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Renal adenosine A_1 receptor binding characteristics and mRNA levels during the development of acute renal failure in the rat

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1 The binding characteristics and mRNA levels for renal adenosine A_1 receptors were investigated in normal rats and rats with acute renal failure (ARF) induced by either glycerol or $HgCl_2$.

2 Saturation isotherms determined from the binding of [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]-DPCPX), a selective adenosine A_1 antagonist, to renal membranes of untreated rats gave values of 0.62 nM for the equilibrium dissociation constant (K_d) and 19.9 fmol mg^{-1} protein for the density of binding sites (B_{max}). No saturable binding was observed with [^{3}H]-2-(p-(carboxylethyl)-phenylethylamino)-5′-N-ethylcarboxamido adenosine ([^{3}H]-CGS 21680), a selective adenosine A_{2a} agonist.

3 By contrast to time-matched controls, renal membranes obtained from rats 16 and 48 h following the induction of ARF with glycerol, showed statistically significant increases (2–4 fold) in both B_{max} and K_d for the binding of [^{3}H]-DPCPX. No significant changes in the binding characteristics of [^{3}H]-DPCPX were noted with membranes from rats 48 h following the production of ARF with $HgCl_2$.

4 Adenosine A_1 receptor mRNA levels were significantly elevated 0.5, 16 and 48 h following induction of ARF with glycerol, whilst no change was noted in mRNA levels for β-actin at the same time points. No statistically significant changes in adenosine A_1 receptor or β-actin mRNA levels were noted 48 h after the induction of ARF with $HgCl_2$.

5 This study indicates that glycerol-induced ARF in the rat is associated with an increase in renal adenosine A_1 receptor density which appears to result from increased transcription of the gene for this receptor. An increase in adenosine A_1 receptor density in renal resistance vessels may explain, at least in part, the enhanced renal vasoconstrictor response to adenosine in glycerol-induced ARF that was noted in a previous study.

Keywords: Acute renal failure; glycerol; $HgCl_2$; adenosine A_1 receptor; mRNA levels; [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine; β-actin

Introduction

Adenosine affects various aspects of renal function; namely, renal blood flow and its distribution within the kidneys, renin and erythropoietin secretion, glomerular filtration rate, sodium excretion and urine flow (Collis *et al*., 1994). In addition, adenosine appears to be an important haemodynamic mediator of some forms of acute renal failure (ARF) (Collis *et al*., 1994). Administration of adenosine antagonists has been shown to ameliorate acute renal dysfunction induced in animals by myohaemoglobinuria produced by intramuscular glycerol injection (Bidani & Churchill, 1983; Bowmer *et al*., 1986; Kellett *et al*., 1989), cisplatin (Knight *et al*., 1991), ischaemia (Lin *et al*., 1986) and hypoxia (Gouyon & Guignard, 1988). Furthermore, adenosine may mediate the acute impairment of renal function produced by radiocontrast media since the adverse effects of contrast media can be reversed in both animals (Arend *et al*., 1987) and man (Erley *et al*., 1994) by the adenosine antagonist theophylline. However, investigations in rats have shown that ARF induced by mercuric chloride ($HgCl_2$) (Rossi *et al*., 1990), gentamicin (Kellett *et al*., 1988) and cyclosporine (Panjehshahin *et al*., 1991) is unaffected by treatment with adenosine antagonists. It therefore appears that adenosine plays an important role in the pathogenesis of some but not all forms of ARF.

The renal vasoconstrictor response to adenosine is progressively enhanced in rats during the development of glycerol-induced ARF; but not in ARF produced by $HgCl_2$ (Gould *et al*., 1995). These findings suggest that increased renal vasoconstriction to adenosine may contribute to the pathophysiological actions of adenosine during the development of ARF. Renal vasoconstriction is mediated by the adenosine A_1 receptor subtype (Spielman & Arend, 1991), whilst vasodilatation in the rat kidney is produced by stimulation of the A_2 receptor subtype (Agmon *et al*., 1993). A study of adenosine receptor expression in the rat kidney has indicated that the adenosine A_2 receptor is of the A_{2a} receptor subtype (Weaver & Reppert, 1992). One possible explanation for the enhanced renal vasoconstriction to adenosine in glycerol-induced ARF is upregulation of adenosine A_1 receptors and/or downregulation of A_{2a} receptors. Therefore, the principal aim of the present study was to characterize and compare the binding characteristics of adenosine A_1 and A_{2a} receptors in renal membranes from rats with ARF induced by either glycerol or $HgCl_2$. In addition, adenosine receptor mRNA levels were also measured in these forms of ARF in order to investigate whether any changes in receptor density were associated with altered receptor gene expression.

Methods

Induction of acute renal failure

The method for induction of ARF with glycerol has been described previously (Bowmer *et al*., 1982). Male Wistar rats (220–300 g) were deprived of drinking water for 24 h, and ARF was produced by an intramuscular injection of 50% v/v glycerol in sterile saline (10 ml kg^{-1}). Control animals were dehydrated and injected with sterile saline (10 ml kg^{-1}). Immediately after injection of either glycerol or saline, rats were allowed free access to drinking water. $HgCl_2$-induced ARF was produced by subcutaneous administration of 2 mg kg^{-1} $HgCl_2$ in sterile saline (2 ml kg^{-1}); whereas control rats received an equivalent volume of saline. In addition, some studies were conducted with rats which had received no treatment.

