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Differential Expression of Renal Adenosine A_1 Receptors Induced by Acute Renal Failure

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ABSTRACT. The distribution of renal adenosine A_1 receptors was investigated in rats with glycerol- or mercuric chloride ($HgCl_2$)-induced acute renal failure. Receptors were localised by autoradiography using [^{3}H]8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX), a selective A_1 adenosine receptor antagonist. In saline-injected control animals, significant labelling with [^{3}H]DPCPX was detected in glomeruli, the inner stripe of outer medulla, and the inner medulla. Sixteen hours following induction of glycerol-induced acute renal failure (ARF), a 34% increase in labelling in glomeruli was noted compared to saline-injected controls, and by 48 hr, glomerular labelling had increased by 200%. In addition, 48 hr following glycerol injection, significant labelling was now detected in the cortical labyrinth and medullary rays whilst, in the inner medulla, labelling had decreased by 34%. By contrast to glycerol-induced ARF, the only significant change noted 48 hr following induction of $HgCl_2$-induced ARF was a 39% decrease in labelling in the inner medulla. It is concluded that glycerol-induced ARF results in differential expression of renal adenosine A_1 receptors with increased expression in the cortex and reduced expression in the inner medulla. Increased density of A_1 receptors in glomeruli may account, at least in part, for the increased renal vasoconstrictor response to adenosine and depressed glomerular filtration rate noted previously in this type of acute renal failure. BIOCHEM PHARMACOL **59**;6:727–732, 2000.

KEY WORDS. kidney; adenosine A_1 receptor; [^{3}H]8-cyclopentyl-1,3-dipropylxanthine; autoradiography; acute renal failure; mercuric chloride

Adenosine is involved in the regulation of various aspects of renal function including renal blood flow, glomerular filtration rate, and sodium reabsorption [1, 2]. In addition to physiological roles within the kidney, adenosine is an important haemodynamic mediator of some forms of ARF†. Administration of adenosine antagonists, particularly selective A_1 adenosine receptor antagonists, has been shown to ameliorate acute renal dysfunction induced in animals by myohaemoglobinuria (produced by intramuscular glycerol injection) [3–5], cisplatin [6], ischaemia [7], or hypoxia [8]. Furthermore, adenosine mediates, at least in part, the renal haemodynamic effects of radiocontrast media since, in both animals [9] and man [10], the adenosine antagonist theophylline reduces the fall in glomerular filtration rate produced by injection of contrast media. However, studies in the rat have shown that adenosine antagonists do not affect the extent and duration of the acute impairment in renal function produced by $HgCl_2$ [11, 12], gentamicin [13], or cyclosporine [14]. 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 $HgCl_2$ [12]. These findings indicate that adenosine's pathophysiological role in some forms of ARF is a consequence of its enhanced renal vasoconstrictor action. Renal vasoconstriction is mediated by the adenosine A_1 receptor subtype [1], and the binding capacity (B_{max}) of A_1 receptors and levels of receptor mRNA in whole kidneys are increased in glycerol- but not $HgCl_2$-induced ARF [15]. These findings suggest that enhanced renal vasoconstriction in glycerol-induced ARF is a consequence of up-regulation of A_1 receptors in the renal vasculature. However, increases in B_{max} and mRNA levels were noted with renal membranes and total mRNA prepared from whole kidneys that would contain material from both vascular and tubular elements [15]. The current study employed autoradiography to further investigate renal A_1 adenosine expression in rats with glycerol- and $HgCl_2$-induced ARF with the aim of identifying regional changes in receptor density.

MATERIALS AND METHODS

Materials

[^{3}H]DPCPX (120 Ci/mmol) was obtained from DuPont. DPCPX, polyethylenimine, phenylmethylsulphonyl fluo-

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† *Abbreviations:* ARF, acute renal failure; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; and $HgCl_2$, mercuric chloride.

