Micropuncture Study of Ion and Water Reabsorption Regulation Range in the Distal Tubule of Triton Nephron

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Micropuncture of the distal tubule in triton nephron and ultramicroanalysis of samples showed that vasotocin stimulates transport of Ca^{2+}, Na^{+}, Mg^{2+}, and Cl^{-} from the nephron lumen and increases permeability of the tubular wall for water. Prostaglandin E_2 suppresses these processes.

Key Words: *micropuncture; triton; vasotocin; indomethacin; prostaglandin E_2*

Each physiological function, including kidney activity, is characterized by a wide range of variations. The higher is the effectiveness of kidney function, the wider is the range of differences in urine composition and the narrower is the range of variations in physicochemical properties of internal fluids [8]. It is interesting to assay qualitatively the range of changes in study parameters, determine the extent of physiological regulation, and estimate the responding sites for regulatory factors in various regions of renal tubules. Hormones, transmitters, and autacoids are involved in the regulation of kidney function. Analysis of urine composition cannot provide the answer to specific questions. Changes in the reabsorption of ions in the proximal tubule during urine formation can be partly compensated by function of the distal nephron and collecting tubules. The effect of physiologically active substances, neurohypophyseal hormones, and autacoids [1,5,9] on absorption of water and ions in the distal nephron attracts much attention. This study will allow evaluating the range of variations in the concentration and reabsorption of substances in the individual nephron segment. Here we studied transport of water and ions in the initial region of the distal renal tubule after treatment with the neurohypophyseal hormone arginine-vasotocin (AV) and autacoid prostaglandin (PGE_2). Prostaglandin synthesis was suppressed with cyclooxygenase inhibitor indomethacin.

MATERIALS AND METHODS

Experiments were performed on *Triturus vulgaris L.* tritons weighing 1.8-2.4 g (Leningrad region). The study was conducted in the fall and winter. The animals were narcotized by immersion into 0.1% tricaine (Sigma). Midline incision was made to access the surface of the kidney. The distal tubule was punctured, and the fluid was obtained under free-flow conditions [6]. A pipette was used to collect fluid samples from the glomerulus. Indomethacin (1 μM, Sigma), PGE_2 (0.03 nM, Sigma), or AV (1 nM, Sigma) was put on the surface of the punctured tubule under oil in a volume of 0.5 μl. Fluid was repeatedly collected by micropuncture of the distal tubule after 10 min. Sodium ferricyanide was used to evaluate the relative reabsorption of fluid. Ferricyanide and inulin have the same concentration indexes when administered simultaneously to the animal [6]. Plasma ferricyanide concentration should not exceed 1-2 mmol/liter. Sodium ferricyanide in isotonic solution (50 μmol/ml, 20 μl) was injected intramuscularly 40 min before micropuncture.

The concentrations of Na, Ca, Mg, Fe, and Cl in samples were measured on a Cameca M.S.-46 electron microanalyzer [6]. After the experiment 4-5 drops of each sample from the tubule and glomerulus or

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standard solution were placed on a beryllium plate under mineral oil using a calibrated micropipette (0.1 nl). The plate was emerged in cold chloroform for 20 sec to remove mineral oil. The samples were dried in air, rehydrated on a cooling table at 4°C, frozen by immediate contact with liquid nitrogen-pretreated metal, and lyophilized in a refrigeration chamber at -25-30°C for 7-10 days. During the analysis the probe of a microanalyzer was defocused by 70-90 μ. The beam intensity corresponded to a current strength of 60-80 nA (accelerating voltage 20 kV). The ratios between the signal of the standard solution containing the measured element in a concentration of 1 mmol/liter in a total volume of 0.1 nl and background signal of beryllium plate were the following: 1.1 for Na^+, 0.98 for Ca^{2+}, 0.61 for Mg^{2+}, 0.69 for Cl^-, and 1.71 for Fe^{2+}. Under these conditions the method sensitivity was 10^{-13} M.

