Evidence for bradykinin as a stimulator of thirst

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Cadnapaphornchai, Melissa A., Boris Rogachev, Sandra N. Summer, Yung-Chang Chen, Lajos Gera, John M. Stewart, and Robert W. Schrier. Evidence for bradykinin as a stimulator of thirst. Am J Physiol Renal Physiol 286: F875–F880, 2004. —Angiotensin-converting enzyme inhibition (ACEI) with captopril has been shown to increase water intake and urine output in rats, but the mechanism is unknown. ACEI impairs the conversion of ANG I to ANG II, a dipsogenic hormone, and impairs the degradation of bradykinin. The goal of this study was to examine the role of bradykinin in the polydipsia and polyuria associated with ACEI. Male Sprague-Dawley rats received captopril (CPT; 20 mg kg–1 day–1) in ground chow for 48 h. Water intake, food intake, and urine output were monitored and compared with control rats (CTL), rats receiving captopril treatment with limited water intake (CPT-LIM), and rats receiving captopril treatment with ad libitum water intake plus 24-h treatment with the bradykinin antagonist B-9430 (CPT-BK1). CPT rats consumed significantly more water and produced more urine vs. CTL. Urine osmolality was significantly decreased in CPT rats vs. CTL. Inner medullary aquaporin-2 (AQP2) protein abundance was also markedly decreased in CPT rats vs. CTL. These findings were reversed in CPT-LIM rats, suggesting captopril-induced primary polydipsia. CPT-BK1 rats demonstrated parameters no different from CTL despite ad libitum water intake. Mean arterial pressure and 24-h creatinine clearance did not differ among groups. We conclude that ACEI with captopril induces primary polydipsia despite impaired production of the dipsogen ANG II and that this primary increase in water intake is likely the cause of the decreased protein abundance of inner medullary AQP2. Furthermore, this dipsogenic effect was reversed by antagonism of bradykinin, thus implicating this hormone in thirst regulation in the rat.

an Angiotensin-converting enzyme inhibition; aquaporin

an Angiotensin HAS BEEN SHOWN in several experimental studies to be dipsogenic. In this regard, ANG I or II infusion into several species has been shown to increase water intake with resultant polyuria (9, 25). On this background, however, are the provocative observations that several angiotensin-converting enzyme inhibitors (ACEI) have been shown to cause polydipsia and polyuria (2, 15, 23). This suggests the possibility that bradykinin may be responsible for the polyuric state, because ACE inhibition also inhibits kininase II with resultant decreased bradykinin degradation. With an intact kininase II system, the infusion of bradykinin does not result in increased water intake (19). However, when exogenous bradykinin has been infused during acute ACE inhibition, polydipsia and polyuria have been observed (10). These results suggest that inhibition of kininase II-mediated bradykinin degradation is necessary for bradykinin to induce polyuria and polydipsia.

The present study was undertaken to further examine the potential effects of endogenous bradykinin on water metabolism. The polyuric effect of the ACE inhibitor captopril was therefore examined in the presence and absence of bradykinin inhibition. To distinguish between primary polyuria vs. polydipsic effect, studies controlling water intake during ACE inhibition were performed. Mean arterial pressure and 24-h creatinine clearance were evaluated to exclude systemic and renal effects of ACEI on water excretion. Finally, the role of endogenous bradykinin in the polyuric/polydipsic state was examined by combining the ACE inhibitor with a bradykinin antagonist.

EXPERIMENTAL PROTOCOL

Materials

The experimental protocol was approved by the UCHSC Animal Institutional Care and Use Committee. Male Sprague-Dawley rats weighing 250–350 g (Charles River Laboratories, Portage, MI) were used for all experiments. The rats were housed in a controlled environment and kept in filter-top microisolators and were allowed to acclimate to altitude for 2 wk. Animals were allowed free access to tap water and food (0.44% sodium and 22.5% protein, ProLab 3000; Agway, Syracuse, NY) until the start of the study. For the study, animals were housed individually in metabolic cages (Nalgene, Nalge, Rochester, NY). To acclimate them to the metabolic cages, rats were housed in the cages continuously for 7 days before balance experiments. These cages provide separation of urine and feces with the combination of a collecting funnel and a separating cone in the lower chamber. Daily water and food intake and urine output were recorded. Animals were subjected to 12:12-h light-dark cycles and were maintained at constant ambient temperature.