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To assess the severity of ARF, plasma urea concentrations were measured at various times after the induction of renal failure. Urea was assayed by reaction with diacetylmonoxime with a commercial diagnostic kit (Sigma Chemical Co., Poole, U.K.).

Preparation of renal membranes

Groups of four rats were anaesthetized with sodium thiobutabarbitone (180 mg kg^{-1}, i.p.), both kidneys were removed from each animal and freeze clamped in liquid nitrogen. Kidneys were either processed immediately or stored at $-70°C$ for no longer than 2 days. The kidneys were pooled, pulverized in a mortar and homogenized in 2 volumes of ice-cold 0.25 M sucrose-buffer, pH 7.4, containing 50 mM Tris base and 0.1 mM phenylmethylsulphonyl fluoride. The homogenate was centrifuged at 1000 *g* for 10 min at 4°C; the supernatant was recovered and centrifuged at 9000 *g* for 20 min at 4°C. The supernatant was recentrifuged at 105,000 *g* for 60 min at 4°C. The resultant pellet was resuspended in buffered sucrose and used immediately for assay. Protein concentration was determined by the method of Lowry *et al*. (1951) with bovine albumin as the standard.

Binding assays

All incubations were carried out at 4°C in 50 mM Tris buffer, pH 7.4, containing 0.25 mM sucrose and 5 u ml^{-1} adenosine deaminase (Freissmuth *et al*., 1987). All binding experiments were performed with 500 μg of protein suspended in 500 μl of buffer.

Association and dissociation studies

Association was initiated by addition of [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]-DPCPX) to give a final concentration of 0.4 nM. At various times from 5 min to 5 h, binding was terminated by rapid filtration through Whatman GF/C glass fibre filters housed in an Ilacon Cell Harvester. Glass fibre filters were pretreated by overnight soaking in 1% v/v polyethylenimine (Bruns *et al*., 1983). Filters were washed with 4×2.5 ml of ice-cold buffer, dried, transferred to scintillation vials and counted for radioactivity. Dissociation was initiated after 5 h incubation by addition of sufficient unlabelled DPCPX to give a final concentration of 1 μM. Binding was stopped at the times indicated in Figure 2 and bound [^{3}H]-DPCPX assayed as described for the association experiments.

Saturation isotherms and competition studies

To study the dependence of binding on ligand concentration, incubations were carried out for 5 h in the presence of 0.08 to 12 nM [^{3}H]-DPCPX or 0.8 to 80 nM [^{3}H]-2-(p-(carboxylethyl)-phenylethylamino)-5′-N-ethylcarboxamido adenosine ([^{3}H]-CGS 21680). Non-specific binding was determined in parallel in the presence of 1 mM theophylline. Some competition experiments were performed with membranes incubated for 5 h with 0.4 nM [^{3}H]-DPCPX and either unlabelled DPCPX or CGS 21680 present at concentrations between 0.001 to 1.0 μM.

RNA preparation

Rats were anaesthetized with sodium thiobutabarbitone (180 mg kg^{-1}, i.p.) and both kidneys removed. Kidneys were immediately freeze clamped in liquid nitrogen and stored at $-70°C$ until use. Total cellular RNA was isolated by the acid-guanidium thiocyanate-phenol-chloroform extraction method of Chromoczynski & Sacchi (1987) and adjusted to approximately 1 μg μl^{-1} in diethylpyrocarbonate-treated water.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Two micrograms of total RNA were reverse transcribed in a total volume of 20 μl which included: 50 mM Tris buffer (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 μM dithiothreitol, 200 u of M-MLV reverse transcriptase and 0.2 μg of oligo $(dT)_{12-18}$ primer. The reaction was carried out at 37°C for 10 min. Five microlitres of the reverse transcribed material were then subjected to PCR in a total volume of 50 μl containing 10 pmol of each primer, 50 μM dNTPs, 10 mM Tris buffer (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 0.1% triton X-100, 1 μCi α-[^{32}P]-dCTP (specific activity; 3000 Ci $mmol^{-1}$) and 2.5 u of *Taq* DNA polymerase prepared according to the method of Pluthers (1993). The adenosine A_1-receptor-specific primers were 5′-CTCGCCATTGCTGTGGATCGA-3′ and 5′-GTGTGT-GAGGAAGATGGCGAT-3′, designed to generate a 540 b.p. PCR product and β-actin-specific primers were 5′-TT-GTAACCAACTGGGACGATATGG-3′ and 5′-GATCT-TGATCTTCATGGTGCTTAGG-3′, designed to generate a 740 b.p. product. The tubes were subjected to 30 cycles: 94°C for 40 s; 64°C for 1 min and 72°C for 1 min. PCR for β-actin employed a 'touchdown' PCR protocol (Don *et al*., 1991) with the primer annealing temperature ranging from 69°C to 61°C. After PCR, a 10 μl aliquot of each sample was electrophoresed on a 1% agarose gel with Tris-acetate-EDTA buffer (Sambrook *et al*., 1989) and the PCR product was visualized and quantitated with a FujiBAS 1000 PhosphorImager.

