J. Pharm. Pharmacol. 1992, 44: 109–113
Received May 17, 1991

© 1992 J. Pharm. Pharmacol.

Further Characterization of the Protective Effect of 8-Cyclopentyl-1,3-dipropylxanthine on Glycerol-induced Acute Renal Failure in the Rat

M. R. PANJEHSHAHIN, T. S. MUNSEY, M. G. COLLIS*, C. J. BOWMER AND M. S. YATES

*Department of Pharmacology, Worsley Medical and Dental Building, The University of Leeds, Leeds LS2 9JT, UK and *Bioscience II, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

Abstract—In the rat, treatment with the alkylxanthine 8-cyclopentyl-1,3-dipropylxanthine (CPX) at a dose of 0·1 mg kg^{-1} antagonizes adenosine-induced falls in renal blood flow and reduces the severity of glycerol-induced acute renal failure. Treatment of glycerol-injected rats with 0·03 mg kg^{-1} of CPX resulted in no significant improvements in a range of indices of renal function. However, treatment with 0·1 or 0·3 mg kg^{-1} doses of CPX did significantly ameliorate acute renal failure although there were no significant differences in the degree of protection of renal function afforded by these two doses. In glycerol-injected rats, 0·1 or 0·3 mg kg^{-1} CPX administered either as a single dose or repeated doses every 12 h for two days did not inhibit renal phosphodiesterase. Thus the beneficial effects of CPX can be produced by doses that have no effect on renal phosphodiesterase activity whereas 0·1 mg kg^{-1} of CPX has been shown previously to antagonize the actions of adenosine. The findings provide further evidence that the beneficial effect of CPX in glycerol-induced acute renal failure is a consequence of adenosine antagonism and not phosphodiesterase inhibition.

Churchill & Bidani (1982) have proposed that adenosine is an important mediator of the haemodynamic changes that occur within the kidney in various forms of acute renal failure (ARF). This proposal is supported by studies in the rat which have shown that treatment with the weak adenosine antagonist theophylline improves renal function in ARF induced by either cisplatin (Heidemann et al 1989), myohaemoglobinuria (Bidani & Churchill 1983), ischaemia (Lin et al 1986) or hypoxaemia (Gouyon & Guignard 1988). However, in addition to blocking adenosine receptors, theophylline is a phosphodiesterase inhibitor (Smellie et al 1979) and consequently the mechanism by which theophylline exerts its beneficial effects is uncertain. Further support for the role of adenosine in the pathophysiology of ARF was provided by the findings that the severity of glycerol-induced ARF was reduced by treatment with either 8-phenyltheophylline or 8-cyclopentyl-1,3-dipropylxanthine (CPX) (Bowmer et al 1986; Kellett et al 1989). These alkylxanthines are more potent adenosine antagonists than theophylline, with CPX possessing selectivity for the A_1 adenosine receptor subtype (Collis et al 1984, 1989; Martinson et al 1987). Both 8-phenyltheophylline and CPX have been reported as being ineffective as phosphodiesterase inhibitors (Smellie et al 1979; Scotini et al 1983; Martinson et al 1987). However, more recent investigations have demonstrated that 8-phenyltheophylline is a more potent inhibitor of cAMP phosphodiesterase in erythrocytes and skeletal muscle than theophylline (Nicholson & Wilke 1989) and that CPX is a potent inhibitor of cAMP phosphodiesterase in opossum kidney cells (Coulson & Scheinman 1989). These studies raise the possibility that phosphodiesterase inhibition may indeed play a role in the beneficial effects of alkylxanthines in ARF.

Correspondence: M. S. Yates, Department of Pharmacology, Worsley Medical and Dental Building, The University of Leeds, Leeds LS2 9JT, UK.

In the present study we have further characterized the protective effects of CPX in glycerol-induced ARF. We have determined to what extent the beneficial actions of CPX in ARF are dose-dependent and have assessed the ability of these doses of CPX to inhibit renal phosphodiesterase activity in-vivo. We have previously examined the ability of CPX in-vivo to antagonize adenosine-induced falls in heart rate and renal blood flow (Kellett et al 1989). Therefore, a comparison of the results of the present study with the findings of our previous investigation (Kellett et al 1989) should indicate whether adenosine antagonism or phosphodiesterase inhibition accounts for the protective effect of CPX in glycerol-induced ARF.

