It is difficult to give an unambiguous estimate of the role of this decrease in δ-aminolevulinate synthetase activity discovered in postradiation changes in the cytochrome P-450 level because this effect is observed against the background of elevation of the "free" heme level.

The results are thus evidence that significant disturbances of heme metabolism take place in the liver of irradiated animals; however, they do not give the final answer to the question of the role of the observed effects in the fall in the microsomal cytochrome P-450 level.

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GEL FILTRATION STUDY OF CYTOPLASMIC cAMP RECEPTORS IN THE KIDNEYS OF RATS OF DIFFERENT AGES

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KEY WORDS: ontogeny; rat kidney; cAMP reception.

The ability of the kidney to participate in the maintenance of water and electrolyte homeostasis in albino rats develops most rapidly during the period when maternal feeding ceases. During this same period the animal develops the ability to respond to regulatory influences of peptide hormones such as ADH, parathyroid hormone, and calcitonin. Sensitivity to these hormones is determined both by maturity of the receptor–adenylate cyclase complex and by the state of the intracellular mechanisms participating in realization of the cAMP effect [2, 6]. An important, and in many cases the sole, contribution to cytoplasmic cAMP reception is made by regulatory subunits of cAMP-dependent protein kinases of various types [5]. The role of cAMP-dependent protein kinases in realization of the hormonal effects and their structure in adult animals has now been comprehensively studied [1, 5, 8, 9]. However, there is extremely little information on the ontogenetic changes in protein-kinase complexes. The study of the molecular weight of receptor complexes with the property of specific reception of cAMP, and formed in the cytosol of the kidneys of animals with an immature and hormonally competent kidney, with different cAMP concentrations in the medium, can contribute to our understanding of the pathways of ontogeny of the intracellular mechanisms of action of cAMP.

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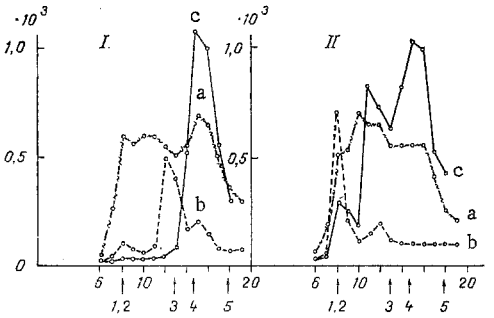

Fig. 1. Gel filtration profiles of cAMP receptors of kidney cytosol of rats aged 15 days (II) and 60 days (I), with different cAMP concentrations in medium. Abscissa, No. of fraction; ordinate, radioactivity (in cpm). Arrows indicate plane of elution of markers: 1) blue dextran, 2) BSA dimer, 3) BSA, 4) ovalbumin, 5) cytochrome c. a) Minimal cAMP concentration, b) 10^{-9} M cAMP, c) 10^{-8} M cAMP.

In the investigation described below the method of gel filtration was used to estimate the molecular weight of proteins receiving cAMP in the kidney cytosol of rats aged 15 and 60 days.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats of both sexes aged 15 and 60 days. Fresh kidneys, obtained from decapitated animals, were homogenized in the cold in 3 volumes of 0.01 M K-phosphate buffer, pH 6.8, 1 mM EDTA, 2 mM 2-mercaptoethanol. In experiments in which the cAMP constant had to be reduced to a minimum, homogenization was carried out with activated charcoal (1 mg/ml). All procedures were carried out in the cold. The homogenate was filtered through Kapron and centrifuged for 1 h at 20,000g [7]. The supernatant, in a volume of 0.3 ml, was applied to a column with Sephadex G-100 (1.6 × 40 cm); volume of the fractions was 3.5 ml. To determine the binding profile of cAMP in experiments in which its level was minimal, fractions 0.2 ml in volume were incubated for 18 h with $0.5 \cdot 10^{-8}$ Ci [^{3}H]-cAMP (45 Ci/mmole), after which the receptor–cAMP complexes were separated from unbound ligands on columns with Bio-Gel P-2 (0.8 × 2.5 cm). In experiments in which cAMP was present in the medium, [^{3}H]-cAMP was added to the homogenate up to the required concentration, after which radioactivity was determined in the fractions after gel filtration. The binding constant was estimated by Scatchard's method [10]. Protein kinase activity was determined by the method in [6]. The column was calibrated by the use of bovine serum albumin (BSA), ovalbumin, cytochrome c, and blue dextran. The molecular weight of proteins possessing receptor for enzyme activity was calculated using the linear dependence of the logarithm of effluent volume on molecular weight [3].

EXPERIMENTAL RESULTS

Soluble cAMP-dependent protein kinase is a tetrameric enzyme consisting of two catalytic subunits, which are evidently the same for all types of the enzyme, and two regulatory units, specific for each type [8]. Elution profiles of specific cAMP receptors, obtained by chromatography of the cytosol of the kidneys of rats aged 15 and 60 days with different concentrations of ligands, indicate significant dependence of the molecular weight of the receptor complexes on the cAMP concentration in the medium (Fig. 1). In the presence of a minimal cAMP concentration, irrespective of the animals' age the receptors are eluted in a large plateau-like peak, extending over the region of molecular weights from 37 kilodaltons and above. Such a profile can evidently be explained not only by different kinds of receptors, but also by their instability, assuming that they form complexes consisting of several macromolecules. In fact, evidence has now been obtained to show that by the action of cAMP, complexes of subunits of cAMP-dependent protein kinase of types R_2 and R_2C may be formed [4]. This effect of cAMP probably explains the appearance of the characteristic receptor peaks in experiments with concentrations of ligand of 10^{-9} and 10^{-8} M. An increase in cAMP concentration caused a decrease in the size of the receptor peaks with high molecular weight in rats of both age groups, and an increase in size of the peaks of efflux of receptor complexes with a lower molecular weight. On the addition of cAMP to the medium in concentrations of

