Abolished tubuloglomerular feedback and increased plasma renin in adenosine A1 receptor-deficient mice

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Received 3 August 2001; accepted in final form 14 August 2001

Brown, Russell, Anna Ollerstam, Björn Johansson, Ole Skött, Samuel Gebre-Medhin, Bertil Fredholm, and A. Erik G. Persson. Abolished tubuloglomerular feedback and increased plasma renin in adenosine A1 receptor-deficient mice. Am J Physiol Regulatory Integrative Comp Physiol 281: R1362–R1367, 2001.—The hypothesis that adenosine acting on adenosine A1 receptors (A1R) regulates several renal functions and mediates tubuloglomerular feedback (TGF) was examined using A1R knockout mice. We anesthetized knockout, wild-type, and heterozygous mice and measured glomerular filtration rate, TGF response using the stop-flow pressure (Psf) technique, and plasma renin concentration. The A1R knockout mice had an increased blood pressure compared with wild-type and heterozygote mice. Glomerular filtration rate was similar in all genotypes. Proximal tubular Psf was decreased from 36.7 ± 1.2 to 25.3 ± 1.6 mmHg in the A1R+/+ mice and from 38.1 ± 1.0 to 27.4 ± 1.1 mmHg in A1R+/- mice in response to an increase in tubular flow rate from 0 to 35 nl/min. This response was abolished in the homozygous A1R−/− mice (from 39.1 ± 4.1 to 39.2 ± 4.5 mmHg). Plasma renin activity was significantly greater in the A1R knockout mice [74.2 ± 14.3 milli-Goldblatt units (mGU)/ml] mice compared with the wild-type and A1R+/- mice (36.3 ± 8.5 and 34.1 ± 9.6 mGU/ml), respectively. The results demonstrate that adenosine acting on A1R is required for TGF and modulates renin release.

A 1R knockout mice; angiotensin; renin release; micropuncture

THE KIDNEY PERFORMS ITS IMPORTANT functions in controlling extracellular fluid volume balance and blood pressure level partly through the tubuloglomerular feedback (TGF) and renin release mechanisms. The TGF operates within the juxtaglomerular apparatus and couples distal tubular flow to afferent arterial tone and, thus, the glomerular filtration rate (GFR). The TGF is a negative feedback system where an increase in salt concentration at the distal tubule is sensed by the macula densa cells, which release a mediator that is transmitted to the glomerular microvasculature causing a constriction of the afferent arteriole (27). It is also well described that when salt delivery and salt concentration decrease at the macula densa site, renin release is increased (30), and there is a significant inverse relationship between end distal tubular NaCl concentration and the plasma renin concentration (15).

In 1982, Osswald and colleagues (22) hypothesized that renal hemodynamics were under metabolic control of local blood flow and proposed adenosine, with a vasoconstrictor response in renal circulation, as the mediator of the signal in the TGF. Adenosine has been suggested as TGF mediator, not only because of its constrictive effects on the afferent arteriole but also its inhibitory effect on renin release (16, 24). The effects of adenosine are mediated via A1, A2A, A2B, and A3 receptors, which are members of the G protein-coupled receptor family (4, 5). Earlier studies have also indicated the existence of both A1 and A2A receptors in the kidney. They have been found to be widely distributed throughout the kidney, in the renal vasculature, juxtaglomerular apparatus, glomeruli, tubules, and collecting ducts (31, 35). The adenosine A1 receptor (A1R) has been shown to constrict afferent arterioles (36), contract mesangial cells in the glomerulus (21), inhibit renin release, to be involved in the responsiveness of the TGF system (23), and decrease the release of noradrenaline while increasing its actions (8, 9).