Quantitation of adenosine A_1 receptor mRNA levels with RT-PCR

Quantitation of adenosine A_1 receptor and β-actin mRNA levels was performed by RT-PCR according to the method of Robinson & Simon (1991). Standard curves were generated with appropriate dilutions of adenosine A_1 receptor and β-actin cRNA. These were produced by *in vitro* transcription of adenosine A_1 receptor and β-actin cDNAs in pSPORT2 and pBluescript II, respectively, with a T7 RNA polymerase-based transcription kit.

Materials

[^{3}H]-DPCPX and [^{3}H]-CGS21680 were obtained from Du Pont (Stevenage, Hertfordshire, U.K.). DPCPX and polyethylenimine were obtained from Sigma Chemical Co. (Poole, Dorset U.K.) and CGS 21680 from Research Biochemicals Interna-

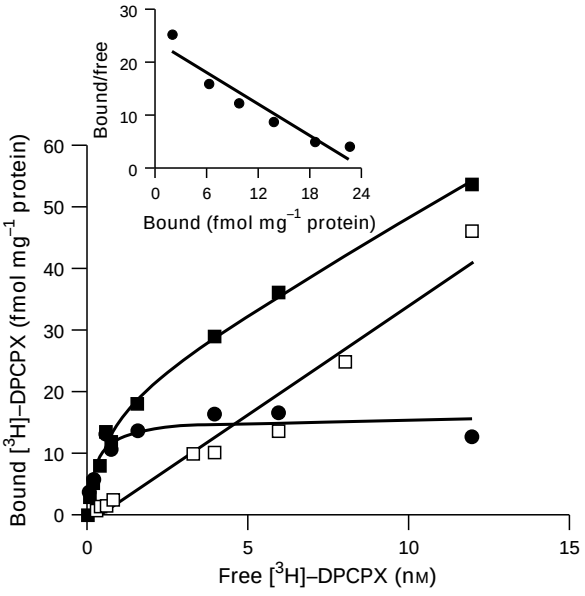

Figure 1 Total (■), specific (●) and non specific (□) binding of [^{3}H]-DPCPX to renal cell membranes from untreated rats. Points are means obtained from 2 experiments carried out with 6 replicates. Cell membranes were obtained from the kidneys of 8 rats. The insert shows a Scatchard plot produced from specific binding data.

tional (Natick, MA, U.S.A). α[^{32}P]-dCTP (2′-deoxycytidine-5′-triphosphate) was obtained from ICN Pharmaceuticals Ltd. (Thame, Oxfordshire, U.K.). All oligonucleotide primers were obtained from Genosys Biotechnologies Ltd. (Cambridge, U.K.). M-MLV reverse transcriptase and pSPORT2 were obtained from Gibco-BRL (Life Technologies, Renfrewshire, Scotland). pBluescript II was obtained from Stratagene Ltd. (Cambridge, U.K.). The Ambion mMESSAGE mMACHINE *In Vitro* Transcription Kit was obtained from ams Biotechnology (Oxfordshire, U.K.).

Analysis of data

Data from binding experiments were analysed by non-linear least squares regression analysis (PCNONLIN, Statistical Consultants Inc., 1986); and the goodness of fit, was determined statistically from an *F*-test on the sum of squares of the residuals. Estimates of K_d, B_{max} and K_i were obtained from the fitting procedures. Data are given as mean ± s.e. mean and statistical comparison of means was made by use of Student's unpaired *t* test or one way analysis of variance (ANOVA) with means compared by Scheffe's test.

Results

Binding studies

Binding of [^{3}H]-DPCPX to renal cell membranes from untreated rats

Saturation and competition experiments Figure 1 shows the equilibrium binding of [^{3}H]-DPCPX to renal membranes. Specific binding was saturable and isotherms were best described by assuming interaction with a single class of homogeneous binding sites. Furthermore, a linear Scatchard plot was obtained on transformation of binding data (Figure 1). The estimates of equilibrium dissociation constant, K_d, and density of binding sites, B_{max} were 0.62 nM and 19.9 fmol mg^{-1} protein, respectively (Table 1). Bound [^{3}H]-DPCPX was displaced by unlabelled DPCPX and the K_i for inhibition was 0.69 ± 0.14 nM. However, the selective adenosine A_{2a} receptor agonist CGS 21680 did not inhibit binding even when the concentration of CGS 21680 (1.0 μM) was three orders of magnitude greater than that of [^{3}H]-DPCPX (0.4 nM).

Kinetic experiments The specific binding of [^{3}H]-DPCPX at 4°C to renal membranes was slow; the equilibrium, at a ligand concentration of 0.4 nM, was reached after 2 h and binding appeared stable for a further 4 h (Figure 2a). The kinetics of association were mono-exponential with a half-time of 31 ± 3 min. The rate constants k_{on} k_{off} were estimated to be 0.015 ± 0.0013 nM^{-1} min^{-1} and 0.017 ± 0.0018 min^{-1}, respectively. The ratio of k_{on} to k_{off} gave a K_d of 1.13 nM that is similar to the dissociation constant of 0.62 nM obtained from the saturation isotherms (Table 1). Addition of 1.0 μM DPCPX resulted in dissociation of specifically bound [^{3}H]-DPCPX; but since the plot of 1nB/Beq versus time was non-linear (Figure 2b), the kinetics of dissociation appeared more complex than those of association. However, the data in Figure 2b could be described by a bi-exponential rate equation which gave k_{off} values of 0.0058 ± 0.0020 and 0.087 ± 0.038 min^{-1}.

