Adenosine A1 Receptor Antagonist Blunts Urinary Potassium Excretion, But Not Renal Hemodynamic Effects, Induced by Carbonic Anhydrase Inhibitor in Rats

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A1 Receptor Blockade and Carbonic Anhydrase Inhibition

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Abbreviations:
Repeated-measures analysis of variance (RM-ANOVA), urine excretion rate (UV), urinary excretion rate of sodium (UNaV), urinary excretion rate of potassium (UKV), renal blood flow (RBF), glomerular filtration rate (GFR), mean arterial blood pressure (MABP), heart rate (HR), vehicle (Vh), 1,3-dipropyl-8-cyclopentylxanthine or DPCPX (DP), acetazolamide (AZ), angiotensin AT1 receptor blocker (ARB), angiotensin II (ANGII), A1 adenosine receptor (A1AR), tubuloglomerular feedback (TGF), carbonic anhydrase (CA), carbonic anhydrase inhibitor (CAI), afferent arteriole (aff art), angiotensin converting enzyme (ACE), renin-angiotensin system (RAS), NHE (sodium-hydrogen exchanger), AQP (aquaporin), dimethylsulfoxide (DMSO), proximal tubule (PT).

Recommendation for Section Assignment:
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Abstract

Acetazolamide (AZ) is a carbonic anhydrase inhibitor with diuretic actions at the proximal tubule. Clinical use of AZ is limited, in part, due to the urinary potassium loss and decrease of renal hemodynamic function that accompanies the drug. There is recent interest in A1 adenosine receptor (A1AR) antagonists, a novel class of diuretic agents that does not cause loss of potassium or tubuloglomerular feedback-(TGF)-mediated-reductions of renal hemodynamics. We tested whether the A1AR-antagonist, DPCPX, could attenuate the adverse effects normally associated with use of AZ. RBF and urine output were measured during two consecutive 40-min periods in anesthetized rats. In the first period, vehicle or DPCPX was infused. DPCPX alone increased urine output and sodium excretion, but did not significantly alter potassium output or RBF. In the second period, the initial infusion of vehicle or DPCPX was continued and either AZ or its vehicle was administered. AZ alone increased urinary excretion of both sodium and potassium, and decreased RBF. DPCPX significantly attenuated the AZ-induced increase of potassium excretion by 50%, but did not blunt the renal hemodynamic response to AZ. In a separate study, angiotensin AT1 receptor blockade also failed to blunt the renal hemodynamic response to AZ. In summary, A1AR-antagonists may be useful diuretic agents alone or in combination with other conventional diuretic agents. The decrease of RBF that occurred in response to carbonic anhydrase inhibition was not attenuated by either A1AR-blockade or AT1 receptor-blockade and does not appear to be mediated by a TGF-dependent mechanism.
Introduction

Diuretics are recommended as first-line therapy for the treatment of edema and hypertension (Chobanian et al., 2003). However, a recent and controversial epidemiologic report outlined data linking diuretic use to the incidence of end-stage renal disease (Hawkins and Houston, 2005). The effect, if true, may be a consequence of potential detrimental effects of diuretics on GFR and RBF which could augment the progression of pre-existing renal disease.

In addition, diuretic-induced alterations of renal hemodynamics limit the efficacy of these agents. For example, carbonic anhydrase inhibition (CAI) attenuates sodium reabsorption in the proximal tubule (PT) resulting in a diuretic effect; however, the diuretic effect is self-limiting, in part, due to a marked decrease in the GFR and RBF. The CAI-induced decreases of GFR and RBF are believed to be mediated through activation of tubuloglomerular feedback (TGF) (Tucker et al., 1978). An increase of sodium chloride delivery to tubular macula densa cells, as occurs following inhibition of proximal sodium reabsorption, stimulates the release of a putative chemical mediator that induces vasoconstriction of the adjacent afferent arteriole. An increase of resistance in the afferent arteriole reduces GFR and RBF, resulting in decreased filtration of sodium into the tubular lumen of that nephron, thus opposing the desired diuretic effect of drug treatment. Furthermore, these renal hemodynamic effects alter the bioavailability of the diuretics at their sites of action since the drugs must be filtered or actively secreted into the tubular lumen in order to inhibit sodium transport.
An additional concern regarding the use of diuretics relates to their concomitant effects on potassium. Diuretics that inhibit sodium reabsorption upstream of the collecting duct (i.e., PT, thick ascending limb of Henle’s loop, or distal convoluted tubule) cause hypokalemia. In contrast, diuretics that block sodium reabsorption in the collecting duct, either through direct inhibition of sodium channels or through antagonism of aldosterone, inhibit the secretion of potassium.