Experimental Protocol

Effect of bradykinin antagonism on water metabolism. These studies were conducted to assess the independent effect of the nonselective bradykinin antagonist B-9430 on water metabolism. Rats were acclimated to metabolic cages as described above. All rats received powdered rat chow without additives (15 g/day) and water ad libitum. The food supply was replenished every 24 h. Six rats (BK1) received B-9430, 4 mg/kg subcutaneously for 8 h (volume 0.2 ml), for 48 h. Six control rats (CTL) received no injections. A water balance study was performed during the final 24 h of the study, with water intake recorded, urine output collected under light mineral oil to avoid evaporation, and volume recorded. Urine from the 24-h balance study was frozen for analysis of osmolality and creatinine concentration. On conclusion of the water balance study, the rats were killed by decapitation to avoid any influence of anesthesia on plasma arginine
EVIDENCE FOR BRADYKININ AS A STIMULATOR OF THIRST

vasopressin (AVP) concentration. Trunk blood was collected, and kidneys were harvested on ice for Western blot analysis of renal aquaporins.

Effect of combined bradykinin antagonism and ACE inhibition on water metabolism and renal aquaporin protein abundance. Thirty-two different rats were utilized for these experiments. Rats were acclimated to altitude and to metabolic cages as described above. During the 2 wk before the study, mean arterial pressure was assessed by tail-cuff measurement (IITC/Life Science Instruments, Woodland Hills, CA) 3 days/wk between 8 and 11 AM. Tail-cuff measurements were assessed 10 times/rat during each training session. This was done to acclimate the rats to the measurement technique.

This experimental protocol lasted for 4 days and included a 2-day baseline period and a 2-day treatment period. Powdered rat chow (15 g/day) was provided during the baseline period. Daily body weight, water intake, food intake, and urine volume were recorded during the 48-h baseline period. Subsequently, the 32 rats were divided into 4 treatment groups of 8 rats each: CTL; captopril treatment with ad libitum water intake (CPT); captopril treatment with restricted water intake (CPT-LIM); or combined treatment with captopril and the bradykinin antagonist B-9430 with ad libitum water intake (CPT-BKI).

For the ensuing 48 h of the experimental protocol, rats in the CPT, CPT-LIM, and CPT-BKI study groups received captopril (20 mg/kg body wt) daily mixed in 15 g of powdered chow. CTL rats received 15 g/day of ground rat chow without additives. Only rats consuming body wt) daily mixed in 15 g of powdered chow. CTL rats received (CPT-BKI).

Western Blot Analysis

Water was provided ad libitum except for the CPT-LIM study group, in which daily water intake was restricted to the amount consumed during the baseline period. Water intake and urine output were recorded every 24 h.

In the final 24 h of the study, CPT-BKI rats received B-9430, 4 mg/kg subcutaneously every 8 h (volume 0.2 ml). CTL, CPT, and CPT-LIM rats received an injection of normal saline (0.2 ml sc every 8 h). A water balance study was performed during the final 24 h of the study, with water intake recorded, urine output collected under light mineral oil to avoid evaporation, and volume recorded. Urine from the 24-h balance study was frozen for analysis of osmolality and creatinine concentration. The food supply was replenished every 24 h.

On conclusion of the balance study, mean arterial pressure was assessed by tail-cuff measurement in all rats between 8 and 11 AM to exclude a hemodynamic effect of captopril treatment on water metabolism. Within 1 h of mean arterial pressure measurement, the rats were killed by decapitation. Trunk blood was collected, and kidneys were harvested on ice for Western blot analysis of renal aquaporins.

