Bruns, R. F., Lawson-Wendling, K., Pugsley, T. A. (1983) A rapid filtration assay for soluble receptors using polyethylenimine-treated filters. Anal. Biochem. 132: 74–81

Garbarg, M., Yeramian, E., Korner, H., Schwartz, J.-C. (1985) Biochemical studies of cerebral H_1-receptors. In: Ganellin, C. R., Schwartz, J.-C. (eds) Advances in the Biosciences, Vol. 51, Frontiers in Histamine Research, Pergammon, Oxford. pp 19–25

Gavish, M., Chang, R. S. L., Snyder, S. H. (1979) Solubilization of histamine H-1, GABA and benzodiazepine receptors. Life Sci. 25: 783–790

Janski, A. M., Cornell, N. W., Yeh, H. J. P. (1980) Preparation of water-soluble digitonin that retains its ability to complex with cholesterol. Fed. Proc. 39: P1721

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Toll, L., Snyder, S. H. (1982) Solubilization and characterization of histamine H_1-receptors in brain. Ibid 257: 13593–13601

Treherne J. M., Young, J. M. (1988a) [^{3}H]-(+)-*N*-Methyl-4-methyldiphenhydramine ([^{3}H]-QMDP), a quaternary radioligand for the histamine H_1-receptor. Br. J. Pharmacol. in press

Treherne, J. M., Young, J. M. (1988b) Temperature-dependence of the kinetics of the binding of [^{3}H]-(+)-*N*-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) to the histamine H_1-receptor: comparison with the kinetics of [^{3}H]-mepyramine. Ibid. in press

Wallace, R. M. (1983) Ph.D. Thesis, University of Cambridge, pp 56–67

Wallace, R. M., Young, J. M. (1983) Temperature-dependence of the binding of [^{3}H]mepyramine and related compounds to the histamine H_1 receptor. Mol. Pharmacol. 23: 60–66

J. Pharm. Pharmacol. 1988, 40: 733–735
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Amelioration of glycerol-induced acute renal failure in the rat with 8-phenyltheophylline: timing of intervention

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Abstract—The importance of timing and duration of 8-phenyltheophylline (8-PT) administration in determining its beneficial action in glycerol-induced acute renal failure (ARF) was investigated by examining the effects of a single dose of 8-PT given immediately following (0 h) glycerol injection and at 1 and 3 h after glycerol injection. 8-PT when given at 0 h significantly lowered plasma urea and creatinine concentrations and significantly increased inulin clearance when compared both to untreated animals and those that received the vehicle for the drug. By contrast, 8-PT when administered at 1 h afforded no protective effect on renal function and, when injected at 3 h, the only significant effect was lowered plasma creatinine levels when compared to untreated rats; at this latter time it did not lower plasma urea levels or improve inulin clearance. None of the 8-PT injections attenuated the increase in kidney weight associated with ARF or reduced the kidney damage as assessed by histological examination. The results show that a single administration of 8-PT made immediately following glycerol injection can ameliorate the biochemical and functional indices of impaired renal function, but does not produce an improvement in kidney morphology.

Recent studies in rats have demonstrated that administration of the alkylxanthines theophylline or 8-phenyltheophylline (8-PT) can reduce the severity of acute renal failure (ARF) produced by intramuscular glycerol injection (Bidani & Churchill 1983; Bowmer et al 1986). This beneficial effect has been attributed to the antagonistic action of these two xanthines at adenosine receptors, particularly those which mediate vasoconstriction of the afferent renal arterioles (Churchill & Bidani 1982). Although both theophylline and 8-PT possess diuretic activity (Brater et al 1983; Collis et al 1986), the salutary action of these compounds in ARF is unlikely to be dependent on a diuretic effect mediated via an action on the renal tubule since both the "tubular diuretics" frusemide and hydrochlorothiazide increase the severity of glycerol-induced ARF (Bidani et al 1987; Yates et al 1987).

During our recent study in which we compared the effects of hydrochlorothiazide and 8-PT in ARF (Yates et al 1987), it was noted that a single injection of 8-PT given 24 h after the initiation of glycerol-induced ARF did not alter plasma urea or creatinine levels. The protective effects observed with 8-PT in ARF have occurred when this drug was given twice daily for 2 days with the initial injection made immediately after glycerol administration (Bowmer et al 1986; Yates et al, 1987). This suggests that the timing and/or duration of administration of 8-PT is important in determining its effect in ARF. We have investigated this by examining the effects of a single dose of 8-PT or its vehicle, injected at various intervals after the induction of ARF, on biochemical, functional and morphological indices of renal function.