Whereas diuretic therapy is generally effective, there are clinical situations in which relative diuretic resistance occurs. For example, loop diuretics are often less effective in heart failure patients (Brater, 1998), perhaps secondary to impaired renal hemodynamics and enhanced proximal tubular sodium reabsorption in this pathological condition (Kramer et al., 1999). In addition, resistance to diuretic therapy may develop during chronic treatment due to upregulation of sodium transporters at the various tubular segments (Na et al., 2003; Kim, 2004).

In view of the limitations described above, it would be ideal to have a diuretic regimen that: 1) inhibits sodium reabsorption at multiple tubular sites, including the PT, to offset the development of diuretic resistance, 2) does not cause significant perturbations of renal potassium handling, and 3) does not activate TGF-mediated reductions of RBF and GFR. Multiple agents may be required to achieve the ideal diuretic goals. In this study, we evaluated whether addition of an A1 adenosine receptor (A1AR) antagonist
could offset the unwanted effects of CAI, while enhancing the desirable diuretic/natriuretic responses.

A1AR antagonists represent a novel class of agents for potential use in the treatment of hypertension and edema (Welch, 2002). Indeed, these drugs produced diuretic effects and blood pressure reductions in salt-sensitive (Nomura et al., 1995; Uehara et al., 1995) and genetically hypertensive rats (Kost et al., 2000). In addition, A1AR antagonists induced diuresis without worsening renal or cardiac function in a rat model of heart failure (Jackson et al., 2001). Interestingly, A1AR antagonists produced diuresis and natriuresis of greater magnitude than thiazide diuretics (Gellai et al., 1998), but without significant potassium wasting or reductions of RBF and GFR. Furthermore, clinical trials in a limited number of subjects demonstrated that A1AR antagonists produced natriuretic and hypotensive effects in essential hypertensive patients (van Buren et al., 1993) and attenuated the furosemide-induced decline of renal hemodynamic function in heart failure patients (Gottlieb et al., 2002). The effects appear to be mediated through blockade of tubular A1AR at multiple sites along the tubule and blockade of vascular A1AR in the afferent arteriole (Figure 1). This is consistent with data indicating that adenosine is a potent vasoconstrictor of the afferent arteriole and, perhaps, the chemical mediator of TGF (Osswald et al., 1982).

In this study, we postulated that the combination of A1AR-blockade with CAI, both of which primarily target the PT, would induce a robust diuretic and natriuretic effect, devoid of adverse renal hemodynamic or kaliuretic effects.
Methods

Animals. Male Sprague Dawley rats were obtained from Harlan Laboratories (Indianapolis, Indiana) and allowed to acclimate to the university animal care facility for a minimum of two weeks prior to study. Room temperature, relative humidity and the light cycle were maintained at approximately 22°C, 50%, and 6 AM to 6 PM, respectively. Rats were allowed unhindered access to tap water and Harlan Teklad 8604 diet pellets containing 0.3% sodium and 1% potassium. Acute experiments were performed when rats were approximately 11-13 weeks of age and weighed between 330 to 380 grams. Procedures involving the rats were approved by the university animal care and use committee in compliance with the NIH guidelines for care and use of laboratory animals.

