Natriuretic effect of adenosine A\textsubscript{1}-receptor blockade in rats

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Abstract

**Background.** Many effects of adenosine on renal function have been identified. The development of adenosine receptor blockers has made it possible to identify which of these effects are exerted by endogenous adenosine. At least four adenosine receptor subtypes, denoted A\textsubscript{1}, A\textsubscript{2a}, A\textsubscript{2b}, and A\textsubscript{3} are currently known. In the present study the selective A\textsubscript{1} receptor blocker 1,3-dipropyl-8\{-2-(5,6-epoxy)norbanyl\}xanthine (CVT-117) was used to assess the effect of A\textsubscript{1} activation by endogenous adenosine on renal function in rats.

**Methods.** Clearance studies were performed before and after administration of 0.1 mg/kg and 0.8 mg/kg of CVT-117 in separate groups of rats and before and after administration of vehicle in time-control rats. Measurements of heart rate before and after administration of exogenous adenosine confirmed effective A\textsubscript{1} receptor blockade.

**Results.** At both the lower and higher doses, A\textsubscript{1} receptor blockade with CVT-117 increased fractional sodium excretion and urine flow rate without altering GFR. The increase in sodium excretion following A\textsubscript{1} blockade was not accompanied by increases in the excretion of phosphate or potassium.

**Conclusion.** These results show that endogenous adenosine promotes sodium retention by activation of A\textsubscript{1} receptors.

**Key words:** adenosine; diuretic; glomerular filtration rate; receptor; sodium

Introduction

Numerous effects of adenosine on renal function have been identified by administration of adenosine to animals and application of adenosine to kidney cells [1,2]. The development of adenosine receptor blockers has made it possible to identify which of these effects are exerted by endogenous adenosine [3]. There are now known to be at least four adenosine receptor subtypes, denoted A\textsubscript{1}, A\textsubscript{2a}, A\textsubscript{2b}, and A\textsubscript{3}. The most frequently examined effects of adenosine on the kidney, including constriction of the afferent arteriole and mesangial cell contraction, suppression of renin release, stimulation of proximal tubule cell phosphate uptake, and inhibition of thick ascending limb chloride reabsorption, appear to be mediated by the A\textsubscript{1} receptor [1,2,4–7]. The current study therefore examined the effect of adenosine A\textsubscript{1} receptor blockade on renal function in rats. Studies were carried out with 1,3-dipropyl-8\{-2-(5,6-epoxy)norbanyl\}xanthine (CVT-117), which is the most selective A\textsubscript{1} receptor antagonist described to date [8].

**Subjects and methods**

Renal function was assessed in male Sprague-Dawley rats weighing 360–420 g and maintained on standard laboratory chow. Rats were anaesthetized with Inactin, 100 mg/kg, i.p., and placed on a temperature-regulated table. A PE-50 tubing catheter was inserted in the left femoral artery and used for blood sampling and monitoring of mean arterial pressure and heart rate using an electronic transducer connected to a direct writing recorder. After tracheostomy PE-50 catheters were inserted in the right and left internal jugular veins for infusion of rat plasma, saline, and inulin (Iso-Tex Diagnostics, Friendswood, TX). Plasma was infused in an amount equal to 1% body weight over 40–45 min, followed by a reduction of the infusion rate to 0.5 ml/h for the duration of the study. Saline was infused at a rate to 1.2 ml/h throughout the study. After 40 min, inulin was added to the saline to achieve an infusion rate of 60 mg/h following a loading dose of 25 mg. At the same time, lithium chloride was added to achieve an lithium infusion rate of 0.8 mEq/h following a loading dose of 0.3 mEq. A PE-10 catheter was inserted in the left ureter for collection of urine.

Clearance studies were carried out beginning 120 min after anaesthesia. Baseline measurements were first made over a 40-min period. Separate groups of rats then received bolus infusions of either 0.1 mg/kg or 0.8 mg/kg of CVT-117 while a third group of time-control rats received only vehicle (200 ml of 1 mM DMSO in saline). After a 10-min equilibration period, measurements of renal function were repeated over second 40-min clearance period. During each period, a 350-μl arterial blood sample was obtained for
determination of haematocrit and plasma inulin, electrolyte, and protein concentrations.

Following clearance studies, the efficacy of A1 receptor blockade was assessed by infusion of test doses of adenine and the adenine agonist, (--)-N6-phenylisopropyladenosine (NPIAd, Sigma, St Louis, MO). Blood pressure and pulse were first recorded before and 15 s after a bolus infusion of adenine, 200 µg i.v. A period of 20 min was then allowed for blood pressure and pulse to return to baseline before repeat measurements before and 1 min after a bolus infusion of NPIAd 200 µg i.v.

