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AGE DIFFERENCES IN HORMONAL REGULATION OF Na^+,K^+-ATPASE ACTIVITY IN THE RAT RENAL CORTEX

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One of the chief regulators of sodium transport in the renal tubules is aldosterone. The effect of aldosterone is due to regulation of expression of aldosterone-dependent genes as a result of interaction between hormone–receptor complexes and the nucleus of the target cell; aldosterone is known to bind with receptors of two types: high-affinity mineralocorticoid and low-affinity glucocorticoid [12]. The most important of the proteins induced by aldosterone is the enzyme Na^+,K^+-ATPase, responsible for active transport of Na^+ and K^+ ions through the basolateral membrane of the epithelial cells of the nephron. Na,K-ATPase is a tetramer composed of two types of subunits, α and β. In mammals born blind (including rats), definitive morphological and functional formation of the kidneys is complete in the postnatal period, and at an early age the epithelium of the nephron is insensitive to the regulatory action of aldosterone. The ability of rat kidneys to change Na^+ reabsorption in response to aldosterone is manifested only at the end of the period of weaning, i.e., after the 20th-25th day of postnatal life [1, 13]. It was shown previously that mineralocorticoid receptors are present in the cytosol of the renal cortex of 10-day-old rats [2, 4]. However, nothing has previously been published on the localization of the mineralocorticoid receptors along the nephron in early postnatal ontogeny. In adult animals the main target for the action of aldosterone is known to be

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the distal convoluted tubules and cortical collecting tubules [8]. It is there that the aldosterone-regulated reabsorption of sodium takes place. It can be tentatively suggested that in rats in the preweaning period, the hormonal resistance of the kidneys is determined either by the pattern of location of mineralocorticoid receptors in different segments of the nephron, developing at the level of regulation of gene transcription and (or) translation of aldosterone-induced proteins.

The aim of this investigation was to study specific aldosterone binding in the distal convoluted tubules and cortical collecting tubules of the nephron of 10-day-old rat kidneys, while the kidney is still insensitive to aldosterone, and in adult animals. The second part of the work was devoted to a study of age differences in hormonal regulation of activity of Na,K-ATPase, as the principal aldosterone-induced protein that determines its physiological effect.

