CHANGES IN CYTOPLASMIC PROTEIN SPECTRUM OF KIDNEY CELLS OF RATS WITH NEUROGENIC RENAL DYSTROPHY AND THEIR RELATIONSHIP TO ALDOSTERONE RECEPTOR FUNCTION

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KEY WORDS: aldosterone; reflex dystrophy of the kidneys; cytoplasm; receptor proteins

It was shown previously that division or chemical stimulation of the sciatic nerve lead to changes in structure and function of the glands of internal secretion and also in the sensitivity of organs of nonendocrine origin, which undergo reflex dystrophy (RD), to hormones [1, 2, 7]. The sensitivity of the kidneys to aldosterone has been studied in detail with respect to sodium reabsorption in the intact animal [4], on the isolated organ [5], and in kidney slices [3], during the development of neurodystrophy, connected mainly with marked weakening of specific accumulation of the hormone in the cytoplasm, nuclei and chromatin of the renal tubule cells [6].

Since we know that the protein receptor complex in the cytoplasm of the target cells for mineralocorticoid hormones and, in particular, for aldosterone, consists of four subunits [10-12], disturbance of the specific accumulation of aldosterone might be connected with changes in the conformational structure of this receptor complex, the degree of oligomerization of its subunits, their electrophoretic mobility, and also the molecular environment of the receptor apparatus. The aim of the present investigation was accordingly to study whether this hypothesis is plausible.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used. Disturbance of the trophic state of the kidneys was caused by the method described previously [6]. Three days before the experiment, the control and experimental animals underwent bilateral adrenalectomy, in order to remove all traces of the endogenous hormone from the aldosterone receptors. [^{3}H]-aldosterone was injected intraperitoneally in a dose of 36 ng/100 g body weight. For differential specific binding from the nonspecific part, simultaneously with labeled aldosterone the animals were given an injection of 500-fold excess of the stable hormone. The rats were killed by decapitation 10 min after injection of the hormone. The cytosol was separated by differential centrifugation at 105,000*g* for 90 min in standard buffer solution (0.32 M sucrose, 25 mM Tris-HCl, pH 7.7, 3 mM $MgCl_2$, and 1 mM PMSF, with the addition of 15 mM sodium molybdate to stabilize the structure of the receptor complex in the cytosol [8]. The protein concentration in the cytosol was determined by Bradford's method [9]. To obtain the fraction of high-molecular-weight proteins, which must include the aldosterone receptor complex, the cytosol was fractionated by gel-filtration on Sephadex G-75. The conditions of gel-filtration were: column measuring 0.5 × 30 cm, quantity of cytosol applied 1.5 ml, rate of elution 0.5 ml/min, volume of eluate collected 1.5 ml per tube, composition of the eluate the same as for isolation of the cytosol but without sucrose, elution time 90 min, including rinsing the column. For the further study of the high-molecular-weight spectra of the cytosol gel-filtration on a high-performance liquid chromatograph (HPLC; from "Voters," USA) was used. Fractionation was carried out on a column for proteins, peptides,

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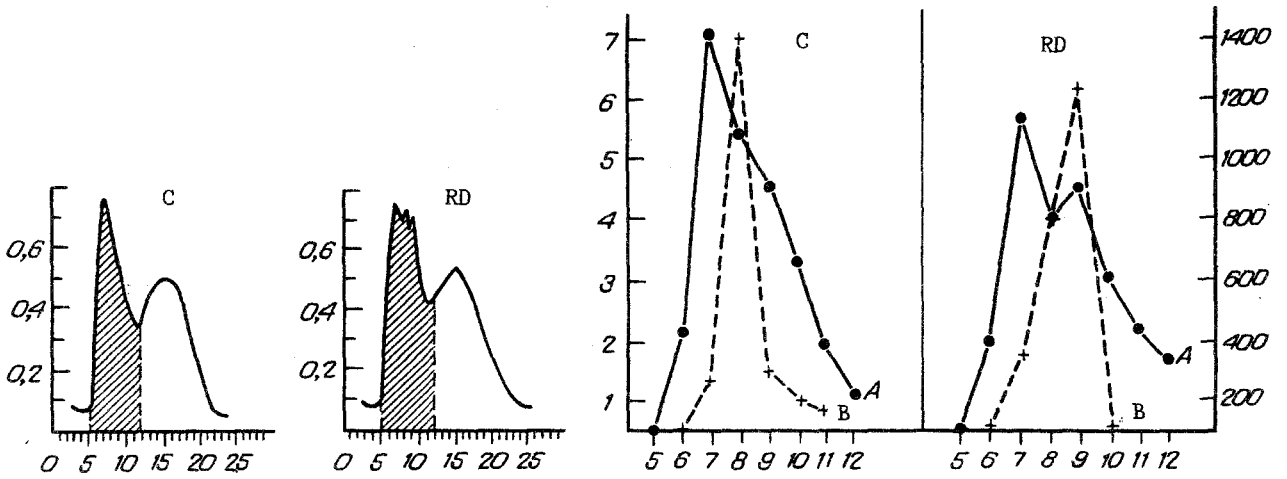

