MORPHOLOGICAL AND FUNCTIONAL RESPONSE OF THE ADRENAL CORTEX TO REPARATIVE OSTEOGENESIS AND TO THYROCALCITONIN

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The combination of morphological and functional changes in the adrenal cortex after injection of thyrocalcitonin (TCT) and after experimental fracture was studied in 150 male albino rats. An increase in the functional activity of the gland was found during reparative osteogenesis. During administration of TCT (5 units daily) a state of hyperfunction develops in the first 5 days. Subsequent saturation with TCT caused inhibition of the adrenal and, in particular, of its glucocorticoid function. The inhibitory effect of TCT on the adrenal cortex, it is suggested, may be one of the mechanisms accelerating the course of repair processes in bone tissue.

KEY WORDS: *thyrocalcitonin*; *adrenal glands*; *reparative osteogenesis*.

Combined experimental and clinical studies of thyrocalcitonin (TCT) have demonstrated its positive effect on the course of posttraumatic regeneration [9, 10]. The stimulant effect of the hormone is due to changes in phosphorus and calcium metabolism [2] and metabolic conversions of the collagen of the bone matrix [3, 10]. Considering the role of adrenal hormones in the regulation of reparative osteogenesis and in the compensation of disturbed physiological mechanisms during the period of adaptation to stress, it was decided to study the effect of TCT on this component of the endocrine system. No information on this question could be found in the accessible literature.

The object of this investigation was to study the adrenal cortex in animals during administration of TCT and in animals at various stages of reparative osteogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on 150 male Wistar rats divided into three groups: group 1) control — intact animals; group 2) animals receiving 2.5 units TCT twice a day for 30 days; group 3) animals in which a similar fracture of the right tibia was produced. The rats were decapitated 5, 15, and 30 days after the beginning of the experiment. Paraffin sections were stained by Heidenhain's method, by Ciaccio's method for lipids, and by Backhus's method for ascorbic acid. For electron-microscopic investigation the gland tissue was fixed with 3% glutaraldehyde, postfixed with osmium, and embedded in Epon. In all the animals the intensity of oxidation—reduction reactions was determined in the adrenal tissue by Warburg's manometric method. Karyometry was performed and the height of all the zones of the adrenal cortex measured by means of a screw-operated ocular micrometer. The volume of the nuclei was calculated by the equation for an ellipsoid of rotation.

EXPERIMENTAL RESULTS

Injection of TCT in the early stages caused a response of all zones of the adrenal cortex of the animals, as shown by an increase in the height and area of the zones and in the volume of the cell nuclei (Table 1). Thickening of the capsule and of the connective-tissue layers between the bands of epithelial cells and increased vascularization of the glands could be found. The response of cells of the zona fasciculata to exogenous TCT was stronger. The number of dark cells was increased. Their nuclei were enlarged, the chromatin in them was distributed regularly, and the nucleoli also were irregular in shape. The cytoplasm became honeycombed (Fig. la, b, c). The total number of lipid inclusions in the zona fasciculata

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was less than in the control. The residual material was scattered among the cytoplasm in tiny granules. A decrease in the lipid content in the adrenal cortex has been shown to be evidence of its activation with a simultaneous increase in its height [1]. The histotopography of ascorbic acid deposits was changed. In the cells of the inner part of the zona glomerulosa accumulation of the vitamin was observed. In the zona fasciculata and zona reticularis the number of granules was considerably reduced. In the fascicular cells in this period an increase in the agranular cytoplasmic reticulum was observed and its small cisterns were in close contact with the mitochondria. The discharge of mitochondrial vesicles into the cytoplasm was observed and the lipid inclusions were few in number (Fig. 1d).

Further administration of the hormone (15th day of the experiment) caused inhibition of functional activity of the adrenal cortex (Fig. 1e). The volume of the nuclei and height of the zona fasciculata were significantly less than in the control. The fascicular cells were shrunken and their cytoplasm homogenized. The staining properties of the nuclei were weaker and they varied considerably in shape. Pseudanophilic material accumulated in the zona fasciculata, masking the cytoplasm of the cells. Considerable deposits of silver at the boundary between the zona glomerulosa and zona fasciculata were mainly beneath the cell membrane. The number of mitochondria in the adrenocorticocytes was reduced, and they contained fewer vesicular structures (Fig. 1f). Most mitochondria had both membranes intact, and the remaining vesicles were not connected with them (type 2 in Demin's classification). The matrix was translucent and the mitochondrial membranes invaginated, which is regarded as an indicator of inhibition of function [15]. The integrity of the plasma membranes indicated the slowest method of liberation of the mitochondrial vesicles [5]. The presence of emptied organoids under these circumstances could be the result of inhibition of the formation of the internal vesicular structures. The less close contact of the mitochondria with lipid inclusions, the regular round shape of those inclusions, and the appearance of vacuolation in the central parts, which were observed in the animals of this group, point to a disturbance of the normal methods of lipid mobilization. Similar changes were described by Schwartz et al. [16] in animals with depressed adrenal glycocorticoid function. The increase in the number of microsomes, possibly the source from which mitochondria are formed [5, 11, 13, 14], under the influence of TCT in this case, accompanied by a general decrease in the number of mitochondria, could be confirmation of the inhibition of secretory function. The saccular dilatation of most cisterns of the endoplasmic reticulum and the enlargement of the perinuclear space can be explained by a disturbance of the liberation of the secreted material, for it is on the calcium level that this process depends.

