Synergistic effects of nitric oxide and prostaglandins on renal escape from vasopressin-induced antidiuresis

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Submitted 4 February 2002; accepted in final form 8 October 2002

Murase, Takashi, Ying Tian, Xiao Ying Fang, and Joseph G. Verbalis. Synergistic effects of nitric oxide and prostaglandins on renal escape from vasopressin-induced antidiuresis. Am J Physiol Regul Integr Comp Physiol 284: R354–R362, 2003. First published October 10, 2002; 10.1152/ajpregu.00065.2002.—Recent results from our laboratories, we showed that escape from vasopressin-induced antidiuresis is accompanied by marked downregulation of kidney aquaporin-2 (AQP2) and AVP V2 receptors. The present studies evaluated the effect of nitric oxide (NO) and PG synthesis blockade on escape from antidiuresis. dDAVP-infused rats were water loaded (WL) for 5 days. l-NAME, an NO synthesis inhibitor, or diclofenac, a cyclooxygenase inhibitor, was infused subcutaneously beginning 1 day before WL. As early as 2 days after WL, urine volume increased and urine osmolality decreased, indicating the onset of escape. Endogenous NO synthesis, measured as urinary NO2 + NO3 excretion, was significantly increased in the WL group compared with the non-WL controls during all 5 days of WL. l-NAME (20 mg·kg−1·day−1) markedly decreased urine volume on days 4 and 5 of WL, indicating inhibition of the escape phenomenon. Kidney AQP2 protein was significantly increased by this dose of l-NAME as well. A lower dose of l-NAME (10 mg·kg−1·day−1) or diclofenac (2.5 mg·kg−1·day−1) did not significantly affect the escape phenomenon by itself, but the combination of l-NAME and diclofenac showed a marked inhibitory effect on the escape phenomenon, which was also accompanied by a significant increase in kidney AQP2 expression. These results therefore suggest that renal NO and PG both play important roles in escape from AVP-induced antidiuresis by acting synergistically to downregulate kidney AQP2 expression.

IN ANIMAL MODELS of the syndrome of inappropriate antidiuretic hormone secretion (SIADH), sustained administration of vasopressin and water results in water retention and a secondary natriuresis, leading to progressive hyponatremia. However, after several days, this is then followed by increased free water excretion despite sustained administration of vasopressin (6, 9, 12, 13, 20, 29). This phenomenon is known as “vasopressin escape” from antidiuresis. With the onset of vasopressin escape, water excretion increases despite continued administration of vasopressin, allowing water balance to be reestablished and the serum sodium to be stabilized at a steady, albeit decreased, level.

Studies over the years have provided convincing evidence that vasopressin escape is secondary to body fluid expansion. Cowley et al. (6) reported that vasopressin escape did not occur in dogs in which total body weight was precisely servo-controlled by adjusting fluid intakes, so that an expansion of body fluid volume was prevented. Similarly, Gross et al. (12) observed that when a positive water balance was prevented by matching the rate of hypotonic fluid infusion to the urine output in rats, vasopressin escape did not occur. Hall et al. (13) showed that vasopressin escape did not occur in dogs in which renal perfusion pressure was servo-controlled, suggesting that vasopressin escape is mediated by increased renal perfusion pressure that causes both diuresis and natriuresis. In previous studies from our laboratories, we showed that escape from vasopressin-induced antidiuresis is associated with a marked vasopressin-independent decrease in kidney aquaporin-2 mRNA and protein expression (9), as well as vasopressin V2 receptor binding (29), and that this downregulation of aquaporin-2 does not appear to be closely related to either plasma or tissue osmolality (20). These combined results indicate that extracellular fluid (ECF) volume expansion is critical for the onset of vasopressin escape. However, the mechanisms by which the water retention induces escape remain unclear.