Surgical Procedures. On the day of the acute experiment, each rat was removed from its home cage, anesthetized with thiobutabarbitral (Inactin®, 100 mg/kg, IP), and placed in dorsal recumbency on an isothermal pad (Braintree Scientific, Braintree, MA). Body temperature was monitored with a rectal thermal probe (Model 8402-20; Cole-Parmer Instrument Co., Vernon Hills, IL) and temperature was maintained at approximately 37°C by adjustment of an overhead heat lamp. A short section of polyethylene tubing (PE-240) was placed in the trachea to facilitate respiration, and a PE-50 catheter was inserted into the right carotid artery to permit measurement of heart rate (HR) and mean arterial blood pressure (MABP) via a digital pressure analyzer (Model BPA; Micro-Med Inc., Louisville, KY). The right jugular vein was cannulated with one PE-10 and two PE-
50 catheters to permit infusion of saline, vehicle, or drug as appropriate. Isotonic 0.9% saline was infused at 2.5 ml/hr via a jugular catheter throughout the acute experiment. A ventral midline incision was made through the skin and linea alba to expose the abdominal cavity. The intestines were slightly deflected using saline-soaked gauze to expose the left kidney. The left renal artery was carefully isolated from the surrounding fat and connective tissue, and a transit-time flow probe (Model 1RB; Transonic System Inc., Ithica, NY) was placed around the renal artery and attached to a flow meter (Model T206; Transonic) to measure RBF. Space between the artery and probe was filled with sterile lubricating jelly as an acoustic couplant. The left ureter was carefully isolated and cannulated with PE-10 tubing to permit urine collection. Acute experimental protocols were initiated following a 60-min stabilization period.

**Experimental Protocol #1; DPCPX and Acetazolamide.** Following a 60-min postsurgical stabilization period, rats were randomly selected to receive an infusion of the A1AR-antagonist, DPCPX, at 10 µg/kg/min, or its vehicle, DMSO, at 0.65 µl/min via the jugular venous PE-10 catheter. Given the relatively low infusion rate of DPCPX and its approximate 1000-fold selectivity for A1AR vs other AR (Fredholm et al., 2001), we believe this dose provided selective A1AR-blockade in our study. In addition, a slow infusion rate for DPCPX and its vehicle was utilized since DMSO at higher infusion rates can induce hemolysis. In addition, a short section of narrow-lumen PE-10 catheter was utilized to reduce dead space in the line given the slow infusion rate. The infusion of DPCPX or DMSO was maintained throughout two consecutive 40-min clearance periods designated as period 1 and period 2. In period 1, there were 16 rats that
received DPCPX and 17 rats that received its vehicle, DMSO. Urine volume was collected throughout the entire 40-min period, and MABP, HR, and RBF were recorded at 5-min intervals, then averaged over the 40 min. Data obtained in period 1 were subjected to statistical analysis to determine the effect of DPCPX alone as compared to DMSO on the measured parameters. At the completion of period 1, rats within each group (DPCPX or DMSO) were randomly assigned to receive either acetazolamide (20 mg/kg, IV) or its vehicle (2M NaOH; 1 ml/kg, IV) via a slow bolus injection lasting approximately 5 min. Completion of this bolus injection marked the beginning of period 2. Urine volume and hemodynamic parameters were measured throughout the 40-min period as described above. There were 8-9 rats in each of the four groups that received the following treatment regimen for period 1/period 2: DMSO vehicle and NaOH vehicle (Vh/Vh); DMSO vehicle and acetazolamide (Vh/AZ); DPCPX and NaOH vehicle (DP/Vh); DPCPX and acetazolamide (DP/AZ). At completion of the study, rats were administered a lethal intravenous bolus injection of KCl, and the left kidney was removed and weighed.

