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Sympathetic Regulation of Aquaporin Water Channels in Rat Kidney

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Whether there exists a sympathetic neural regulation on the aquaporin (AQP) channels in the kidney was examined. Male Sprague-Dawley rats were used. They were renal nerve denervated by stripping the nervous and connective tissues passing along the renal artery and vein, and painting these vessels with 10% phenol solution through a midline abdominal incision. Three days later, the expression of AQP1-4 proteins in the denervated kidneys was determined. The content of norepinephrine was found significantly decreased following the denervation. Accordingly, the expression of AQP2 proteins was markedly decreased. The expression of AQP3 and AQP4 was also slightly but significantly decreased, while that of AQP1 was not. Neither the basal nor the AVP-stimulated accumulation of cAMP was significantly affected in the denervated kidney. It is suggested that the sympathetic nervous system has a tonic stimulatory effect on AQP channels in the kidney.

Key Words: Aquaporins, Sympathetic nervous system, Kidney

INTRODUCTION

The discovery of aquaporin (AQP) water channels has increased our understanding of how water moves across the permeable epithelia. In the kidney, at least seven isoforms of AQP channels have been known (Kwon et al, 2001). The critical role of AQP1, which is highly expressed in the proximal tubule and descending thin limb, in concentrating the urine has been well known (Ma et al, 1998). AQP2 is expressed in the principal cell of the collecting duct, contributing to reabsorption of the tubular load left by the proximal nephron (Fushimi et al, 1993; Nielsen et al, 1993). Water reabsorption across the basolateral membrane of the collecting duct is in turn mediated by AQP3 and AQP4 (Nielsen & Agre, 1995). The physiological significance of other AQP channels remains largely undetermined.

As with other biological systems, AQP channels are to be regulated. For instance, AQP2 is short-term and long-term regulated by arginine vasopressin (AVP)/cAMP pathway to increase the osmotic water permeability in the collecting duct. The short-term regulation occurs as a result of an exocytic insertion of the cytoplasmic AQP2 vesicles into the apical membrane (Nielsen et al, 1995; Yamamoto et al, 1995), whereas the long-term regulation is to increase its total abundance (Terris et al, 1996). A role for AVP/cAMP pathway in the regulation of AQP3 has been also suggested by a marked increase of its expression in response to AVP infusion (Ecelbarger et al, 1995). However, the regulatory mechanisms of AQP channels other than AVP/cAMP pathway have not been established.

On the other hand, it has long been known that the decreased cardiac output unloads the baroreceptors and activates the sympathetic nervous system in congestive heart failure (Gaffney & Braunwald, 1963). More recently, the water retention in congestive heart failure has been found associated with an increased release of AVP and subsequently increased expression of AQP2 channels in the collecting duct (Nielsen et al, 1997; Xu et al, 1997). Taken together, an interaction between the sympathetic nervous system and AQP2 channels in the kidney is suggested. An interaction, if any, may also in part account for the denervation diuresis which is unrelated to systemic or intrarenal hemodynamic changes (Bello-Reuss et al, 1975; Rogenes & Gottschalk, 1982).

The present study was aimed to examine whether AQP channels in the kidney are regulated by the sympathetic nervous system. Rats were renal nerve denervated to deplete the neuronal storage of sympathetic neurotransmitters, and the expression of AQP proteins in the kidney was determined.

METHODS

Renal nerve denervation

Male Sprague-Dawley rats (200～250 g) were used. All procedures conformed to the *Institutional Guidelines for Laboratory Animal Care and Use.* The rats were anesthetized with ketamine (50 mg/kg, intraperitoneally), and their left renal nerves were stripped of the nervous

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ABBREVIATIONS: AQP, aquaporin; AVP, arginine vasopressin.

and connective tissue passing to and along the course of the renal artery and vein, and these vessels were painted with 10% phenol solution through a midline abdominal incision. Control rats received only a midline incision, the renal nerves being left intact.

Three days later, systolic blood pressure in a conscious state was indirectly measured by tail-cuff method. The trunk blood was then taken by decapitation in a conscious state to determine the plasma levels of norepinephrine and AVP. The kidneys were removed, and stored at $-70^{\circ}C$ until assayed.

