA showed no difference in binding to control and hypothyroid tissues.

DISCUSSION

The present results are in good agreement with previous studies (Sasano et al., 1989; Ahi et al., 2007) reporting low affinity of both mannose- and fucose-specific lectins to adrenal cortical parenchyma. However, strong labelling of the adrenal medulla with SBA, which was documented by the latter authors, was not seen in the present study, and these discrepancies may depend on differences in fixative protocols, as well as on the age of experimental animals.

From our recent findings on reciprocal tendencies in the rearrangement of DMan/LFuc, and DGal/DGalNAc determinants within the adrenal parenchyma, it can be hypothesized that hypothy-
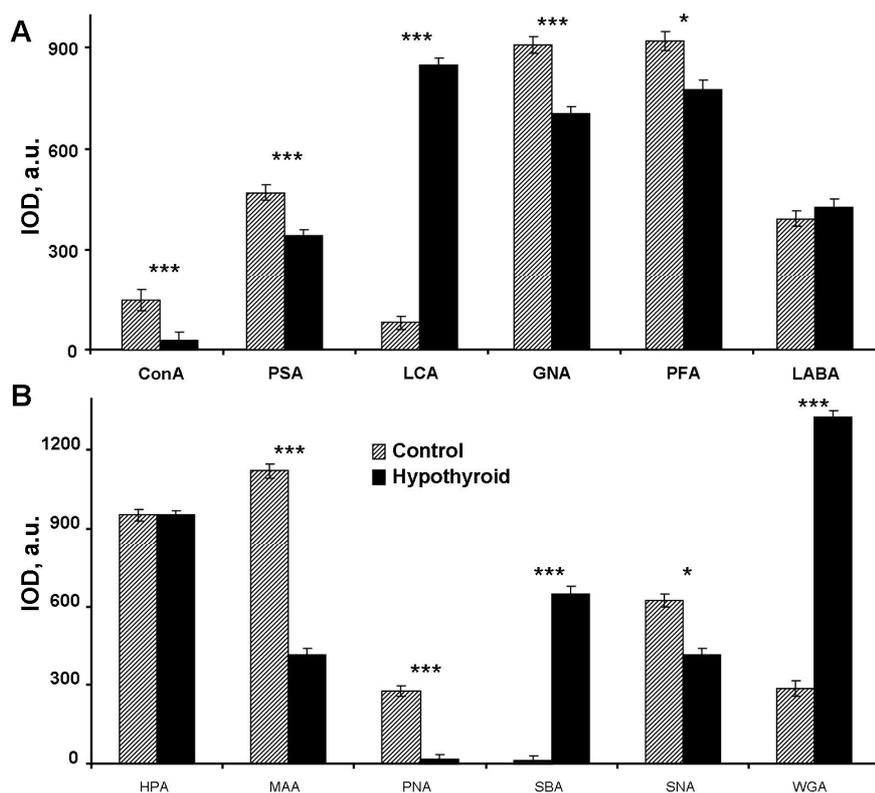
Fig. 6. Optic density of lectin-reactive glycoconjugates in adrenal gland samples of control and hypothyroid rats. * p < 0.05; *** p < 0.001.

roidism-induced impairments of carbohydrates include unmasking of DGal and DGalNAc residues due to incomplete final glycosylation patterns of oligomannosidic N-glycans, as well as of fucose-containing glycoconjugates, the primary structure of which has not yet been deciphered. It is likely that the prominent redistribution of lectin receptor sites in the adrenal cortex revealed in our studies reflects histochemical manifestations of the developing hypothyroidism-induced cortical insufficiency (Prystupjuk, 2011).

It can also be speculated that the increased count of cortical cells with strong lectin-reactive cytoplasmic granularity is due to an imbalance in the biosynthesis, accumulation, and secretion by these cells of biologically active substances. This notion is supported by the observations of others who detected the accumulation of RCA, WGA, and Con A-reactive cytoplasmic granularity within human adrenal gland cells manifesting hypercorticism (Sasano et al., 1989) and PNA-positive granularity in pheochromocytomas (Moorghen and Carpenter, 1991).

Our recent results on the selective LABA binding to vascular endothelium of adrenal gland support the observations of Sokurenko and Chaikovsky (2012), who reported this lectin reactivity with spinal cord vasculature. However, the present findings are somewhat different from those Hrytsevych et al. (2012) on the intensive labelling of rat oe-

sophageal vascular endothelium with mannose-specific GNA, LCA, PSA, and Con A. These discrepancies probably rely on organ-dependent cellular heterogeneity of rat vascular endothelium as detected by HPA and GS-I lectin probes (Smolkova et al., 2001). Vasodilatation in the adrenal medulla, perivascular oedema, and enhanced LABA reactivity appear to reflect certain priorities existing in the hypothyroidism-induced impairments of adrenal glands.

Conclusions

1. Hypothyroidism has significant effects on glycoconjugate processing in the rat adrenal gland, decreasing αDMan/αLFuc and increasing βDGal/DGalNAc exposure; this trend was accompanied by uncoupling of the synthesis and secretion of physiologically active substances.

2. Most significant hypothyroidism-induced changes were detected within the adrenal gland vascular bed, with predominant impairments of medullary blood vessels.

3. Redistribution of lectin-binding sites in the adrenal parenchyma in hypothyroidism was more relevant in the cortical region, especially in the zona glomerulosa, in comparison with the medulla.

4. Fucose-specific lectin from Laburnum anagyroides bark demonstrated strong affinity towards endothelium and, therefore, can be recom-

mended as a reliable histochemical label of the rat vascular bed.

ACKNOWLEDGEMENTS

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