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Effect of the adenosine antagonist 8-phenyltheophylline on glycerol-induced acute renal failure in the rat

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1 8-Phenyltheophylline (8-PT) (10 mg kg^{-1}) or its vehicle (1 ml kg^{-1}) were administered intravenously or intraperitoneally twice daily over 48 h to rats with acute renal failure (ARF) induced by intramuscular (i.m.) injection of glycerol.

2 Rats treated with 8-PT i.v. had significantly lower plasma urea and creatinine levels at 24 and 48 h compared to untreated animals.

3 The vehicle also reduced plasma urea and creatinine when compared to untreated controls. However, plasma urea levels in 8-PT-treated rats were significantly lower than in vehicle-treated animals at 24 and 48 h after both i.v. and i.p. administration. Plasma creatinine concentrations also tended to be lower in the 8-PT-treated group.

4 [^{3}H]-inulin clearance at 48 h after i.m. glycerol was significantly greater in rats dosed i.p. with 8-PT compared to either untreated or vehicle treated rats.

5 Examination of kidneys taken from rats 48 h after i.m. glycerol showed that 8-PT treatment significantly reduced renal damage and kidney weight compared to the untreated or vehicle-treated groups.

6 In a 7 day study all the rats which received 8-PT i.p. survived whilst in the vehicle and untreated groups the mortality rates were 12 and 21% respectively.

7 In a separate series of experiments 8-PT (10 mg kg^{-1}; i.v. or i.p.) was found to antagonize adenosine-induced bradycardia in conscious rats for up to 5 h.

8 There is no clear explanation for the partial protection afforded by the vehicle but it may be related to either its alkalinity or an osmotic effect produced by the polyethylene glycol component.

9 The protective effect of 8-PT in rats with ARF was probably the result of adenosine antagonism.

Introduction

Although there have been many clinical and experimental investigations into the factors which initiate and maintain acute renal failure (ARF) there is still no generally accepted hypothesis that fully accounts for its pathogenesis. The initial stages of ARF in man are characterized by a decrease in renal blood flow (RBF) (Börner & Klinkman, 1980). A reduction in RBF is also thought to be the primary mechanism by which glycerol injection induces a decrease in glomerular filtration rate (GFR) and ARF in experimental animals (Stein *et al.*, 1978). In this experimental model, the initial reduction in RBF appears to be a consequence of a reduced cardiac output and of an increase in renal vascular resistance (Hsu *et al.*, 1977). However, 24–48 h after the initiation of glycerol-induced ARF, renal blood flow returns to normal whilst GFR is still depressed (Stein *et al.*, 1978). Kurtz *et al.* (1976) have suggested that this persistent decrement in GFR in the presence of a normal RBF is due to an increase in pre-glomerular resistance and a decrease in post-glomerular resistance.

The initial decrease in RBF that occurs in ARF could alter renal metabolism. For example, Trifillis *et al.* (1981) found that levels of adenine nucleotides (ATP, ADP and AMP) were decreased by 55% after the initiation of glycerol-induced ARF in the rat. One consequence of low cellular ATP levels is the activation of 5′ nucleotidase, the enzyme that converts AMP to adenosine (Woods *et al.*, 1970). Churchill & Bidani (1982) have proposed that adenosine may play an

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important role in the pathogenesis of ARF. This idea is supported by the enhanced release of adenosine from the ischaemic kidney (Miller *et al.*, 1978) and by the observation that both ARF and adenosine evoke pre-glomerular constriction and post-glomerular dilatation (Churchill, 1982; Osswald, 1984). Bidani & Churchill (1983) have also found that the weak adenosine antagonist theophylline (as aminophylline) can ameliorate some of the biochemical indices of glycerol-induced ARF.

The mechanism by which aminophylline exerts its beneficial effects in ARF is not certain. This is because, in addition to blocking adenosine receptors, aminophylline is also a phosphodiesterase inhibitor (Smellie *et al.*, 1979). In the present study we have examined the effects of another adenosine antagonist, 8-phenyltheophylline (8-PT) on the biochemical, functional and morphological indices of glycerol-induced renal failure. 8-PT was chosen for this study since it is 20–30 times more potent as an adenosine antagonist than theophylline (Collis *et al.*, 1984) and because it has no effect on phosphodiesterase (Smellie *et al.*, 1979). A preliminary account of this work has been presented to the British Pharmacological Society (Bowmer *et al.*, 1985).