The relative reabsorption (%) of fluid (FR_{H_2O}) and electrolytes (FR_X) was calculated by the equations: $FR_{H_2O}=(1-GF_{Fe}/TF_{Fe})\times100$, where GF_{Fe} and TF_{Fe} are the concentrations of iron in the glomerular capsule and tubular fluid, respectively; and $FR_X=(1-TF/GF)_X/(TF/GF)_{Fe})\times100$, where $(TF/GF)_X$ and $(TF/GF)_{Fe}$ are the concentration indexes for ion and iron, respectively.

The results were analyzed by Student's *t* test. Statistical treatment sometimes included the comparison of populations with paired samples ($\Delta\pm S_\Delta$).

RESULTS

Under conditions of free flow the concentrations of ions in the initial region of the distal renal tubule decreases due to their reabsorption (in mmol/liter): Na^+ from 62.9 to 34.6±5.6 mmol/liter, Cl^- from 65.0 to 25.6±7.7 mmol/liter, Ca^{2+} from 1.44 to 0.76±0.32

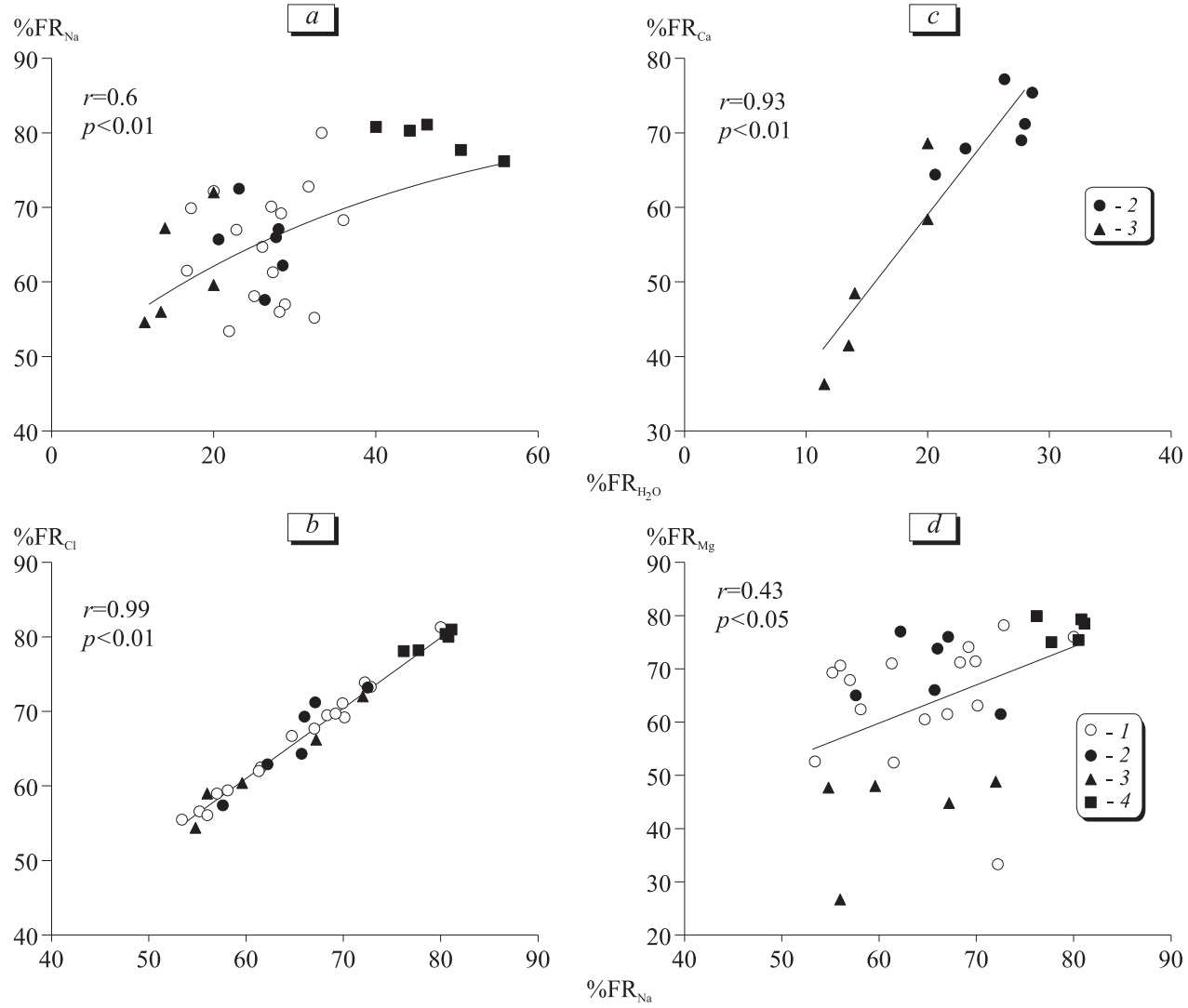

Fig. 1. Ratio between reabsorbed fractions (RF) of ions and fluid in the distal tubule of triton nephron: control (*1*), 10^{-6} M indomethacin (*2*), 10^{-7} prostaglandin E_2 (*3*), and 10^{-9} M arginine-vasotocin (*4*).

mmol/liter, and Mg^{2+} from 1.97 to 0.96±0.29 mmol/liter. The concentration of ions in the nephron lumen depends on the rate of ion absorption through the tubular wall, passive efflux of ions into the nephron from interstitial space along the electrochemical gradient, fluid flow rate in the tubule, and intensity of water absorption after reabsorption of substances, which determine the increase in the concentration of substances in the tubular fluid.

These processes depend on a variety of regulatory factors that modulate the function of ion channels, cotransporters, ion pumps, and other cell mechanisms of transepithelial transport [2,12,13].

A relationship was revealed between fractional reabsorption of Na^+ and reabsorption of fluid in the initial portion of the distal tubule ($p<0.01$, Fig. 1, *a*). Reabsorption of Na^+ and Cl^- increased and attained a maximum under the influence of AV. Application of PGE_2 to the outer surface of the tubules had an opposite effect (Fig. 1, *a*, *b*). These data show that the test substances produce different effects on the function of the distal tubule and allow evaluating the range of variations in function of the tubule. A close correlation exists between reabsorption of Na^+ and Cl^- ($r=0.99$, $p<0.01$, Fig. 1, *b*). Absorption was maximum after administration of AV, but minimum under the influence of PGE_2.

