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STATE OF COLLAGEN METABOLISM DURING IMMOBILIZATION AND ELECTROPUNCTURE

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Analysis of the parameters of collagen metabolism is widely used to assess the state of the connective tissue in various diseases. However, little attention has been paid to the study of changes in the metabolism of the corresponding biopolymers which may be induced by the accompanying stress.

It was accordingly decided to study parameters of collagen metabolism in the course of repeated immobilizations and electropunctures, which were used as strong and weak stressors respectively.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 150-200 g; 16 intact rats served as the control. The experimental animals were immobilized for 8 h daily in constricting cages for up to 5 days (series I, 30 rats) or subjected to electropuncture for 20 min (4-5 V, 50 Hz) in the zone of the stomach, by the method of Zakhar'in and Gede, for up to 8 days (series II, 40 rats). Some animals were exposed to electropuncture of the skin, accompanied after the 4th day of the experiment by simultaneous immobilization, as described above (series III, 30 rats). Electropuncture was applied 1 h before immobiliation. The apparatus used in the experiments was built at Ustinov Mechanical Institute.

The animals were killed under ether anesthesia at different stages of the experiments. Proteolytic activity (PA) was determined in the blood plasma [8]. Parameters of collagen metabolism, namely free, peptide-bound, and protein-bound hydroxyproline (FH, PEBH, and PRBH respectively) — in the blood plasma were studied with the aid of chloramine B and T and p-dimethylaminobenzaldehyde [2, 3, 6], as described later. Blood plasma in a volume of 2 ml was mixed with 1 ml of 6% TCA and 1 ml of 57% $HClO_4$. The mixture was centrifuged for 5-6 min at 1000 g. Exactly half of the supernatant was neutralized with 6 N NaOH until a pale purple color was obtained with phenolphthalein (tube 1). The other half was hydrolyzed in a boiling waterbath for 40 min, cooled, and neutralized (tube 2). The residue was treated with 1 ml each of distilled water, 6% TCA, and 57% $HClO_4$. The resulting mixture was hy-

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TABLE 1. Changes in Plasma Biochemical Parameters during Immobilization and Electropuncture (M ± m)

Experimental conditions	Number of experiments	FH	PEBH	PRBH	PA, μg α-amino nitrogen/ml plasma/h
Control	16	13,7±0,6	8,1±0,9	44,6±0,8	7,1±0,2
Immobilization					
1 day	10	17,0±0,9*	7,4±0,4	49,1±3,2	18,7±0,5*
3 days	10	23,6±1,9*	10,7±0,9*	56,3±4,3*	24,8±1,2*
5 days	10	39,5±2,1*	7,4±1,0	32,2±3,8*	17,3±0,8*
Electropuncture					
1 day	10	21,6±1,9*	10,0±1,3	45,8±1,4	8,7±0,9
3 days	9	15,3±1,1	8,9±0,8	44,0±1,6	8,4±0,7
5 days	10	13,8±1,5	8,7±0,8	46,0±1,8	8,0±0,5
8 days	10	12,9±1,7	9,1±1,2	44,1±2,2	7,4±0,6
Electropuncture + immobilization					
1 day	10	20,6±1,9*	10,4±0,2*	57,0±2,5*	9,2±0,6*
3 days	10	15,0±1,2	9,2±1,2	49,8±3,1	8,2±0,9
5 days	10	14,9±0,9	8,7±1,0	48,6±4,4	7,8±0,6

Legend. *P < 0.05.