Materials and Methods

Materials

[^{3}H(G)]Inulin (180–215 mCi g^{-1}) of stated radioactive purity 98–99% and *p*-[glycyl-1-^{14}C]aminohippuric acid (46·4–57·6 mCi $mmol^{-1}$, purity 97·5–98%) were both obtained from NEN Research Products and were used without further purification. CPX was synthesized by Dr R. James of ICI. Kits for the assay of creatinine and urea were obtained from Pierce and Warriner and BDH Ltd, respectively.

Induction of ARF

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 i.m. injection of 50% v/v glycerol in sterile 0·9% NaCl (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.

Determination of phosphodiesterase activity

Experimental groups. Renal phosphodiesterase activity was determined either after a single injection or repeated injections of CPX. Rats were randomly assigned to 3 groups. Group 1 consisted of rats which received no treatments and were used to estimate basal phosphodiesterase activity. Group 2 comprised rats injected i.m. with saline as above and which immediately received one of three treatments via the tail vein: 0·1 or 0·3 mg kg^{-1} CPX, or the vehicle for CPX (1·0 mL kg^{-1}; 1·0% v/v DMSO, 0·75% v/v 1 M NaOH in saline). Group 3 consisted of rats injected i.m. with glycerol and which then received one of the three treatments as for group 2. Treated rats were studied 0·5, 1 and 6 h after i.v. injection.

Repeated i.v. injections of CPX (0·1 or 0·3 mg kg^{-1}) or vehicle were given to rats which had received glycerol i.m. The first injection of CPX or vehicle was made immediately following glycerol administration with subsequent injections 12, 24 and 36 h later. One group of glycerol-injected rats received no treatment. Rats were studied 1 h after the last CPX or vehicle injection or in the case of the untreated group 37 h after glycerol injection. Immediately before the study, a blood sample (0·5 mL) was taken by cardiac puncture under ether anaesthesia.

Phosphodiesterase assay. Rats were killed by cervical dislocation and the kidneys removed, cleared of adipose tissue and homogenized at 0°C in ten volumes of 50 mM Tris/HCl buffer, pH 7·5, with 5 strokes of a Teflon-glass homogenizer. The homogenate was centrifuged at 9000 *g* for 10 min and the supernatant was assayed for phosphodiesterase activity by the method of Arch & Newsholme (1976). A 30 μL sample (about 30 μg of protein) of homogenate was added to an incubation medium (100 μL) containing 50 mM Tris/HCl, pH 7·5, 6 mM $MgCl_2$, 2·5 mM dithiothreitol, 50 μg mL^{-1} of 5′-nucleotidase, 230 μg mL^{-1} of bovine albumin, 10 μM cAMP and 0·075 μCi (0·4 μM) [^{3}H]cAMP. The reaction was carried out at 30°C for 10 min and was stopped by addition of 1·2 mL of anion exchange resin slurry (Dowex X8 200–400 mesh; resin to water 1 : 1 v/v, pH 5·0). This mixture was centrifuged at 9000 *g* for 5 min and 200 μL of supernatant were added to 10 mL of scintillation fluid (Optiphase Safe, LKB) and counted for tritium in a Packard Tri-Carb 1500 liquid scintillation counter. The protein content of homogenates was determined using the Biuret assay with bovine albumin as the standard.

Evaluation of CPX treatment in glycerol-induced ARF

Experimental protocol. Before administration of glycerol or saline, a 0·75 mL control blood sample (0 h) was taken from the tail vein. Immediately after glycerol injection, rats were treated with either CPX (0·03, 0·1 or 0·3 mg kg^{-1}) or vehicle (1·0 mL kg^{-1}) administered via the tail vein. Following i.m. saline injection, rats received 0·3 mg kg^{-1} of CPX i.v. 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 anaesthetized and the clearances of [^{14}C]*p*-aminohippuric acid (CL_{PAH}) and [^{3}H]inulin CL_{IN} determined. Following clearance measurements a blood sample (1·0 mL) was taken from the carotid cannula. At the end of the experiment rats were killed by an overdose of anaesthetic and the kidneys removed, weighed and prepared for histology.