Much of the early work relied on the use of pharmacological tools, including the rather nonspecific antagonist theophylline. We have developed an A1R knockout mouse (12), and, because this deletion is very selective, we have investigated if there are alterations in the TGF mechanism and/or in the renin release. We therefore performed clearance, stop-flow pressure (Psf), and renin release measurements on these knockouts and their healthy littermates. To study if the blood pressure and renal functions were not more dependent on the renin-angiotensin system in the A1R knockout mice than in their wild-type controls, an ANG II recep-

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tor type 1 (AT₁) blocker was administered. The results from our present study indicate that adenosine, through the A₁R, has a key role in the TGF mechanism and in renin release.

METHODS

Genotyping of A₁R-deficient mice. The A₁R knockout mice were generated as described by Johansson and coworkers (12). The A₁R mice used in experiments were siblings from matings of A₁R+/− mice of a 50% C57BL, 50% 129/OlaHsd background. A₁R adenosine-receptor knockout mice were genotyped with Southern blot analysis. DNA from tail biopsies was digested with BamHI, run on an electrophoresis gel and probed with a BamHI XhoI fragment derived from the immediate 5′ vicinity of the targeted exon. A wild-type allele generated a 20-kb fragment that hybridized, whereas in the targeted allele there was instead a 9-kb hybridizing fragment.

Surgical procedures. The experiments were performed on female mice weighing 20–25 g. The animals had free access to food and tap water. Anesthesia was induced by spontaneous inhalation of isoflurane (Forene, Abbott Scandinavia, Kista, Sweden). The inhalation gas was a mixture of 40% oxygen and 60% nitrogen, which contained ~2.2% isoflurane. The mice were placed on a servo-regulated heating pad to maintain body temperature at 37.5°C. Catheters were inserted into the carotid artery and the jugular vein for blood pressure measurements and infusion of maintenance fluid (0.9% NaCl and 2% albumin, 0.35 ml/h), respectively. The bladder was catheterized for urine collection. For stop-flow measurements, the left kidney was exposed through a subcostal flank incision. The kidney was dissected free from surrounding tissue, placed in a Lucite cup, and fixed in a 3% glutaraldehyde solution. The surface of the kidney was covered with paraffin oil to prevent drying during the experiments. Stop-flow measurements were started after a 45-min equilibration period.

Whole kidney clearance measurements. After the surgical procedures were completed, the mice were given a bolus infusion of 0.5 μCi [3H]methoxy-inulin in 0.08 ml of maintenance fluid. Five microcuries per milliliter was then added to the maintenance fluid for continuous infusion. Total kidney urine flow rate and sodium and potassium excretion were determined from urine samples taken through a catheter in the bladder after a 45-min equilibration period and 40 min of control sampling, the mice were given a 10 μg/kg bolus dose of candesartan (AstraZeneca, Mölndal, Sweden), an ANG II AT₁-receptor blocker, and allowed to stabilize for 30 min, followed by a 40-min sampling period. Urine volumes were determined gravimetrically. Urinary sodium and potassium concentrations were determined by flame photometry (FLM3, Radiometer, Copenhagen, Denmark). Blood samples (15 μl) were taken 20 min into each sampling period. The samples were centrifuged, and aliquots of plasma and urine were analyzed in a multichannel gamma counter (MR 300 Automatic Liquid Scintillation System, Kontron). Inulin clearance was then calculated as a measure of GFR. At the conclusion of each experiment, the kidneys were removed, cleaned of any surrounding tissue, and weighed.

Psf measurements. TGF characteristics were determined by stop-flow technique. Randomly chosen proximal tubular segments on the kidney surface were punctured with a sharpened glass pipette (3–5 μm OD) filled with a 1 M NaCl solution stained with Lissamine green. The pipette was connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT) to determine the proximal tubular Psf. By injections of stained fluid, the tubular distribution of the kidney surface was defined. In nephrons for which more than three proximal segments were identified, a second pipette (7–9 μm OD) was inserted in the last accessible segment of the proximal tubule. This pipette was filled with an artificial ultrafiltrate (in mM: 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 4 NaHCO₃, 7 urea, and 2 g/L Lissamine green, pH 7.4) and connected to a microperfusion pump (Hampel, Frankfurt, Germany). Between these two pipettes a solid wax block was placed with a third pipette (7–9 μm OD). The pressure upstream to the block, the proximal tubular Psf, was determined at different perfusion rates (0–35 nl/min) in the loop of Henle.

