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Decreased Expression of Aquaporin-2 Water Channels in the Kidney in Rats Treated with Reserpine

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Whether there exists a sympathetic neural mechanism regulating the expression of aquaporin (AQP) water channels in the kidney was investigated. Male Sprague-Dawley rats were treated with reserpine (1 mg/kg, *IP*), and the expression of AQP1-4 proteins was determined in the kidney one day thereafter. Following the treatment with reserpine, the systolic blood pressure measured in a conscious state was significantly decreased in the experimental group compared with that in the control (83 ± 8 vs 124 ± 6 mmHg; n=6 each, $P < 0.05$). The expression of AQP2 proteins was decreased in the cortex, outer medulla, and inner medulla. The decrease of AQP2 proteins was in parallel in the membrane and the cytoplasmic fractions, suggesting a preserved AQP2 targeting. No significant changes were observed in the expression of AQP1, AQP3, or AQP4. Neither basal nor AVP-stimulated formation of cAMP was significantly altered. These results suggest that the sympathetic nervous system has a tonic stimulatory effect specifically on the expression of AQP2 water channels in the kidney.

Key Words: Aquaporin-2, Reserpine, Sympathetic nervous system

INTRODUCTION

Recent advances in molecular biology have characterized aquaporin (AQP) channels that allow rapid movement of water across the permeable epithelia. In the kidney at least 7 aquaporins are expressed at distinct sites. Among them, AQP1 is highly expressed in the proximal tubule and descending thin limb (Sabolic et al, 1992). Its critical role in concentrating the urine has been found in transgenic mice lacking AQP1 (Ma et al, 1998). AQP2 is expressed in the principal cell of the collecting duct, contributing to normally reabsorb 10~20% of the tubular load not reabsorbed by the proximal nephron (Fushimi et al, 1993; Nielsen et al, 1993). AQP3 and AQP4 are both present in the basolateral membrane of collecting duct principal cells, representing exit pathways for water reabsorbed apically via AQP2 (Ecelbarger et al, 1995; Terris et al, 1995). The physiological functions of AQP6, AQP7, and AQP8 remain rather poorly defined.

As with other biological systems, AQP channels are to subject to regulation. For instance, AQP2 is short-term and long-term regulated by arginine vasopressin (AVP)/cAMP pathway to increase the osmotic water reabsorption 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, 1993; Sabolic et al, 1995; Yamamoto et al, 1995), whereas the long-term regulation is to increase its total abundance (Terris & Ecelbarger, 1996). A role of AVP/cAMP pathway in the regulation of AQP3 has been also suggested by a marked increase of its expression in the collecting duct in response to AVP infusion (Ecelbarger et al, 1995).

In congestive heart failure (CHF), the water retention and hyponatremia is associated with selectively increased expression and enhanced membrane tar-

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geting of AQP2 in the kidney (Nielsen et al, 1997). This finding indicates a major role of AQP2 in the water retention in CHF. In addition, in CHF, the decreased cardiac output unloads the baroreceptors and activates the sympathetic nervous system (Gaffney & Braunwald, 1963), and hence stimulates the release of AVP through a separate pathway that overrides the osmotic pathway (Schrier & Martin, 1998). In this context, there may exist an interaction among the sympathetic nervous system, AVP, and AQP2. The increased AQP2 expression in CHF may be attributed to an increased sympathetic tone as well as to an increased AVP release. However, the regulatory mechanisms of AQP channels other than AVP/cAMP pathway have not been defined.

The present study examined the hypothesis that AQP channels in the kidney are regulated by the sympathetic nervous system. Rats were treated with reserpine to deplete neuronal storage granules of sympathetic neurotransmitters, and their expression of AQP1-4 proteins was determined in the kidney.

METHODS

Depletion of sympathetic neurotransmitters

Male Sprague-Dawley rats (200~250 g) were used. They were treated with reserpine (1 mg/kg, *IP*) to deplete neuronal storage granules of sympathetic neurotransmitters. The rats treated with solvent (2% acetic acid) served as control. On the next day, systolic blood pressure was indirectly measured in a conscious state by tail-cuff method. It was significantly decreased in the reserpine-treated compared with that in the control (83 ± 8 vs 124 ± 6 mmHg; n=6 each, $P < 0.05$). Their kidneys were then taken following decapitation under a conscious state, and stored at $-70^{\circ}C$ until assayed. All procedures conformed to the Institutional Guidelines for Laboratory Animal Care and Use.