Binding of [^{3}H]-DPCPX to renal cell membranes from rats with acute renal failure

Plasma urea concentrations Plasma urea concentrations remained unchanged 30 min after glycerol injection. However, 16 and 48 h after the induction of ARF with glycerol there were statistically significant ($P<0.05$) elevations, 500 and 560% respectively, in plasma urea concentrations relative to saline-injected controls (Table 2). Similarly, there was a statistically significant ($P<0.05$) increase in plasma urea concentration (590%) 48 h after the administration of $HgCl_2$ relative to the control group.

Table 1 Binding characteristics of renal adenosine A_1 receptors in untreated rats and rats injected with either saline, glycerol or $HgCl_2$

Group	K_d (nM)	B_{max} (fmol mg^{-1} protein)
Untreated	0.62 ± 0.17	19.9 ± 2.5
Saline (i.m.) 0.5 h	0.58 ± 0.08	11.9 ± 1.3†
Glycerol (i.m.) 0.5 h	0.39 ± 0.06	11.2 ± 0.7
Saline (i.m.) 16 h	0.30 ± 0.02	7.5 ± 1.1††
Glycerol (i.m.) 16 h	0.73 ± 0.05*	20.6 ± 2.0*
Saline (i.m.) 48 h	0.33 ± 0.02	13.1 ± 2.6
Glycerol (i.m.) 48 h	1.20 ± 0.05*	31.2 ± 2.6*
Saline (s.c.) 48 h	0.51 ± 0.06	18.2 ± 2.1
$HgCl_2$ (s.c.) 48 h	0.82 ± 0.16	22.7 ± 5.9

Values are given as estimate ± s.e. estimate (8 d.f.) from 3 experiments. Each experiment was with 4 to 6 replicates with pooled membranes from 4 rats. †$P<0.05$, ††$P<0.01$ relative to untreated rats; *$P<0.01$ relative to saline controls. (Student's *t* test).

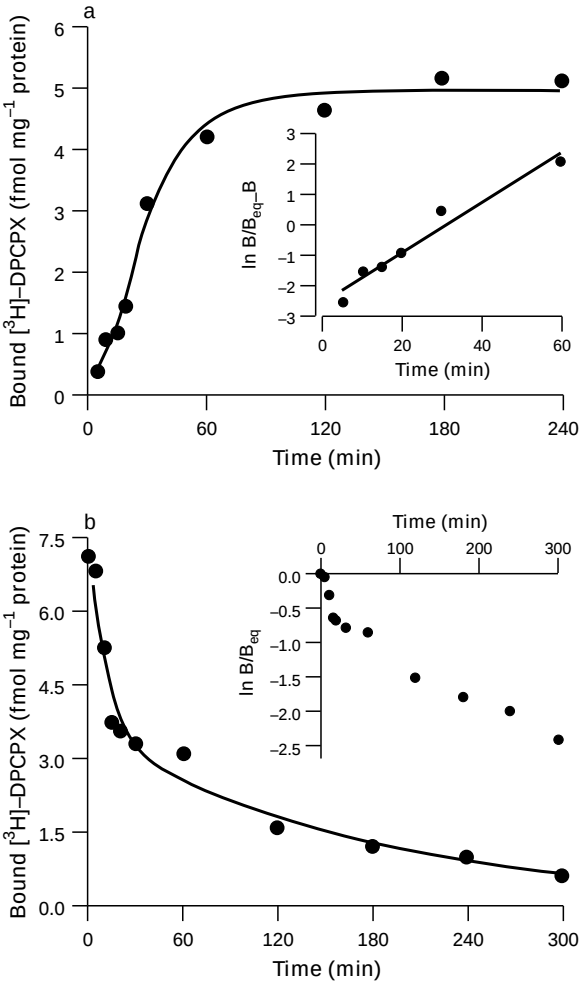

Figure 2 Association (a) and dissociation (b) kinetics for [^{3}H]-DPCPX binding to renal cell membranes. Association was carried out with 0.4 nM [^{3}H]-DPCPX and dissociation was induced by addition of 1 μM cold ligand. Points are the means of 2 experiments carried out with 4 replicates. Cell membranes were produced from the kidneys of 8 animals. The inserts show plots of $\ln B/B_{eq} - B$ or $\ln B/B_{eq}$ versus time where B and B_{eq} are the concentrations of specifically bound drug at a given time and specifically bound drug at equilibrium, respectively.

