Requirement of Intact Adenosine A1 Receptors for the Diuretic and Natriuretic Action of the Methylxanthines Theophylline and Caffeine

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ABSTRACT

Although the diuretic and natriuretic effects of the methylxanthines caffeine and theophylline are well established, the mechanisms responsible for these effects are unclear and may be related to inhibition of phosphodiesterases and/or antagonism of adenosine receptors. With regard to the latter, pharmacological blockade of A1 receptors can induce diuresis and natriuresis by inhibition of proximal tubular reabsorption. To elucidate the role of the A1 receptor in renal actions of methylxanthines, experiments were performed in A1 receptor knockout (A1R−/−) and littermate wild-type (A1R+/+) mice. Urinary excretion was determined in awake mice in metabolic cages over 3 h in response to theophylline (as theophylline/ethylenediamine, 45 mg/kg), caffeine (45 mg/kg), or vehicle (0.9 ml/30 g b.wt. of 0.85% NaCl) given by oral gavage. Theophylline and caffeine elicited a diuresis and natriuresis (in absolute terms and related to urinary creatinine excretion) in A1R+/+ but not in A1R−/− mice. In a second series, the renal effect of intravenous application of theophylline (30 mg/kg) was determined in clearance experiments under anesthesia. This study revealed that the blunted diuretic and natriuretic effect of theophylline in A1R−/− mice was not due to different responses in blood pressure or glomerular filtration rate. The data indicate that an intact A1 receptor is necessary for caffeine- and theophylline-induced inhibition of renal reabsorption causing diuresis and natriuresis. This is consistent with the assumption that A1 receptor blockade mediates these effects.

Caffeine and theophylline are members of the methylxanthine family that are very widely consumed and exert well described behavioral effects. In the kidney, methylxanthines induce diuresis and natriuresis, an effect first described by Davis and Shock (1949). The mechanisms by which methylxanthines act as diuretics and natriuretics are not completely understood. Since methylxanthines are nonselective adenosine receptor antagonists (Fredholm et al., 2001), it is conceivable that the changes in urine excretion are the result of inhibition of the renal actions of endogenous adenosine.

Adenosine is an important regulator of kidney function and is involved in the regulation of glomerular filtration rate (GFR) (Osswald, 1975), medullary blood flow (Zou et al., 1999), and renal water and electrolyte transport (Kupper et al., 1951; Fulgraff et al., 1969). Adenosine is the mediator of the tubuloglomerular feedback (Osswald et al., 1980; Thomas et al., 2000; Sun et al., 2001), and it affects renin release (Osswald et al., 1978). Moreover, both nonselective and selective A1 receptor antagonists have been shown to increase renal fluid and Na+ excretion in animal studies (Knight et al., 1993; Wilcox et al., 1999), healthy volunteers (Brater et al., 1983; Balakrishnan et al., 1993, 1996), and in hypertensive patients (van Buren et al., 1993). The observed diuresis and natriuresis in response to selective A1 receptor blockade was caused by inhibition of proximal tubular reabsorption (Knight et al., 1993; van Buren et al., 1993; Wilcox et al., 1999). In accordance, in vitro studies in the proximal tubule showed that activation of A1 receptor can stimulate transport of fluid, HCO3− as well as Na+−glucose and Na+−phosphate symport (Coulson et al., 1991; Takeda et al., 1993; Cai et al., 1994, 1995). The intracellular signaling pathways that contribute to these changes in tubular transport are still under investigation but may include reductions of intracellular cAMP levels (Kost et al., 2000), increases of intracellular Ca2+ (Di Sole et al., 2003), and calcineurin homologous pro-

ABBREVIATIONS: GFR, glomerular filtration rate.
tein-mediated regulation of Na\(^{+}\)-H\(^{+}\)-exchanger NHE3 (Di Sole et al., 2004).

