Cytochrome P-450-dependent metabolism of arachidonic acid in the kidney of rats with diabetes insipidus

Albert Sarkis,1 Osamu Ito,2 Takefumi Mori,1,3 Masahiro Kohzuki,2 Sadayoshi Ito,3 Joseph Verbalis,4 Allen W. Cowley Jr.,1 and Richard J. Roman1

1Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin; 2Department of Internal Medicine and Rehabilitation Science, 3Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; and 4Department of Medicine, Georgetown University, Washington, District of Columbia

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Sarkis, Albert, Osamu Ito, Takefumi Mori, Masahiro Kohzuki, Sadayoshi Ito, Joseph Verbalis, Allen W. Cowley Jr., and Richard J. Roman. Cytochrome P-450-dependent metabolism of arachidonic acid in the kidney of rats with diabetes insipidus. Am J Physiol Renal Physiol 289:F1333–F1340, 2005. First published July 12, 2005; doi:10.1152/ajprenal.00188.2005.—This study compared the renal metabolism of arachidonic acid in Brattleboro (BB) (vasopressin deficient) and Long-Evans (LE) control rats and the effects of a cytochrome P-450 (CYP) inhibitor 1-aminobenzotriazole (ABT) on renal function in these animals. The production of 20-hydroxyeicosatetraenoic acid (20-HETE) by renal cortical and outer medullary microsomes was significantly greater in BB than in LE rats (155 ± 16 vs. 92 ± 13 and 59 ± 7 vs. 33 ± 3 pmol·min⁻¹·mg protein⁻¹). Renal cortical epoxygenase activity was not different in these strains. The expression of CYP4A proteins was 58 and 78% higher in the renal cortex and outer medulla of BB than in LE rats. Chronic treatment of BB rats with a vasopressin type 2 receptor agonist for 1 wk normalized the renal production of 20-HETE. Chronic blockade of the formation of 20-HETE and EETs with ABT had little effect on renal function in LE rats. However, urine flow increased by 54% and urine osmolality decreased by 33% in BB rats treated with ABT. Plasma levels of oxytocin fell significantly from 7.2 ± 1.3 to 3.9 ± 1.0 pg/ml. The effects of ABT in BB rats were attenuated by chronic infusion of oxytocin (0.7 ng·min⁻¹·100 g⁻¹) to maintain fixed high plasma levels of this hormone. These results indicate that the expression of CYP4A protein and the renal formation of 20-HETE are elevated in the kidney of BB rats due to a lack of vasopressin and that chronic blockade of the formation of 20-HETE and EETs with ABT promotes water excretion in vasopressin-deficient BB rats by reducing the circulating levels of oxytocin, which is a weak vasopressin agonist. Brattleboro rats; 20-hydroxyeicosatetraenoic acid; cytochrome P-450; epoxygenase activity; renal hemodynamics.

ARACHIDONIC ACID (AA) is metabolized by cytochrome P-450 (CYP) enzymes in the kidney to produce epoxygenase products (EETs), 20-hydroxyeicosatetraenoic acid (20-HETE), and dihomoxyeicosatetraenoic acids (DiHETEs). These compounds have been reported to play an important role in the control of both renal tubular and vascular function (26). EETs are potent endothelial-derived vasodilators in the renal circulation (17, 47). They also inhibit sodium and water reabsorption in the collecting duct (40). In contrast, 20-HETE is produced by vascular smooth muscle cells and is a potent constrictor of renal arterioles (5, 19, 23). Inhibition of the synthesis of 20-HETE attenuates the vasoconstrictor response of renal arterioles to elevations in transmural pressure in vitro (16, 18) and autoregulation of renal blood flow and tubuloglomerular feedback responses in vivo (8, 48). At the level of the renal tubules, 20-HETE inhibits sodium reabsorption in the proximal tubule and thick ascending limb of the loop of Henle (6, 32, 34, 39).

