Expression of adenosine receptors in the preglomerular microcirculation

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Jackson, Edwin K., Chongxue Zhu, and Stevan P. Tofovic. Expression of adenosine receptors in the preglomerular microcirculation. Am J Physiol Renal Physiol 283: F41–F51, 2002; 10.1152/ajprenal.00232.2001.—The purpose of this study was to systematically investigate the abundance of each of the adenosine receptor subtypes in the preglomerular microcirculation vs. other vascular segments and vs. the renal cortex and medulla. Rat preglomerular microvessels (PGMVs) were isolated by iron oxide loading followed by magnetic separation. For comparison, mesenteric microvessels, segments of the aorta (thoracic, middle abdominal, and lower abdominal), renal cortex, and renal medulla were obtained by dissection. Adenosine receptor protein and mRNA expression were examined by Western blotting, Northern blotting, and RT-PCR. Our results indicate that compared with other vascular segments and renal tissues, A1 and A2B receptor protein and mRNA are abundantly expressed in the preglomerular microcirculation, whereas A2A and A3 receptors protein and mRNA are barely detectable or undetectable in PGMVs. We conclude that, relative to other vascular and renal tissues, A1 and A2B receptors are well expressed in PGMVs, whereas A2A and A3 receptors are notably deficient. Thus A1 and A2B receptors, but not A2A or A3 receptors, may importantly regulate the preglomerular microcirculation.

in these processes in vascular smooth muscle cells from conduit arteries (5, 7).

Despite the potential physiological importance of adenosine receptors in the preglomerular microcirculation, there are no published studies characterizing the abundance of the four known adenosine receptor subtypes in the preglomerular microcirculation. As summarized in Table 1, adenosine receptors in the kidney have been investigated in at least 14 different studies employing a variety of methods, including ligand binding in isolated membranes, autoradiography, Northern blotting, in situ hybridization, RT-PCR, immunocytochemistry and Western blotting. However, none of these studies defined the relative expression of adenosine receptors in the preglomerular microcirculation.

Given the probable physiological importance of adenosine receptors in the preglomerular microcirculation and the recently accelerated efforts to develop specific and potent adenosine-receptor agonists and antagonists for the treatment of renal diseases (23, 24) and diuretic (10), we felt that it was critical to investigate carefully and thoroughly the expression of adenosine receptor subtypes in the preglomerular microcirculation. To accomplish this objective, we isolated renal preglomerular microvessels (PGMVs) using iron oxide loading with magnetic separation and measured the expression of A1, A2A, A2B, and A3 receptor protein using Western blotting with quantitative densitometry. We also measured adenosine receptor mRNA expression with Northern blotting and/or RT-PCR. As a reference, we calibrated the quantities of the various adenosine receptor subtypes in the preglomerular microcirculation to the expression of the receptors in the renal cortex, renal medulla, mesenteric microvessels, and various segments of the aorta (thoracic, middle abdominal, and lower abdominal).

METHODS

Isolation of PGMVs. Adult male Wistar rats were obtained from Taconic Farms (Germantown, NY), housed at the University of Pittsburgh Animal Facility, and fed Prolab RMH 3000 (PMI Feeds, St. Louis, MO) containing 0.26% sodium
Table 1. Summary of studies that investigated the expression of adenosine receptors in the kidney