Reversibility of captopril-induced polydipsia and polyuria. Additional studies were performed to assess the reversibility of polydipsia and polyuria in rats receiving captopril. For these studies, 10 rats were acclimated to altitude and to metabolic cages as described above. Drinking water was provided ad libitum. For the initial 2 days of study (“baseline period”), rats received powdered chow at 15 g/day without additives. Food and water intake and urine output were recorded daily. Urine was collected on day 2 and frozen for later assessment of urine osmolality. On days 3–4 of the study period (“treatment period”), all rats received captopril at 20 mg/kg · day−1 in the daily supply of ground chow. In the final 24 h of the treatment period (day 4), food and water intake and urine output were recorded, and urine was collected and frozen for later assessment of urine osmolality. On days 5–6 of the study period (“recovery period”), rats again received ground chow without additives. In the final 24 h of this treatment period (day 6), food and water intake and urine output were recorded, and urine was collected and frozen for later assessment of urine osmolality. At the conclusion of the recovery period, the rats were killed by decapitation. Trunk blood was collected, and kidneys were harvested on ice.

Drugs

Captopril was obtained from Sigma (St. Louis, MO). B-9430, a nonselective bradykinin B1- and B2-receptor antagonist, was a kind gift of Drs. Lajos Gera and John Stewart (4, 21, 22). B-9430 is ~100 times as potent at B2 receptors as at B1 receptors (4). B1 receptors are not constitutively present but instead are expressed in response to chronic inflammation.

Tissue Preparation for Immunoblotting

At the time of death, kidneys were dissected on ice and inner medullary regions were removed. These inner medullary samples were immediately homogenized in ice-cold isolation solution containing 250 mM sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, with 0.1% vol protease inhibitors (0.7 μg/ml pepstatin, 0.5 μg/ml leupeptin, 1 μg/ml aprotinin) and 200 μM phenylmethylsulfonyl fluoride. Protein concentration was determined for each sample by the Bradford method (Bio-Rad, Richmond, CA).

Western Blot Analysis

Western blot analysis was performed to examine inner medullary expression of aquaporins (AQP)-2, -3, and -4. SDS-PAGE was performed on 12% acrylamide gels. After transfer by electroblot to polyvinylidene difluoride membrane (Millipore, Bedford, MA), blots were blocked overnight at 4°C with 5% nonfat dry milk in calcium- and magnesium-free PBS (PBS(−)) and then probed with primary antibody for 24 h at 4°C. AQP-3 antibody was a kind gift of Dr. Mark Knepper (Bethesda, MD). AQP-4 antibody was obtained from Alpha Diagnostic International (San Antonio, TX). The specificity of these antibodies has been previously documented (7, 24, 26). After washing with blot buffer containing PBS(−) with 0.1% Tween 20 (J. T. Baker, Phillipsburg, NJ), the membranes were exposed to secondary antibody for 1.5 h at room temperature. Subsequent detection of AQP was carried out by enhanced chemiluminescence (Amersham, Arlington Heights, IL) according to the manufacturer’s instructions. Prestained protein makers were used for molecular mass determinations.

Densitometric results were reported as integrated values (area × density of band) and expressed as a percentage compared with the mean value in CTL rats (100%). The blots shown are representative of blots obtained from all samples. Membranes were stained with Coomassie blue to ensure equal loading.

Biochemical Analysis

Serum and urine osmolalities were determined by freezing point depression (Advanced Instruments, Norwood, MA). Serum and urine creatinine were measured (Beckman Instruments), and results were used to calculate 24-h creatinine clearance as an estimate of glomerular filtration rate. Plasma AVP concentrations were measured by radioimmunoassay as described previously (14). Plasma renin activity was measured by radioimmunoassay. At the end of the balance study, plasma renin activity was measured for comparison in the CPT vs. CPT-BKI groups.