drolyzed for 6 h is a boiling waterbath, cooled, 0.2-0.4 g of activated charcoal was added, the mixture was shaken, then centrifuged for 5-6 min at 1000 g, after which the transparent part of the contents of the tube was neutralized (tube 3). The control tube 4 contained 2 ml of water. Into each of the four tubes was poured 0.5 ml of 7% chloramine B or T in 0.1 M phosphate buffer, pH 8.0. After 4-4.5 min, 0.5 ml of a 10% solution of p-dimethylaminobenzaldehyde in 94-96% ethanol and 0.5 ml of 57% $HClO_4$ were added to the mixture. The contents were then stirred, heated in a boiling waterbath for 80-90 sec, cooled, and treated with 0.5 ml of a saturated solution of NaCl, 1 ml CCl_4, and 3 ml of n-butanol. The contents of the tubes were shaken, centrifuged for 10 min at 1000 g, and the top phase was subjected to photometry at a wavelength of 550-560 nm. The concentration of FH (tube 1), FH + PEBH (tube 2), and PRBH (tube 3) was calculated by means of a calibration curve and expressed in micromoles/liter of blood plasma. The concentration of PEBH was found as the difference between the concentrations of hydroxyproline in tubes 1 and 2.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that levels of FH, PEBH, and PRBH in the blood plasma averaged 13.7, 8.1, and 44.6 μmoles/liter respectively, only a little different from data in the literature [3, 7].

In the course of repeated immobilizations for 8 h the blood FH level rose progressively (by 24-188%). Fluctuating changes were found in the PEBH and PRBH levels. Since the blood FH level reflects collagen breakdown [4] whereas the PEBH and PRBH levels reflect intensification of fibrillogenesis in connective tissue [4, 9, 10], it can be concluded that during repeated exposure to immobilization stress, catabolism predominates in collagen metabolism. The simultaneous rise in the plasma PA level does not contradict this conclusion.

During the action of a weak stressor (electropuncture) changes in the parameters of collagen metabolism were not significant. Only after the first exposure was a transient increase (by 23%) observed in the blood FH level. Greater differences were observed in the individual PEBH concentration, possibly on account of differences in the degree of response of the animals to this stressor.

A powerful stressor is known to cause a sharp increase in the blood catecholamine and corticosteroid levels. These substances activate catabolic reactions in the metabolism of connective-tissue biopolymers, including collagen [1, 5]. This fact may explain the disturbances discovered in collagen metabolism during immobilization stress.

To judge from the character of the changes in the blood biochemical parameters, electropuncture has no harmful action of collagen metabolism. However, as an unusual stimulus, it evidently stimulates compensatory powers of the experimental animal, or it prevents the development of profound changes in collagen metabolism under conditions of strong stress.

The observed rise in the blood CO level during repeated immobilizations may thus be included among the list of tests providing information about lasting disturbances of metabolism of connective-tissue biopolymers during stress.

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INTERACTION OF PROSTAGLANDINS E_2 WITH RECEPTORS AND THEIR EFFECT ON ADENYLATE CYCLASE ACTIVITY IN HUMAN THYROID GLAND TISSUE

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Prostaglandins of the E_2 group (PGE_2) have an action on thyroid gland cells similar to the stimulating effect of thyrotrophic hormones (TSH) [5, 13]. The writers previously determined the kinetic characteristics of specific binding of TSH and PGE_2 with isolated cells of euthyroid tissue of the human thyroid gland (TG) [1] and studied the dynamics of changes in intracellular PGE_2 and cyclic nucleotide levels at the initiating stage of transmission of the thyrotrophic signal [2]. It could be concluded from the results that PGE_2 play a modulating role in the realization of the effect of TSH on target cells in the normal human TG. However, the role of these compounds under pathological conditions and, in particular, in thyrotoxicosis, has been studied extremely inadequately. We know that TG may be to some degree outside the control of TSH. Whether under these circumstances PGE_2 retain their stimulating effect on thyroid function is not known, but some workers regard these compounds as directly connected with the development of thyrotoxicosis [10, 15].

The aim of this paper was to compare parameters of ligand-binding capacity and affinity of receptors for PGE_2, the basal level of PGE_2, and the effect of PGE_2 on adenylate cyclase activity in euthyroid and thyrotoxic tissue of the human TG.

EXPERIMENTAL METHOD

Histologically unchanged paranodal TG tissue, obtained at operations on patients with modular euthyroid goiter, and thyroid tissue from patients with diffuse toxic goiter (thyrotoxicosis) were used. Isolated thyrocytes were obtained by the method in [7]. PG-receptor interaction was studied by the use of ^3H-PGE_2, with specific activity of 150 Ci/mmole (England), by the method described previously [1]. Affinity constants and ligand-binding capacities of the receptors were determined by the method in [6]. Preliminary chromatographic

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