<table>
<thead>
<tr>
<th>Reference/(No.)</th>
<th>Method</th>
<th>Tissue</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu and Churchill (43)</td>
<td>Ligand binding using DPCPX</td>
<td>Rat kidney membranes</td>
<td>Detected A2 binding</td>
</tr>
<tr>
<td>Freissmuth et al. (9)</td>
<td>Ligand binding using HPIA</td>
<td>Rabbit cortical glomeruli, microvessels, and tubules purified by sucrose gradient</td>
<td>Detected A1 binding in glomeruli and microvessels</td>
</tr>
<tr>
<td>Weber et al. (40)</td>
<td>Autoradiography using HPIA</td>
<td>Guinea pig kidney</td>
<td>Detected A1 binding in renal medulla</td>
</tr>
<tr>
<td>Weaver and Reppert (39)</td>
<td>Northern blot using HPIA</td>
<td>Pig proximal tubular BBMs and BLMs</td>
<td>No A1 binding in BBMs or BLMs; binding in BLMs that was A3-like</td>
</tr>
<tr>
<td>Blanco et al. (2)</td>
<td>Ligand binding using NECA and CGS-21680</td>
<td>Human glomeruli</td>
<td>Detected A1 binding</td>
</tr>
<tr>
<td>Toya et al. (37)</td>
<td>Ligand binding using CCPA</td>
<td>Microdissected rat nephron segments</td>
<td>Detected A1 mRNA in glomeruli, collecting duct, and thick ascending limb with weak signals in proximal tubules</td>
</tr>
<tr>
<td>Yamaguchi et al. (44)</td>
<td>RT-PCR</td>
<td>Microdissected rat outer medullary descending vasa recta</td>
<td></td>
</tr>
<tr>
<td>Kreisberg et al. (15)</td>
<td>RT-PCR</td>
<td>Pig proximal tubular BBMs and BLMs</td>
<td>No A1 binding in BBMs or BLMs; binding in BLMs that was A3-like</td>
</tr>
<tr>
<td>Gould et al. (11)</td>
<td>Ligand binding using DPCPX and CGS-21680</td>
<td>Pig proximal tubular BBMs and BLMs</td>
<td>No A1 binding in BBMs or BLMs; binding in BLMs that was A3-like</td>
</tr>
<tr>
<td>Smith et al. (34)</td>
<td>Autoradiography using DPCPX</td>
<td>Rat kidney membranes</td>
<td>Detected A1, but not A2A, binding</td>
</tr>
<tr>
<td>Halimi et al. (12)</td>
<td>RT-PCR</td>
<td>Rat kidney membranes</td>
<td>Detected A1 mRNA</td>
</tr>
<tr>
<td>Zou et al. (46)</td>
<td>Western blotting</td>
<td>Rat kidney cortical and medullary membranes</td>
<td>Detected A1 mRNA</td>
</tr>
<tr>
<td>Smith et al. (33)</td>
<td>Autoradiography using DPCPX</td>
<td>Rat kidney</td>
<td>Detected A1 binding in glomeruli and medulla</td>
</tr>
<tr>
<td>Smith et al. (32)</td>
<td>Immunocytochemistry</td>
<td>Rat kidney</td>
<td>Detected A1 receptors in afferent arterioles, mesangial cells, proximal tubules, and collecting ducts</td>
</tr>
</tbody>
</table>

HPIA, (4R,6S)-3-(4-hydroxyphenylisopropyl)-adenosine; NECA, 5’-(N-ethylcarboxamido)adenosine; CCPA, 2-chloro-N6-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; BBMs and BLMs, brush-border membranes and basolateral membranes, respectively.

Isolation of other tissues. Microvessels (second- and third-order branches) from the mesenteric vascular bed and renal cortical tissue, renal medullary tissue, and total aorta and segments from the thoracic, midabdominal, and lower abdominal aorta were removed, placed in ice-cold PBS, and dissected from surrounding adipose and connective tissue.

Protein extraction. Immediately after the tissues were obtained, the tissues were frozen in liquid nitrogen and ground into a powder on liquid nitrogen with a mortar and pestle. The ground tissues were placed in a tube with 0.5 ml SDS buffer (50 mM Tris, pH 7.0, 2% SDS, 10% glycerol) containing protease inhibitors (2 μg/ml antipain, 1 μg/ml aprotinin, 2 μg/ml leupeptin, 1 mg/ml phenylmethylsulfonyl fluoride) and homogenized in a tight glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 12,000 rpm at 4°C for 10 min, and the supernatant was recovered. Protein in the supernatant was determined by the copper bicinechonic acid method, and samples were stored at −20°C.

Western blotting. Laemmli buffer was added to samples, and they were placed in boiling water for 5 min and then chilled immediately on ice. Samples (30 μg protein/well) were loaded onto a 7.5–10% acrylamide gel and subjected to SDS-PAGE using the Bio-Rad minigel system. Proteins were then electroblotted onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was blocked with 5% milk for 1 h and incubated for 3 h at room temperature or at 4°C overnight with the first antibody [anti-A1 receptor
antibody (catalogue no. A-268, diluted 1:1,000 in PBS containing 0.5% Tween 20, Sigma); anti-A2A receptor, anti-A2B receptor and anti-A3 receptor antibodies (catalogue nos. AB1559P, AB1589P, and AB1590P, respectively, diluted 1:500 in PBS containing 0.5% Tween 20, Chemicon, Temecula, CA). Membranes were washed three times in PBS containing 0.5% Tween 20 solution and then incubated at room temperature for 1 h with horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody (Amer sham, Arlington Heights, IL) at 1:5,000 dilution. Membranes were exposed to films, and the signals were detected by a Supersignal Substrate kit (Pierce, Rockford, IL).

