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Altered regulation of renal nitric oxide, atrial natriuretic peptide and cyclooxygenase systems in aldosterone escape in rats

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ABSTRACT

The present study was aimed to determine whether there is an altered role of local nitric oxide (NO), atrial natriuretic peptide (ANP) and cyclooxygenase (COX) systems in the kidney in association with the aldosterone escape. Male Sprague–Dawley rats were used. Aldosterone (200 μg/day) was infused through entire time course. The control group was kept on a low sodium diet (0.02 mEq/day), and the experimental group was supplied with a higher sodium diet (2.0 mEq/day). Four days after beginning the regimen, the kidneys were taken. The protein expression of NO synthase (NOS) and COX isoforms was determined by semiquantitative immunoblotting. The mRNA expression of components of ANP system was determined by real-time polymerase chain reaction. The activities of soluble and particulate guanylyl cyclases were determined by the amount of cGMP generated in responses to sodium nitroprusside and ANP, respectively. There developed aldosterone escape in the experimental group. Accordingly, the renal content and the urinary excretion of NO increased. The expression of nNOS was increased in the inner medulla. Neither the expression of eNOS nor that of iNOS was changed. The expression and the catalytic activity of soluble guanylyl cyclase remained unaltered. The mRNA expression of ANP was increased. Neither the expression of NPR-A or NPR-C nor the activity of particulate guanylyl cyclase was altered in the papilla. The protein expression of COX-2 was increased in the inner medulla, while that of COX-1 remained unchanged. In conclusion, the upregulation of nNOS, ANP, and COX-2 may be causally related with the aldosterone escape.

1. Introduction

The regulatory mechanisms which are responsible for overriding the sodium-retaining effects of mineralocorticoids have been termed as "escape" [1,2]. The escape has been attributed to the expansion of the effective vascular volume induced by salt and water retention and the resultant increase in arterial pressure, leading to increased sodium excretion [3]. It has now become clear that there is an activation of various local natriuretic systems including nitric oxides (NO), atrial natriuretic peptides (ANP), and prostaglandins (PG) to facilitate the escape [4–6].

The net effect of NO system in the kidney is to promote natriuresis and diuresis, contributing to the renal adaptation to variations of salt intake [7]. An increased synthesis of NO stimulated by increased renal perfusion pressure has been shown to contribute to the escape from mineralocorticoids [4]. However, there may be a controversy concerning the causative role of NO in aldosterone escape [8]. Moreover, the regulation of different isoforms of NO synthases (NOS) has not been established in aldosterone escape.

ANP may influence tubular sodium reabsorption through changes in medullary blood flow and sodium reabsorption [9]. An increase of plasma ANP levels was suggested as a mediator of aldosterone escape [5]. However, the natriuretic response to ANP does not correlate well with its plasma levels after acute intravenous saline infusion [10]. In addition, a substantial increase in circulating ANP for several days by chronic intravenous ANP infusion produces no changes or trivial increases in urinary sodium excretion [11]. These observations suggest a role of local ANP system, rather than circulating ANP, in renal handling of sodium homeostasis.

The urinary PG excretion is also increased along with the mineralocorticoid escape, and a transient sodium retention may be induced by indomethacin [12]. The synthesis of PG is catalyzed by cyclooxygenases (COX), two different isoforms which have been characterized [13]. Although COX-1 and COX-2 share similar enzymatic properties, they differ markedly with respect to cellular expression pattern and regulation [14,15]. They may be differentially regulated in aldosterone escape.

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Table 1
Renal functional data (Protocol 1).

	Control ($n=8$)	Escape ($n=8$)
Body weight (g)	258 ± 7	264 ± 21
UO (mL/d)	11.9 ± 1.3	11.6 ± 2.1
P_{cr} (mg/dL)	0.20 ± 0.05	0.20 ± 0.07
C_{cr} (mL/min)	2.3 ± 0.5	2.5 ± 0.5
P_{Na} (mEq/L)	142.6 ± 0.9	144.7 ± 0.8*
$U_{Na} \times UO$ (mEq/d)	0.22 ± 0.02	1.82 ± 0.47*
FE_{Na} (%)	0.05 ± 0.01	0.35 ± 0.11*
Plasma aldosterone (ng/dL)	878 ± 209	912 ± 153

Values are expressed as mean ± SEM. These values are measured at the last day of experiments (on day 4). UO, urine output; P_{cr}, plasma creatinine; C_{cr}, creatinine clearance; P_{Na}, plasma sodium; $U_{Na} \times UO$, daily urinary sodium excretion; FE_{Na}, fractional excretion of sodium. *$p<0.05$ compared with control.

The present study was aimed to determine whether there is an altered regulation of local NO, ANP and COX systems in the kidney in association with the aldosterone escape.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 180–200 g were used. Rats were kept in a temperature-controlled room regulated on a 12:12-h light–dark cycle. The study was approved by the Ethics Committee of Chonnam National University Medical School.

In Protocol 1, the escape was induced as described by previous investigators [16]. The rats were anesthetized with ketamine (90 mg/kg)/xylazine (10 mg/kg) and subcutaneously implanted with osmotic minipumps (Alzet, Model 2ML2; Palo Alto, CA, USA) delivering 200 μg per day of aldosterone (Sigma; St. Louis, MO, USA). The minipump was sustained throughout the experiment. The intake of sodium was initially maintained at a very low level (0.02 mEq/d) by ration feeding of measured amounts of a gelled mixture of low-Na food (Ziegler Brothers; Gardner, PA, USA) and water. After 3 days, they were then divided into 2 groups: the one was switched to a higher Na intake (2.0 mEq/d, aldosterone escape group), whereas the other was kept on the same low intake (0.02 mEq/d, control group).