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

*Determination of [^{3}H]inulin and [^{14}C]*p*-aminohippuric acid clearances.* Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). The trachea was cannulated to maintain a clear airway and rats allowed to breathe spontaneously throughout the experiment. Cannulae were inserted into the left jugular vein and right carotid artery and the animals were heparinized (500 int. units kg^{-1}, i.v.). The single injection method of Hall et al (1977) was then used to measure simultaneously the clearances of [^{3}H]inulin (100 mg kg^{-1}; 20 μCi kg^{-1}, i.v.) and [^{14}C]*p*-aminohippurate (40 mg kg^{-1}; 4 μCi 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. The degree of necrosis and presence of casts were scored out of 5.

Statistical methods

Results are expressed as mean ± s.e.m. Statistical comparisons of phosphodiesterase activity, plasma creatinine and urea concentrations, clearance determinations and total kidney weight data were made by either an unpaired Student's *t*-test or one-way analysis of variance (ANOVA). Following analysis of variance, means were compared, where appropriate, using Scheffé's test. Statistical analysis of the histological damage score was made by a two-sided Wilcoxon test.

Results

Phosphodiesterase activity

Fig. 1 shows mean phosphodiesterase activity of homogenates obtained from untreated rats (0 h) and from saline-injected rats up to 6 h after a single bolus dose of CPX (0·1 or 0·3 mg kg^{-1}; i.v.) or its vehicle. In comparison with the vehicle-injected group, rats which received 0·1 mg kg^{-1} CPX showed no significant change ($P > 0·05$) in phosphodiesterase activity. Administration of 0·3 mg kg^{-1} CPX resulted in a 30% decrease ($P < 0·05$) in enzyme activity 1 h after injection compared with the activity in the vehicle-treated group at the same time. However, this higher dose of CPX did not produce any inhibition of enzyme activity at 0·5 or 6 h (Fig. 1). CPX administration at doses of either 0·1 or 0·3 mg kg^{-1} to glycerol-injected rats did not result in any significant

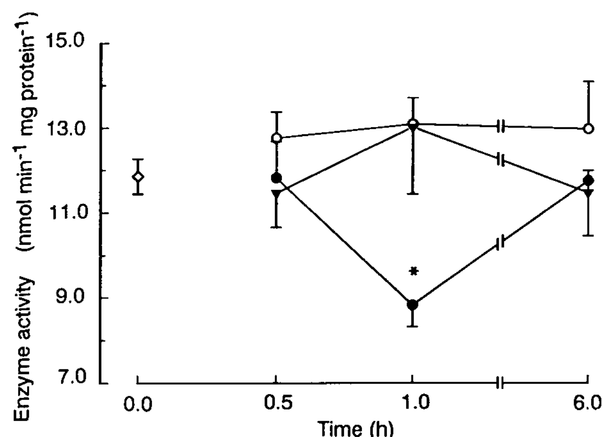

FIG. 1. Renal cAMP phosphodiesterase activity in saline-injected rats at various times after i.v. injection of 8-cyclopentyl-1,3-dipropylxanthine, 0·1 mg kg^{-1} (○), 0·3 mg kg^{-1} (●) or its vehicle, 1·0 mL kg^{-1} (v). The activity of renal phosphodiesterase in untreated rats is shown at 0 h (▼). Results are given as mean ± s.e.m. (n = 6) *$P < 0{\cdot}05$ relative to enzyme activity 1 h after vehicle injection (ANOVA).

change ($P > 0{\cdot}05$, ANOVA) in phosphodiesterase activity at any time point compared with activity in the vehicle group (Table 1). There was a general decline in enzyme activity over the 6 h in glycerol-injected rats which was noticeable in groups given vehicle and 0·3 mg kg^{-1} of CPX.

There was no significant difference ($P > 0{\cdot}05$, ANOVA) in phosphodiesterase activity between glycerol-injected rats which received repeated injections of either vehicle or CPX, 0·1 or 0·3 mg kg^{-1} (Table 2). The plasma urea concentrations determined in these animals showed that treatment with either 0·1 or 0·3 mg kg^{-1} CPX resulted in significant falls in urea levels compared with the untreated group (Table 2). This indicates that treatment with CPX improves renal function in rats with glycerol-induced ARF and this is dealt with in detail in the following section.

Renal function after treatment with CPX

Saline-injected rats. Treatment of saline-injected rats with CPX (0·3 mg kg^{-1}) resulted in no change in plasma urea or creatinine concentrations (Fig. 2). In addition CL_{IN} and CL_{PAH} (Fig. 3) and kidney weight and morphology (Table 3) in these CPX treated animals were unchanged compared with saline-injected rats which received no treatment. These results indicate that in normal rats CPX treatment with the highest dose used in this study did not have any detectable effect on the various indices employed to assess renal function.

