Amelioration of glycerol-induced acute renal failure in the rat with 8-cyclopentyl-1,3-dipropylxanthine

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1 Previous studies have shown that 8-phenyltheophylline (8-PT), a non-selective antagonist at adenosine A₁- and A₂-receptors, can ameliorate the severity of glycerol-induced acute renal failure (ARF) in the rat. In the present study we have examined the effects of an antagonist with selectivity for adenosine A₁-receptors (8-cyclopentyl-1,3-dipropylxanthine, CPX) on the development of ARF.

2 In the anaesthetised rat 8-PT (4 mg kg⁻¹, i.v.) and CPX (0.1 mg kg⁻¹, i.v.) antagonised adenosine-evoked responses which are thought to be mediated via A₁-receptors (bradycardia and decrease in renal blood flow). The agonist dose-ratio produced by CPX was equal to or greater than that found with 8-PT (heart rate and renal blood flow respectively). The hypotensive response to adenosine which is predominantly due to A₂-receptor activation was also antagonised by 8-PT, whereas CPX was a much less effective antagonist of this response.

3 Administration of CPX (0.1 mg kg⁻¹, i.v.; twice daily for two days) significantly attenuated the increase in plasma levels of urea and creatinine, the increased kidney weight and the renal tubule damage observed in rats 2 days following induction of ARF with intramuscular glycerol injection. In addition treatment with CPX significantly enhanced the clearances of inulin and *p*-aminohippurate.

4 After glycerol injection, the mortality rate over 7 days in untreated and vehicle-treated rats was 43% and 21% respectively. In contrast, all animals treated with CPX survived over the 7 day observation period.

5 These results support the suggestion that adenosine is an important factor in the development of ARF and indicate that this effect of the purine is likely to be mediated via an adenosine A₁-receptor.

Introduction

Recent studies in the rat have demonstrated that administration of the alkylxanthine 8-phenyltheophylline (8-PT) can reduce the severity of acute renal failure (ARF) produced by intramuscular glycerol injection (Bowmer *et al.*, 1986; Yates *et al.*, 1987; Bowmer *et al.*, 1988). We have suggested that the beneficial effects of 8-PT are due to its ability to block adenosine receptors, particularly those which mediate vasoconstriction of the afferent renal arterioles. This proposal is supported by the observation that in the initial stage of glycerol-induced ARF renal blood flow is reduced (Stein *et al.*, 1978) and the resultant ischaemia is likely to evoke release of adenosine (Miller *et al.*, 1978). In addition, ARF is associated with a fall in glomerular filtration rate, an effect which is also produced by an infusion of aden-

osine (Churchill, 1982). Furthermore, blockade of adenosine uptake with dipyrindamole augments the impairment in renal function associated with various models of ARF (Arend *et al.*, 1987; Lin *et al.*, 1987).

The renal vasculature possesses both A₁- and A₂-adenosine receptors and activation of A₁-receptors mediates arteriolar constriction, whereas activation of A₂-receptors mediates arteriolar dilatation (Murray & Churchill, 1984). Thus, an A₁-selective antagonist may prove as beneficial in the treatment of ARF as 8-PT which has been found to block both sub-classes of adenosine receptor (Collis *et al.*, 1985). We have pursued this idea by studying 8-cyclopentyl-1,3-dipropylxanthine (CPX) which has been shown, *in vitro*, to be a selective A₁-antagonist (Haleen *et al.*, 1987; Collis *et al.*, 1989). Initial studies were concerned with evaluating the ability of CPX to antagonise adenosine-induced

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responses *in vivo* mediated predominantly by A_1 -receptors (heart rate and renal blood flow) or A_2 -receptors (blood pressure). In further experiments we investigated the effect of CPX on biochemical, functional and morphological indices of glycerol-induced ARF.

Methods

Evaluation of adenosine antagonism

Measurement of blood pressure and heart rate Male Wistar rats (300–350 g) were anaesthetised with thiobutabarbitalone (120 mg kg^{-1} , i.p.). Polyethylene cannulae were inserted into the left jugular vein for drug administration, into the left carotid artery for measurement of blood pressure and into the trachea. The rats were artificially respired ($90 \text{ strokes min}^{-1}$, 3.5–4.0 ml tidal volume) with air supplemented with oxygen. Body temperature was monitored via a rectal probe and maintained at $37\text{--}38^\circ\text{C}$ by heat lamps. Systemic blood pressure was recorded directly via a pressure transducer (Bell and Howell L221) and heart rate was derived electronically from the blood pressure signal.

After a 15–30 min stabilisation period, adenosine dissolved in saline (0.9% w/v NaCl) was administered via the venous catheter ($20\text{--}2000 \mu\text{g kg}^{-1}$, in 4–400 μl) with a micrometer controlled syringe. Blood pressure and heart rate were allowed to return to control values after each administration of adenosine.