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 mM sucrose, 1 mM ethylenediamine tetraacetic acid (EDTA), 0.1 mM 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 g, 10 min; 10,000 g, 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), 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 videodensitometer (Bioneer; Cheongwon, Korea).

Differential centrifugation

The targeting of AQP2 proteins was assessed by comparing the magnitudes of their expression in the membrane-enriched fraction and in the cytoplasmic fraction. The tissue homogenate of the whole kidney was centrifuged at low-speed spins (1,000 g for 10 min) to remove cell debris and nuclear fragments. The supernatant was further centrifuged at 17,000 g (high-density fraction, HD) for 20 min to yield membrane-enriched pellets. The supernatant was centrifuged again at 100,000 g (low-density fraction, LD) for 1 hr to obtain a cytoplasmic vesicle-enriched pellet. The expression of AQP2 was separately determined in both fractions. A decrease in HD/LD ratio reflects an inhibited targeting of AQP2.

Membrane preparation and adenylyl cyclase activity

The inner medulla was dissected and homogenized in ice-cold homogenizing buffer (50 mM Tris-HCl, pH 8.0, containing 1 mM EDTA, 0.2 mM PMSF, and

250 mM sucrose), centrifuged at 1,000 g and 100,000 g in succession. The resulting pellet was used as a membrane preparation. Protein concentrations were determined by bicinchonic acid assay kit (BioRad; Hercules, CA, USA).

Adenylyl cyclase activity provoked by AVP was determined by the method of 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 mM Tris-HCl, pH 7.6, containing 1 mM ATP, 20 mM phosphocreatine, 0.2 mg/ml creatine phosphokinase, 6.4 mM $MgCl_2$, 1 mM 3-isobutyl-1-methylxanthine, 0.02 mM GTP). After 15 min, the reaction was stopped by cold application of solution consisting of 50 mM sodium acetate, pH 5.0, and centrifuged at 1,000 g and at 4°C for 10 min.

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). Standards or samples were taken up in a final volume of 100 μl of 50 mM sodium acetate buffer (pH 4.8). One hundred μl of dilute cAMP antiserum (Calbiochem-Novabiochem; San Diego, CA, USA) and ^{125}I-ScAMP-TME (10,000 cpm/100 μl) were then added and 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 interassay coefficients of variation were 5.0 ±1.2 (n=10) and $9.6\pm1.9\%$ (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 $\pm$ SEM. The statistical significance of differences between the groups was determined using unpaired *t*-test.

RESULTS

Expression of AQP proteins

The expression of AQP2 proteins was determined in the cortex, outer medulla, and inner medulla of the kidney. Anti-AQP2 antibody recognized 29 kDa and ~42 kDa bands, corresponding to nonglycosylated and glycosylated AQP2, respectively. The AQP2 expression was significantly decreased in the cortex, outer medulla, and inner medulla following the treatment with reserpine (Fig. 1). However, the decrease was in parallel in HD and LD, suggesting a preserved targeting (Fig. 2).

Figs. 3-5 show the expression of AQP1, AQP3, and AQP4, respectively. Anti-AQP1 antibody recognized