Previous studies indicated that the renal production of EETs and/or 20-HETE is altered in diabetes and in genetic and experimental models of hypertension (1, 7, 21, 24, 25, 28, 41). The release of 20-HETE in renal and peripheral blood vessels is stimulated by ANG II (5), phenylephrine (20a), endothelin (37), and serotonin (3). There is considerable evidence that 20-HETE contributes to the vasoconstrictor responses to these hormones by modulating potassium channel activity and calcium influx (1). Arginine vasopressin (AVP), similar to ANG II and endothelin, is also a potent vasoconstrictor that activates phospholipase A2 and releases AA (10, 31). Blockade of CYP activity with SnCl₂ attenuates the vasoconstrictor response to vasopressin in isolated, perfused kidney of rats (45). However, the effects of AVP on the expression of CYP4A enzymes and the renal production of 20-HETE and EETs remain poorly understood.

Therefore, the present study compared the expression of CYP4A enzymes and the renal production of EETs and 20-HETE in the kidney of vasopressin-deficient Brattleboro (BB) and a vasopressin-replete Long-Evans (LE) rats. The effects of acute and chronic inhibition of the formation of EETs and 20-HETE with 1-aminobenzotriazole (ABT) on renal tubular and vascular function were compared in BB and LE rats. We also studied the effects of fixing plasma levels of oxytocin by intravenous infusion on the renal response to ABT in BB and LE rats.

METHODS

General. Experiments were performed on 9- to 10-wk-old male BB (n = 62) or LE (n = 46) rats purchased from Harlan Sprague Dawley Laboratories (Indianapolis, IN). The rats were housed in the Animal Care Facility at the Medical College of Wisconsin, which is approved by the American Association for the Accreditation of Laboratory Animal Care. Food (catalog no. 5001, Labdiet Purina, Richmond, IN) and water were provided ad libitum. All protocols were approved by the Animal Care Committee of the Medical College of Wisconsin.

Protocol 1: expression of CYP4A protein and renal formation of 20-HETE and EETs. These experiments were performed on groups of BB and LE rats that received daily injections of either ABT (50 mg/kg ip) or vehicle (0.9% NaCl solution) for 3 days or a group of BB rats...
that received dDAVP (2 mg/1) in the drinking water for 1 wk. At the end of the treatment period, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The kidneys were rapidly removed and the renal cortex and outer medulla were collected and homogenized separately in 3 ml of a 10 mM potassium buffer (pH 7.7) containing 250 mM sucrose, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). Microsomes were prepared as previously described (14). Briefly, homogenates were centrifuged at 3,000 g for 5 min to remove large tissue fragments, and the supernatants were centrifuged at 9,000 g for 15 min to remove mitochondria and nuclei. The supernatant was spun at 100,000 g for 1 h to collect membrane fragments and endoplasmic reticulum. The microsomal pellets were resuspended in the 100 mM potassium buffer (pH 7.25) containing 30% glycerol, 1 mM dithiothreitol, and 0.1 mM PMSF. The protein concentration of the microsome samples was measured using the Bradford method (Bradford) with bovine gamma globulin (Bio-Rad Laboratories, Hercules, CA) as a standard.

The renal metabolism of AA was determined by incubating the microsomes prepared from the renal cortex (0.25 mg protein) or outer medulla (0.5 mg protein) with [14C]AA (10.2 pmol/ml, 10 μM, American Life Sciences, Arlington Heights, IL) in 1 ml of a 100 mM potassium phosphate buffer (pH 7.4) containing 10 mM MgCl2, 1 mM EDTA, 1 mM NADPH, and an NADPH-regenerating system (10 mM isocitrate and 0.4 U/ml isocitrate dehydrogenase) at 37°C for 30 min. The reaction was terminated by acidification to pH 4.0 with 1 M formic acid. Metabolites of AA were extracted twice with 3 ml of ethyl acetate, and the extract was dried under N2. The metabolites were separated by using a 2 × 250-mm C18-reverse-phase HPLC column (Supelcosil LC18, cat. no. 57935, Supelco, Bellfonte, PA) at a flow rate of 0.3 ml/min using a linear elution gradient ranging from acetoniitrile/water/acetic acid (50/50/0.1 vol/vol/vol) to acetonitrile/acetic acid (100/0.1 vol/vol) over a 40-min period. The radioactivity products were monitored using a radioactive flow detector (model A-120, Radiomatic Instrument, Tampa, FL), and the production rates of the various metabolites were expressed as picomoles of metabolites formed per minute per milligram of protein.