Preliminary studies demonstrated that repeatable dose-response curves for adenosine in terms of its hypotensive and bradycardic responses could be obtained in this preparation. Dose-response curves to adenosine were then repeated in rats before and 15 min after administration of CPX (0.1 mg kg^{-1} , i.v.) or 8-PT (4 mg kg^{-1} , i.v.). CPX was prepared at 0.1 mg ml^{-1} in 1% v/v DMSO, 0.75% v/v 1 M NaOH in saline, and 8-phenyltheophylline (8-PT) at 10 mg ml^{-1} in 50% v/v 0.1 M NaOH and polyethylene glycol 400. These vehicles had no significant effects on adenosine-evoked vasodilatation and bradycardic responses in the anaesthetised rat.

Measurement of renal blood flow Male Wistar rats (200–250 g) were anaesthetised with thiobutabarbitalone (120 mg kg^{-1} , i.p.) and the trachea cannulated. A polyethylene cannula was inserted into the left jugular vein to allow a continuous infusion of 6% w/v dextrose (2.4 ml h^{-1}) delivered by a Sage Infusion pump. The right carotid artery was exposed and a cannula was passed down the artery and into the thoracic aorta to a position just above the branch of the left renal artery. The correct position of the cannula was verified at the end of the experiment on

post mortem examination. A flank incision was made and an electromagnetic flow probe (internal circumference 2.0 mm; Carolina Medical Electronics) was placed around the left renal artery. This was connected to an electromagnetic flow meter (Model FM 501, Carolina Medical Electronics) for a direct reading of renal blood flow (RBF). A polyethylene cannula was inserted into the left femoral artery and attached to a transducer for the measurement of blood pressure and heart rate.

Forty-five min after the completion of surgery, increasing bolus doses of adenosine (0.165 to $500 \mu\text{g}$ dissolved in saline) were given via the carotid cannula in a constant volume of $50 \mu\text{l}$ and the resultant fall in RBF was recorded. After initial doses of adenosine, rats received CPX (0.1 mg kg^{-1} , i.v.) or 8-PT (4 mg kg^{-1} , i.v.) and ten minutes later, responses to adenosine were recorded. In order to assess the shift of the dose-response curve produced by CPX or 8-PT, the dose-ratio of adenosine required to reduce RBF by 1.0 ml min^{-1} was estimated.

Evaluation of 8-cyclopentyl-1,3-dipropylxanthine in glycerol-induced acute renal failure

Induction of acute renal failure The method for inducing ARF has been described previously (Bowmer *et al.*, 1982). Briefly, male Wistar rats (200–300 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 rats were similarly dehydrated but injected with saline (10 ml kg^{-1}) instead of glycerol. Immediately after injection of either saline or glycerol, the drinking water was returned for the remainder of the experiment.

Experimental protocol Before the administration of glycerol or saline, a 0.75 ml control blood sample (0 h) was taken from the tail vein. Further blood samples were taken at 24 h from the tail vein and at 48 h from a carotid cannula following clearance determinations. Immediately after glycerol or saline injection, rats were treated with either CPX (0.1 mg kg^{-1}) or vehicle (1.0 ml kg^{-1} ; 1.0% v/v DMSO, 0.75% v/v 1 M NaOH in saline), administered via the tail vein. Additional doses of CPX or vehicle were also given i.v. at 12, 24 and 36 h after the initial dose. A group of saline-injected and a group of glycerol-injected rats received no treatment. At 48 h after injection of saline or glycerol, rats were anaesthetised and the clearances of [^{14}C]-*p*-aminohippuric acid (C_{PAH}) and [^3H]-inulin (C_{IN}) determined. At the end of the experiment rats were killed by an overdose of anaesthetic, the kidneys removed, weighed and prepared for histology.

Figure 1 The effect of 8-phenyltheophylline (8-PT) and 8-cyclopentyl-1,3-dipropylxanthine (CPX) on the heart rate (a) and mean blood pressure (b) response evoked by adenosine (i.v.) in the anaesthetised rat. (●) Control ($n = 6$), (▲) CPX 0.1 mg kg^{-1} ($n = 6$), (■) 8-PT 4 mg kg^{-1} ($n = 6$). Results are given as mean and vertical lines indicate s.e.mean.

In a separate series of experiments, a group of glycerol-injected rats and a group of rats treated with either vehicle or CPX as above were studied over a 7 day period. Blood samples were taken from the tail vein prior to and on the 2nd and 5th days after glycerol injection. A further blood sample was taken from a carotid cannula following determinations of C_{PAH} and C_{IN} on the 7th day.

Measurement of plasma urea and creatinine concentrations Plasma urea and creatinine concentrations were determined by standard spectrophotometric assays: urea by reaction with diacetyl monoxime and creatinine by reaction with picrate in alkaline solution.