Methods

Induction of acute renal failure

The method for the production of ARF has been described in detail (Bowmer *et al.*, 1982). Male Wistar albino rats (220–330 g) were dehydrated for 24 h and ARF was produced by intramuscular injection of 50% v/v glycerol in sterile saline (0.9% w/v NaCl), 10 ml kg^{-1} body weight. Control rats were similarly dehydrated but injected with sterile saline only, 10 ml kg^{-1}.

Experimental protocol

Immediately after injection of glycerol or saline, rats were given a bolus dose of either 8-PT (10 mg kg^{-1}) or vehicle (1 ml kg^{-1} of polyethylene glycol: 0.1 M NaOH; 50:50 v/v) via the tail vein. Three further injections were made at 12, 24 and 36 h after the initial dose of 8-PT or vehicle. Some glycerol-injected rats received no treatment and were allowed to develop ARF without any intervention. Blood samples (about 0.7 ml) were taken from the tail vein before (0 h) and at 24 and 48 h after the injection of glycerol or saline.

In a separate series of experiments glycerol-injected rats received either 8-PT or vehicle administered i.p. using the same dose and regime described for i.v. administration. Again a group of glycerol-injected rats were given no treatment. Blood samples (0.7 ml) were taken from the tail vein at 0, 48 h and 7 days after injection of glycerol.

Further experiments were conducted 48 h after injection of either saline or glycerol to determine the clearance of [^{3}H]-inulin in rats which had received either vehicle or 8-PT i.p. using the dosage regime stated above.

[^{3}H]-inulin clearance (C_{IN})

The single-injection method of Hall *et al.* (1977) was used to measure the clearance of [^{3}H]-inulin (100 mg kg^{-1}; 20 μCi kg^{-1} i.v.) from plasma of rats anaesthetized with sodium pentobarbitone (60 mg kg^{-1}).

Plasma creatinine and urea

Standard spectrophotometric assays were used: creatinine by reaction with alkaline picrate solution and urea by reaction with diacetylmonoxime (Henry *et al.*, 1974).

Kidney histology

The rats used in the first series of experiments were killed 48 h after injection of glycerol or saline. Longitudinal sections were cut from one kidney of each rat and stained with haematoxylin and eosin. The sections were examined by a pathologist who was unaware of the treatment which the donor animal had received. The degree of renal damage was assessed according to the following scoring system.

(a) Necrosis:	
occasional necrotic tubular cells	: score 1
occasional necrotic tubules	: score 2
25% cortical tubules necrotic	: score 3
33% cortical tubules necrotic	: score 4
50% cortical tubules necrotic	: score 5
(b) Damaged tubules and casts:	
occasional damaged tubules	: score 1
occasional damaged tubules and some casts	: score 2
30% of tubules with casts	: score 3
50% of tubules with casts	: score 4
51–100% tubules with casts	: score 5

The two scores for each kidney were added to give the total damage score (maximum 10).

Evaluation of 8-phenyltheophylline as an adenosine antagonist

Wistar rats (200–260 g) were anaesthetized with halothane and vinyl catheters were surgically implanted in the right jugular vein and thoracic aorta (via the left carotid artery). After a recovery period of 24 h, the

rats were placed in open-ended perspex tubes which allowed them to stand comfortably. The aortic blood pressure was recorded directly via a pressure transducer (Bell and Howell L221) and displayed on a chart recorder (Devices MX 2). Heart rate was derived from the blood pressure trace. When not in use, the catheters were filled with sterile saline (0.9% NaCl) containing heparin (50 u ml^{-1}).

Adenosine, dissolved in saline, was infused for a period of 1 to 2 min via the jugular vein catheter. The infusion was terminated when a stable heart rate had been achieved. Adenosine infusions were performed before and 1, 3, 5, 8 and 24 h after i.v. or i.p. injections of 8-PT (10 mg kg^{-1}) or its vehicle (1 ml kg^{-1}).

Materials

[9-^{3}H]-inulin (180 mCi g^{-1}) of stated radioactive purity 98% was obtained from New England Nuclear Ltd and used without further purification. 8-PT was purchased from Calbiochem Ltd and was dissolved in polyethylene glycol (Sigma Chemical Co.): 0.1 M NaOH (50:50 v/v) to give a final concentration of 10 mg ml^{-1}. Dissolution was achieved with the aid of gentle stirring at 50°C for about 20 min. Adenosine and inulin were obtained from Sigma Chemical Co. and reagents for the assay of creatinine and urea were bought from Pierce and Warriner Ltd and BDH respectively.