Plasma renin measurements. Immediately following anesthesia, a blood sample was taken from the carotid artery, centrifuged, and the plasma was frozen to −85°C. Plasma renin concentration was measured by RIA of ANG I using the antibody-trapping technique (17). Briefly, 10 μl plasma from each sample was serially diluted between 50- and 1,000-fold. Five microliters of each dilution were incubated in duplicates for 24 h together with rabbit ANG I antibody and renin substrate (−1,200 ng ANG I/ml) from 24 h nephrectomized rats and from which renin had been extracted by affinity chromatography. The reaction was stopped by addition of 1 ml cold barbital buffer, ANG I tracer was added, and an RIA was performed. Only results with linearity in serial dilutions were accepted. Renin values were standardized with renin standards obtained from the Institute for Medical Research (MRC, Holly Hill, London, UK) and are expressed in standard Goldblatt units (GU).

Statistics. The results are presented as means ± SE. The data were tested for significance with the Student’s t-test for paired or unpaired observations. When multiple groups were compared, one-way ANOVA was employed. The Bonferroni test for pairwise multiple comparisons was used to allow for more than one comparison with the same variable. This states a significance level of P/M, where M is the number of comparisons to be made. Statistical significance was defined as P < 0.05.

RESULTS

There were no differences in mean body or total kidney weights between the studied mice (Table 1). Mean arterial blood pressure (MAP) remained stable for all genotypes throughout the experiments. The A₁R knockout mice had an increased MAP compared with wild-type and heterozygote mice both in mice where GFR was measured and in animals where Psf determinations were made.

After the administration of the AT₁-receptor blocker candesartan, MAP decreased in all three genotypes by 2.5, 15.0, and 21.2 mmHg in the

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ArR+/+</th>
<th>ArR+/−</th>
<th>ArR−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>24 ± 1</td>
<td>26 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Total kidney wt, g</td>
<td>0.293 ± 0.03</td>
<td>0.301 ± 0.01</td>
<td>0.285 ± 0.01</td>
</tr>
<tr>
<td>Kidney wt/body wt</td>
<td>0.012 ± 0.001</td>
<td>0.012 ± 0.001</td>
<td>0.011 ± 0.0002</td>
</tr>
</tbody>
</table>

Values are given as means ± SE (n = no. of mice). A₁R, adenosine receptor type 1.
Table 2. MAPs and renal excretion rates in anesthetized wild-type, heterozygote, and knockout A1R mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MAP, mmHg</th>
<th>MAP cand, mmHg</th>
<th>ΔMAP, mmHg</th>
<th>GFR/kidney wt, ml-min⁻¹·g⁻¹</th>
<th>ΔGFR/kidney wt, ml-min⁻¹·g⁻¹</th>
<th>Na⁺ excretion, μmol/min⁻¹·g⁻¹</th>
<th>K⁺ excretion, μmol/min⁻¹·g⁻¹</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1R+/+</td>
<td>85 ± 1</td>
<td>63 ± 2*</td>
<td>-22 ± 2</td>
<td>0.78 ± 0.06</td>
<td>-0.11 ± 0.10</td>
<td>0.19 ± 0.05</td>
<td>1.03 ± 0.12</td>
<td>8</td>
</tr>
<tr>
<td>A1R+/-</td>
<td>90 ± 3</td>
<td>75 ± 4†</td>
<td>-15 ± 2</td>
<td>0.71 ± 0.08</td>
<td>0.11 ± 0.11</td>
<td>0.41 ± 0.13</td>
<td>1.02 ± 0.20</td>
<td>8</td>
</tr>
<tr>
<td>A1R−/-</td>
<td>97 ± 2</td>
<td>74 ± 4†</td>
<td>-21 ± 4</td>
<td>0.71 ± 0.06</td>
<td>-0.13 ± 0.08</td>
<td>0.40 ± 0.08</td>
<td>1.09 ± 0.07</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are given as means ± SE (n = no. of mice). MAP, mean arterial pressure; cand, candesartan; ΔMAP, change in MAP after administration of candesartan; GFR, glomerular filtration rate. *P < 0.05 vs. +/+; †P < 0.05 vs. same genotype before candesartan.