Norepinephrine assay

The frozen kidneys were minced and homogenized. The tissue proteins obtained were put into a tube along with 5% perchloric acid and 1.5 mol/L sodium acetate and stirred up. The suspension was immediately centrifuged at 10,000 xg at 4°C for 10 min, and the protein-free supernatant was passed through 0.22 μm membrane filter. The norepinephrine levels were determined by high-performance liquid chromatography (Hitachi; Tokyo, Japan) with electrochemical detection (Coulochem II, ESA; Chelmsford, MA, USA): A 20 μL sample was injected, the electrochemical detector was set at 70 mV and 50 nA, and separation was carried out on a column (Waters Associates; Milford, MA, USA; 4.6 × 150 mm ID). The signal from the detector was recorded on a Hitachi Integrator (L-7500). The mobile phase consisted of 0.1 mol/L potassium phosphate buffer (pH 3.1 ~ 3.3) containing 10% methanol, 0.4 mg/mL sodium heptanesulfonate and 100 μmol/L ethylenediamine tetraacetic acid (EDTA)-Na_2. The flow rate was 0.7 mL/min.

Western blot analysis

The cortex, outer medulla, and inner medulla from the frozen kidneys were dissected, and homogenized at 3,000 rpm in a solution containing 250 mmol/L sucrose, 1 mmol/L EDTA, 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 10 mM Tris-HCl buffer at pH 7.6. Large tissue debris and nuclear fragments were removed by two low speed spins in succession (1,000 xg for 10 min; 10,000 xg for 10 min). Protein samples were loaded and electrophoretically size-separated with a discontinuous system consisting of 12.5% polyacrylamide resolving gel and 5% polyacrylamide stacking gel. The proteins were then electrophoretically transferred to a nitrocellulose membrane at 40 V for 3 hr. The membranes were washed in Tris-based saline buffer (pH 7.4) containing 0.1% Tween-20 (TBST; Amresco; Solon, OH, USA), and blocked with 5% nonfat milk in TBST for 1 hr. They were then incubated with affinity-purified anti-rabbit polyclonal antibodies against AQP1 (1 : 1,000), AQP2 (1 : 1,000), AQP3 (1 : 750), and AQP4 (1 : 1,000) (AQP1-3, Alomone Lab, Jerusalem, Israel; AQP4, Alpha Diagnostic, San Antonio, TX, USA) in 0.2% nonfat milk/TBST for 2 ~ 3 hr at room temperature. The membranes were again incubated with a horseradish peroxidase-labeled goat anti-rabbit IgG (1 : 1,200) in 2% nonfat milk in TBST for 1 hr. The bound antibody was detected by enhanced chemiluminescence (Amersham; Little Chalfont, Buckinghamshire, UK) on hyperfilm. Relative protein levels were determined by analyzing the signals of autoradiograms using the transmitter scanning video-densitometer (Bioneer; Cheongwon, Korea).

Table 1. Tissue levels of norepinephrine

	Control	Denervated
Plasma (ng/mL)	0.56 ± 0.11	0.54 ± 0.12
Kidney (μg/g)	0.16 ± 0.01	0.06 ± 0.01*

Numbers of rats were 6 each. *p < 0.01 vs. control.

Membrane preparation and adenylyl cyclase activity

The inner medulla was dissected and homogenized in ice-cold homogenizing buffer (50 mmol/L Tris-HCl, pH 8.0, containing 1 mmol/L EDTA, 0.2 mmol/L PMSF, and 250 mmol/L sucrose), centrifuged at 1,000 xg and 100,000 xg in succession. The resulting pellet was used as membrane preparation. Protein concentrations were determined by bicinchonic acid assay kit (BioRad; Hercules, CA, USA).