Determination of $[\text{}^3\text{H}]$ -inulin and $[\text{}^{14}\text{C}]$ -p-aminohippuric acid clearances Rats were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} , i.p.). The trachea was cannulated to maintain a clear airway and rats allowed to breath spontaneously throughout the experiment. Cannulae were inserted into the left jugular vein and right carotid artery and the

animals were heparinised (500 i.u. kg^{-1} , i.v.). The single injection method of Hall *et al.* (1977) was then used to measure simultaneously the clearances of $[\text{}^3\text{H}]$ -inulin (100 mg kg^{-1} ; $20 \mu\text{Ci kg}^{-1}$, i.v.) and $[\text{}^{14}\text{C}]$ -p-aminohippurate (40 mg kg^{-1} ; $4 \mu\text{Ci kg}^{-1}$, i.v.).

Kidney histology At the end of the experiment, kidneys were removed, cleared of adherent tissue and their weights recorded. The kidneys were bisected longitudinally, washed and placed in formal saline (BDH). A longitudinal section was cut from one kidney of each rat and stained with haematoxylin and eosin. Sections were examined by a pathologist, who was unaware of the treatment the donor animal had received, and the extent of renal damage was assessed according to the scoring system previously described (Bowmer *et al.*, 1986). The degree of necrosis and presence of casts were each scored out of 5. The two scores for each kidney were added to give the total damage score (maximum 10).

Statistical methods Results are expressed as mean $\pm$ s.e.mean. Statistical comparison of creatinine, urea, C_{IN} , C_{PAH} and total kidney weight data were made by either an unpaired Student's *t* test or one way analysis of variance (ANOVA) with means being compared by the method of Least Significant Difference (Snedecor & Cochran, 1967). Statistical analysis of the histological damage score was made by a two-sided Mann-Whitney test. Other comparisons utilised an unpaired Student's *t* test.

Materials

Adenosine and 8-PT were purchased from Sigma Chemical Co. $[\text{}^3\text{H}(\text{G})]$ -inulin ($180\text{--}215 \text{ mCi g}^{-1}$) of stated radioactive purity 98–99% and p -[glycyl- ^{14}C]-aminohippuric acid ($46.4\text{--}57.6 \text{ mCi mmol}^{-1}$; purity 97.5–98%) were both obtained from New England Nuclear Ltd and were used without further purification. CPX was synthesised by Dr R. James of ICI. Kits for the assay of creatinine and urea were obtained from Pierce and Warriner and BDH Ltd, respectively.

Results

Evaluation of adenosine antagonism

Blood pressure and heart rate studies Adenosine evoked dose-related decreases in both mean blood pressure and heart rate of anaesthetised rats, such that at a dose of $600 \mu\text{g kg}^{-1}$ there was a reduction of about 30 and 50%, respectively, in these variables (Figure 1). CPX alone had no effect on resting heart rate (control = 371 ± 9 , CPX = 357 ± 9 beats

Figure 2 Decreases in renal blood flow (RBF) in response to bolus injections of adenosine given into the thoracic aorta of an anaesthetised rat before (●) and 10 min (■) following 8-cyclopentyl-1,3-dipropylxanthine (0.1 mg kg⁻¹ i.v.).

min⁻¹; $n = 6$), although mean arterial blood pressure was slightly, but significantly, decreased (control = 145 ± 7 , CPX = 129 ± 7 mmHg; $n = 6$, $P < 0.05$). Similarly, 8-PT did not significantly alter resting heart rate (control = 348 ± 9 , 8-PT = 337 ± 8 beats min⁻¹; $n = 6$), but it did produce a significant reduction in mean arterial blood pressure (control = 124 ± 7 , 8-PT = 111 ± 8 mmHg; $n = 6$, $P < 0.05$).

Both CPX (0.1 mg kg⁻¹) and 8-PT (4 mg kg⁻¹) significantly antagonised bradycardic responses evoked by all doses of adenosine (Figure 1). These effects of CPX and 8-PT were similar in magnitude as the log dose-ratios measured at a 15% decrease in heart rate were not significantly different (8-PT = 0.84 ± 0.10 , CPX = 0.75 ± 0.11). 8-PT also antagonised the hypotensive response evoked by all doses of adenosine. However, CPX was less potent in this respect and only caused a significant ($P < 0.05$) reduction in the response evoked by $600 \mu\text{g kg}^{-1}$ of the purine (Figure 1).

