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The Protective Effect of Glycine in Cisplatin Nephrotoxicity: Inhibition with N^G-Nitro-L-arginine Methyl Ester

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Abstract—The effect of glycine on the acute changes in renal haemodynamics and nephrotoxicity produced by cisplatin was investigated in the rat. Cisplatin (6·0 mg kg^{-1}, i.v.) injection in anaesthetized rats produced, over a period of 2 h, falls of approximately 50% in renal blood flow (RBF) and the clearance of [^{3}H]inulin (CL_{IN}), effects which were prevented by co-administration of glycine (1·0 g kg^{-1}). Infusion of the nitric oxide (NO) synthase-inhibitor N^G-nitro-L-arginine methyl ester, L-NAME (10 μg min^{-1} kg^{-1}, i.v.), abolished glycine's ability to maintain RBF in cisplatin-injected rats whilst partially inhibiting the ability of glycine to preserve CL_{IN}. Treatment of cisplatin-injected rats with glycine (1·0 g kg^{-1}, i.v.) significantly ameliorated the nephrotoxic effects of cisplatin (6·0 mg kg^{-1}) as judged by improvements in a range of indices of renal function which included plasma urea and creatinine concentrations, urine output, sodium excretion, CL_{IN} and the clearance of [^{14}C]*p*-aminohippurate. Administration of L-NAME (1·0 mg kg^{-1}, i.v.) to rats which received cisplatin and glycine significantly inhibited the reno-protective effect of glycine. However, L-NAME administration to rats which were treated only with cisplatin did not result in any potentiation of cisplatin nephrotoxicity. The findings of this study suggest that glycine can block the acute falls in RBF and C_{IN} produced by cisplatin by a mechanism which involves the production of NO. Furthermore, the results indicate that these renal haemodynamic actions of glycine are responsible, at least in part, for the ability of this amino acid to ameliorate cisplatin nephrotoxicity.

Glycine has been shown to exert protective effects in various forms of acute renal injury. This amino acid protects against anoxic injury (Weinberg et al 1987) and ouabain toxicity (Weinberg et al 1990) in rabbit dispersed proximal tubules. Furthermore, Heyman et al (1991) have shown in-vivo that glycine infusion also significantly ameliorates the nephrotoxic actions of the anticancer agent cisplatin in the rat and that this effect is not a consequence of an obvious pharmacokinetic interaction. The mechanisms involved in the renal protective effects of glycine have not been elucidated, although in cisplatin nephrotoxicity the beneficial effects of glycine may be a result of a haemodynamic action. Cisplatin treatment has been shown to produce a fall in renal blood flow (RBF) in man, dog and rat (Offerman et al 1984; Winston & Safirstein 1985; Daugaard et al 1987). Glycine produces increases in renal blood flow and glomerular filtration rate (GFR) in isolated perfused kidneys and in rats with normal renal function by a mechanism which, at least in part, involves the production of nitric oxide (NO) (El Sayed et al 1991; King et al 1991). Consequently, glycine's ability to ameliorate cisplatin nephrotoxicity may be due to a functional antagonism of the renal vasoconstriction induced by the anticancer agent.

The aim of the present study was to examine this possibility by investigating, in both acute and chronic experiments, the effects of glycine on renal function in cisplatin-treated rats. In some of these experiments, animals were also treated with the NO synthase-inhibitor N^G-L-arginine methyl ester (L-NAME). These experiments were conducted in order to assess the contribution of renal haemodynamic changes to the protective effect of glycine and, in addition, to determine the extent of the role of NO in the renal haemodynamic effects of glycine in cisplatin-treated rats.

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Materials and Methods

Materials

Glycine, L-NAME, inulin, *p*-aminohippuric acid and cisplatin were purchased from Sigma Chemical Co., Poole, UK. [^{3}H(G)]Inulin (201 mCi g^{-1}) and *p*-[glycyl-1-^{14}C]aminohippuric acid (43 mCi $mmol^{-1}$) were obtained from DuPont NEN Research Products, Stevenage, UK. The stated radiochemical purity of each isotope was greater than 98%. Reagent kits for assay of creatinine and urea were bought from Pierce & Warriner, Chester, and BDH Ltd, Lutterworth, respectively.