Adenylyl cyclase activity was determined by the method of Bar (Bar, 1975), with a slight modification. The reaction was started by adding the membrane fraction, of which protein contents were 20, 10 and 10 μg for the renal cortex, outer medulla, and inner medulla, respectively, in 100 μL working solution (50 mmol/L Tris-HCl, pH 7.6, containing 1 mmol/L ATP, 20 mmol/L phosphocreatine, 0.2 mg/mL creatine phosphokinase, 6.4 mmol/L MgCl2, 1 mmol/L 3-isobutyl-1-methylxanthine, 0.02 mmol/L GTP). After 15

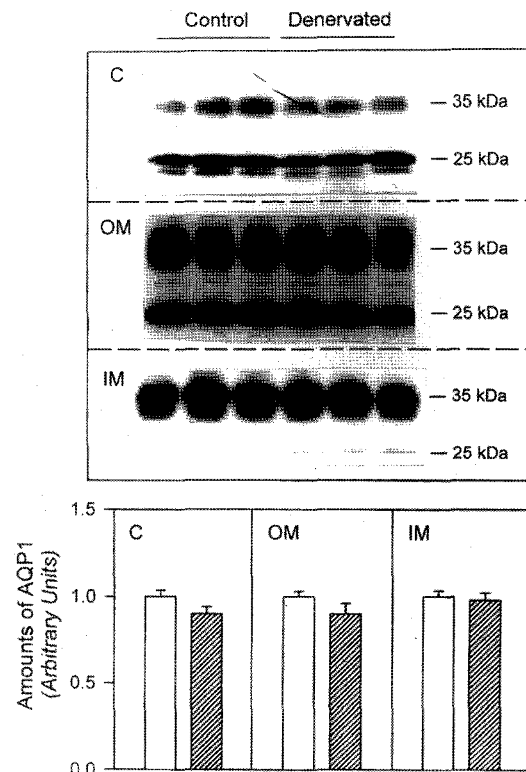

Fig. 1. Representative autoradiograms and densitometric analysis of AQP1 in the cortex (C), outer medulla (OM), and inner medulla (IM) of the denervated kidney. Anti-AQP1 antibody recognized 25 kDa and ~35 kDa bands, corresponding to nonglycosylated and glycosylated AQP1, respectively. Symbols are: (□) control; (▨) experimental. Each column represents mean ± SEM of 6 rats.

min, the reaction was stopped by the application of cold solution (50 mmol/L sodium acetate, pH 5.0), and centrifuged at 1,000 xg for 10 min at 4°C.

cAMP was then measured in the supernatant by equilibrated radioimmunoassay. Iodinated 2-O-monosuccinyl-adenosine 3,5-cyclic monophosphate tyrosyl methyl ester (^{125}I-ScAMP-TME) was prepared as described previously (Steiner et al, 1969). One hundred μL of dilute cAMP antiserum (Calbiochem-Novabiochem; San Diego, CA, USA) and ^{125}I-ScAMP-TME (10,000 cpm/100 μL) were added to standards or samples in a final volume of 100 μL of 50 mmol/L sodium acetate buffer (pH 4.8), and the mixture was incubated for 15 hr at 4°C. The bound form was separated from the free form by charcoal suspension, and the supernatant was counted in gamma counter (Packard Instrument; Meriden, CT, USA). All samples in one experiment were analyzed in a single assay. Nonspecific binding was <2.0%. The 50% intercept was at 16.5±0.8 fmol/tube (n=10). The intra- and inter-assay coefficients of variation were 5.0±1.2 (n=10) and 9.6±1.9% (n=10), respectively. Results were expressed as moles of cAMP generated per mg protein per min.

Drugs and statistical analysis

Drugs were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless stated otherwise. Results are expressed as mean±SEM. The statistical significance of differences between the groups was determined using unpaired t-test.

RESULTS

Norepinephrine levels

Systolic blood pressure was not significantly altered by the unilateral renal denervation (121±6 mmHg in the experimental vs 113±8 mmHg in the control; n=6 each). The plasma concentrations of norepinephrine did not significantly differ between the two groups. On the contrary, the tissue contents of norepinephrine in the denervated kidney were significantly decreased (Table 1).