It has been reported that urinary PGE2 excretion is increased concomitant with the onset of escape and that prevention of this increase in urinary PGE2 excretion with indomethacin delays the onset of escape (12). Moreover, kidney nitric oxide (NO) and PGs have been reported to antagonize the antidiuretic effect of vasopressin (14, 21). Both NO and PG have also been considered to be involved in pressure diuresis and volume expansion-induced natriuresis (16, 19, 26). These findings suggest the possibility that kidney NO and PG may somehow be involved in the mechanisms of vasopressin escape as well. In the present study, we evaluated the effects of NG,N-nitro-L-arginine methyl ester...
ter (L-NAME), an NO synthetase inhibitor, and diclofenac, a PG synthetase inhibitor, on renal aquaporin-2 expression and the onset and maintenance of escape during vasopressin-induced antidiuresis.

MATERIALS AND METHODS

Animals and escape model. Male Sprague-Dawley rats (300–350 g; Taconic Farms, Germantown, NY) were maintained under the controlled conditions (24°C, lights on 0600–1800). Under light methoxyflurane (Mefotane) anesthesia, osmotic mini-pumps were implanted subcutaneously (model 2002; Alzet, Palo Alto, CA) to deliver 5 ng/h of dDAVP (Rhone-Poulenc Rorer, Collegeville, PA). After 4 days of dDAVP administration, during which time all rats received ad libitum pelleted chow and water, the experimental groups were water loaded by substituting daily feedings of a liquid formula (AIN-76; Bio Serve, Frenchtown, NJ in a volume of 70 ml), whereas non-water-loaded control group received AIN-76 powder. This amount of liquid diet provides sufficient calories for weight maintenance in adult rats. Thus, to maintain their caloric intake, the rats were forced to consume substantial quantities of water as well. According to the manufacturer’s information, the sodium, potassium, and chloride contents of the AIN-76 liquid diet are 0.0113, 0.0054, and 0.0110 meq/l, respectively. The rats were maintained in metabolic cages, allowing quantitative urine collections. All procedures were carried out in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Animals and an animal study protocol approved by the Georgetown University Animal Use and Care Committee.

Effect of L-NAME and diclofenac on escape from vasopressin-induced antidiuresis. Separate water-loaded groups were infused with L-NAME (10 or 20 mg·kg⁻¹·day⁻¹) or diclofenac (2.5 mg·kg⁻¹·day⁻¹) via osmotic mini-pumps (model 2001; Alzet, Palo Alto, CA), which were implanted 1 day before the start of water loading. This dose of diclofenac has been reported to effectively decrease kidney PGE₂ production in rats (28). Because diclofenac has a potential problem to cause gastrointestinal bleeding, ranitidine (10 mg·kg⁻¹·day⁻¹), Sigma Chemical, St. Louis, MO) was administered subcutaneously via osmotic mini-pumps to all groups in the experiments using diclofenac. Rats were confirmed that they did not have gastrointestinal bleeding at the time of death. L-NAME (Sigma) was dissolved in saline, and diclofenac (Sigma) was dissolved in 50% DMSO. Urine volume and osmolality were measured daily. On day 5 after water loading, rats were euthanized by decapitation and both kidneys were rapidly removed and frozen in dry ice. The kidneys were stored at −80°C until processing for immunoblotting. Plasma samples were collected from trunk blood at the time of decapitation for measurement of plasma sodium concentration and osmolality.

Urinary NOₓ excretion measurement. Because urinary NOₓ excretion measurement cannot be affected by dietary NOₓ intake in the food (10), the same amount of food was given to the water-loaded group as to the liquid diet group and to the non-water-loaded group as to the powder diet group by pair feeding. Urine was collected into a container with penicillin G (2,000 IU; Bristol-Myers Squibb, Princeton, NJ) and streptomycin (2,000 IU; Sigma) to inhibit bacterial growth. Thereafter, the urine was centrifuged, and an aliquot was stored at −70°C until measurement of NOₓ. Urinary NOₓ was measured by chemiluminescence (Sievers Instruments, Boulder, CO). NO₃ was catalytically converted to NO₂, from which NO was evolved under acid hydrolysis and reacted with O₃, yielding photons that were measured using a photomultiplier tube (31).