Table 1. Renal cAMP phosphodiesterase activity (nmol min^{-1} (mg $protein)^{-1}$) in glycerol-injected rats at various times after i.v. administration of a single bolus dose of 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·1 or 0·3 mg kg^{-1}) or its vehicle (1·0 mL kg^{-1}).

	Treatment groups		
		CPX	
Time (h)	Vehicle-treated	0·1 mg kg^{-1}	0·3 mg kg^{-1}
0·5	12·3 ± 1·05	14·4 ± 1·61	13·7 ± 0·94
1·0	12·3 ± 0·53	12·1 ± 1·66	12·4 ± 0·69
6·0	8·98 ± 0·78	12·9 ± 1·14	9·03 ± 1·27

Results are given as mean ± s.e. mean (n = 6).

Table 2. Renal cAMP phosphodiesterase activity and plasma urea concentrations in glycerol-injected rats treated with 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·1 or 0·3 mg kg^{-1}) or vehicle (1·0 mL kg^{-1}) i.v. twice daily for 2 days.

Group	Phosphodiesterase activity (nmol min^{-1} (mg $protein)^{-1}$)	Plasma urea (mg dL^{-1})
No treatment	13·5 ± 0·99	171 ± 22
Vehicle treatment	12·6 ± 1·60	153 ± 18
CPX (0·1 mg kg^{-1})	16·2 ± 1·60	71 ± 18*
CPX (0·3 mg kg^{-1})	14·4 ± 1·20	68 ± 19*

Results are given as mean ± s.e.m. (n = 6). *$P < 0{\cdot}05$ relative to no treatment group (ANOVA).

Glycerol-injected rats. Following i.m. injection of glycerol, plasma urea and creatinine levels increased six- to sevenfold by 48 h (Fig. 2). At the end of this 48 h period, CL_{IN} and CL_{PAH} were reduced by 78 and 68%, respectively, compared with saline-injected rats which received no treatment (Fig. 3). Furthermore, kidney weight was significantly increased and

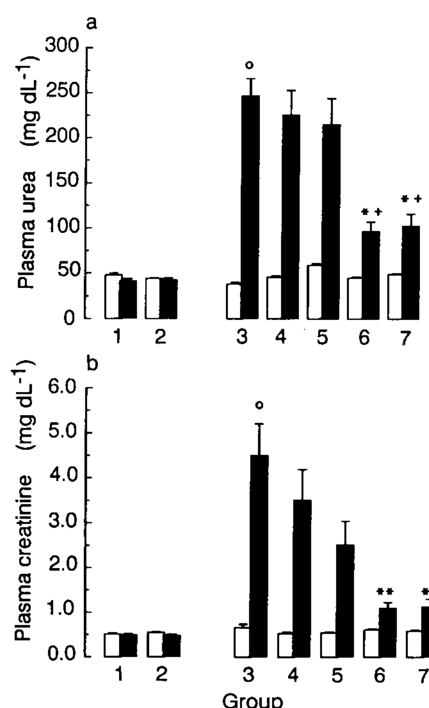

FIG. 2. Plasma urea (a) and creatinine (b) concentrations in rats immediately before (open columns) and 2 days after (filled columns) i.m. injection of saline or glycerol. Rats were treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·03, 0·1 or 0·3 mg kg^{-1}) or its vehicle (1·0 mL kg^{-1}) twice daily i.v. for 2 days. Key to groups: 1, saline-injected, no treatment; 2, saline-injected + CPX 0·3 mg kg^{-1}; 3, glycerol-injected, no treatment; 4, glycerol-injected + vehiccle; 5, glycerol-injected + CPX 0·03 mg kg^{-1}; 6, glycerol-injected + CPX 0·1 mg kg^{-1}; 7, glycerol-injected + CPX 0·3 mg kg^{-1}. Columns represent means and vertical bars s.e.m. (n = 12). ○ $P < 0{\cdot}001$ relative to group 1 (Student's *t*-test). *$P < 0{\cdot}05$; **$P < 0{\cdot}01$ relative to group 4 (ANOVA). +$P < 0{\cdot}05$ relative to group 5 (ANOVA).

Table 3. Total kidney weight and histological damage score in saline- and glycerol-injected rats treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·03, 0·1 or 0·3 mg kg^{-1}) or vehicle (1·0 mL kg^{-1}) i.v. twice daily for 2 days.