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Materials and methods

Materials

8-PT, inulin and polyethylene glycol 400 were obtained from Sigma Chemical Co. [G-^{3}H]Inulin (180mCi g^{-1}) of stated radioactive purity >98% was obtained from New England Nuclear Ltd and was used without further purification. Reagents for the assay of creatinine and urea were purchased from Pierce and Warriner and BDH Ltd, respectively.

Methods

Induction of acute renal failure. The method for production of ARF has been previously described in detail (Bowmer et al 1982). Male Wistar albino rats (250–300 g) were deprived of water for 24 h and ARF was produced by i.m. injection of 50% v/v glycerol in sterile saline (0·9% w/v NaCl), 10 mL kg^{-1}.

Experimental protocol. Glycerol-injected rats received either no treatment or a single injection of 8-PT (10 mg kg^{-1} i.p.) or vehicle (1·0 mL kg^{-1} i.p. of 50% v/v polyethylene glycol 400 in 0.1 M NaOH) immediately after (0 h) glycerol injection. Further groups of rats received a single injection of 8-PT (10 mg kg^{-1} i.p.) at 1 h or 3 h after glycerol adminstration.

Blood samples (about 0·7 mL) were taken from the tail vein before (0 h) and 24 h after glycerol administration. Forty eight hours after glycerol injection rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) and cannulae placed in the left jugular vein and right carotid artery. The single-injection method of Hall et al (1977) was then used to measure the clearance of [^{3}H]inulin (100 mg kg^{-1}; 20 μCi kg^{-1} i.v.) from plasma (C_{IN}). At the end of the experiment a blood sample was taken from the carotid artery (for the determination of creatinine and urea) and the kidneys were removed, weighed, bisected longitudinally and placed in formal-saline (BDH Ltd).

Plasma creatinine and urea. Standard spectrophotometric assays

Table 1. Plasma urea and creatinine at 48 h in glycerol-injected rats after treatment with a single i.p. injection of 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) or vehicle (1·0 mL kg^{-1}).

	No treatment	Vehicle at 0 h	8-PT at 0 h	8-PT at 1 h	8-PT at 3 h
Plasma urea (mg dL^{-1})					
0 h	40±3	35±2	44±2	36±2	42±1
24 h	233±22	202±12	166±20**	186±15	266±15
48 h	390±38	295±31*	189±33***†	316±35	471±25
Plasma creatinine (mg dL^{-1})					
0 h	0·56±0·05	0·55±0·05	0·48±0·03	0·54±0·02	0·53±0·03
24 h	4·50±0·14	2·73±0·26***	2·09±0·31***	4·08±0·20	3·58±0·19**
48 h	6·46±0·34	3·76±0·43***	2·44±0·44***†	5·45±0·30	5·39±0·35*

Results are given as mean±s.e. mean, n=12.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$ relative to rats with no treatment.
† $P<0·05$ rats given 8-PT at 0 h relative to vehicle treated rats.
Statistical analysis was performed using ANOVA.

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

Kidney histology. A longitudinal section was 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 a scoring system previously reported (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).

Analysis of results. Results are expressed as mean±s.e. mean. Statistical comparisons of plasma urea and creatinine concentrations, C_{IN} and kidney weight were made by one-way analysis of variance (ANOVA) after which the means were compared by the Method of Least Significant Difference (Snedecor & Cochran 1967). The statistical comparison of data obtained after vehicle administration at 0 h was only made with data from untreated rats and from rats which received 8-PT at 0 h. There was no significant correlation between kidney weight and body weight, so kidney weight was not expressed as a function of body weight. Statistical analysis of the histological damage score was made by a one-sided Mann Whitney test.

Results

Following injection of glycerol, mean plasma urea and creatinine concentrations in untreated rats increased by about 10 and 9-fold, respectively at 48 h (Table 1). In rats treated with vehicle immediately after glycerol injection (0 h) levels of urea at 48 h and creatinine at 24 and 48 h were significantly lower ($P<0·05$) than in rats that received no treatment. 8-PT when administered to rats at 0 h resulted in plasma concentrations of urea and creatinine that were significantly lower ($P<0·01$) than in untreated rats at both 24 and 48 h and significantly lower ($P<0·05$) than in vehicle treated rats at 48 h. By contrast, rats that received 8-PT 1 h following glycerol administration had plasma urea and creatinine levels which were not significantly different ($P>0·05$) from concentrations obtained in untreated rats. Similar results for plasma urea were obtained for rats given 8-PT 3 h after glycerol injection, but creatinine levels at 24 and 48 h showed modest reductions when compared to untreated animals ($P<0·05$).