Immunoblotting. Left whole kidneys were homogenized in chilled membrane-isolation solution containing 250 mM sucrose, 10 mM triethanolamine, 1 μg/ml leupeptin (Bachem California, Torrence, CA), and 0.1 mg/ml phenylmethylsulfonyl fluoride (United States Biochemical, Cleveland, OH) adjusted to pH 7.6. Protein concentration was measured by BCA protein assay reagent kit (Pierce Chemical, Rockford, IL). All samples were then diluted with isolation solution to a protein concentration of 2 μg/μl and then diluted to 1 μg/μl with NOVEX sample buffer (NOVEX, San Diego, CA). SDS-PAGE was carried out on 12% Tris-Glycylamide minigels using an Xcell II Mini-Cell electrophoresis apparatus (NOVEX). The proteins were transferred from the gels electrophoretically to nitrocellulose membranes using a Bio-Rad mini-Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories, Hercules, CA). The nitrocellulose membranes were probing with the affinity-purified rabbit polyclonal antibodies directed against rat aquaporin-2 (L127) (24). The secondary antibody was donkey anti-rabbit IgG conjugated to horseradish peroxidase (No. 31458; Pierce Chemical) used at a concentration of 0.16 μg/ml. To visualize sites of antibody-antigen reaction, blots were exposed to a lumino-based enhanced chemiluminescence reagent (LumiGLO; Kirkegaard and Perry Laboratories, Gaithersburg, MD) before exposure to X-ray film (FUJI FILM, Kanagawa, Japan). Relative quantitation of the band densities from immunoblots was carried out by densitometry using NIH image software.

Statistics. Data are presented as means ± SE. Urine data were statistically analyzed by two-way repeated-measures ANOVA followed by Student-Newman-Keuls multiple comparison. Results from immunoblot densitometry were analyzed by one-way ANOVA followed by Student-Newman-Keuls multiple comparison. Differences were considered statistically significant at P < 0.05.

RESULTS

Time course of urine volume and urine osmolality. Figure 1A shows the time course of changes in daily urine volume in an initial group of rats. Urine volume began to increase by day 2 of water loading despite sustained administration of dDAVP. Figure 1B shows the time course of changes in urine osmolality in the same experiment. Urine osmolality was significantly decreased by day 2 of water loading. These results indicate that escape from vasopressin-induced antidiuresis began 2 days after the onset of water loading.

Effects of water loading on urinary NOₓ and NO₃ excretion. Figure 2 shows daily urinary NO₂ and NO₃ excretion after water loading. Endogenous NO synthesis, measured as urinary NOₓ, was significantly increased in the water-loaded group compared with the non-water-loaded controls during all 5 days of water loading and peaked on day 2 (WL = 6.8 ± 0.9, control = 4.7 ± 0.4 μmol/day, P < 0.05). L-NAME (20 mg·kg⁻¹·day⁻¹) significantly decreased urinary NOₓ and NO₃ excretion on all days to levels that were the same as, or less than, those of the non-water-loaded controls.

Effects of L-NAME on urine volume and osmolality. Figure 3 shows the effects of L-NAME (20 mg·kg⁻¹·day⁻¹) on urine volume and urine osmolality. Rats ate 70 ml of liquid diet on the first day but drank
only ~10 ml on days 2 and 3 of water loading, likely because of anorexia caused by the induced hyponatremia, and the food intake began to recover thereafter (Table 1). There were no differences in the food intake between the L-NAME-treated group and the water-loaded control group. As in the initial time course studies, urine volume increased and urine osmolality decreased by 2 days after the start of water loading. One group of the water-loaded rats was implanted with an osmotic mini-pump that delivered L-NAME (20 mg·kg⁻¹·day⁻¹) 1 day before the start of water loading. Values are expressed as means ± SE. *P < 0.05 compared with control group.