A1R+/+, A1R+/-, and A1R−/- genotypes, respectively (Table 2). GFRs were similar in all genotypes (Table 2), although candesartan induced no significant differences in GFR (A1R+/+, -0.11 ± 0.10; +/−, -0.11 ± 0.11 and −/−, -0.13 ± 0.08 ml·min⁻¹·g⁻¹). Na⁺ excretion was higher in the A1R+/+ and −/− mice compared with the wild-type mice, whereas K⁺ excretion was similar for all groups. There was no significant difference in urine flow rate between the different genotypes (A1R+/+, 2.53 ± 0.29; A1R+/-, 2.84 ± 0.45; A1R−/-, 3.22 ± 0.47 μl·min⁻¹·g⁻¹). After candesartan administration, the urine flow rates remained unchanged (A1R+/+, 2.13 ± 0.056; A1R+/-, 2.64 ± 0.58; A1R−/-, 2.63 ± 0.55 μl·min⁻¹·g⁻¹).

Table 3 summarizes the Psf measurements in the three genotypes of the A1R knockout strain. Proximal tubular Psf was decreased from 36.7 ± 1.2 to 25.3 ± 1.6 mmHg (ΔPsf 11.4 ± 1.1 mmHg) in the A1R+/+ mice and from 38.1 ± 1.0 to 27.4 ± 1.1 mmHg (ΔPsf 10.6 ± 1.3 mmHg) in A1R+/- mice in response to an increase in tubular flow rate from 0 to 35 nl/min. This response was completely abolished in the homozygous A1R-deficient mice as shown in the original recording obtained from A1R+/+ and A1R−/- mice (Fig. 1). Mean Psf in the A1R−/- mice was 39.1 ± 4.1 mmHg at 0 perfusion and 39.2 ± 4.5 mmHg (ΔPsf 1.0 ± 0.8 mmHg; P < 0.001 vs. +/+; +/−) when the perfusion rate was increased to 35 nl/min.

Table 3. Tubuloglomerular feedback characteristics in wild-type, heterozygote, and knockout A1 R mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MAP, mmHg</th>
<th>Psf, mmHg</th>
<th>Psf = 0, mmHg</th>
<th>Psf = 35, mmHg</th>
<th>ΔPsf, mmHg</th>
<th>Mice/Tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1R+/+</td>
<td>92 ± 3</td>
<td>13.2 ± 1.6</td>
<td>36.7 ± 1.2</td>
<td>25.3 ± 1.6</td>
<td>11.4 ± 1.1</td>
<td>5/7</td>
</tr>
<tr>
<td>A1R+/-</td>
<td>90 ± 3</td>
<td>14.7 ± 1.3</td>
<td>38.1 ± 1.0</td>
<td>27.4 ± 1.1</td>
<td>10.6 ± 1.3</td>
<td>3/6</td>
</tr>
<tr>
<td>A1R−/-</td>
<td>102 ± 1*</td>
<td>14.5 ± 2.0</td>
<td>39.1 ± 4.1</td>
<td>39.2 ± 4.5</td>
<td>1.0 ± 0.8*</td>
<td>4/7</td>
</tr>
</tbody>
</table>

Values are given as means ± SE. Psf, free-flow pressure; Psf = 0, proximal tubular stop-flow pressure; Psf = 35, proximal tubular stop-flow pressure during 35 nl/min perfusion; ΔPsf, maximal proximal stop-flow pressure response. *P < 0.001 vs. +/+ and +/−.