Glycerol-induced acute renal failure Typical binding isotherms for the interaction of [^{3}H]-DPCPX with renal membranes isolated from rats with glycerol-induced ARF or time-matched controls are depicted in Figure 3. Analysis of saturation isotherms obtained from binding to cell membranes from saline-injected controls revealed that at 0.5 and 16 h, there were significant ($P<0.01$) decreases in B_{max} in comparison to untreated animals (Table 1). K_d values at 16 and 48 h in saline-injected rats were also lower than K_d from untreated animals but these differences did not attain statistical significance ($P>0.05$). A comparison of saturation isotherms obtained after the induction of ARF with time-matched saline controls, showed no statistically significant ($P>0.05$) change in B_{max} at 0.5 h; but by contrast, at 16 and 48 h after the induction of ARF, there were

Table 2 Plasma urea concentrations in rats following injection of saline, glycerol or mercuric chloride ($HgCl_2$)

Binding studies

Group ($n=12$)	*Plasma urea* (mg 100 ml^{-1})	*Group* ($n=12$)	*Plasma urea* (mg 100 ml^{-1})
Saline (i.m.) 0.5 h	47±8	Glycerol (i.m.) 0.5 h	40±6
Saline (i.m.) 16 h	38±2	Glycerol (i.m.) 16 h	231±17*
Saline (i.m.) 48 h	39±6	Glycerol (i.m.) 48 h	258±67*
Saline (s.c.) 48 h	32±2	$HgCl_2$ (s.c.) 48 h	220±40*

Quantitation of mRNA studies

Group ($n=5$)	*Plasma urea* (mg 100 ml^{-1})	*Group* ($n=5$)	*Plasma urea* (mg 100 ml^{-1})
Saline (i.m.) 0.5 h	52±5	Glycerol (i.m.) 0.5 h	64±13
Saline (i.m.) 16 h	51±4	Glycerol (i.m.) 16 h	216±30*
Saline (i.m.) 48 h	41±2	Glycerol (i.m.) 48 h	299±89*
Saline (s.c.) 48 h	37±2	$HgCl_2$ (s.c.) 48 h	377±23**

Values are given as mean±s.e.mean. *$P<0.05$, **$P<0.001$ (Student's t test) relative to respective saline-injected control group.

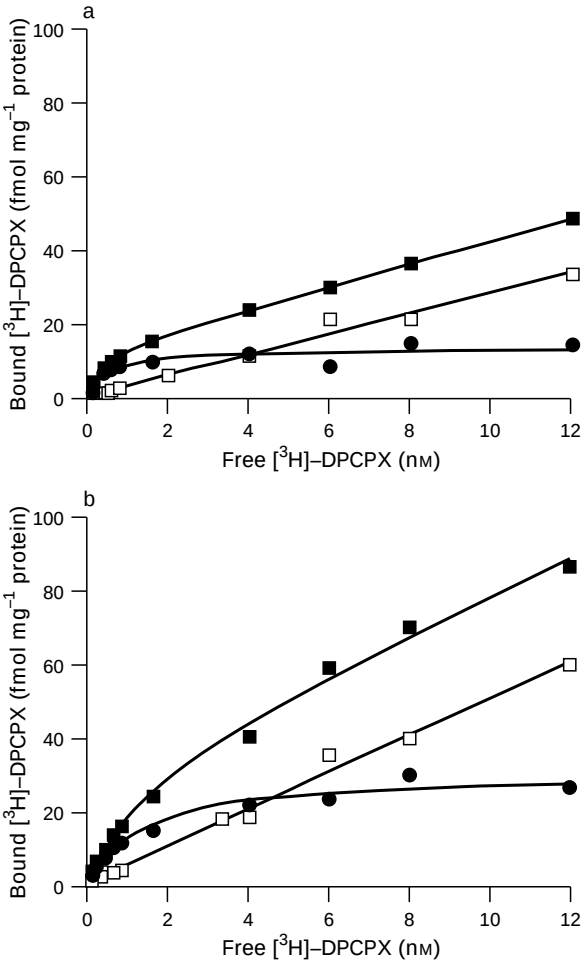

Figure 3 Total (■), specific (●) and non specific (□) binding of [^{3}H]-DPCPX to renal cell membranes 48 h after injection of saline (a) and glycerol (b). Points are the means obtained from 3 experiments carried out with 4 replicates. Cell membranes were obtained from the kidneys of 12 rats. Non specific binding of [^{3}H]-DPCPX to cell membranes from both groups of rats ranged from about 26% of total binding at 0.4 nM to 75% at 12 nM.

