Effects of adenosine receptor antagonists on the responses to contrast media in the isolated rat kidney

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ABSTRACT

Contrast media can induce both a decrease in renal blood flow and a reduction in glomerular filtration rate (GFR) when administered to both experimental animals and humans. In the present study we have examined the role of adenosine in mediating these effects using the isolated perfused rat kidney. Kidneys were perfused with a 6.7%-(w/v)-albumin-based perfusate supplemented with glucose and amino acids (n = 6 per group). They were exposed to diatrizoate [20 mg of iodine (mgI)/ml; osmolality 1650 mOsm/kg of water] or iotrolan (20 mgI/ml; osmolality 320 mOsm/kg of water) in the presence or absence of theophylline (10.8 l g/ml), or to diatrizoate in the presence or absence of a specific adenosine A1 receptor antagonist (KW-3902; 2 l g/ml) or a specific A2 receptor antagonist (KF17837; 6 l g/ml). Diatrizoate (n = 6) produced a fall in GFR from 0.65 ± 0.04 to 0.42 ± 0.03 ml [min]−1 [g]−1 (P < 0.05); renal perfusate flow (RPF) also declined, from 36.5 ± 3.8 to 22.0 ± 3.2 ml [min]−1 [g]−1 (P < 0.05). Iotrolan (n = 6) produced a fall in GFR from 0.64 ± 0.02 to 0.48 ± 0.04 ml [min]−1 [g]−1 (P < 0.05) and in RPF from 33.3 ± 3.8 to 24.0 ± 3.0 ml [min]−1 [g]−1 (P < 0.05). Theophylline (10.8 µg/ml) prevented the fall in GFR caused by either diatrizoate (baseline, 0.63 ± 0.05 ml [min]−1 [g]−1; diatrizoate + theophylline, 0.60 ± 0.04 ml [min]−1 [g]−1) or iotrolan (baseline, 0.64 ± 0.04 ml [min]−1 [g]−1; iotrolan + theophylline, 0.67 ± 0.05 ml [min]−1 [g]−1), but did not affect the decreases in RPF caused by either agent. KW-3902 (2 µg/ml) also prevented the fall in GFR produced by diatrizoate (baseline, 0.66 ± 0.05 ml [min]−1 [g]−1; diatrizoate + KW-3902, 0.61 ± 0.05 ml [min]−1 [g]−1), while the fall in RPF remained unaffected. KF17837 (6 µg/ml) had no effect on the decreases in either GFR or RPF induced by diatrizoate (n = 6 per group). The results suggest a role for adenosine acting at the A1 receptor in mediating the decrease in GFR induced by contrast media. This effect is independent of a change in renal vascular resistance, and possibly secondary to mesangial cell contraction causing a decrease in the ultrafiltration coefficient.

INTRODUCTION

Contrast media (CM) nephrotoxicity remains an important clinical problem, despite the use of modern low-osmolar CM. Patients with pre-existing renal insufficiency are particularly at high risk of developing CM nephrotoxicity. [1–3] The pathogenesis of CM nephrotoxicity is not fully understood and could be multifactorial [4–7]. Alterations in renal haemodynamics and direct tubular toxicity caused by CM are thought to be

Key words: adenosine, antagonists, contrast media, nephrotoxicity, glomerular filtration rate (GFR), rat kidney.
Abbreviations: CM, contrast media; GFR, glomerular filtration rate; IPRK, isolated perfused rat kidney; mgI, mg of iodine; RPF, renal perfusate flow.
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the primary factors in the effects on renal function [4–8]. CM can induce both a decrease in renal blood flow and a reduction in glomerular filtration rate (GFR) [4–9]. These effects could be due to modulation of the intrarenal synthesis and release of vasoactive mediators [10], such as nitric oxide (NO) [11], prostaglandins [12], endothelin [13] and adenosine [14]. We have investigated in the past the role of endothelin and NO in mediating the renal effects of CM [13,15]. These studies have shown that endothelin plays a crucial role in mediating the renal haemodynamic effects of CM. They have also shown that the release of NO from the endothelium of the renal blood vessels is not affected by CM. However, other studies have indicated that CM may reduce the activity of NO synthase in the renal cortex and decrease the synthesis of NO, leading to an increase in the resistance of the renal vasculature [16,17].

