ELSEVIER

Biochemical Pharmacology

Biochemical Pharmacology 61 (2001) 237–244

Short communication

Immunolocalisation of adenosine A_1 receptors in the rat kidney

Jane A. Smith, Asipu Sivaprasadarao, Timothy S. Munsey, Christopher J. Bowmer, Michael S. Yates*

School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK

Received 18 January 2000; accepted 20 June 2000

Abstract

The location of adenosine A_1 receptors in the rat kidney was investigated using immunolabelling with antibodies raised to a 15-amino-acid sequence near the C-terminus of the receptor (antibody I) and to a 14-amino-acid sequence in the second extracellular loop (antibody II). In the cortex, antibody I bound to adenosine A_1 receptors in mesangial cells and afferent arterioles, whilst antibody II bound to receptors in proximal convoluted tubules. In the medulla, both antibodies bound to receptors in collecting ducts and the papillary surface epithelium. These observations provide support for the diverse functional roles previously proposed for the adenosine A_1 receptor in the kidney. The labelling of distinct but different structures in the cortex by antibodies raised to different amino acid sequences on the A_1 receptor protein suggests that differing forms of the receptor are present in this region of the kidney.

Keywords: Kidney; Adenosine A_1 receptor; Afferent arteriole; Immunocytochemistry; Mesangial cells; Proximal tubule; Collecting ducts

1. Introduction

Adenosine is a paracrine agent in many tissues where it acts as a potential regulator that balances cell oxygen demand with delivery. In the kidney, adenosine has diverse actions that include modulation of renal blood flow and its distribution within the kidneys, glomerular filtration rate, transport function, and urine flow [1,2]. The presence of adenosine receptors in the kidney is well-established [1], although their functional significance is less clear. A_1 receptors may mediate tubuloglomerular feedback [3] and, in conjunction with the A_2 subtype, regulate vasomotor tone in the outer medullary descending vasa recta [4]. The adenosine A_1 receptor subtype also mediates antidiuretic and antinatriuretic effects, as demonstrated by the diuresis and natriuresis produced in both man and the rat by selective A_1 adenosine antagonists [5,6]. Adenosine receptors located on the apical membrane of tubule cells influence electrolyte transport in the distal nephron: sodium channels are regulated by stimulatory A_1 and inhibitory A_2 receptors [7] and calcium reabsorption is stimulated by A_1 receptors [8].

In addition to physiological roles within the kidney, adenosine is implicated in the pathogenesis of some forms of renal dysfunction and acute renal failure. Adenosine mediates, at least in part, the adverse effects of radiocontrast media [9,10] and bacterial lipopolysaccharide [11,12] on renal haemodynamics. Administration of adenosine antagonists, particularly selective A_1 adenosine receptor antagonists, has been shown to ameliorate acute renal dysfunction induced in animals by intramuscular glycerol injection [13,14], cisplatin [15], ischaemia [16], or hypoxia [17]. Moreover, it has recently been reported that the selective A_2 agonist DWH-146e reduces ischaemia–reperfusion injury in rat kidney [18].

We have recently shown that alterations in dietary sodium intake and glycerol-induced acute renal failure affect expression of adenosine A_1 receptors in the kidney [19,20]. In these studies, autoradiographic techniques were used to identify and assess the density of adenosine A_1 receptors. The resulting autoradiograms allowed receptors to be mapped in particular regions of the kidney, but the resolution was insufficient to locate receptors at the cellular level. The aim of this study was to employ immunocytochemical techniques, which permit greater resolution than autoradiography, to study the cellular location of adenosine A_1 receptors in the normal rat kidney.

* Corresponding author. Tel.: +44-113-233.4316; fax: +44-113-233.4228.

E-mail address: m.s.yates@leeds.ac.uk (M.S. Yates).

0006-2952/01/$ – see front matter
PII: S0006-2952(00)00532-3

2. Materials and methods

2.1. Materials

Anti-rabbit immunoglobulin G fluorescein isothiocyanate (FITC)-labelled antibody, anti-α-smooth muscle actin Cy3- labelled antibody, electrophoresis, and enhanced chemiluminescence reagents were purchased from Sigma. Horseradish peroxidase-conjugated anti-rabbit antibody was obtained from Bio-Rad. Sulfo-link™, coupling gel was obtained from Pierce.

2.2. Preparation of antiserum

A peptide sequence corresponding to a region near the carboxyl terminus (amino acids 309–323; CQPEPPIDEDLPEEK) of the rat adenosine A_1 receptor was synthesised and covalently linked to keyhole limpet haemocyanin. A New Zealand white rabbit was immunised with the conjugated peptide at two weekly intervals (performed by Genosys International) using Freund's complete and incomplete adjuvant. Test bleeds were performed at 5, 7, 9, and 11 weeks and analysed by enzyme-linked immunosorbant assays. The antiserum collected at week 11 (antibody I) was affinity-purified using a peptide–agarose column prepared using Sulfo-link™ gel. In addition, a second affinity-purified antibody to the rat adenosine A_1 receptor was used in the study. This antibody (antibody II, Alpha Diagnostics Inc.) was raised to the second extracellular loop (third extracellular domain) of the rat adenosine A_1 receptor (amino acids 163 to 176, GEPVIKCEFEKVIS).