EXPERIMENTAL METHOD

Male Wistar rats aged 10 days and 2 months were used. Aldosterone reception was studied on isolated distal convoluted tubules and cortical collecting tubules, obtained by microdissection from perfused kidneys by the method in [8]. Specific aldosterone binding was determined by competitive displacement. Isolated segments of tubules (20 mm of each per sample) were incubated for 20 min at 37°C with ^{3}H-aldosterone (10^{-9} M; 46 Ci/mmole, from "Amersham," Great Britain) in the presence of unlabeled aldosterone (10^{-7} M; from "Sigma," USA), and without it. Glucocorticoid receptors were blocked by the use of the synthetic glucocorticoid RU-38486 (10^{-7} M; "Roussel Uclaf," France). After the end of incubation the sample was washed twice to remove unbound hormone and the residue was solubilized with 1% Triton X-100 solution and transferred into scintillation flasks. Synthetic binding was determined as the difference between total and nonspecific binding in the presence of the competitor, and expressed in fmoles ^{3}H-aldosterone/mm length of tubule. Na,K-ATPase activity was determined in the membrane fraction of the renal cortex of adrenalectomized rats. Triple hormonal induction was carried out on the adult animals 24 h, and on the 10-day-old animals 5 h after the operation, with an interval of 3 h between sessions. All the rats were divided into three groups: animals of group 1 (control) were given intraperitoneal injections of 8% alcoholic physiological saline; animals of group 2 received injections of aldosterone (5 μg/100 g body weight) in 8% alcoholic physiological saline; animals of group 3 were given combined injections of aldosterone in the same dose and spironolactone (5 mg/100 g body weight). The animals were killed 2 h after the last injection by decapitation, the membrane fraction was isolated from the kidneys by the method in [10], and frozen at −70°C until required for determination. Activity of Na,K-ATPase was determined by measuring the amount of inorganic phosphate liberated during hydrolysis of ^{32}P-γ-ATP [7]. Activity of Na,K-dependent ouabain-inhibited ATPase was determined as the difference between total ATPase activity and activity of ouabain-sensitive ATPases. Nonenzymic hydrolysis of ATP in a medium not containing membrane fraction was estimated in preliminary experiments, and the kinetic curve of ^{32}P accumulation also was plotted. The membrane fraction was incubated for 10 min at 30°C in incubation medium (50 mM NaCl; (sic) mM KCl; 10 mM $MgCl_2$; 1 mM EGTA; 100 mM Tris-HCl; 3.45 mM Na_2-ATP; 5 μCi ^{32}P-γ-ATP; 148 PBq/mmole in the presence and absence of 1 mM ouabain. The reaction was stopped by addition of cold 10% TCA solution. To separate ^{32}P from unhydrolyzed ATP, centrifuge microcolumns 20 μl in volume, filled with activated charcoal, 5% in 0.1 M phosphate buffer, were used, with a "Beckman" centrifuge (80,000 rpm) for 1 min. The filtrate thus formed was transferred into scintillation flasks and their radioactivity determined by liquid scintillation counting. Na,K-ATPase activity was calculated as the difference between the increase in total ATPase activity, during the 10 min after the beginning of incubation, and the increase in enzymic activity of ouabain-insensitive ATPases, and expressed in μmoles inorganic phosphate (P_i)/mg protein/h/100 μl. The protein concentration in the membrane fraction was determined by Bradford's method [5]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The experiments revealed the presence of specific mineralocorticoid receptors in the isolated distal convoluted tubules and the cortical collecting tubules of the renal nephron of 10-day-old rats. Specific aldosterone binding in these parts of the nephron did not differ significantly in 10-day-old and adult rats, namely 0.26 ± 0.04 ($p = 9$) and 0.22 ± 0.03 ($p = 8$) fmoles ^{3}H-aldosterone/mm length of tubule respectively (Fig. 1).