In the kidney, adenosine can induce both vasoconstriction and vasodilatation through A₁ and A₂ receptors respectively, and is a mediator of the tubuloglomerular feedback response [18]. The A₂ receptors are further subdivided into high-affinity A₂ₐ and low-affinity A₂ₐ receptors, based on pharmacological criteria [18]. A previous study has shown that the reduction in GFR induced by CM in dogs with renal insufficiency can be attenuated by pretreatment with theophylline, a non-selective antagonist of adenosine receptors, and abolished by a selective A₁ receptor antagonist [14]. The same study also demonstrated that the initial renal vasodilatation induced by CM is mediated by adenosine A₂ receptors. Clinical experience, although limited, also suggests that theophylline, a non-selective adenosine receptor antagonist, may offer some protection against the renal impairment associated with CM administration [19,20]. However, in one study a significant reduction in creatinine clearance remained following administration of high-osmolar CM, in spite of the prophylactic use of theophylline, suggesting that there may be other mechanisms involved [19].

In the present study we have examined the effects of a non-selective adenosine receptor antagonist, theophylline, a selective adenosine A₁ receptor antagonist, KW-3902 [8-(noradamant-3-yl)-1-3-dipropylxanthine] [21], and a selective adenosine A₂ receptor antagonist, KF17837 [E,1,3-dipropyl-8-(3,4-dime-thoxystyryl)-7-methylxanthine] [22], on the renal response to both high-osmolar (diatrizoate; 1650 mOsM/kg of water) and iso-osmolar (ioteolran; 320 mOsM/kg of water) CM in the isolated perfused rat kidney (IPRK). This ex vivo system allows the study of the direct local effects of exogenous substances on the kidney without any interference by systemic and nervous influences. This model has been utilized successfully by our group to study the pathophysiology of the renal effects of CM [8,9,13,23], amino acids [24,25] and vasoactive mediators [26].

**METHODS**

**IPRK model**

Male Wistar rats (400–450 g; University of Sheffield strain) were anaesthetized with thiopentone sodium (125 mg/kg, intraperitoneal). The right ureter was cannulated for the collection of urine. The right renal artery was cannulated by means of a non-ischaemic technique via the superior mesenteric artery. The right kidney was then removed and attached to a recirculating perfusion apparatus, as described previously [13,23]. The kidney was perfused ex vivo at a constant pressure of 100 mmHg (recorded continuously from within the renal artery) with an oxygenated (95%O₂/5% CO₂) perfusion medium based on Krebs–Henseleit solution containing 6.7% (w/v) BSA, 5.5 mM glucose and 14 mM mixed amino acids. [¹⁴C]Inulin (1 μCi; 37,000 Bq) was added to the perfusate. The perfusion solution temperature was maintained at 37 °C. Urine and perfusate samples were collected at 5 min intervals.

GFR was assessed as the renal clearance of [¹⁴C]inulin. Renal perfusate flow (RPF) was monitored by means of the continuous recording of pump speed. Both parameters were expressed per g wet weight of the non-perfused kidney, as well as a percentage of the value obtained in the 5 min before addition of CM. GFR and RPF were both stable in this preparation for a period of 150 min in experiments carried out in the absence of any CM or antagonist (GFR, 0.62 ± 0.05 to 0.63 ± 0.06 ml·min⁻¹·g⁻¹; RPF, 29.5 ± 0.7 to 30.8 ± 0.9 ml·min⁻¹·g⁻¹; n = 6), as observed previously [9,13,23].

**Experimental protocol**

Each kidney was perfused for 120 min. The first 40 min were an equilibration period, followed by an 80 min experimental period during which serial measurements of RPF and GFR were made. A continuous infusion of angiotensin II (5 ng/min) was sustained throughout the experiment to enhance the vascular tone of the preparation [13,23].

A 10 ml aliquot of high-osmolar diatrizoate or isoosmolar ioteolran was added to the perfusate reservoir at 20 min into the experimental period (thereafter regarded as time 0) to give a concentration of 20 mg of iodine (mgI)/ml in the perfusate. This dose has been used previously by our laboratory as one that elicits a significant renal response in the IPRK, and was not chosen to simulate clinical doses. Renal function was monitored for a further 60 min. Experiments with each contrast medium were repeated after pretreatment with pharmacologically effective doses of theophylline (10.8 µg/ml; KW-3902 (2 µg/ml; 5.6 µM) or KF17837 (6 µg/ml; 14.5 µM) [21,22], which were added to the perfusion system 10 min before the addition of contrast medium.

Ex vivo pilot studies in an identical preparation of the
isolated rat kidney showed that the inhibitory affinity against adenosine-induced vasoconstriction has the order KW-3902 > KF17837 > theophylline. The potency of KW-3902 was approx. 20 times that of KF17837, while the potency of KF17837 was approx. 2 times that of theophylline. Hence it was demonstrated that KF17837 had a lower affinity for the $A_1$ receptor than KW-3902. In previous binding studies in rat tissues the selectivity of the $A_2$ antagonist KW-3902 for the $A_1$ receptor gave a $K_i$ ratio ($A_1/A_2$) of 0.0011, while KF17837 gave a $K_i$ ratio ($A_1/A_2$) of 62, indicating a much greater degree of selectivity of KF17837 for the $A_2$ receptor [22].