Expression of AQP channels

The expression of AQP1-4 proteins was determined in the denervated kidney. The expression of AQP1 was not significantly affected (Fig. 1). The expression of AQP2 was not significantly altered in the cortex, but was decreased in the outer medulla and inner medulla (Fig. 2). The expression of AQP3 was decreased in the outer and inner

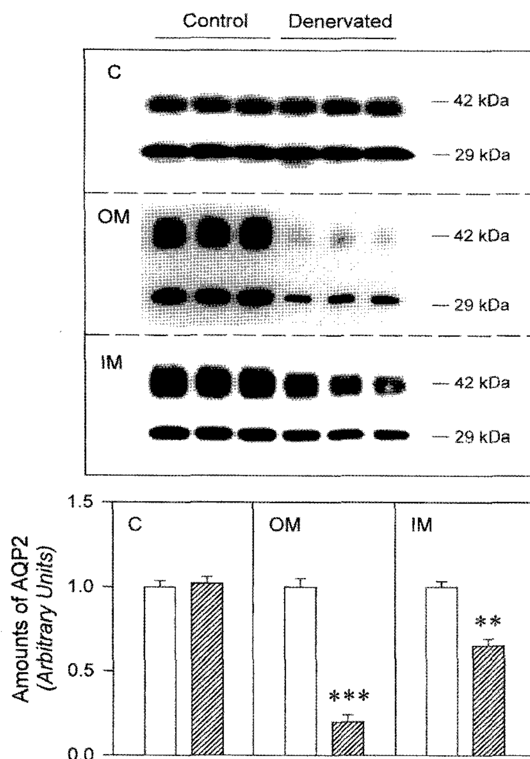

Fig. 2. Representative autoradiograms and densitometric analysis of AQP2 in the cortex (C), outer medulla (OM), and inner medulla (IM) of the denervated kidney. Anti-AQP2 antibody recognized 29 kDa and ~42 kDa bands, corresponding to nonglycosylated and glycosylated AQP2, respectively. Symbols are: (□) control; (▨) experimental. Each column represents mean±SEM of 6 rats. *p<0.05; **p<0.01; ***p<0.001 vs. control.

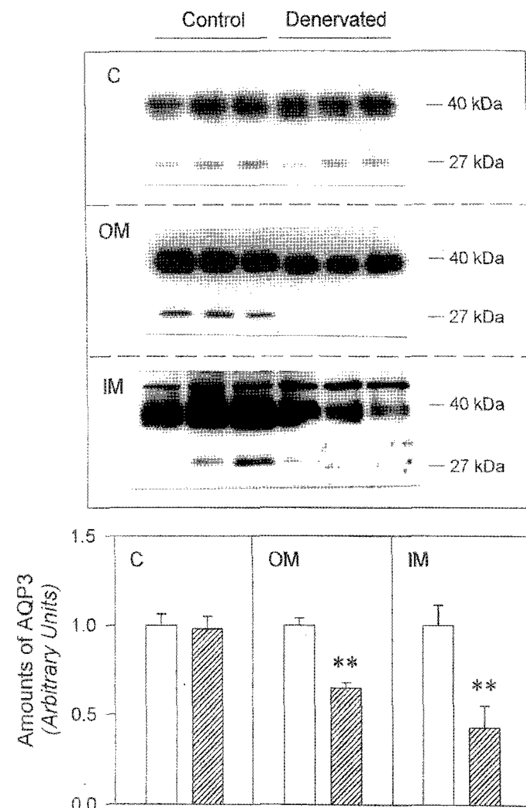

Fig. 3. Representative autoradiograms and densitometric analysis of AQP3 in the cortex (C), outer medulla (OM), and inner medulla (IM) of the denervated kidney. Anti-AQP3 antibody recognized 27 kDa and ~40 kDa bands, corresponding to nonglycosylated and glycosylated AQP3, respectively. Symbols are: (□) control; (▨) experimental. Each column represents mean±SEM of 6 rats. *p<0.05; **p<0.01; ***p<0.001 vs. control.

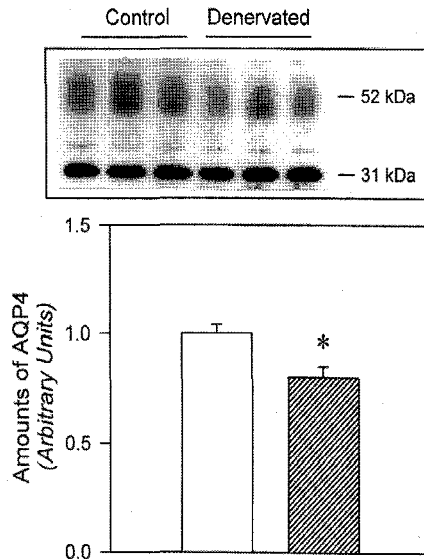

Fig. 4. Representative autoradiograms and densitometric analysis of AQP4 in the inner medulla of the denervated kidney. Anti-AQP4 antibody recognized 31 kDa and 52 kDa bands, corresponding to nonglycosylated and glycosylated AQP4. Symbols are: (□) control; (▨) experimental. Each column represents mean±SEM of 6 rats. *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs. control.

medulla (Fig. 3). The expression of AQP4 in the inner medulla was slightly but significantly decreased (Fig. 4).