Statistical Analysis

Statistical analysis was performed using ANOVA followed by Tukey’s post hoc test or nonparametric methods where appropriate. Results are expressed as means ± SE. P < 0.05 was considered significant.

RESULTS

Effect of Bradykinin Antagonism on Water Metabolism

No significant differences were noted between the CTL and BKI groups for body weight, food or water intake, urine output, urine osmolality, serum creatinine, 24-h creatinine
EVIDENCE FOR BRADYKININ AS A STIMULATOR OF THIRST

F877
clearance, or plasma AVP concentration (Table 1). No significant differences were noted between the two groups for inner medullary protein abundance of AQP2 [CTL 100 ± 12 vs. BKI 95 ± 11% CTL mean, P not significant (NS)]; AQP3 (CTL 100 ± 17 vs. BKI 112 ± 10% CTL mean, P NS); or AQP4 (CTL 100 ± 15 vs. CTL-BKI 138 ± 21% CTL mean, P NS).

Effect of Captopril Treatment on Mean Arterial Pressure and 24-h Creatinine Clearance

There were no significant differences in body weight among the study groups at the initiation of the 24-h balance study. Mean arterial pressure, serum creatinine, and 24-h creatinine clearances were assessed at the conclusion of the study and were similar between the study groups (Table 2).

Effect of Captopril Treatment on Water Metabolism During Ad Libitum Water Intake

Treatment with captopril for 48 h resulted in increased water intake (Fig. 1A), increased urine output (Fig. 1B), decreased urine osmolality (Fig. 2A), and decreased plasma AVP concentration (Fig. 2B) compared with CTL animals. There was a mild decrease in serum osmolality in CPT rats (288 ± 2 vs. 293 ± 1 mosmol/kgH2O, P NS) compared with CTL. Inner medullary AQP2 protein abundance was significantly decreased in the captopril-treated rats (52 ± 7 vs. 100 ± 12% CTL mean, P < 0.05) vs. CTL (Fig. 3A). Inner medullary AQP3 and AQP4 protein abundance were not significantly different in the captopril-treated rats compared with CTL (Fig. 3, B and C).

Effect of Captopril Treatment on Water Metabolism During Water Restriction

With daily water intake limited to the volume consumed in the baseline period, captopril-treated rats (CPT-LIM) demonstrated water intake (29 ± 2 vs. 29 ± 2 ml/day, P NS), urine output (8 ± 2 vs. 8 ± 2 ml/day, P NS), serum osmolality (292 ± 2 vs. 293 ± 1 mosmol/kgH2O, P NS), and urine osmolality (2,547 ± 183 vs. 2,415 ± 384 mosmol/kgH2O, P NS) similar to CTL. Plasma AVP concentration was not significantly increased in CPT-LIM rats (1.95 ± 0.26 vs. 1.61 ± 0.11 pg/ml, P NS) compared with CTL. Inner medullary AQP2 protein abundance was not significantly increased in CPT-LIM rats (112 ± 1 vs. 100 ± 12%, P NS) compared with CTL.

Table 1. Effect of isolated bradykinin antagonist on water metabolism

|                  | CTL (n = 6) | BKI (n = 6) | P
|------------------|------------|------------|---
| Body weight, g   | 303 ± 7    | 302 ± 13   | NS
| Food intake, g/d | 15 ± 0     | 15 ± 0     | NS
| Water intake, ml/d | 25 ± 1   | 23 ± 1     | NS
| Urine output, ml/d | 10 ± 1    | 10 ± 1     | NS
| Urine osmolality, mosmol/kgH2O | 1,770 ± 89 | 1,879 ± 130 | NS
| Serum creatinine, mg/dl | 0.42 ± 0.03 | 0.38 ± 0.04 | NS
| 24-h Creatinine clearance, ml/min | 1.71 ± 0.18 | 2.25 ± 0.23 | NS
| Plasma AVP, pg/ml | 1.02 ± 0.10 | 1.22 ± 0.22 | NS