Forty eight hours after glycerol injection the mean C_{IN} of untreated rats was 0·14±0·02 mL min^{-1}/100 g. Treatment of rats with vehicle at 0 h resulted in a C_{IN} which was similar to untreated rats (Fig. 1). By contrast, animals which received 8-PT

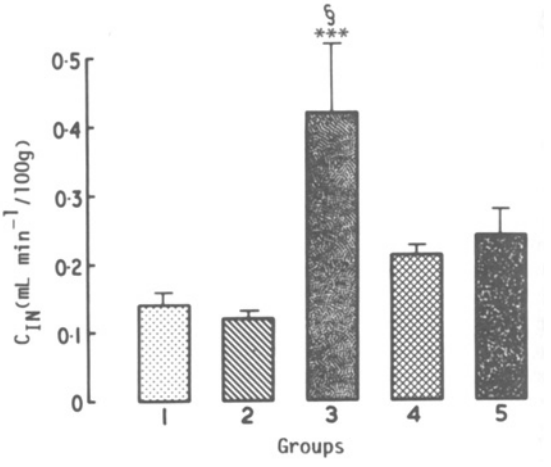

FIG. 1. The clearance of [^{3}H]inulin (C_{IN}) at 48 h in glycerol-injected rats after treatment with a single i.p. injection of vehicle (1·0 mL kg^{-1}) or 8-phenyltheophylline (8-PT, 10 mg kg^{-1}). Key to groups: (1) no treatment; (2) vehicle at 0 h; (3) 8-PT at 0 h; (4) 8-PT at 1 h; (5) 8-PT at 3 h. Columns represent mean values with vertical bars showing s.e. means, n=12. *** $P<0·001$ relative to group 1. § $P<0·001$ group 3 relative to group 2. Statistical analysis was performed using ANOVA.

at 0 h had a threefold greater C_{IN} ($P<0·001$) when compared with either untreated or vehicle treated rats (Fig. 1). C_{IN} in rats given 8-PT at 1 or 3 h was greater than in the untreated group but this difference was not significant ($P>0·05$).

Untreated rats, 48 h after induction of ARF, had a mean kidney weight of 2·9±0·1 g. Treatment with either vehicle at 0 h or 8-PT given at any of the times after glycerol injection resulted in kidney weights which were not significantly different than those weights recorded in untreated rats (Table 2). Data from histological examination of kidneys taken from rats after various treatments are also shown in Table 2. Neither the vehicle

Table 2. Effect of a single i.p. dose of 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) and vehicle (1·0 mL kg^{-1}) on the total kidney weight and renal damage associated with glycerol-induced acute renal failure.

Group	Total kidney weight (g)	Damage score
No treatment	2·93±0·12	6·17±0·11
Vehicle at 0 h	2·87±0·10	6·42±0·15
8-PT at 0 h	2·73±0·11	6·17±0·34
8-PT at 1 h	2·78±0·06	6·75±0·22
8-PT at 3 h	2·95±0·15	6·50±0·20

Results are given as mean±s.e. mean, n=12.

at 0 h nor 8-PT administered at any of the three time-points had a significant effect on the morphological damage score.

Discussion

The findings of this study show that in ARF the protective effect afforded by a single injection of 8-PT, is greatest when its administration immediately follows glycerol injection. This supports the work of Bidani & Churchill (1983) who investigated the effect of single injections of aminophylline on the development of glycerol-induced ARF. They also found that protection was most pronounced when aminophylline was given at the time of glycerol injection, but in their study the severity of renal failure was judged solely by serum creatinine concentrations whereas in the present study a range of parameters was evaluated.

In glycerol-induced ARF there is an initial reduction in renal blood flow which is principally a result of a fall in cardiac output (Hsu et al 1977). It has been proposed that in various forms of experimentally induced ARF, adenosine, released during ischaemia, evokes pre-glomerular constriction and post-glomerular dilatation which produces decreases in filtration fraction and glomerular filtration rate (Churchill & Bidani 1982). The time of onset of renal ischaemia in the glycerol model of ARF is not accurately known. In the study of Hsu et al (1977) renal blood flow was found to be reduced 3 h after the induction of glycerol-induced ARF but no measurements of renal blood flow were made before this. By contrast to 8-PT administered immediately following glycerol injection, 8-PT given at 1 and 3 h after glycerol injection was not effective in ameliorating ARF. One possible explanation for this is that adenosine plays a very early role in the pathogenesis of ARF and that the antagonist is less effective when administered once adenosine levels are elevated. Confirmation of this would require an evaluation of the temporal relationship between renal haemodynamics and adenosine levels following the induction of ARF.

