AGE DIFFERENCES IN RAT ADRENAL CAPILLARY ULTRASTRUCTURE FOLLOWING ACTH ADMINISTRATION AND IN INSULIN SHOCK

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During aging adaptive changes develop in the tissues and organs, accompanied by biochemical, physiological, and morphological changes [5, 6]. Secretion of glucocorticocoids and catecholamines by the adrenals has been shown to change very little with age [3, 11].

Considering that the adrenal capillaries play an important part in hormone transport into the blood stream in various extremal states, it was decided to study ultrastructural age differences in the capillary wall during stimulation of adrenal cortical and medullary function in adult and old rats.

EXPERIMENTAL METHOD

Ten adult (8 months) and 10 old (26 months) male albino rats were used. In the experiments of series I adult and old rats were given a single injection of ACTH (4 units/100 g body weight, intramuscularly) and were decapitated 40 min later at a time of maximal stimulation of adrenocortical function [6]. In the experiments of series II rats were given an injection of insulin (20 i.u./100 g body weight, intraperitoneally) and they were killed 3-4 h later, at the time of development of insulin shock [4]. The adrenals of intact rats served as the control. Material was fixed in 3% glutaraldehyde solution in phosphate buffer (pH 7.4) postfixed in 1% osmic acid solution, dehydrated, and embedded in Epon-812 resin. Ultrathin sections were stained with lead citrate and studied in the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

After injection of ACTH and in insulin shock changes in capillary ultrastructure were found in all layers of the adrenals in the adult rats. A very slight degree of translucency of the matrix of the cytoplasm was found in the endotheliocytes with widening of the mitochondrial cristae (Fig. 1A). The capillaries of the medulla contained endotheliocytes with numerous free ribosomes and polysomes. The small mitochondria had a dense matrix and the tubules of the rough endoplasmic reticulum were moderately dilated (Fig. 1B, C). These changes in endotheliocyte ultrastructure are evidence of increased activity of energy and protein metabolism in the cells, required to provide for increased functional activity of the capillaries [2]. Meanwhile, in insulin shock, lysosomes formed in the cytoplasm of the endotheliocytes and lipofuscin granules accumulated (Fig. 1D). The number of pores and fenestrae 40 nm in diameter was increased in the cortical capillaries and many micropinocytotic vesicles 30-40 nm in diameter and vents and canals were formed in the endothelium in the medullary capillaries. Numerous microprojections could be seen on the luminal surface of the endotheliocytes and evaginations or extrusions formed in the direction of the basal layer of the cytoplasm [10]. The basal layer of the capillaries was unevenly widened from 340 to 650 nm, with foci of hydration of the extracellular components (Fig. 1A, C). According to some workers these ultrastructural changes in the capillaries play an important role in an increase in capillary permeability [1, 8-10].

Consequently, both after injection of ACTH and in insulin shock the ultrastructural changes in the adrenal capillaries of adult rats promoted intensification of transcapillary exchange in the cortex and medulla and so facilitated regulation of adrenal homeostasis.

After injection of ACTH into the old animals well-marked folding of the surface of the endotheliocyte nuclei was observed in the adrenal capillaries with condensation of heterochromatin around the periphery of the nucleus; marked hydration, dilatation of the tubules of the rough endoplasmic reticulum, an increase in the number of lysosomes, residual bodies, and lipofuscin granules, and swelling of the mitochondria with destruction of the cristae were observed in the