Adenylyl cyclase activity

The plasma concentration of AVP did not significantly differ between the experimental and control groups (0.364 ±0.042 pg/mL vs 0.386±0.055 pg/mL; n=6 each). The adenylyl cyclase activity in response to AVP in the denervated kidney did not significantly differ between the experimental and control groups (Fig. 5).

DISCUSSION

Following the renal denervation, the tissue content of norepinephrine was significantly decreased. Accordingly, the expression of AQP2-4 channels was significantly decreased, although that of AQP1 was not significantly altered. This finding may substantiate our previous observations that the expression of AQP2 channels was significantly decreased in the kidney by the treatment with reserpine to deplete the tissue storage of catecholamines (Lee et al, 2001). However, since the systemic blood pressure was markedly decreased in this study, the altered expression of AQP channels may not only be related with a decreased sympathetic activity, but also with an altered systemic or intrarenal hemodynamics. The systemic blood pressure remained unaltered in the present study, and the altered regulation of AQP channels cannot be attributed to an altered intrarenal hemodynamics.

A regulatory role of AVP/cAMP pathway in the expression of AQP2 and AQP3 channels has been well known (Ecelbarger et al, 1995; Terris et al, 1996). A decrease of their expression is related with a decreased release of AVP and subsequent formation of cAMP. The pressor sensitivity to

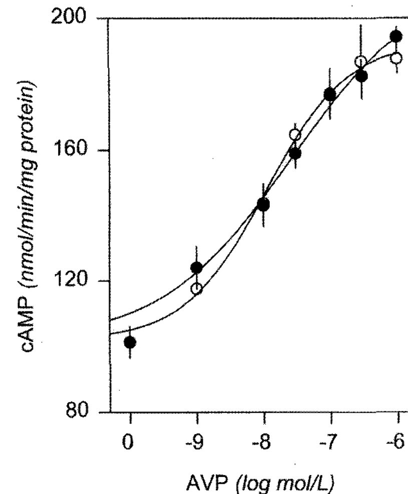

Fig. 5. Adenylyl cyclase activity stimulated by AVP in the inner medulla of the denervated kidney. Symbols are: (○) control; (●) experimental. Each point represents mean±SEM of 6 experiments.

AVP may be increased in patients with impaired sympathetic activity (Gavras, 1991). Therefore, a decreased sympathetic activity in the denervated kidney should result in an increased sensitivity to AVP. However, the plasma AVP level did not significantly differ between the renal denervated and control groups. Nor was the adenylyl cyclase activity, either basal or AVP-stimulated, significantly altered by the renal denervation. Therefore, the decreased expression of AQP channels following the denervation may not be related to an altered activity of AVP/ cAMP pathway. A specific role of the sympathetic nervous system may exist in the regulation of AQP channels in the kidney.

In response to normal physiological stimuli, changes in efferent renal sympathetic nerve activity contribute importantly to homeostatic regulation of renal hemodynamics, and tubular solute and water transport. A unilateral renal denervation increases the urinary flow and sodium excretion in the ipsilateral kidney, and decreases them in the contralateral kidney (Colindres et al, 1980). Furthermore, the denervation natriuresis and diuresis may occur without significant changes in glomerular filtration rate, renal blood flow and distribution of intrarenal blood flow (Nomura et al, 1976; Rogenes & Gottschalk, 1982). The denervation diuresis may then in part be attributed to a decreased tubular reabsorption, for which the decreased expression of AQP channels may in part be responsible.

In summary, the renal denervation decreased the expression of AQP2-4 channels, along with decreased tissue levels of norepinephrine and no changes of systemic blood pressure or local adenylyl cyclase activity. It is suggested that the sympathetic nervous system has a tonic stimulatory effect on AQP channels in the kidney.

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