Fig. 1. General chromatographic spectrum of proteins. a) Elution profile of cytoplasmic proteins of kidney cells of control (C) rats and animals with RD of the kidney (RD). High-molecular-weight region of spectrum is shaded. Abscissa, serial Nos. of tubes; ordinate, optical density at 280 nm (relative units); b) protein concentration (ordinate, left, in mg/ml, curve A) and specific binding of [^{3}H]-aldosterone (ordinate, right, cpm, curve B) in eluate of high-molecular-weight region of spectrum of cytosolic protein fraction from kidney cells of control rats (C) and animals with RD of the kidney (RD). Abscissa, serial Nos. of tubes.

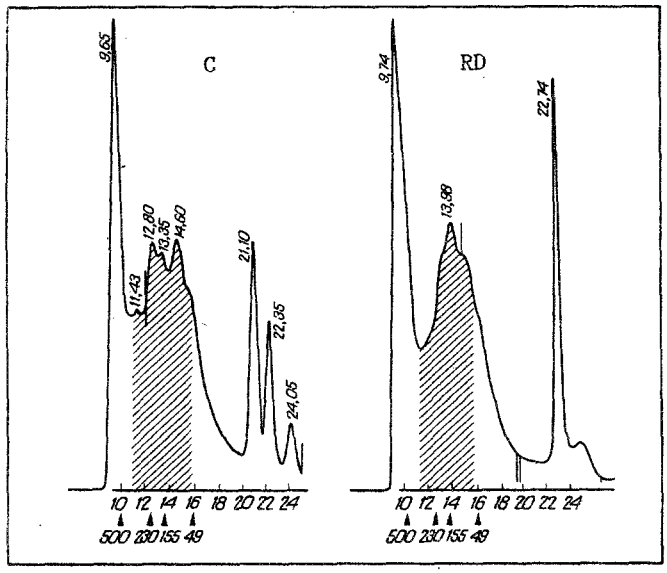

Fig. 2. Spectra of maximal nonreceptor-functional peak of cytoplasmic proteins from liver cells of control rats and rats with RD of the kidney (RD). Here and in Fig. 3, high-molecular-weight region of spectrum is shaded; abscissa, time of emergence of proteins in eluate (in min); numbers below time scale indicate molecular weight of marker proteins (in kD).

nucleic acids, and amino acids (of the Protein Pack 300 SW type), measuring 7.5 mm × 30 cm, with a resolving power for molecular weight of between 10 and 400 kD. A carefully filtered eluate of the following composition was used: 50 mM Tris, 0.1 N NaCl, pH 7.2. The rate of elution was 0.5 ml/min. Aliquots (as protein) of the control and experimental cytosol were used.