Acute effects of cisplatin on renal function in anaesthetized animals

Male albino Wistar rats, 200–250 g, were anaesthetized with thiobutabarbitone (180 mg kg^{-1}, i.p.) and cannulae inserted into the trachea to facilitate spontaneous ventilation, left jugular vein for saline infusion and drug administration, and right carotid artery for measurement of mean arterial blood pressure (MAP) via a pressure transducer (Druck PDCR 75). In some experiments a cannula was placed in the femoral vein for infusion of L-NAME. The abdomen was opened by a midline incision and a cannula was inserted into the bladder for collection of urine and an ultrasonic perivascular flow probe (model 2SB, Transonic Systems Inc., USA) was placed around the left renal artery. The probe was connected to a small-animal flowmeter (T206 Transonic Systems Inc.) to record mean renal blood flow (RBF). Body temperature was maintained at 37°C using a rectal thermometer and heating lamps. On completion of surgery, 2 mL saline (0·9% NaCl)

containing 0·35 μCi mL^{-1} [^{3}H]inulin was administered via the jugular vein. This solution was infused for the remainder of the experiment at a rate of 100 μL min^{-1}. A 60-min equilibration period was then allowed for stabilization of urine flow.

Each experiment consisted of a control collection period of 30 min followed by six 20-min clearance periods during which urine was collected into pre-weighed tubes, and a blood sample (0·1 mL) was taken at the midpoint of each urine collection so the renal clearance of [^{3}H]inulin (CL_{IN}) could be estimated. Blood samples were centrifuged and the plasma separated for subsequent analysis. The erythrocytes were suspended in an equal volume of isotonic saline and transfused back into the animal. Following the control collection period, rats received one of the following treatments intravenously, cisplatin (6 mg kg^{-1}, 2 mg mL^{-1} in saline); cisplatin (6 mg kg^{-1}) with glycine (1 g kg^{-1}, 5 mL kg^{-1} in saline) in which cisplatin was given as a bolus dose at the midpoint of the injection of glycine which was made over 3 min; cisplatin (6 mg kg^{-1}) with glycine (1 g kg^{-1}) and an infusion of L-NAME (10 μg kg^{-1} min^{-1}; 10 μL min^{-1} in saline) which commenced 60 min before the cisplatin/glycine injection and continued throughout the experiment; cisplatin (6 mg kg^{-1}) and the same infusion of L-NAME (10 μg kg^{-1} min^{-1}). In experiments involving glycine treatment, the timing of the first test clearance period commenced from the start of the glycine injection. The infusion of L-NAME was selected on the basis of preliminary experiments in normal rats, which showed that 10 μg kg^{-1} min^{-1} of L-NAME inhibited the increases in RBF and CL_{IN} produced by glycine (1 g kg^{-1}). These findings confirm previous studies (King et al 1991; Cernadas et al 1992) which demonstrated that treatment with NO synthase-inhibitors blocked the increase in renal plasma flow and GFR induced by glycine.

Cisplatin-induced nephrotoxicity (8 day study)

Male Wistar albino rats (200–250 g) were injected intravenously via the tail vein with either saline (3 mL kg^{-1}), cisplatin (6 mg kg^{-1}, 2 mg mL^{-1} in saline); cisplatin (6 mg kg^{-1}) followed immediately by glycine (1 g kg^{-1}, 5 mL kg^{-1} in saline injected over 3 min); cisplatin (6 mg kg^{-1}) followed immediately by dextrose (0·43 g kg^{-1}, 5 mL kg^{-1} in saline injected over 3 min); cisplatin (6 mg kg^{-1}) followed immediately by glycine (1 g kg^{-1}) and L-NAME (1 mg kg^{-1}, 1 mL kg^{-1} in saline); or cisplatin (6 mg kg^{-1}) followed immediately by L-NAME (1 mg kg^{-1}). The dose of dextrose was isosmotic with the glycine dose whilst the bolus dose of L-NAME has been shown to be effective in inhibiting NO synthesis in-vivo (Szabó et al 1993).

Rats were placed in metabolic cages for a 24-h urine collection 3 days following the various treatments (day 2) and a blood sample (0·75 mL) was taken from the tail vein on day 4. Animals were also placed in metabolic cages for urine collection over 24 h on day 7. On day 8, rats were anaesthetized and the clearances of [^{3}H]inulin (CL_{IN}) and [^{14}C]*p*-aminohippuric acid (CL_{PAH}) determined. At the end of the experiment, a final blood sample (1 mL) was taken from the carotid artery and rats were killed with an overdose of anaesthetic.