The expression of CYP4A protein was determined by Western blot analysis. An aliquot of microsomal protein isolated from the renal cortex (10 μg protein) or outer medulla (25 μg protein) of BB and LE rats and BB rats treated with dDAVP was solubilized at 94°C for 5 min in Laemml sample buffer containing 5% mercaptoethanol and separated by electrophoresis on 7.5% SDS polyacrylamide gels (Bio-Rad) for 1.0 h at 200 V. The proteins were transferred to nitrocellulose membranes, and the membranes were blocked overnight in a buffer containing 10 mM Tris-HCl, 150 mM NaCl, 0.08% Tween 20, and 10% nonfat dry milk. The membranes were incubated for 2 h with a 1:2,000 dilution of a goat polyclonal antibody raised against rat CYP4A (cat. no. 299230, Daiichi Pure Chemicals, Tokyo, Japan), rinsed several times with buffer, and then incubated with a 1:4,000 dilution of a horseradish peroxidase-coupled, anti-goat secondary antibody (cat. no. SC 2020, Santa Cruz Biolaboratory, Santa Cruz, CA) for 1 h. The blots were developed using an enhanced chemiluminescent kit (West Pico, Pierce, Rockford, IL), exposed to X-ray film, and the relative intensities of the bands in the 50- to 52-kDa range for CYP4A proteins were quantified using an Eagle Eye imaging system (Stratagene, La Jolla, CA) and Un-Scan It software (Silk Scientific, Orem, UT).

Protocol 2: effect of chronic blockade of 20-HETE and EETs with ABT on sodium and water excretion in conscious BB and LE rats. BB (n = 14) and LE (n = 14) rats were placed in metabolic cages. After 3 days of acclimatization, a control 24-h urine sample was collected for measurement of baseline urine flow, sodium excretion, urinary osmolality, and creatinine clearance. The rats were then given daily injections of ABT (50 mg/kg ip) to inhibit the renal formation of 20-HETE and EETs. On the third day of treatment, a 24-h urine sample was collected and a blood sample was collected for measurement of plasma blood-urea-nitrogen and creatinine levels.

The effects of ABT on the response to dehydration were determined in a separate group of BB (n = 6) and LE (n = 6) rats. In these experiments, water was restricted for 4 h in BB and for 8 h in LE rats, and a urine sample was collected for another 4 h in BB and 16 h in LE rats. The rats were allowed to recover from dehydration for 4 days and then were treated with daily intraperitoneal injections of ABT (50 mg·kg⁻¹·day⁻¹). After 3 days, water was again restricted and urine samples were recollected.

Protocol 3: effects of oxytocin on the renal response to ABT in BB and LE rats. Plasma levels of oxytocin were measured in four BB rats to determine whether the increase in urine flow and fall in urinary osmolality in BB rats treated with ABT was due to a fall in circulating levels of oxytocin. In these experiments, a chronic catheter was implanted in the femoral artery. After a 3-day recovery period, a control blood sample (0.5 ml) was collected. The rats then were treated with daily intraperitoneal injections of ABT (50 mg/kg). After 3 days, a blood sample was recollected.

Experiments were also performed in separate groups of eight BB and eight LE rats to determine whether fixing circulating oxytocin at high levels by chronic intravenous infusion would block the effects of ABT in BB rats. These rats were surgically prepared with a chronic arterial catheter as described above. In addition, an osmotic pump (model 2002, Alzet Osmotic Pumps, Cupertino, CA) was implanted in the peritoneal cavity to deliver oxytocin at a dose of 0.7 mg·min⁻¹·100 g body wt⁻¹ throughout the experiment. After a 3-day recovery period, a control 24-h urine sample was collected for measurement of baseline urine flow, sodium excretion, urinary osmolality, and creatinine clearance. A blood sample (0.5 ml) was also collected from the femoral artery to measure oxytocin levels. The rats were then given daily injections of ABT (50 mg/kg ip). On the third day of treatment, a 24-h urine sample was collected and an experimental blood sample was collected for measurement of oxytocin levels.

Blood levels of oxytocin were measured using two different assays. The low endogenous levels of oxytocin in the BB rats were determined by a proprietary radioimmunoassay after acetone-ether extraction as described previously (43). The much higher plasma levels of oxytocin in the oxytocin-infused LE and BB rats were measured using an less sensitive but commercially available EIA kit (Assay Designs, Ann Arbor, MI).