Inulin content of plasma and urine was assessed by the anthrone method. Sodium, potassium and lithium concentrations in plasma and urine were assessed by flame photometry. Plasma and urine phosphorus concentrations were measured by a commercial analyser (Ektachem, Kodak, Rochester, NY). Plasma protein concentration was determined by refractometry. GFR and fractional clearance values for sodium, potassium, phosphorous, and lithium were calculated by standard formulae. The significance of changes observed between the groups following A1 blockade and vehicle infusion was assessed by one-factor ANOVA and Fisher’s exact test. Values are expressed as the mean ± SD throughout and a P < 0.05 was considered statistically significant.

Results

The effects of A1 receptor blockade on renal function are summarized in Table 1. GFR remained stable in rats subjected to A1 receptor blockade and in time control rats which received only vehicle. Both the lower (0.1 mg/kg) and higher (0.8 mg/kg) doses of the A1 receptor blocker caused marked increases in urine flow rate (V) and fractional sodium excretion (FeNa). In contrast, urine flow and sodium excretion remained nearly stable in time control rats. Of note, natriuresis and diuresis following selective A1 receptor blockade in our study were not associated with increases in the fractional excretion of potassium or phosphorous. An insignificant increase in the fractional excretion of lithium was observed in each group of rats, including the time controls, while values for haematocrit (Hct) and plasma protein concentration (CA) remained stable.

The effects of A1 receptor blockade on changes in blood pressure and pulse following administration of exogenous adenine and NPIAd are summarized in Table 2. Both adenine and NPIAd caused marked hypotension and bradycardia in time-control rats. As expected, selective A1 receptor blockade with CVT-117 limited the reduction in heart rate but not the reduction in blood pressure. The reduction in heart rate was completely prevented by the higher dose of CVT-117 and largely prevented by the lower dose of CVT-117.

Discussion

The physiological effects of adenine are mediated by a least four receptor subtypes, denoted A1, A2a, A2b, and A3 [3]. The current study examined whether endogenous adenine controls renal function by activation of the A1 receptor. This receptor subtype has a higher affinity for adenine than the A2 receptors and has been more frequently identified as a mediator of the effects of exogenous adenine on renal function. A1 blockade was achieved by administration of 1,3-dipropyl-82 mercapto-5,6-epoxy norbancalin xanthine (CVT-117), which is the most selective A1 receptor antagonist described to date [8]. Results showed that A1 blockade increased sodium excretion and urine flow rate without altering blood pressure. These findings are consistent with those of recent studies which have examined the effects of other A1 receptor antagonists on renal function in the rat [9,10]. Similar results were also reported by Kuan et al. [11] using two different, but less potent selective A1 antagonists. The authors observed an increase in urine volume from 6 µl/min to 23 µl/min and an increase in fractional sodium excretion from 0.3 to 1.69% using the A1 receptor antagonists DPCPX. In comparison, the higher dose of CVT-117 led to similar increases in urine flow from 9 to 25 µl/min and FENa from 0.5 to 2.0%. The current study, like that of Munger and Jackson [10] was carried out in anaesthetized rats which had received plasma to replace surgical volume losses. Together with the results of Mizumoto et al. [9] in unanaesthetized rats, these findings suggest that A1 receptor activation by endogenous adenine exerts an antinatriuretic effect in the kidney of volume-replete animals, which is similar to

Table 1. Summary of renal function studies

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>MAP (mmHg)</th>
<th>Hct (%)</th>
<th>CA (g/dl)</th>
<th>GFR (ml/min)</th>
<th>V (µl/min)</th>
<th>FeNa (%)</th>
<th>FeK (%)</th>
<th>FePO4 (%)</th>
<th>FeLi (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>386 ± 15</td>
<td>127 ± 8</td>
<td>43 ± 2</td>
<td>5.9 ± 0.2</td>
<td>2.08 ± 0.17</td>
<td>9 ± 3</td>
<td>0.5 ± 0.4</td>
<td>37 ± 10</td>
<td>7 ± 4</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>384 ± 24</td>
<td>124 ± 9</td>
<td>42 ± 2</td>
<td>6.1 ± 0.2</td>
<td>2.06 ± 0.19</td>
<td>10 ± 3</td>
<td>0.8 ± 0.5</td>
<td>36 ± 9</td>
<td>9 ± 5</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>Baseline</td>
<td>393 ± 17</td>
<td>130 ± 8</td>
<td>43 ± 1</td>
<td>6.0 ± 0.3</td>
<td>2.07 ± 0.22</td>
<td>16 ± 3*</td>
<td>1.3 ± 0.5*</td>
<td>37 ± 6</td>
<td>8 ± 5</td>
<td>33 ± 2</td>
</tr>
</tbody>
</table>