Analysis of results

Results are expressed as mean ± s.e.mean. Statistical comparison of creatinine, urea, C_{IN} and total kidney weight was made by one-way analysis of variance (ANOVA) and means were compared by the method of Least Significant Difference (Snedecor & Cochran, 1967). There was no significicant correlation between total kidney weight and body weight, so kidney weight was not expressed as a fraction of body weight. Statistical analysis of the histological damage score was made by a one-sided Mann Whitney test. The effect of 8-PT on responses to injected adenosine was analysed using Student's *t* test for paired observations.

Results

Adenosine antagonism by 8-phenyltheophylline

Intravenous infusion of adenosine (0.2 to 3.75 mg kg^{-1} min^{-1}) caused bradycardia (16 ± 8.1 to 244 ± 16 beats min^{-1}) and a decrease in diastolic pressure (6.3 ± 3.8 to 67.5 ± 6.3 mmHg) in the conscious rat. Infusion of doses greater than 3.75 mg kg^{-1} min^{-1} evoked dysrhythmia. An infusion rate of 1 mg kg^{-1} min^{-1} was used in subsequent studies since this dose evoked a marked but submaximal bradycardia.

The effect of 8-PT (10 mg kg^{-1}), administered, i.v. or i.p., and of its vehicle (1 ml kg^{-1}) on responses evoked by adenosine infusion was examined. The control heart rate of the four groups of rats used did not differ significantly (ANOVA) either before or after administration of 8-PT or its vehicle (Table 1). There was also no significant difference between the control heart rate responses evoked by adenosine in the four groups of animals (Table 1). The vehicle for 8-PT had no significant effect on the bradycardia evoked by adenosine but 8-PT (10 mg kg^{-1}) attenuated this

Table 1 Effect of 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) on adenosine-induced bradycardia in the conscious rat

Parameter	*Treatment*			
	Vehicle i.v.	*8-PT i.v.*	*Vehicle i.p.*	*8-PT i.p.*
Control heart rate (beats min^{-1})	473 ± 21	496 ± 15	441 ± 18	481 ± 14
Heart rate 1 h after treatment	486 ± 15	476 ± 17	438 ± 15	447 ± 18
Change in heart rate evoked by adenosine (1 mg kg^{-1} min^{-1})				
0 h	−116 ± 10.5	−108 ± 9	−103 ± 15	−109 ± 12
1 h	−123 ± 7.6	+10 ± 7***	−125 ± 14	−35 ± 7.5**
3 h	−130 ± 9.7	+2 ± 12***	−105 ± 13	−32 ± 13***
5 h	−101 ± 16	−60 ± 21*	−87 ± 16	−90 ± 7.3
8 h	—	−70 ± 17	—	−111 ± 9
24 h	−102 ± 8	−107 ± 11	−109 ± 12	−111 ± 10
n	6	8	10	10

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; relative to response evoked by adenosine at 0 h.

Table 2 Plasma urea and creatinine in saline and glycerol-injected rats treated by i.v. injection of either vehicle (1.0 ml kg^{-1}) or 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) twice daily for 2 days

	Saline-injected			*Glycerol-injected*	
	Vehicle treated ($n = 6$)	*8-PT treated* ($n = 6$)	*No treatment* ($n = 12$)	*Vehicle treated* ($n = 12$)	*8-PT treated* ($n = 12$)
Plasma urea ($mg\,dl^{-1}$)					
0 h	51 ± 3	42 ± 5	48 ± 1	52 ± 2	49 ± 3
24 h	40 ± 2	37 ± 3	257 ± 19	187 ± 23*	112 ± 11***‡
48 h	45 ± 1	42 ± 2	317 ± 37	210 ± 46*	108 ± 10***†
Plasma creatinine ($mg\,dl^{-1}$)					
0 h	0.68 ± 0.01	0.71 ± 0.10	0.68 ± 0.01	0.66 ± 0.03	0.69 ± 0.04
24 h	0.81 ± 0.05	0.76 ± 0.07	3.29 ± 0.31	2.68 ± 0.33	1.92 ± 0.08***
48 h	0.72 ± 0.05	0.80 ± 0.05	3.61 ± 0.48	2.38 ± 0.49*	1.42 ± 0.10***

Mean ± s.e. mean and number of rats in parentheses.
*$P < 0.05$; ***$P < 0.001$ relative to glycerol-injected no treatment rats.
†$P < 0.05$; ‡$P < 0.01$ relative to glycerol-injected vehicle treated rats.

response. This antagonist effect was significant 1, 3 and 5 h after i.v. administration and 1 and 3 h after i.p. administration (Table 1).