2.3. Membrane preparation

The specificity of the antisera was verified by immunoblotting with brain membrane preparations. Brain membranes were used to characterise binding of the antibodies, since there is a higher level of expression of adenosine A_1 receptors in brain than in kidney [21,22]. Male Wistar rats (200–250 g) were killed by cervical dislocation. Brains were rapidly removed, freeze clamped in liquid nitrogen, and stored at −70° until required. Brains were homogenised with a Potter Teflon homogeniser at setting 6 in 4 volumes of 0.25 M sucrose, 100 mM sodium dihydrogen phosphate, pH 7.4, and 0.1 mM phenylmethylsulphonyl fluoride. Homogenates were centrifuged at 4° for 30 min at 7500 *g*. Supernatants were passed through mesh (150-μm pore size) prior to centrifuging at 100,000 *g* for 1 hr at 4°. The resulting pellet was resuspended in PBS (140 mM NaCl, 5 mM KCl, 3 mM Na_2HPO_4, 3 mM KH_2PO_4, pH 7.4) and stored at −70° until required. Protein concentration in brain cell membranes was determined by the method of Lowry *et al*. [23] with BSA as the standard.

2.4. SDS–PAGE and immunoblotting

SDS–PAGE was performed according to Laemmli [24]. Cell membrane preparations (brain, 80 μg) were diluted 2:1 in SDS sample buffer (0.125 M Tris–HCl [pH 6.8], 4% SDS, 0.02% [w/v] bromophenol blue, 10% β-mercaptoethanol, 20% glycerol) at room temperature for 15 min. Proteins were separated on a 10% polyacrylamide gel followed by electrophoretic transfer to nitrocellulose membranes. The membranes were blocked with 5% non-fat milk powder in PBS for 1 hr at room temperature followed by an overnight incubation at 4° with primary antibodies I or II (1:500 dilution in PBS containing 1% milk powder). Membranes were washed three times in PBS containing 0.2% Tween 20, after which goat anti-rabbit peroxidase-labelled secondary antibody (1:3000 dilution in PBS containing 1% milk powder) was added for 1 hr at room temperature. The nitrocellulose membranes were washed three times in PBS containing 0.2% Tween 20 and peroxidase activity detected using enhanced chemiluminescence reagents and Kodak X-Omat™ film.

2.5. Immunocytochemical staining of oocytes

Dumont stage V or stage VI *Xenopus* oocytes were injected with 50 nL adenosine A_1 receptor cRNA (20–30 ng) and maintained at 19° in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 5 mM Tris–HCl (pH 7.4), 100 U/mL of penicillin, 100 μg/mL of streptomycin). Three days after cRNA injection, oocytes were fixed in methanol for 4 hr at −20° and cryopreserved at 4° in PBS containing 30% sucrose. A further three days later, oocytes were embedded in octyl transferase and 20-μm sections cut at −18° with a cryostat (Bright). Sections were mounted onto slides with Vectashield antifade mountant, allowed to air-dry for 5 min, and then blocked in PBS containing 1% BSA at 4° for 2 hr. Sections were incubated overnight at 4° with either antibody I or II, diluted 1:50 or 1:500, respectively, with 3% BSA in PBS (antibody dilution buffer). Following incubation with antibody, sections were washed three times with PBS containing 0.1% Tween 20 and anti-rabbit immunoglobulin G–fluorescein isothiocyanate conjugate added at 1:100 in antibody dilution buffer for 1 hr at room temperature. A coverslip, with a drop of Vectashield, was lowered on to the slide and the edges sealed with DePeX mounting medium (BDH). Sections were viewed using an inverted microscope (Nikon Diaphot 300) and images were collected using a cooled CCD camera (Hamamatsu Photonics). All the imaging was controlled by Improvision software that included Openlab 1.7.7 (Image Processing & Vision Company Ltd.) and operated on a 8600 Macintosh Power PC. To provide controls, sections were cut from uninjected oocytes and exposed to primary and secondary antibodies.

2.6. Immunocytochemical studies of rat kidney

Male Wistar rats (200–250g) were anaesthetised with sodium thiopentone (120 mg kg^{-1}, i.p.) and a cannula placed in the abdominal aorta caudal to the left renal artery. A ligature was placed on the abdominal aorta above the right renal artery, and both kidneys were perfused retrogradely via the aortic cannula with sterile saline (0.9% w/v NaCl) for 1 min. The kidneys were rapidly removed, sliced longitudinally, and immersed in 0.5 M sucrose in PBS for 2 min at 4°. Kidneys were frozen in isopentane precooled in liquid nitrogen. Tissue sections (5 μm) were cut at −18° with a cryostat (Bright), thaw-mounted onto poly-L-lysine-coated slides, and stored at −70° until required.

The frozen kidney sections (10–15 sections from the kidneys of 3 rats) were allowed to air-dry for 1 hr and fixed with acetone for 3 min at room temperature. Fixed sections were washed twice with PBS and blocked at room temperature for 20 min with 0.3% BSA, 0.1% Triton X-100 in PBS containing 0.1% Tween 20, followed by a 30-min block in 25% goat non-immune serum in PBS containing 0.1% Tween 20. Sections were incubated overnight at 4° with antibodies I or II, diluted 1:500 with antibody dilution buffer or, in the case of control sections, antibodies I or II pre-adsorbed with the corresponding antigenic peptide, or antibody dilution buffer alone. All controls were without labelling. A number of sections were also incubated with an anti α-smooth muscle actin Cy3-conjugated antibody (1:200, 2 hr). Following this, sections were washed three times with PBS containing 0.1% Tween 20 and anti-rabbit immunoglobulin G–fluorescein isothio-cyanate conjugate added at 1:100 in antibody dilution buffer for 1 hr at room temperature. The sections were washed 3 times, mounted with Vectashield antifade mountant, and sealed with DePeX mounting medium (BDH). Slides were viewed and images collected as described above.