Estimation of [^{3}H]inulin and [^{14}C]p-aminohippuric acid clearances

Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and cannulae were inserted into the trachea, left jugular vein and right carotid artery. Animals were heparinized (200 units kg^{-1}) and the clearances of [^{3}H]inulin (100 mg kg^{-1}, 20 μCi kg^{-1}, i.v.) and [^{14}C]*p*-aminohippuric acid (40 mg kg^{-1}, 4 μCi kg^{-1}, i.v.) dissolved in saline, were then determined simultaneously by the single injection method of Hall et al (1977).

Urine and plasma analysis

Urine concentrations of sodium were measured by flame photometry and levels of [^{3}H]inulin and [^{14}C]*p*-aminohippuric acid (8-day nephrotoxicity studies only) were determined by liquid scintillation counting. Plasma levels of creatinine and urea were assayed by standard colorimetric procedures, creatinine by reaction with picrate in alkaline solution and urea by reaction with diacetyl monoxime.

Analysis of results

Results are expressed as mean ± s.e.m. Comparison of means between groups was made using either a non-paired Student's *t*-test or, where appropriate, by one-way analysis of variance with means compared by Scheffe's test.

Results

Renal function in anaesthetized animals

There were no statistically significant differences ($P > 0·05$) in MAP amongst the four groups of rats before injection of cisplatin and either glycine or L-NAME. Furthermore, administration of cisplatin alone resulted in no significant changes in MAP during the course of the experiment from a control level of 100 ± 7 mmHg (n = 6). By contrast, when rats were given both cisplatin and glycine, there was a fall in MAP, 1 min after the start of glycine injection, to 78 ± 8 mmHg (n = 6) which was significantly lower ($P < 0·05$) than the pressure noted in rats 1 min following injection of cisplatin (101 ± 6 mmHg, n = 6). Five minutes after glycine injection MAP had returned to levels which were not significantly ($P > 0·05$) different from those noted in the cisplatin group. Although from 10–110 min, MAP in rats given cisplatin and glycine was 10% lower than that in the cisplatin group, at the end of the experiment (110 min) there was no statistically significant ($P > 0·05$) difference in MAP between the groups (cisplatin 97 ± 6 mmHg, n = 6; cisplatin/glycine 90 ± 2 mmHg, n = 6). Rats which received cisplatin, glycine and L-NAME also exhibited a marked but transient reduction in MAP 1 min after administration of cisplatin and glycine, with MAP reduced to 57 ± 5 mmHg (n = 6). By contrast with the small fall in MAP observed in cisplatin/glycine animals from 10 to 110 min, MAP gradually increased in rats given these compounds and infused with L-NAME, such that values at times from 30 to 110 min were significantly ($P < 0·05$) higher than those recorded in animals which received only cisplatin and glycine. For example, at 70 min, MAP in rats given cisplatin/glycine/L-NAME was 111 ± 3 mmHg (n = 6) compared with a value of 92 ± 1 mmHg (n = 6) in animals receiving cisplatin/glycine. MAP

was higher in rats which received cisplatin and L-NAME compared with the cisplatin group. At 70 min, for instance, MAP in rats which received cisplatin/L-NAME was 112±4 mmHg (n=6) compared with a pressure of 102±6 mmHg (n=6) in animals given cisplatin, although at no time were any of these differences statistically significant ($P>0.05$).

Fig. 1 shows that following cisplatin injection there was a gradual decline in both RBF and CL_{IN}, such that by 110 min these measures of renal function had decreased to about 50% of control values. These changes were accompanied by a gradual diuresis and natriuresis (Fig. 2). At the end of the experiment, urine flow and sodium excretion were increased 6-fold relative to flow and excretion before cisplatin injection. Administration of glycine with cisplatin elicited a rapid 68% increase in RBF which by 10 min had returned to pre-injection levels, but remained significantly ($P<0.05$) greater than RBF in cisplatin-treated animals during the periods from 50 to 110 min (Fig. 1). CL_{IN} in the cisplatin/glycine group showed a similar pattern of change to RBF. CL_{IN} initially increased ($P<0.05$) with subsequent values remaining 2-fold higher than in the cisplatin group. Furthermore, Fig. 2 shows a rapid and marked increase in urine flow occurred after injection of cisplatin/glycine which declined to levels noted in cisplatin-injected rats after 50 min. Similar changes were seen for sodium excretion, but in this case, excretion did not return to levels found in cisplatin rats until 110 min. Injections of cisplatin and glycine to rats receiving an infusion of L-NAME evoked a transient fall in RBF. The initial rise in RBF observed in the cisplatin/glycine group was not observed and there was no sustained elevation of RBF. Similarly, L-NAME infused into rats given cisplatin and glycine also attenuated the elevations in CL_{IN} noted in the cisplatin/glycine group with significant differences ($P<0.05$) occurring between these groups during the clearance periods 0–20, 80–100 and 100–120 min (Fig. 1). However, by contrast with RBF, L-NAME-infusion in rats given cisplatin and glycine did not return CL_{IN} to values recorded in rats injected with cisplatin only. L-NAME infusion to animals which received cisplatin and glycine significantly ($P<0.05$) potentiated the diuretic and natriuretic effects observed in the

