Cellular localization of adenine receptors in the rat kidney and their functional significance in the inner medullary collecting duct

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Am J Physiol Renal Physiol 305: F1298–F1305, 2013. First published August 28, 2013; doi:10.1152/ajprenal.00254.2013.—The G1-coupled adenine receptor (AdeR) binds adenine with high affinity and potentially reduces cellular cAMP levels. Since cAMP is an important second messenger in the renal transport of water and solutes, we localized AdeR in the rat kidney. Real-time RT-PCR showed higher relative expression of AdeR mRNA in the cortex and outer medulla compared with the inner medulla. Immunoblots using a peptide-derived and affinity-purified rabbit polyclonal antibody specific for an 18-amino acid COOH-terminal sequence of rat AdeR, which we generated, detected two bands between ~30 and 40 kDa (molecular mass of native protein: 37 kDa) in the cortex, outer medulla, and inner medulla. These bands were ablated by preadsorption of the antibody with the immunizing peptide. Immunofluorescence labeling showed expression of AdeR protein in all regions of the kidney. Immunoperoxidase revealed strong labeling of AdeR protein in the cortical vasculature, including the glomerular arterioles, and less intense labeling in the cells of the collecting duct system. Confocal immunofluorescence imaging colocalized AdeR with aquaporin-2 protein to the apical plasma membrane in the collecting duct. Functionally, adenine (10 µM) significantly decreased (P < 0.01) 1-deamino-8-D-arginine vasopressin (10 nM)-induced cAMP production in membrane preparations of native tissues and cell lines in high density cultures. AdeR, tentatively designated as a P0 (zero) receptor, is a purine receptor coupled to Gi, and, hence, it potentially inhibits adenyl cyclase, thus lowering intracellular cAMP levels. Accordingly, Müller and associates (10) showed that adenine dose dependently inhibited forskolin-stimulated adenyl cyclase activity and cAMP production in membrane preparations of NG108–15 cells as well as in intact cells, thus establishing the functionality of this receptor in native cells. They also established the rank order of potency of selected ligands for this receptor as adenine > 2-fluoroadenine > 7-methyladenine > 1-methyladenine >> N6-dimethyladenine (33a) and studied the interaction of a variety of synthetic adenine derivatives with AdeRs in cultured neuronal (1321N1 astrocytoma) or human embryonic kidney-293 cells (3). Furthermore, in these studies, neither the adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine nor the P2 recep-
tor antagonist suramin could change the inhibitory effect of adenine on isoproterenol-induced cAMP formation in human 1321N1 astrocytoma cells stably expressing mouse AdeRs (33a). Using site-directed mutagenesis, the same group (20) showed that two amino acid residues (Asn^{194} and Leu^{201}) are crucial for the activation of rat AdeRs (20). Finally, they recently cloned and characterized a new adenine receptor in the mouse, designated Ade1R, as opposed to the previously described mouse AdeR, Ade2R (31).

Important functions of the kidney, such as the transport of water and Na\(^+\) across the tubular cells, are mediated through cAMP or Ca\(^{2+}\) signaling. Apart from circulating hormones, such as vasopressin and aldosterone, these signaling pathways are known to be modulated by autocrine/paracrine agents, such as extracellular ATP, endothelins, and prostaglandins (PGE\(_2\)) through their respective G protein-coupled receptors. Our laboratories have long-standing interest in the autocrine/paracrine regulation of the function of the distal nephron (3, 9, 17, 19, 20).