3. Results

3.1. Immunoblots

Representative immunoblots of brain membranes are shown in Fig. 1. The antibody to a peptide sequence near the C-terminus of the rat adenosine A_1 receptor (antibody I) detected two strongly staining bands at 35 and 37 kDa and a weak band at 39 kDa, together with three further bands clustered at 68, 74, and 79 kDa (Fig. 1A). Antibody II, raised to a peptide sequence in the second extracellular loop of the receptor, induced a signal at 79 kDa (Fig. 1B).

3.2. Immunocytochemical detection of adenosine A_1 receptors in *Xenopus* oocytes

In order to eliminate the possibility that the two antibodies recognise proteins other than the adenosine A_1 receptor, we expressed the A_1 receptor in *Xenopus* oocytes by injecting cRNA corresponding to the receptor. Sections taken from these oocytes showed labelling of cell membranes with both antibodies. (Fig. 2, A and B). Intracellular binding was also detected, which was improbably due to receptor protein present in the endoplasmic reticulum. By contrast, neither antibody I or II bound to the cell membranes of uninjected oocytes (Fig. 2, C and D).

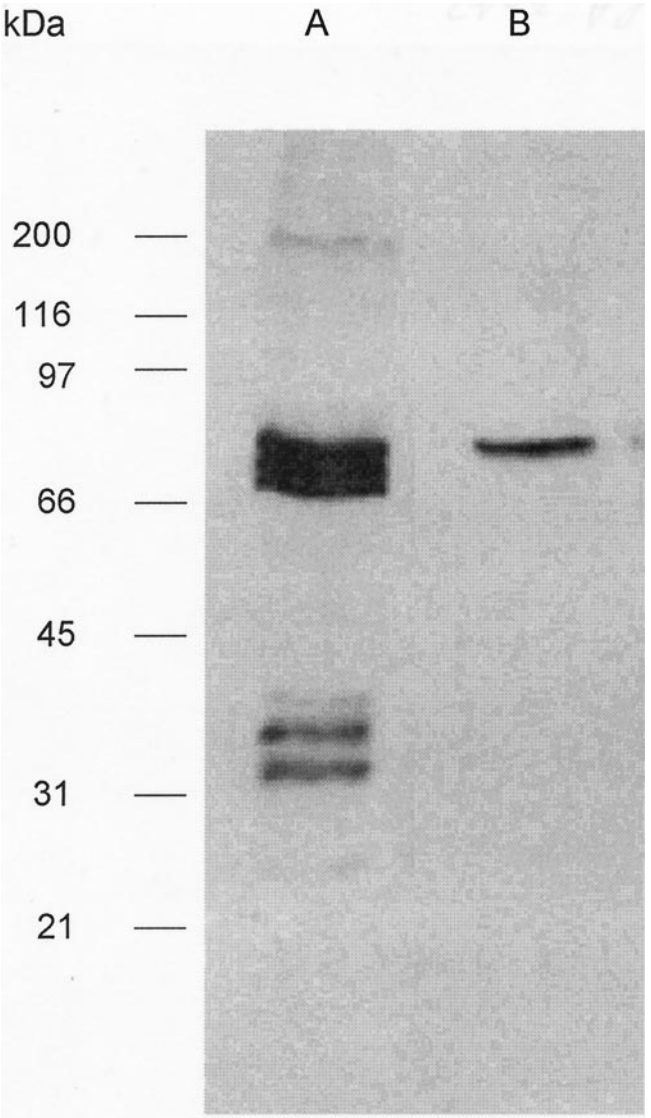

Fig. 1. Immunoblots of rat brain membranes using affinity-purified antibodies to the rat adenosine A_1 receptor raised to (A) a 15-amino-acid sequence near the C-terminus (antibody I) and to (B) a 14-amino-acid sequence in the second extracellular loop (antibody II). The blots are representative of 5 similar blots prepared using brain membranes from 10 rats. Adsorption with the appropriate immunising peptide removed bands identified by both antibodies.

3.3. Immunocytochemical localisation of adenosine A_1 receptors in the kidney

Both antibodies detected structures in the cortex and medulla. Antibody I bound to glomerular structures (Fig.