Evaluation of effects of 8-phenyltheophylline on glycerol-induced acute renal failure

(1) *Forty-eight hour study, i.v. treatment* Rats injected with saline and given either 8-PT or vehicle showed no significant difference in creatinine and urea levels at 24 and 48 h after injection (Table 2). Mean total kidney weight was also not significantly different, in 8-PT- or vehicle-treated rats, from those injected with saline and given no further treatment (Figure 1). Treatment with 8-PT, however, caused a significant ($P < 0.01$) degree of damage to kidney morphology when compared with kidneys from vehicle-treated rats (Table 3).

Following the injection of glycerol, mean plasma

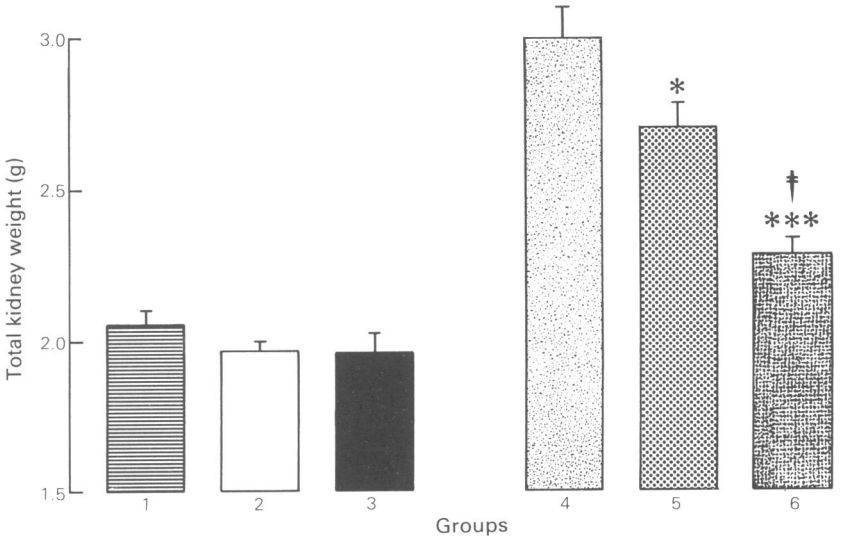

Figure 1 Total kidney weight of saline and glycerol-injected rats 48 h after treatment with either vehicle (1.0 ml kg^{-1}) or 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) twice daily i.v. Key to groups: (1) saline-injected, no treatment ($n = 12$); (2) saline-injected, vehicle-treated ($n = 6$); (3) saline-injected, 8-PT-treated ($n = 6$); (4) glycerol-injected, no treatment ($n = 12$); (5) glycerol-injected, vehicle-treated ($n = 12$); (6) glycerol-injected, 8-PT-treated ($n = 12$). Columns represent mean values with vertical bars showing s.e.means.
*$P < 0.05$, ***$P < 0.001$ relative to group 4; ‡$P < 0.001$ relative to group 5.

Table 3 Effect of 8-phenyltheophylline (8-PT, 10 mg kg^{-1} i.v.) on the renal damage associated with glycerol-induced renal failure

Group	n	*Damage score*
Saline-injected vehicle treated	6	1.5 ± 0.2
Saline-injected 8-PT-treated	6	3.7 ± 0.9†
Glycerol-injected no treatment	12	7.7 ± 0.4
Glycerol-injected vehicle-treated	12	8.3 ± 0.4
Glycerol-injected 8-PT-treated	12	6.5 ± 0.5*‡

†$P < 0.01$ relative to saline-injected vehicle-treated.
*$P < 0.05$ relative to glycerol-injected no treatment.
‡$P < 0.01$ relative to glycerol-injected vehicle-treated. Kidneys were fixed and sectioned 48 h after injection of glycerol or saline. Maximum possible damage score = 10.

creatinine and urea concentrations were increased almost 5 fold at both 24 and 48 h (Table 2). Mean kidney weight increased by about 50% in rats injected with glycerol (Figure 1) and these kidneys exhibited a high degree of morphological damage (Table 3).