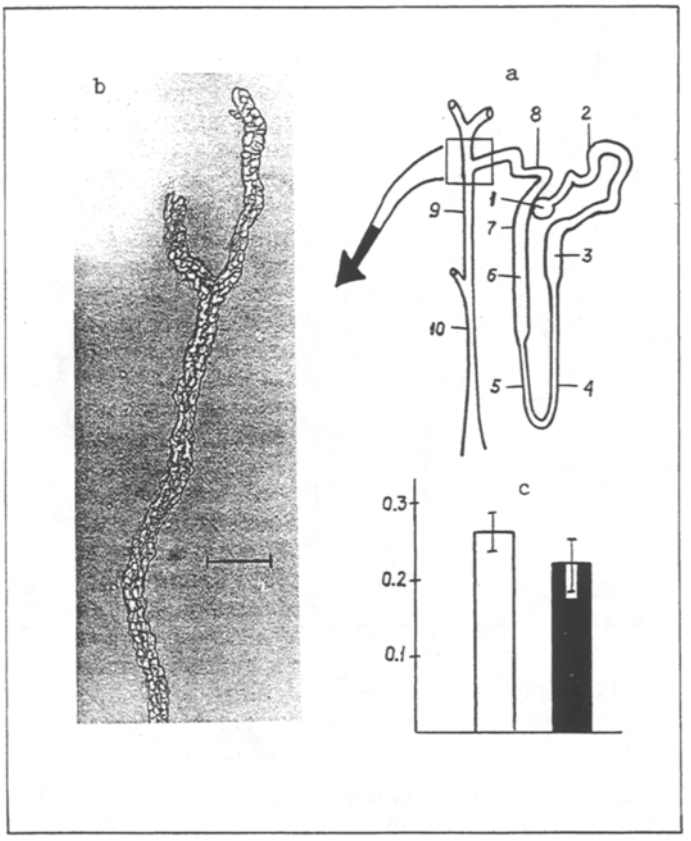

Fig. 1. Specific aldosterone binding in isolated segments of rat renal nephron: a) diagram of structure of mammalian renal nephron: 1) glomerulus; 2) proximal convoluted tubule; 3) proximal straight tubule; 4) thin descending part of Henle's loop; 5) thin ascending part of Henle's loop; 6) medullary thick ascending part; 7) cortical thick ascending part; 8) distal convoluted tubule; 9) cortical collecting tubule; 10) medullary collecting tubule; b) fragment of distal convoluted tubule and cortical collecting tuhule obtained by microdissection (length of section 100 μ, magnification 170); c) specific binding of ^{3}H-aldosterone ($1 \cdot 10^{-9}$ M) in isolated segments of rat renal nephrons. Abscissa, binding (in fmoles ^{3}H-aldosteroneimm length of tubule). Unshaded columns – 10-day-old rats; black columns – adult rats.

The writers showed previously that specific aldosterone binding in the cortical cytosol of "immature" kidneys is higher than in adult animals [2]. This was evidently due to the disproportionate growth of individual segments of the nephron observed during ontogeny. For instance, it has been shown that the relative fraction of distal tubules in which mineralocorticoid receptors are concentrated is greater in 10-day-old rats than in adult rats [9].

Thus, at the age of 10 days, specific receptors capable of binding aldosterone are already present in the distal convoluted tubules and cortical collecting tubules of rats. Consequently, the hormonal resistance of the kidneys in the preweaning period can hardly be linked with age differences in the localization of mineralocorticoid receptors along the nephron. It can therefore be postulated that absence of hormonal competence of the kidneys of 10-day-old rats is due to immaturity of the postreceptor stages in the action of aldosterone and, in particular, differences in regulation of Na,K-ATPase activity.

Na,K-ATPase activity in the membrane fraction of kidneys of the control group of animals was thus found to increase significantly with age from 0.39 ± 0.06 in young rats to 0.72 ± 0.10 μmole P_i/mg protein/h/100 μl ($p < 0.05$) in adult rats (Fig. 2). Induction by aldosterone causes a significant increase in Na,K-ATPase activity in both 10-day-old and adult animals, by about three times compared with the control level, to 1.53 ± 0.26 and 2.44 ± 0.28 μmoles P_i/mg protein/h/100 μl ($p < 0.01$; $p < 0.05$) respectively (Fig. 2). However, it is not known to which type of

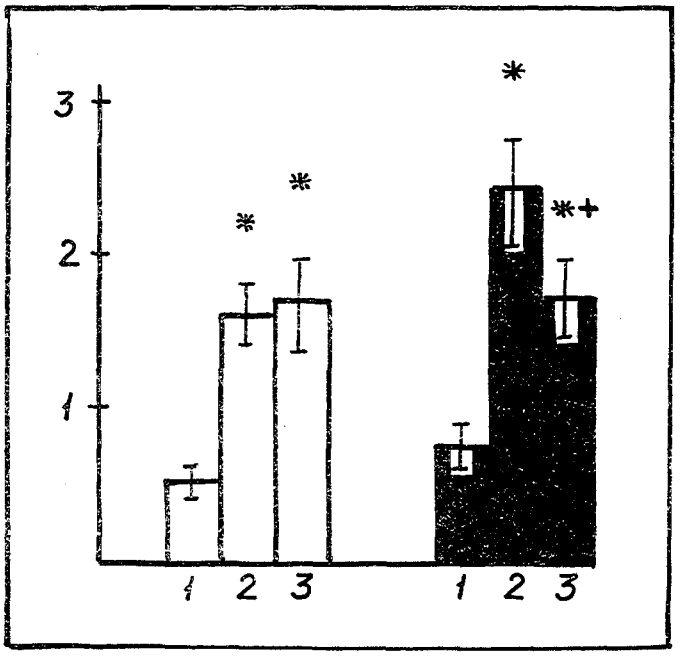

Fig. 2. Na,K-ATPase activity in membrane fraction of renal cortex of control group of rats (1); of rats induced by aldosterone (2), and rats induced by aldosterone and spironolactone (3). Abscissa, activity in μmoles P_i/mg protein/h/100 μl. Unshaded columns – 10-day-old rats; black columns – adult rats. Asterisk indicates effect of hormonal induction compared with control level ($p < 0.05$); plus sign indicates effect of spironolactone on action of aldosterone ($p < 0.01$).

