Sodium intake determines the role of adenosine A2 receptors in control of renal medullary perfusion in the rat

Leszek Dobrowolski, Elżbieta Kompanowska-Jezierska, Agnieszka Walkowska and Janusz Sadowski

Laboratory of Renal and Body Fluid Physiology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Abstract

Background. In the kidney, adenosine (ADO) can induce either vasoconstriction or vasodilatation, mediated by A1 or A2 receptors, respectively. The vasodilator influence may be of special importance in the renal medulla which operates at low tissue pO2 levels and is susceptible to ischaemic damage. It has not been established if ADO induced vasodilatation is modified by salt intake.

Methods. We examined effects of stimulation or inhibition of ADO receptors (A2R) on perfusion of the renal cortex and medulla on low- or high-sodium intake (LS, HS). Effects of suprarenal aortic ADO (0.03 mmol/kg/h), A2R agonist (DPMA), 0.08–0.4 mmol/kg/h, or antagonist (DMPX), 1.7 μmol/kg/h, were examined in anaesthetized rats maintained on LS (0.15% Na) or HS (4% Na) diet for 3 weeks. Whole kidney blood flow (RBF) and the perfusion (laser-Doppler) of the superficial cortex and outer and inner medulla (OM-BF, IM-BF) were measured.

Results. In LS rats neither drug changed renal perfusion. In HS rats ADO increased RBF 18% and OM-BF 16% whereas IM-BF 16% increased after DPMA 18% and decreased after DMPX 13%; neither drug consistently changed perfusion of the cortex.

Conclusions. On HS intake, medullary perfusion is controlled by ADO vasodilator (A2) receptors, which may help provide adequate oxygen to the medulla, the zone which normaly operates under relative hypoxia. On LS intake, the vasodilator and vasoconstrictor effects are probably in balance and ADO has little role in control of intrarenal circulation.

Keywords: adenosine; adenosine A2 receptors; laser-Doppler measurement; renal blood flow; renal medullary blood flow; salt intake

Introduction

There is increasing evidence that extracellular adenosine (ADO) contributes to the control of intrarenal circulation. In the kidney, ADO-dependent vasoconstriction is mediated by A1 and vasodilatation by A2a and A2b receptor types [1]. There is evidence that some aspects of ADO metabolism and, possibly, of its functional role depend on salt intake. On high-sodium diet interstitial ADO concentration increases and so does the density of vasodilator A2 receptors (A2R), especially in the renal medulla [2–4]. Simultaneously, the activity of vasoconstrictor A1R decreases [3,5].

In contrast to the studies of receptor density or distribution, investigations of functional effects of ADO receptor stimulation or inhibition on intrarenal circulation were few in number and, have so far been conducted only in animals on standard diet [6,7].

In the present study, we compared the effects of ADO itself as well as of an agonist and an antagonist of A2AR in anaesthetized rats maintained previously on low- or high-sodium diet (LS, HS, respectively). The focus was on the renal medulla; we reasoned that perfusion thereof should be most effectively controlled because this zone is particularly vulnerable to hypoperfusion and hypoxia [6]. The blood flow through the inner and outer medullary layer was measured separately.

Methods

The experimental procedures were approved by the First Ethical Committee, Warsaw. Male Wistar rats were fed an LS (0.15% Na, w/w) or HS (4% Na, w/w) diet (SSNIFF...
GmbH, Stoeß, Germany) for 3 weeks before the experiment. In our earlier studies this duration of the exposure to high or low sodium intake was found to effectively modify renal circulatory responses to purine active agents [8,9]. The animals had free access to water until the day of the experiment. They were anaesthetized with thiopental (Sandoz GmbH, Kundl, Austria), 100 mg/kg i.p. which provided stable anaesthesia for at least 4 h. The rats’ body temperatures were maintained at about 37°C by means of a heating pad and, to compensate for fluid losses, 3% bovine albumin in Ringer solution was infused via the femoral vein at 2.3 ml/h.