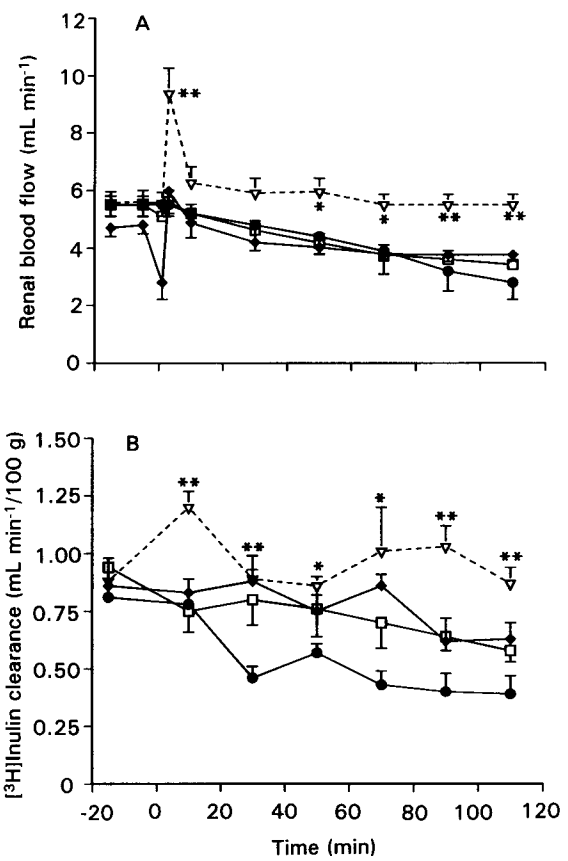

FIG. 1. A. Renal blood flow (RBF) and B. [^{3}H]inulin clearance (CL_{IN}) in anaesthetized rats treated intravenously with cisplatin (●; 6·0 mg kg^{-1}) and either glycine (▽; 1·0 g kg^{-1}) or N^G-nitro-L-arginine methyl ester (□; L-NAME, 10 μg kg^{-1} min^{-1}) or cisplatin+glycine+L-NAME (◆). Results are given as mean and vertical bars indicate s.e.m. (n=6). Cisplatin and glycine were administered at time 0 whereas L-NAME infusion commenced 60 min before these injections. $*P<0.05$, $**P<0.01$ relative to cisplatin. RBF values in the cisplatin+glycine+L-NAME group are significantly different ($P<0.05$) from the cisplatin+glycine group at times from 1 min onwards. CL_{IN} values in the cisplatin+glycine+L-NAME group are significantly different ($P<0.05$) from the cisplatin+glycine group in clearance periods 0–20, 80–100 and 100–120 min. CL_{IN} values in the cisplatin+L-NAME group are significantly different ($P<0.05$) from the cisplatin group in clearance periods 20–40 and 60–80 min.