Extraction of RNA, RT-PCR, preparation of cDNA probes, and Northern blotting. Total RNA was isolated from rat brains using TRIzol reagent solution (GIBCO Life Technologies, Carlsbad, CA), and this material was used to prepare cDNA probes for rat A1, A2A, A2B, and A3 receptor mRNA and for rat β-actin mRNA. By using the primer sequences listed in Table 2, RNA (0.5 μg) was reverse transcribed and amplified using a Titanium One-Step RT-PCR Kit (Clontech, Palo Alto, CA). Each PCR cycle (a total of 30 cycles) consisted of denaturing at 94°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 60 s. RT-PCR products were separated on a 1.2% agarose gel. The RT-PCR products were extracted from the agarose gel and immediately ligated into pCR II vector (Invitrogen, Carlsbad, CA), which was used to transform competent bacteria. Gel electrophoresis of specific restriction enzyme digests (Table 2) of the vector containing the inserted cDNA was consistent with the expected fragmentation pattern. Furthermore, the inserted cDNA in the subcloned plasmids was sequenced to confirm that our procedure did indeed yield the appropriate cDNA probes. For probe preparation, plasmids extracted from transformed bacteria were digested with EcoRI restriction enzyme, and the expected cDNA fragments were harvested from the agarose gel and labeled with 32P using the method of random priming (Roche Diagnostics, Indianapolis, IN).

Total RNA (10 μg) from kidney tissues and vascular tissues was isolated with TRIzol reagent, denatured, and loaded onto a 1.2% agarose-formaldehyde gel. After electrophoresis, RNA was transferred to a nylon membrane and fixed by exposure to ultraviolet light. Membranes were prehybridized for 30 min at 60°C with ExpressHyb hybridization solution (Clontech). Denatured cDNA probe was added to prewarmed hybridization solution to give a final concentration of 1,000,000 counts·min⁻¹·ml⁻¹. Membranes were incubated with the probe solution with continuous rotation at 63°C for 1 h. After incubation with probe solution, membranes were washed three times with solution I (2% standard sodium citrate, 0.05% SDS) at room temperature, and then washed in solution II (0.1% standard sodium citrate, 0.1% SDS) at 50°C. Blots were developed using the PhosphorImage system (Molecular Dynamics, Sunnyvale, CA). Blots then were stripped and rehybridized with β-actin cDNA probe and redeveloped.

Data analysis. For Western blot analysis, band densities were quantitatively measured using Scion-image software. Background signals were obtained in each lane and subtracted from the band densities to correct for the background signal. For Northern blot analysis, band radioactivity was determined using the PhosphorImage system (Molecular Dynamics), and values were normalized to (i.e., divided by) the signal for β-actin. Data were analyzed with a one-factor analysis of variance followed by a Fisher’s least significant difference test if the overall P value from the analysis of variance was significant. The criterion of significance was P < 0.05, and all data are expressed as means ± SE.

RESULTS

Figures 1 and 2 show Western blot analysis and quantitative densitometry results using the anti-A1 receptor antibody. Western blot analysis revealed a single, dense band at ~38 kDa that corresponded closely to the nominal mass of the A1 receptor (36.5 kDa) (16) and to the 38-kDa signal obtained by Rivkees et al. (29) and Cheng et al. (3) using anti-A1 receptor antibodies. All vascular tissues examined (thoracic aorta, middle abdominal aorta, lower abdominal aorta, mesenteric microvessels, and PGMVs) gave a strong 38-kDa signal; however, quantitative densitometry demonstrated that PGMVs expressed significantly (P < 0.05), albeit only modestly, more A1 receptors per milligram protein compared with all other vascular tissues (Fig. 1). Although the three aortic segments expressed similar levels of A1 receptors, the mesenteric microvessels expressed significantly, but only slightly, more A1 receptors than did the middle abdominal and lower abdominal aorta (Fig. 1). As shown in Fig. 2, PGMVs and renal cortical tissue expressed similar numbers of A1 receptors, whereas renal medullary tissue expressed approximately twice the number of A1 receptors per milligram protein compared with either the renal cortex or PGMVs (P < 0.05). The approximate doubling of A1 receptor protein expression in the renal medulla compared with the renal cortex or PGMVs was associated with an approximate doubling of the expression of A1 receptor mRNA in the renal medulla compared with the cortex and PGMVs (Fig. 3). However, unlike receptor protein expression, receptor mRNA expression was similar in the aorta (thoracic +