A tracheal cannula ensured free airways. The drugs (see subsequent text) were infused via a suprarenal aortic catheter; another catheter, placed in the upper aorta, was used for measurement of systemic blood pressure (MAP). The left kidney was exposed from a subcostal flank incision and placed in a plastic holder similar to that used for micropuncture studies; the ureter was cannulated to ensure unobstructed urine outflow and for timed urine collection. Urine volume was determined gravimetrically and urinary sodium by flame photometry (Jenway PFP7, Essex, UK).

A cuff probe placed on the renal artery and connected with a transonic flowmeter (Type T106, Transonic System Inc, Ithaca, NY, USA) was used for measurement of total renal blood flow (RBF), which was also taken as an index of whole cortex perfusion. The blood perfusion of the renal superficial cortex (CBF) and outer and inner medulla (OM-BF, IM-BF), were measured separately as laser-Doppler fluxes using Periflux 4001 system (Perimed AB, Jarfalla, Sweden). For CBF, a PF 407 probe was placed on the kidney surface and for OMBF and IMBF two needle probes (PF 402), were inserted into the kidney to the depth of 3 and 5 mm. Since the size of the kidney was larger in rats fed high-salt (compared with low-salt) diet, based on postmortem measurements of the width of individual renal zones in LS vs HS rats, the depth of probe insertion was increased by 0.5–0.7 mm in the latter animals. After each experiment, the position of the medullary probes was verified at the kidney’s cross-section.

**Experimental protocols**

At the end of surgical preparations and after placement of laser-Doppler probes, the infusion of albumin was replaced by isotonic saline at 2.3 ml/h. Simultaneously, a suprarenal aortic infusion of ADO, given during a 15 min experimental period at 0.03 mmol/kg/h, was verified at the kidney’s cross-section. The depth of probe insertion was increased by 0.5–0.7 mm in the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats, the width of individual renal zones in LS vs HS rats. This was followed by recovery periods as described above. We checked before that smaller doses of ADO (0.003 mmol/kg/h), equimolar with the active doses of ATP (adenosine 5’-triphosphate disodium salt) [8] did not change renal perfusion in LS or HS rats.

**Effects of DPMA.** During two 30 min experimental periods the rats received first 0.08 and then 0.4 mmol/kg/h of the drug. Thereafter the drug was replaced by saline for recovery periods. This dosage was previously found to induce a decrease in MAP comparable with that seen after an A1R agonist [9].

**Effects of DMPX.** During a 30–45 min experimental period the rats received DMPX at 1.7 μmol/kg/h. Thereafter, the drug was replaced by saline for recovery periods. In an additional series, DMPX was given in rats maintained on LS or HS diet for 1 week before experiment. We used here the dose based on that applied in another whole kidney study [7].

**Statistics**

The significance of changes within one group over time was first evaluated by repeated measures analysis of variance (ANOVA), followed by Student’s test for dependent variables. Differences in mean values between groups were first analysed by the classical one-way ANOVA followed by modified Student’s t-test for independent variables, using Bonferroni’s correction for multiple comparisons. P < 0.05 was taken to indicate significance of differences.

**Results**

There was no significant difference in body weight between rats on LS and HS diet (310 ± 4 and 300 ± 4 g, respectively), whereas kidney weight was significantly (11%) higher in the latter group (1.52 ± 0.03 vs 1.36 ± 0.03 g in LS rats).

The difference in MAP between HS compared with LS rats (131 ± 4 vs 121 ± 3 mmHg, respectively), calculated from the pooled baseline data of the three groups shown in Table 1 (n = 21 for either diet), was of borderline significance (P = 0.056). The corresponding differences in the baseline renal haemodynamic parameters were insignificant. The baseline sodium excretion calculated jointly for the three groups (ADO, DPMA, DMPX) differed significantly between LS and HS rats and was 0.6 ± 0.2 vs 3.0 ± 0.6 μmol/min, respectively. The respective values for urine flow were 5.2 ± 1.0 vs 21.4 ± 2.7 μl/min.

