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

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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, because of 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 do not cause loss of potassium or tubuloglomerular feedback- (TGF) mediated reductions of renal hemodynamics. We tested whether the A1AR antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) could attenuate the adverse effects normally associated with use of AZ. Renal blood flow (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 II type 1 (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 seem to be mediated by a TGF-dependent mechanism.
sites of action because the drugs must be filtered or actively secreted into the tubular lumen 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 up-regulation 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 seem to be mediated through blockade of tubular A1AR at multiple sites along the tubule and blockade of vascular A1AR in the afferent arteriole (Fig. 1). This is consistent with data indicating that adenosine is a potent vasoconstrictor of the afferent arteriole and, perhaps, the chemical mediator of TGF (Oswald 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.
Acetazolamide inhibits carbonic anhydrase 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 seems 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 seems to increase sodium uptake through epithelial sodium channels. The sites at which adenosine has been postulated to act are indicated by a star.
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), and 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 used in this study; six were provided access to normal tap water, whereas the other six were provided tap water containing the ARB L158809 (7.5 mg/l) for 1 week. L158809 is highly potent and exhibits a >10,000-fold selectivity for AT1 receptors compared with angiotensin II type 2 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 was also a single oral dose of 0.3 mg/kg administered to conscious rats was shown to provide 75 to 85% blockade of ANGII- (0.1 μg/kg i.v. bolus) induced pressor responses with a 24-h duration of action (Siegl et al., 1992).

Both the control and ARB-treated rats were surgically prepared as described above. After a 60-min postsurgical 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 i.v.) 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 used in this study achieved adequate blockade of AT1 receptors. After 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.

Statistical Analysis. Criterion for significance was \( p < 0.05 \). Data obtained during period 1 of the first protocol were compared using unpaired \( t \) tests to assess the effect of DPCPX alone compared with that of DMSO. The interaction of DPCPX and AZ in protocol 1 was determined by two-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 least significant difference test. Data obtained in protocol 2 also were analyzed by two-factor, repeated measures ANOVA. In this case, factor A = treatment group (i.e., plus/minus ARB treatment), and factor B = period (i.e., baseline versus AZ versus ANGII). Where appropriate, the ANOVA was followed up by a post hoc comparison with Fisher’s least significant difference 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 (NCSS, Kaysville, UT).

Chemicals. Acetazolamide, DPCPX, DMSO, angiotensin II, and thiobutabarbital 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 compared with 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 seemed to be a slight elevation of heart rate (367 ± 8 versus 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 used 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, 2 M 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 with Vh/AZ rats; however, the AZ-induced increase of UNaV (i.e., \( \Delta \)UNaV; period 2 minus period 1) was not significantly different between the two groups. This in-
indicates that DPCPX and AZ have an additive effect on UNaV. Despite the increased UNaV in DP/AZ compared with 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, and angiotensin II) in one group of rats that received ARB for 1 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 \pm 3 \text{ mm Hg}) compared with control rats (110 \pm 3 \text{ mm Hg}). AZ induced a significant decrease of RBF in both the control rats and in rats treated for 1 week with the ARB, and the magnitude of the AZ-induced RBF decrease was similar in both groups (Fig. 5). The ARB treatment seemed 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 seemed to reduce both UV and UNaV compared with 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 seems 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 seemed 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 with 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 using murine gene deletion models (i.e., sodium-hydrogen exchanger 3/−/− and aquaporin 1/−/−) 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 aquaporin 1/−/− knockout mouse with an A1AR/−/− knockout mouse produced a model with reduced proximal fluid reabsorption but absent TGF responses (Hashimoto et al., 2004b). 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 re-
absorption 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 used in our study may have been inadequate to block A1AR. However,

Fig. 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 with control. Rats were pretreated for 1 week prior to the acute experiment. There were six rats in each group. Left panels depict mean ± S.E.M. for values obtained at baseline (open), following acetazolamide treatment (AZ; solid), or during angiotensin II infusion (ANGII; hatched). Data were analyzed by two-factor, repeated measures ANOVA with post hoc comparisons. *, significant difference of AZ period from baseline period; and #, 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; *, significant difference of response to AZ or ANGII if detected between control and ARB-treated rats. RM-ANOVA, repeated measures ANOVA.

Fig. 6. Effect of acetazolamide and angiotensin II on urine and sodium excretion in rats pretreated with an angiotensin receptor blocker compared with control. Data were analyzed and significant differences indicated as described in Fig. 5. RM-ANOVA, repeated measures ANOVA.
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). After 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., 2004a). Our data, using pharmacological blockade of A1AR, are consistent with these findings. Interestingly, Hashimoto et al. (2004a) 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 et al. (2004a) described above, we designed an experiment in which we treated rats with the ARB L158809 for 1 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 differ from that of Hashimoto et al. (2004a) in mice. However, our results are in general agreement with Deng et al. (2002), who reported that in the rat, angiotensin-converting enzyme 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. (2004a) 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 compared with 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 seems to be more dependent upon the endogenous RAS and thus more sensitive to RAS inhibition in mice compared with 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). Although 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 plasma renin concentration 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 seem 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.

**References**


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