Beside antagonizing adenosine receptors, methylxanthines are known to inhibit phosphodiesterases, an effect reflected in the enhanced urinary excretion of cAMP (Fredholm et al., 1978, 1984; Coulson and Scheinman, 1989). In fact, it has been suggested that inhibition of phosphodiesterases in the proximal tubule may contribute to the diuretic and natriuretic effects of methylxanthines (Fredholm et al., 1978; Coulson and Scheinman, 1989).

The present study was performed to further assess the role of A\(_1\) receptors in methylxanthine-induced diuresis and natriuresis. To this end, the renal excretory effects of caffeine and theophylline were studied in conscious A\(_1\) receptor knockout (+/−) and littermate wild-type (+/+ ) mice. To exclude the possibility that differences in the responses of blood pressure or GFR may account for different excretory effects of methylxanthines between genotypes, additional experiments assessed the effect of theophylline on blood pressure and GFR in a two-period clearance experiment in anesthetized A\(_1\) receptor +/+ and −/− mice. Experiments were designed to test the hypothesis that methylxanthines should be without effect in mice lacking the A\(_1\) receptor if blockade of A\(_1\) receptors is the mode of action of this class of agents.

Materials and Methods

Chemicals

α-Chloralose, aminophylline (1,3-dimethylxanthine, compared with 1,2-ethanediame 2:1), bovine serum albumin, and NaCl were from Sigma-Aldrich (Taufkirchen, Germany). Caffeine (1,3,7-trimethylxanthine) was obtained from Carl Roth (Karlsruhe, Germany) and ketamine from Curamed Pharma GmbH (Karlsruhe, Germany). \(^{3}H\)Inulin was purchased from BioTrend (Köln, Germany) and Ultima Gold from PerkinElmer (Rodgau-Ju¨ gesheim, Germany).

Animals

Animal experimentation was conducted in accordance with the German Law on the Protection of Animals including approval by the local Ethical Committee. Mice were housed at a 12-h light/dark cycle with free access to food (Altromin 1324; Altromin, Lage, Germany) and tap water. A\(_1\) receptor +/− and +/+ littermates were from a subcolony of the original strain generated by Sun et al. (2001) that is kept at the Institute of Pharmacology and Toxicology, University of Tübingen, Germany (Vallon et al., 2004). Mice have always been reproduced by heterozygous crossing. Therefore, the genetic background of the animals was a mix of 129Sv/J and C57BL/6. Genotyping was done by polymerase chain reaction from ear tissue DNA using A\(_1\) receptor and neo-A\(_1\) receptor-specific primers as previously described (Sun et al., 2001).

Experimental Protocol

Metabolic Cage Experiments in Awake Mice. A\(_1\) receptor +/+ and −/− mice were randomized to acute application of caffeine (45 mg/kg b.wt.), theophylline (as theophylline/ethylenediamine, 45 mg/kg), or vehicle (0.9 ml/30 g b.wt. of 0.85% NaCl) given by oral gavage as described (Sim and Hopcroft, 1976; Cooling and Sim, 1977). The mice were then placed in metabolic cages (Tecniplast, Hohenpeissenberg, Germany) for quantitative urine collections over 3 h without access to food or water as described (Yao et al., 2004). Thereafter, mice were anesthetized with isoflurane to take a ~70-µl blood sample from the retro-orbital plexus for determination of Na\(^{+}\) K\(^{-}\) and arterial hematocrit. In experiments with theophylline, 500 µl of blood was taken for additional determination of plasma theophylline concentration.