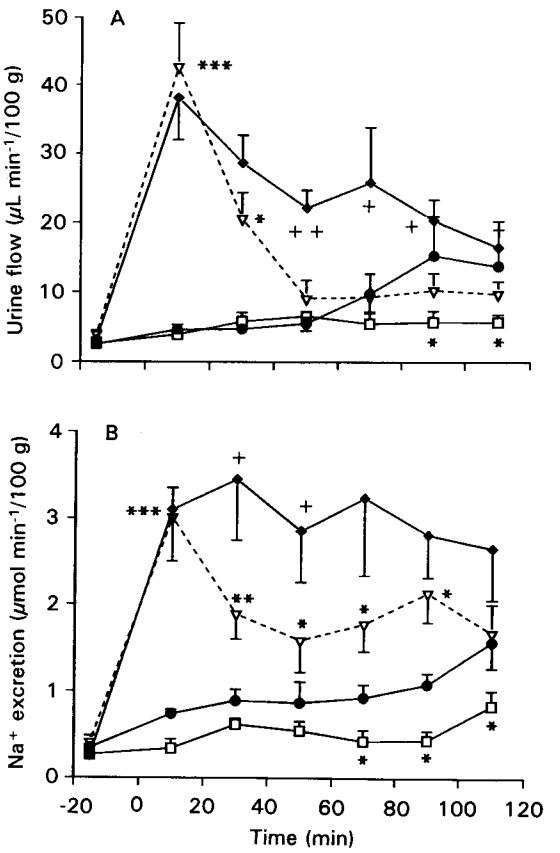

FIG. 2. A. Urine flow and B. sodium excretion in anaesthetized rats treated intravenously with cisplatin (●; 6·0 mg kg^{-1}) and either glycine (▽; 1·0 g kg^{-1}) or N^G-nitro-L-arginine methyl ester (□; L-NAME, 10 μg kg^{-1} min^{-1}) or cisplatin+glycine+L-NAME (◆). Results are given as mean and vertical bars indicate s.e.m. (n=6). Cisplatin and glycine were administered at time 0 whereas L-NAME infusion commenced 60 min before these injections. $*P<0.05$, $**P<0.01$, $***P<0.001$ relative to cisplatin, $^+P<0.05$, $^{++}P<0.01$ relative to cisplatin+glycine.

Table 1. Plasma urea and creatinine concentrations and urinary excretion in saline and cisplatin-injected rats.

	Days	Treatment groups					
		Saline	Cisplatin	Cisplatin + dextrose	Cisplatin + glycine	Cisplatin + glycine + L-NAME	Cisplatin + L-NAME
Plasma urea (mg/100 mL)	4	36 ± 1	146 ± 14†††	161 ± 25	49 ± 4***	74 ± 5^{+}	97 ± 4^{+}
	8	30 ± 2	151 ± 7†††	188 ± 26	48 ± 7***	78 ± 6^{+}	92 ± 6^{+}
Plasma creatinine (mg/100 mL)	4	0·56 ± 0·02	2·07 ± 0·25†††	3·60 ± 0·28^{+}	0·61 ± 0·04***	1·22 ± 0·04^{++}	1·30 ± 0·08^{++}
	8	0·63 ± 0·08	1·73 ± 0·19†††	2·66 ± 0·50^{+}	0·60 ± 0·07***	1·43 ± 0·15^{++}	1·46 ± 0·10
Urine output (mL/100 g/24 h)	4–5	3 ± 1	21 ± 1†††	15 ± 1^{+}	5 ± 1***	13 ± 1^{++}	17 ± 1
	7–8	3 ± 1	17 ± 1†††	12 ± 1^{+}	6 ± 1**	13 ± 1^{++}	17 ± 1
Sodium excretion (mmol/100 g/24 h)	4–5	0·65 ± 0·04	0·43 ± 0·05†	0·27 ± 0·04^{+}	0·65 ± 0·10*	0·56 ± 0·06	0·48 ± 0·06
	7–8	0·86 ± 0·05	0·38 ± 0·05††	0·40 ± 0·07	0·51 ± 0·06	0·62 ± 0·08	0·64 ± 0·03^{+}

Results are shown as mean ± s.e.m. (n = 8). Doses were as follows: cisplatin, 6 mg kg^{-1}; dextrose, 0·43 g kg^{-1}; glycine 1 g kg^{-1} L-NAME, 1 mg kg^{-1}. †$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$ relative to saline-injected rats (t-test). $^{+}P < 0·05$, $^{++}P < 0·01$ relative to cisplatin only; *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ relative to cisplatin + dextrose; $^{+}P < 0·05$, $^{++}P < 0·01$ relative to cisplatin + glycine.

cisplatin/glycine group (Fig. 2). By comparison with rats given cisplatin, infusion of L-NAME to cisplatin-injected animals resulted in no change in RBF, significantly ($P < 0·05$) higher CL_{IN} values during the clearance periods 20–40 and 60–80 min, and significantly lower ($P < 0·05$) urine flows and sodium excretion from 60 to 120 min.