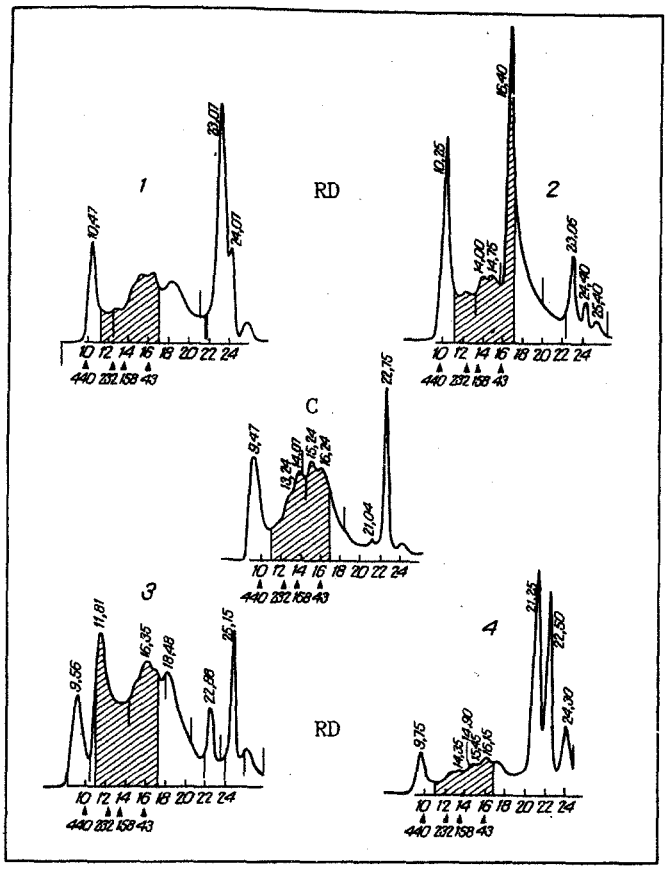

Fig. 3. Spectra of maximal receptor-functional peak of cytoplasmic proteins from kidney cells of control rats (C) and animals with RD of the kidney (RD). Explanation in text.

EXPERIMENTAL RESULTS

The elution profile of proteins of the cytosolic fraction from rat kidney cells on Sephadex G-75 (Fig. 1a) shows that the character of the curve in the high-molecular-weight (shaded) part of the spectrum in the control differs from the character of the corresponding part of the curve in the cytosol from kidney cells of rats with RD of the kidney. This may perhaps be because of redistribution of the proteins by molecular weight in the cytosol of kidney cells of the experimental animals (RD) due to possible aggregation and dissociation as a result of excessive pathological stimulation carried along nerve fibers from the stump of the damaged sciatic nerve to the kidney cells. Nevertheless, after fractionation of the cytosol on Sephadex G-75 high-molecular-weight proteins were found in both experimental and control groups in tubes Nos. 5-12. The protein concentration and specific binding of [^{3}H]-aldosterone with receptors were determined in each of these tubes. As Fig. 1b shows, and as we expected, maximal protein concentrations in the experimental (RD) and control groups did not coincide with the maxima of specific binding of labeled aldosterone. The point on the protein concentration curve corresponding to maximal specific aldosterone binding we called the receptor-functional peak, and the point corresponding to the maximal protein concentration — the nonreceptor-functional peak. The greatest degree of specific binding in the experimental group, moreover, occurred in tubes 8 and 9, whereas in the control it was in tube 8. Consequently, the maximal receptor load in the cytosol of the experimental group is probably carried by the group 2 proteins, but the group 1 proteins in the control. These facts indirectly confirm the presence of changes in the high-molecular-weight region of the spectrum of cytosolic proteins which have maximal receptor function relative to aldosterone (Fig. 1a).

The most typical spectra of the maximal nonreceptor-functional peak of cytosolic proteins from cells of the control and experimental (RD) animals are given in Fig. 2. They show that in RD of the kidney cells the number of peaks of high-molecular-weight proteins (the shaded part of the spectrum) is reduced and marked changes take place in the spectrum of low-molecular-weight compounds. It can be tentatively suggested that under the influence of excessive and distorted

stimulation transmitted along nerve fibers from the neuroma of the central stump of the damaged sciatic nerve changes take place in the conformational structure, the degree of oligomerization and, possibly, of other physicochemical characteristics of the cytosolic proteins which are not aldosterone receptors in the kidney cells of the rats. This state of affairs reflects disturbance of the molecular environment of the aldosterone–receptor complex in the cytosol, which may affect interaction of aldosterone with the receptor complex and may ultimately prevent the physiological manifestation of the action of the hormone on the target cell.