Table 2. Adenosine receptor PCR primers and cDNA sizes

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Accession No.</th>
<th>Primer</th>
<th>Nucleotides</th>
<th>Sequence 5’–3’</th>
<th>Product Size</th>
<th>Restriction Digest</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>M64299</td>
<td>Forward</td>
<td>118–139</td>
<td>CAGATAGTGACGGCTGAGTCTG</td>
<td>790</td>
<td>BglII</td>
</tr>
<tr>
<td>A2A</td>
<td>L08102</td>
<td>Reverse</td>
<td>907–886</td>
<td>GGTAGATAGGACGCTGAGGAG</td>
<td>615</td>
<td>XbaI</td>
</tr>
<tr>
<td>A2B</td>
<td>M91446</td>
<td>Forward</td>
<td>1197–1218</td>
<td>AGGTCTGCTATGTCCTGAGAG</td>
<td>1281</td>
<td>PsI</td>
</tr>
<tr>
<td>A3</td>
<td>M94152</td>
<td>Reverse</td>
<td>1811–1790</td>
<td>CCCCCTGACTAAGCTGAGTGG</td>
<td>640</td>
<td>HindIII</td>
</tr>
<tr>
<td>β-Actin</td>
<td>M12481</td>
<td>Forward</td>
<td>25–45</td>
<td>GTGCGCGCGCTTAAGCAGCATC</td>
<td>540</td>
<td>BglII</td>
</tr>
</tbody>
</table>

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Fig. 1. Top: Western blot analysis of proteins (30 μg/lane) using an anti-A1 receptor antibody. Samples were from the thoracic aorta (lanes 1–3), middle abdominal aorta (lanes 4–6), lower abdominal aorta (lanes 7–9), mesenteric microvessels (lanes 10–12), and preglomerular microvessels (PGMVs; lanes 13–15) from 3 different rats. Bottom: bands shown in top panel were subjected to quantitative densitometry and analyzed by 1-factor ANOVA followed by Fisher’s least significant difference test. Values are means ± SE (n = 3). MW, molecular weight.

Fig. 2. Top: Western blot analysis of proteins (30 μg/lane) using an anti-A1 receptor antibody. Samples were from the renal medulla (lanes 1–4), renal cortex (lanes 5–8), and PGMVs (lanes 9–12) from 4 different rats. Bottom: bands shown in top panel were subjected to quantitative densitometry and analyzed by 1-factor ANOVA followed by Fisher’s least significant difference test. Values are means ± SE (n = 4).
Middle abdominal + lower abdominal) compared with PGMVs.

Multiple bands were detected when vascular samples were stained with the anti-A2A receptor antibody (Fig. 4). However, a dense, dominant band was detected at ~45 kDa in all of the vascular tissues except the PGMVs in which the 45-kDa band was barely detectable. Most likely, this 45-kDa band represents staining of the A2A receptor because the nominal mass of the A2A receptor is at 44.7 kDa (16) and because a similar band was reported by Rosin et al. (43 kDa) (17) and Piersen et al. (30 kDa) (16). However, the Western blot analysis appeared convincing, and the 52-kDa protein detected in this study corresponded closely to the 50- to 55-kDa protein detected by Puffinbarger et al. (28) using an anti-A2B receptor antibody. Also, preabsorption of the anti-A2B receptor antibody with a blocking peptide to the anti-A2B receptor antibody abolished the 52-kDa signal (data not shown). Most likely, this high-molecular-mass species represents a glycosylated form of the A2B receptor. Importantly, the expression of the A2B receptor was similar among all the vascular tissues examined (Fig. 7) and was similar among renal cortical tissue, renal medullary tissue, and PGMVs (Fig. 8). Northern blot analysis revealed a slightly, albeit significantly (P < 0.05), lower A2B receptor mRNA expression in PGMVs compared with aorta (total), renal medulla, or renal cortex (Fig. 9).