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TABLE 1. Plasma urea concentrations in rats at various times following injection of saline or induction of acute renal failure (ARF) with either glycerol or mercuric chloride ($HgCl_2$)

Saline groups	Plasma urea (mg/100 mL)	ARF groups	Plasma urea (mg/100 mL)
(i.m.) 0.5 hr	17 ± 3	Glycerol 0.5 hr	18 ± 1
(i.m.) 16 hr	10 ± 1	Glycerol 16 hr	69 ± 7*
(i.m.) 48 hr	14 ± 2	Glycerol 48 hr	132 ± 13†
(s.c.) 48 hr	13 ± 1	$HgCl_2$ 48 hr	164 ± 12†

Results are given as means ± SEM. *P* values (Student's *t*-test) are relative to respective saline-injected rats.
*$P < 0.05$.
†$P < 0.001$.

ride, and adenosine deaminase (type V) were obtained from Sigma Chemical Co.

Animal Preparation

The method for induction of ARF with glycerol has been described previously [16]. Male Wistar rats (220–300 g) were deprived of drinking water for 24 hr, and ARF was produced by an intramuscular injection of 50% v/v glycerol in sterile saline (10 mL/kg). Control animals were dehydrated and injected with sterile saline (10 mL/kg). 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 $HgCl_2$ in sterile saline (2 mL/kg), whereas control rats received an equivalent volume of saline [12]. 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 using a diagnostic kit supplied by Sigma Chemical Co.

Preparation of Tissues

Rats were anaesthetised with sodium thiopentone (120 mg/kg 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 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 0.1 M sodium phosphate buffer, pH 7.4, containing 0.32 M sucrose for 2 min at 4°. Kidneys were then frozen in isopentane cooled in liquid nitrogen. Tissue sections (20 μm) were cut at −18° with a cryostat (Bright), thaw-mounted onto poly-L-lysine-coated slides and stored at −70° until required. Protein concentration in tissue sections was determined by the method of Lowry *et al.* [17] with bovine albumin as the standard. Sections of kidney were taken from rats 30 min, 16 hr, and 48 hr following intramuscular injection with glycerol or saline, and 48 hr following subcutaneous injection with $HgCl_2$. These times were selected to correspond with times used in previous studies of glycerol and $HgCl_2$-induced ARF that investigated renal vascular reactivity to adenosine [12] and renal adenosine A_1 receptor binding and mRNA levels [15].

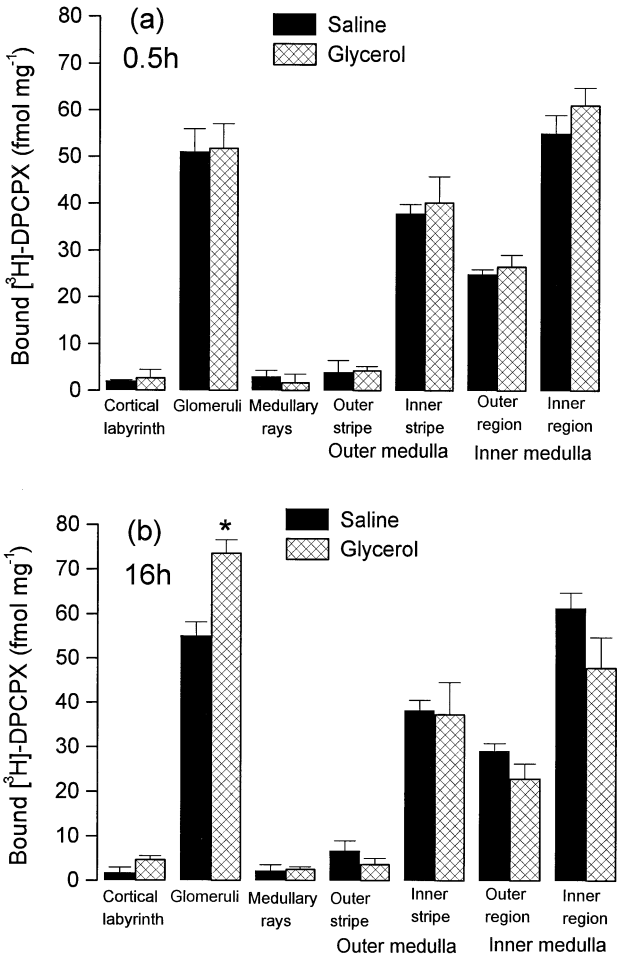

FIG. 1. Binding density of [^{3}H]DPCPX to various regions of kidneys of rats 0.5 hr (a) and 16 hr (b) following saline or glycerol injection. Binding density was determined in 20-μm sections of the kidney. Ten readings of grain density (subsequently converted to binding density) were made in each kidney region with the exception of glomeruli, where 20 readings were taken, for at least 3 sections per rat with 3–5 rats per group. Columns show mean values + SEM (N = 3–5). *$P < 0.01$ relative to the respective saline-injected group (Student's *t*-test).