**Experimental Protocol #2; AT1 Receptor Blockade and Acetazolamide.** This protocol was included in our study after we had observed that A1AR-blockade with DPCPX did not attenuate the decrease of RBF associated with AZ treatment. We reasoned that the hemodynamic response secondary to AZ may be mediated by angiotensin II (ANGII) production, and if so, we should observe an attenuated RBF response to AZ during treatment with an angiotensin AT1 receptor blocker (ARB). Twelve male Sprague Dawley rats were utilized in this study; six were provided access
to normal tap water while the other six were provided tap water containing the ARB, L158809 (7.5 mg/L) for one week. L158809 ([5,7-dimethyl-2-ethyl-3-[2'-(1H-tetrazol-5yl)[1,1’]-biphenyl-4-yl]methyl]-3H-imidazo[4,5-b]pyridine) is highly potent and exhibits a >10,000-fold selectivity for AT1 receptors compared to AT2 receptors (Chang et al., 1992). We estimated that 7.5 mg/L of L158809 in drinking water should provide a daily dose of greater than 0.3 mg/kg for the week leading up to the acute experiment. In a previous experiment, L158809 as a single oral dose of 0.3 mg/kg administered to conscious rats was shown to provide 75-85% blockade of ANGII (0.1 µg/kg, i.v. bolus)-induced pressor responses with a 24-hr duration of action (Siegl et al., 1992).

Both the control and ARB-treated rats were surgically prepared as described above. Following a 60-min post-surgical stabilization period, parameters such as urine excretion rate, MABP and RBF were recorded over three consecutive 40-min collection periods. Period 1 served as the baseline period. At the completion of period 1, AZ (20 mg/kg, IV) was administered by slow bolus injection, and parameters were measured for the following 40 min (period 2; AZ). During the final collection period (period 3; ANGII), rats were administered an intravenous infusion of ANGII at 15 ng/min to assess whether the ARB treatment regimen utilized in this study achieved adequate blockade of AT1 receptors. Following period 3, rats were administered a lethal intravenous bolus injection of KCl, and the left kidney was removed and weighed.

Sample Analysis. Urine excretion rate was determined gravimetrically by weighing the collection tubes before and after each of the 40-min collection periods. Sodium and
potassium concentrations were measured by flame photometry (Model IL943; Instrumentation Laboratory, Lexington, MA). The urine excretion rate (UV), RBF, and excretion rates of sodium (UNaV) and potassium (UKV) were normalized to gram of kidney weight (g kid).

**Statistical Analysis.** Criterion for significance was p<0.05. Data obtained during period 1 of the first protocol were compared by unpaired t-test to assess the effect of DPCPX alone compared to that of DMSO. The interaction of DPCPX and AZ in protocol 1 was determined by 2-factor, repeated measures ANOVA where factor A=treatment group (i.e., plus/minus DPCPX) and the repeated factor B=period (i.e., plus/minus acetazolamide). Changes of period 1 to period 2 were analyzed by one-way ANOVA. Where appropriate, the ANOVA was followed up by a post-hoc comparison with Fisher’s LSD test. Data obtained in protocol 2 also were analyzed by 2-factor, repeated measures ANOVA. In this case, factor A=treatment group (i.e., plus/minus ARB treatment) and factor B=period (i.e., baseline vs AZ vs ANGII). Where appropriate, the ANOVA was followed up by a post-hoc comparison with Fisher’s LSD test to assess the effect of AZ over the baseline period, and the effect of ANGII over the AZ period. In addition, the AZ-induced change in parameters was compared between control and ARB-treated rats by t-test. Analysis of data was performed with the Number Cruncher Statistical System (Kaysville, UT).
**Chemicals.** Acetazolamide, DPCPX, DMSO, angiotensin II, and Inactin were purchased from Sigma-Aldrich (St. Louis, MO). L158809 was generously provided by the Merck Research Labs (Rahway, NJ).
Results

**Experimental Protocol #1; DPCPX and Acetazolamide.** DPCPX produced a significant diuretic and natriuretic effect in period 1 of this study as compared to DMSO (Fig. 2). Despite the near-doubling of UV and UNaV, DPCPX did not significantly alter UKV or RBF in these rats (Fig. 2). In the DPCPX-treated group, there also appeared to be a slight elevation of heart rate (367± 8 vs. 341± 10 beats/min in control, p=0.05). The increase of UV and UNaV, along with the tendency for an increase of heart rate, indicated that the infusion rate of DPCPX utilized in this study was adequate to produce blockade of A1AR in the kidney and heart.