Protocol 4: effects of blockade of 20-HETE and EETs with ABT on renal function in anesthetized BB rats. Further experiments were performed to determine the mechanism by which ABT increases urine flow and free water clearance in BB rats. Two groups of rats were studied. In group 1, six BB rats were treated with daily intraperitoneal injections of ABT (50 mg/kg) for 3 days to block the renal production of 20-HETE. In group 2, six BB rats received intraperitoneal injections of vehicle for 3 days. Both groups of rats were then anesthetized with thiobutabarbital (100 mg/kg body wt ip) and ketamine (30 mg/kg im) and surgically prepared for clearance experiments. The rats were placed on a heating table to maintain body temperature at 37°C, and a cannula was placed in the trachea to facilitate breathing. The left jugular vein was cannulated for intravenous infusions, and the left carotid artery was catheterized to measure mean arterial pressure. The vasopressin-deficient BB rats received an intravenous infusion of hypotonic 0.45% NaCl at a rate of 8 ml/h to replace fluid losses and maintain a water diuresis. [3H]inulin (2 μCi/ml) and lithium chloride (10 mM) were included in the infusion solution to measure glomerular filtration rate (GFR) and the fractional excretion of lithium (FELi). A flow probe was placed around the left renal artery to measure renal blood flow (RBF) using a transit-time flowmeter (model T106, Transonic Systems). The left ureter was catheterized for the collection of urine.

After a 60-min equilibration period, urine and plasma samples were collected during two 15-min clearance periods. In addition, the vehicle-treated BB rats were given a bolus intravenous injection of ABT (50 mg/kg). After a 60-min equilibration period, urine and plasma
concentration. Urine and plasma Na concentration was calculated as follows: measured using the Jaffe reaction in an autoanalyzer concentration,

samples were assayed for the renal metabolism of AA.

Mean values ± SE are presented. Numbers in parentheses indicate the number of rats studied per group. *Significant difference from the corresponding value in LE rats. †Significant difference from the corresponding value in BB rats.

Expression of CYP4A protein and renal formation of 20-HETE and EETs. A comparison of CYP-dependent metabolism of AA in microsomes prepared from the renal cortex and outer medulla of BB and LE rats is presented in Fig. 1. The production of 20-HETE by renal cortical microsomes was significantly greater in BB than in LE rats; however, no difference was observed in the production of EETs or DiHETEs. The production of 20-HETE by microsomes prepared from the outer medulla was also significantly greater in BB rats than in LE rats (P < 0.05). We did not detect formation of EETs or DiHETE by the outer medullary microsomes.

Chronic treatment of BB rats for 1 wk with dDAVP reduced the production of 20-HETE in both cortical and outer medullary microsomes of BB rats to the same levels seen in LE rats. However, dDAVP did not alter the formation of EETs and DiHETEs (Fig. 1). The production of 20-HETE by renal cortical or outer medulla microsomes fell by more than 90% in BB and LE rats chronically treated with ABT. Renal cortical epoxygenase activity was also reduced to a similar extent in BB and LE rats treated with ABT (Fig. 1).

The expression of CYP4A protein in microsomes prepared from the renal cortex and outer medulla of BB and LE rats is presented in Fig. 2. The expression of CYP4A protein was 58 and 78% greater in the renal cortex and outer medulla of BB rats compared with the levels seen in LE rats. Chronic
treatment of the BB rats with dDAVP reduced the expression of CYP4A protein in the renal cortex and outer medulla (Fig. 3).