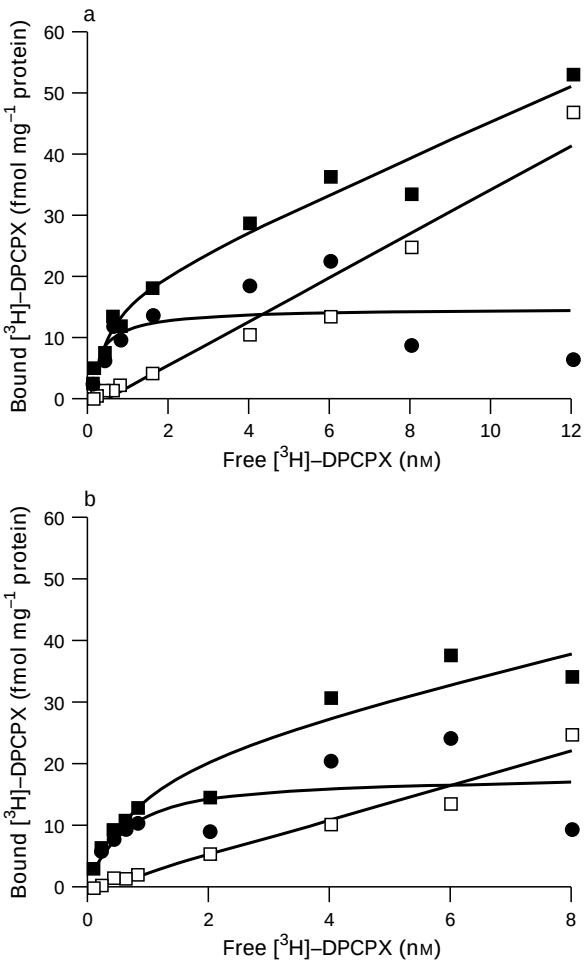

Figure 4 Total (■), specific (●) and non specific (□) binding of [^{3}H]-DPCPX to renal cell membranes 48 h after injection of saline (a) and $HgCl_2$ (b). Points are the means obtained from 3 experiments carried out with 4 replicates. Cell membranes were obtained from the kidneys of 12 rats. Non specific binding of [^{3}H]-DPCPX to cell membranes from both groups of rats ranged from about 11% of total binding at 0.4 nM to 71% at 12 nM.

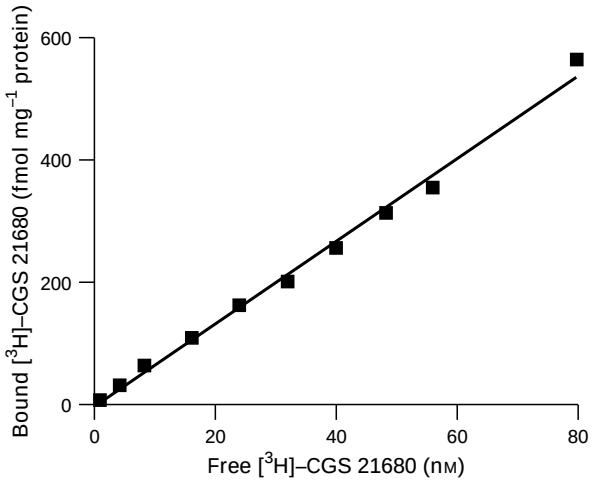

Figure 5 Binding of [^{3}H]-CGS21680 to renal cell membranes. Points are the means obtained from 2 experiments carried out with 6 replicates. Cell membranes were produced from the kidneys of 8 animals.

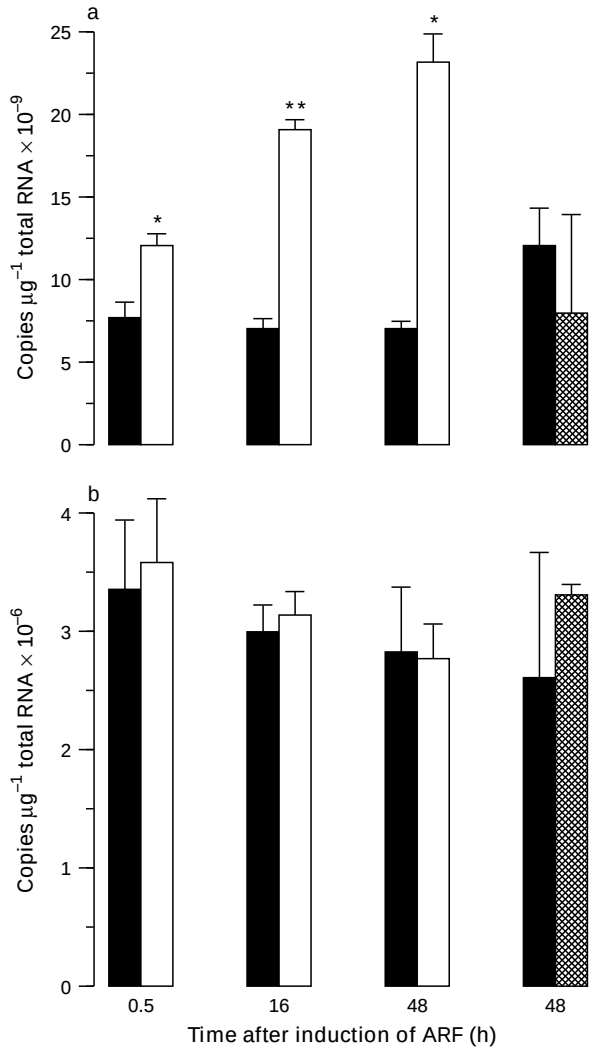

Figure 6 Levels of adenosine A_1 receptor (a) and β-actin (b) mRNA in the kidneys of rats following the induction of acute renal failure (ARF) with either glycerol or $HgCl_2$. Solid columns indicate mRNA levels in saline-injected controls; open columns, levels in glycerol-injected rats and hatched columns, levels in $HgCl_2$-injected rats. Columns show mean values $\pm$ s.e.mean. $^*P<0.05$; $^{**}P<0.001$ relative to respective saline-injected control group (Student's t test).