Rat AdeR sequence used to synthesize the immunizing peptide compared with the corresponding peptide sequences previously reported standard methods (13, 16), and the antibody generation was outsourced to Invitrogen. The 19-amino acid peptide sequence CGSFHRQKHTQLMVIQRA (with an added NH\(_2\)-terminal cysteine) designed by us corresponds to 293- to 310-amino acid residues of the Rat musculus G protein-coupled receptor (rc 56.1.3 gene, GenBank Accession No. AJ311952.1) published by Bender et al. (1). This peptide sequence has 85% homology with the corresponding peptide sequence of the Mus musculus G protein-coupled AdeR (mAd2R, GenBank Accession No. DQ386867) published by von Kügelgen et al. (33a) or 78% homology with mAd1R (GenBank Accession No. JN662396) (Table 2). After solid-phase synthesis, the peptide was purified by HPLC. The purified peptide was conjugated to keyhole limpet hemocyanin through the NH\(_2\)-terminal cysteine. Using standard protocol of Freund’s complete and incomplete adjuvant, two rabbits were immunized with the peptide-keyhole limpet hemocyanin conjugate. Both rabbits developed antibody titers of >1:25,000, as determined by ELISA using microplates coated with the immunizing peptide. Antisera were affinity purified on an agarose bead column containing immunobilized immunizing peptide (Pierce Sulfolink Immobilization Kit, Thermo Scientific, Rockford, IL). IgG fractions of the preimmune sera were prepared using Pierce protein A agarose columns (Thermo Scientific) for use in control experiments.

### Table 1. Nucleotide sequences of the primer pairs used in real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession No.</th>
<th>Primer Position</th>
<th>Primer Sequence</th>
<th>Amplicon Size, bp</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat AdeR</td>
<td>AJ_311952.1</td>
<td>Nucleotides 639–660</td>
<td>5’-GCGCTCTGCTGCTAGATCGT-3’</td>
<td>62</td>
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<td>Nucleotides 680–700</td>
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<td>78</td>
<td>Ref. 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleotides 18–37</td>
<td>5’-CAACGGGAGTACAACCTCC-3’</td>
<td>207</td>
<td>Ref. 36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleotides 205–224</td>
<td>5’-CGGATACCCGACATCACC-3’</td>
<td>207</td>
<td>Ref. 36</td>
</tr>
</tbody>
</table>

### Table 2. Rat AdeR sequence used to synthesize the immunizing peptide compared with the corresponding peptide sequences of the two types of mouse AdeRs

<table>
<thead>
<tr>
<th>Position of Amino Acid Residues</th>
<th>Amino Acid Residues</th>
<th>Position of Amino Acid Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat AdeR</td>
<td>G S F R H Q* K* H Q T L K M V L* Q R* A</td>
<td>310</td>
</tr>
<tr>
<td>Mouse Ade1R</td>
<td>G S F R H H R L* Q* H Q T L K M V L* Q S* A</td>
<td>311</td>
</tr>
<tr>
<td>Mouse Ade2R</td>
<td>G S F R H R L* K* H Q T L K M V L* Q S* A</td>
<td>311</td>
</tr>
</tbody>
</table>

*Amino acid residues that do not match.
AdeR EXPRESSION IN THE RAT KIDNEY

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Synthesis and characterization of PSB-08162. PSB-08162 [3-methylamino-N-(9H-purin-6-yl) propanamide dihydrochloride], a selective antagonist of AdeR, was synthesized, purified, and characterized in the laboratory of C. Müller at the University of Bonn (Bonn, Germany). The compound was validated for its selective antagonism toward rat AdeR stably transfected in 1321N1 astrocytoma cells. Details of the synthesis and characterization of PSB-08162, which constitute a doctoral thesis work, will be published elsewhere.

Detection of AdeR protein. The immunoblot approach established in our laboratory was used for the detection of AdeR protein in the brain and kidney (36, 39, 40). Briefly, the rat brain and kidney cortex, outer medullary, and inner medullary tissue samples were homogenized in a buffer containing protease inhibitors, and protein contents of the homogenates were determined and then solubilized in Laemmli sample buffer. Proteins in the solubilized samples were size fractionated by electrophoresis on 12% polyacrylamide gels under denaturing conditions. Proteins in the gel were electrotransferred to nitrocellulose membranes. After being blocked with 2% goat serum (Jackson ImmunoResearch Labs, West Grove, PA) in blocking buffer, membranes were incubated with affinity-purified AdeR antibody (1:500 dilution) or the antibody preadsorbed with a molar excess (1:2) of the immunizing peptide overnight at 4°C. After washoff of the primary antibody, membranes were incubated with peroxidase-conjugated secondary antibody to rabbit IgG (Dako North America, Carpinteria, CA). Sites of antigen-antibody reaction were detected by a chemiluminescence reaction and captured on X-ray film.