The addition of antagonists to the perfusate did not affect either GFR or RPF, which remained stable during this pretreatment period. Measurements of $[^{14}\text{C}]$inulin clearance and RPF were expressed as a percentage of the value obtained in the 5 min before addition of CM.

### Materials

Iotrolan (Iovist 300; Schering AG, Berlin, Germany), diatrizoate (Urografin 325; 300 mgI/ml; diluted with sterile water; Schering AG), thiopentone sodium (Intraval; May & Baker, Dagenham, Essex, U.K.), $[^{14}\text{C}]$inulin (Amersham International, Little Chalfont, Bucks., U.K.), mixed amino acids (Vamin 14; Kabi Pharmacia, Uppsala, Sweden), angiotensin II, DMSO and theophylline (Sigma, Poole, Dorset, U.K.) were from the suppliers indicated. KW-3902 and KF17837 were supplied by Dr Fumio Suzuki (Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co. Ltd, Shizuoka, Japan). Both these antagonists were dissolved in DMSO/0.9 % NaCl. The final concentration of DMSO was not greater than 0.8 %.

### Statistical analysis

Results were expressed as absolute values and as a percentage of control values, and are means ± S.E.M. Within-group comparisons were made by performing a Dunnet’s $t$-test, and between-group comparisons were made by performing analysis of variance using Excel or Minitab software. A change was considered significant at $P < 0.05$.

### RESULTS

#### Effects of diatrizoate in the IPRK

Diatrizoate alone produced biphasic changes in both GFR ($[^{14}\text{C}]$inulin clearance) and RPF, consisting of a transient increase followed by a sustained decrease (Figures 1A and 2A). At 1 h after the addition of diatrizoate (20 mgI/ml) to the perfusion system, GFR had declined from 0.65 ± 0.04 to 0.42 ± 0.03 ml·min$^{-1}$·g$^{-1}$ ($n = 6, P < 0.05$) and RPF had declined from 36.5 ± 3.8 to 22.0 ± 3.2 ml·min$^{-1}$·g$^{-1}$ ($n = 6, P < 0.05$) (Figures 1B and 2B).

#### Effects of theophylline on the renal response to diatrizoate

Theophylline itself had no significant effect on renal function in the IPRK; only a slight non-significant increase in basal RPF of 4.5 ± 2.9 ml·min$^{-1}$·g$^{-1}$ ($n = 12$) was observed. In the presence of theophylline, the sustained reduction in GFR following diatrizoate treat-
ment was abolished. GFR was $0.63 \pm 0.05$ ml min$^{-1}$ g$^{-1}$ at baseline and $0.60 \pm 0.04$ ml min$^{-1}$ g$^{-1}$ at the end of the observation period ($n = 6$) (Figures 1A and 1B). By contrast, the decline in RPF was unaffected by theophylline pretreatment, with diatrizoate administration resulting in a decrease in RPF from a baseline value of $34.5 \pm 4.0$ ml min$^{-1}$ g$^{-1}$ to $19.9 \pm 4.1$ ml min$^{-1}$ g$^{-1}$ at the end of the experiment (Figures 2A and 2B).

**Effects of iotrolan in the IPRK**

Iotrolan (20 mg/l/ml) alone produced a biphasic effect on GFR: a transient increase followed by a sustained decrease (Figure 3A). At 1 h after the addition of iotrolan to the perfusion system, GFR had declined from $0.64 \pm 0.02$ to $0.48 \pm 0.04$ ml min$^{-1}$ g$^{-1}$ ($n = 6, P < 0.05$) (Figure 3B). Unlike diatrizoate, iotrolan produced only a sustained decrease in RPF, without an initial transient increase in the flow (Figure 4A). At 1 h after the addition of iotrolan, RPF had declined from $33.3 \pm 3.8$ to $24.0 \pm 3.0$ ml min$^{-1}$ g$^{-1}$ ($n = 6, P < 0.05$) (Figure 4B).