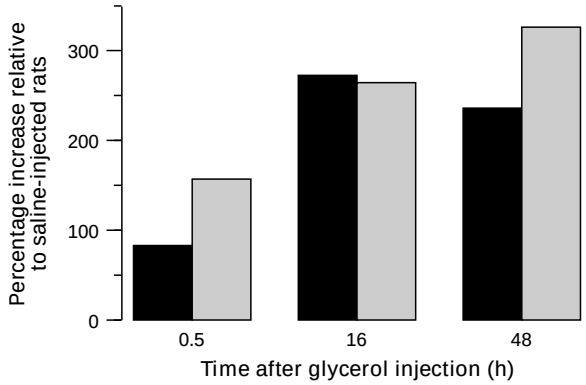

Figure 7 The percentage increase in receptor density, B_{max} (solid columns) and mRNA levels (shaded columns) for the adenosine A_1 receptor in kidneys of rats during the development of glycerol-induced acute renal failure. The percentage increases are relative to values from respective time-matched saline-injected groups and are derived from mean data shown in Table 1 and Figure 6.

statistically significant ($P<0.01$) increases in B_{max} (Table 1). A similar pattern of change occurred for K_d. Thirty minutes after glycerol injection there was no detectable change in K_d. However, when compared to time-matched controls, statistically significant ($P<0.01$) increases in K_d were found after 16 and 48 h of ARF (Table 1).

$HgCl_2$-induced ARF Figure 4 shows some representative binding isotherms for the interaction of [^{3}H]-DPCPX with renal membranes from rats given either $HgCl_2$ or saline. Table 1 lists the values of K_d and B_{max} obtained. By contrast to the increase in binding parameters found for rats 48 h after the initiation of glycerol-induced ARF, no statistically significant ($P>0.05$) changes in either K_d or B_{max} were detected 48 h after the induction of ARF by $HgCl_2$ in comparison to saline-injected controls. Furthermore, B_{max} and K_d for saline-injected controls were not significantly ($P>0.05$) different from values estimated from the untreated group (Table 1).

Binding of [^{3}H]-CGS21680 to renal cell membranes The relationship between bound and unbound [^{3}H]-CGS21680 is shown in Figure 5. Binding exhibited a linear dependence on ligand concentration and no saturable binding was detected.

Quantitation of adenosine A_1 receptor mRNA levels

The plasma urea concentrations in rats with ARF used in these experiments showed similar increases to those noted in the binding experiments (Table 2). Renal adenosine A_1 receptor mRNA levels were significantly ($P<0.05$) elevated at 0.5, 16 and 48 h following induction of glycerol-induced ARF when compared to time-matched controls (Figure 6a). Levels of adenosine A_1 receptor mRNA increased progressively during the development of ARF such that at 48 h there was a three fold increase relative to saline-injected rats. However, whilst mRNA levels at 48 h were significantly higher than those measured at 0.5 h ($P<0.05$, ANOVA) there was no significant difference ($P>0.05$, ANOVA) between mRNA levels at 48 and 16 h. The relationship between increases in B_{max} for the binding of [^{3}H]-DPCPX to renal adenosine A_1 receptors and receptor mRNA is shown in Figure 7. In general, the increases in receptor density and mRNA levels show a similar pattern, although the increase in receptor mRNA at 0.5 h and 48 h is proportionally greater. By contrast to rats with glycerol-induced ARF, no statistically significant ($P>0.05$) changes in adenosine A_1 receptor mRNA levels were detected 48 h following injection of $HgCl_2$ compared to saline controls (Figure 6a). Furthermore, there were no significant ($P>0.05$) differences in the levels of β-actin mRNA in kidneys of rats with either glycerol or $HgCl_2$-induced ARF compared to their respective controls (Figure 6b).

Discussion

In the present study, specific binding of [^{3}H]-DPCPX to cell membranes from the rat kidney was found. The labelled binding sites appear to be adenosine A_1 receptors since binding was saturable; the equilibrium K_d was similar to both the K_d calculated from the kinetic experiments and the K_i for unlabelled DPCPX, and binding was not inhibited by the selective A_{2a} agonist CGS 21680. Moreover, the estimate of K_d (0.62 nM) is comparable to that found for the A_1-receptor subtype in tubule membranes from rat medullary thick ascending limb (0.25 nM, Weber *et al*., 1990) and membranes from other rat tissues, such as brain (0.42 nM, Bruns *et al*., 1987; 0.45 nM, Klotz *et al*., 1990). Although the distribution of adenosine A_1 receptors in rat kidney was not examined here, Weaver & Reppert (1992) found rat adenosine A_1 receptor mRNA was most abundant in the juxtaglomerular apparatus of cortical nephrons, and collecting ducts in the medulla and papilla. Moreover, ligand binding and autoradiography studies with either cell membranes or thin sections of guinea-pig (Weber *et al*., 1988), human (Palacios *et al*., 1987), pig (Blanco *et al*., 1992) and rabbit kidneys (Freissmuth *et al*., 1987) suggest that A_1-receptors are principally located in the medulla and glomeruli of the mammalian kidney.

The association of [^{3}H]-DPCPX with rat renal membranes was described by a mono-exponential function. This indicates binding had a single kinetic element and indeed, the Scatchard plot for equilibrium binding appeared linear. By contrast, analysis of dissociation data revealed two kinetic states. Casado *et al*. (1994) also observed that the interaction of [^{3}H]-DPCPX with membranes from the cortex of the pig brain exhibits two distinct kinetic components, and suggested that these are not detected on analysis of binding isotherms because the K_d values for the two states are similar. Casado *et al*. (1994) found the presence of the two kinetic components seemed to be related to the ionic strength of the buffer used; when the kinetics of binding were measured with 9 mM Tris-HCl buffer both association and dissociation kinetics had two components, but with a 50 mM Tris buffer, the concentration used in the present study, only dissociation data showed two kinetic phases and, with a 108 mM buffer, both the association and dissocation data were described by a single exponential phase.