Table 1. Food intake after L-NAME treatment

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>WL, ml</td>
<td>61 ± 1</td>
<td>17 ± 4</td>
<td>13 ± 4</td>
<td>20 ± 5</td>
<td>36 ± 5</td>
</tr>
<tr>
<td>L-NAME, ml</td>
<td>59 ± 1</td>
<td>11 ± 2</td>
<td>14 ± 2</td>
<td>18 ± 2</td>
<td>33 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE. WL (n = 4), water loaded; L-NAME (n = 4), water loaded treated with N⁶-nitro-ʟ-arginine methyl ester (L-NAME). Food intake was expressed as volume of liquid diet (ml). L-NAME (20 mg·kg⁻¹·day⁻¹) was infused via osmotic mini-pumps from 1 day before the start of water loading. There are no significant differences between WL and L-NAME groups on each day. Because non-water-loaded rats were not fed with liquid diet, they were not included here.
indicating ongoing renal escape from vasopressin-induced antidiuresis. Infusion of a high dose of L-NAME (20 mg·kg⁻¹·day⁻¹) did not affect urine volume and osmolality on days 1 and 2; however, urine volume markedly decreased and urine osmolality slightly increased on day 3 of water loading and thereafter, indicating inhibition of the progress of the escape process. Plasma sodium concentration was also slightly increased in the L-NAME-treated group compared with the water-loaded control group (Table 2). Urinary sodium excretion was markedly increased on day 2 in water-loaded rats causing negative balances (Fig. 4A). In the L-NAME-treated group, urinary sodium excretion tended to decrease on day 3 of water loading and thereafter compared with the water-loaded controls. Negative potassium balances were also seen on all days (Fig. 4B). Consequently, the total sodium and potassium balances between days 1 and 5 were negative in all groups, but the electrolyte loss was significantly decreased in the L-NAME-treated group compared with the water-loaded control group (Table 3).

Effects of L-NAME on kidney aquaporin-2 protein expression after water loading. Figure 5A shows immunoblots of rat whole kidney homogenates on day 5 of water loading probed with anti-aquaporin-2. The aquaporin-2 protein bands, which are decreased in the water-loaded groups compared with the non-water-loaded controls, appeared to be decreased to a lesser degree by treatment with L-NAME (20 mg·kg⁻¹·day⁻¹). Densitometry confirmed that L-NAME significantly increased aquaporin-2 protein expression compared with the water-loaded controls (175 ± 7% of water-loaded controls, P < 0.05; Fig. 5B).

Effects of L-NAME on urine volume and osmolality in non-water-loaded rats. L-NAME (20 mg·kg⁻¹·day⁻¹) slightly increased urine volume and decreased urine osmolality in non-water-loaded rats, but these changes were not significant (data not shown). Aquaporin-2 immunoblots showed that this dose of L-NAME (20 mg·kg⁻¹·day⁻¹) did not affect kidney aquaporin-2 expression in non-water-loaded rats (data not shown).

Effects of combination of L-NAME and diclofenac on urine volume and osmolality after water loading. There were no significant differences in the food intake among the treatment groups (Table 4). Figure 6 shows the effects of a lower dose of L-NAME (10 mg·kg⁻¹·day⁻¹) and diclofenac (2.5 mg·kg⁻¹·day⁻¹) on urine volume and osmolality after water loading. L-NAME (10 mg·kg⁻¹·day⁻¹) or diclofenac (2.5 mg·kg⁻¹·day⁻¹) did not significantly affect the urine volume and osmolality by themselves. However, the combination of L-NAME and diclofenac markedly decreased urine volume and increased urine osmolality on day 3 of water loading and thereafter, indicating inhibition of the progress of the escape process. Despite this inhibition of escape, there were no significant differences in

Table 2. Effects of L-NAME on plasma sodium after water loading

<table>
<thead>
<tr>
<th>Control (4)</th>
<th>Water Loaded (4)</th>
<th>Water Loaded + L-NAME (4)</th>
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<tbody>
<tr>
<td>Plasma sodium, meq/l</td>
<td>137.4 ± 0.3</td>
<td>98.5 ± 0.4*</td>
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Values are expressed as means ± SE. Plasma samples were collected after 5 days of water loading. L-NAME (20 mg·kg⁻¹·day⁻¹) was infused via osmotic mini-pumps from 1 day before the start of water loading. *P < 0.05 compared with non-water-loaded control. **P < 0.05 compared with water-loaded control. Numbers of rats for each group are indicated in parenthesis.