*P < 0.05 vs baseline; n = 5 each group. BW, body-weight; MAP, mean arterial pressure; Hct, haematocrit; CA, plasma protein concentration; GFR, glomerular filtration rate; V, urine flow; Fe, fractional excretion.
the conclusion of Kuano et al. [11] who found that endogenous adenosine with A<sub>1</sub> receptors directly enhances the tubular reabsorption of filtered sodium. A<sub>1</sub> blockade with CVT-117 had no effect on GFR in the current study. Previous studies have suggested that A<sub>1</sub> receptor activation by adenosine is an important mediator of tubuloglomerular feedback [12]. These results suggest that A<sub>1</sub> blockade could increase the GFR by removing the brake on filtration imposed by solute delivery to the macula densa. Balakrishnan et al. [13] recently reported that selective A<sub>1</sub> blockade with 200 mg of the drug FK453 not only increased sodium excretion and urine flow rates but also significantly increased GFR 2 h after infusion into normal humans. Notably, the dose of A<sub>1</sub> receptor antagonist required to increase the GFR was larger than that required to induce natriuresis. This effect on GFR could not be observed, however, when patients with chronic renal disease and a mean baseline creatinine clearance of 115 ml/min were subjected to exactly the same study [14]. In the current study, however, administration of a dose of CVT-117 larger than that required to induce natriuresis and sufficient to block the A<sub>1</sub> effect of a large dose of exogenous adenosine did not increase GFR. This result is in accord with those of previous studies of A<sub>1</sub> blockade in rats [9,10]; however, the influence of anaesthesia as explanation of the different responses of GFR and the differences in phosphate and lithium excretion to selective A<sub>1</sub> blockade in human and rat remains to be determined. Another explanation would be that there is a different response or site of action of A<sub>1</sub> receptor antagonists among species. Yet another alternative explanation would be that the effect of FK 453 on lithium and phosphate excretion is not mediated via A<sub>1</sub> blockade or that CV-117 lacks these unspecific actions.

The finding of natriuresis unaccompanied by an increase in GFR indicates that A<sub>1</sub> blockade reduces tubule sodium reabsorption in the rat. Within the nephron, A<sub>1</sub> receptors have been found to be most prominent in collecting duct and thick ascending limb segments but A<sub>1</sub> receptor message has also recently been detected in proximal convoluted and straight tubules [13,16]. Reports that A<sub>1</sub> receptor activation stimulates phosphate uptake by cultured proximal tubule cells and Na-3HCO<sub>3</sub> cotransport activity in isolated proximal convoluted tubules suggest that endogenous adenosine could promote proximal phosphate and sodium reabsorption [5,6,17]. In the current study, however, A<sub>1</sub> receptor blockade did not increase fractional phosphate reabsorption. A<sub>1</sub> receptor blockade did slightly increase the fractional excretion of lithium, but the change observed was not significantly greater than seen in time controls. It should be noted that adenosine A<sub>1</sub> may alter proximal epithelial transport in part by counteracting the effects of peptide hormones such as PTH [1,2,6,18]. The effects of A<sub>1</sub> blockade on proximal phosphate and volume reabsorption which are predicted by in vitro studies might therefore become apparent in vivo under appropriate conditions of hormone stimulation.

A<sub>1</sub> blockade increased sodium excretion without increasing potassium excretion in the current study. Similar results were obtained in previous studies of A<sub>1</sub> blockade in rats [9,10]. These findings suggest that the natriuretic effect of A<sub>1</sub> blockade in volume-replete rats is due, at least in part, to reduction of sodium reabsorption in the distal nephron. Studies in isolated inner medullary collecting ducts have shown that A<sub>1</sub> receptor activation antagonizes the effect of AVP on water permeability in this nephron segment [18]. Enhancement of the effect of AVP could explain why urine flow increased less than sodium excretion during A<sub>1</sub> blockade. The hypothesis that A<sub>1</sub> blockade also decreases sodium reabsorption in the collecting duct remains to be tested in isolated tubule segments.

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**References**

2. McCoy DE, Bhattacharya S, Olson BA, Levier DG, Arend LJ,

### Table 2. Response to exogenous adenosine and NPIAd

<table>
<thead>
<tr>
<th>Pulse (min&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>MAP (mmHg)</th>
</tr>
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<tbody>
<tr>
<td>Adenosine</td>
<td>NPIAd</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>413 ± 25</td>
</tr>
<tr>
<td>post</td>
<td>261 ± 95*</td>
</tr>
<tr>
<td>CVT-117 (0.1 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>404 ± 40</td>
</tr>
<tr>
<td>post</td>
<td>356 ± 28</td>
</tr>
<tr>
<td>CVT-117 (0.8 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>401 ± 8</td>
</tr>
<tr>
<td>post</td>
<td>395 ± 17</td>
</tr>
</tbody>
</table>

*P<0.05 vs baseline; n= 5 each group.


8. Belardinelli L, Shroyock JC, Zhang Y et al. 1,3-Dipropyl-8-[2-(5,6-epoxy)norbomyl]xanthine, a potent, specific and selective A<sub>1</sub> adenosine receptor antagonist in the guinea pig heart and brain and in DDT1MF-2 cells. *J Pharmacol Exp Ther* 1995; 275: 1167–1176


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