The rats were maintained individually in the metabolic cage to allow urine collections for the measurement of Na^+ and creatinine. Under anesthesia with isoflurane, blood samples were collected from the inferior vena cava and analyzed for plasma levels of Na^+ and creatinine. The right kidney was removed, dissected into three zones [cortex/outer stripe of outer medulla (Cortex/OSOM), inner stripe of outer medulla (ISOM) and inner medulla], and processed for immunoblotting. The left kidney was removed and assayed for mRNA expression by real-time polymerase chain (PCR) reaction.

In Protocol 2, the experiment was made to investigate the effects of different levels of sodium intake, with no aldosterone infused. The intake of sodium was initially maintained at a very low level (0.02 mEq/d) for 4 days. Thereafter, they were divided into 2 groups: the one was kept on low sodium intake (0.02 mEq/d, NaCl-restricted group), and the other was switched to a higher sodium intake (2.0 mEq/d, NaCl-replete group). The kidneys were taken and processed for immunoblotting and real-time PCR.

2.2. Semiquantitative immunoblotting

The dissected cortex/OSOM, ISOM and inner medulla were homogenized in ice-cold isolation solution containing 0.3 M sucrose, 25 mM imidazole, 1 mM ethylenediaminetetraacetic acid (EDTA), 8.5 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF), with pH 7.2. The homogenates were centrifuged at 1000 *g* for 15 min at 4 °C to remove whole cells, nuclei and mitochondria. The total protein concentration was measured by BCA assay kit (Pierce; Rockford, IL, USA). All samples were adjusted with isolation solution to reach the same final protein concentrations. They were then dissolved at 65 °C for 15 min in SDS-containing sample buffer and stored at −20 °C. To confirm equal loading of protein, an initial gel was stained with Coomassie blue. SDS-PAGE was performed on 9 or 12% polyacrylamide gels.

The proteins were transferred by gel electrophoresis (Bio-Rad, Mini Protean II; Hercules, CA, USA) onto nitrocellulose membranes (Amersham Pharmacia Biotech, Hybond ECL RPN3032D; Little Chalfont, UK). The blots were subsequently blocked with 5% milk in PBS with tween 20 (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and incubated overnight at 4 °C with primary antibodies, followed by incubation with secondary anti-rabbit (Dako; Glostrup, Denmark) or anti-mouse (Dako) horseradish peroxidase-conjugated antibodies. The labeling was visualized by an enhanced chemiluminescence system. The monoclonal antibodies to eNOS, nNOS, and iNOS (Transduction Laboratories; Lexington, KY, USA), soluble guanylyl cyclase (sGC; Santa Cruz Biotechnology; Santa Cruz, CA, USA), and COX-1, and -2 (Cayman Chemical; Ann Arbor, MI, USA) were commercially obtained.

2.3. Colorimetric assay of nitrite/nitrate

As an index of synthesis of NO, its stable metabolites (nitrite/nitrate, NOx) were measured by a colorimetric NO assay kit (Oxford Biochemical; Oxford, MI, USA). Microplate was used to perform enzyme reactions in vitro. For spectrophotometric assay of nitrite with griess reagent, 80 μl MOPS (50 mM)/EDTA (1 mM) buffer and

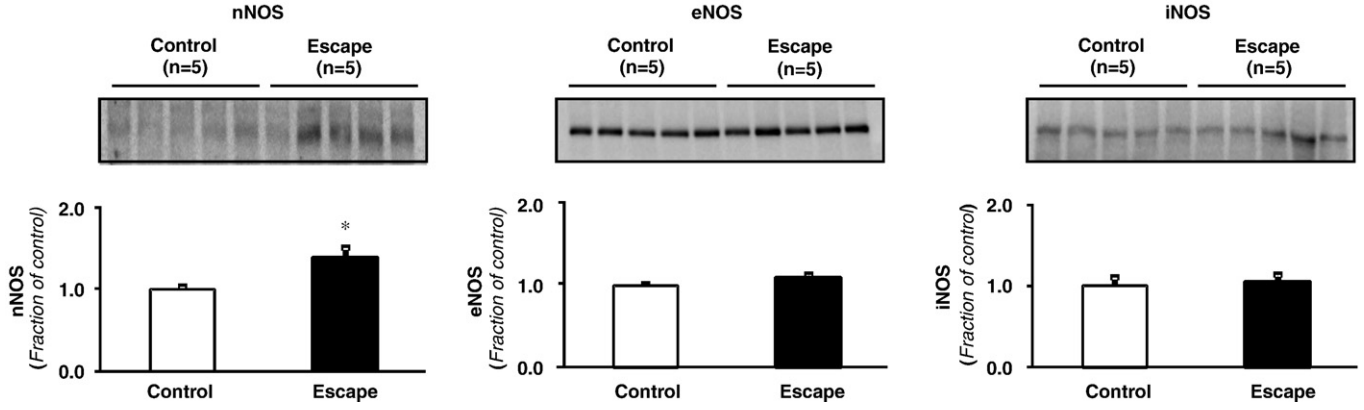

Fig. 1. Semiquantitative immunoblotting of nNOS, eNOS and iNOS in the inner medulla. *$p<0.05$ compared with control.