Table 3. Effects of L-NAME on sodium and potassium balances during water loading

<table>
<thead>
<tr>
<th></th>
<th>Na⁺, meq</th>
<th>K⁺, meq</th>
<th>Na⁺ and K⁺, meq</th>
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<tbody>
<tr>
<td>WL</td>
<td>-2.26 ± 0.15</td>
<td>-4.15 ± 0.24</td>
<td>-6.41 ± 0.18</td>
</tr>
<tr>
<td>L-NAME</td>
<td>-1.85 ± 0.14</td>
<td>-3.49 ± 0.13*</td>
<td>-5.34 ± 0.20*</td>
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</table>

Values are presented as means ± SE. L-NAME, water loaded treated with L-NAME (20 mg·kg⁻¹·day⁻¹). Sodium and potassium balances were calculated from sum of dietary intake and urinary losses between days 1 and 5. *P < 0.05 compared with water-loaded group.
plasma sodium among the groups (Table 5). In the L-NAME- and diclofenac-treated group, urinary sodium excretion tended to decrease on day 3 of water loading and thereafter compared with the water-loaded controls (Fig. 7A). The total sodium and potassium balances between days 1 and 5 were negative in all groups, but the negative balance tended to be less in the L-NAME + diclofenac-treated group (Table 6).

Effects of L-NAME and diclofenac on kidney aquaporin-2 protein expression after water loading. Figure 8A shows immunoblots of rat whole kidney homogenates on day 5 of water loading probed with antiaquaporin-2. Neither L-NAME (10 mg·kg⁻¹·day⁻¹) nor diclofenac (2.5 mg·kg⁻¹·day⁻¹) significantly affected kidney aquaporin-2 protein expression by themselves. However, the combination of L-NAME and diclofenac appeared to increase the aquaporin-2 bands compared with the water-loaded controls. Densitometry confirmed that the combination of L-NAME and diclofenac significantly increased kidney aquaporin-2 expression (166 ± 5% of water-loaded controls, *P < 0.05; Fig. 8B).

![Figure 5](image)

**Fig. 5.** Effects of L-NAME treatment on aquaporin-2 (AQP2) protein expression after water loading. C, non-water-loaded control; WL + L-NAME, water-loaded group treated with L-NAME. A: Western immunoblots of whole kidney homogenates from Sprague-Dawley rats after water loading for 5 days (all rats received dDAVP by minipumps). L-NAME (20 mg·kg⁻¹·day⁻¹) was administered via osmotic mini-pumps implanted 1 day before the start of water loading. Each lane was loaded with sample from a different rat (7 µg total protein/lane). Blots were probed with anti-AQP2 antibody (120 ng/ml). The 29-kDa band is the nonglycosylated form and 40-kDa band is the glycosylated form of AQP2. B: summary of densitometry data (sum of both 29- and 40-kDa bands) from the immunoblots. Values represent the mean value of each group expressed as a percentage of the average value of the non-water-loaded control group. *P < 0.05 compared with control group; †P < 0.05 compared with water-loaded group. Water loading markedly decreased kidney AQP2 protein expression, and this effect was significantly blunted by L-NAME treatment.

![Figure 6](image)

**Fig. 6.** Effects of L-NAME and/or diclofenac on urine volume (A) and urine osmolality (B) after water loading. All rats were implanted with dDAVP osmotic mini-pumps at day −4 and had access to pelleted rat chow and water ad libitum. On day 0, all rats were water loaded by substituting daily feedings of a liquid formula for the pelleted rat chow. Experimental groups were implanted with osmotic mini-pumps that delivered L-NAME (10 mg·kg⁻¹·day⁻¹) and/or diclofenac (2.5 mg·kg⁻¹·day⁻¹) 1 day before the start of water loading. Values are expressed as means ± SE. *P < 0.05 compared with water-loaded group.

