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Blunted Indomethacin-Induced Downregulation of Aquaporins by Nitric Oxide Synthesis Inhibition in Rats

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The present study was aimed to determine whether nitric oxide (NO) plays a role in the regulation of aquaporin (AQP) channels in the kidney. Male Brattleboro rats (250~300 g body weight) were used. The experimental group was treated with N^G-nitro-L-arginine methyl ester (L-NAME, 100 mg/L drinking water) for 1 week, and cotreated with indomethacin (5 mg/kg, twice a day, i.p.) for the last two days. Control groups were treated with either L-NAME for 1 week, indomethacin for 2 days, or without any drug treatment. The abundance of AQP1, AQP2 and AQP3 proteins in the kidney was determined by Western blot analysis. Indomethacin downregulated AQP channels, whereas L-NAME by itself showed no significant effects on them. The indomethacin-induced downregulation of AQP2 and AQP3 was significantly blunted in L-NAME-treated rats, while that of AQP1 was not affected. These results suggest that endogenous NO, when stimulated, may downregulate AQP channels that are specifically regulated by AVP/cAMP pathway in the kidney.

Key Words: AQP water channels, Indomethacin, N^G-nitro-L-arginine methyl ester

INTRODUCTION

Epithelial water transport occurs through aquaporin (AQP) channels. As in other biological systems, they are subject to nervous and/or hormonal regulation. For instance, among the isoforms localized in the kidney, AQP2 and AQP3 channels are short-term and long-term regulated by vasopressin (AVP)/cAMP pathway (DiGiovanni et al, 1994; Ecelbarger et al, 1995). A specific excitatory role of sympathetic nerves in the regulation of AQP channels has been also demonstrated (Lee et al, 2006).

A role of nitric oxide (NO) in mediating urinary water excretion has been well documented. The increased urinary excretion in response to water loading is mediated by NO (Peterson et al, 1997). A preserved NO production is critical for pressure-diuretic/natriuretic mechanisms (Garcia-Estan et al, 1996). On the other hand, NO-induced urinary water excretion occurs through inhibiting AVP-stimulated osmotic water permeability in the collecting duct, in association with a decreased cAMP formation resulted from activation of protein kinase that is dependent on generation of cGMP, the second messenger to NO (Gracia et al, 1996). Furthermore, NO synthase (NOS) and AQP2 immunoreactivities are colocalized in the principal cell of the collecting duct (Wang et al, 1998). Taken together, there may exist an interplay between NO and AQP channels in the kidney.

The present study was undertaken to determine whether endogenous NO plays a role in the regulation of AQP channels in the kidney. The expression of AQP channels was determined in rats treated with indomethacin to stimulate the endogenous formation of NO, since it has been known that cyclooxygenase inhibition significantly increases urinary nitrite excretion in rats (Criado et al, 1999).

METHODS

Animals

Male Brattleboro rats weighing 250~300 g were purchased from Harlan (Indianapolis, IN, USA). The experimental group was treated with N^G-nitro-L-arginine methyl ester (L-NAME, 100 mg/L drinking water) for 1 week, and cotreated with indomethacin (5 mg/kg, twice a day, i.p.) for the last two days. Control groups were treated with either L-NAME only for 1 week, indomethacin only for 2 days, or without any drugs. Indomethacin was dissolved in Na_2CO_3 (10 mM). All procedures were performed in accordance with Institutional Guidelines for Laboratory Animal Care and Use.

Western blot analysis

The kidneys were rapidly isolated following decapitation in a conscious state, and kept at −70°C until assayed. The

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ABBREVIATIONS: AQP, aquaporin; NO, nitric oxide; NOS, nitric oxide synthase; L-NAME, N^G-nitro-L-arginine methyl ester; AVP, arginine vasopressin.

inner medulla was dissected from the frozen kidney. It was homogenized at 3,000 rpm in a solution containing 250 mM sucrose, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride and 10 mM Tris-HCl buffer, at pH 7.6. The homogenate was centrifuged at 1,000×g for 15 min to remove unbroken cells, nuclei and mitochondria. The supernatant was then centrifuged at 100,000×g for 1 h to obtain a pellet containing membrane fractions enriched for both plasma membranes and intracellular vesicles. The pellet was resuspended in homogenizing solution for protein blotting.

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 overnight to a nitrocellulose membrane at 20 V. The membrane was 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 h, and incubated with affinity-purified anti-rabbit polyclonal AQP1, AQP2 and AQP3 antibodies (1:750, Alomone Lab; Jerusalem, Israel) in 2% nonfat milk/TBST for 1 h at room temperature. It was then incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (1:1,200) in 2% nonfat milk in TBST for another 1 h. 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).

Drugs and statistical analysis

Drugs were purchased from Sigma Chemical Company (St. Louis, MO, USA) unless stated otherwise. Results are expressed as mean±SEM. Statistical significance was determined by one-way ANOVA followed by Newman-Keuls test.