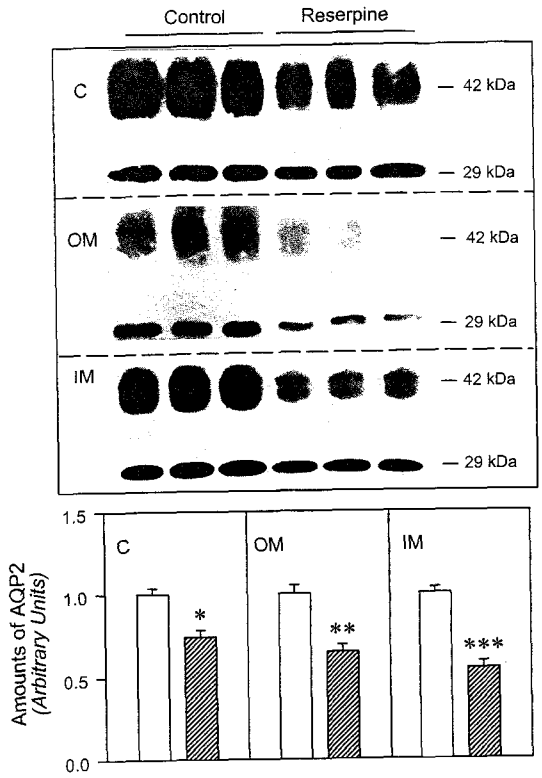

Fig. 1. The representative autoradiograms and densitometric analysis of AQP2 in the cortex (C), outer medulla (OM), and inner medulla (IM). The open column represents the control, and the hatched column depicts the reserpine-treated (mean $\pm$ SEM of 6 rats each). Amounts of AQP are in arbitrary units, in which unit 1.0 is given as an average of the expression in the control group. *P< 0.05, **P<0.01, ***P<0.001; vs control.

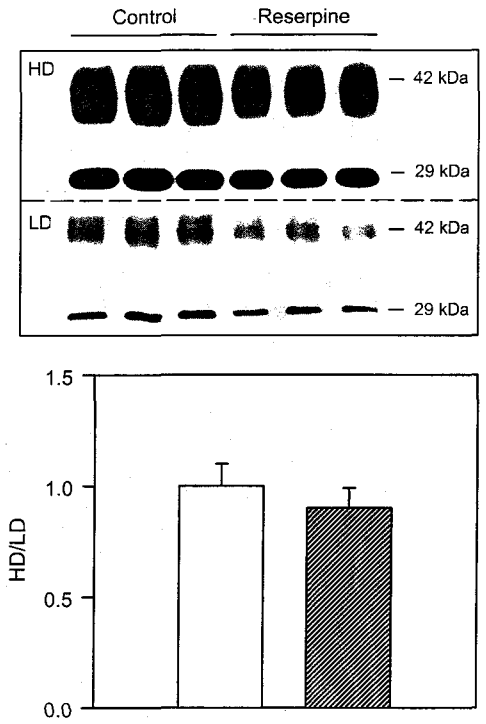

Fig. 2. The expression of AQP2 in the high-density fraction (HD) and the low-density fraction (LD). Each column represents the ratio of HD/LD (mean ± SEM of 6 rats).

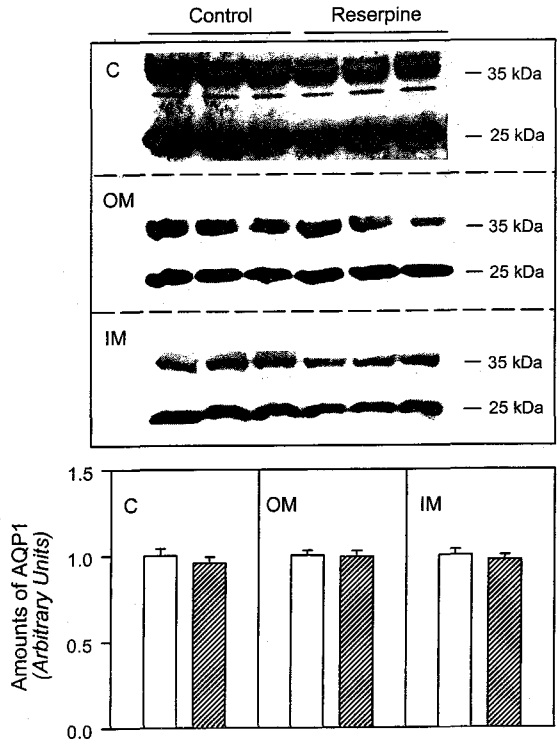

Fig. 3. The representative autoradiograms and densitometric analysis of AQP1 in the cortex (C), outer medulla (OM), and inner medulla (IM). The open column represents the control, and the hatched column depicts the reserpine-treated (mean ± SEM of 6 rats each).