Spectra of the maximal receptor-functional peak of cytosolic proteins from kidney cells of control rats and four groups of animals with sciatic nerve injury (RD: 1, 2, 3, 4) are shown in Fig. 3. Protein spectra of the receptor-functional peak in the experiment, although differing in detail from one another in their structure in the different groups of animals, differ considerably in principle from the spectrum of the receptor-functional peak in the control. This was to be expected, for the reaction of the corresponding types of cell populations in different groups of animals to the action of the same stimulus may be manifested differently under identical conditions, depending on genetic differences between the individuals. The fundamental difference in the character of the spectra in the control and experiment is quite evident. It is reflected, first, in the fact that three peaks of almost equal size (Fig. 3, C, shaded) are present in the high-molecular-weight region of the spectrum in the control (after fractionation for 15, 16, and 17 min), whereas in the experiment in this region of the spectrum either a sharp increase in the intensity of some peaks (Fig. 3, RD, 2 and 3) or a no less significant decrease (Fig. 3, RD, 4), or, again, smoothing of the peaks (Fig. 3, RD, 1) are observed; second, in the fact that three peaks of different size and shape are always present in the low-molecular-weight region of the spectrum in the experiment (see Fig. 3, RD, 1, 2, 3, and 4, at the 21st-25th minutes of fractionation), whereas in the control, the peaks in this part of the spectrum always have the same configuration (Fig. 3, C). Maximal specific binding of ^{3}H-aldosterone was observed in this apparently heterogeneous spectrum of the receptor-functional peak of the cytostolic protein. Consequently, receptor proteins of aldosterone or the hormone–receptor complex are present in this high-molecular-weight fraction. Although the present investigation cannot identify which of these proteins bears a receptor function, it is possible to reach a definite conclusion on the character of changes in the spectrum in the experimental tests and the size and shape of individual protein peaks of the receptor-functional complex, and also regarding the proteins which constitute the closest molecular environment of the hormone-receptor assembly. In other words, both the nearest molecular environment and distant molecular assemblies of the cytosol of cells affected by RD are modified, and it is unlikely that these changes in the proteins are not reflected in the function of the receptor apparatus for mineralocorticoid hormones.

The results of this investigation thus indicate that RD of rat kidney cells developing after sciatic nerve injury is accompanied by changes in the cytosolic protein spectrum of these cells. Changes in the spectrum of cytoplasmic high-molecular-weight proteins, which include proteins of the receptor complex for aldosterone, may perhaps be the result of a disturbance of their conformational structure and degree of oligomerization, as well as of other physicochemical parameters. It must be recalled that changes in the macro- and micromolecular environment of the aldosterone receptor complex due to neurodystrophic changes in the cells may be reflected not only in the work of this complex, but also in the state of other metabolic mechanisms of cells maintaining the functional activity of the neuron and intracellular transmission of the aldosterone stimulus.

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EXPERIMENTAL PROTEIN-DEFICIENCY DIABETES AND ITS CORRECTION

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In 1985, in addition to insulin-dependent and insulin-independent types of diabetes mellitus, a new type of this disease was introduced into its classification by a WHO expert group, namely diabetes mellitus connected with protein deficiency (PDD) [3]. The reason for introducing this clinical class of diabetes was the recognition of the precise clinical features of this disease, its severity, and its high incidence in some tropical countries. This new category of diabetes comprises two subclasses: fibrocalculous pancreatic diabetes and pancreatic diabetes linked with protein deficiency [3]. This last type of diabetes is characterized by resistance to the development of ketosis, partial resistance to the action of insulin, and a high degree of emaciation. Information on the new type of diabetes [3] and data relating to the successful use of transplantation of fetal pancreas in recent years to correct the disturbance of carbohydrate metabolism in type I diabetes [2] were the basis for the present investigation.

Its aim was to create a model of diabetes associated with protein deficiency and to determine the effect of transplantation of the fetal pancreas in diabetic rats.

EXPERIMENTAL METHOD

Experiments were carried out on 79 noninbred albino rats: 49 males and 30 females. The rats were deprived of animal proteins (cheese, meat, milk) for 68-224 days. At the end of this period of protein deprivation (PD) the rats were put back on a more balanced diet including animal proteins. There were nine series of experiments, which differed in the duration of PD and the age composition of the rats at the beginning of PD (Table 1). Implantation of the fetal pancreas (IFP) subcutaneously into the ear was carried out on 22 rats. In series I-VI, IFP was carried out on the day when PD ended, in series VII it was carried out 2.5 months after the end of PD, and in series VIII and IX, IPA was not performed. The control consisted of two series (X and XI) of experiments with short-term PD. The state of the rats during the experiments was determined by regular weighing (once a week), observation of changes in carbohydrate metabolism (blood sugar level, glycosuria), and also by biochemical tests of the urine by rapid methods using strips: 1) Glucoprofile, 2) Glucofan, 3) Albufan. At the end of the observations the internal organs and remains of the transplanted pancreas were subjected to pathological and histological investigation. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

As Table 1 shows, 12 of the 79 protein-deprived rats developed diabetes (15.19%). Diabetes did not develop in all series of experiments. However, in those series (II-IV) in which diabetes was not observed, a larger number of the animals

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