In vehicle-treated glycerol-injected rats, plasma urea levels were significantly lower ($P < 0.05$), at 24 and 48 h when compared to glycerol-injected rats (Table 2). By contrast, creatinine levels were not significantly different at 24 h; but at 48 h creatinine was significantly lower ($P < 0.05$) than in animals treated with glycerol alone (Table 2). Total kidney weight was also significantly less ($P < 0.05$) in this group of rats when compared to that of glycerol-injected rats (Figure 1). However, there was no significant difference in the severity of the morphological damage that was observed (Table 3).

Table 2 shows that at both 24 and 48 h, urea concentrations were substantially reduced ($P < 0.05$) in the 8-PT-treated glycerol-injected group when compared to either the glycerol-injected rats or the vehicle-treated glycerol-injected animals. Creatinine levels were significantly lower at both 24 and 48 h compared to glycerol-injected rats; but although the mean levels of creatinine were also lower than those in the vehicle-treated group, these differences were not statistically significant. However, total kidney weight was significantly less ($P < 0.001$) in the 8-PT-treated group when compared to either untreated or vehicle-treated glycerol-injected rats (Figure 1). In addition, 8-PT caused a significant reduction in renal damage when compared to that in the untreated ($P < 0.05$) and in the vehicle-treated ($P < 0.01$) glycerol-injected rats (Table 3).

(2) *Seven day study, i.p. treatment* This study was undertaken in order to determine whether the vehicle effect was peculiar to i.v. administration and to assess the effect of 8-PT on recovery from ARF. Table 4 shows plasma creatinine and urea concentrations at 48 h and 7 days following i.m. injection of glycerol in untreated, vehicle- and 8-PT-treated rats. At 48 h a similar pattern emerged to that seen previously when the vehicle or 8-PT was given i.v. (Table 2). Creatinine and urea levels were significantly decreased ($P < 0.05$) in both the treated groups and, in addition, levels of these metabolites were substantially less ($P < 0.01$) in the 8-PT group when compared to those in the vehicle-treated animals. Between 48 h and 7 days mortality in the untreated rats was 21% (15 animals survived out of 19) whereas in the vehicle- and 8-PT-treated rats it was 12% (15 animals survived out of 17) and 0% respec-

Table 4 Plasma urea and creatinine in rats with glycerol-induced acute renal failure treated by i.p. injection of either vehicle (1.0 ml kg^{-1}) or 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) twice daily for 2 days

	Glycerol-injected rats		
	No treatment ($n = 15$)	*Vehicle-treated* ($n = 15$)	*8-PT-treated* ($n = 15$)
Plasma urea (mg dl^{-1})			
0 h	44 ± 2	49 ± 3*	48 ± 2
48 h	252 ± 34	190 ± 29**	87 ± 6***‡
7 days	121 ± 27	76 ± 10**	46 ± 3**†
Plasma creatinine (mg dl^{-1})			
0 h	0.68 ± 0.03	0.72 ± 0.03	0.70 ± 0.06
48 h	2.96 ± 0.46	2.13 ± 0.30**	1.20 ± 0.07***§
7 days	1.40 ± 0.27	0.93 ± 0.08*	0.79 ± 0.05**

Mean ± s.e.mean and number of rats in parentheses.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ relative to glycerol-injected no treatment.
†$P < 0.05$; §$P < 0.01$; ‡$P < 0.001$ relative to glycerol-injected vehicle treated.

tively. In the animals surviving to 7 days, mean levels of creatinine and urea had almost returned to control values in the 8-PT-treated group but only in the case of urea levels was there any significant different ($P < 0.05$) between this group and vehicle-treated rats. Total kidney weight at 7 days after glycerol injection was significantly less in both vehicle- ($P < 0.05$) and 8-PT-treated groups ($P < 0.001$). However, kidney weight was much less ($P < 0.001$) in the 8-PT group than in the vehicle-treated group (Figure 2).