25 kDa and ~35 kDa bands, corresponding to nonglycosylated and glycosylated AQP1, respectively. Anti-AQP3 antibody recognized 27 kDa and ~40 kDa bands, corresponding to nonglycosylated and glycosylated AQP3, respectively. Anti-AQP4 antibody recognized 31 kDa and ~52-40 kDa bands, corresponding to nonglycosylated and glycosylated AQP3. None of them were significantly altered by the treatment with reserpine.

Adenylyl cyclase activity

Fig. 6 shows cAMP generation in response to increasing doses of AVP in the inner medulla. Neither basal nor AVP-evoked cAMP formation was significantly changed by the reserpine-treatment.

DISCUSSION

Following the treatment with reserpine, the blood pressure was significantly decreased, indicating an overall depression of the sympathetic activity. Accordingly, the expression of AQP2 proteins was significantly decreased in the kidney. AQP2 channels are short-term regulated as a result of an exocytic insertion of the cytoplasmic vesicles into the apical membrane (Nielsen et al, 1993; Sabolic et al, 1995; Yamamoto et al, 1995). However, the decrease of AQP2 expression was parallel in the membrane and the cytoplasmic fractions, suggesting a preserved targeting. An altered long-term regulation with no changes in the short-term regulation has been noted in some acquired forms of nephrogenic diabetes insipidus such as cisplatin-induced nephropathy (Kim et al, 2001), lithium-induced nephropathy (Marples et al, 1995), and chronic renal failure induced by surgical renal mass reduction (Kwon et al, 1998). However, it is uncertain whether an initial change of AQP2 targeting is transiently passed by and only an altered abundance becomes apparent.

The transcriptional regulation of AQP2 exerted by AVP/cAMP pathway is mediated by cAMP-dependent phosphorylation and subsequent binding to cAMP response element (CRE) of CRE-binding pro-

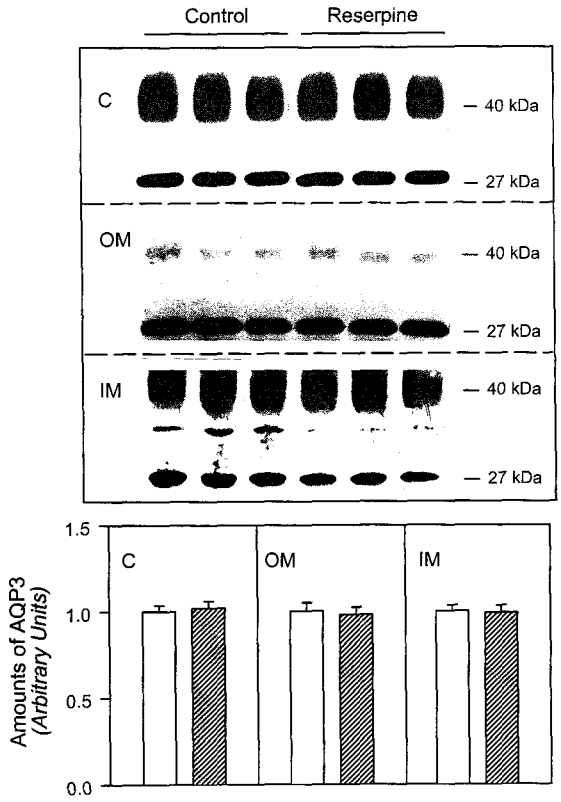

Fig. 4. The representative autoradiograms and densitometric analysis of AQP3 in the cortex (C), outer medulla (OM), and inner medulla (IM). The open column represents the control, and the hatched column depicts the reserpine-treated (mean ± SEM of 6 rats each).

