Short-term effect of aldosterone on renal sodium transport and tubular Na-K-ATPase in the rat

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Abstract. The short-term effect of one single injection of aldosterone on the renal sodium transport on one hand, and the Na-K-ATPase activity on the other hand, was studied in chronic adrenalectomized rats. Sodium transport was estimated by clearances, and Na-K-ATPase was measured in microdissected fragments of the nephron, according to our microtechnique previously described. Five to eight days after adrenalectomy, only 30% of the initial enzyme activity was recovered in the cortical collecting tubule (CCT). Administration of aldosterone completely restored the ATP-ase activity within three hours. Adrenalectomy also curtailed by 20–45% the activity of other nephron segments but aldosterone had no stimulatory effect on them. Sodium-reabsorption also increased after the hormone injection, following the same time (0.5 < t½ < 1 h) and dose dependencies (0.8 < K½ < 0.9 µg/kg) as those observed for the enzyme activity in the CCT. It is concluded that the short-term stimulation of Na-K-ATPase in the collecting tubule, after an acute administration of aldosterone, may be responsible for the simultaneous increase in sodium transport.

Key words: Adrenalectomized rats - Sodium clearance - Nephron microdissection - Mineralocorticoids

Introduction

The involvement of the sodium pump in the effect of mineralocorticoids on the renal transport of sodium is still controversial. A relationship between the mineralocorticoid status and the renal Na-K-ATPase activity was first mentioned by Chignell et al. [3, 4] who observed a decrease of the enzyme activity in rat kidney homogenates after adrenalectomy. This initial observation was confirmed by several authors [2, 5, 14, 15, 17–19, 21, 22, 28, 30–32, 36]. However, conclusions concerning the effect of corticosteroids on the Na-K-ATPase are conflicting: whereas some studies favorizes the hypothesis of a direct action of the hormones upon the Na-K-ATPase [15, 21, 32] others sustain that sodium entry in the tubular cell is a necessary intermediate step for this action [2, 14, 18, 19, 22, 28, 36].

The discrepancy between these results may be ascribed to methodological variations. The high doses of corticoids used in some of these studies might result in the occupancy of both mineralocorticoid and glucocorticoid receptors, and secondarily induce their respective physiological effects. Also, most of the Na-K-ATPase determinations reported in literature were performed in homogenates or in membrane suspension. Detection of the steroid effect on these preparations might be masked by the heterogeneity of the structures and the lack of responsiveness of some of them.

The purpose of the present study was to examine the time- and dose-response of the renal Na-K-ATPase activity, to a range of physiological doses of aldosterone, in microdissected segments of nephrons from adrenalectomized rats. Results were compared to the corresponding changes in sodium reabsorption measured in another series of adrenalectomized animals receiving the same doses of aldosterone.

Results indicate that aldosterone administration provokes an increase of the Na-K-ATPase activity in the cortical collecting tubule exclusively. The enzyme stimulation and the increase in sodium transport are closely correlated in time, and although the complete induction of Na-K-ATPase activity occurs later than the peak of the sodium retaining effect, both phenomena are initiated concomitantly, and are dose-related to aldosterone in a similar way.

Methods

Animals

Experiments were performed on male Wistar rats weighing 180–270 g and fed a standard laboratory diet. Animals were adrenalectomized under light ether anesthesia, through a lumbar approach, and were allowed to recover from surgery for 5–8 days before study. They had free access to both tap water and a solution of 0.9 % NaCl during this period. Sham-operated animals were kept under similar conditions. Adequacy of adrenalectomy was assessed prior to the study by the registration of the body weight: the adrenalectomized rats gained less weight than sham-operated controls during the recovery period (sham-operated: 5.0 ± 0.3 g/day ± SE, n = 25; ADX: 2.1 ± 0.2, n = 92; P < 0.001). In some experiments blood samples were taken immediately after death for aldosterone measurement.

Renal function

Glomerular filtration rate and sodium and potassium excretion were measured in sham-operated and adrenalectomized rats. The animals were anesthetized with Inactin (Byk Gulden Pharmazeutica, Konstanz, FRG; 10 mg/100 g body wt. i.p.) and a tracheostomy was performed. Polyethylene catheters (PE 50) were introduced into a jugular vein for
solution infusion, into a carotid artery for blood sampling and into the bladder. After administration of a priming dose of insulin (2 - 3 μCi of [3H]-insulin in 1 ml of 0.9% NaCl solution), animals were perfused with 0.9% saline solution containing [3H]-insulin (2 - 3 μCi/ml) at a rate of 40 μl/min⁻¹. After 60-min equilibration period, urine was collected for 5 - 7 consecutive 30-min periods. Blood was obtained from the carotid artery in the middle of each collection period. The body temperature of animals was monitored within physiological range during the whole experiment. Aldosterone was injected through the jugular vein at the appropriate concentrations (0.5, 1.0, 2.0, 5.0 or 10.0 μg/kg body wt.), in 100 - 200 μl of saline at the beginning of the third collection period. Urinary sodium and potassium were determined by flame photometry (Eppendorf, Hamburg, FRG) and plasma and urine labeled insulin were measured using a liquid scintillation spectrometer (Model LS 7500, Beckman, Paris, France). Counting efficiency was monitored by the sample ratio method. Glomerular filtration rate (GFR) was measured by the clearance of inulin, and the excretion of sodium and potassium were expressed as a function of GFR determined during the same period.