Autoradiography

Tissue sections were pre-incubated at 37° in the presence of 1 U/mL adenosine deaminase [18], 0.1 mM phenylmethylsulphonyl fluoride, and 0.005% (v/v) polyethylenimine for 30 min, followed by a 4-hr incubation at 4° in the presence of 0.3 nM [^{3}H]DPCPX. Non-specific binding was determined by incubating consecutive tissue sections with 1 mM theophylline. Sections were washed twice for 15 min in ice-cold 50 mM Tris buffer, pH 7.4, rinsed in distilled water, and dried in a stream of cold air. The sections were apposed to coverslips coated in LM1 nuclear emulsion (Amersham International) and left to expose at 4° for 12 weeks in lightproof boxes. After exposure, the emulsion-

coated coverslips were developed in Kodak-D19 (Kodak) for 4 min at 18°, rinsed for 30 secs in distilled water, fixed for 5 min in Ilford Hypam (Ilford), and finally rinsed in running water for 10 min. Consecutive sections were counterstained using haematoxylin and eosin. Autoradiograms and counterstained sections were viewed under dark- and bright-field illumination, respectively, using a Leitz Dialux 20 microscope. Autoradiographic grain densities were quantified with a Leitz MPV compact microscope photometer. Images were captured using a Leica Quantimet 500+ image processing and analysis system. With the exception of glomeruli, ten readings of grain density were made in each kidney region for at least 3 sections per rat. Twenty readings of grain density were made for glomeruli in each section, as the sectioning process resulted in a greater variation in grain density in such small discrete structures. The average background grain density was also determined and subtracted from sample values. Grain density was converted to the quantity of bound ligand by comparison with autoradiograms of ^3H standards (Amersham International) [19]. Renal structures and regions were identified as described by The Renal Commission for The International Union of Physiological Sciences [20]. These regions/structures were the cortical labyrinth (excluding glomeruli), glomeruli, medullary rays, outer stripe of the outer medulla, inner stripe of the outer medulla, and inner medulla (divided into outer and inner regions of equivalent size based on autoradiographic grain density).

Analysis of Data

Data are given as means ± SEM and statistical comparison of means was made using Student's unpaired *t*-test. Differences were taken to be significant if $P < 0.05$.

RESULTS

Plasma Urea Concentration

Plasma urea concentration remained unchanged 30 min after glycerol injection. However, 16 and 48 hr after glycerol injection, there were 7- and 9-fold elevations, respectively, in plasma urea concentration relative to saline-injected controls (Table 1). Similarly, there was a 12-fold increase in plasma urea concentration 48 hr after the administration of $HgCl_2$ relative to the control group. There was no significant difference between plasma urea concentrations 48 hr following glycerol and $HgCl_2$ injection. These data indicate that injection of either glycerol or $HgCl_2$ reduced renal function and, at 48 hr, the degree of impairment of renal function was comparable.

Autoradiography

SALINE-INJECTED CONTROLS. Autoradiograms prepared from kidney sections taken from saline-injected controls for either glycerol- or $HgCl_2$-injected groups showed appreciable specific labelling with [^3H]DPCPX to glomeruli, inner stripe of the outer medulla, and both inner and outer regions of the inner medulla (see Figs. 1 and 2).