<table>
<thead>
<tr>
<th>Table 4. Food intake after L-NAME and/or diclofenac treatment</th>
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<tr>
<td>WL, ml</td>
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<tr>
<td>L-NAME, ml</td>
</tr>
<tr>
<td>Diclofenac, ml</td>
</tr>
<tr>
<td>L-NAME + diclofenac, ml</td>
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Values are presented as means ± SE. WL, n = 4; L-NAME, n = 3; diclofenac, (n = 4) water loaded with diclofenac; L-NAME + diclofenac, (n = 3) water loaded with L-NAME and diclofenac. Food intake is expressed as a volume of liquid diet (ml). There are no significant differences among each group on any days.

**DISCUSSION**

To study the potential roles of NO and PG in the escape from vasopressin-induced antidiuresis, we first evaluated NO synthesis during water loading. Urinary PGE₂ excretion has been reported to increase concomitantly with the onset of escape (7, 12). However, there have been no reports about changes in NO synthesis after water loading. Our results showed that endogenous NO synthesis, measured as urinary NO₂ + NO₃...
excretion, was significantly increased after water loading. This upregulation of NO synthesis was seen as early as 1 day after initiation of water loading and peaked on day 2, which coincided with the onset of escape. After 3 days, NO synthesis decreased somewhat, which could be because of the decrease in body water retention as a result of increased urine flow due to the ongoing escape. Thus, endogenous NO synthesis after water loading may be related to the ECF volume status. Although it is still unclear what the sources of NO or PG actually are, it seems reasonable to hypothesize that these vasoactive substances are synthesized in the vasculature. In this regard, it is notable that Hall et al. (13) showed that vasopressin escape did not occur in dogs in which renal perfusion pressure was servo-controlled, suggesting that vasopressin escape is mediated by increased renal perfusion. Increased renal perfusion pressure due to ECF volume expansion would be expected to cause stretching of renal vascular endothelial cells, as well as glomerular mesangial cells (1, 32). It has been reported that shear stress stimulates the production of NO and PG through stretch-activated ion channels in vascular endothelial cells (4, 5, 25, 27, 33).

In the present study, we showed that L-NAME has inhibitory effects on vasopressin escape, although predominantly during the later stages of escape (i.e., days 3-5). This may be explained as follows. This dose of L-NAME has been reported to significantly increase blood pressure (2). It is well known that elevation of renal blood pressure increases urine flow and sodium excretion, which has been termed pressure natriuresis (22). Such increases in blood pressure would be enhanced by increased ECF volume. Therefore, it is possible that the antidiuretic effect of dDAVP was overcome by a pressure diuresis produced by the hypertensive effect of L-NAME. However, any L-NAME-induced increases in blood pressure would be expected to be maximal on day 1 or day 2 after water loading when water retention was maximal, whereas the effects of L-NAME were seen mainly during the later stages of escape. Interestingly, L-NAME did not significantly decrease urine flow in non-water-loaded rats treated with dDAVP infusion. This suggests that inhibitory effects of L-NAME on vasopressin escape are probably not caused by non-

### Table 5. Effects of L-NAME and/or diclofenac on plasma sodium after water loading

<table>
<thead>
<tr>
<th></th>
<th>WL (4)</th>
<th>WL + L-NAME (3)</th>
<th>WL + Diclofenac (4)</th>
<th>WL + L-NAME + Diclofenac (3)</th>
</tr>
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<tbody>
<tr>
<td>Plasma sodium, meq/l</td>
<td>98.5 ± 1.4</td>
<td>100.4 ± 0.7</td>
<td>99.2 ± 1.1</td>
<td>99.6 ± 2.0</td>
</tr>
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</table>

Values are expressed as means ± SE. All groups were water loaded (WL) for 5 days. L-NAME (10 mg·kg⁻¹·day⁻¹) and/or diclofenac (2.5 mg·kg⁻¹·day⁻¹) were infused via osmotic mini-pumps from 1 day before the start of water loading. There are no significant differences among each group. Numbers of rats for each group are indicated in parenthesis.