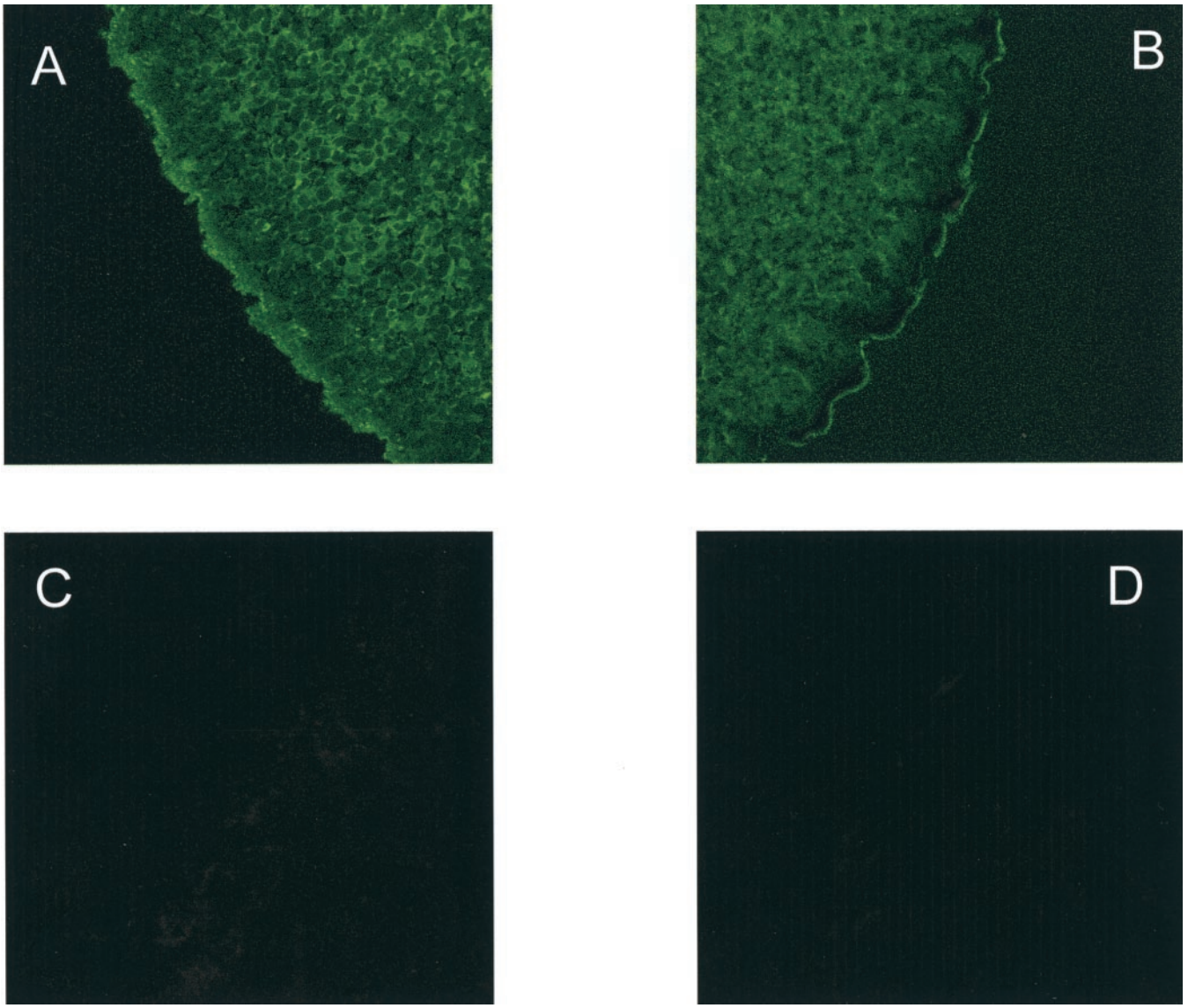

Fig. 2. Immunolabelling of sections taken from *Xenopus* oocytes injected with 20–30 ng adenosine A_1 receptor cRNA. Labelling in (A) was with affinity-purified antibodies to the rat adenosine A_1 receptor raised to a 15-amino-acid sequence near the C-terminus (antibody I), whilst labelling in (B) was with antibody raised to a 14-amino-acid sequence in the second extracellular loop (antibody II). No labelling was detected in uninjected oocytes exposed to either antibody I (C) or antibody II (D). Anti-rabbit immunoglobulin G fluorescein isothiocyanate-labelled antibody was used to visualise antibodies to adenosine A_1 receptors. Labelled sections are representative of 10–12 similarly prepared sections from 3 oocytes.

3A) but to no other sites within the cortex (Fig. 3D). The glomerular structures labelled by antibody I were single mesangial cells in the glomerular tuft and the afferent arteriole just within or adjacent to the glomerulus (Fig. 3A). Binding of antibody to α-smooth muscle actin confirmed the identify of the afferent arteriole (Fig. 3B). By contrast to the pattern of binding with antibody I, no structures were labelled in or adjacent to glomeruli by antibody II (Fig. 3C), but binding was noted in the proximal convoluted tubules in the cortical labyrinth (Fig. 3E).

Although the A_1 adenosine receptor antibodies labelled different structures in the cortex, the pattern of labelling was similar in the medulla. Labelling with both antibodies was found along the length of collecting ducts in the inner medulla (Fig. 4, A and B) and on the papillary surface epithelium (Fig. 4, C and D).

4. Discussion

This study has, using immunocytochemistry, shown the presence of adenosine A_1 receptors at various sites within the renal cortex and medulla. These observations support the functional roles proposed for the adenosine A_1 receptor in the kidney, as discussed below. Antibody to the peptide sequence near the C-terminus of the receptor protein (antibody I) detected three bands in the 35–39 kDa range. The molecular mass of the adenosine A_1 receptor using immu-