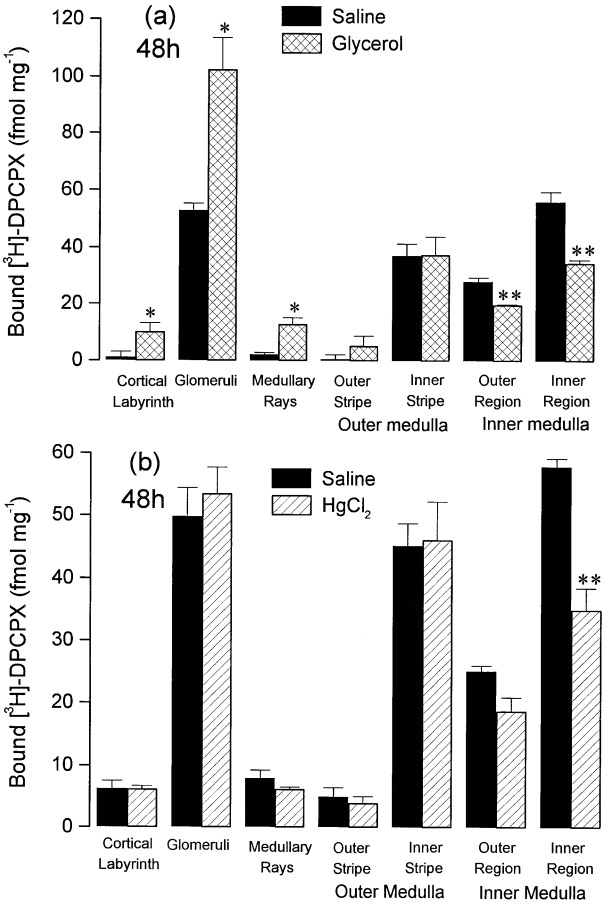

FIG. 2. Binding density of [^3H]DPCPX to various regions of kidneys of rats 48 hr following induction of acute renal failure by injection of either glycerol (a) or $HgCl_2$ (b). Binding density was determined in 20-μm sections of the kidney. Ten readings of grain density (subsequently converted to binding density) were made in each kidney region with exception of glomeruli, where 20 readings were taken, for at least 3 sections per rat with 3–5 rats per group. Columns show mean values + SEM (N = 3–5). *$P < 0.05$; **$P < 0.01$ relative to the respective saline-injected group (Student's *t*-test).

GLYCEROL-INDUCED ACUTE RENAL FAILURE. The pattern of expression of cortical A_1 receptors changed during the development of glycerol-induced ARF. Whilst no changes were noted at 30 min (Fig. 1a), at 16 hr there was a 34% increase in [^3H]DPCPX bound to glomeruli compared to saline-injected controls (Fig. 1b). Forty-eight hours following glycerol injection, further increases in labelling in the cortex were noted, with appreciable labelling now recorded in the cortical labyrinth and medullary rays, i.e. 10- and 6-fold increases, respectively, compared to the low density of labelling noted in saline-injected controls (Fig. 2a). Moreover, labelling in glomeruli showed a 2-fold increase. The enhanced labelling in the cortex 48 hr following glycerol injection is illustrated in typical autoradiograms