Investigation of the adrenals after TCT administration for 30 days revealed a tendency for the above-mentioned changes to disappear. The decrease in the morphometric indices relative to the control values continued in this period also, whereas the intensity of tissue respiration of the adrenal fell 15 and 30 days after the beginning of TCT administration.

In animals in a state of reparative osteogenesis, marked structural and metabolic changes were found in the tissues of their adrenal gland. Five days after the beginning of experimental osteogenesis the glands reacted by increased vascularization and the active utilization of steroid precursors, as reflected morphologically in the predominance of cells with dark, finely granular cytoplasm and an increase in the size of the perikaryon and in the diameter of the nuclei and nucleoli. In the outer part of the zona fasciculata solitary cells which could be regarded as loci of deposition of the original materials for steroid formation, could be identified. Investigation of the histotopography of the ascorbic acid deposits confirmed this tendency. Both the number and size of the silver granules in the adrenocorticocytes were reduced. Virtually no deposition of silver along the course of the capillaries was observed. Investigation of the velocity of the biochemical reactions in homogenates of the gland indicated intensification of oxidation—reduction reactions.

In the course of reparative regeneration, at the moment of formation of chondroid-cartilaginous callus (15th day of the experiment) marked absence of synchronization was observed between the functional activity of individual areas of the adrenal cortex. The number of large pale cells was increased. Concentrations of them were found in all zones of the cortex, indicating activation of new mechanisms of morphological and physiological reorganization. The dark cells has significantly ($P < 0.001$) larger nuclei and nucleoli than in the control, and their relatively homogeneous cytoplasm possessed a marked degree of basophilia. The content of ascorbic acid granules in the cells of the zona fasciculata was a little smaller than at the previous time of the investigation. Deposits of silver were discovered