**Effects of theophylline on the renal response to iotrolan**

In the presence of theophylline, the effect of iotrolan on GFR was abolished, and no significant change was observed ($0.64 \pm 0.04$ ml min$^{-1}$ g$^{-1}$ at baseline;
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Figure 4 Effects of theophylline on the iotrolan-induced fall in RPF
(A) Time course showing the effects of iotrolan (20 mg/l/ml) on RPF (% of control) in the IPRK in the absence (■) and presence (●) of theophylline (10.8 μg/ml). Significance of differences: *P < 0.05 compared with baseline values. (B) Effect of iotrolan (20 mg/l/ml) on RPF (ml·min⁻¹·g⁻¹) in the IPRK during the final 5 min of perfusion. Open bar, control; stippled bar, iotrolan alone; hatched bar, iotrolan in the presence of theophylline (10.8 μg/ml). Significance of differences: *P < 0.05 compared with control group. Results are means ± S.E.M. (n = 6 per group).

Figure 5 Effects of KW-3902 on the diatrizoate-induced fall in GFR
(A) Time course showing the effects of diatrizoate (20 mg/l/ml) on GFR (% of control) in the IPRK in the absence (■) and presence (●) of KW-3902 (2 μg/ml). Significance of differences: *P < 0.05 compared with baseline values. (B) Effect of diatrizoate (20 mg/l/ml) on GFR (ml·min⁻¹·g⁻¹) in the IPRK during the final 5 min of perfusion. Open bar, control; stippled bar, diatrizoate alone; hatched bar, diatrizoate in the presence of KW-3902 (2 μg/ml). Significance of differences: *P < 0.05 compared with control group; ns, not significant. Results are means ± S.E.M. (n = 6 per group).

Effects of the selective adenosine A₁ receptor antagonist KW-3902 on the renal response to diatrizoate
KW-3902 itself had no effect on renal function in the IPRK. In the presence of KW-3902, diatrizoate had no effect on GFR (0.66 ± 0.05 ml·min⁻¹·g⁻¹ at baseline; 0.61 ± 0.05 ml·min⁻¹·g⁻¹ at the end of the observation period; n = 6) (Figures 5A and 5B). The reduction in RPF induced by diatrizoate was unaffected by KW-3902; a decrease from 34.8 ± 4.0 ml·min⁻¹·g⁻¹ at baseline to 26.4 ± 3.7 ml·min⁻¹·g⁻¹ at the end of the experiment (n = 6, P = 0.05) was observed (Figures 6A and 6B).

Effects of the selective adenosine A₂ receptor antagonist KF17837 on the renal response to diatrizoate
KF17837 itself had no effect on renal function in the IPRK. In the presence of KF17837, no change was observed in the diatrizoate-induced decreases in both GFR and RPF.
GFR and RPF (Figures 7 and 8; n = 6 per group). The maximal initial transient increase in GFR observed following diatrizoate was reduced by KF17837, although this did not reach statistical significance (Figure 7).

**DISCUSSION**

In the present study we have demonstrated an important role for adenosine in mediating the decrease in GFR induced by both diatrizoate (high-osmolar CM) and iotrolan (iso-osmolar CM) in the IPRK. Theophylline, a non-selective adenosine antagonist, prevented the decreases in GFR induced by diatrizoate and iotrolan, but had no effect on the changes in RPF. The affinities of theophylline for adenosine receptors, as assessed by $K_i$ (dissociation constant) values, are: $A_1$, 8.5 μM; $A_2A$, 25.3 μM; $A_2B$, 4.8 μM [27]. Therefore theophylline at a concentration of 10.8 μg/ml (60 μM) should block the effects of adenosine at $A_1$, $A_2A$ and $A_2B$ receptors. Theophylline is a non-specific antagonist of adenosine receptors. In addition, it can also inhibit cyclic nucleotide phosphodiesterase, leading to accumulation of cAMP and hence causing vasodilatation. However, this occurs
only at theophylline concentrations in the millimolar range [18] and is unlikely to be a factor in the pharmacological effect of theophylline in the present experiments, where a dose in the micromolar range was employed.

KW-3902, a selective adenosine A1 receptor antagonist, also prevented the decrease in GFR induced by diatrizoate, but had no effect on the change in RPF, although there did appear to be a trend in favour of KW-3902. The possibility cannot be ruled out that the effect may be merely a type II error and a consequence of inherent variation in the system, although the group sizes used are sufficient to give a high study power in such experiments. The selective adenosine A2 receptor antagonist KF17837 did not alter either the reduction in GFR or the change in RPF induced by diatrizoate.