Table 2. Effect of captopril treatment on mean arterial pressure and 24-h creatinine clearance

|                  | CTL | CPT | CPT-LIM | CPT-BKI | P
|------------------|-----|-----|---------|---------|---
| Body weight, g   | 314 ± 8 | 315 ± 9 | 304 ± 12 | 303 ± 7 | NS
| MAP, mmHg        | 106 ± 1 | 103 ± 3 | 106 ± 3 | 105 ± 2 | NS
| Serum creatinine, mg/dl | 0.36 ± 0.03 | 0.39 ± 0.03 | 0.37 ± 0.02 | 0.35 ± 0.03 | NS
| 24-h Ccr, ml/min | 1.62 ± 0.15 | 2.35 ± 0.26 | 2.05 ± 0.31 | 1.63 ± 0.21 | NS

Values are means ± SE. CPT, captopril treatment with ad libitum water intake; CPT-LIM and CPT-BKI, captopril treatment with limited water intake and injection of B-9430, respectively; MAP, mean arterial pressure; Ccr, creatinine clearance. No significant differences were noted between study groups for body weight, MAP, serum creatinine, or 24-h Ccr.

Effect of Captopril Treatment and Bradykinin Antagonism on Water Metabolism

With the addition of the bradykinin antagonist B-9430 to captopril treatment (CPT-BKI), captopril-induced alterations in water intake (Fig. 1A) and urine output (Fig. 1B) were returned to CTL levels. Serum osmolality was returned to CTL levels with bradykinin inhibition (CPT-BKI 292 ± 2 vs. CTL 293 ± 1 mosmol/kgH2O, P NS). Urine osmolality was increased in CPT-BKI rats compared with CPT (Fig. 2A), but this did not reach significance. Plasma AVP concentration was comparable in CPT-BKI and CTL rats (Fig. 2B). Inner medullary AQP2, -3, and -4 protein abundance were not significantly different in CPT-BKI rats vs. CTL rats (Fig. 3, A–C). Plasma renin activity in the CPT vs. CPT-BKI was no different (8.5 ± 0.4 vs. 8.3 ± 0.7 mg·ml⁻¹·h⁻¹, P NS).

Fig. 1. Water intake (A) and urine output (B) were significantly increased in captopril-treated (CPT) rats compared with either control (CTL) rats or rats receiving captopril treatment with ad libitum water intake plus 24-h treatment with the bradykinin antagonist B-9430 (CPT-BKI).
Captopril treatment was associated with a significant increase in water intake and urine output and a significant decrease in urine osmolality compared with baseline (Fig. 4). These parameters returned to normal within 48 h of discontinuation of captopril. Plasma AVP concentration also returned to normal in this time period (baseline 1.7 ± 0.7 vs. recovery 2.3 ± 0.8 pg/ml, P NS).

**DISCUSSION**

It has been recognized for many years that exogenous bradykinin administration in the setting of ACE inhibition is associated with polydipsia and polyuria (10). However, the role of endogenous bradykinin in mediating this effect has not been well defined. Therefore, the goal of the present study was to assess the effect of bradykinin antagonism on water metabolism and renal AQP water channels in rats receiving 48-h treatment with captopril.

The present observations confirmed previous reports that captopril treatment is associated with polydipsia and polyuria (2, 15, 23). Such findings could relate either to a primary increase in thirst or to primary polyuria with a compensatory increase in water intake. Previous investigators have suggested that the renal kallikrein-kinin system acts to impair the hydrosmotic effect of AVP (8, 12, 13), resulting in a form of nephrogenic diabetes insipidus. Kinins are known to promote solute-free water excretion in dogs receiving AVP infusions (3) and to decrease AVP-stimulated water reabsorption in amphibian urinary bladder (11) and rabbit cortical collecting duct (20). Bradykinin B2 receptor gene knockout mice also demonstrate increased urinary concentration in response to AVP (1), indicating that endogenous kinins acting via B2 receptors oppose the antidiuretic effect of AVP.