The infusion of DPCPX or DMSO was continued into period 2, and rats in each group were further subdivided by random assignment to AZ or vehicle groups (Fig. 3). Of note, there were no “within-group” differences at baseline in the DPCPX or DMSO groups (post-hoc; p>0.05). In period 2, rats were administered a slow bolus of the CAI, acetazolamide (Vh/AZ and DP/AZ), to block proximal tubular sodium reabsorption, or its vehicle, 2M NaOH (Vh/Vh or DP/Vh). AZ induced a significant increase of UNaV in both the DMSO-treated (Vh/AZ) and DPCPX-treated (DP/AZ) groups (Fig. 3). The absolute UNaV was significantly greater in the DP/AZ rats compared to Vh/AZ rats, however the AZ-induced increase of UNaV (i.e., ΔUNaV; period 2 minus period 1) was not significantly different between the two groups. This indicates that DPCPX and AZ have an additive effect on UNaV. Despite the increased UNaV in DP/AZ compared to Vh/AZ rats, the UKV was significantly lower in the DP/AZ group, and the AZ-induced increase...
of UKV was attenuated by more than 50% in the DPCPX group (Fig. 3). The diuretic response (i.e., increase of UV) to AZ was similar in the DMSO- (Vh/AZ) and DPCPX- (DP/AZ) treated rats (Fig. 4). In addition, AZ produced a significant and similar decrease of RBF in both the DMSO and DPCPX groups (Fig. 4). The observation that A1AR-blockade with DPCPX failed to attenuate the RBF decrease induced by AZ lead us to perform the experimental protocol #2.

**Experimental Protocol #2; AT1 Receptor Blockade and Acetazolamide.** This protocol consisted of three periods (i.e., baseline, acetazolamide, angiotensin II) in one group of rats that received ARB for one week and another group of age-matched control rats. At baseline, control and ARB-treated rats had similar RBF; however, MABP was significantly (p<0.05) reduced in ARB-treated rats (101±3 mm Hg) as compared with control rats (110±3 mm Hg). AZ induced a significant decrease of RBF in both the control rats and in rats treated for one week with the ARB, and the magnitude of the AZ-induced RBF decrease was similar in both groups (Fig. 5). The ARB treatment appeared to provide adequate blockade of AT1 angiotensin receptors since intravenous infusion of ANGII (15 ng/min) in period 3 of the study produced a significant reduction of RBF from the previous period in control, but not in ARB-treated rats. In addition, ANGII infusion raised MABP in control but not in the ARB-treated rats (Fig. 5).

The UV and UNaV in control and ARB-treated rats were similar at baseline (Fig. 6). However, in response to AZ, ARB treatment significantly augmented UNaV, and tended
to increase UV (Fig. 6). ANGII infusion appeared to reduce both UV and UNaV compared to the previous period in the ARB-treated rats, but not in control rats (Fig 6).
Discussion

A significant finding from this study was that A1AR-blockade attenuated AZ-induced urinary excretion of potassium. In addition, the AZ-induced renal vascular response was not prevented by A1AR-blockade or by pretreatment with an angiotensin AT1 receptor blocker. It appears that in the rat, the renal hemodynamic effect of CAI is mediated independently of either adenosine’s actions at the A1AR or angiotensin’s actions at the AT1 receptor.