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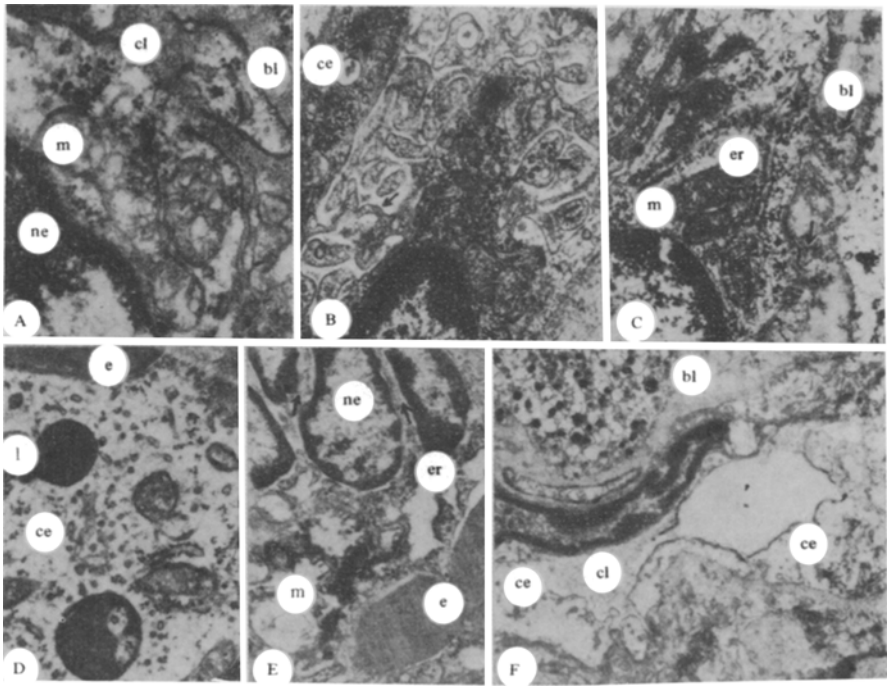

Fig. 1. Ultrastructural changes in capillary endotheliocytes of adrenal cortex and medulla in adult and old rats after ACTH injection and in insulin shock. A) ACTH, rat aged 8 months: translucency of matrix in cytoplasm of endotheliocytes, swelling of mitochondrial cristae in cortical capillary, 39,000 X; B) the same: accumulation of free ribosomes and polysomes in cytoplasm of endotheliocyte, formation of numerous microprojections of plasmalemma of capillary endotheliocyte in medulla, 24,000 X; C) the same: dilatation of tubules of rough endoplasmic reticulum in cytoplasm of endotheliocyte, appearance of extrusions (arrow) on its basal surface, and loosening of substance of basal layer of medullary capillary, 26,000 X; D) insulin shock, rat aged 8 months: accumulation of lysosomes in cytoplasm of endotheliocyte of cortical capillary, 30,200 X; E) ACTH, rat aged 26 months: deep invaginations can be seen on surface of nucleus (arrows), sharp dilatation of tubules of rough cytoplasmic reticulum, swelling of mitochondria with destruction of cristae in endotheliocyte of cortical capillary, 11,200 X; F) insulin shock, rat aged 26 months: marked edema of cytoplasm of endotheliocyte of medullary capillary, 7,800 X. bl) Basal layer; l) lysosome; m) mitochondrion; cl) capillary lumen; ce) cytoplasm of endotheliocyte; e) erythrocyte; ne) nucleus of endotheliocyte; er) endoplasmic reticulum.

cytoplasm of the endotheliocyte (Fig. 1E). These observations indicate predominance of trophic disturbances in the organelles of the endotheliocyte and, consequently, a reduction in the powers of adaptation of the adrenal capillary cells of old rats. Changes in the endotheliocytes were even more demonstrative in insulin shock, when the trophic and destructive changes in the organelles developed against the background of edema of the cell cytoplasm (Fig. 1F).

After injection of ACTH and in insulin shock reactive changes are thus observed in all components of the tissue-blood barrier in the adrenal capillaries of adult and old animals, but compensatory and adaptive processes are more marked in adult rats, whereas in old animals trophic disturbances and destructive changes develop in the organelles of the endotheliocytes, and were particularly severe in insulin shock.

Considering that the plasmalemma of the capillary endotheliocytes participates directly in the formation of fenestral pores, micropinocytotic vesicles, and extrusions [9, 12], it can be tentatively suggested that depression of morphogenetic processes in the plasma membranes of the endotheliocytes plays an important role in the disturbance of transcapillary exchange in the adrenals of old rats in extremal states. This, in turn, develops as a result of trophic disturbances and destructive changes reflecting a decrease in energy-forming and protein-synthesizing metabolism in the capillary endotheliocytes of old animals. These changes facilitate the development of pathological processes in the adrenal glands themselves in old animals.