### Table 6. Effects of L-NAME and/or diclofenac on sodium and potassium balances during water loading

<table>
<thead>
<tr>
<th></th>
<th>Na⁺, meq</th>
<th>K⁺, meq</th>
<th>Na⁺ and K⁺, meq</th>
</tr>
</thead>
<tbody>
<tr>
<td>WL</td>
<td>−1.06 ± 0.27</td>
<td>−4.12 ± 0.33</td>
<td>−6.08 ± 0.60</td>
</tr>
<tr>
<td>L-NAME</td>
<td>−1.46 ± 0.49</td>
<td>−3.83 ± 0.26</td>
<td>−5.28 ± 0.49</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>−1.96 ± 0.03</td>
<td>−3.86 ± 0.16</td>
<td>−5.82 ± 0.19</td>
</tr>
<tr>
<td>L-NAME + Diclofenac</td>
<td>−1.46 ± 0.18</td>
<td>−3.20 ± 0.32</td>
<td>−4.65 ± 0.44</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. L-NAME, water loaded with L-NAME (10 mg·kg⁻¹·day⁻¹); diclofenac, water loaded with diclofenac (2.5 mg·kg⁻¹·day⁻¹); L-NAME + diclofenac, water loaded with L-NAME and diclofenac. Sodium and potassium balances were calculated from sum of dietary intake and urinary losses between days 1 and 5. There are no significant differences among each group on any days.

Fig. 7. Effects of L-NAME and/or diclofenac on sodium (A) and potassium (B) balances during water loading. D, water-loaded group with diclofenac; N + D, water-loaded group with L-NAME and diclofenac. All rats were implanted with dDAVP osmotic mini-pumps at day −4 and had access to pelleted rat chow and water ad libitum. On day 0, rats were water loaded by substituting daily feedings of a liquid formula for the pelleted chow. Experimental groups were implanted with osmotic mini-pumps that delivered L-NAME (10 mg·kg⁻¹·day⁻¹) and/or diclofenac (2.5 mg·kg⁻¹·day⁻¹) 1 day before the start of water loading. Daily sodium and potassium balances were calculated from dietary intake and urinary losses, ignoring fecal losses that were assumed to be small and constant across groups. Values are expressed as means ± SE. There are no significant differences in sodium intake and excretion as well as potassium among each group on any days.

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and/or diclofenac (2.5 mg/kg) 

vasopressin-stimulated cAMP synthesis by increasing intracellular cGMP, indicating that NO has an antidiuretic effect via a vasopressin-independent mechanism. However, this study did not report the effects of NO on vasopressin-induced aquaporin-2 translocation. A second potential mechanism is that L-NAME might increase aquaporin-2 expression indirectly by modulating renal hemodynamics. L-NAME is known to vasoconstrict the renal artery and reduce renal blood flow by decreasing local synthesis of NO, a potent vasodilator (2). As mentioned above, because an increase in renal perfusion appears to be critical for vasopressin escape, it is possible that L-NAME might reverse downregulation of aquaporin-2, in part, by decreasing renal blood flow. Together with our previous reports showing marked downregulation of kidney aquaporin-2 expression during vasopressin escape (9, 20), the results from the present studies suggest that downregulation of aquaporin-2 plays a pivotal role in the escape phenomenon. However, we cannot exclude the possibility of other mechanisms. For example, factors such as wash-out of the medullary osmotic concentration gradient, local changes in renal blood flow, changes in systemic blood pressure, and other factors that are known to influence renal concentrating ability may also play an important role in the escape phenomenon. Further studies will be required to assess the relative contributions of these factors to vasopressin escape.

It is interesting that the plasma sodium concentration did not decrease further after L-NAME (20 mg·kg⁻¹·day⁻¹) or L-NAME + diclofenac treatment despite marked decreases in urine volume. Because decrease in urine volume would result in more water retention in the body, it would be expected that the plasma sodium concentration would decrease in these rats as a result of plasma dilution. To address this point, we calculated the sodium and potassium balances during the experiments. The urinary sodium excretion was markedly increased on day 2 resulting in negative sodium balance, which is characteristic of SIADH. The urinary sodium excretion of L-NAME (20 mg·kg⁻¹·day⁻¹) or L-NAME + diclofenac group tended to decrease on days 3, 4, and 5 compared with the water-loaded control group. The total sodium and potassium balance between days 1 and 5 was negative in all groups, but it was higher in the L-NAME (20 mg·kg⁻¹·day⁻¹) group compared with the control group. It was also higher, although not statistically significant, in the L-NAME + diclofenac group. These results indicate that rats in the L-NAME (20 mg·kg⁻¹·day⁻¹) or L-NAME + diclofenac group have more sodium in their bodies at the end of experiments compared with the control group, and this may explain why the plasma sodium concentrations did not decrease further in the L-NAME (20 mg·kg⁻¹·day⁻¹) and the L-NAME + diclofenac groups despite additional water retention caused by a decrease in urine volume.