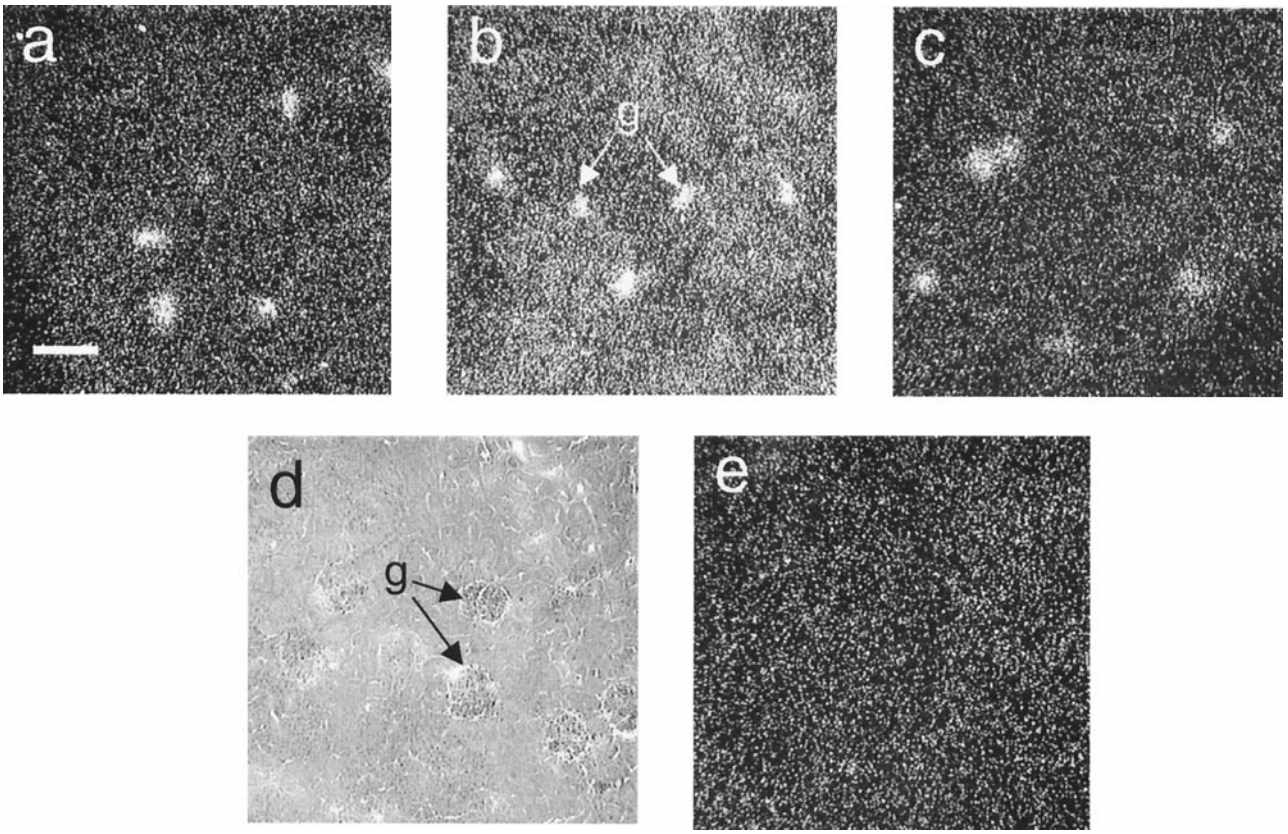

FIG. 3. Effect of acute renal failure on the distribution of adenosine A_1 receptors in the rat renal cortex. Panels (a–c) are autoradiograms showing the distribution of silver grains following incubation for 12 weeks with 0.3 nM [^{3}H]DPCPX. The autoradiograms were prepared from kidney sections taken 48 hr following i.m. saline injection (a) or induction of ARF with either injection of i.m. glycerol (b) or s.c. mercuric chloride (c). A consecutive section counterstained with haematoxylin and eosin from a saline-injected rat is shown in (d), whilst non-specific binding was assessed by determining the binding of [^{3}H]DPCPX in the presence of 1 mM theophylline (e). Key: g, glomeruli. Scale bar in A represents 200 μm with all images taken at the same magnification.

(Fig. 3). Changes in labelling were also noted in the medulla 48 hr following glycerol injection. However, by contrast to increases in receptor density observed in the cortex, decreases ranging from 29–38% were noted in both regions of the inner medulla (Fig. 2a). This reduced labelling is shown in typical autoradiograms in Fig. 4.

MERCURIC CHLORIDE-INDUCED ACUTE RENAL FAILURE. By comparison to glycerol-induced ARF, induction of ARF with $HgCl_2$ produced few changes in renal adenosine A_1 receptor labelling. The only statistically significant change noted 48 hr following induction of $HgCl_2$-induced ARF was a 39% decrease in labelling in the inner region of the inner medulla (Fig. 2b). The pattern of A_1 receptor distribution in kidneys of rats injected with $HgCl_2$ is illustrated in typical autoradiograms (Figs. 3c and 4c).