Clearance Experiments under Anesthesia. Mice were anesthetized with α-chloralose (120 mg/kg b.wt. i.p., 2 µg/g b.wt.) and ketamine (100 mg/kg b.wt. i.m., 2 µg/g b.wt.) and prepared for clearance experiments as described (Rieg et al., 2004). Briefly, mice were placed on an operating table with a servo-controlled heating plate (RT, Effenberger, München, Germany) to maintain body temperature at 37.5°C. The trachea was cannulated with polyethylene tubing and 100% oxygen blown toward the tracheal tube throughout the experiment. The femoral artery was cannulated for blood pressure measurement and blood sample withdrawal. The jugular vein was cannulated for continuous maintenance infusion of 2.25% bovine serum albumin in 0.85% NaCl at a rate of 0.5 ml/b/g h.b.wt. For assessment of two-kidney glomerular filtration rate, \(^{3}H\)Inulin was added to this infusion to deliver 5 µCi/b/g h.b.wt. A polyethylene catheter was placed into the bladder via a suprapubic incision and served for collection of urine. The above preparation took about 35 min, and the mice were allowed another 60 min to stabilize before the experiment was started. Quantitative urine collections were made in a basal period (P1, 30 min) and in a consecutive second period (P2, 30 min). After completion of P1 and 5 min before starting P2, theophylline (30 mg/kg b.wt. in 2.25% bovine serum albumin/0.85% NaCl) was applied (1 µg/g b.wt., as a bolus over 2 min). Plasma samples (50 µl) were drawn midway in each period. Blood pressure and heart rate were monitored continuously (P23dB; Gould-Statham, Oxnard, CA). After completion of P2, 500 µl of blood was taken for determination of plasma theophylline concentration.

Analysis of Plasma and Urine Samples. Urinary flow rate was determined gravimetrically. Na\(^{+}\) and K\(^{-}\) concentrations in plasma and urine were analyzed in 5- and 10-µl samples, respectively, by flame photometry (ELEX 6361; Eppendorf, Hamburg, Germany). Urine Cl\(^{−}\) was measured with a chlorideometer 6610 by electrometric titration (Eppendorf) and creatinine in urine with a standard creatinine assay (Wako Chemicals GmbH, Neuss, Germany). Plasma theophylline concentration was assessed with a particle-enhanced turbidimetric inhibition immunoassay (Dade Behring, Newark, DE). Two-kidney GFR was determined by \(^{3}H\)Inulin clearance. Concentrations of \(^{3}H\)Inulin in plasma and urine were measured in 5-µl samples by liquid-phase scintillation counting in 5-ml liquid scintillation cocktail in a model 2550TR β-counter (Canberra Packard, Frankfurt, Germany). Ca\(^{2+}\), phosphate, and glucose were determined by enzymatic assays (Kit 11489216, 11489348, and 11447513, respectively; Roche Diagnostics, Mannheim, Germany).

Data Analysis. Data are expressed as means ± S.E.M. Unpaired Student’s t test was performed to analyze for statistical differences between groups. Paired t test has been performed to analyze for statistical differences within groups between P1 and P2 in clearance experiments. p < 0.05 has been considered statistically significant.

Results

Acute Effects of Caffeine in Conscious Mice. As summarized in Table 1, there were no significant differences in any of the measured systemic parameters or absolute renal excretion between vehicle-treated A\(_1\) receptor +/+ and −/− mice except for a modestly higher arterial hematocrit in A\(_1\) receptor −/− mice. Caffeine significantly increased urinary excretion of fluid, Na\(^{+}\), Cl\(^{−}\), and Ca\(^{2+}\) in A\(_1\) receptor +/+ mice, both in absolute terms (Table 1) and in relation to urinary creatinine excretion (Fig. 1), the latter indicating an effect on renal reabsorption. In contrast, caffeine did not significantly alter urinary excretion of fluid, Na\(^{+}\), Cl\(^{−}\), or Ca\(^{2+}\) in A\(_1\) receptor −/− mice. Absolute K\(^{-}\) excretion tended to be higher in caffeine-treated +/+ versus vehicle-treated +/+ mice (p = 0.08), whereas K\(^{-}\) excretion related to urinary creatinine was not different. Glucose excretion in absolute
terms and related to creatinine excretion was significantly increased in response to caffeine in both A1 receptor wild-type (+/+) and knockout (−/−) mice when compared with vehicle-treated animals (see Table 1 and Fig. 1). Excretion of phosphate was not significantly affected in response to caffeine in A1 receptor wild-type or knockout mice. 