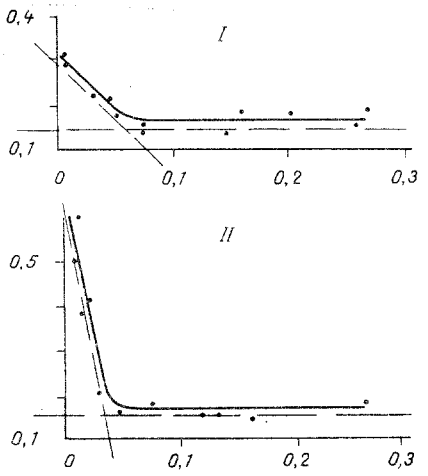

Fig. 2. Binding of cAMP with receptors as a function of cAMP concentration between Scatchard coordinates. Abscissa, concentration of bound ligand (in pM); ordinate, ratio of concentrations of ligand (bound/free). Remainder of legend as to Fig. 1.

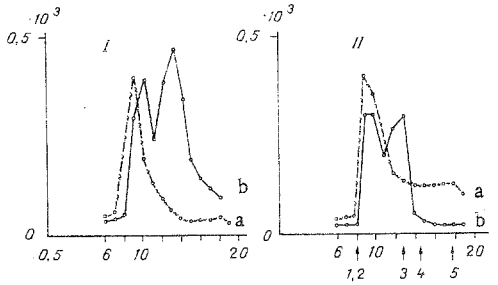

Fig. 3. Gel filtration profiles of protein kinase activity of kidney cytosol from rats aged 15 and 60 days with different cAMP concentrations in medium. a) Minimal cAMP concentration, b) 10^{-8} M cAMP. Remainder of legend as to Fig. 1.

10^{-9} and 10^{-8} M significant differences were observed in the elution profiles of cAMP receptors in animals of different ages: With cAMP in a concentration of 10^{-9} M receptors in young animals were concentrated mainly in a peak with molecular weight of 130 kilodaltons and over, whereas in adult animals the molecular weight was about 85 kilodaltons. An increase in the cAMP concentration to 10^{-8} M caused further changes in the reception profile for rats of the two age groups, toward a fall of molecular weight in the region of about 37 kilodaltons. However, whereas in adult animals with a high concentration of ligands virtually all receptors came out in this peak, a considerable proportion of receptors in the cytosol of the immature kidney had a molecular weight of about 85 and 130 kilodaltons or more.

The appearance of receptor complexes with different molecular weights under the influence of cAMP in the cell cytosol from mature and immature kidneys suggests that during development of the kidney the properties of cAMP receptors change substantially. This hypothesis is confirmed by the results of analysis of affinity of the receptors for cAMP (Fig. 2). It can be concluded from the character of the Scatchard plots that the pool of high-affinity receptors in each experimental group is very homogeneous, but the affinity constants differ significantly: $(1.0 \pm 0.2) \cdot 10^9$ M^{-1} ($n = 4$) and $(0.20 \pm 0.05) \cdot 10^9$ M^{-1} ($n = 5$) respectively at the ages of 15 and 60 days. Activity of the enzyme and affinity of the regulatory subunits for cAMP depend on the degree of aggregation of the enzyme which, in turn, depends on the cAMP concentration in the medium [5]. The results of determination of

the profile of protein kinase activity in experiments with high and low cAMP concentrations showed that association of protein kinase activity with cAMP receptors is possible with a low concentration of the ligand, and dissociation if its concentration is increased (Fig. 3). With a low cAMP concentration protein kinase activity was found in the peak of the dead volume with mol. wt. of 130 kilodaltons or more, whereas in the presence of a high cAMP concentration it was found in peaks with mol. wt. of about 110 and 65 kilodaltons. Catalytic subunits in these peaks are perhaps in the form of a complex with certain proteins that are not receptors for cAMP, which explains the difference in their molecular weight. The experimental results thus give grounds, in our opinion, for a number of suggestions concerning ontogenetic changes in cytoplasmic cAMP reception in the kidney. On the basis of estimation of the affinity constants and the results of gel filtration of protein kinases in the presence of a low cAMP concentration it can be concluded that reception of cAMP in each age group is effected mainly by regulatory subunits of one particular type that is characteristic of the given age. cAMP receptors are evidently mainly macromolecular complexes in which, besides regulatory subunits of cAMP-dependent protein kinases, there are also nonreceptor proteins. During maturation of kidney function the ability of the receptor complexes to dissociate under the influence of cAMP increases, and this may be connected with the development of sensitivity of the renal epithelium to the action of hormones.

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CHANGES IN PURINE METABOLISM IN MOUSE MACROPHAGES DUE TO A NEW SYNTHETIC ANALOG OF MURAMYL DIPEPTIDE

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KEY WORDS: macrophages; immunomodulator; 5'-nucleotidases; adenosine deaminase.

Recently the attention of many investigators has been concentrated on the study of the immunomodulating properties of muramyl dipeptide (N-acetyl-muramyl-L-alanyl-D-isoglutamine; MDP), a minimal chemical component of the glycopeptide of bacterial cell walls, which exhibits adjuvant activity [4]. This preparation increases the cytolytic activity of mouse peritoneal macrophages in vivo and in vitro against both bacteria [14] and tumor cells [7]. It was shown previously that N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP), a new analog of MDP, has a marked antitumor action, and with respect to several parameters,

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