Effect of chronic blockade of 20-HETE and EETs with ABT on sodium and water excretion in conscious BB and LE rats. A comparison of the effects of ABT on urine flow and \( U_{\text{osm}} \) in BB and LE rats is presented in Fig. 4. Urine flow increased by 54\%, and \( U_{\text{osm}} \) decreased by 33\% (\( P < 0.001 \)) in BB rats treated with ABT. Sodium excretion was not significantly altered (1.7 ± 0.1 vs. 1.9 ± 0.2 \( \mu \text{eq/day} \)), and potassium excretion remained unchanged (3.8 ± 0.3 vs. 3.5 ± 0.3 \( \mu \text{eq/day} \)). The effect of ABT to promote water excretion in BB rats was associated with a significant elevation in \( C_{\text{H2O}} \) and \( C_{\text{cr}} \) (Fig. 5). \( C_{\text{H2O}} \) rose by 80\%, and \( C_{\text{cr}} \) increased by 45\%. In contrast, ABT had little effect on renal function in LE rats. Urine flow, sodium excretion, \( C_{\text{H2O}} \), and \( C_{\text{cr}} \) were not significantly different in LE rats treated with ABT or vehicle (Figs. 4 and 5). However, ABT treatment significantly decreased \( U_{\text{osm}} \) in ABT-treated LE rats.

Effect of ABT on the response to dehydration in BB and LE rats. A comparison of the effects of ABT on the response of BB and LE rats to dehydration is presented in Table 1. \( U_{\text{osm}} \) increased and urine flow and sodium excretion fell in BB rats following water restriction. Blockade of the formation of 20-HETE and EETs with ABT attenuated the increase in \( U_{\text{osm}} \) and the decrease in sodium excretion following dehydration in BB rats. However, ABT had no effect on the fall in urine flow following dehydration in the BB rats. In LE rats, dehydration alone did not affect \( U_{\text{osm}} \) or urine flow while it decreased urinary sodium excretion. ABT attenuated \( U_{\text{osm}} \) response to dehydration in LE rats but it did not affect urine flow or urinary sodium excretion following dehydration in LE rats treated with ABT.

Effects of oxytocin on the renal response to ABT in BB and LE rats. The effects of fixing plasma levels of oxytocin by intravenous infusion on the renal response to ABT are presented in Fig. 4. Baseline urine flow decreased significantly in BB rats infused with oxytocin. This was associated with a significant increase in \( U_{\text{osm}} \). The increase in urine flow and the fall in \( U_{\text{osm}} \) produced by ABT were significantly attenuated in BB rats that were chronically infused with oxytocin. In contrast, infusion of oxytocin had no effect on the baseline urine flow or \( U_{\text{osm}} \) in LE rats and it did not alter the response to ABT.

The effects of oxytocin infusion on \( C_{\text{H2O}} \) and \( C_{\text{cr}} \) in LE and BB rats are presented in Fig. 5. Baseline \( C_{\text{H2O}} \) significantly decreased in BB rats infused with oxytocin. The increase in \( C_{\text{H2O}} \) and \( C_{\text{cr}} \) produced by ABT was significantly attenuated in BB rats infused with oxytocin. In contrast, exogenous administration of oxytocin had no effect on baseline \( C_{\text{H2O}} \) and \( C_{\text{cr}} \) in LE rats or the response to ABT.

The effects of chronic blockade of 20-HETE and EETs with ABT on plasma oxytocin levels in BB rats are presented in Fig. 6. Plasma levels of oxytocin fell significantly by 46\% in BB rats that were treated with ABT. Plasma levels of oxytocin increased in BB and LE rats chronically infused with oxytocin. Plasma oxytocin levels averaged 425 ± 111 and 323 ± 41 pg/ml in BB and LE rats, respectively. These levels remained unchanged after administration of ABT.

Effects of blockade of 20-HETE and EETs with ABT on renal function in anesthetized BB rats. Additional experiments were performed to determine whether the increases in urine flow, \( C_{\text{H2O}} \), and \( C_{\text{cr}} \) in BB rats treated with ABT were due to changes in RBF or GFR. There was no significant difference in any of the measured parameters in anesthetized BB rats treated with ABT for 3 days to block renal 20-HETE levels vs. the vehicle-treated rats in which 20-HETE production was acutely blocked with an intravenous bolus injection of ABT. Therefore, the data from these two groups were pooled and presented together in Table 2. Urine flow, sodium excretion, RBF, GFR, \( F_{\text{E}_{\text{Na}^+}} \), and \( F_{\text{E}_{\text{Li}^+}} \) were similar in BB rats treated with ABT or vehicle. However, potassium excretion significantly decreased by 53\% after ABT treatment compared with control period.