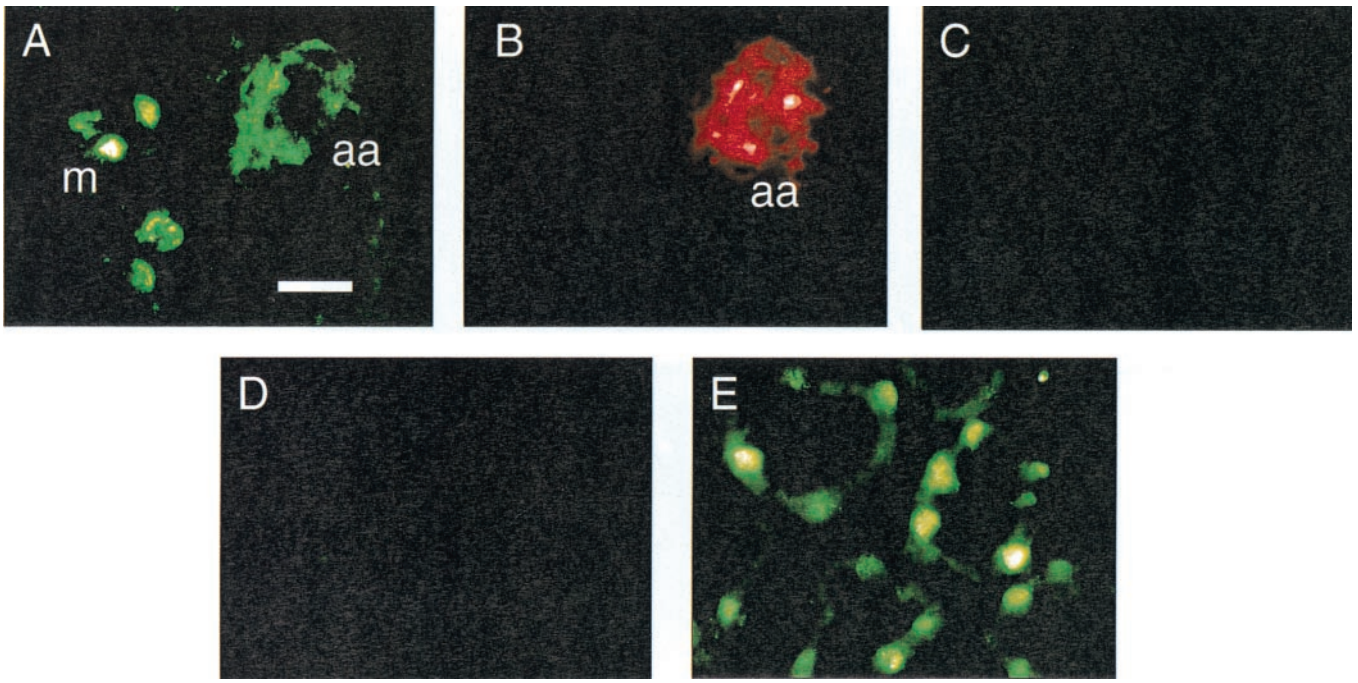

Fig. 3. Immunolabelling of adenosine A_1 receptors and α-smooth muscle actin in sections from the rat renal cortex. (A) Glomerulus after exposure to antibody I showing labelling of adenosine A_1 receptors in mesangial cells (m) and afferent arteriole (aa); (B) labelling of α-smooth muscle actin in the afferent arteriole; (C) glomerulus after exposure to antibody II with no adenosine A_1 receptors labelled; (D) proximal convoluted tubules after exposure to antibody I with no adenosine A_1 receptors identified; and (E) proximal convoluted tubules after exposure to antibody II showing labelling of adenosine A_1 receptors. Images shown in (A) and (B) were taken with the same section. Both antibodies were raised to peptide sequences of the rat adenosine A_1 receptor, antibody I to a 15-amino-acid sequence near the C-terminus and antibody II to a 14-amino-acid sequence in the second extracellular loop. Anti-rabbit immunoglobulin G fluorescein isothiocyanate-labelled antibody was used to visualise antibodies to adenosine A_1 receptors. α-Smooth muscle actin was visualised in (B) using Cy3-labelled antibody. The labelled sections are representative of 10–15 similarly prepared sections from 3 rats. Incubation of sections with antibody pre-adsorbed with corresponding antigenic peptide resulted in no labelling (data not shown). Scale bar in (A) represents 25 μm, with all images taken at the same magnification.

noblots has been estimated as 38 kDa in rat brain membranes [25] and 39 kDa in rat kidney membranes [26]. The molecular mass of the deglycosyated receptor in rat brain has been reported as 32 kDa [27] or 30 kDa [28], with the receptor undergoing glycosylation with different sugar moieties [28]. Antibody I might therefore bind to the receptor in the brain in its various glycosylated forms. A previous study using immunoblots to identify adenosine A_1 receptor in pig brains detected receptor dimer as well as the monomer [29]. Similarly, antibody I bound to three bands in the adenosine A_1 receptor dimer range (68–79 kDa). Antibody to the peptide sequence in the second extracellular loop (antibody II) only detected the receptor dimer in brain membranes, as it only bound to one band at 79 kDa. Using antibody to adenosine A_1 receptor from the same source (Alpha Diagnostics Inc.), Zou *et al*. [26] detected receptor monomer at 39 kDa in membranes prepared from either rat renal cortex or medulla.

Glycosylation often occurs on the second extracellular loop of the receptor [30], the location of the peptide sequence to which antibody II was raised. Consequently, glycosylation might impede binding of antibody II, whilst interaction of antibody I to the C-terminus should be relatively unaffected. Such an explanation may account for the differences in immunostaining produced by the antibodies, with antibody I detecting more glycosylated forms of the receptor than antibody II. Future studies using deglycosylated samples may help to clarify how glycosylation affects binding of the antibodies. By contrast to the differing immunoblots, both antibodies clearly detected adenosine A_1 receptor protein heterologously expressed in the cell membrane of *Xenopus* oocytes. The Western blots and oocyte studies confirm the specificity of the antibodies for the adenosine A_1 receptor, although they may bind to different forms of the receptor when expressed in rat tissues.