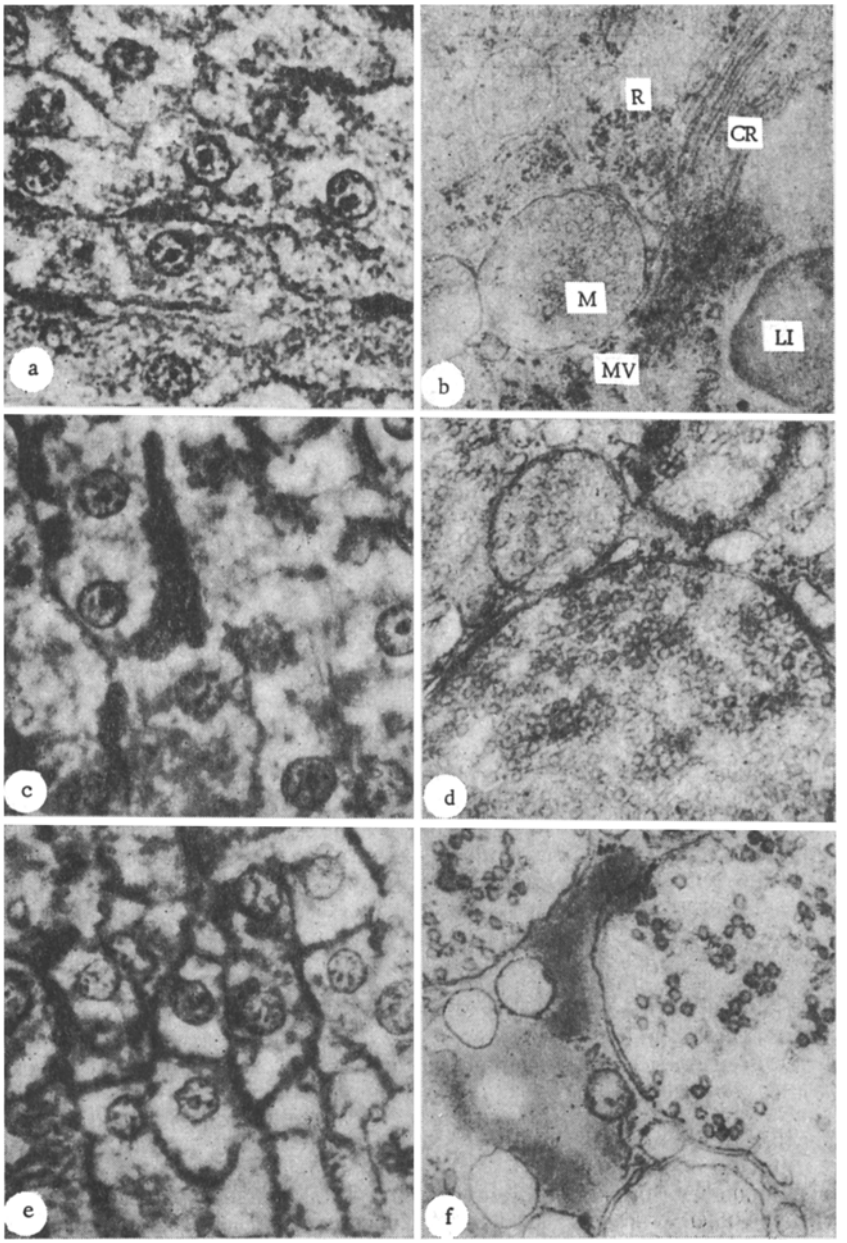

Fig. 1. Adrenocorticocytes of zona fasciculata during TCT administration. a, b) Cells of zona fasciculata of intact animal; c) hypertrophy of nuclei and nucleoli after TCT administration for 5 days; d) rupture of mitochondrial membrane and discharge of vesicles into cytoplasm of adrenocorticocytes 5 days after beginning of TCT administration; e) hypochromic nuclei and nucleoli of fascicular cells after TCT administration for 15 days; f) mitochondria deficient in vesicular contents after TCT administration for 15 days. a, c, e) Stained by Bouin–Heidenhain method (1100×); b, d, f) glutaraldehyde–osmium, Epon (30,000×). M) Mitochondria, MV) mitochondrial vesicles; R) ribosomes; CR) cytoplasmic reticulum, LI) lipid inclusions.

both extra- and intracellularly. The lipid inclusions were fairly large but few in number. Their distribution in the zona fasciculata was typically mosaic in character. During replacement of the chondroid-cartilaginous callus by bony structures (the 30th day of reparative osteogenesis) the adrenals were in a state of mild hyperfunction.

These experiments thus showed that administration of TCT for 5 days causes changes indicative of the functional activity of the adrenal cortex. The morphological equivalence of hypersecretion of the gland was an increase in size of the zones of nuclei and nucleoli; predominance of dark over pale cells, a decrease in the levels of lipids and ascorbic acid,

TABLE 1. Dynamics of Morphometric Indices of Zona Fasciculata of Adrenal Cortex Under Experimental Conditions

Index	Control (n=20)	TC (M ± m) administration			Fracture		
		5 days (n=23)	15 days (n=18)	30 days (n=17)	5 days (n=18)	15 days (n=18)	30 days (n=18)
Height of zona fasciculata, μ	423,1±5,6	448,5±2,6	286,0±3,9	326,1±3,1	516,3±3,2	481,0±4,1	502,4±3,1
P		<0,001	<0,001	<0,001	<0,001	<0,001	**<0,001**
Volume of nuclei, μ^3	127,6±3,7	140,0±3,6	102,7±3,0	118,3±2,6	160,8±3,5	144,1±3,7	138,5±2,8
P		<0,01	<0,001	<0,05	<0,001	<0,001	<0,05

and the presence of a considerable number of small cisternae of endoplasmic reticulum and liberation of mitochondrial vesicles into the cytoplasm. Under conditions of prolonged saturation of the body with TCT the secretory activity of the glandular epithelium of the adrenal fell. This was expressed as a decrease in the morphometric indices, the accumulation of sudanophilic material, the appearance of mitochondria with continuous membranes, a decrease in the number of vesicular elements in the cytoplasm, and dilatation of the cisternae of the endoplasmic reticulum. One probable aspect of the influence of TCT on metabolic activity of the adrenocorticocytes is connected with changes in phosphorus and calcium homeostasis by the hormone. An increase in the calcium ion concentration is known to stimulate corticosteroid secretion [12]. The deficiency of ultrafiltered calcium ions caused by TCT evidently leads to the development of the opposite effect, aggravated by the signs of increasing hypothyroidism [8].

A number of features reflecting different degrees of increased functional activity of the adrenal cortex were discovered during reparative osteogenesis. Excessive production of glucocorticoids, found during the first 15 days of healing, leads to the development of the catabolic phase of protein and calcium metabolism [4]. The protein breakdown observed under these circumstances and the increased excretion of osteotropic minerals, especially in the early stages of repair, create unfavorable conditions for local reactions in the region of regeneration [6]. The formation of endosteal cancellous bone is inhibited and the formation of primary bony callus and mineralization of the protein matrix are delayed [7]. In this connection a lowering of the functional activity of the zona fasciculata, discovered during TCT administration to the intact animals, could prove useful during posttraumatic regeneration as one mechanism accelerating morphological repair.

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