(3) *Clearance of [^{3}H]-inulin (C_{IN}), 48 h i.p. treatment*
Mean C_{IN} values in rats injected with saline and dosed i.p. with either vehicle or 8-PT were 0.99 ± 0.10 and 1.07 ± 0.01 ml min^{-1} 100 g^{-1} body wt. ($n = 8$) respectively. These values were not significantly different from that obtained for untreated saline-injected rats (1.08 ± 0.05 ml min^{-1} 100 g^{-1} body wt.; $n = 8$). Plasma creatinine and urea concentrations along with C_{IN} at 48 h after injection of glycerol in untreated, vehicle and 8-PT-dosed rats are listed in Table 5. The effect of either vehicle or 8-PT treatment on plasma creatinine and urea levels was similar to that observed previously (Tables 2 and 4). C_{IN} was significantly greater ($P < 0.05$) in both vehicle- and 8-PT-treated rats when compared to untreated rats. In addition C_{IN} was greater ($P < 0.001$) in the 8-PT-treated group than in their vehicle-treated counterparts.

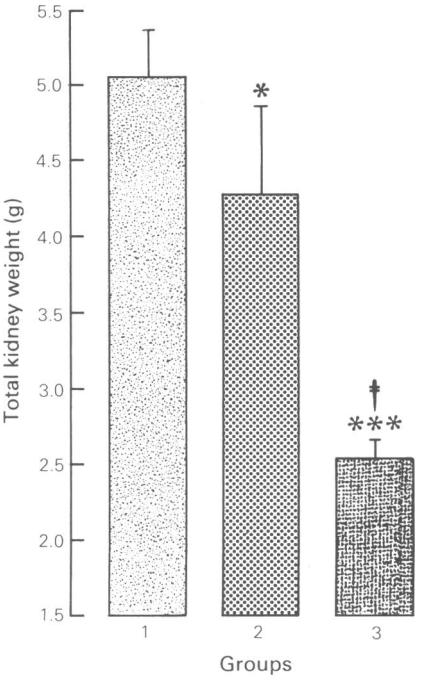

Figure 2 Total kidney weight of glycerol-injected rats 7 days after treatment with either vehicle (1.0 ml kg^{-1}) or 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) twice daily i.p. for 48 h. Key to groups: (1) no treatment; (2) vehicle treated; (3) 8-PT treated. Columns represent mean values with vertical bars showing s.e.means ($n = 15$).
*$P < 0.05$, ***$P < 0.001$ relative to group 1; ‡$P < 0.001$ relative to group 2.

Discussion

In the present study, glycerol was used to induce ARF in the rat. ARF was characterized by elevated plasma levels of urea and creatinine, decreased plasma clearance of [^{3}H]-inulin, extensive renal ultrastructural damage and mortality. The severity of the glycerol-induced ARF was markedly reduced by 8-PT and to a lesser extent by its vehicle.

The beneficial effect of 8-PT in rats with ARF was evident from the biochemical, functional and morphological lesions associated with this disorder. Plasma urea was significantly less in the 8-PT-treated group of rats with ARF than in either the vehicle-treated or the untreated group. Plasma creatinine was also less in the 8-PT-treated rats with ARF than in untreated or vehicle-treated rats. However, this did not always achieve statistical significance in the latter case. The major source of plasma creatinine is muscle (Baron, 1973) and damage to skeletal muscle following injection of glycerol may mean that plasma creatinine levels are not a totally reliable index of renal function in this model of ARF.

The plasma clearance of [^{3}H]-inulin was significantly greater in rats given 8-PT when compared to either vehicle-treated or untreated rats. This suggests that glomerular filtration rate was improved in animals treated with 8-PT. Total kidney weight was also considerably less in the 8-PT-treated rats with ARF than in either the vehicle-treated or the untreated groups. This indicates that there was less oedema in the glycerol-injected 8-PT rats, and is consistent with the presence of fewer casts and damaged tubules in these animals. Furthermore, there were no deaths up to 7 days in the 8-PT-treated group whereas mortalities occurred in both the vehicle-treated and the untreated groups.

The beneficial effects of 8-PT in glycerol-induced renal failure, may be due to adenosine receptor blockade. The dose of 8-PT used in these studies attenuated the bradycardia evoked by a large dose of adenosine for up to 5 h. This observation does not necessarily mean that this dose of 8-PT was blocking the renovascular effects of endogenous adenosine. Previous studies have shown, however, that 10 mg kg^{-1} of 8-PT is effective in blocking the systemic vascular effects of the purine (Collis *et al.*, 1984).

