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EFFECT OF ANTITHYROID ANTIBODIES ON THYROID HORMONE SECRETION

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Extensive factual material has now accumulated on the discovery of circulating antithyroid autoantibodies in patients with various thyroid gland diseases, but their pathogenetic role has not been finally established. Several investigations suggest that circulating autoantibodies have a different point of application in the pathogenesis of autoimmune thyroiditis from direct injury to the thyroid gland tissues, namely their effect on secretion and peripheral utilization of thyroid gland hormones [3, 4].

The object of the present investigation was accordingly to study secretion of thyroid hormones both in animals with experimental autoimmune thyroiditis and in rats with passive immunization with immune sera and with antithyroid antibodies.

EXPERIMENTAL METHOD

Experiments were carried out on Chinchilla rabbits weighing 3 kg and on noninbred rats weighing 180 g. The rabbits were divided into four groups: 1) healthy rabbits not receiving thyroid-stimulating hormone (–TSH), 2) immunized rabbits (–TSH), 3) healthy rabbits (+TSH), 4) immunized rabbits (+TSH).

The experimental rats were kept for 11 days on an iodine-free diet and the animals were divided into five groups: 1) healthy rats (+TSH) – control, 2) immunized (+TSH), 3) receiving serum of healthy rats (+TSH) – control, 4) receiving serum of immunized rats (+TSH), 5) receiving antibodies (+TSH).

Autoimmune thyroiditis was reproduced in the rabbits and rats by the method of Witebsky and Rose [5]. The rabbits received a single injection of 3 units TSH, and measurements were made 24 h after its injection. Rats were passively immunized with sera and antibodies twice, with an interval of 3 days between injections, each in a volume of 1 ml intraperitoneally. Each animal received 50 microunits TSH intraperitoneally 30 min before sacrifice. To assess thyroid secretion, the method of counting colloid droplets in 100 cells, measurement of acid protease activity, pH 3.8, and radioimmune determination of the concentrations of serum triiodothyronine (T_3) and total thyroxine (T_4) by means of kits from the Radiochemical Centre, Amersham (England), was used.

EXPERIMENTAL RESULTS

Proteolytic enzyme activity in the thyroid gland of healthy rabbits was 19.6 ± 1.7 mg tyrosine/g weight of gland, whereas in the animals with autoimmune thyroiditis it was reduced to 6.1 ± 0.6 mg tyrosine/g weight of gland (Table 1). Injection of 3 units TSH into healthy rabbits increased activity of the enzyme to 26.1 ± 0.01 mg tyrosine/g weight of gland, and this was used as the control for activity of this hormone because a manifestation of the early action of TSH is known to be proteolysis of colloid. A single injection of TSH into rabbits with autoimmune thyroiditis led to an increase in acid protease activity.

Colloid endocytosis and secretion of hormones into the blood were studied in rats kept on an iodine-free diet. Without stimulation of healthy rats with TSH the colloid endocytosis

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TABLE 1. Secretory Function of the Thyroid Gland in Animals with Experimental Thyroiditis

Group of animals	No. of animals	Activity of proteolytic enzymes, mg tyrosine/g wt. of gland	Group of animals	No. of animals	No. of colloid droplets per 100 cells	T_3, ng/100 ml	T_4, µg/100 ml
Healthy rabbits (– TSH)	11	19,6±1,7	Healthy rats (– TSH)	4	11±1	54,7±2	5,48±0,6
Rabbits with autoimmune thyroiditis (– TSH)	5	6,1±0,6 $P<0,001$	Healthy rats (+ TSH)	6	160±14,8	164,4±2,3	14,8±0,35
Healthy rabbits (+ TSH)	11	26,1±0,01 $P<0,001$	Rats with autoimmune thyroiditis (+ TSH)	5	106±16 $P<0,001$	74,9±15 $P<0,001$	0,96±0,1 $P<0,001$
Rabbits with autoimmune thyroiditis (+ TSH)	9	21,5±1,9 $P<0,02$					

TABLE 2. Secretion of the Rat Thyroid Gland under the Influence of Immune Factors

Group of animals	No. of rats	No. of colloid droplets per 100 cells	T_3, ng/100 ml	T_4, µg/100 ml
Rats with autoimmune thyroiditis (+ TSH)	5	160±14,8	164±2,3	14,8±0,35
Serum of healthy rats (+ TSH)	5	174±9,4 $P<0,5$	157,8±2 $P<0,05$	18,2±0,73 $P>0,01$
Serum of immunized rats (+ TSH)	6	97±12,5 $P<0,001$	107,5±14,5 $P<0,01$	4,64±0,45 $P>0,001$
Antithyroid antibodies (IgG γ_2)	6	119±7,8 $P>0,01$	140±3 $P<0,001$	1,58±0,2 $P>0,001$

was expressed as single colloid drops; the serum T_3 concentration was 54.7 ± 2 ng/100 ml, total thyroxine was 5.48 ± 0.6 µg/100 ml. Injection of 50 microunits TSH into healthy animals stimulated colloid endocytosis and the secretion of thyroid hormones into the blood stream (Table 1). Similar stimulation of the thyroid gland of immunized rats also increased the liberation of colloid droplets from the follicles, but they were fewer in number than in the control. Radioimmune investigations showed that in this disease it is stimulation of thyroxine secretion which is most severely affected, for after injection of TSH it was virtually absent: only 0.96 ± 0.1 µg/100 ml compared with 14.8 ± 0.35 µg/100 ml in the control.