DISCUSSION

The current study indicates that ARF is associated with changes in the regional expression of adenosine A_1 receptors within the kidney. Greater changes in intrarenal distribution of receptors were noted in rats with ARF induced with glycerol compared to those given $HgCl_2$. A previous study of rats with glycerol-induced ARF, a model of myohaemoglobinuric ARF, found increases in renal A_1 receptor density and mRNA levels [15]. Statistically significant increases in receptor density of up to 2.7-fold were noted 16 and 48 hr following glycerol injection [15]. This study was conducted with cell membranes extracted from the whole kidney and therefore did not allow localisation of any changes in receptor density.

The current study revealed that the increase in receptor density in glycerol-induced ARF is restricted to the cortex, with increases in labelling with [^{3}H]DPCPX noted in the discrete structures of the glomeruli as well as in the general areas of the cortical labyrinth and medullary rays. The increase in density of labelling throughout the cortex suggests up-regulation of receptors in both tubular and vascular structures. A functional study using the selective A_1 agonist N^6-cyclohexyladenosine demonstrated the presence of A_1 receptors in the afferent arteriole [21]. These receptors mediate vasoconstriction, with the distal segment of the arteriole, which is partly contained within the glomerulus, exhibiting the greatest constrictor response [21]. Increased density of A_1 receptors in afferent arterioles, which account for the majority of preglomerular resistance [22], could explain the enhanced renal vasoconstrictor