By contrast to [^{3}H]-DPCPX, [^{3}H]-CGS 21680 did not label any specific binding sites on membranes from whole rat kidney, but there is evidence from functional studies (Agmon *et al*., 1993) and from investigations of adenosine receptor gene expression (Weaver & Reppert, 1992) that A_{2a}-receptors are present in the rat kidney. Failure to detect specific binding is unlikely to be due to inappropriate experimental conditions because specific binding to membranes from whole rat brain has been found with the same conditions employed here (unpublished observations). It is possible, therefore, that with membranes from the whole kidney the density of receptors was below the limit of detection with [^{3}H]-CGS 21680.

Glycerol-induced ARF was accompained by significant increases in B_{max} values for the binding of [^{3}H]-DPCPX to renal cell membranes. No significant change in B_{max} was noted from binding studies with kidneys of rats with $HgCl_2$-induced ARF, although there were similar elevations in plasma urea concentrations in the two forms of ARF, indicative of an equivalent degree of acute renal dysfunction. A previous study of renal vascular responses to adenosine showed that falls in renal blood flow produced by close renal arterial injections of adenosine, mediated by A_1 receptors (Spielman & Arend, 1991), were significantly enhanced 16 and 48 h but not 0.5 h following induction of ARF with glycerol (Gould *et al*., 1995). By contrast, no change in the renal constrictor response to adenosine was noted 48 h after the induction of ARF with $HgCl_2$ (Gould *et al*., 1995). The time-course for the increase in adenosine A_1 receptor density in rats with glycerol-induced ARF, i.e. significant increases at 16 and 48 h but not 0.5 h, was similar to that of the enhanced renal constrictor response to adenosine (Gould *et al*., 1995). Thus, the increased constrictor response to adenosine in glycerol-induced ARF may result from an increase in renal adenosine A_1 receptor density. However, binding studies were conducted with renal cell membranes obtained from the whole kidney and vascular resistance segments comprise only a small proportion of renal tissue. As discussed previously, *in situ* hybridization studies of A_1 receptor mRNA indicate that A_1 receptors in the rat kidney are located in the collecting ducts and glomeruli (Weaver & Reppert, 1992). Whilst the latter structure contains a high proportion of vascular elements, it is possible that the increase in density of adenosine A_1 receptors in the whole kidney in glycerol-induced ARF is a result, at least in part, of a change in receptor number in tubular structures which comprise a large proportion of renal mass.

Adenosine and angiotensin II produce vasoconstriction of afferent arterioles in a synergistic manner (Schnermann *et al*., 1991) and therefore, the elevated plasma renin activity noted 8–48 h following induction of glycerol-induced ARF (Hollenberg *et al*., 1983) may also contribute to the enhanced renal constrictor effects of adenosine. Moreover, the increase in B_{max} for the binding of [^{3}H]-DPCPX to renal cell membranes from rats with glycerol-induced ARF was accompanied by an increase in K_d indicating a reduction in affinity of renal adenosine A_1 receptors. The functional significance of such a change in affinity states of adenosine A_1 receptors for the renal vasoconstrictor effect of adenosine is difficult to predict, particularly when, as in rats with glycerol-induced ARF, it is associated with an increase in receptor density.

In addition to changes in adenosine A_1 binding characteristics in rats with glycerol-induced ARF, the saline-injected control animals showed statistically significant decreases in B_{max} in relation to untreated rats. This indicates that 24 h of water deprivation *per se* induces changes in the density of adenosine A_1 receptors in the kidney. This is further supported by the absence of any such changes in the saline-injected control group for experiments with $HgCl_2$-induced ARF, since these rats were not subjected to withdrawal of water. The decrease in adenosine A_1 receptor density may result from the action of vasopressin since levels of this hormone are elevated in the dehydrated state (Robertson & Berl, 1996).

Measurement of mRNA levels for the adenosine A_1 receptor revealed that these were significantly increased in kidneys of rats with glycerol-induced ARF at all the time points examined. By contrast, 48 h following the induction of ARF with $HgCl_2$, no significant change in mRNA levels was noted. The elevations in B_{max} and mRNA levels for renal adenosine A_1 receptors in rats with glycerol-induced ARF were of a similar magnitude and time course with the exception of 0.5 h (Figure 7). At this earliest time point, there was a statistically significant rise in mRNA levels but not in B_{max}. However, it could be anticipated that a rise in mRNA levels would occur before mRNA translation and insertion of the receptor protein in the cell membrane. The results of the mRNA measurements indicate that the increase in receptor density of renal A_1 receptors in glycerol-induced ARF is a function of mRNA translation and not due to a reduction in receptor internalisation. Moreover, the absence of any change in adenosine A_1 mRNA levels in the kidneys of rats with $HgCl_2$-induced ARF together with no change in mRNA levels for β-actin, an abundant cytoskeletal protein, provides evidence that the increase in A_1 receptor density is not part of a general response of the kidney to insult.

In conclusion, this study has shown that glycerol-induced ARF in the rat is associated with an increase in renal adenosine A_1 receptor density which appears to result from increased transcription of the gene for this receptor. An increase in adenosine A_1 receptor density in renal resistance vessels may explain, at least in part, the enhanced renal vasoconstrictor response to adenosine in glycerol-induced ARF.

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