A1AR-blockade alone increased UV and UNaV without significantly altering UKV or RBF. The lack of a kaliuretic effect is in agreement with published data (Knight et al., 1993; Kuan et al., 1993). Natriuretic responses to A1AR antagonists were previously shown to be accompanied by increased fractional excretion of lithium (Knight et al., 1993) and decreased fluid reabsorption in the PT (Wilcox et al., 1999). It also seems likely that some portion of the natriuretic effect of A1AR antagonists occurs secondary to blockade of sodium reabsorption in the collecting duct system (Ma and Ling, 1996; Macala and Hayslett, 2002) where sodium reabsorption normally drives potassium secretion. Interactions at both early and late tubular segments may account for the balanced effects of A1AR-blockade on renal potassium handling observed in this and other studies. Also consistent with published data, we did not observe a decrease of RBF associated with the natriuretic effect of A1AR-blockade. Given their diuretic effect in the PT, one might expect these agents to trigger TGF producing a decrease of RBF. However, adenosine is believed to be the chemical mediator of TGF, and murine gene
knockout models that lack A1AR do not exhibit intact TGF responses (Brown et al., 2001; Sun et al., 2001). Likewise, pharmacological blockade of A1AR in the afferent arteriole appeared to inhibit TGF-mediated reductions of RBF despite increased delivery of NaCl out of the PT induced by A1AR-blockade in this tubular region.

**A1AR-blockade attenuated the UKV induced by acetazolamide.** It was previously reported that A1AR-blockade had additive natriuretic effects with furosemide, and that the combination did not induce UKV beyond that of furosemide alone (Gellai et al., 1998). Our results are similar in that we also found an additive natriuretic response with the combination of DPCPX and AZ. When compared to AZ alone, the combination produced a lesser kaliuretic response, indicating that A1AR-blockade blunted the UKV associated with AZ treatment despite augmented sodium output. Collectively, these data indicate that A1AR antagonists may enhance UNaV and attenuate UKV when used in combination with other diuretic agents.

**A1AR-blockade did NOT blunt the decrease of RBF induced by acetazolamide.** Studies utilizing murine gene deletion models (i.e., NHE3 -/- and AQP1 -/-) have demonstrated that reduced proximal sodium and water reabsorption results in diminished GFR and RBF relative to wild-type mice (Schnermann et al., 1998; Lorenz et al., 1999). Crossing of the AQP1 -/- knockout mouse with an A1AR -/- knockout mouse produced a model with reduced proximal fluid reabsorption, but absent TGF responses (Hashimoto et al., 2004a). Despite reduced proximal fluid reabsorption, the single nephron GFR was essentially normal in these double knockout mice indicating that
A1AR-linked TGF interactions can lead to a decrease of RBF and GFR in situations where proximal tubular fluid reabsorption is impaired.

In view of the studies described above, we had postulated that AZ-induced inhibition of proximal sodium and fluid reabsorption would initiate a TGF-mediated reduction of RBF through activation of A1AR on afferent arterioles in untreated rats, and that A1AR-blockade would abolish the response in DPCPX-treated rats. Our observation that pharmacological A1AR-blockade with DPCPX did not attenuate the AZ-induced RBF decrease was unexpected. One possible explanation is that the infusion rate of DPCPX utilized in our study may have been inadequate to block A1AR. However, we observed a significant diuretic/natriuretic response to the infused DPCPX, and an attenuation of AZ-induced kaliuresis. In addition, heart rate tended to be elevated in the DPCPX-treated rats indicative of systemic A1AR-blockade.

Another possible explanation for the inability of DPCPX to block AZ-induced renal hemodynamic changes is that the adenosine-A1AR interaction may not mediate TGF. Indeed there is controversy regarding the identity of the TGF mediator, with some data suggesting that ATP may play a prominent role (Nishiyama and Navar, 2002). For example, macula densa cells released ATP during perturbations of luminal NaCl concentrations (Bell et al., 2003), and renal interstitial ATP levels increased, whereas levels of adenosine did not change, in response to AZ-induced natriuresis (Nishiyama et al., 2000). Following release from macula densa, ATP may directly activate purinergic receptors to induce afferent arteriolar vasoconstriction. However, there are data
indicating that ATP undergoes conversion to adenosine via the catalytic activity of nucleotidases, and the resulting adenosine activates TGF through A1AR stimulation (Thomson et al., 2000; Castrop et al., 2004).