The study of the mechanism of the inhibition of secretion thus found in autoimmune thyroiditis necessitated a study of the humoral factors of immunity: serum containing antibodies and, separately, antibodies in the form of the $IgG\gamma_2$ fraction. These experiments were conducted on albino rats.

Injection of serum of healthy rats into the animals stimulated resorption of colloid from the lumen of the follicles, and a uniform outflow of colloid drops was found in all thyroid gland preparations (Table 2). Injections of serum of immunized rats into the experimental rats inhibited endocytosis and depressed secretion of T_3 and, in particular, of T_4. On injection of antibodies against rat thyroid gland a decrease in colloid endocytosis and T_3 secretion and distinct inhibition of liberation of total thyroxine, the concentration of which was 1.58 ± 0.2 µg/100 ml, were observed. Some differences in colloid endocytosis in the experimental rats must be noted. Among the many follicles containing a reduced number of colloid droplets there were some with a sharply increased outflow of colloid material, possible evidence of a disturbance of the apical cell membrane.

The decrease in colloid endocytosis and proteolytic enzyme activity in animals with autoimmune thyroiditis, coupled with the depressed reaction to TSH, may be the result of hypofunction of the gland and the insufficient liberation of this hormone [1, 2]. It must evidently be considered that antithyroid antibodies which, as the writers' previous observations showed, depress iodination of thyroglobulin through interaction with the active centers of the antigen, which leads to the obtaining of thyroglobulin deficient in iodoamino acids, must be considered to be responsible for the inhibition of excretion of thyroid hormones into the blood stream.

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INJURY AND REPAIR OF HEART MUSCLE DNA IN EMOTIONAL-PAIN-INDUCED STRESS

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In emotional-pain-induced stress (EPS) high catecholamine concentrations cause activation of lipid peroxidation in the myocardium and labilization of lysosomal enzymes, which damage the membranes of the myocardial cells [1]. It is possible that lysosomal enzymes and lipid hydroperoxides forming active free radicals injure not only membranes, but also DNA.

The object of this investigation was to study the state of polymerization of single DNA strands and the reparative synthesis of DNA in the myocardium of rats exposed to EPS.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. The animals were exposed for 6 h to EPS [4] and then decapitated at various times after the end of exposure. Control animals were killed at the same time. A group of five animals was used to isolate each preparation of nuclei [10] and DNA [8].

For sedimentation analysis, a suspension of nuclei in solution containing 0.1 M NaCl, 0.01 M EDTA, and 0.01 M Tris-HCl buffer, pH 7.4 ($4 \cdot 10^6$ nuclei in 1 ml) was applied in a volume of 1 ml to an equal volume of 1 N NaOH in a 36-ml centrifuge tube and allowed to stand for 12 h at 20°C. A linear (5-20%) sucrose gradient containing 0.2 N NaOH was poured in a volume of 34 ml into the bottom of the centrifuge tubes, starting with a low concentration. The nuclear lysate under these circumstances was displaced from the top of the gradient. The gradients were centrifuged in the SW-27 rotor of the Beckman L-65 centrifuge for 5 h at 25,000 rpm. Fractions, each of 2.4 ml, collected from the bottom of the centrifuge tube, were analyzed in UV light at 260 nm against the corresponding control solutions, which is a modification of the method in [9]. DNA of animal cells are known to contain alkaline-labile bonds, as a result of rupture of which stable DNA subunits are formed [7]. At the same time, deproteinization of the DNA and dissociation of the DNA-membrane complex take place [5], as confirmed by spectral data (Fig. 1), which point to liberation of DNA in the course of centrifugation from the other nuclear components remaining on the top of the gradient (fraction 15). During work with nondividing cells and tissues with a low mitotic index, incorporating radioactive precursors of DNA only weakly, sedimentation constants and molecular weight of the DNA can be determined by extrapolation to zero dilution, because of overloading of the gradient that is unavoidable even when a sensitive fluorescent label [6] is used. For this reason, in the present investigation the analysis was limited to qualitative evaluation of the change in the state of polymerization of the DNA, just as is usually done.

Reparative synthesis of DNA was assessed *in vivo* and *in vitro*. To determine reparative synthesis of DNA *in vivo* the animals were given an intraperitoneal injection of hydroxyurea

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