Renal blood flow Adenosine given via the thoracic aorta elicited dose-dependent falls in blood flow to the left kidney. The results of a typical experiment

are shown in Figure 2. Administration of the vehicle (1.0 ml kg⁻¹) for either alkylxanthine did not affect the response of renal blood flow to adenosine. Injection of CPX (0.1 mg kg⁻¹) did not alter basal renal blood flow (control = 5.13 ± 0.33 , CPX = 5.15 ± 0.36 ml min⁻¹; $n = 6$). Similarly administration of 8-PT (4 mg kg⁻¹) had no effect on basal renal blood flow (control = 5.60 ± 0.28 , 8-PT = 5.21 ± 0.47 ml min⁻¹; $n = 7$). Both alkylxanthines produced shifts to the right of the adenosine dose-response curve. The mean log-dose-ratio for the shifts produced by CPX was 1.47 ± 0.18 ($n = 6$) which was significantly greater ($P < 0.001$) than the shift produced by 8-PT (1.06 ± 0.07 ; $n = 7$). When larger doses of adenosine (50–500 µg) were given after CPX, falls in mean arterial blood pressure of up to 50 mmHg were noted. These changes in blood pressure occurred after the peak decreases in renal blood flow. By contrast, when the same doses of adenosine were injected after 8-PT, only small falls in mean arterial pressure were observed (5–10 mmHg). Administration of adenosine following either CPX or 8-PT resulted in only minor changes in heart rate (increases of 5–10 beats min⁻¹) which occurred in the dose range 165–500 µg.

Treatment with 8-cyclopentyl-1,3-dipropylxanthine

Two day study

Saline-injected rats The plasma urea levels of the CPX-treated group were significantly lower before any injections (0 h) and 24 h later compared to the untreated group (Table 1). The biological significance of this is questionable since the differences are relatively small and in addition, there was no difference in plasma creatinine concentrations between the two groups (Table 2). Kidney weight and morphology were unaffected by CPX treatment (Figure 4, Table 3). In addition, there was no change in C_{IN} , but by contrast, C_{PAH} was significantly increased by almost 50% (Figure 3).

Table 1 Plasma urea concentrations (mg 100 ml⁻¹) in saline and glycerol-injected rats treated by i.v. injection of either vehicle (1.0 ml kg⁻¹) or 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.1 mg kg⁻¹) twice daily for 2 days

	Saline-injected		Glycerol-injected		
	No treatment ($n = 8$)	CPX ($n = 8$)	No treatment ($n = 15$)	Vehicle ($n = 15$)	CPX ($n = 15$)
0 h	41 ± 1	33 ± 1^b	42 ± 1	45 ± 3	40 ± 1
24 h	38 ± 1	35 ± 1^a	213 ± 15^c	217 ± 15	$95 \pm 10^{*†}$
48 h	43 ± 3	39 ± 1	262 ± 19^c	273 ± 22	$94 \pm 8^{*†}$

Mean $\pm$ s.e.mean and number of rats in parentheses.

^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$, relative to saline-injected no treatment group (t test).

^{*} $P < 0.001$ relative to glycerol-injected no treatment group (ANOVA).

[†] $P < 0.001$ relative to glycerol-injected vehicle-treated group (ANOVA).

Table 2 Plasma creatinine concentrations ($\text{mg } 100 \text{ ml}^{-1}$) in saline and glycerol-injected rats treated by i.v. injection of either vehicle (1.0 ml kg^{-1}) or 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.1 mg kg^{-1}) twice daily for 2 days

	Saline-injected			Glycerol-injected	
	No treatment (<i>n</i> = 8)	CPX (<i>n</i> = 8)	No treatment (<i>n</i> = 15)	Vehicle (<i>n</i> = 15)	CPX (<i>n</i> = 15)
0 h	0.58 ± 0.04	0.57 ± 0.03	0.49 ± 0.01	0.52 ± 0.03	0.49 ± 0.02
24 h	0.56 ± 0.02	0.56 ± 0.01	3.07 ± 0.25^c	3.69 ± 0.29	$1.08 \pm 0.09^{*†}$
48 h	0.62 ± 0.06	0.55 ± 0.03	3.23 ± 0.41^c	4.15 ± 0.46	$0.85 \pm 0.08^{*†}$

Mean $\pm$ s.e.mean and number of rats in parentheses.

^c $P < 0.001$ relative to saline-injected no treatment group (*t* test).

^{*} $P < 0.001$ relative to glycerol-injected no treatment group (ANOVA).

[†] $P < 0.001$ relative to glycerol-injected vehicle treated group (ANOVA).

Glycerol-injected rats Following an injection of glycerol, plasma urea and creatinine concentrations increased five to six fold within 24 h and increased even further by 48 h (Tables 1 and 2). At the end of this 48 h period, C_{IN} and C_{PAH} were both reduced by about 75% compared to untreated, saline-injected rats (Figure 3). Moreover, kidney weight and histological damage were significantly increased after glycerol administration (Figure 4; Table 3). In glycerol-injected rats treated with vehicle, no changes above those produced by glycerol-injection alone were seen in any indices of renal function measured.