Acute Effects of Theophylline in Conscious Mice.

Like caffeine, theophylline significantly increased urinary flow rate (UV) and urinary concentrations of Na⁺, K⁺, Cl⁻, Ca²⁺, phosphate, and glucose related to urinary creatinine concentration in metabolic cage experiments of caffeine- and vehicle (veh)-treated A1 receptor wild-type (+/+) and knockout (−/−) mice. *p < 0.05 versus veh same genotype; §p < 0.05 versus wild-type veh.

**Table 1**

Response to application of caffeine (caff, 45 mg/kg b.wt.) or vehicle (veh, 0.85% NaCl, 0.9 ml/30 g b.wt.) by oral gavage in A1 receptor wild-type (+/+) and knockout (−/−) mice.

<table>
<thead>
<tr>
<th></th>
<th>−/− Veh (n = 6)</th>
<th>−/− Caff (n = 7)</th>
<th>−/+ Veh (n = 6)</th>
<th>−/+ Caff (n = 7)</th>
</tr>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>28 ± 1</td>
<td>28 ± 1</td>
<td>28 ± 1</td>
<td>28 ± 1</td>
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<tr>
<td>[Na⁺] plasma (mmol/l)</td>
<td>147 ± 3</td>
<td>144 ± 1</td>
<td>148 ± 2</td>
<td>148 ± 1</td>
</tr>
<tr>
<td>[K⁺] plasma (mmol/l)</td>
<td>5.5 ± 0.3</td>
<td>5.2 ± 0.5</td>
<td>5.2 ± 0.2</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>Arterial hematocrit (%)</td>
<td>53.5 ± 1</td>
<td>53.2 ± 0.4</td>
<td>53.2 ± 0.4</td>
<td>51 ± 0.9</td>
</tr>
<tr>
<td>Fluid excreted (%) of dosed</td>
<td>61 ± 3</td>
<td>129 ± 16</td>
<td>67 ± 5</td>
<td>69 ± 7</td>
</tr>
<tr>
<td>UV (μl/min)</td>
<td>2.7 ± 0.1</td>
<td>6.1 ± 0.8</td>
<td>3.1 ± 0.3</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>AE Na⁺ (nmol/min)</td>
<td>301 ± 18</td>
<td>832 ± 73</td>
<td>314 ± 17</td>
<td>435 ± 81</td>
</tr>
<tr>
<td>AE K⁺ (nmol/min)</td>
<td>114 ± 10</td>
<td>199 ± 36</td>
<td>154 ± 21</td>
<td>150 ± 19</td>
</tr>
<tr>
<td>AE Cl⁻ (nmol/min)</td>
<td>358 ± 21</td>
<td>824 ± 73</td>
<td>374 ± 22</td>
<td>420 ± 70</td>
</tr>
<tr>
<td>AE Ca²⁺ (nmol/min)</td>
<td>3.6 ± 0.3</td>
<td>13.2 ± 2.0</td>
<td>4.8 ± 1.1</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>AE glucose (nmol/min)</td>
<td>2.5 ± 0.4</td>
<td>6.2 ± 0.6</td>
<td>2.3 ± 0.3</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>AE phosphate (nmol/min)</td>
<td>31 ± 8</td>
<td>38 ± 14</td>
<td>36 ± 11</td>
<td>40 ± 13</td>
</tr>
</tbody>
</table>

UV, urinary flow rate; AE, absolute urinary excretion.

* p < 0.05 versus veh same genotype.

† p < 0.05 versus wild-type veh.