Group	Total kidney weight (g)	Damage score
Saline-injected		
No treatment	$2·18 \pm 0·06$	0·0
CPX (0·3 mg kg^{-1})	$2·17 \pm 0·07$	0·0
Glycerol-injected		
No treatment	$3·00 \pm 0·15$#	$2·75 \pm 0·22$§
Vehicle treatment	$3·04 \pm 0·10$	$2·79 \pm 0·21$
CPX (0·03 mg kg^{-1})	$2·91 \pm 0·13$	$2·83 \pm 0·11$
CPX (0·1 mg kg^{-1})	$2·70 \pm 0·07$	$2·17 \pm 0·11$*†
CPX (0·3 mg kg^{-1})	$2·58 \pm 0·09$	$1·75 \pm 0·25$**‡

Results are given as mean ± s.e.m. (n = 12). Maximum possible damage score = 5. #$P < 0·001$ relative to saline-injected no treatment (Student's *t*-test). §$P < 0·0001$ relative to saline-injected no treatment (Wilcoxon test). *$P < 0·05$; **$P < 0·01$ relative to glycerol-injected vehicle treatment (Wilcoxon test). † $P < 0·01$; ‡ $P < 0·001$ relative to glycerol-injected CPX (0·03 mg kg^{-1}) (Wilcoxon test).

histological damage was evident in glycerol-injected rats when compared with the untreated saline-injected group (Table 3). Vehicle treatment of glycerol-injected rats resulted in no significant changes ($P > 0·05$) in any of the indices of renal function above those produced by glycerol alone.

Treatment of glycerol-injected rats with 0·03 mg kg^{-1} CPX resulted in small improvements in most indices of renal

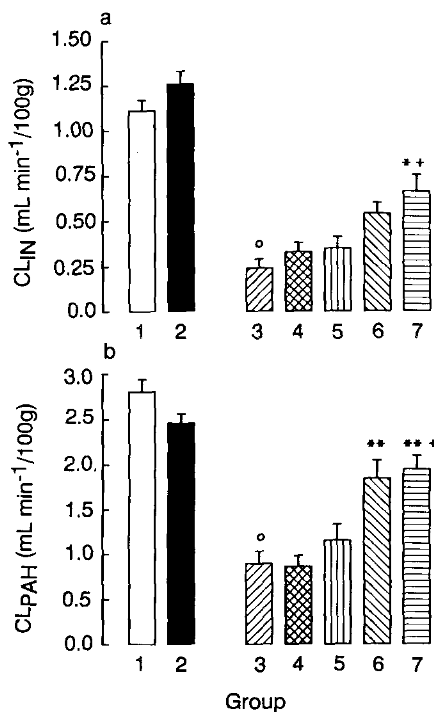

FIG. 3. The clearances of (a) [^{3}H]inulin and (b) [^{14}C]*p*-aminohippurate determined in rats 2 days after i.m. injection of saline or glycerol. Rats were treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·03, 0·1 or 0·3 mg kg^{-1}) or its vehicle (1·0 mL kg^{-1}) twice daily i.v. for 2 days. Key to groups: 1, saline-injected no treatment; 2, saline-injected + CPX 0·3 mg kg^{-1}; 3, glycerol-injected no treatment; 4, glycerol-injected + vehicle; 5, glycerol-injected + CPX 0·03 mg kg^{-1}; 6, glycerol-injected + CPX 0·1 mg kg^{-1}; 7, glycerol-injected + CPX 0·3 mg kg^{-1}. Columns represent means and vertical bars s.e.m. (n = 12). ° $P < 0·001$ relative to group 1 (Student's *t*-test). *$P < 0·05$; **$P < 0·01$ relative to group 4 (ANOVA). + $P < 0·05$ relative to group 5 (ANOVA).