Staining vascular (Fig. 10) or renal (Fig. 11) samples with the anti-A3 receptor antibody gave rise to only one clear-cut band at ~52 kDa, which is more massive than the nominal molecular mass of the A3 receptor (36.2 kDa) (16). However, Western blot analysis appeared convincing, and the 52-kDa protein detected in this study corresponded closely to the 52-kDa protein detected by Zou et al. (46) using an anti-A3 receptor antibody. Most likely, this high-molecular-mass species represents a glycosylated form of the A3 receptor. Importantly, compared with either all other vascular tissues (Fig. 10) or the renal medulla or renal cortex (Fig. 11), PGMVs expressed few A3 receptors. A3 receptor mRNA could not be detected in PGMVs using Northern blotting (data not shown). Moreover, even when mRNA from PGMVs was reverse transcribed and subjected to 30 cycles of amplification by PCR, ethidium bromide staining failed to detect a signal for A3 receptor cDNA (Fig. 6), whereas a clear signal was obtained when renal cortical tissue similarly was subjected to RT-PCR.

DISCUSSION

An important finding of this study is the abundant level of expression of A1 receptors in PGMVs. In this regard, relative to other vascular segments, PGMVs express slightly more A1 receptor protein per milligram total protein, and the expression of A1 receptor protein by PGMVs is similar to that of the renal cortex. It is notable, however, that the most abundant expression of A1 receptor protein was observed in the renal medulla, which expressed approximately twice the level of A1 receptor protein compared with the renal cortex or PGMVs. The similar levels of expression of A3 receptor protein in vascular elements and renal cortex and the higher levels of expression of A1 receptor protein in the renal medulla is consistent with the Northern blot
**Fig. 4.** Top: Western blot analysis of proteins (30 μg/lane) using an anti-A2A receptor antibody. Samples were from the thoracic aorta (lanes 1–3), middle abdominal aorta (lanes 4–6), lower abdominal aorta (lanes 7–9), mesenteric microvessels (lanes 10–12), and PGMVs (lanes 13–15) from 3 different rats. Bottom: bands shown in top panel were subjected to quantitative densitometry and analyzed by 1-factor ANOVA followed by Fisher’s least significant difference test. Values are means ± SE (n = 3).

**Fig. 5.** Top: Western blot analysis of proteins (30 μg/lane) using an anti-A2A receptor antibody. Samples were from the renal medulla (lanes 1–4), renal cortex (lanes 5–8), and PGMVs (lanes 9–12) from 4 different rats. Bottom: bands shown in top panel were subjected to quantitative densitometry and analyzed by 1-factor ANOVA followed by Fisher’s least significant difference test. Values are means ± SE (n = 4).
studies. In this regard, the medulla expressed twice as much A1 receptor mRNA compared with the aorta, cortex, or PGMVs, all of which expressed similar levels of A1 receptor mRNA.

These results imply an important physiological role for A1 receptors in the preglomerular microcirculation. In agreement with this interpretation are the reports that A1-receptor agonists vasoconstrict the PGMVs (31), potentiate angiotensin II-induced changes in renal vascular resistance (42), reduce the glomerular filtration rate (20), and inhibit renin release (21). Also consistent with this inference are the findings that A1-receptor antagonists inhibit tubuloglomerular feedback (31), stimulate renin release (26), and reduce vasoconstriction induced by certain nephrotoxins (14). Finally, recent studies indicate a total lack of tubuloglomerular feedback responses in A1 receptor knockout mice (35).

The role of A1 receptors in other vascular segments, such as the aorta and mesentery, is presently unclear; however, the present results suggest that A1 receptors may play yet-to-be determined physiological roles in other segments of the vascular tree. Our results also imply an important physiological role for A1 receptors in the renal medulla. Indeed, it is conceivable that the lack of effect of A1-receptor antagonists on potassium excretion (14) may be because of blockade of A1 receptors in the collecting tubules of the renal medulla, a site where potassium secretion is under strong regulation.