LITERATURE CITED

1. Ya. L. Karaganov, Arkh. Anat., No. 1, 15 (1972).
2. V. F. Kondalenko, Byull. Eksp. Biol. Med., No. 12, 88 (1974).
3. V. N. Slavnov and G. V. Valueva, in: Endocrinology. Republican Interinstitute Collection [in Russian], No. 6, Kiev (1976).
4. N. A. Smitten, The Sympatho-Adrenal System in Vertebrate Phylogeny and Ontogeny [in Russian], Moscow (1972).
5. A. S. Stupina, in: Textbooks of Gerontology [in Russian], Moscow (1978), p. 143.
6. V. V. Frol'kis, The Nature of Aging. Biological Mechanisms of Development of Aging [in Russian], Moscow (1969).
7. V. V. Frol'kis, Regulation, Adaptation, and Aging [in Russian], Leningrad (1970).
8. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, The Microcirculation [in Russian], Moscow (1977).
9. V. A. Shakhlamov, Capillaries [in Russian], Moscow (1971).
10. K. Ichev, Arkh. Anat., No. 2, 56 (1970).
11. R. Kvetnansky et al., Mech. Aging Dev., 7, 209 (1978).
12. G. E. Palade, Circulation, 24, 368 (1961).

RHYTHM OF DNA SYNTHESIS IN HUMAN ADIPOCYTES

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Physiologists have long been of the opinion that during functional loading of average intensity only a proportion of the structural units of a given type in a tissue or organ participates in the corresponding activity. For a long time this view rested on an essentially indirect piece of evidence: the ability of organs and tissues to change their level of functional activity considerably in organisms in different states. More recently direct evidence of differences in the structural and functional state of homonymous cells has appeared. This evidence consists chiefly of histochemical and immunohistochemical data on enzyme activity and the content of glycogen and certain other substances [1, 3, 6], and also the very clearly demonstrable difference in the phases of liberation of secretion in glandular cells [2]. Pathologists are well aware of the different degrees of severity of lesions in different parts or individual cells of the same tissue in many diseases. This fact is nowadays also explained by the unequal functional state of the cells. Since cell activity is controlled by the genetic apparatus, differences in this activity in neighboring cells ought to be linked with corresponding differences in regulating influences. No direct proof in support of this hypothesis has so far been published. This situation is most probably due to the fact that qualitative changes in RNA (base composition, size of molecules) are by their nature unsuitable for analysis by morphological methods; biochemistry, however, cannot detect differences in single cells. In some tissues, however, states arise in which structural and functional differences between homonymous cells are so considerable that they give rise to quantitative changes in the level of RNA synthesis that are detectable by electron-microscopic autoradiography. The results of such observations on fibroblasts and mast cells have been published by Sarkisov et al. [5]. Data on adipocytes are reported in the present communication.

EXPERIMENTAL METHOD

The unchanged fatty areolar tissue of two men aged 55 and 59 years undergoing operations for lung cancer and desmoid was studied. Pieces of adipose tissue measuring 1 × 1 × 0.5 mm were incubated for 90 min at 37°C in medium No. 199 with ^{3}H-uridine in a dose of 100 μCi/ml (specific activity 26 Ci/mmole). After incubation the pieces were washed with cold medium No. 199 and phosphate buffer, pH 7.4, to remove unincorporated ^{3}H-uridine. After fixation with 2.5% glutaraldehyde solution and 1% osmium tetroxide solution the material was embedded in Epon. Semithin sections were coated with M emulsion and exposed for 3 days. Regions for ultrathin section cutting were chosen on the basis of the results of analysis of autoradiographs on semithin sections. Electron-microscopic autoradiographs were prepared by the method described previously [4, 5].

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