Cisplatin-induced nephrotoxicity

Cisplatin administration produced 2·5- to 5-fold increases in the levels of plasma urea and creatinine on days 4 and 8 following its injection compared with saline-injected rats (Table 1). The urine collected from these animals on days 4–5 and 7–8 showed that, in comparison with the saline controls, cisplatin evoked a marked polyuria which was associated with significant reductions in sodium excretion. In addition, Fig. 3 shows that on day 8, CL_{IN} and CL_{PAH} in cisplatin-injected rats were reduced to 22 and 37%, respectively, of saline control values.

Treatment of cisplatin-injected rats with dextrose-saline solution (isosmotic to glycine solution) resulted in plasma urea levels which were not significantly different ($P < 0·05$) from rats given cisplatin alone, although on both days 4 and 8, plasma creatinine concentrations were significantly ($P < 0·05$) higher (Table 1). In addition, by comparison with cisplatin-injected rats, urine output was significantly lower ($P < 0·05$) during both collection periods and sodium excretion reduced during the first collection period in the group given cisplatin and dextrose. CL_{IN} was 66% lower ($P < 0·05$) in the cisplatin/dextrose group compared with rats injected with cisplatin, whilst there was no significant difference ($P > 0·05$) in CL_{PAH} values between the two groups (Fig. 3). Overall, these findings show that the isosmotic vehicle for glycine does not result in any beneficial effect on renal function in cisplatin-treated rats.

Glycine treatment of cisplatin-injected rats resulted in statistically significant improvements in the range of indices of renal function monitored, in comparison with the cisplatin/dextrose group. Treatment with the amino acid produced reductions in plasma urea of 70 and 74% and in plasma creatinine of 83 and 77% on days 4 and 8, respectively

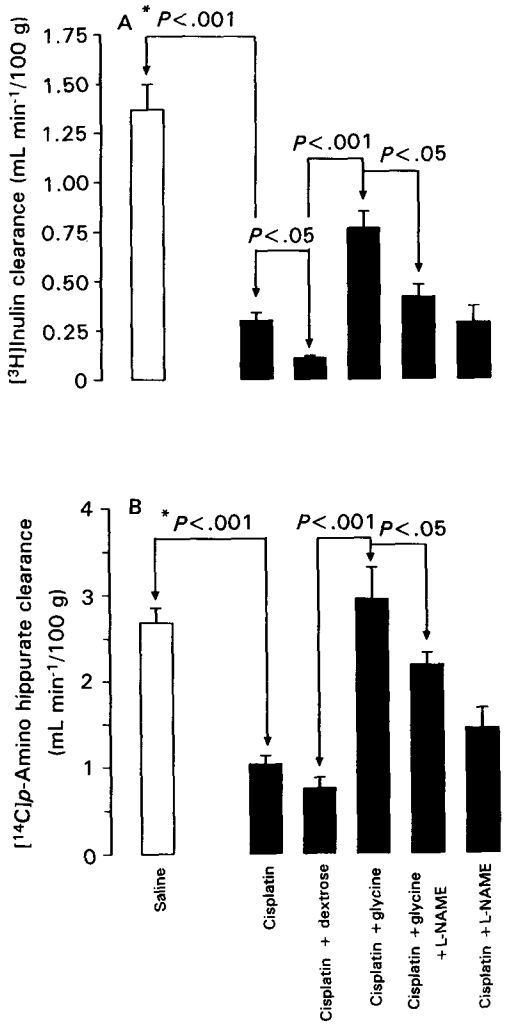

FIG. 3. A. [^{3}H]Inulin clearance (CL_{IN}) and B. [^{14}C]p-aminohippurate clearance in rats on day 8 after cisplatin or saline injections. Columns represent means with vertical bars s.e.m. (n = 8). Statistical comparisons were made using analysis of variance except * which indicates t-test.