Plasma urea and creatinine concentrations in glycerol-injected rats treated with CPX were significantly lower at 24 and 48 h than those seen in vehicle and untreated rats. In contrast to these two groups, the elevated urea and creatinine levels in the CPX treated group did not increase further from 24 to

Table 3 Effect of i.v. treatment with either vehicle (1.0 ml kg^{-1}) or 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.1 mg kg^{-1} i.v.) administered twice daily for 2 days on kidneys from rats injected with saline or glycerol

Group	<i>n</i>	Damage score
Saline-injected no treatment	8	0.1 ± 0.3
Saline-injected CPX-treated	8	0.0 ± 0
Glycerol-injected no treatment	15	6.2 ± 0.2^c
Glycerol-injected vehicle-treated	15	5.5 ± 0.2
Glycerol-injected CPX-treated	15	$4.7 \pm 0.2^{*†}$

^c $P < 0.001$ relative to saline-injected no treatment.

^{*} $P < 0.001$ relative to glycerol-injected no treatment.

[†] $P < 0.05$ relative to glycerol-injected vehicle-treated (two tailed Mann-Whitney test).

Kidneys were fixed and sectioned 48 h after injection of either glycerol or saline. Maximum possible damage score = 10.

Figure 3 The clearances of (a) [^3H]-inulin (C_{IN}) and (b) [^{14}C]-*p*-aminohippurate (C_{PAH}) 48 h after injection of either saline or glycerol. Rats were given vehicle (1.0 ml kg^{-1}) or 8-cyclopentyl-1,3-dipropylxanthine (CPX) (0.1 mg kg^{-1}) twice daily i.v. for 2 days. Key to groups: (1) saline-injected, no treatment (*n* = 8); (2) saline-injected, CPX treated (*n* = 8); (3) glycerol-injected, no treatment (*n* = 15); (4) glycerol-injected, vehicle-treated (*n* = 15); (5) glycerol-injected, CPX-treated. Columns represent means and vertical bars s.e.mean. † $P < 0.01$; ‡ $P < 0.001$ relative to group 1 (*t* test). * $P < 0.001$ relative to group 3; † $P < 0.001$ relative to group 4 (ANOVA).

Figure 4 Total kidney weight of rats 48 h following an injection of either saline or glycerol. Rats were given vehicle (1.0 ml kg^{-1}) or 8-cyclopentyl-1,3-dipropylxanthine (CPX) (0.1 mg kg^{-1}) twice daily i.v. for 2 days. Key to groups: (1) saline-injected, no treatment ($n = 8$); (2) saline-injected, CPX-treated ($n = 8$); (3) glycerol-injected, no treatment ($n = 15$); (4) glycerol-injected, vehicle-treated ($n = 15$); (5) glycerol-injected, CPX-treated ($n = 15$). Columns represent means and vertical bars s.e.mean. * $P < 0.001$ relative to group 1 (t test). * $P < 0.001$ relative to group 3; † $P < 0.001$ relative to group 4 (ANOVA).

48 h (Tables 1 and 2). In rats injected with glycerol, C_{IN} was significantly greater in CPX-treated rats compared to vehicle and untreated animals and was 70% of the mean control value from saline-injected rats (Figure 3a). C_{PAH} was similarly larger in CPX-treated rats (Figure 3b) compared to untreated or vehicle-treated rats. The kidney weights of the CPX-treated group were significantly less than in the vehicle and untreated groups (Figure 4). Moreover, the degree of morphological damage associated with glycerol injection was also reduced ($P < 0.001$) following treatment with CPX (Table 3).

Seven day study Two days following glycerol injection, plasma urea and creatinine concentrations were much lower in the CPX-treated rats compared to untreated and vehicle treated animals (Tables 4 and 5). These findings are similar to those noted in the 48 h study (Tables 1 and 2). Between 2 and 5 days following the glycerol injection, mortality in untreated rats was 43% (8 out of 14 survived) and in vehicle-treated rats the mortality rate was 21% (11 out of 14 survived). In these two groups of rats there

Table 4 Plasma urea concentrations ($\text{mg } 100 \text{ ml}^{-1}$) in glycerol-injected rats treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.1 mg kg^{-1}) or vehicle (1.0 ml kg^{-1}) i.v. twice daily for 2 days and studied over 7 days

	No treatment ($n = 14$)	Vehicle ($n = 14$)	CPX ($n = 14$)
Day 0	43 ± 3	49 ± 2	47 ± 2
Day 2	215 ± 18 ($n = 8$)	210 ± 23 ($n = 11$)	$130 \pm 10^{**\dagger}$ ($n = 14$)
Day 5	163 ± 27	168 ± 29	$81 \pm 9^{*\dagger}$
Day 7	77 ± 11	100 ± 23	54 ± 5

Results are given as mean $\pm$ s.e.mean.

* $P < 0.05$; ** $P < 0.01$ relative to no treatment group (ANOVA).

† $P < 0.01$ relative to vehicle-treated group (ANOVA).

were no further fatalities between 5 and 7 days. In contrast, all 14 rats in the CPX group survived to 7 days. On day 5, plasma urea and creatinine concentrations were significantly reduced in the CPX-treated rats compared to the surviving animals in the untreated and vehicle-treated groups (Tables 4 and 5). Urea and creatinine levels on day 7 in the CPX-treated rats were also lower than the survivors in the other two groups, but the only statistically significant difference was that plasma creatinine levels in the CPX group were less than in the vehicle-treated rats.