Fluid absorption depended on Ca^{2+} reabsorption (Fig. 1, *c*). Minimum values were obtained in experiments with PGE_2. After blockade of autacoid secretion with indomethacin, the data were distributed to the upper part of this diagram (Fig. 1, *c*). These data suggest that PGE_2 and, probably, other eicosanoids serve as the regulators of Ca^{2+} reabsorption in this region of the nephron.

It was interesting to compare Na^+ and Mg^{2+} reabsorption (Fig. 1, *d*) in view of known relation of transport of these ions with Mg^{2+} secretion in the kidney of marine bony fishes and migratory fishes [10]. Our previous experiments showed that the increase in secretion of Mg^{2+} in fish nephron is accompanied by an equimolar increase in reabsorption of Na^+ [10]. The higher is Na^+ absorption, the higher is the amount of Mg^{2+} transported by tubular cells from the lumen to blood ($p<0.05$), *i.e.* in triton kidney this process resembles cotransport, but not antiport.

Chemical evolution of nonapeptides in vertebrates (vasotocin, hydrin 2, and vasopressin) was not accompanied by changes in their functional role [3]. These hormones are involved in the regulation of water-salt metabolism in amphibians, reptilians, birds, and vertebrates [5,11]. It should be emphasized that not only neurohypophyseal hormones, but also autacoids (*e.g.*, eicosanoids) regulate intracellular transport of water and ions in renal tubules and other osmoregulatory organs [7]. These substances act as functional antagonists of neurohypophyseal hormones [13], decrease osmotic permeability [2,9], and inhibit transport of Na^+ [5,13] and Mg^{2+} [1]. The study of the distal tubule in triton kidney showed that the indexes of ion transport correspond to opposite points on the same line. These results suggest that the hormone (AV) and autacoid (PGE_2) regulate activity of the same cells, but produce different effects on their physiological functions. AV stimulates ion transport and increases permeability for water, while PGE_2 suppresses both processes. Experiments on the distal tubule of the nephron allowed us to develop a new approach to study the range of variations in ion concentration under physiological regulation of kidney function.

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