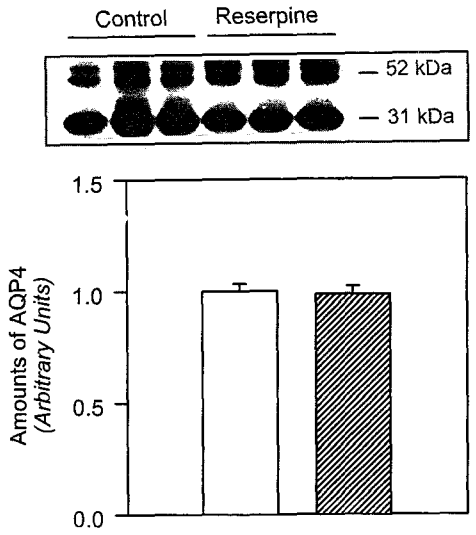

Fig. 5. The representative autoradiograms and densitometric analysis of AQP4 in the inner medulla. The open column represents the control, and the hatched column depicts the reserpine-treated (mean ± SEM of 6 rats each).

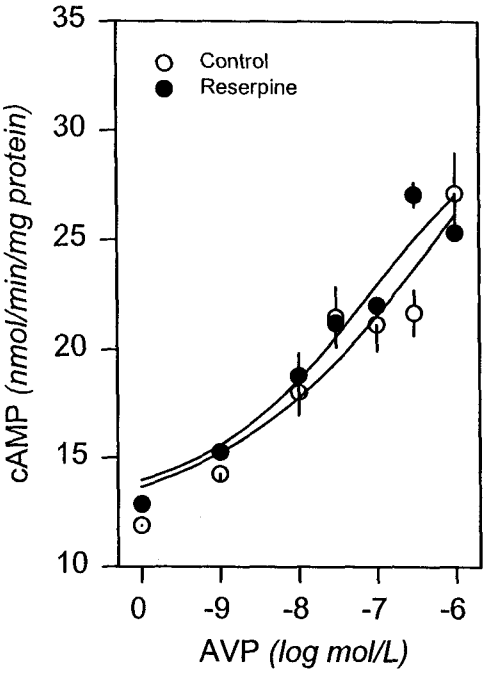

Fig. 6. cAMP formation stimulated by AVP in the inner medulla. Each point represents mean ± SEM of 6 experiments.

tein in the promoter region of AQP2 gene (Matsumura et al, 1997). Therefore, the expression of AQP2 proteins may be augmented by an increased release of AVP and subsequently enhanced formation of cAMP. For instance, in CHF, the upregulation of AQP2 proteins associated with water retention and hyponatremia (Nielsen et al, 1997; Xu et al, 1997) can be related to an increased release of AVP stimulated through a separate pathway that overrides the osmotic pathway (Schrier & Martin, 1998).

On the other hand, it has been known in CHF that the decreased cardiac output unloads the baroreceptors and activates the sympathetic outflow (Gaffney & Braunwald, 1963). In patients with an impaired sympathetic activity, an interaction between AVP and α-adrenergic function has been suggested, in which the pressor sensitivity to AVP is increased (Gavras, 1991). In this context, one may speculate that an altered sympathetic tone has an indirect effect through AVP on the expression of AQP2 channels at least in certain pathophysiological states. The attenuated sympathetic activity may decrease the responsiveness to AVP, resulting in an altered regulation of AQP2 channels. However, neither the basal

formation nor the stimulated accumulation in response to AVP of cAMP was significantly affected by the treatment with reserpine. Therefore, the decreased expression of AQP2 may not be attributed to an altered activity of AVP/cAMP pathway.

On the other hand, recent studies have also demonstrated that the expression of AQP3 proteins is markedly increased in the collecting duct in response to water restriction or AVP infusion, but not that of AQP1 or AQP4 (Ecelbarger et al, 1995; Terris et al, 1996). This finding suggests that a long-term regulation of AQP3 is also exerted by AVP/cAMP pathway. However, unlike AQP2, the expression of AQP3 was not significantly altered by the treatment with reserpine. Neither the expression of AQP1 nor that of AQP4 was significantly altered. These results suggest that the decreased expression AQP2 cannot be attributed to a nonspecific effect of reserpine. Furthermore, the differential effect on different isoforms of AQP channels suggests that the sympathetic nervous system has a specific effect on the regulation of AQP2.

In summary, the present study showed that the treatment with reserpine decreased the expression of AQP2, while it did not affect the expression of AQP1, AQP3, or AQP4. It is suggested that the sympathetic nervous system has a tonic and specific stimulatory effect on the expression of AQP2.

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