C_{IN} and C_{PAH} in rats which received CPX were significantly greater than the clearance values recorded in either the untreated or vehicle-treated animals (Figure 5). The mean total kidney weight of rats injected with CPX ($3.87 \pm 0.18 \text{ g}$, $n = 14$) was significantly smaller ($P < 0.01$, ANOVA) than the mean

Table 5 Plasma creatinine concentrations ($\text{mg } 100 \text{ ml}^{-1}$) in glycerol-injected rats treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.1 mg kg^{-1}) or vehicle (1.0 ml kg^{-1}) i.v. twice daily for 2 days and studied over 7 days

	No treatment ($n = 14$)	Vehicle ($n = 14$)	CPX ($n = 14$)
Day 0	0.50 ± 0.04	0.59 ± 0.03	$0.44 \pm 0.03\$$
Day 2	3.34 ± 0.60 ($n = 8$)	3.58 ± 0.50 ($n = 11$)	$1.36 \pm 0.13^{**\$}$ ($n = 14$)
Day 5	2.11 ± 0.41	2.30 ± 0.58	$0.77 \pm 0.09^{*\dagger}$
Day 7	1.17 ± 0.20	1.54 ± 0.38	$0.53 \pm 0.04^\dagger$

Results are given as mean $\pm$ s.e.mean.

* $P < 0.05$; ** $P < 0.01$ relative to no treatment group (ANOVA).

† $P < 0.01$; § $P < 0.001$ relative to vehicle-treated group (ANOVA).

Figure 5 The clearances of (a) [³H]-inulin (C_{IN}) and (b) [¹⁴C]-*p*-aminohippurate (C_{PAH}) 7 days after glycerol injection. Rats were treated with either 8-cyclopentyl-1, 3-dipropylxanthine (CPX, 0.1 mg kg⁻¹ i.v.) or vehicle (1.0 ml kg⁻¹, i.v.) twice daily for 2 days following glycerol injection. Columns represent mean values and vertical bars s.e.mean. Group 1 received no treatment ($n = 8$), group 2 vehicle ($n = 11$) and group 3 CPX ($n = 14$). * $P < 0.05$, ** $P < 0.01$ relative to group 1; ‡ $P < 0.01$; § $P < 0.001$ relative to group 2 (ANOVA).

weight noted in untreated (5.29 ± 0.35 g, $n = 8$) or vehicle-treated rats (4.98 ± 0.29 g, $n = 11$).

Discussion

The results of this study demonstrate that the A₁-selective adenosine receptor antagonist CPX is effective in ameliorating some of the biochemical, functional and morphological correlates of ARF in the rat. These observations support the results of our previous investigation with 8-PT (Bowmer *et al.*, 1986) and together with those of Bidani & Churchill (1983), with theophylline, they provide further evidence that adenosine antagonism is beneficial in

ARF. The present findings also imply that it is activation of the A₁ sub-type of adenosine receptor which is involved in the pathogenesis of this disorder.

In vitro studies have shown that CPX is equi-effective with 8-PT as an A₂-receptor antagonist (Collis *et al.*, 1989), but that it has a 30–100 fold higher affinity for the A₁-receptor. In the present study we provide *in vivo* evidence that CPX is A₁-selective at 0.1 mg kg⁻¹. Thus CPX antagonised the bradycardic and the renal vasoconstrictor response evoked by adenosine in the anaesthetised animal. The bradycardic response is mediated via A₁-receptors in the sino-atrial node and perhaps on adrenergic nerve terminals innervating the node (Hedquist & Fredholm, 1979; Brown & Collis, 1983; Collis & Saville, 1984). The renal vasoconstrictor response is also elicited via A₁-receptors located on the afferent renal arterioles (Murray & Churchill, 1984). In contrast, CPX had little effect on hypotensive responses evoked by adenosine. This response is due mainly to intraluminal adenosine acting to relax vascular smooth muscle via an A₂-receptor (Collis & Brown, 1983). The small degree of antagonism of the hypotensive response to larger doses of the purine by CPX may have been due to blockade of a pre-junctional inhibitory effect of adenosine on adrenergic vascular neurotransmission or to an A₁-mediated decrease in cardiac output. By contrast to CPX, a dose of the non-selective antagonist 8-PT, which was chosen to match the effect of CPX on heart rate responses to adenosine, also antagonised the hypotensive effects of the purine at all doses administered.