It is necessary to consider the possibility that 8-PT

Table 5 Plasma urea, creatinine and [^{3}H]-inulin clearance (C_{IN}) at 48 h in glycerol-injected rats treated by i.p. injection of either vehicle (1.0 ml kg^{-1}) or 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) twice daily for 2 days

	Glycerol-injected rats		
	No treatment ($n = 8$)	*Vehicle-treated* ($n = 8$)	*8-PT-treated* ($n = 8$)
Plasma urea (mg dl^{-1})	342 ± 30	211 ± 48*	114 ± 37***
Plasma creatinine (mg dl^{-1})	5.03 ± 0.60	2.50 ± 0.64**	1.45 ± 0.30***
C_{IN} (ml min^{-1} 100 g^{-1} body wt.)	0.13 ± 0.02	0.35 ± 0.04*	0.60 ± 0.09***†

Mean ± s.e.mean and number of rats in parentheses.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ relative to glycerol-injected no treatment.
†$P < 0.01$ relative to glycerol-injected vehicle-treated.

ameliorated ARF by a systemic cardiovascular effect. Some alkylxanthines, such as theophylline, have cardiotonic effects and can increase cardiac output (Hudlicka *et al.*, 1981; Collis *et al.*, 1984). Since one of the early features of glycerol-induced ARF is a reduction in cardiac output (Hsu *et al.*, 1977), its restoration could be protective. However, this cannot be the case for 8-PT since it has little cardiotonic effect (Collis *et al.*, 1984) probably because it does not inhibit phosphodiesterase (Smellie *et al.*, 1979).

Diuretic agents have been shown to ameliorate haemodynamically induced ARF (Levinsky *et al.*, 1980). It is not known whether 8-PT has diuretic activity, but it is a possibility since this property has been demonstrated for other alkylxanthines that block adenosine receptors (Baer *et al.*, 1983). Thus it is possible that a diuretic effect, mediated by adenosine receptor blockade, may have contributed to the beneficial effect of 8-PT in ARF.

We cannot exclude the possibility that part of the protective effect of 8-PT stems from a reduction of muscle damage at the site of glycerol injection which thereby lessens the severity of renal failure. This seems unlikely, however, because the related alkylxanthine theophylline has been shown to ameliorate the impairment in renal function induced by radiocontrast media (Bakris & Burnett, 1985) and amphotericin B (Heidemann *et al.*, 1983). In both these instances of nephrotoxicity muscle damage is not involved yet theophylline was still protective.

The vehicle for 8-PT (sodium hydroxide and polyethylene glycol) had a protective effect on the development of ARF. This effect was observed in relation to plasma creatinine and urea, C_{IN}, total kidney weight and mortality. By contrast, histology revealed that the vehicle did not improve kidney morphology. Why the vehicle should attenuate some facets of ARF is not clear. High sodium loads given to rats prior to the development of ARF can ameliorate the condition (McDonald *et al.*, 1969; Wilkes & Hollenberg, 1982; Yates *et al.*, 1983). The total sodium load given to each rat in the present experiment was 0.2 mmol kg^{-1} day^{-1} for two days. A sodium load of this magnitude is relatively small compared to those used to attenuate ARF (McDonald *et al.*, 1969; Yates *et al.*, 1983).

The vehicle for 8-PT is highly alkaline. Alkaline diuresis has been shown to prevent the development of ARF that results from the injection of either haemoglobin or myoglobin in the rabbit (Baker & Dodds, 1925; Perri & Gorini, 1952) and both of these haemproteins have been implicated in the development of glycerol-induced ARF (Carroll *et al.*, 1965; Preuss *et al.*, 1975). Thus the alkalinity of the 8-PT vehicle may be a factor in this protective effect against ARF. It is also possible that the protective effect afforded by the vehicle results from its polyethylene glycol component since this substance has been shown in the rat kidney to prevent tubular cell swelling associated with ischaemic injury (Leaf *et al.*, 1983).

The results of the present study demonstrate that a potent adenosine antagonist can ameliorate glycerol-induced ARF. The pathogenesis of glycerol-induced ARF is likely to be multifactorial (Stein *et al.*, 1978: Trifillis *et al.*, 1981); but the present results and those of Bidani & Churchill (1983) point to a role for adenosine in this model.

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