**Fig. 1.** Urinary flow rate (UV) and urinary concentrations of Na⁺, K⁺, Cl⁻, Ca²⁺, phosphate, and glucose related to urinary creatinine concentration in metabolic cage experiments of caffeine- and vehicle (veh)-treated A₁ receptor wild-type (+/+) and knockout (−/−) mice.
excretion of fluid, Na\(^+\), K\(^+\), Cl\(^-\), and Ca\(^{2+}\) excretion of \(A_1\) receptor \(+/+\) mice in both absolute terms (see Table 2) as well as in relation to urinary creatinine excretion (Fig. 2). The effect of theophylline observed in \(A_1\) receptor \(+/+\) mice, however, was completely absent in \(-/-\) mice. Importantly, plasma theophylline concentrations were not different between \(A_1\) \(+/+\) versus \(-/-\) mice (21 ± 4 and 21 ± 4 mg/l, N.S.). Absolute excretion of glucose tended to be higher in theophylline- versus vehicle-treated wild-type mice (\(p = 0.08\)), whereas glucose excretion related to urinary creatinine was essentially unaltered. Glucose excretion in \(A_1\) \(-/-\) mice was insensitive to theophylline. Excretion of phosphate was not significantly affected by theophylline in absolute terms or when related to urinary creatinine in either genotype.

### Discussion

The main observation of the present study is that mice lacking the \(A_1\) receptor do not exhibit the diuresis and natriuresis typically elicited by the application of the methylxanthines caffeine or theophylline. The plasma concentrations of theophylline established in the present experiments were close to the therapeutic range (8–20 mg/l). Since caffeine was applied in the same dose as theophylline, we assume caffeine plasma concentrations to be in a comparable range. Earlier data have shown that a concentration of about 8 mg/l is established by drinking 5 to 7 cups of coffee/day (Biagioni et al., 1991). Our data indicate that an intact \(A_1\) adenosine receptor is necessary for the diuretic and natriuretic effects to occur consistent with the notion that methylxanthine-induced diuresis and natriuresis is the consequence of \(A_1\) receptor blockade.

Several observations indicate that methylxanthines increase renal fluid and Na\(^+\) excretion mainly by affecting tubular reabsorption rather than blood pressure or glomerular filtration rate implying that the absence of an effect in \(A_1\) receptor \(-/-\) mice is due to the lack of a tubular target site for methylxanthines. First, the metabolic cage experiments show that the diuretic and natriuretic effects of the methylxanthines observed in wild-type mice were still present when the rates of urinary excretion of fluid and Na\(^+\) were related to urinary creatinine, consistent with a primary effect on renal reabsorption. Second, the changes of mean arterial blood pressure, heart rate, and GFR caused by theophylline were similar in \(A_1\) receptor \(+/+\) and \(-/-\) mice whereas the diuresis and natriuresis occurred only in wild-type mice.

### Previous Studies

Previous studies have reported unchanged renal blood flow (Munger and Jackson, 1994; Gellai et al., 1998; Kost et al., 2000) and GFR (Osswald, 1975; Munger and Jackson, 1994; Gellai et al., 1998; Wilcox et al., 1999; Zou et al., 1999) in response to methylxanthines or \(A_1\) receptor antagonists. Increases of mean arterial blood pressure and heart rate after administration of methylxanthines have been related to enhanced release of catecholamines and blockade of adenosine receptors. Activation of presynaptic \(A_1\) receptors in the sympathetic nerve endings inhibits noradrenaline release and thereby reduces heart rate and blood pressure. Both effects could be blocked with theophylline (Robertson et al., 1981; Fredholm, 1984). A dose-related increase in heart rate and systolic blood pressure was found in healthy human subjects in which i.v. administration of theophylline/ethylenediamine (aminophylline) elicited similar theophylline plasma concentrations as in the present study. The authors further found increased plasma epinephrine and norepinephrine levels and concluded that the cardiovascular effects are mediated by stimulation of the sympathetic nervous system (Vestal et al., 1983). Furthermore, rats treated with selective \(A_{2A}\) receptor antagonists as well as \(A_{2A}\) receptor knockout mice displayed an elevated blood pressure and heart rate (Ledent et al., 1997; Monopoli et al., 1998). Since methylxanthines are unselective adenosine receptor antagonists it seems possible that \(A_2\) receptor blockade, possibly of