It is possible that the lack of any notable beneficial effects of 8-PT administered at 1 and 3 h is a result of a detrimental effect on renal function of the associated vehicle which would mask the ameliorating effects of the alkylxanthine. However, when the vehicle is administered at 0 h there is a significant improvement in some indices of renal function, as discussed later, and in a limited number of experiments ($n=5$) we have found no evidence for a deleterious effect of a single injection of vehicle on inulin clearance and plasma urea and creatinine concentrations when given at 1 and 3 h after glycerol injection (unpublished results).

By comparison to a previous study in which 8-PT was administered twice daily for 2 days (Yates et al 1987), the single injection of 8-PT at 0 h produced a similar degree of amelioration of ARF as judged by plasma urea and creatinine levels and C_{IN} although it failed to reduce kidney weight, a reflection of the degree of oedema. Furthermore, it also failed to limit kidney damage as assessed by histological examination. This indicates that the protective effect of 8-PT on kidney morphology arises from its repeated administration. This particular beneficial effect of 8-PT may not be related to adenosine antagonism since the xanthine enprofylline when administered twice daily for 2 days to rats with glycerol-induced ARF, at a dose devoid of adenosine antagonism, reduced the severity of morphological damage without improving any other index of renal function (Yates et al 1987).

The vehicle for 8-PT when given as a single dose at 0 h lowered levels of plasma urea and creatinine compared to untreated rats. These changes were, however, less than those seen with a single administration of 8-PT. We obtained similar findings in rats with ARF when the vehicle was administered twice daily for 2 days (Bowmer et al 1986; Yates et al 1987). However, by contrast to a single dose of vehicle, multiple doses resulted in an increase in C_{IN} and a reduction in kidney weight in comparison with rats which received no treatment (Bowmer et al 1986; Yates et al 1987). The protective effect of the vehicle may be due to the polyethylene glycol component (Yates et al 1987) which, by virtue of its hyperoncotic and impermeant nature could reduce cell swelling and oedema in damaged kidneys (Frega et al 1979; Leaf et al 1983). Thus some of the protective effects afforded by the vehicle may be a consequence of a prolonged hyperoncotic effect.

In conclusion the present study of glycerol-induced ARF shows that a single administration of 8-PT made immediately following glycerol injection can ameliorate the biochemical and functional correlates of impaired renal function. However, in order to obtain an improvement in kidney morphology it would appear necessary to give repeated injections of 8-PT during the course of ARF.

References

Bidani, A. K., Churchill, P. C. (1983) Aminophylline ameliorates glycerol-induced acute renal failure in rats. Can. J. Physiol. Pharmacol. 61: 567–571

Bidani, A. K., Churchill, P. C., Packer, W. (1987) Theophylline-induced protection in myoglobinuric acute renal failure: further characterization. Ibid. 65: 42–45

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

Brater, D. C., Kaojarern, S., Chennavasin, P. (1983). Pharmacodynamics of the diuretic effects of aminophylline and acetazolamide alone and combined with furosemide in normal subjects. J. Pharmacol. Exp. Ther. 227: 92–97

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

Collis, M. G., Baxter, G. S., Keddie, J. R. (1986) The adenosine receptor antagonist, 8-phenyltheophylline, causes diuresis and saliuresis in the rat. J. Pharm. Pharmacol. 38: 850–852

Frega, N. S., Dibona, D. R., Leaf, A. (1979) The protection of renal function from ischemic injury in the rat. Pflügers Arch. 381: 159–164

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

Henry, R. J., Cannon, D. C., Winkelman, J. W. (1974) Clinical Chemistry Principals and Techniques. 2nd ed. Harper and Row: London, pp 503–563

Hsu, C. H., Kurtz, T. W., Waldinger, T. P. (1977) Cardiac output and renal blood flow in glycerol-induced acute renal failure in the rat. Circ. Res. 40: 178–182

Leaf, A., Cheung, J. Y., Mills, J. W., Bonventre, J. V. (1983). Nature of the cellular insult in acute renal failure in: Brenner, B.M., Lazarus, J.M. (eds). Acute Renal Failure. W.B. Sanders Company. Philadelphia, pp 2–20

Snedecor, G. W., Cochran, W. V. (1967) Statistical Methods 6th ed. Iowa State University Press, Ames, Iowa, pp 271–275

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. J. Pharm. Pharmacol. 39: 803–808