ADO infusion significantly decreased MAP in the HS group only, while after selective stimulation of A2R with DPMA the decrease was seen irrespective of the diet. The decrease in MAP after DPMA was associated with a significant increase in HR, from 370 ± 9 to 386 ± 12 beats/min in LS, and from 366 ± 10 to 387 ± 12 beats/min in HS rats. Thus, the characteristic pattern of inverse responses of HR and MAP to A2R stimulation, in agreement with earlier reports [10], confirmed the selectivity of...
Table 1. Effects of adenosine (ADO), selective A2a receptor agonist (DPMA) and selective A2a receptor antagonist (DMPX) on mean arterial blood pressure (MAP) and renal blood perfusion parameters in rats maintained on low- or high-sodium diet (LS, HS).

<table>
<thead>
<tr>
<th></th>
<th>basal</th>
<th>ADO</th>
<th>recovery</th>
<th>basal</th>
<th>DPMA</th>
<th>recovery</th>
<th>basal</th>
<th>DMPX</th>
<th>recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>LS</td>
<td>121 ± 3</td>
<td>118 ± 4</td>
<td>120 ± 4</td>
<td>131 ± 3</td>
<td>120 ± 3</td>
<td>124 ± 4</td>
<td>112 ± 4</td>
<td>111 ± 4</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>132 ± 5</td>
<td>127 ± 4a</td>
<td>128 ± 6</td>
<td>139 ± 9</td>
<td>123 ± 10a</td>
<td>125 ± 9</td>
<td>119 ± 3</td>
<td>116 ± 3</td>
</tr>
<tr>
<td>RBF (ml/min/g)</td>
<td>LS</td>
<td>7.0 ± 0.6</td>
<td>7.3 ± 0.8</td>
<td>6.9 ± 0.6</td>
<td>8.0 ± 0.9</td>
<td>7.7 ± 0.9</td>
<td>7.5 ± 1.0</td>
<td>8.4 ± 0.8</td>
<td>8.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>7.6 ± 0.9</td>
<td>9.0 ± 1.1a</td>
<td>8.2 ± 1.1</td>
<td>8.2 ± 1.7</td>
<td>7.4 ± 1.5</td>
<td>7.0 ± 1.5</td>
<td>9.7 ± 0.5</td>
<td>9.0 ± 0.6</td>
</tr>
<tr>
<td>RVR (mm Hg min/ml)</td>
<td>LS</td>
<td>18.0 ± 1.3</td>
<td>17.0 ± 1.6</td>
<td>18.3 ± 1.6</td>
<td>17.6 ± 2.3</td>
<td>16.8 ± 2.0</td>
<td>18.5 ± 2.8</td>
<td>14.1 ± 1.0</td>
<td>14.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>20.3 ± 3.8</td>
<td>16.5 ± 2.9a</td>
<td>18.9 ± 4.9</td>
<td>16.5 ± 2.0</td>
<td>15.1 ± 1.6</td>
<td>16.5 ± 2.4</td>
<td>12.5 ± 0.8</td>
<td>13.3 ± 1.1</td>
</tr>
<tr>
<td>CBF (perfusion units)</td>
<td>LS</td>
<td>667 ± 62</td>
<td>652 ± 75</td>
<td>631 ± 62a</td>
<td>660 ± 61</td>
<td>637 ± 74</td>
<td>659 ± 79</td>
<td>508 ± 37</td>
<td>544 ± 33a</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>749 ± 40</td>
<td>800 ± 40</td>
<td>757 ± 48</td>
<td>735 ± 98</td>
<td>706 ± 114</td>
<td>696 ± 119</td>
<td>596 ± 40</td>
<td>554 ± 42</td>
</tr>
<tr>
<td>OM-BF (perfusion units)</td>
<td>LS</td>
<td>343 ± 69</td>
<td>304 ± 76</td>
<td>317 ± 73</td>
<td>144 ± 14</td>
<td>148 ± 17</td>
<td>151 ± 19</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>223 ± 42</td>
<td>254 ± 43a</td>
<td>207 ± 37</td>
<td>215 ± 40</td>
<td>207 ± 32</td>
<td>215 ± 38</td>
<td>206 ± 42</td>
<td>181 ± 42a</td>
</tr>
<tr>
<td>IM-BF (perfusion units)</td>
<td>LS</td>
<td>308 ± 27</td>
<td>301 ± 38</td>
<td>292 ± 30</td>
<td>171 ± 15</td>
<td>184 ± 11</td>
<td>191 ± 18</td>
<td>178 ± 31</td>
<td>214 ± 25</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>209 ± 23</td>
<td>238 ± 23a</td>
<td>200 ± 26</td>
<td>214 ± 34</td>
<td>251 ± 38a</td>
<td>228 ± 38</td>
<td>165 ± 15</td>
<td>144 ± 14a</td>
</tr>
</tbody>
</table>