Fig. 3. Effects of dDAVP on the expression of CYP4A protein in the renal cortex and outer medulla of BB rats. A: lanes 1 through 4 were loaded with microsomes (20 \( \mu \text{g} \)) prepared from the renal cortex of BB rats. Lanes 6 through 9 were loaded with microsomes (20 \( \mu \text{g} \)) prepared from the outer medulla of BB rats. Lane 5 was loaded with microsomes (20 \( \mu \text{g} \)) prepared from the liver of a Sprague-Dawley rat that expresses all 3 isoforms of CYP4A. B: lanes 1 through 4 were loaded with microsomes (50 \( \mu \text{g} \)) prepared from the outer medulla of BB rats. Lanes 6 through 9 were loaded with microsomes (50 \( \mu \text{g} \)) prepared from the outer medulla of BB rats chronically treated with dDAVP (2 mg/l in drinking water) for 3 days. Lane 5 was loaded with microsomes (20 \( \mu \text{g} \)) prepared from the liver of a Sprague-Dawley rat that expresses 3 isoforms of CYP4A. Values are means ± SE from 4 rats per group. *Significant difference from the corresponding value in BB rats.
DISCUSSION

Recent studies have indicated that CYP metabolites of AA play an important role as second messengers in the regulation of the tubular and vascular effects of peptide hormones and growth factors (1, 3, 37, 40). For example, ANG II and endothelin stimulate the renal formation of 20-HETE, which contributes to the renal vasoconstrictor and natriuretic actions of these hormones (11, 12, 35). The expression of CYP4A enzymes and the formation of 20-HETE are also elevated in the kidney of ANG II-infused hypertensive rats (1), and 20-HETE contributes to the development of hypertension and end-organ damage in this model (1, 27, 28). Vasopressin is another vasoconstrictor peptide that activates phospholipase A2 and releases AA (31). Previous studies have established that vasopressin stimulates the formation of PGE2 in mesangial and tubular epithelial cells and increases the urinary excretion of PGE2 (20, 44, 46). PGE2 modulates both the tubular and vascular responses to vasopressin (9, 15, 38). However, much less is known about the possible effect of vasopressin on the formation of EETs and 20-HETE in the kidney or whether CYP metabolites of AA contribute to the effects of AVP on renal vascular and tubular function. Thus the present study examined the effects of vasopressin on the CYP-dependent metabolism of AA in the kidney by comparing the expression of CYP4A protein and the formation of EETs and 20-HETE in microsomes prepared from the kidneys of vasopressin-deficient BB and control LE rats. We also examined the effects of acute and chronic blockade of the renal formation of EETs and 20-HETE with ABT on renal function in these rats.

The present results indicate that expression of CYP4A protein and renal production of 20-HETE is elevated in both the renal cortex and outer medulla of vasopressin-deficient BB rats. Chronic treatment of BB rats with a V2 receptor agonist restored urine-concentrating ability and returned the expression of CYP4A protein and the renal production of 20-HETE in the renal cortex and outer medulla to levels that are not different from those seen in control LE rats. These findings suggest that vasopressin downregulates the renal expression of CYP4A protein and the production of 20-HETE via an action on the V2 receptor. This finding is somewhat surprising in view of previous results indicating that acute administration of vasopressin...
pressin stimulates the release of 19- and 20-HETE from isolated, perfused kidneys of spontaneously hypertensive rats (SHR) (33) and that vasopressin stimulates the formation of EETs by rabbit thick ascending limbs (42) and cortical collecting ducts (13) in vitro. The reasons for the differences in results remain to be determined. They may reflect species or strain differences (rats vs. rabbits; SHR vs. BB rats), differences in the acute vs. chronic effects of vasopressin, and/or differences in the effects of vasopressin on V1 vs. V2 receptors. It also remains unclear as to what cell types are responsible for the effect of dDAVP on the expression of EETs and 20-HETE in the renal cortex, as V2 receptors are expressed in the medullary thick ascending limb and the collecting duct while the proximal tubules are the primary cell type that produces 20-HETE and EETs in the renal cortex. Further studies in microdissected nephron segments will be required to address the issue.