Visualisation of A_1 receptors in the afferent arteriole in the cortex, using antibody I, confirms the functional study of Holtz and Steinhausen [31], who noted constriction of the arteriole in response to application of cyclohexyladenosine, an adenosine A_1 receptor agonist. Contraction of cultured mesangial cells has also been reported in response to stimulation of adenosine A_1 receptors [32], a finding supported by immunocytochemical detection of the receptor in this cell type, although no signal for α-smooth muscle actin was detected. Failure to localise α-smooth muscle actin to mesangial cells is not unexpected, since an investigation by Adyel *et al*. [33] found that this protein was not expressed in kidney biopsy specimens from normal kidneys as used in this study, although expression was recorded in specimens from patients with glomerulonephritis.

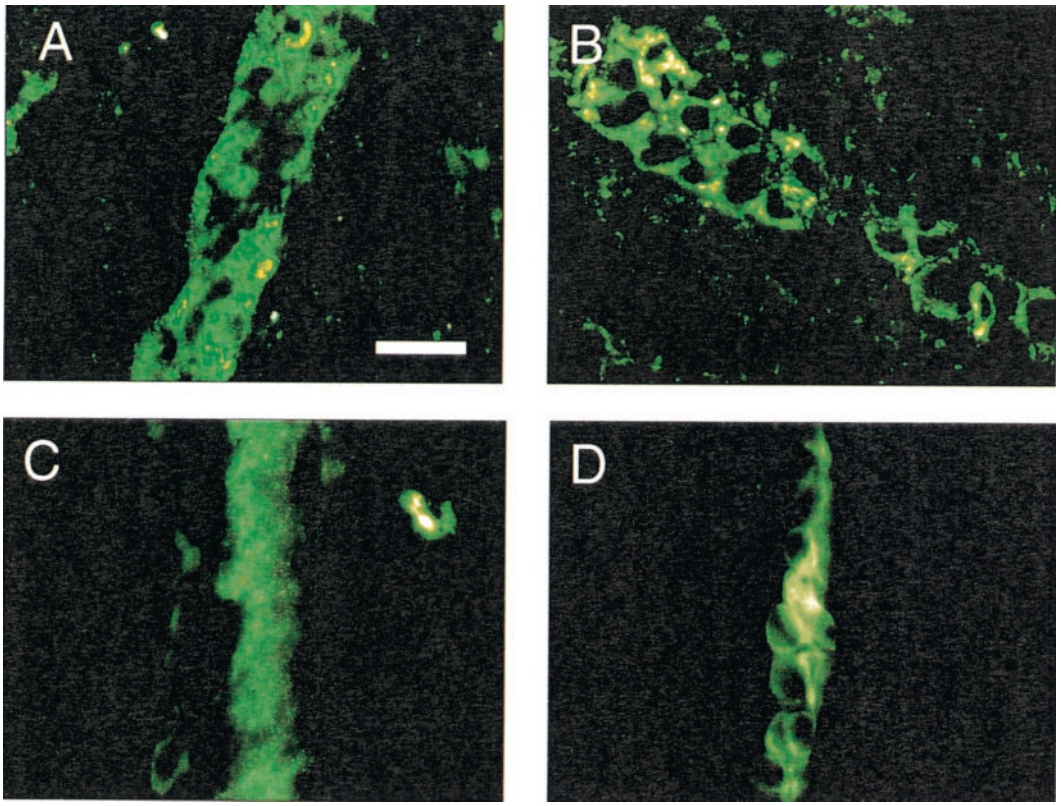

Fig. 4. Immunolabelling of sections from the rat renal medulla showing medullary collecting ducts (A and B) and papillary surface epithelium (C and D). Labelling in (A) and (C) was with affinity-purified antibodies to the rat adenosine A_1 receptor raised to a 15-amino-acid sequence near the C-terminus (antibody I), whilst labelling in (B) and (D) was with antibody raised to a 14-amino-acid sequence in the second extracellular loop (antibody II). Anti-rabbit immunoglobulin G fluorescein isothiocyanate-labelled antibody was used to visualise antibodies to adenosine A_1 receptors. The labelled sections are representative of 10–15 similarly prepared sections from 3 rats. Incubation of sections with antibody pre-adsorbed with corresponding antigenic peptide resulted in no labelling (data not shown). Scale bar in (A) represents 25 μm, with all images taken at the same magnification.

By contrast to our previous autoradiographic studies [19, 20], adenosine A_1 receptors were identified, using antibody II, in the proximal convoluted tubule. Previous autoradiographic investigations in guinea-pig and human kidneys [34] or *in situ* hybridisation studies of the rat kidney [35] have also failed to detect adenosine A_1 receptor protein or mRNA in proximal tubules. However, adenosine A_1 receptor mRNA has been detected in the proximal tubule, cortical collecting duct, and cortical thick ascending limb of the rat using the reverse transcriptase–polymerase chain reaction [36]. There is also functional evidence for the presence of adenosine A_1 receptors in the proximal tubule. For example, studies of rabbit proximal tubules indicate that endogenous adenosine, via an adenosine A_1 receptor, stimulates basolateral Na^+-HCO_3^- cotransport [37]. Moreover, studies in both rat and man suggest that the diuresis produced by selective adenosine A_1 receptor antagonists such as 1,3-dipropyl-8-cyclopentylxanthine and FK-453 ((+)-(*R*)-[(*E*)-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)acryloy]-2-piperidine ethanol) is primarily evoked by an action on the proximal tubule, with some activity at more distal nephron segments [5,6].