The affinities of both selective antagonists for receptor types other than adenosine receptors and phosphodiesterases has been studied; both showed no affinity [22,28], although specific receptors such as those for angiotensin II and endothelin were not tested. The specificity of the antagonists was not tested in this model per se, although the inhibitory effects of the three antagonists used were tested against the vasoconstrictive response to adenosine in the IPRK preparation. The ability to inhibit a vasoconstrictive response was in the rank order KW-3902 > KF17837 > theophylline. None of the adenosine antagonists studied afforded protection against the fall in RPF induced by diatrizoate, suggesting that this phenomenon is independent of the involvement of adenosine. However, the results suggest that the decrease in GFR induced by CM is mediated by adenosine through A1 receptors, and that A2 receptors have no role in mediating the observed effects. The prevention by theophylline of the decrease in GFR caused by the isosmolar iotrolan suggests that the release of adenosine is not an entirely osmolar-dependent response to CM.

In an in vivo study of the effects of adenosine receptor antagonists on renal responses to the low-osmolar contrast medium iohexol, Arakawa et al. [14] found that iohexol-induced decreases in GFR and effective RPF could be ameliorated with theophylline or the A1 antagonist KW-3902. The present study on the IPRK confirms this beneficial effect of theophylline and KW-3902 on the fall in GFR, but we did not see a protective effect of these agents on the fall in RPF. The discrepancy between our observations and those of Arakawa et al. [14] cannot be fully explained, and may reflect differences in experimental approach. Previous studies have shown differences in the renal response to adenosine between in vivo and ex vivo experimental models [29,30]. While exogenous adenosine induces vasodilatation in the IPRK, infusion of adenosine in vivo in the dog produced a brief renal vasoconstriction [31]. This was followed by a return of the renal blood flow to a value at or above the control level [31]. Despite the return of blood flow toward pre-infusion levels during the continued infusion of adenosine, GFR generally remains depressed [31,32]. Since adenosine induces only a transient renal vasoconstriction, it is unlikely to be an important mediator of the sustained decrease in RPF induced by CM. Recent studies have indicated that endothelin is an important mediator of the sustained renal vasoconstriction induced by CM [6,7].

Studies directed at the mechanism of the adenosine-induced fall in GFR have demonstrated a contraction of mesangial cells and a fall in glomerular hydrostatic pressure resulting from a preglomerular vasoconstriction.
and a more slowly developing postglomerular vasodilation [33]. Adenosine constricts afferent glomerular arterioles and mesangial cells via the A1 receptor and dilates efferent arterioles via the A2 receptor [33–36]. The effect on mesangial cells would cause a decrease in the ultrafiltration coefficient (Kf), which is a product of the filtering surface area and its permeability to water. An alteration in Kf due to mesangial cell contraction and/or glomerular permeability can alter GFR without a need for alterations in RPF [37]. The ability to prevent the decrease in GFR without altering the RPF in the present study suggests that adenosine may exert its effects predominantly on the glomerular mesangial cells following CM administration.

It has been observed previously using adenosine antagonists [35,38] and/or angiotensin II antagonists/angiotensin-converting enzyme inhibitors [35,38,39] that adenosine and angiotensin II co-operate in a mutually dependent and synergistic fashion to produce vasoconstriction in the renal microvasculature, particularly via afferent arteriolar constriction [35]. Indeed, it has been proposed that the ability of endogenous adenosine to reduce renal blood flow is dependent on the presence of angiotensin II and a functioning angiotensin II receptor system [38,40]. In this respect the angiotensin II pre-constricted state of our IPRK preparation may provide ideal conditions under which to investigate adenosine-induced renal responses. However, despite these favourable conditions, adenosine antagonists failed to produce statistically significant amelioration of the reduction in RPF produced by CM. This provides further support for a role of mesangial cell contraction, as opposed to renal vasoconstriction, in CM-induced falls in GFR, as discussed above.

We have demonstrated previously in IPRK studies that the CM-induced falls in both RPF and GFR can be completely abolished by endothelin antagonists [13,23]. The results of these studies suggest that endothelin is a crucial mediator of the renal haemodynamic effects of CM [6,7]. The biological interaction between adenosine and endothelin has not been adequately investigated. A previous study has shown that the stimulation of adenosine A1 receptors evokes the release of endothelin from rat thyroid FRTL-5 cells [41]. More recently, it was reported that endothelin-induced bronchoconstriction is enhanced by adenosine [42]. However, the mechanism of interaction between endothelin and adenosine in the kidney is not known. It is feasible that adenosine can induce the release of endothelin in the kidney both directly, by stimulating the A1 receptors, and indirectly, secondary to renal vasoconstriction. Clinically, blocking the effects of endothelin with ET1 receptor antagonists may prove to be more worthwhile than blocking those of adenosine with A1 antagonists, as endothelin antagonism would tend to prevent not only the fall in GFR, but also that in renal perfusion, induced by CM.

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