selective effects such as renal toxicity and further supports the idea that increased NO synthesis after water loading may be an important contributing factor to the induction and/or maintenance of vasopressin escape.

The results of the present study also showed that the inhibitory effect of L-NAME on vasopressin escape was accompanied by a significant blunting of the water-loading-induced decreases in kidney aquaporin-2 protein expression. There are two possibilities for this inhibitory mechanism of L-NAME. One is that L-NAME acts directly on collecting duct cells to reverse an inhibitory effect of NO on the antidiuretic effect of vasopressin. Vasopressin regulates kidney collecting duct water permeability via aquaporin-2 by stimulating its intracellular shuttling (23) or by increasing its abundance (8). Both of these mechanisms have been reported to be induced by increases in cellular cAMP levels (15, 18). It has been reported that NO inhibits vasopressin-stimulated cAMP synthesis by increasing cGMP in kidney collecting ducts (11). Therefore, it is possible that NO might downregulate kidney aquaporin-2 expression by inhibiting cellular cAMP production, in which case L-NAME would reverse this downregulation of aquaporin-2 expression by inhibiting NO synthesis. Recently, Bouley et al. (3) reported that NO stimulates insertion of aquaporin-2 in renal epithelial cells by increasing intracellular cGMP, indicating that NO has an antidiuretic effect via a vasopressin-independent mechanism. However, this study did not report the effects of NO on vasopressin-induced aquaporin-2 translocation. A second potential mechanism is that L-NAME might increase aquaporin-2 expression indirectly by modulating renal hemodynamics.
Perhaps most importantly, the results of the present study suggest that there is a potentially important interaction between NO and PG in maintaining renal escape from vasopressin-induced antidiuresis. Neither a lower dose of L-NAME (10 mg·kg⁻¹·day⁻¹) nor diclofenac significantly affected vasopressin escape when they were administered separately. However, it is striking that the combination of L-NAME and diclofenac produced a marked inhibitory effect on the escape phenomenon, which was also accompanied by significant increase in kidney aquaporin-2 expression. It has been reported that diclofenac causes a decrease in glomerular filtration rate (GFR) (17, 30), and we cannot deny the possibility that the decrease in urine flow may be caused by the decrease in GFR. However, aquaporin-2 expression was increased by diclofenac when it was administered with L-NAME. Therefore, it seems reasonable to think that a decrease in GFR is not the only contributor to the observed decrease in urine volume. Although the present study was not designed to elucidate the mechanism of this interaction between NO and PG, it seems possible that since both NO and PG are potent vasodilators, an inhibition of either NO or PG alone might be overcome by a compensatory increase in the activity of the other. In this case, inhibition of NO or PG individually would not effectively inhibit vasopressin escape. However, when infused at a higher dose, L-NAME alone did inhibit vasopressin escape to some degree. This may be because the vasoconstricting activity of L-NAME is more potent than the vasodilating effect of PG, and consequently renal blood flow was significantly decreased by the higher dose of L-NAME alone. Another possibility is that L-NAME activates mechanisms other than modulating renal hemodynamics, such as direct effects on renal collecting duct cells. In this case, compensation by PG might not occur or may be less potent with higher doses of L-NAME.

In conclusion, results from the present study indicate that renal NO and PG both play important roles in escape from vasopressin-induced antidiuresis and suggest that they might act synergistically to downregulate kidney aquaporin-2 expression during escape from vasopressin-induced antidiuresis.

We thank Dr. M. A. Knepper (LKEM, National Institutes of Health, Bethesda, MD) for providing the anti-aquaporin-2 antibody. This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-38094.

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