An additional explanation for our observation is that the renal hemodynamic response to CAI may occur independent of the TGF mechanism. An earlier study demonstrated that the CAI, benzolamide, induced a similar-magnitude decrease of GFR and RBF in both A1AR -/- knockout and wild-type mice, despite the absence of TGF responses in the A1AR -/- knockout mice (Hashimoto et al., 2004b). Our data, utilizing pharmacological blockade of A1AR, are consistent with these findings. Interestingly, Hashimoto et al also found that acute AT1 angiotensin receptor blockade significantly attenuated renal hemodynamic responses to benzolamide in both groups of mice indicating that activation of the RAS may be responsible for the CAI-induced RBF response.

**Angiotensin AT1 receptor blockade did NOT prevent the acetazolamide-induced renal hemodynamic response in rats.** Based on the work by Hashimoto and colleagues described above, we designed an experiment in which we treated rats with the ARB, L158809, for one week and examined the response to AZ. Contrary to our expectation, we found that the decrease of RBF in response to AZ was not attenuated by ARB treatment. To verify blockade of AT1 angiotensin receptors, the rats were infused with ANGII during the final period of the study. We found that ANGII caused a significant reduction of RBF and increase of MABP in control, but not in ARB-treated
rats. In addition, baseline MABP was reduced in ARB-treated rats. Collectively, our data indicate that vascular AT1 receptors were indeed blocked by ARB treatment, but this did not diminish the renal hemodynamic response to AZ.

Our renal hemodynamic data in rats obviously differs from that of Hashimoto et al in mice (Hashimoto et al., 2004b). However, our results are in general agreement with Deng and colleagues (Deng et al., 2002) who reported that, in the rat, ACE-inhibitor treatment did not block the RBF decrease induced by benzolamide. Interestingly, plasma and kidney ANGII concentrations in the rats were not significantly increased after benzolamide infusion (Deng et al., 2004), whereas Hashimoto et al found that plasma renin concentrations were elevated in mice after CAI. These data indicate that the RAS may play a greater role in mediating the renal hemodynamic effects of CAI in mice as compared to rats. Of note, there are recognized species-differences regarding the contribution of the RAS to maintenance of blood pressure (Cholewa et al., 2005), with mice having elevated plasma renin concentrations relative to other species. Furthermore, blood pressure appears to be more dependent upon the endogenous RAS and thus more sensitive to RAS inhibition in mice as compared to rats. The clinical implications of the species differences noted here and in other studies are difficult to predict given that RAS function is qualitatively similar in mice and rats despite quantitative differences in individual components of the system.

Our experiments provide evidence that neither adenosine acting at A1AR, nor ANGII acting at AT1 receptors, mediates the acute decrease of RBF following CAI in the rat. A
limitation of our study is that we did not assess GFR and cannot directly address the role of A1AR, AT1 receptors, or TGF in mediating CAI-induced changes of GFR. It has been suggested that the GFR decrease following CAI may be explained by an increase of hydrostatic pressure in the tubules due to enhanced fluid delivery to distal segments, resulting in decreased net filtration pressure in the glomeruli (Leyssac et al., 1994).

While this may account for reported alterations in GFR, it seems unlikely to explain the decrease of RBF. Further work is needed to identify the mechanism(s) responsible for renal hemodynamic changes following CAI in the rat.

In summary, A1AR-blockade alone produced diuresis and natriuresis without significant perturbations of UKV or RBF. When combined with a CAI, A1AR-blockade had additive effects on UNaV and attenuated the AZ-induced UKV. These data indicate that A1AR antagonists are likely to inhibit sodium reabsorption in multiple sites along the tubule and may be useful diuretic agents alone or in combination with other conventional diuretic agents. The decrease of RBF that occurred in response to CAI was not inhibited by A1AR-blockade and did not appear to be mediated by a TGF/A1AR-dependent mechanism. In the mouse, the response may be mediated through the RAS, however our data and that of others indicate that the renal hemodynamic response to CAI in the rat occurs independent of the RAS.
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Footnotes