CPX treatment had little effect on renal function in normal rats except for an increase in C_{PAH} . We have no immediate explanation for this change in C_{PAH} , but C_{PAH} is a measure of effective renal plasma flow and an increase would indicate a rise in renal blood flow. However, in the studies designed to assess adenosine antagonism, CPX did not affect basal renal blood flow when recorded directly with an electromagnetic flow probe. Recent studies with a non-xanthine adenosine antagonist, CGS 15943A, have also demonstrated antagonism of adenosine-evoked renal vasoconstriction without an effect on basal renal blood flow (Callis *et al.*, 1989). Moreover, the duration of action of CPX (0.1 mg kg⁻¹) in the conscious rat is 3–5 h (Collis *et al.*, unpublished results) whilst the increase in C_{PAH} was noted 12 h following the last dose of CPX. Thus the increase in C_{PAH} may not be directly related to adenosine antagonism. It is noteworthy that CPX administration resulted in no morphological damage in normal rats (Table 3). This is in contrast to 8-PT, since we found that this alkylxanthine produced a significant degree of kidney damage in normal rats

compared to treatment with its vehicle (Bowmer *et al.*, 1986).

In glycerol-treated rats, the progression of ARF over a 48 h and a 7 day period was similar to that previously described in this model of renal failure (Bowmer *et al.*, 1986). Thus, plasma urea and creatinine were elevated with decreases in C_{IN} and C_{PAH} . There was morphological evidence of renal damage, with tubular necrosis and cast formation and an increase in kidney weight, probably due to oedema, and deposition of haem pigments in the renal tubules. CPX treatment caused a marked and significant reduction in the severity of ARF over the 48 h period following glycerol injection. This amelioration of ARF was seen for all the variables measured. The beneficial effects of treatment with CPX for the first 2 days following glycerol administration persisted over the following 5 days. There was an appreciable mortality rate over this period in untreated (43%) and vehicle-treated (21%) rats but no deaths were noted in CPX-treated animals. The lower mortality rate in the vehicle-treated rats is surprising since vehicle treatment did not produce significant improvements in any of the indices of renal function when compared to untreated rats. Furthermore, at 7 days there was no significant difference in plasma urea, plasma creatinine, C_{IN} , C_{PAH} and kidney weight between the surviving animals in the untreated and vehicle groups. Those animals from the untreated and vehicle-treated groups which survived to 7 days were clearly recovering from ARF as plasma urea and creatinine levels and C_{IN} together with C_{PAH} were returning towards normal levels. However, rats which had been treated with CPX showed a more complete recovery.

The results of this study support the hypothesis put forward by Churchill & Bidani (1982) that adenosine is an important factor in the pathogenesis of glycerol-induced ARF. It is postulated that adenosine has a deleterious effect by increasing afferent arteriolar resistance, probably via an action at the A_1 -adenosine receptor. However, adenosine can also

have vasodilator effects in the kidney via A_2 -receptor activation (Murray & Churchill, 1984). This prompts the question, is an A_1 -selective adenosine antagonist more effective at ameliorating ARF than a non-selective antagonist? Some indication of the answer to this question can be gained from a comparison of the results obtained in this study with those we observed in a previous study with the non-selective antagonist 8-PT (Bowmer *et al.*, 1986).

To make a comparison between the effects of CPX and 8-PT in rats with ARF, it is necessary to show that the A_1 -antagonistic potency of the dose of CPX (0.1 mg kg^{-1}) used in this study was similar to that of the dose of 8-PT (10 mg kg^{-1}) used previously. Data in the present paper show that 4 mg kg^{-1} 8-PT is equi-effective with 0.1 mg kg^{-1} of CPX in terms of blocking A_1 -mediated bradycardic responses to adenosine and is slightly but significantly less potent as an antagonist of A_1 -mediated renal vasoconstriction. However, extrapolation of the dose-ratios obtained in the renal blood flow studies indicates that 10 mg kg^{-1} 8-PT and 0.1 mg kg^{-1} CPX would produce similar degrees of antagonism of the renal vasoconstrictor effects of adenosine. Thus, a comparison of the effects of 0.1 mg kg^{-1} CPX with those previously found for 10 mg kg^{-1} 8-PT (Bowmer *et al.*, 1986) appears valid. When compared to data from vehicle-treated rats with ARF, CPX caused a 66% decrease in plasma urea, an 80% decrease in plasma creatinine and a 300% increase in inulin clearance at the 48 h time point. The effect of 8-PT on these parameters was consistently less at this time after the induction of ARF (-50% , -40% and $+70\%$, respectively). This comparison, therefore, suggests that selective A_1 -adenosine receptor blockade may be more effective in ameliorating ARF than non-selective antagonism of both A_1 - and A_2 -receptors. However, confirmation of this would require a more detailed study of the relationship between dose, blockade of adenosine responses and beneficial effect of A_1 -selective and non-selective adenosine antagonists in ARF.