Figure 1. Original recordings of the proximal tubuli stop-flow pressures (Psf) during 0 and 35 nl/min (solid bars) in the adenosine A1 receptor wild-type (A1R+/+; A) and knockout mice (A1R−/-; B).
lation but also from adenosine $A_{2A}$ receptors (29). Adenosine $A_{2A}$ receptor (and possibly also $A_{2B}$ receptor) stimulation gives rise to vasodilatation (Tang et al., Ref. 32). In the afferent arterioles, the $A_1$R predominates, which produces a vasoconstriction upon activation from adenosine (32). The administration of N$^\circ$-cyclopentyladenosine, a selective $A_1$R agonist, demonstrated that adenosine-mediated reduction of cortical and medullary blood flow was mediated by the $A_1$R (1). A recent publication by Nishiyama et al. (20) concludes that adenosine $A_{2A}$ receptor-mediated vasodilatation partly buffers adenosine-induced vasoconstriction in both pre- and postglomerular segments of the renal microvasculature. Studies in the isolated afferent arterioles have shown that adenosine constricts the vessels, and this constriction is most pronounced in the distal region of the afferent arteriole, closest to the glomerulus (13, 36). It is known that within this region, the TGF-activated contraction will occur (19). Even in humans, administration of adenosine has been shown to reduce GFR without affecting the systemic blood pressure (2).

The TGF mechanism operates by sensing the distal load to the macula densa cells and adjusting the tone of the afferent arteriole and the rate of renin release. The sensing step of the load involves the detection of the NaCl concentration via an Na-K-2Cl cotransport mechanism (25). The increase in electrolyte load may elevate the metabolic rate and the consumption of ATP and generation of adenosine as described above. The results from the present paper clearly show the absence of a TGF response, with no detectable drop in $P_{\text{sf}}$ after increased distal delivery of fluid. This finding is entirely in line with the original suggestion made by Osswald and associates (22), where they see adenosine as a mediator of the TGF mechanism rather than as a modulator. From a modulator one would not expect a total absence of response. Earlier studies by Schermsmann (26) showed that the luminal administration of a $A_1$R agonist increased the TGF response, and, in another study, he and coworkers (28) found that inhibitors of $A_1$Rs impaired the TGF response measured with $P_{\text{sf}}$ technique in rats. It could be argued that the lack of a difference in the TGF response between the $A_1R^{+/+}$ and the $A_1R^{+/-}$ indicates that adenosine has a permissive role on the TGF instead of acting as a mediator of TGF. However, the connection between agonist concentration, receptor number, and response is often complex and nonlinear (14). That there is no difference in the TGF response between the $A_1R^{+/+}$ and the $A_1R^{+/-}$ could be due to a surplus of receptors, spare receptors, in the wild-type mice.

Weihprecht and coworkers (36) showed a synergism between the vasoconstrictive action of adenosine with that by ANG II, as shown earlier for other vasoconstrictor stimuli (8). Another indication of the functional interactions between ANG II and adenosine was described by Traynor et al. (34), who found a marked reduced constrictor response of $A_1$R agonist N$^\circ$-cyclohexyladenosine in ANG II type 1A receptor knockout mice. In the present study inhibition of the ANG II AT1 receptor with candesartan gave rise to a similar degree of reduction in blood pressure and GFR in the $A_1$R knockout animals compared with their controls. The absence of a synergistic effect between the ANG II and adenosine might be explained by the increased plasma renin concentration that could give rise to an increased production of ANG II, which in turn would give a similar reduction in GFR after the administration of candesartan. The increase in plasma renin concentration found in the present paper in the $A_1$R knockout mice is also completely in line with earlier findings using nonelective adenosine receptor antagonists (22). Studies by Itoh (11) suggested that adenosine was released from the macula densa cells in response to increased distal delivery of fluid and that the adenosine, via the activation of the $A_1$R, decreased renin release. In contrast, activation of $A_2$ receptors might stimulate renin release (18). More recently Lorenz et al. (16) found an inhibitory effect of adenosine in the macula densa-mediated renin secretion in isolated perfused juxtaglomerular apparatus from rabbit kidneys. Thus from the present investigation and earlier studies we conclude that there is an important inhibition on renin release through activation of the $A_1$R.