receptor aldosterone exerts its effect in the immature kidney. To examine this problem, we used a model of combined induction of the enzyme by aldosterone and spironolactone. Spironolactone is an aldosterone antagonist, it binds with type I receptors to form an inactive complex, which does not penetrate into the nucleus [11]. The results showed that spironolactone significantly depresses growth of Na,K-ATPase activity induced by aldosterone in adult animals, and does not lead to any similar effect on 10-day-old rats (Fig. 2).

Regulation of expression of mRNA of the α- and β-subunits of Na,K-ATPase was investigated in the writers' laboratory on the same model of hormonal induction, and it was found that spironolactone also causes a decrease in the mRNA concentration of both subunits in adult animals without affecting it in 10-day-old rats [3]. Thus age differences in hormonal regulation of expression of mRNA of α- and β-subunits of the enzyme are in agreement with our own observations on regulation of activity of the enzyme.

In adult animals the effect of aldosterone is evidently realized mainly through interaction with mineralocorticoid receptors, although a certain part of the signal also is conducted through glucocorticoid receptors. The absence of an inhibitory effect of spironolactone on 10-day-old rats is evidence that glucocorticoid receptors are mainly involved in regulation of activity of the enzyme. In other words, although in rats of preweaning age mineralocorticoid receptors capable of interacting with aldosterone are already present, they do not participate in this period of development in hormone-controlled sodium reabsorption. Possibly mineralocorticoid receptors, combined with aldosterone, induce another domain of the genes, and thereby regulate processes of growth and differentiation of kidney tissue. We know, for instance, that aldosterone can induce thymidine kinase synthesis in the kidneys of 22-day-old rats [6]. Thymidine kinase is an enzyme participating in DNA synthesis, and which can directly affect ontogenetic processes in the genome. It can therefore be postulated that aldosterone in early ontogeny performs a different function from that in the adult state.

The results thus suggest that age differences discovered in the mechanism of hormonal regulation of Na,K-ATPase may lie at the basis of the absence of a physiological reaction of the kidney to aldosterone in early postnatal ontogeny.

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CORRELATION BETWEEN CELL COMPOSITION OF THE SPLEEN AND CHANGES IN SPLENOCYTE CHEMILUMINESCENCE AFTER LASER IRRADIATION

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KEY WORDS: laser radiation; semiconductor laser; mouse splenocytes; chemiluminescence

Low-intensity laser therapy (He–Ne laser, λ = 632.8 nm, and various semiconductor lasers, λ = 800-900 nm) has been successfully used in the treatment of diseases connected with various kinds of inflammatory processes [4, 6]. In particular, laser irradiation leads to more rapid healing of wounds, trophic ulcers, and burns. During wound healing an active role is played by neutrophils and macrophages, which rid the injured part of infection, and also by lymphocytes and fibroblasts. During phagocytosis active forms of oxygen (AFO) are formed: the superoxide anion-radical, hydroxyl radical, hydrogen peroxide, etc., which perform a bactericidal function [2, 8]. It has also been shown that certain populations of lymphocytes [9], epidermal cells [5], and others have the ability to generate AFO. The appearance of AFO can be recorded by measuring the chemiluminescence (Chl) which accompanies this process, and which is magnified many times over in the presence of luminol.

Intact cells possess spontaneous chemiluminescence (SChl), which reflects the initial state of metabolic processes in the cell [2]. Under the influence of various stimuli, the chemiluminescent response may alter, i.e., the quantity of AFO generated by cells can increase or decrease [2]. The writers showed previously [3, 7] that irradiation by low-intensity red light (λ = 632.8 nm) within the dose range 100-300 J/m^2 stimulates AFO formation in mouse spleen cells. The aim of this investigation was to study the action of infrared laser radiation on chemiluminescence of splenocytes and to study how the effects of irradiation depend on the cell composition of the irradiated suspension.

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