(Table 1). Urine output was reduced by 66 and 50% and sodium excretion increased by 141 and 28% during the collections on days 4–5 and 7–8, respectively. In addition, glycine-treated rats showed marked increases in CL_{IN} (600%) and CL_{PAH} (288%) on day 8 (Fig. 3). When L-NAME was administered along with glycine to cisplatin-injected rats there was a reduction in the protective effect of glycine as demonstrated by significant increases ($P<0.05$) in plasma urea and creatinine and urine output and significant decreases ($P<0.01$) in CL_{IN} and CL_{PAH} compared with the glycine/cisplatin group. However, with the exception of urine output, attenuation of the protective effects of glycine by L-NAME was far from complete since the levels of plasma urea and creatinine and values of CL_{IN} and CL_{PAH} did not attain the values observed in cisplatin-injected animals treated with dextrose. Furthermore, there was no significant difference ($P>0.05$) in sodium excretion between cisplatin-injected rats treated with glycine and those treated with both glycine and L-NAME. Treatment of cisplatin-injected rats with L-NAME resulted in no significant change ($P<0.05$) in urine output, CL_{IN} or CL_{PAH} when compared with rats given only cisplatin, although this NO-synthase inhibitor did produce significant ($P<0.05$) falls in urea levels on days 4 and 8, creatinine on day 4 and a significant increase ($P<0.05$) in sodium excretion on days 7–8 (Table 1) relative to the cisplatin group.

Discussion

The results of this study show that cisplatin produces a substantial fall in RBF and CL_{IN} within 2 h of administration. Cisplatin treatment has been shown previously to produce a fall in RBF in man, dog and rat (Offerman et al 1984; Winston & Safirstein 1985; Daugaard et al 1987). This decrease in RBF occurs within 3 h in man (Offerman et al 1984), but has only been noted in the dog and rat 48–72 h following cisplatin administration (Winston & Safirstein 1985; Daugaard et al 1986, 1987). The decline in RBF and CL_{IN} was prevented by a bolus dose of glycine ($1.0\ g\ kg^{-1}$), an effect which appeared to be independent of pronounced blood pressure changes since, despite an initial transient fall, MAP in cisplatin-injected rats treated with glycine was not significantly different at the end of the experiment from the value noted in rats given cisplatin only. This supports a previous report of increases in RBF and glomerular filtration rate evoked by glycine which occur in the absence of any change in systemic blood pressure (Cernadas et al 1992).

The preservation of RBF and CL_{IN} in cisplatin-injected rats by glycine was inhibited by an infusion of the NO-synthase inhibitor L-NAME ($10\ \mu g\ kg^{-1}\ min^{-1}$). These findings support previous studies in normal rats (King et al 1991; Cernadas et al 1992) in which administration of N^G-monomethyl-L-arginine was shown to block glycine-induced elevations in glomerular filtration rate and provide further evidence for the importance of NO in the renal response to glycine. We have shown that L-NAME but not D-NAME, the enantiomer of L-NAME which is inactive against NO synthase, when infused in normal rats at a dose of $10\ \mu g\ kg^{-1}\ min^{-1}$ produces falls in RBF and CL_{IN} (Li et al 1994). Similar findings in rats have been reported by Lahera et al (1991) following infusion of L-NAME ($10\ \mu g\ kg^{-1}\ min^{-1}$). By contrast, when L-NAME was given to rats injected with cisplatin there was no potentiation of cisplatin-induced falls in RBF and CL_{IN}. There is no obvious explanation for the lack of any renal constrictor response to L-NAME in cisplatin-treated rats. However, these findings indicate that glycine's ability to protect RBF and CL_{IN} in cisplatin-treated rats is a result of glycine stimulating NO production and that the ability of L-NAME to block the glycine response is not a result of the renal vasoconstrictor action of L-NAME itself. The source of NO is the guanidine moiety of L-arginine (Palmer et al 1988) and thus the ability of glycine to increase RBF and CL_{IN} may be a consequence of a direct or indirect stimulation of NO synthase. Cernadas et al (1992) proposed that glycine acts indirectly as a nitrogen source for the synthesis of L-arginine, therefore providing endogenous L-arginine for NO formation.

In the study of King et al (1991), infusion of N^G-monomethyl-L-arginine only partially blocked the increase in filtration induced by glycine but abolished the increase in RBF. Similarly, the present study, using the NO synthase inhibitor L-NAME, showed incomplete blockade of glycine's ability to preserve CL_{IN} in cisplatin-injected rats, but L-NAME completely blocked glycine-induced maintenance of RBF. This suggests that by contrast with the renal hyperaemic response to glycine, the hyperfiltration action involves an additional NO-independent mechanism. A study with the rat isolated kidney has shown that the ability of a mixed amino acid solution to reverse the time-dependent fall in CL_{IN} was partially inhibited by either indomethacin or sulpiride (El Sayed et al 1991). These findings indicate that possible mediators of glycine's hyperfiltration action, in addition to NO, are prostanoids and dopamine. Alternatively, as suggested by King et al (1991), the incomplete blockade of glycine's ability to increase glomerular filtration rate in the presence of NO-synthase inhibition may be a consequence of afferent and efferent arterioles differing in their ability to produce or respond to stimuli for NO production.