References

- AREND, L.J., BAKRIS, G.L., BURNETT, J.C., MEGERIAN, C. & SPIELMAN, W.S. (1987). Role for intrarenal adenosine in the renal hemodynamic response to contrast media. *J. Lab. Clin. Med.*, **110**, 406–411.
- BIDANI, A.K. & CHURCHILL, P.C. (1983). Aminophylline ameliorates glycerol-induced acute renal failure in the rat. *Can. J. Physiol. Pharmacol.*, **61**, 567–571.
- BOWMER, C.J., YATES, M.S. & EMMERSON, J. (1982). The effect of acute renal failure on the pharmacokinetics of indocyanine green in the rat. *Biochem. Pharmacol.*, **31**, 2531–2538.
- BOWMER, C.J., COLLIS, M.G. & YATES, M.S. (1986). Effect of the adenosine antagonist 8-phenyltheophylline on glycerol-induced acute renal failure in the rat. *Br. J. Pharmacol.*, **88**, 205–212.
- BOWMER, C.J., COLLIS, M.G. & YATES, M.S. (1988). Amelioration of glycerol-induced acute renal failure in the rat with 8-phenyltheophylline: timing of intervention. *J. Pharm. Pharmacol.*, **40**, 733–735.
- BROWN, C.M. & COLLIS, M.G. (1983). Adenosine A_1 receptor mediated inhibition of nerve stimulation-induced contractions of the rabbit portal vein. *Eur. J. Pharmacol.*, **93**, 277–282.
- CALLIS, J.T., KUAN, C.-J., BRANCH, K.R., ABELS, B.C., SABRA, R., JACKSON, E.K. & BRANCH, R.A. (1989). Inhibition of renal vasoconstriction induced by intrarenal

- hypertonic saline by the nonxanthine adenosine antagonist CGS 15943A. *J. Pharmacol. Exp. Ther.*, **248**, 1123–1129.
- CHURCHILL, P.C. (1982). Renal effects of 2-chloroadenosine and their antagonism by aminophylline in anaesthetised rats. *J. Pharmacol. Exp. Ther.*, **222**, 319–323.
- CHURCHILL, P.C. & BIDANI, A.K. (1982). Hypothesis: adenosine mediates hemodynamic changes in renal failure. *Med. Hypoth.*, **8**, 275–285.
- COLLIS, M.G. & BROWN, C.M. (1983). Adenosine relaxes the aorta by interacting with an A_2 receptor and an intracellular site. *Eur. J. Pharmacol.*, **96**, 61–69.
- COLLIS, M.G., PALMER, D.B. & SAVILLE, V.L. (1985). Comparison of the potency of 8-phenyltheophylline as an antagonist at A_1 and A_2 adenosine receptors in atria and aorta from the guinea-pig. *J. Pharm. Pharmacol.*, **37**, 278–280.
- COLLIS, M.G. & SAVILLE, V.L. (1984). An investigation of the negative chronotropic effect of adenosine on the guinea-pig atrium. *Br. J. Pharmacol.*, **83**, 413P.
- COLLIS, M.G., STOGGALL, S.M. & MARTIN, F.M. (1989). Apparent affinity of 1,3-dipropyl-8-cyclopentylxanthine for adenosine A_1 and A_2 receptors in isolated tissues from guinea-pig. *Br. J. Pharmacol.*, **97**, 1274–1278.
- HALEEN, S.J., STEFFEN, R.P. & HAMILTON, H.W. (1987). PD116,948, a highly selective A_1 adenosine receptor antagonist. *Life Sci.*, **40**, 555–561.
- HALL, J.E., GUYTON, A.C. & FARR, B.M. (1977). A single injection method for measuring glomerular filtration rate. *Am. J. Physiol.*, **232**, F72–F76.
- HEDQUIST, P. & FREDHOLM, B. B. (1979). Inhibitory effect of adenosine on adrenergic neuroeffector transmission in the rabbit heart. *Acta. Physiol. Scand.*, **105**, 120–122.
- LIN, J.-J., CHURCHILL, P.C. & BIDANI, A.K. (1987). The effect of dipyridamole on the initiation phase of postischemic acute renal failure in rats. *Can. J. Physiol. Pharmacol.*, **65**, 1491–1495.
- MILLER, W.L., THOMAS, R.A., BERNE, R.M. & RUBIO, R. (1978). Adenosine production in the ischaemic kidney. *Circ. Res.*, **43**, 390–397.
- MURRAY, R.D. & CHURCHILL, P.C. (1984). Effects of adenosine receptor agonists in the isolated perfused rat kidney. *Am. J. Physiol.*, **247**, H343–H348.
- SNEDECOR, G.W. & COCHRAN, W.V. (1967). *Statistical Methods*. 6th edn., pp. 271–275. Ames Iowa, USA: Iowa State University Press.
- STEIN, J.H., LIFSCHITZ, M.D. & BARNES, L.D. (1978). Current concepts on the pathophysiology of acute renal failure. *Am. J. Physiol.*, **234**, F171–F181.
- YATES, M.S., BOWMER, C.J., KELLETT, R. & COLLIS, M.G. (1987). Effect of 8-phenyltheophylline, enprofylline and hydrochlorothiazide on glycerol-induced acute renal failure in the rat. *J. Pharm. Pharmacol.*, **39**, 803–808.

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