Detection of adenosine A_1 receptors in the glomerulus and proximal tubule was by different antibodies: antibody I bound to receptors in the afferent arteriole and mesangial cells, whilst antibody II bound to receptors in the proximal tubule, with no evidence of any overlap between the binding of the two antibodies in the cortex. The binding of antibodies raised to different peptide sequences of the receptor protein could occur if there are subtypes of the receptor arising from splice variants, although none have been reported for the rat adenosine A_1 receptor. Two transcripts for the human adenosine A_1 receptor have been identified, but these transcripts have the same coding sequence [38]. A further possible explanation for the differential binding of the two antibodies is the degree and nature of glycosylation of the receptors at tubular and glomerular sites. Since the glycosylation state affects immunoreactivity of the rat adenosine A_1 receptor [28], antibodies I and II may selectively bind to different glycosylated forms of the receptor.

Antibodies to adenosine A_1 receptor also bound to structures in the inner medulla, with the pattern of receptor-binding sites similar to that recorded in our previous autoradiographic studies [19,20]. The most likely candidates for the labelled tubular structures in the inner medulla are the collecting ducts, since *in situ* hybridisation studies showed that adenosine A_1 receptor mRNA in the rat kidney was most abundant in these tubular elements [35]. One potential role for adenosine A_1 receptors in collecting tubules is to

influence sodium channel function and therefore sodium excretion (see Ref. 19). Adenosine A_1 receptors were also found on the papillary surface epithelium. Transporter proteins, such as H^+- K^+-ATPase [39] and vasopressin V_2 receptors [40], have been identified in this epithelium, indicating that it has similar transport properties to the collecting duct. Therefore, as in the collecting duct, adenosine A_1 receptors may influence sodium channel function in the papillary surface epithelium. Both antibody I and II bound to collecting ducts and the papillary surface epithelium. This suggests that either the medullary receptors are a mixture of the forms detected in the cortex by each antibody or the adenosine A_1 receptor present in the medulla binds both antibodies.

In conclusion, this study has, for the first time, used immunocytochemistry to localise adenosine A_1 receptors in the kidney. Receptors were identified at the afferent arteriole, mesangial cells, proximal convoluted tubule, medullary collecting ducts, and papillary surface epithelium. The labelling of distinct and different structures in the cortex by antibodies raised to different amino acid sequences on the A_1 receptor protein suggests that differing forms of the receptor are present in this region of the kidney.

Acknowledgment

We wish to thank Veronique Lapostolle for injecting oocytes.