The present findings, however, support a primary increase in thirst with ACEI rather than nephrogenic diabetes insipidus.
Specifically, primary polydipsia is implied by the observations that ACEI results not only in increased water intake and urine output but also in decreased urine osmolality and plasma AVP concentrations. These findings are not explained by captopril-mediated hypotension or by altered renal function, as mean arterial pressure and 24-h creatinine clearance were similar between all study groups. Further support that primary polydipsia occurs with captopril treatment comes from the observation that controlled water intake (CPT-LIM) returns urine output and osmolality and plasma AVP concentrations to levels seen in CTL rats. The polydipsic effect of captopril on thirst reversed within 48 h after cessation of captopril administration.

Our results demonstrated that administration of B-9430 in the absence of ACE inhibition does not affect water metabolism. ACEI is associated with increased endogenous bradykinin concentrations due to impaired degradation of bradykinin by kininase II. Additionally, prior experiments suggest that ACEI can induce cross talk between the B2 receptor and ACE on plasma membranes, thus delaying the sequestration of the B2 receptor and thereby potentiating the actions of bradykinin (5). To further define the role of bradykinin in captopril-mediated primary polydipsia, water metabolism was evaluated in the present study during simultaneous administration of captopril and the nonselective bradykinin receptor antagonist B-9430. Bradykinin receptor antagonism in the setting of captopril treatment resulted in a return of water intake, urine output, urine and serum osmolality, and plasma AVP concentration to CTL levels, implying that stimulation of bradykinin receptors is responsible for the primary polydipsia associated with ACEI. ANG-(1–7) metabolites of ANG I, which increase during ACEI, may also potentiate the action of bradykinin on the B2 receptor via binding to the active site of ACE (5, 6, 16).

The increase in fluid intake in the rat when bradykinin has been added to captopril was associated with an increase in plasma renin activity (18) and thus is compatible with the angiotensin spillover theory of the dipsogenic effect of exogenous bradykinin (9). In the present study, however, plasma renin activity was no different between the CPT and the CPT-BKI groups, thus implicating a dipsogenic role for bradykinin. This result extends the previous observation of Rowland and Fregly (18) whereby the bradykinin antagonist HOE-140 reduced the increased fluid intake that occurred with acute or chronic administration of captopril and bradykinin by 65–70%. The angiotensin receptor blocker losartan also reversed the increase in water intake with captopril and bradykinin, thus leading the authors to conclude that both angiotensin and bradykinin are involved in thirst regulation in the rat.

To further extend our observations, Western immunoblotting techniques were utilized to assess the effect of bradykinin antagonism on renal inner medullary AQP2, -3, and -4 protein abundance. It is known that chronic AVP stimulation of the renal V2 receptor on the basolateral membrane of the principal cell of the collecting duct induces an increase in the protein abundance of AQP2 in the inner medulla. Alternatively, states of chronic AVP suppression, such as psychogenic polydipsia or central diabetes insipidus, are associated with downregulation of inner medullary AQP2 (reviewed in Ref. 17). As anticipated, the primary polydipsia associated with ACEI resulted in a significant decline in inner medullary AQP2 protein abundance in the absence of any changes in AQP3 or -4. With the reversal of primary polydipsia during combined ACEI and bradykinin antagonism, inner medullary AQP2 protein abundance also returned to CTL levels.

In summary, the present results demonstrate that the polyuric effect of ACEI occurs secondary to increased fluid intake, suppression of AVP, and decreased AQP2 in the collecting duct. The attenuation of this polydipsic effect by a bradykinin antagonist incriminates endogenous bradykinin in thirst regulation in the rat, as has been shown with exogenous bradykinin. The effect of the bradykinin antagonist occurred in the absence of changes in mean arterial pressure, renal function, or plasma renin activity. Thus along with angiotensin, bradykinin should be considered as a dipsogenic factor in the rat.

GRANTS
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REFERENCES