function with the exception of histological damage (Figs 2, 3, Table 3). However, in comparison with vehicle-treated rats, none of these changes were statistically significant ($P > 0·05$). By contrast, treatment with CPX at a dose of 0·1 mg kg^{-1} produced significant reductions in plasma urea and creatinine concentrations compared with vehicle-treated rats. CL_{IN} and CL_{PAH} in rats treated with this dose of CPX were 63 and 114% greater, respectively, than the clearance values recorded in the vehicle-treated group although only the increase in CL_{PAH} was statistically significant ($P < 0·01$). The kidney weight in the CPX (0·1 mg kg^{-1}) treated group was some 11% lower than the kidney weight noted in vehicle-treated rats, but the difference was not statistically significant ($P > 0·05$). However, CPX treatment at 0·1 mg kg^{-1} resulted in a significant reduction in kidney damage as assessed by histological examination (Table 3). Although there were considerable improvements in all indices of renal function in rats treated with 0·1 mg kg^{-1} compared with those which received the lower dose of 0·03 mg kg^{-1} when statistical comparisons were made, only plasma urea concentrations and histological damage score were significantly lower ($P < 0·05$).

Treatment with the highest dose of CPX (0·3 mg kg^{-1}) resulted in a similar pattern of beneficial effects on renal function as noted with the 0·1 mg kg^{-1} dose. These improvements were characterized by significant decreases in plasma urea and creatinine levels (Fig. 2) and histological damage score (Table 3) and significant increases in CL_{IN} and CL_{PAH} (Fig. 3) compared with vehicle-treated rats. As seen with the 0·1 mg kg^{-1} dose the kidney weight of rats treated with 0·3 mg kg^{-1} was lower than in vehicle-treated rats but this difference failed to achieve statistical significance ($P > 0·05$). A comparison of the results of 0·3 mg kg^{-1} treatment with the data from treatment at 0·1 mg kg^{-1} showed that, although in some instances treatment with the higher dose resulted in some further improvements in renal function, e.g. CL_{IN}, there were no statistically significant differences ($P > 0·05$). However, a comparison of the results of 0·3 mg kg^{-1} treatment vs treatment at 0·03 mg kg^{-1} showed that treatment with CPX at the higher dose produced a significant reduction in plasma urea and significant increases in CL_{IN} and CL_{PAH} ($P < 0·05$).

Discussion

The results of this study have shown that in-vivo CPX administered at a dose of 0·3 mg kg^{-1} resulted in significant inhibition of renal phosphodiesterase whereas administration of 0·1 mg kg^{-1} was without effect on enzyme activity. The inhibitory effect of the higher dose of CPX was noted only in saline-injected rats and at only one time point (1 h). In glycerol-injected animals no inhibition of enzyme activity was detected after either administration of a single dose or repeated doses of CPX. There are conflicting reports with regard to the potency of CPX as a phosphodiesterase inhibitor. Coulson & Scheinman (1989) have found that, in cultured opossum kidney cells, CPX is an effective inhibitor of cAMP phosphodiesterase with a greater potency than 1-methyl-3-isobutylxanthine while Martinson et al (1987), using porcine coronary artery, have shown CPX to be ineffective as an inhibitor of this enzyme. It is difficult to

compare the present results using the rat kidney with these previous studies since, first, it is probable that cAMP phosphodiesterases from different tissues and renal phosphodiesterases from different species show varying sensitivities to inhibition by xanthines (Amer & Kreighbaum 1975; Coulson & Scheinman 1989) and second, in these enzyme studies with opossum and porcine tissue, CPX was added to the homogenates whilst in the present investigation CPX was administered to the rat and the kidney subsequently removed to determine phosphodiesterase activity. Thus we are able to make an assessment of the inhibitory effect of CPX in-vivo. Furthermore, the studies involving repeated administration of CPX to glycerol-injected rats show the extent to which enzyme activity might be altered in the experiments designed to examine the protective effects of CPX in ARF.

By contrast to the results from the phosphodiesterase assay, treatment with either 0·1 or 0·3 mg kg^{-1} of CPX produced significant improvements in renal function in rats with glycerol-induced ARF. We have previously demonstrated in-vivo that administration of 0·1 mg kg^{-1} of CPX antagonizes adenosine-induced falls in renal blood flow to the extent of producing a 30-fold shift to the right of the adenosine dose–response curve (Kellett et al 1989). In view of these results, the lack of any detectable inhibitory action on renal phosphodiesterase activity of both 0·1 and 0·3 mg kg^{-1} of CPX in glycerol-injected rats, together with the beneficial effects of both doses in glycerol-induced ARF indicate that the protective effects of CPX are a consequence of adenosine antagonism and not phosphodiesterase inhibition. This conclusion is supported by previous studies which demonstrated that, whilst theophylline and 8-phenyltheophylline were effective in reducing the severity of cisplatin and glycerol-induced ARF, respectively, treatment with the alkylxanthine enprofylline was ineffective (Yates et al 1987; Heidemann et al 1989). Enprofylline is a more potent inhibitor of phosphodiesterase than theophylline (Fredholm 1985) but has a very low affinity for adenosine receptors (Collis et al 1984).