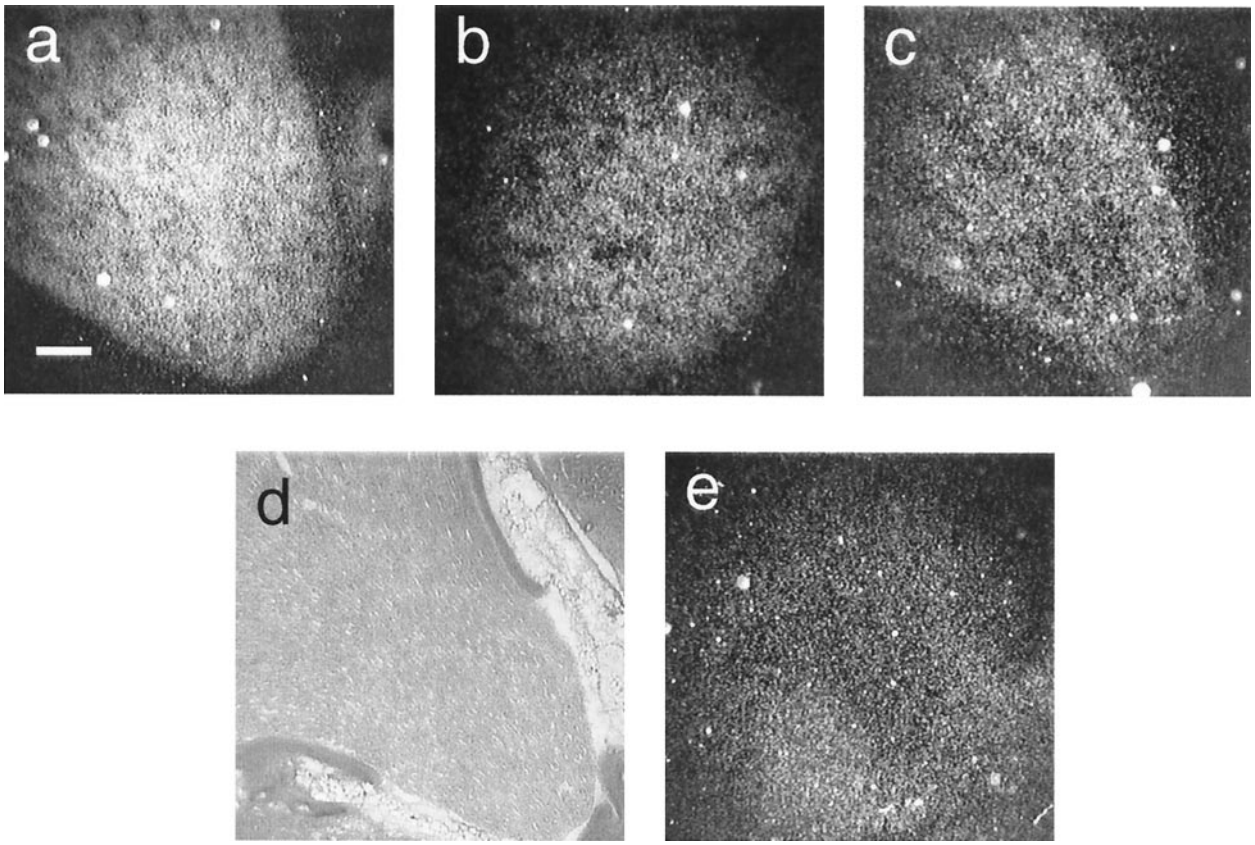

FIG. 4. Effect of ARF on the distribution of adenosine A_1 receptors in the rat renal inner medulla. Panels (a–c) are autoradiograms showing the distribution of silver grains following incubation for 12 weeks with 0.3 nM [^{3}H]DPCPX. The autoradiograms were prepared from kidney sections taken 48 hr following i.m. saline injection (a) or induction of ARF with either injection of i.m. glycerol (b) or s.c. mercuric chloride (c). A consecutive section counterstained with haematoxylin and eosin from a saline-injected rat is shown in (d), whilst non-specific binding was assessed by determining the binding of [^{3}H]DPCPX in the presence of 1 mM theophylline (e). Scale bar in A represents 500 μm with all images taken at the same magnification.

response to adenosine noted previously in glycerol-induced ARF [12]. No such accentuated renal vasoconstrictor response to adenosine was noted in rats with $HgCl_2$-induced ARF [12], and no alteration in the cortical density of [^{3}H]DPCPX labelling was detected in the kidneys of rats with this form of nephrotoxicity. In addition to the afferent arteriole, A_1 receptors mediating contraction have been identified in mesangial cells [23]. Up-regulation of A_1 receptors present in mesangial cells could contribute to the increased [^{3}H]DPCPX binding detected in the glomeruli of rats with glycerol-induced ARF.

By contrast to increased labelling in the cortex, a decrease in labelling was noted in the inner medulla of rats with glycerol-induced ARF. Moreover, the only change in adenosine receptor distribution observed in rats with $HgCl_2$-induced ARF was reduced labelling of the inner medulla. The most likely candidates for the labelled structures in the medulla are the collecting ducts, since *in situ* hybridisation studies showed that A_1 receptor mRNA in the rat kidney was most abundant in these tubular elements [24]. The magnitude of the reduction in [^{3}H]DPCPX labelling in the inner medulla (~38% in the inner region of the inner medulla) is similar to that observed previously (47%) for the inner medulla of kidneys from animals fed a high sodium chloride diet [25]. A high salt diet (4% sodium chloride content compared to 0.4% in a normal diet) results in a marked diuresis and increased sodium excretion [25]. Diuresis was observed 48 hr following the induction of glycerol-induced ARF [26], whilst a diuresis and natriuresis were found by Taria *et al.* [27] in rats 48 hr following $HgCl_2$ injection. Furthermore, since stimulation of A_1 receptors in the inner medullary collecting duct increases the availability of sodium channels [28], the effect of A_1 receptor down-regulation would be increased sodium excretion. Thus, the reduction in density of adenosine A_1 receptors in the inner medulla of rats with both glycerol- and $HgCl_2$-induced ARF may represent an adaptive response to the increased delivery of salt to distal regions of the nephron.

In conclusion, autoradiographic studies suggest that glycerol-induced ARF is associated with differential expression of renal adenosine A_1 receptors, with up-regulation in the cortex, particularly in glomeruli, and down-regulation in the inner medulla. The only change in regional distribution of A_1 receptors in the kidneys of rats with $HgCl_2$-induced ARF is a reduction in density in the inner medulla. Up-regulation of A_1 receptors in glomeruli is most probably due to increased expression of receptors in the sections of afferent arterioles contained within glomeruli and mesan-

gial cells. Such increases in receptor density may account for the increased renal vasoconstrictor response to adenosine and depressed glomerular filtration rate noted previously in this type of ARF and explain, at least in part, why this form of ARF can be ameliorated by treatment with adenosine antagonists.

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