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Figure Legends

Figure 1. Illustration of renal sodium transport and tubuloglomerular feedback mechanisms. Acetazolamide inhibits carbonic anhydrase (CA) and thus sodium reabsorption primarily in the proximal tubule and to some extent in the thick ascending limb of Henle’s loop. Increased delivery of sodium chloride to the macula densa is believed to trigger release of ATP and vasoconstriction of the afferent arteriole (Aff Art). In addition, an increase of tubular sodium delivery to the collecting duct drives potassium secretion. Reabsorption of sodium and water in the collecting duct are increased by aldosterone and vasopressin, respectively. Adenosine acting at A1AR stimulates vasoconstriction of the afferent arteriole and appears to enhance proximal tubular sodium reabsorption via luminal sodium/glucose and sodium/phosphate cotransport as well as basolateral sodium/bicarbonate transport. In the collecting duct, adenosine appears to increase sodium uptake through epithelial sodium channels. The sites at which adenosine has been postulated to act are indicated by a star.

Figure 2. Effect of DPCPX on urine output, electrolyte excretion rates, and renal blood flow. Data are shown as mean ± standard error of the mean (SEM) for values obtained in period 1 from DPCPX-treated (n=16) or DMSO vehicle-treated (n=17) groups. Significant differences are indicated as p<0.05 by unpaired t-test.

Figure 3. Interaction between DPCPX and acetazolamide on electrolyte excretion rates. Left panels depict mean ± SEM for values obtained in period 1 from DPCPX-
treated (DP) or DMSO vehicle-treated (Vh) groups that are then administered either acetazolamide (AZ) or its vehicle (Vh) in period 2. There were 8 or 9 rats in each group. Data were analyzed by 2-factor, repeated-measures ANOVA with post-hoc comparisons. "**" indicates a significant within-group difference (p<0.05) in period 2 compared with period 1. Letters “a,b,c,d” depict a significant difference (p<0.05) among groups for values obtained in period 2. For example, “a” indicates significant difference from group a (Vh/Vh), “b” indicates difference from group b (Vh/AZ), etc. Right panels depict the change from period 1 to period 2 induced by acetazolamide or its vehicle. Data were analyzed by one-way ANOVA followed by post-hoc comparisons. Significant differences among groups are depicted by letters “a,b,c,d” as described above.

**Figure 4. Interaction between DPCPX and acetazolamide on urinary excretion rate and renal blood flow.** Left panels depict mean ± SEM for values obtained in period 1 from DPCPX-treated (DP) or DMSO vehicle-treated (Vh) groups that are then administered either acetazolamide (AZ) or its vehicle (Vh) in period 2. Right panels depict the change from period 1 to period 2 induced by acetazolamide or its vehicle. Data were analyzed and significant differences indicated as described in figure 3.

**Figure 5. Effect of acetazolamide and angiotensin II on renal blood flow and mean arterial blood pressure in rats pretreated with an angiotensin receptor blocker compared to control.** Rats were pretreated for one week prior to the acute experiment. There were 6 rats in each group. Left panels depict mean ± SEM for values obtained at baseline (open), following acetazolamide treatment (AZ; solid), or
during angiotensin II infusion (ANGII; hatched). Data were analyzed by 2-factor, repeated-measures ANOVA with post-hoc comparisons. “**” indicates significant difference of AZ period from baseline period, and “#” indicates significant difference of ANGII period from AZ period. Right panels depict the change from period 1 to period 2 induced by AZ for RBF, and the change from period 2 to period 3 induced by ANGII for MABP. Data were analyzed by unpaired t-test and “**” indicates a significant difference of response to AZ or ANGII if detected between control and ARB-treated rats.

**Figure 6.** Effect of acetazolamide and angiotensin II on urine and sodium excretion in rats pretreated with an angiotensin receptor blocker compared to control. Data were analyzed as described in figure 5.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.