### Table 2

**Response to application of theophylline (theo, 45 mg/kg b.wt.) or vehicle (veh, 0.85% NaCl, 0.9 ml/30 g b.wt.) by oral gavage in \(A_1\) receptor wild-type \((+/-)\) and knockout \((-/-)\) mice**

<table>
<thead>
<tr>
<th></th>
<th>+/+ Veh</th>
<th>+/+ Theo</th>
<th>–/- Veh</th>
<th>–/- Theo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>28 ± 2</td>
<td>31 ± 1</td>
<td>28 ± 1</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>[Na(^+)] plasma (nmol/l)</td>
<td>147 ± 1</td>
<td>145 ± 1</td>
<td>147 ± 2</td>
<td>146 ± 1</td>
</tr>
<tr>
<td>[K(^-)] plasma (nmol/l)</td>
<td>4.8 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Arterial hematocrit (%)</td>
<td>50.9 ± 0.7</td>
<td>51.2 ± 0.3</td>
<td>51.8 ± 0.6</td>
<td>49.8 ± 0.5*</td>
</tr>
<tr>
<td>Fluid excreted (% of dosed)</td>
<td>68 ± 7</td>
<td>141 ± 11*</td>
<td>80 ± 6</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>UV (μl/min)</td>
<td>3.2 ± 0.3</td>
<td>7.3 ± 0.6*</td>
<td>3.7 ± 0.3</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>(AE) Na(^+) (nmol/min)</td>
<td>347 ± 50</td>
<td>869 ± 61*</td>
<td>432 ± 31</td>
<td>435 ± 54</td>
</tr>
<tr>
<td>(AE) K(^+) (nmol/min)</td>
<td>152 ± 16</td>
<td>317 ± 30*</td>
<td>174 ± 14</td>
<td>192 ± 16</td>
</tr>
<tr>
<td>(AE) Cl(^-) (nmol/min)</td>
<td>415 ± 52</td>
<td>866 ± 67*</td>
<td>457 ± 38</td>
<td>423 ± 36</td>
</tr>
<tr>
<td>(AE) Ca(^{2+}) (nmol/min)</td>
<td>6.8 ± 1.5</td>
<td>23.2 ± 3.2*</td>
<td>6.6 ± 1.0</td>
<td>7.1 ± 1.4</td>
</tr>
<tr>
<td>(AE) glucose (nmol/min)</td>
<td>2.9 ± 0.5</td>
<td>5.9 ± 1.2</td>
<td>3.0 ± 0.5</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>(AE) phosphate (nmol/min)</td>
<td>15 ± 7</td>
<td>10 ± 5</td>
<td>21 ± 8</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

UV, urinary flow rate; \(AE\), absolute urinary excretion.

* \(p < 0.05\) versus veh same genotype.
the A2A subtype, contributed to the increase in blood pressure and heart rate observed in both A1 receptor+/+ and −/− mice. Methylxanthine-induced inhibition of phosphodiesterase is another possibility that may have been involved.

The present experiments were not designed to localize the diuretic action of methylxanthines along the nephron. Some evidence is provided, however, to support the notion that inhibition of proximal tubular reabsorption could have been involved. Ca2+ reabsorption occurs throughout the nephron. In the proximal tubule, 60 to 70% of the filtered Ca2+ is reabsorbed, and this occurs passively as a consequence of primary reabsorption of Na+ and water. The present exper-

**Fig. 2.** Urinary flow rate (UV) and urinary concentrations of Na+, K+, Cl−, Ca2+, phosphate, and glucose related to urinary creatinine concentration in metabolic cage experiments of theophylline- and vehicle-treated A1 receptor wild-type (+/+) and knockout (−/−) mice. *, p < 0.05 versus vehicle same genotype.