Localization of AdeR protein by immunoperoxidase labeling. Localization of AdeR protein by immunoperoxidase labeling was carried out as previously described with a few modifications (14, 15). Briefly, rat kidney samples were fixed in 10% neutral buffered formalin for 2 days and then embedded in paraffin. After deparaffinization, thin sections (5 µm) were either stained with hematoxylin-eosin for conventional histology or processed for immunoperoxidase labeling. For immunoperoxidase labeling, antigen retrieval was achieved by incubating sections with 1% SDS in PBS (pH 7.4) for 5 min at room temperature. Sections were then washed with PBS and treated for the removal of endogenous peroxidase activity. Afterward, sections were blocked for 1 h at room temperature in blocking buffer (5% BSA and 0.5% Tween 20 in PBS). Sections were then incubated at 4°C overnight with affinity-purified rabbit polyclonal antibody against AdeR in blocking buffer at a dilution of 1:50. To establish the specificity of the AdeR antibody, control kidney sections were incubated in parallel with preimmune IgG (4 µg/ml). After washoff of the primary antibody, sections were incubated with ABC reagent (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA) for 30 min at room temperature followed by color development with 3,3’-diaminobenzidine reagent (Vector Laboratories). After a wash, sections were counterstained with hematoxylin, dehydrated, and mounted with Permount under coverslips. Processed tissue sections were examined under a Reichert microscope, and digital pictures were taken with a Nikon 995 Coolpix camera.

Localization of AdeR protein by confocal immunofluorescence. Localization of AdeR protein by confocal immunofluorescence was performed as previously described (11, 12). Kidneys were fixed in situ by perfusion of 4% paraformaldehyde, and tissue blocks containing all organ regions were then placed in the same fixative overnight at 4°C. Tissue blocks were embedded in paraffin, and 4-µm sections were cut, deparaffinized in toluene, and rehydrated through xylene and graded ethanol. To retrieve antigens, slides were heated for 2 × 10 min in a microwave with medium heat in PBS and allowed to cool for 40 min. Sections were then fixed with 4% paraformaldehyde for 10 min and permeabilized for 10 min with 0.1% Triton X-100 in PBS. Goat serum (1:20 dilution, Jackson ImmunoResearch Labs) in PBS was applied to sections for 1 h to block nonspecific binding. Sections were then probed with AdeR rabbit polyclonal antibody to a dilution of 1:100 overnight followed by an incubation with secondary Alexa fluor 594-conjugated goat anti-rabbit antibody (Invitrogen) at 1:500 dilution for 1 h. Some sections were double labeled first by an incubation with an aquaporin-2 (AQP2) mouse monoclonal antibody (1:500) followed by an incubation for 1 h with goat anti-mouse secondary Alexa fluor 488-conjugated antibody at a dilution of 1:500. After a final wash step, all sections were mounted with Vectashield mounting media containing the nuclear stain 4’,6-diamidino-2-phenylindole (Vector Laboratories) and examined with a Leica TCS SP5 confocal microscope.