Additional experiments were performed to determine the functional significance of changes in the production of 20-HETE on renal function in BB rats. Chronic treatment of BB and LE rats with ABT for 3 days significantly inhibited the synthesis of 20-HETE, EETs, and DiHETEs by renal microsomes in both strains of rats. Chronic blockade of the renal formation of these compounds had no effect on urine flow, sodium excretion, or creatinine clearance in conscious LE rats. However, ABT decreased Uosm in LE rats in both dehydrated and hydrated states. The mechanism for the fall in Uosm remains unknown. One possibility is that ABT might induce a change in the sensitivity of osmoreceptors to release vasopressin in response to dehydration. It may also suggest that ABT has an influence on vasopressin secretion as could be predicted by the work of Negro-Vilar et al. (30) where the authors showed that eicosanoids affect the secretion of both oxytocin and vasopressin. In contrast to ABT-treated LE rats, urine flow and free C\textsubscript{H2O} increased significantly, and urine osmolality fell in BB rats. The diuretic response seen in BB was unexpected, because EETs and 20-HETE are generally thought to inhibit rather than increase tubular reabsorption of sodium and water in the kidney (26, 40).

We next considered whether the increase in free C\textsubscript{H2O} seen in BB rats after ABT could be due to an increase in distal delivery of sodium because of inhibition of sodium transport in the proximal tubule and/or an increase in GFR. Indeed, previous studies have indicated that CYP inhibitors attenuate renal response to vasoconstrictors (4, 11) and that blockade of the CYP system with DBDD increases renal blood flow and GFR (36). Alternatively, C\textsubscript{H2O} could increase in BB rats in the absence of vasopressin, if the permeability of the medullary collecting duct to water was further reduced. To address these possibilities, we studied the effects of acute and chronic blockade of the formation of 20-HETE and EETs with ABT on renal hemodynamics and tubular function. We found that ABT had no effect on RBF, GFR, or the FE\textsubscript{Na+} or FE\textsubscript{Li+}, an index of proximal tubular reabsorption in BB rats. Thus it is unlikely that the increase in water excretion in BB rats was due to a rise in the filtered load or inhibition of proximal sodium reabsorption.

Another possible explanation for the diuretic and natriuretic response seen in BB rats after ABT treatment is that 20-HETE and EETs might affect the secretion of hormones important in the control of sodium and water balance. For example, ABT might inhibit the synthesis of aldosterone, which is dependent on the work of Negro-Vilar et al. (30) where the authors showed that CYP inhibition decreased aldosterone synthesis.

**Table 1. Effects of ABT in response to dehydration in BB and LE rats**

<table>
<thead>
<tr>
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<th>BB Rats (n = 6)</th>
<th>LE Rats (n = 6)</th>
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<tr>
<td></td>
<td>Control</td>
<td>4-h Dehydration</td>
</tr>
<tr>
<td>Uosm, mosmol/kgH\textsubscript{2}O</td>
<td>180 ± 10</td>
<td>388 ± 17*</td>
</tr>
<tr>
<td>UV, ml/day</td>
<td>171 ± 7.1</td>
<td>29 ± 4*</td>
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<tr>
<td>UNa, V, μEq/day</td>
<td>1.99 ± 0.1</td>
<td>1.31 ± 0.1*</td>
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<td>1,709 ± 155</td>
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<td>11.6 ± 1.2</td>
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<td>11.75 ± 1.7</td>
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Values are means ± SE. Uosm, urinary osmolarity; UV, urine flow; UNa, sodium excretion. BB, Brattleboro rats; LE, Long-Evans rats. *Significant difference vs. control. †Significant difference dehydration + ABT vs. dehydration alone.

**Fig. 6. Plasma levels of OT in BB rats (n = 4) before and after administration of ABT (50 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}) for 3 days. *Significant difference from the corresponding control value.**