RBF: whole kidney blood flow; RVR, renal vascular resistance; CBF, OM-BF, IM-BF, cortical, outer- and inner-medullary blood flow (laser-Doppler flux), respectively. Maximal values within and 30 min after cessation (recovery) of the drug administration are given (means ± SEM); n = 7; *significantly different from baseline; †measured in rats maintained on HS diet for 1 week only, n = 4.

DPMA in vivo. A2R inhibition with DMPX did not alter MAP or HR.

ADO altered renal haemodynamics in HS rats only (Table 1). The roughly parallel increases in RBF, OM-BF and IM-BF (16–18%) were significant while the modest 7% increase in CBF was not. There was also a significant reversible decrease in renal vascular resistance (RVR).

Selective stimulation of A2R with DPMA did not alter renal haemodynamics in LS rats. On HS diet, the only significant change was a selective 18% increase in IM-BF.

The most prominent findings, referring to medullary perfusion changes in HS rats, are recapitulated in Figure 1. The increase in OM-BF after ADO, mediated, expectedly, by A2R, contrasted with the decrease observed after inhibition of A2R with DMPX. Interestingly, stimulation of A2R with DPMA did not change OM-BF. In the inner medulla, there was a clear contrast between an increase in perfusion after ADO and DPMA and a decrease after DMPX.

Discussion

Administration of ADO or selective manipulation of ADO A2R activity (stimulation or inhibition) had little effect on renal perfusion in animals on low-salt (LS) intake but definite effects in high salt (HS) rats. In our recent study, selective ADO A1R stimulation distinctly lowered the perfusion of the kidney in HS rats, however, a lesser but distinct change was also seen on low-salt intake [9]. Taken together, the results suggest that the role of ADO in control of intrarenal haemodynamics, especially its effect mediated by A2R, is much more pronounced in HS animals. The reason for the role of high-salt intake is probably complex; it may be related to the attendant increased generation of ADO [2,3] and, perhaps more important, to the prevalence of A2 over A1R in HS animals, as documented in earlier studies [3]. The expression of ADO A2 receptors and intrarenal circulation

Fig. 1. Changes in outer- (OM-BF) and inner- (IM-BF) medullary perfusion after adenosine (ADO), an A2 receptor agonist (DPMA) and an A2 receptor antagonist (DMPX) in rats on high-salt (HS) diet. *Significantly different from pre-treatment control (paired Student t-test); †significantly different from ADO effect (ANOVA, unpaired Student t-test); ‡significantly different from ADO and DMPX effect (ANOVA, unpaired Student t-test). The data for DMPX effect on OM-BF are for rats on HS diet for 1 week. The data for the effect of an A1 receptor agonist (CCPA, dotted line) has been extracted from [9].
A2a receptors in the renal medulla was recently reported to increase after transition from standard to high-salt intake [4].

The focus of the present study was on the role of ADO in control of circulation in the renal medulla; the effects of pharmacological manipulations on the perfusion of the outer and inner layer of this zone are collected in Figure 1. In this study we used DPMA, an established agonist of A2aR, whereas a selective inhibitor of A2aR, DMPX, was used in another group of rats to remove the influence of endogenous ADO on A2R. The suprarenal aortic administration of DPMA and DMPX increased or decreased, respectively, the perfusion of the inner medulla without significantly affecting total renal or cortical blood flow. This indicates the prominent role of A2aR in the control of microcirculation in this region.