Effect of adenine on 1-deamino-8-arginine vasopressin-stimulated cAMP generation in the rat inner medullary collecting duct. The effects of adenine on 1-deamino-8-arginine vasopressin (dDAVP; desmopressin)-stimulated cAMP generation, and the specific involvement of AdeR in it, were determined in freshly prepared fractions enriched in rat inner medullary collecting ducts (IMCD) by the methods previously established in our laboratories (36, 39, 40). Briefly, inner medullas were minced and digested with collagenase and hyaluronidase to obtain small fragments of the IMCD. IMCD fragments in the digest were isolated from the rest of the non-IMCD elements by low-speed centrifugation and washes, aliquoted into microtubes, and then processed for incubation with test substances. All incubations were performed in the presence of 0.5 mM IBMX to inhibit phosphodiesterases. Aliquots of the fractions enriched in IMCD, after being warmed to 37°C, were incubated for 10 min with or without the addition of 10 µM adenine (Sigma Chemical, St. Louis, MO). This was followed by the addition of 10 nM dDAVP (Sigma) to some aliquots, and the incubation was continued for another 20 min at 37°C. PSB-08162 was first dissolved in DMSO and diluted in incubation buffer. PSB-08162 was added to respective aliquots of the IMCD fractions at a final concentration of 20 µM just before fractions were warmed to 37°C. The reaction was stopped by adding chilled 0.1 N HCl. IMCD fragments were pelleted by centrifugation, and cAMP levels in the pellets were quantified by a CoomasiePlus Protein Assay Reagent Kit (Pierce Biotechnology). Measured cAMP levels in the samples were normalized to the protein contents of the respective pellets.

RESULTS

Relative expression of AdeR mRNA in the rat kidney. Using gene-specific primers for rat AdeR, we determined the relative mRNA expression of AdeR in different regions of the rat kidney. Real-time RT-PCR showed expression of AdeR mRNA in all regions of the kidney. However, expression levels were relatively higher in the deep cortical region and outer medulla and lowest in the inner medullary tip. The superficial cortex and base of the inner medulla showed modestly higher levels of AdeR mRNA (Fig. 1).

Characterization of AdeR antibody. To study the expression and intrarenal localization of AdeR protein, affinity-purified rabbit polyclonal antibody specific for an 18-amino acid COOH-terminal sequence of rat AdeR was generated. Immunoblot analysis detected two bands between ~30 and 40 kDa (molecular mass of native protein: 37 kDa) in samples of the rat kidney cortex, outer medulla, and inner medulla and in control brain tissue, which were ablated by preadsorption of the antibody with the immunizing peptide (Fig. 2A). To further confirm antibody specificity, immunoperoxidase labeling of rat kidney sections was performed with or without the AdeR antibody. Intense labeling was found in blood vessels in the rat renal cortex with the affinity-purified rabbit polyclonal AdeR antibody, but there was a lack of labeling in parallel-run kidney sections when the preimmune IgG fraction was used (Fig. 2B).
Expression and immunolocalization of AdeR protein in the rat kidney. By immunofluorescence labeling, we found high levels of expression of AdeR protein in all major regions of the kidney (Fig. 3). The labeling appeared to be localized in tubular as well as vascular structures. In most kidney regions except the tip of the papilla, AdeR protein expression (Fig. 3) was consistent with the RT-PCR data shown in Fig. 1. The predominant expression of AdeR protein in the vasculature and collecting duct system was confirmed by immunoperoxidase labeling. As shown in Fig. 4, A and B, the muscular layers of small- and medium-sized arteries were intensely labeled. In addition, there was clear labeling of glomerular arterioles (Fig. 4B). However, glomerular cells were not la-
beled for AdeR. Weak labeling was found in the proximal tubules. Interestingly, weak but clear labeling was seen over clusters of peritubular interstitial cells in the cortex (Fig. 4E).

In the medulla, there was weak and diffuse labeling of the thick ascending limbs (not shown here). On the other hand, AdeR immunolabeling was clear and continuous along the tubular epithelium in the medullary collecting duct (Fig. 4, C and D). The labeling was predominant at the apical aspects, although diffuse intracellular labeling was seen in the collecting duct cells. Medullary interstitial cells were not labeled for AdeR protein (Fig. 4F).