The present findings show that the protection afforded by CPX treatment in glycerol-induced ARF is restricted to a narrow dose range. The threshold dose for the beneficial actions appears to be in the region of 0·03 mg kg^{-1} since treatment with this dose produced statistically insignificant improvements in renal function. Although for some indices (e.g. CL_{PAH}) treatment with 0·3 mg kg^{-1} of CPX resulted in some small improvements in renal function compared with the effects of 0·1 mg kg^{-1}, these improvements were of no statistical significance. This indicates that the limit to the extent of protection that can be gained with CPX administration corresponds to that resulting from treatment with 0·1 mg kg^{-1}.

In conclusion the results of this study have shown that in rats with glycerol-induced ARF, treatment with the alkylxanthine CPX produces improvements in renal function with maximum beneficial effect achieved with treatment at 0·1 mg kg^{-1}. The findings provide evidence that this beneficial action is not a consequence of renal phosphodiesterase inhibition but is probably a result of adenosine antagonism.

References

Amer, M. S., Kreighbaum, W. E. (1975) Cyclic nucleotide phosphodiesterase: properties, activators, inhibitors, structure-activity relationships and possible role in drug development. J. Pharm. Sci. 64: 1–37

Arch, J. R. S., Newsholme, E. A. (1976) Activities and some properties of adenylate cyclase and phosphodiesterases in muscle, liver and nervous tissues from vertebrates and invertebrates in relation to the control of the concentration of adenosine 3′:5′-cyclic monophosphate. Biochem. J. 158: 603–622

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

Churchill, P. C., Bidani, A. K. (1982) Hypothesis: adenosine mediates hemodynamic changes in renal failure. Med. Hypoth. 8: 275–285

Collis, M. G., Keddie, J. R., Torr, S. R. (1984) Evidence that the positive inotropic effects of alkylxanthines are not due to adenosine receptor blockade. Br. J. Pharmacol. 81: 401–407

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. Ibid. 97: 1274–1278

Coulson, R., Scheinman, S. J. (1989) Xanthine effects on renal proximal tubular function and cyclic AMP metabolism. J. Pharmacol. Exp. Ther. 248: 589–595

Fredholm, B. B. (1985) On the mechanism of action of theophylline and caffeine. Acta Med. Scand. 217: 149–153

Gouyon, J.-B., Guignard, J.-P. (1988) Theophylline prevents the hypoxemia-induced renal hemodynamic changes in rabbits. Kidney Int. 33: 1078–1083

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

Heidemann, H.Th., Müller, St., Mertins, L., Stepan, G., Hoffman, K., Ohnhaus, E. E. (1989) Effect of aminophylline on cisplatin nephrotoxicity in the rat. Br. J. Pharmacol. 97: 313–318

Kellett, R., Bowmer, C. J., Collis, M. G., Yates, M. S. (1989) Amelioration of glycerol-induced acute renal failure in the rat with 8-cyclopentyl-1,3-dipropylxanthine. Ibid. 98: 1066–1074

Lin, J.-J., Churchill, P. C., Bidani, A. K. (1986) Effect of theophylline on the initiation phase of postischemic acute renal failure. J. Lab. Clin. Med. 108: 150–154

Martinson, E. A., Johnson, R. A., Wells, J. N. (1987) Potent adenosine receptor antagonists that are selective for the A_1 receptor subtype. Mol. Pharmacol. 31: 247–252

Nicholson, C. D., Wilke, R. (1989) 8-Phenyltheophylline as an inhibitor of cyclic AMP hydrolysis by cyclic nucleotide phosphodiesterase. J. Auton. Pharmacol. 9: 159–166

Scotini, E., Carpendo, F., Fassina, G. (1983) New derivatives of methyl-xanthines: effect of thiocaffeine, thiotheophylline and 8-phenyltheophylline on lipolysis and on phosphodiesterase activities. Pharmacol. Res. Comm. 15: 131–143

Smellie, F. W., Davis, C. W., Daly, J. W., Wells, J. N. (1979) Alkylxanthines: inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. Life Sci. 24: 2475–2482

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