Tubule preparation

Microdissection and ATPase determinations were performed in animals treated in the same manner as for clearance studies. Aldosterone (or an identical volume of vehicle) was injected through the jugular vein 15 - 30 min after starting the perfusion. The animals were perfused for 0.5 - 3 h following the hormone administration, and heparchinized immediately before death.

Kidney perfusion and tubule microdissection were performed according to the technique previously described [7, 20]. Animals were bled from the abdominal aorta and plasma was separated for aldosterone determination. The left kidney was perfused via the abdominal aorta with an ice-cold collagenase solution (NaCl, 137 mM; KCl, 5 mM; MgSO₄, 0.8 mM; Na₂HPO₄, 0.33 mM; KH₂PO₄, 0.44 mM; MgCl₂, 1 mM; Tris-HCl, 10 mM; CaCl₂, 1 mM; collagenase, 149 U/ml, 0.7 mg/ml and bovine serum albumin, 1.5 mg/ml; pH, 7.4). Small pieces of kidney tissue were cut along the corticomediullary axis, incubated in collagenase solution for 25 min at 37°C and rinsed with cold microdissection solution (identical to the collagenase solution except that albumin and collagenase were omitted and CaCl₂ was 0.25 mM).

The nephron segments were identified according to their topography and morphology criteria, dissected, transferred to a concave bacteriological slide and photographed in order to determine their length (0.5 - 1.5 mm). Samples were stored on ice until the assay.

Na-K-ATPase assay

The procedure for Na-K-ATPase determination was reported in detail elsewhere [7] and will be summarized here. In order to increase membrane permeability and to remove all the cations from the medium, the single pieces of tubule were soaked in distilled water and rapidly frozen on dry ice. After removal of the water, incubation was carried out for 15 - 20 min at 37°C in 1 μl of assay medium and terminated by cooling and adding trichloroacetic acid. Radiolabeled phosphate released by the hydrolysis of [γ-32P]ATP was separated from the nucleotide by adding activated charcoal which binds the unhydrolyzed ATP, and filtered through a Millipore filter (HAWPO 1300, Millipore, Molsheim, France). The eluate containing the inorganic phosphate was collected directly into counting vials.

The composition of the incubation medium for total ATPase measurements was: NaCl, 50 mM; KCl, 5 mM; MgCl₂, 10 mM; EGTA, 1 mM; Tris-HCl, 100 mM; Na₂-ATP, 10 mM; [γ-32P]ATP, 2 - 10 Ci/mmol, in tracer amounts (~5 nCi/μl). For determination of the ouabain-insensitive, magnesium dependent ATPase activity (Mg-ATPase), NaCl and KCl were omitted, Tris-HCl was 150 mM, and ouabain, 1 mM, was added. The pH of both solutions was 7.4.

All determinations were performed in 4 - 7 duplicates and results were expressed as picomoles of inorganic phosphate released per millimeter of tubule length per hour.

Aldosterone determination

Aldosterone was determined in the plasma of sham-operated and adrenalectomized rats given either diluent or 5 μg/kg body wt aldosterone. Blood was collected from the abdominal aorta in a heparinized syringe immediately prior to the death. Aldosterone treated animals were sacrificed 0.5, 1.0 or 3.0 h after intravenous administration of the hormone. Radioimmunoassay of aldosterone was performed according to Pham Huu Trung and Corvol [29].

Statistics

All results are presented as means ± SE and were evaluated by the Student t-test for unpaired data.

Results

Plasma aldosterone concentration

A shown in Table 1, the adrenalectomized animals have low circulating aldosterone. Thirty minutes after the administration of 5 μg/kg aldosterone, the plasma concentration reaches 3-fold higher levels than in the sham-operated animals. At the end of the second 30-min period, the hormone concentration decreased to the control animals level, and completely disappeared within three hours.

Clearance studies

The clearance of inulin and the sodium and potassium excretion related to GFR in seven adrenalectomized (ADX) and nine sham-operated rats remained constant through the whole experiments (Fig 1). Adrenalectomy resulted in a significantly lower glomerular filtration rate (sham-operated: 2.33 ± 0.09 ml/min⁻¹ ± SE, n = 9; ADX: 1.54 ± 0.21 ml/min⁻¹, n = 7; P < 0.005). Sodium excretion expressed as a function of GFR (U₉₄/V/GFR) was relatively high in adrenalectomized animals (4.06 ± 1.00 mEq/min⁻¹ ± SE, n = 7) compared to the control rats (1.15 ± 0.32 mEq/min⁻¹ ± SE, n = 9; P < 0.005). However potassium excretion was similar in the two groups of animals (sham-operated: 1.59 ± 0.21 mEq/min⁻¹, n = 7; ADX: 1.59 ± 0.21 mEq/min⁻¹, n = 7) and the slight increment in U₉₄/V observed after adrenalectomy entirely resulted from the decrease in GFR. In contrast U₉₄/V was higher in ADX compared to sham-operated rats (sham-operated: 2.63 ± 0.68 mEq/min⁻¹ ± SE, n = 9; ADX: 5.87 ± 1.23 mEq/min⁻¹, n = 7; P < 0.05).