RESULTS

The expression of AQP1, AQP2 and AQP3 channels was significantly decreased by the treatment with indomethacin (Figs. 1~3). The indomethacin-induced downregulation of AQP1 was not affected in L-NAME-treated rats (Fig. 1), while that of AQP2 and AQP3 was markedly blunted (Figs. 2 & 3). L-NAME by itself did not significantly affect the expression of AQP channels (Fig. 4).

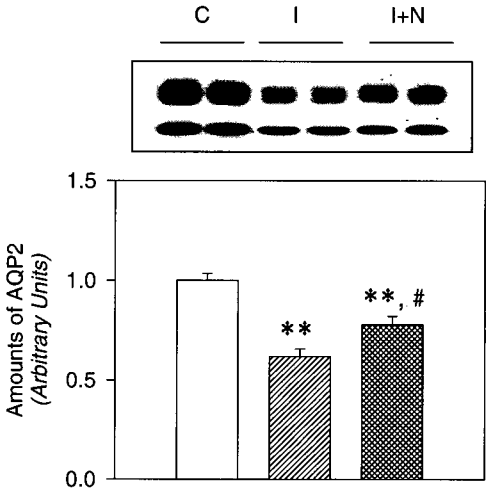

Fig. 2. Expression of AQP2 in control, indomethacin-treated, and indomethacin/L-NAME-treated rats. Anti-AQP2 antibody recognized 29 and 35~50 kDa bands, corresponding to nonglycosylated and glycosylated AQP2, respectively. Each column represents mean ±SEM of 6 rats. **p<0.01 vs. control, #p<0.05 vs. indomethacin-treated.

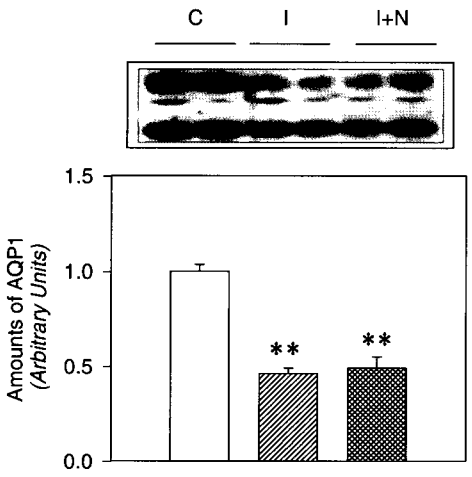

Fig. 1. Expression of AQP1 in the inner medulla of the kidney in control (C), indomethacin-treated (I), and indomethacin/L-NAME (I+N)-treated rats. Anti-AQP1 antibody recognized 29 and 35~50 kDa bands, corresponding to nonglycosylated and glycosylated AQP1, respectively. Representative immunoblots and densitometric data are shown. (□) control, (▨) I, (▩) I+N (mean±SEM of 6 rats each). **p<0.01 vs. control.

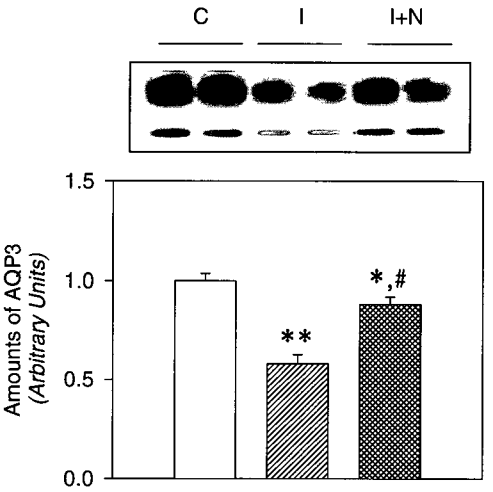

Fig. 3. Expression of AQP3 in control, indomethacin-treated, and indomethacin/L-NAME-treated rats. Anti-AQP3 antibody recognized 27 and 33~40 kDa bands, corresponding to nonglycosylated and glycosylated AQP3, respectively. Each column represents mean ±SEM of 6 rats. **p<0.01 vs. control, #p<0.05 vs. indomethacin-treated.