References

[1] McCoy DE, Bhattacharya S, Olson BA, Levier DG, Arend LJ, Spielman WS. The renal adenosine system: structure, function, and regulation. Semin Nephrol 1993;13:31–40.
[2] Collis MG, Bowmer, CJ, Yates MS, Drugs acting on purinergic receptors. In: Singh BN, Dzau VJ, Vanhoutte PM, Woosley RL, editors. Cardiovascular pharmacology and therapeutics. Lond: Churchill Livingstone, 1994. p. 145–60.
[3] Schnermann J, Weihprecht H, Lorenz JN, Briggs JP. The afferent arteriole—the target for macula densa-generated signals. Kidney Int Suppl 1991;39:S74–7.
[4] Silldorff EP, Kreisberg MS, Pallone TL. Adenosine modulates vasomotor tone in outer medullary descending vasa recta of the rat. J Clin Invest 1996;98:18–23.
[5] Balakrishnan VS, Coles GA, Williams JD. A potential role for endogenous adenosine in control of human glomerular and tubular function. Am J Physiol 1993;265:F504–10.
[6] Knight RJ, Bowmer CJ, Yates MS. The diuretic effect of 8-cyclopentyl-1,3-dipropylxanthine, a selective A_1 adenosine receptor antagonist. Br J Pharmacol 1993;109:271–7.
[7] Ma H, Ling BN. Luminal adenosine receptors regulate amiloride-sensitive Na^+ channels in A6 distal nephron cells. Am J Physiol 1996;270:F798–F805.
[8] Hoenderop JG, Hartog A, Willems PH, Bindels RJ. Adenosine-stimulated Ca^{2+} reabsorption is mediated by apical A_1 receptors in rabbit cortical collecting ducts. Am J Physiol 1998;274:F736–43.
[9] Arend LJ, Bakris GL, Burnett JC, Megerian C, Spielman WS. Role for intrarenal adenosine in the renal hemodynamic response to contrast media. J Lab Clin Med 1987;110:406–11.
[10] Erley CM, Duda SH, Schlepckow S, Koehler J, Huppert PE, Strohmaier WL, Bohle A, Risler T, Osswald H. Adenosine antagonist theophylline prevents the reduction of glomerular filtration rate after contrast media application. Kidney Int 1994;45:1425–31.
[11] Knight RJ, Bowmer CJ, Yates MS. Effect of the selective A_1 adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine on acute renal dysfunction induced by *Escherichia coli* endotoxin in rats. J Pharmacol 1993;45:979–84.
[12] Nishiyama A, Miura K, Miyatake A, Fujisawa Y, Yue W, Fukui T, Kimura S, Abe Y. Renal interstitial concentration of adenosine during endotoxin shock. Eur J Pharmacol 1999;385:209–16.
[13] Bowmer CJ, Collis MG, Yates MS. Effect of the adenosine antagonist 8-phenyltheophylline on glycerol-induced acute renal failure. Br J Pharmacol 1986;88:205–12.
[14] Kellett R, Bowmer CJ, Collis MG, Yates MS. Amelioration of glycerol-induced acute renal failure in the rat with 8-cyclopentyl-1,3-dipropylxanthine. Br J Pharmacol 1989;98:1066–74.
[15] Knight RJ, Collis MG, Yates MS, Bowmer CJ. Amelioration of cisiplatin-induced acute renal failure with 8-cyclopentyl-1,3-dipropylxanthine. Br J Pharmacol 1991;104:1067–8.
[16] Lin J-J, Churchill PC, Bidani AK. Effect of theophylline on the initiation phase of postischemic acute renal failure. J Lab Clin Med 1986;108:150–4.
[17] Gouyon JB, Guignard JP. Theophylline prevents the hypoxemia-induced renal hemodynamic changes in rabbits. Kidney Int 1988;33:1078–83.
[18] Okusa MD, Linden J, Macdonald T, Huang L. Selective A_{2A} adenosine receptor activation reduces ischemia–reperfusion injury in rat kidney. Am J Physiol 1999;277:F404–12.
[19] Smith JA, Whitaker EM, Yaktubay N, Morton MJ, Bowmer CJ, Yates MS. Regulation of renal adenosine A_1 receptors: effect of dietary sodium chloride. Eur J Pharmacol 1999;384:71–9.
[20] Smith JA, Whitaker EM, Bowmer CJ, Yates MS. Differential expression of renal adenosine A_1 receptors induced by acute renal failure. Biochem Pharmacol 2000;59:727–32.
[21] Klotz KN, Keil R, Zimmer FJ, Schwabe U. Guanine nucleotide effects of 8-cyclopentyl-1,3-[^{3}H]dipropylxanthine binding to membrane-bound and solubilized A_1 adenosine receptors of rat brain. J Neurochem 1990;54:1988–94.
[22] Gould J, Morton MJ, Sivaprasadarao A, Bowmer CJ, Yates MS. Renal adenosine A_1 receptor binding characteristics and mRNA levels during the development of acute renal failure in the rat. Br J Pharmacol 1997;120:947–53.
[23] Lowry OH, Rosebrough MJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[24] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;277:680–5.
[25] Rivkees SA, Price SL, Zhou FC. Immunohistochemical detection of A_1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum and basal ganglia. Brain Res 1995;677:193–203.
[26] Zou AP, Wu F, Li PL, Cowley AW. Effect of chronic salt loading on adenosine metabolism and receptor expression in renal cortex and medulla in rats. Hypertension 1999;33:511–6.
[27] Klotz KN, Lohse MJ. The glycoprotein nature of A_1 adenosine receptors. Biochem Biophys Res Commun 1986;140:406–13.
[28] Nakata H. Development of an antiserum to rat-brain A_1 adenosine receptor: application for immunological and structural comparison of A_1 adenosine receptors from various tissues and species. Biochim Biophys Acta 1993;1177:93–98.
[29] Ciruela F, Casado V, Mallol J, Canela EI, Lluis C, Franco R. Immunological identification of A_1 adenosine receptors in brain cortex. J Neurosci Res 1995;42:818–28.

[30] Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev 1998;50:413–92.
[31] Holz FG, Steinhausen M. Renovascular effects of adenosine receptor agonists. Ren Physiol 1987;10:272–82.
[32] Olivera A, Lamas S, Rodriguez-Puyol D, Lopez-Novoa J. Adenosine induces mesangial cell contraction by an A_1 type receptor. Kidney Int 1989;35:1300–5.
[33] Adyel FZ, Makni H, Hachicha J, Jlidi R, Trigui N, Ayadi H. Deposition of IgG anti-actin antibodies on kidneys of glomerular nephropathy patients with an enhanced glomerular alpha smooth muscle actin expression. Nephron 1998;78:410–15.
[34] Palacios JM, Fastbom J, Wiederhold KH, Probst A. Visualization of adenosine A_1 receptors in the human and guinea-pig kidney. Eur J Pharmacol 1987;138:273–6.
[35] Weaver DR, Reppert SM. Adenosine receptor gene expression in rat kidney. Am J Physiol 1992;263:F991–5.
[36] Yamaguchi S, Umemura S, Tamura K, Iwamoto T, Nyui N, Ishigami T, Ishii M. Adenosine A_1 receptor mRNA in microdissected rat nephron segments. Hypertension 1995;26:1181–5.
[37] Takeda M, Yoshitomi K, Imai M. Regulation of Na^+ - $3HCO_3^-$ cotransport in rabbit proximal convoluted tubule via adenosine A_1 receptor. Am J Physiol 1993;265:F511–9.
[38] Ren H, Stiles GL. Posttranscriptional mRNA processing as a mechanism for regulation of human A_1 adenosine receptor expression. Proc Nat Acad Sci USA 1994;91:4864–6.
[39] Ahn KY, Madsen KM, Tisher CC, Kone BC. Differential expression and cellular distribution of messenger RNAs encoding alpha-isoforms and beta-isoforms of Na^+-K^+-ATPase in rat kidney. Am J Physiol 1993;265:F792–801.
[40] Ostrowski NL, Young WS 3rd, Knepper MA, Lolait SJ. Expression of V_{1A} and V_2 receptor messenger-ribonucleic-acid in the liver and kidney of embryonic, developing, and adult-rats. Endocrinology 1993;133:1849–59.