**Table 2. Effects of blockade of 20-HETE and EETs with ABT on renal function in anesthetized BB rats**

<table>
<thead>
<tr>
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<th>BB Rats</th>
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<tbody>
<tr>
<td>RBF, ml·min\textsuperscript{-1}·g kidney wt\textsuperscript{-1}</td>
<td>4.26 ± 0.4</td>
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<tr>
<td>GFR, ml·min\textsuperscript{-1}·g kidney wt\textsuperscript{-1}</td>
<td>1.021 ± 0.04</td>
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<tr>
<td>UV, μl·min\textsuperscript{-1}·g kidney wt\textsuperscript{-1}</td>
<td>77.9 ± 2.8</td>
</tr>
<tr>
<td>UNa, V, μEq·min\textsuperscript{-1}·g kidney wt\textsuperscript{-1}</td>
<td>0.38 ± 0.15</td>
</tr>
<tr>
<td>UK\textsubscript{c}, V, μEq·min\textsuperscript{-1}·g kidney wt\textsuperscript{-1}</td>
<td>0.63 ± 0.12</td>
</tr>
<tr>
<td>FE\textsubscript{Na+}, %</td>
<td>0.250 ± 0.09</td>
</tr>
<tr>
<td>FE\textsubscript{Li+}, %</td>
<td>11.1 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. From acute and chronic administration of ABT were added together and represented under ABT column. *Significant differences vs. control ns. Not significant; RBF, renal blood flow; GFR, glomerular filtration rate; UK\textsubscript{c}, V, potassium excretion; FE\textsubscript{Na+}, fractional excretion of sodium; FE\textsubscript{Li+}, fractional excretion of lithium.
on CYP11B2 activity (aldosterone synthase) (22). A fall in aldosterone levels in vasopressin-deficient BB rats undergoing a water diuresis would be expected to increase urine flow, sodium excretion, and urine osmolality, while the excretion of potassium would decrease. However, this seems to be an unlikely explanation for the diuretic response seen in BB rats chronically treated with ABT, because the urinary excretion of sodium and potassium was not significantly altered. Moreover, in acute studies, urine flow remained unchanged and sodium excretion tended to decrease rather than increase in BB rats treated with ABT. In addition, the timeframe of ABT treatment is too short to lower aldosterone levels. The reason for the fall in potassium excretion seen after acute administration of ABT to BB rats remains to be determined. One possible explanation is that potassium recycling in the thick ascending limb of the loop of Henle is mediated by the ROMK channel. 20-HETE inhibits Na\(^{+}\), K\(^{+}\), 2Cl\(^{-}\) transport and the ROMK transport in this segment of the nephron. ABT inhibits the synthesis of 20-HETE. Blockade of 20-HETE will increase the transepithelial potential across the membrane, thus increasing the passive Na\(^{+}\) and K\(^{+}\) reabsorption, and this may contribute to the decrease in K\(^{+}\) excretion.

Oxytocin levels have been reported to be elevated in vasopressin-deficient BB rats (2). Because oxytocin is a weak vasopressin agonist, it may help maintain some degree of urine-concentrating ability in vasopressin-deficient BB rats. Thus we examined the effects of ABT on plasma oxytocin levels in BB rats. We found that plasma levels of oxytocin fell in BB rats treated with ABT. Moreover, chronic infusion of BB rats with oxytocin attenuated the fall in urine osmolality and the diuretic response in BB rats treated with ABT. These results are consistent with the view that elevated levels of oxytocin has a vaspressin-like effect and that blockade of the CYP-dependent metabolites of AA with ABT promotes diuresis by reducing oxytocin secretion in these animals. This observation is also consistent with previous findings indicating that CYP metabolites of AA, likely EETs, contribute to the secretion of oxytocin and vasopressin in the posterior pituitary gland (30). However, a fall in the levels of oxytocin does not fully explain the diuresis seen in the BB rats treated with ABT. Oxytocin clearly attenuated the response to ABT in BB rats, but some residual response remained. This residual effect may be related to enhanced sodium and potassium reabsorption as it is known that 20-HETE inhibit electrolyte transport in the proximal tubule and the thick ascending limb of the loop of Henle as discussed above.

Perspectives

The present study indicates that the renal formation of 20-HETE is elevated in the kidney of BB rats with diabetes insipidus and that blockade of the formation of EETs and 20-HETE with ABT increases urine flow and C\(_{12}H_{20}\) and decreases U\(_{\text{osm}}\) in BB rats. The diuretic effect of ABT was not associated with an increase in GFR or inhibition of sodium transport in the proximal tubule. Rather, it appears to be due to an inhibition of the release of oxytocin from the posterior pituitary gland, which previous studies have indicated might be dependent of the formation of CYP metabolites of AA (30).

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