Administration of glycine to cisplatin-injected rats induced a marked diuresis and natriuresis which was not blocked by L-NAME, but significantly potentiated. This finding is in contrast to that of Cernadas et al (1992) who reported the diuretic and natriuretic effects of glycine in rats with normal renal function were significantly reduced by the NO-synthase inhibitor N^G-nitro-L-arginine (*N*-L-Arg), indicating an NO mechanism for the effects of glycine on excretory function. The difference between the findings of Cernadas et al (1992) and the present study may be related to the presence of cisplatin or to the dose of glycine, which was $7.5\ mg\ kg^{-1}$ in their investigation compared with $1.0\ g\ kg^{-1}$ in the current experiments. It is likely that the diuretic and natriuretic effect of glycine noted in the present study is a result of an osmotic diuresis which is enhanced by L-NAME infusion. Indeed, Chen et al (1992) have shown that the diuresis and associated increase in sodium excretion produced by an intravenous infusion of mixed amino acids ($1.1\ mg/100\ g\ min^{-1}$) was significantly potentiated by co-infusion of *N*-L-Arg. Since L-NAME alone can produce a diuresis (Lahera et al 1991), the enhancement of the diuretic and natriuretic effects of glycine is probably due to L-NAME's effects on excretory function.

Treatment of cisplatin-injected rats with a bolus dose of glycine produced a significant reduction in the subsequent nephrotoxic actions of cisplatin. This confirms the findings of the protective action of glycine infusion in rats which received cisplatin (Heyman et al 1991). The bolus dose of glycine used in this study was equivalent to the total dose of glycine administered in the investigation of Heyman et al (1991). The protective effect of glycine was reduced by treatment with L-NAME indicating that this effect was, at least in part, related to the production of NO. Attenuation of glycine's beneficial effects by L-NAME was not a result of the NO-synthase inhibitor potentiating cisplatin nephrotoxicity since treatment of rats with both cisplatin and L-NAME, in comparison with rats given cisplatin only, did not result in further deterioration in renal function. On the contrary, treatment of cisplatin-injected rats with L-NAME produced modest improvements in a number of indices of renal function with statistically significant reductions in plasma urea and creatinine compared with rats given cisplatin only.

Since glycine blocks the acute falls in RBF and CL_{IN} produced by cisplatin, it is tempting to attribute the protective effect against cisplatin nephrotoxicity to these acute renal haemodynamic actions. The protective effect of renal vasodilation is supported by the finding that blockade of voltage-dependent calcium channels which increases RBF and glomerular filtration rate in normal animals (Carmines et al 1992) can also ameliorate cisplatin-nephrotoxicity, since nifedipine has been shown to reduce plasma concentrations of urea and creatinine in rats 5 days after injection of 5 mg kg^{-1} cisplatin (Deray et al 1988). However, nifedipine produced a modest protection of renal function with reductions of 32–42% in levels of plasma creatinine and urea (Deray et al 1988), whereas in the present study glycine produced extensive protection of a range of indices of renal function, in particular plasma creatinine concentrations and CL_{PAH} which reached levels noted in control animals injected with saline. This suggests that glycine has renal protective actions in cisplatin nephrotoxicity independent of its renal haemodynamic actions, as indicated by its ability to protect against anoxic injury in-vitro in rabbit dispersed proximal tubules (Weinberg et al 1987). The finding that L-NAME produced only a partial reduction in glycine's beneficial effects also implies that there is a non-haemodynamic component to the protective actions of glycine. Identification of any non-haemodynamic protective actions of glycine in cisplatin nephrotoxicity may be revealed by defining the threshold doses of glycine which produce, on the one hand, reduction of the acute falls in RBF and glomerular filtration rate induced by cisplatin and, on the other, amelioration of cisplatin nephrotoxicity.

In conclusion, the results of this study indicate glycine can block the acute falls in RBF and CL_{IN} produced by cisplatin by a mechanism which involves NO. Furthermore, the results suggest that these renal haemodynamic actions may explain, in part, the ability of glycine to ameliorate cisplatin nephrotoxicity, a toxic effect which limits the therapeutic use of this anticancer agent.

Acknowledgements

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