In contrast to the A1 receptor, the A2A receptor is markedly underexpressed in the preglomerular microcirculation.
circulation. In this regard, A2A receptors are either undetectable or only barely detectable by Western blotting, whereas Western blotting for A2A receptors gives rise to strong signals in other vascular segments, as well as in the renal cortex. This contrast is particularly striking when the levels of expression of A2A receptors in the mesenteric microvessels vs. PGMVs (Fig. 4) and in the renal cortex vs. PGMVs (Fig. 5) are compared. The results of the studies using Western blotting are confirmed by the inability to detect A2A receptor mRNA with either Northern blotting or RT-PCR (Fig. 6). These results imply an unimportant role for A2A receptors in the preglomerular microcirculation. Consistent with this interpretation are the reports that A2A-receptor agonists have little or no effect on the preglomerular microcirculation (1, 41), while strongly dilating efferent arterioles (22) and the vasa recta (1, 45). However, there is evidence that A2A receptors mediate
Fig. 10. Top: Western blot analysis of proteins (30 μg/lane) using an anti-A3 receptor antibody. Samples were from the thoracic aorta (lanes 1–3), middle abdominal aorta (lanes 4–6), lower abdominal aorta (lanes 7–9), mesenteric microvessels (lanes 10–12), and PGMVs (lanes 13–15) from 3 different rats. Bottom: bands shown in top panel were subjected to quantitative densitometry and analyzed by 1-factor ANOVA followed by Fisher’s least significant difference test. Values are means ± SE (n = 3).

Fig. 11. Top: Western blot analysis of proteins (30 μg/lane) using an anti-A3 receptor antibody. Samples were from the renal medulla (lanes 1–4), renal cortex (lanes 5–8), and PGMVs (lanes 9–12) from 4 different rats. Bottom: bands shown in top panel were subjected to quantitative densitometry. Values are means ± SE (n = 4).
dilation of preglomerular vessels in the small population of juxtamedullary nephrons (22).

A3 receptors are particularly densely expressed in mesenteric microvessels, suggesting a quantitatively significant role for A3 receptors in the regulation of intestinal blood flow. Indeed, pharmacological studies indicate that A3-receptor agonists markedly vasodilate the intestinal circulation (13). The high level of expression of A2A receptors in the renal cortex suggests an important role for A2A receptors in renal physiology, an implication that is supported by recent findings that A2A receptors inhibit intrarenal inflammation and renal ischemia-reperfusion injury (24).

A2B receptors are abundantly expressed in the preglomerular microcirculation. In fact, A2B receptors are widely and evenly expressed throughout the vascular system and kidneys, including segments of the aorta, mesenteric microvessels, PGMVs, renal cortex, and renal medulla. A similar conclusion is reached when A2B receptor mRNA abundance is measured by Northern blotting (Fig. 6). In light of recent investigations into the role of A2B receptors in vascular biology, our finding that A2B receptors are present throughout the circulation is of considerable importance. In cultured vascular smooth muscle cells (5, 7), cardiac fibroblasts (4, 8), and mesangial cells (6), A2B receptors strongly suppress cell proliferation, extracellular matrix production, and MAP kinase activation. Thus it appears that A2B receptors negatively regulate vascular remodeling. If this hypothesis is correct, A2B receptors may importantly suppress inappropriate vascular remodeling and fibrosis in the vascular tree, including PGMVs. Therefore, A2B receptors in the preglomerular microcirculation may be targets for drug development aimed at ameliorating the progression of chronic renal failure by inhibiting the abnormal growth of vascular elements and, perhaps, mesangial cells in the kidney.

Like the A2A receptor, but unlike A1 receptors or A2B receptors, the A3 receptor is underexpressed in the preglomerular microcirculation. Figure 10 demonstrates strong signals for the A3 receptor in the thoracic aorta, middle abdominal aorta, lower abdominal aorta, and mesenteric microvessels, but barely detectable signals for the A3 receptor in PGMVs. Similarly, Western blotting of renal cortex, medulla, and PGMVs indicates low expression of A3 receptor protein in PGMVs compared with the renal medulla and cortex. The low signal for the A3 receptor in PGMVs vs. aortic tissue cannot be attributed to the fact that PGMVs are resistance vessels and the aorta is a conduit vessel because the A3 receptor signal is strong in the mesenteric microvessels. The results of the studies using Western blotting are confirmed by the inability to detect A3 receptor mRNA in PGMVs with either Northern blotting or RT-PCR (Fig. 6). The low expression of A3 receptors in the preglomerular microcirculation is consistent with the lack of effects of A3-receptor agonists and antagonists in the kidney (18, 45). However, the abundant expression of A3 receptors in the aorta and mesenteric microvessels relative to PGMVs suggests an as yet unrecognized role for A3 receptors in the extrarenal circulation.

In summary, our results indicate that both A1 receptors and A2B receptors are highly expressed in the preglomerular microcirculation and may be important targets for the development of new drugs to treat renal disease and regulate renal function.

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REFERENCES