To further explore the nature of AdeR-expressing cells in the medullary collecting duct and to study whether AdeR is expressed along with AQP2 protein in cells, we performed double-labeling immunofluorescence of AdeR and AQP2. AdeR immunofluorescence was seen all over medullary collecting duct cells (Fig. 5A, red color), similar to AQP2 immunofluorescence (Fig. 5B, green color). Overlay of the AdeR and AQP2 immunofluorescence profiles clearly showed colocalization of these two proteins in medullary collecting duct cells and revealed apical cell membrane colocalization of AdeR with AQP2 (Fig. 5C, yellow color).

Effect of adenine on dDAVP-stimulated cAMP production in the rat IMCD. Since double-immunofluorescence labeling revealed the colocalization of AdeR and AQP2 protein along the apical membrane of medullary collecting duct cells, we further
explored the functional relevance of renal AdeR expression in IMCD cells. For this, we tested the effect of adenine on dDAVP-induced production of cAMP in ex vivo preparations of the IMCD as well as its blockade by a selective antagonist of AdeR. As shown in Fig. 6, the addition of dDAVP (10 nM) to rat IMCD preparations caused a 2.4-fold increase in cAMP levels (vehicle control: 0.78 ± 0.10 pmol/μg protein vs. dDAVP: 1.91 ± 0.51 pmol/μg protein, P < 0.001), which was significantly reduced by coincubation of dDAVP with 10 μM adenine (0.61 ± 0.21 pmol/μg protein). Thus, these data indicate that adenine is a potent inhibitor of dDAVP-induced cAMP production in IMCD cells. To further explore whether the effect of adenine on dDAVP-induced cAMP production in the IMCD is mediated via AdeR or not, we used PSB-08162, a selective antagonist of AdeR, which was synthesized and characterized by C. Müller and associates. To ensure robust inhibition, in our incubations we used a twofold molar excess of PSB-08162 (20 μM) relative to the adenine concentration (10 μM). As shown in Fig. 6, PSB-08162 significantly (P < 0.01) reversed the inhibitory effect of adenine on dDAVP-induced cAMP production in the IMCD. PSB-08162 alone did not have any effect on basal or dDAVP-induced cAMP levels in the IMCD. These data indicate that the inhibitory effect of adenine is mediated via AdeR and not independent of AdeR, thus establishing the functionality of AdeR in the IMCD.

DISCUSSION

This study describes the expression, cellular localization, and functional significance of AdeRs in the rat kidney. Using gene-specific primers and a peptide-derived polyclonal antibody, we showed that AdeR mRNA and protein are highly expressed in the rat kidney. The predominant AdeR immunolabeling was found in the cortical vasculature and in cells of the collecting duct system. Both medium and small arteries as well as glomerular arterioles were labeled in the cortex. In the collecting duct, AdeR was colocalized to the apical plasma membrane along with AQP2 protein. In parallel, we showed that adenine significantly reduced dDAVP-induced cAMP production in ex vivo preparation of the rat IMCD, and this effect was specifically mediated via AdeR. These findings suggest that adenine may have potential autocrine/paracrine regulatory roles in the renal vasculature and collecting duct. Thus, this study opens a new avenue for exploration of the autocrine/paracrine regulation of renal tubular and vascular functions by the nucleobase adenine. We discuss the significance of our findings in relation to renal physiology or pathophysiology below.

In mammalian systems, adenine in the extracellular milieu is not derived from the degradation of adenine-containing nucleotides. Most cells can synthesize and recycle adenine, which makes adenine a potential autocrine or paracrine agent. Our immunolocalization experiments showed strong labeling for AdeR in the collecting duct system, colocalized with AQP2 protein. This indicates that AdeR is expressed in principal cells of the collecting duct, although its expression in other cells,
such as intercalated cells, was seen in immunofluorescence. Since the cAMP signaling pathway is crucial for the function of collecting ducts, our data suggest that extracellular adenine may play potentially significant autocrine/paracrine regulatory roles in water and/or acid/base transport in the collecting duct. This notion is supported by our findings showing that adenine significantly inhibited dDAVP-stimulated cAMP generation in ex vivo preparations of the IMCD, and this action was specifically mediated via AdeR.