**TABLE 3**
Response to application of theophylline (theo, 30 mg/kg b.wt. i.v.) in A1 receptor wild-type (+/+, n = 5) and knockout (−/−, n = 5) mice anesthetized with α-chloralose/ketamine

<table>
<thead>
<tr>
<th></th>
<th>+/+ Basal</th>
<th>+/+ Theo</th>
<th>−/+ Basal</th>
<th>−/+ Theo</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>97 ± 9</td>
<td>107 ± 7*</td>
<td>94 ± 12</td>
<td>114 ± 11*</td>
</tr>
<tr>
<td>HR (1/min)</td>
<td>477 ± 30</td>
<td>609 ± 35*</td>
<td>459 ± 36</td>
<td>597 ± 40*</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>45 ± 2</td>
<td>44 ± 2</td>
<td>46 ± 1</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>[Na+] plasma (mmol/l)</td>
<td>149 ± 1</td>
<td>151 ± 2</td>
<td>148 ± 2</td>
<td>150 ± 2*</td>
</tr>
<tr>
<td>GFR (μl/min)</td>
<td>207 ± 20</td>
<td>160 ± 21</td>
<td>191 ± 31</td>
<td>156 ± 33</td>
</tr>
<tr>
<td>UV (μl/min)</td>
<td>2.4 ± 0.3</td>
<td>4.6 ± 0.8*</td>
<td>1.6 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>AE Na+ (mmol/min)</td>
<td>77 ± 8</td>
<td>387 ± 102*</td>
<td>61 ± 10</td>
<td>105 ± 32</td>
</tr>
<tr>
<td>FE Na+ (%)</td>
<td>0.3 ± 0.1</td>
<td>1.7 ± 0.5*</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; Hct, arterial hematocrit; UV, urinary flow rate; AE, absolute urinary excretion; FE, fractional urinary excretion.

* p < 0.05 versus P1 same genotype.
responses to caffeine, however, was observed in A1 receptor urinary glucose excretion in wild-type mice. A similar response to theophylline in A1 receptor mediated these effects.

In contrast, a previous micropuncture study showed that basal proximal tubular reabsorption of fluid and Na+ was not different in A1 receptor +/+ and −/− mice indicating effective compensation with chronic inhibition or absence of A1 receptors (Vallon et al., 2004).

Previous studies indicated that glucose reabsorption in the proximal tubule, which is mediated by Na+-dependent glucose transport, can be activated by adenosine (Coulson et al., 1991; Cai et al., 1994). Consistent with this notion, the present study revealed that caffeine significantly increased urinary glucose excretion in wild-type mice. A similar response to caffeine, however, was observed in A1 receptor −/− mice indicating that inhibition of other adenosine receptor subtypes and/or phosphodiesterases participated in this response. In contrast to caffeine, theophylline was lacking a clear-cut effect on renal glucose reabsorption in both wild-type and A1 receptor −/− mice. The reason for this remains unclear. Vice versa, the present experiments revealed an increase in absolute and creatinine-related excretion of K+ in response to theophylline in A1 receptor +/+ mice. This response is consistent with a previous study in humans (Beutler et al., 1991) and most likely the consequence of an enhanced Na+ and fluid load to the distal nephron increasing K+ secretion at this site. Despite similar natriuresis, however, a clear-cut kaliuretic response to caffeine was not evident in A1 receptor −/− mice.

In vitro and in vivo studies have shown that selective A1 receptor antagonists as well as theophylline can reduce re-absorption of phosphate in the proximal tubule (Brater et al., 1983; Colin et al., 1984; Coulson et al., 1991; Balakrishnan et al., 1993; Cai et al., 1994). The present study, however, did not reveal any significant effect of caffeine or theophylline on renal phosphate excretion in either genotype. Taken together, the present study demonstrates that in intact A1 receptors are required for the diuretic and natriuretic effects of the methylxanthines caffeine and theophylline to occur, consistent with the assumption that A1 receptor blockade mediates these effects.

References


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