In the present study there are no significant differences in GFR in the $A_1$R knockout mice compared with their controls, indicating no major changes in the glomerular filtration process. This would indicate that other systems for microvascular control have taken over and regulate glomerular filtration pressure and GFR to the control level. The present paper also demonstrates a modest but significant blood pressure increase of $\pm 10$ mmHg in the $A_1$R knockout mice.

![Fig. 2. Plasma renin concentrations (PRC) in adenosine $A_1$ receptor wild-type (+/+; n = 6), heterozygote (+/-; n = 5), and knockout (-/-; n = 5) mice. *P < 0.0005 vs. +/+ and +/− mice. GU, Goldblatt units.](http://ajpregu.physiology.org/doi/pdf/10.1152/ajpregu.00005.2001)
compared with the wild-type mice. One possible explanation for this increase in blood pressure could be that the increase in renin generates an elevation in ANG II levels, which in turn could raise blood pressure. Therefore, studies were performed to see if the administration of candesartan reduced blood pressure or GFR differently in the different genotypes. We found that on administration of candesartan, MAP was reduced in all the different genotypes to approximately the same extent. Thus an increase in ANG II does not seem to be directly responsible for the increase in MAP. The increase might be mediated by release of other salt balance hormones, such as aldosterone, or possibly dependent on an increase in sympathetic tone. An increase in tone might depend on impaired A1R stimulation on the presynaptic neurons (3, 8).

The present results are also of importance in relation to the human use of caffeine, consumed by more than 80% of the adult population in western countries (6). During normal consumption, levels reached are able to bind to approximately half the A1 receptors. We earlier showed that in A1R+/- mice, receptor number is decreased to half and dose-response curves to adenosine are shifted to the right (12). Hence, this mouse may be a model of long-term caffeine use. It is therefore interesting to note that we found a small increase in MAP in +/− mice, which is similar to that seen in humans consuming caffeine (10). There was also a marked increase in Na+ excretion, which agrees with known effects of caffeine. By contrast, there were no changes in TGF or in plasma renin levels, indicating that long-term caffeine ingestion does not alter these functions. This also shows that Na+ excretion and TGF are independently regulated by adenosine A1 receptors.

To conclude, the present findings, with a completely blocked TGF mechanism in the A1R knockout mice, demonstrate that adenosine is an important mediator of the TGF response. The increase in plasma renin concentration also demonstrates that adenosine has important inhibitory functions in the mechanisms that release renin from the juxtaglomerular apparatus.

Perspectives

Adenosine has multiple effects both in metabolic control and in control of vascular tone. The A1R has been shown to constrain afferent arterioles (36), contract mesangial cells in the glomerulus (21), inhibit renin release, and be involved in responsiveness of the TGF system (23). In this study we demonstrated that adenosine, via the A1 receptor, mediates the TGF and is involved in the control of the renin release. Consequently, A1R is important in controlling the regulation of afferent arteriolar tone and in extracellular fluid volume balance. It will be interesting to study this more closely in the A1R knockout mice, especially during different salt loading situations to challenge the TGF- and renin-angiotensin system.

This study was supported financially by the Swedish Medical Research Council 14X-03522, 14X-02553, and 14X-12587; Danish Medical Research Council 9902742; the Wallenberg Foundation; Ingabritt and Arne Lundberg Foundation; European Commission (EURCAR); the Swedish Society of Medicine; and the Johansson, Thuring and Wiberg Foundations.

REFERENCES