In healthy humans, the plasma concentration of adenine is ~70 nM. However, in patients with chronic renal failure (CRF), plasma adenine levels are elevated to higher than 1 µM (29). The high plasma adenine levels in patients with CRF are directly correlated with the severity of kidney failure, as assessed by serum creatinine concentrations. Interestingly, plasma adenine concentrations in patients with CRF also directly correlated with erythrocyte ATP concentrations and the adenine incorporation rate into the erythrocyte adenine nucleotide pool. Based on these findings, it has been suggested that elevated plasma adenine concentrations, in addition to the accelerated incorporation into the erythrocyte adenine nucleotide pool, may play an important role in the raised ATP concentration in uremic patients (29). A significant reduction in both plasma adenine and erythrocyte ATP was observed immediately after hemodialysis, but 2 days later, the high predialysis plasma adenine and erythrocyte ATP concentrations were restored (29). However, after successful renal transplantation, erythrocyte ATP and plasma adenine concentrations reached control values (29). Interestingly, the ability of blood adenine to increase the erythrocyte ATP concentration is the basis for the inclusion of significant amounts of adenine in CPD-1 blood preservative for the long-term storage of human blood for transfusion (24, 28).

These observations in patients with CRF underscore the importance of adenine and AdeR and the significance of our study. Although the physiological effects of circulating adenine in the kidneys of healthy humans is not known, it is conceivable that elevated blood concentrations of adenine in conditions such as CRF may have significant effects mediated through the recently characterized AdeR depending on the sites of its intrarenal expression. Recently, it has been reported that AdeRs are expressed in primary rat hepatic stellate cells (HSC) as well as T-6 cells, an immortalized rat HSC cell line, and these cells undergo functional changes in response to adenine (34). The adenine-induced functional changes in HSCs are actin reorganization, inhibition of the inositol 1,4,5-trisphosphate-mediated increase in cytosolic Ca²⁺, inhibition of chemotaxis, and upregulation of α-smooth muscle actin and collagenase type I (34). Thus, this report suggests that adenine has a potential signaling role in cellular differentiation. Future studies should investigate whether adenine has a similar signaling role in cellular differentiation in the kidney, especially in conditions such as CRF.

Although in our study on the rat kidney we could not find the expression of AdeR protein in proximal tubular cells, it is possible that AdeR might be expressed in proximal tubular cells of other species. This notion is supported by a report (35) showing that adenine inhibited Na⁺-ATPase in isolated basolateral membranes of proximal tubular cells of the pig kidney via Gt-coupled inhibition of the cAMP signaling pathway. Although bulk absorption of Na⁺ in the proximal tubule is mainly driven by ouabain-sensitive Na⁺-K⁺-ATPase or the Na⁺ pump, however, ouabain-insensitive and furosemide-inhibitable Na⁺-ATPase, often called the second Na⁺ pump, may be involved in fine tuning the Na⁺ reabsorption in the proximal tubule (for a review, see Ref. 26). Experimental evidence suggests that Na⁺-ATPase is a target for angiotensins and that it is increased in the kidneys of spontaneously hypertensive rats, without alterations in the expression of Na⁺-K⁺-ATPase (6, 26). In view of these reports, future studies should investigate the potential role of AdeR on Na⁺ reabsorption in the kidney and intestines of different species.

In conclusion, we localized the AdeR in the rat kidney and showed its functional role in IMCD cells. Based on its cellular localization, the AdeR may play important functional roles in renal hemodynamics and salt and water transport. Thus, this work opens a new avenue to study the role of adenine in renal physiology and pathophysiology based on the precise localization of AdeR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


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