It will be noticed that in vascular preparations, but also in whole kidney studies, effects of ADO receptor activation differed depending on the side of active agent delivery: luminal (intravascular) vs abluminal (interstitial) [1]. Ours is the first demonstration that vasodilator effect of A2R stimulation confined to the medulla occurs when the receptors are accessed from the vascular lumen, however, this is observed on HS but not LS intake. The zonal selectivity of the effect may be the consequence of higher density of A2R in the medulla or even extreme paucity or absence of A2aR in the cortex reported by Liclican et al. [4]. The observation that within the medulla the effect was seen in the inner layer only suggests that the A2R responsible were mainly those located in the descending vasa recta rather than those controlling perfusion of the juxtamedullary glomeruli. Alternatively, the receptors were located mostly in the core vasa recta of the outer medullary vascular bundle, those supplying blood to the inner medullary layer. Before, medullary vasodilation was demonstrated when A2R were accessed from the renal interstitium, cortical [6] or medullary [7]; these effects were seen on standard salt intake.

Also in whole kidney studies and using the same route of active agent administration (suprarenal aortic infusion) we confirmed recently the vast earlier evidence indicating that activation of ADO A1R decreased perfusion of the medulla [9]. The effect was more pronounced on high-salt intake and occurred in parallel with the decreased whole kidney and cortical perfusion. Together, the present and earlier results add to the evidence that ADO A1R and A2R types interplay in control of the renal medullary circulation. The net effect would reflect the balance of vasoconstrictor influence of A1R, distributed and active throughout the kidney, and vasodilator A2R which seem to be localized and active mostly in the medulla. The contribution of ADO to the overall effects of diverse vasoconstrictor and vasodilator agents controlling medullary perfusion seems to be important in animals on HS intake.

Selective manipulation of A1R and A2R activity helped delineate their specific effects on intrarenal perfusion, however, effects of the natural hormone may be thought to be more relevant to the actual functional role of ADO. An increase in medullary perfusion after suprarenal aortic infusion of the hormone, similar to after DPMA, suggests that on high-salt intake the vasodilator influence, probably mediated by A2aR, prevailed over the vasoconstrictor influence of A1R. However, as discussed above, the former receptor type seems to be active mostly or exclusively in the medulla and cannot account for the observed post-ADO increase in perfusion of the cortex (increase in RBF). This effect might depend on activation of A2bR; a low-affinity vasodilator receptor subtype [1] reported to be expressed in the pre-glomerular vessels [1,11]. The same receptors, possibly located in the pre-glomerular arterioles of juxtamedullary glomeruli, could also mediate the ADO-induced hyperaemia of the outer medulla (Figure 1), an effect that was not seen after A2aR stimulation with DPMA.

In summary, the results of ADO receptor activation or inhibition were shown to critically depend on the status of body salt metabolism and, likely, on some aspects of tubular sodium handling. On HS intake, ADO induced vasodilatation in the medulla (mediated by A2aR) and the cortex (possibly mediated by A2bR) prevailed over vasoconstrictor effects of A1R (active throughout the kidney); the net result was increased perfusion. On LS intake, the vasodilator and vasoconstrictor effects of ADO were probably in balance and intrarenal perfusion was not altered. It is likely that activation of the renin-angiotensin system induced by LS intake contributed to neutralization of vasodilator effects of ADO; angiotensin was reported to enhance the effects of A1R [1].

A limitation of this study relates to induction of supranormal plasma levels of ADO or its analogue, which could activate low-affinity receptors (A2b but probably also A2a) that may have no functional role under resting conditions. However, it will be noticed that in the renal medulla the A2aR blockade effectively decreased perfusion, which suggested a basal vasodilator tonic influence mediated by this receptor type. It should also be admitted that effects different from ours could be observed with the active agents delivered from the interstitial rather than from the vascular luminal side. However, there is indirect evidence that ADO acts on A2R located on renal vascular endothelium [1].

Conflict of interest statement. None declared.

References


Received for publication: 22.3.07
Accepted in revised form: 2.5.07