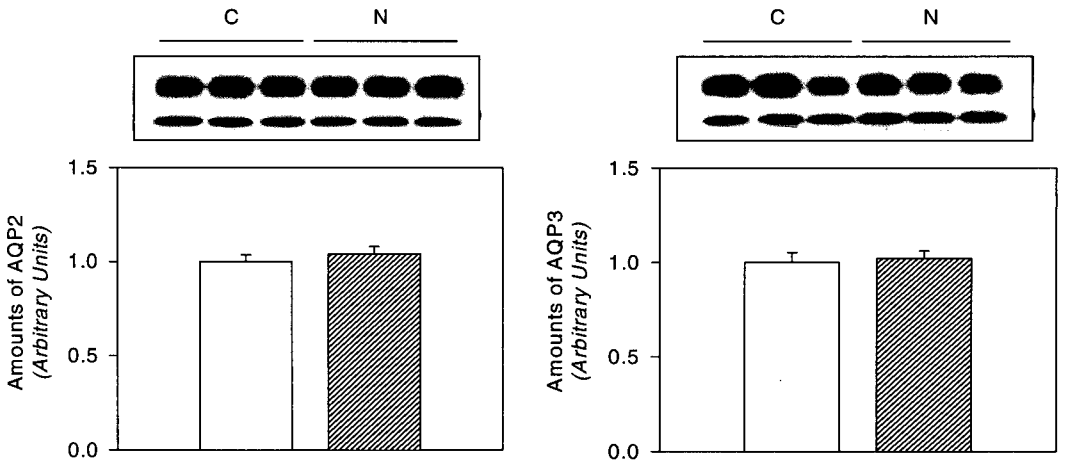

Fig. 4. Expression of AQP2 and AQP3 in control and L-NAME-treated rats. (□) control, (▨) L-NAME (mean±SEM of 6 rats each).

DISCUSSION

Indomethacin decreased the expression of AQP1, AQP2 and AQP3 proteins in the kidney, being consistent with previous findings (Kim et al, 2004; Kim et al, 2005). Furthermore, the indomethacin-induced downregulation of AQP2 and AQP3 channels was blunted in L-NAME-treated rats, while that of AQP1 was not affected. L-NAME may indirectly affect the expression of AQP through modulating renal hemodynamics, since it is known to constrict the renal artery (Bank et al, 1994). However, a decreased renal blood flow may be associated rather with a downregulation of AQP channels (Kim et al, 2000; Lee et al, 2001). It is unlikely that L-NAME increased the expression of AQP channels by modulating the renal blood flow.

AQP2 and AQP3 channels in the kidney are regulated by AVP/cAMP pathway (DiGiovanni et al, 1994; Ecelbarger et al, 1995). The AVP-stimulated cAMP formation may be inhibited by NO/cGMP pathway (Garcia et al, 1996). It is, therefore, speculated that NO downregulates AQP channels which are regulated by AVP/cAMP pathway through inhibiting cellular cAMP production. L-NAME may then reverse the downregulation of AQP through decreasing cGMP generation and subsequently increasing cAMP formation. Indeed, the increased urinary excretion in response to water loading is mediated by NO and is associated with a downregulation of AQP2 (Murase et al, 2003). Furthermore, L-NAME blunts the water loading-induced downregulation of AQP2 (Murase et al, 2003).

However, L-NAME by itself did not significantly affect the expression of AQP. The hemodynamic and diuretic effect of either NO or prostaglandins can be fully compensated during blockade of the other (Salom et al, 1991). The formation of NO may be enhanced when prostaglandin synthesis is inhibited (Criado et al, 1999). The present study may represent a direct effect of blockade of NO synthesis to reverse the downregulation of AQP channels.

The use of non-steroidal anti-inflammatory drugs may be complicated by an extracellular volume expansion (Brater, 1999). We have also shown that indomethacin decreases the urinary flow rate along with a positive water balance (Kim et al, 2004). The indomethacin-induced downregulation of AQP may thus be attributed to a positive water balance. However, a prolonged administration of L-NAME by itself may also result in sodium and water retention (Baylis et al, 1992; Ribeiro et al, 1992). Murase et al (2003) observed that the treatment with inhibitors of both NO and prostaglandin synthesis markedly decreased the urine volume even after water-loading. They further demonstrated that L-NAME decreased the urine volume along with a blunted downregulation of AQP2 in water-loaded animals. Although we did not measure the urine volume in the present study, the blunted downregulation of AQP2 and AQP3 channels may increase the tubular water reabsorption to decrease the urine volume in L-NAME- treated rats. Therefore, the L-NAME- induced blunting of the indomethacin-induced downregulation cannot be attributed to changes in body fluid balance.

Finally, it should be pointed out that NOS and AVP are colocalized in some hypothalamic neurons (Calka & Block, 1993), and NOS are present in the supraoptic and paraventricular nuclei of the hypothalamus and in the posterior pituitary gland (Vanhatalo & Soinila, 1995). However, the effects of NO on the secretion of AVP have been controversial: NO should either stimulate or tonically inhibit the basal release of AVP (Cao et al, 1996; Kadekaro et al, 1997). Nevertheless, we used AVP-deficient Brattleboro rats in the present study, so that the effect of L-NAME cannot be attributed to a modulated AVP secretion.

In summary, the indomethacin-induced downregulation of AQP2 and AQP3 channels was blunted in L-NAME-treated rats, while that of AQP1 was not affected. It is concluded that NO, when its synthesis has been stimulated, may downregulate the expression of AQP channels that are regulated by AVP/cAMP pathway in the kidney.

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