The protective effect of γ-glutamyl L-dopa on the glycerol treated rat model of acute renal failure

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(Received 19 August 1982/6 January 1983; accepted 16 February 1983)

Summary
1. γ-Glutamyl L-dopa, a renal pro-drug for dopamine, was administered to rats before and after injection of glycerol, and to a control group which received water in place of glycerol. A third group of rats was given glycerol but no γ-glutamyl L-dopa.
2. The plasma creatinine in rats given γ-glutamyl L-dopa and glycerol was significantly lower than in rats receiving glycerol alone.
3. The fall in urine creatinine excretion, and polyuria, after glycerol was reduced by γ-glutamyl L-dopa and the natriuresis abolished.
4. γ-Glutamyl L-dopa given alone caused a 4000-fold increase in urine dopamine excretion, associated with a natriuresis.
5. The administration of γ-glutamyl L-dopa reduces the severity of renal failure produced by glycerol.

Key words: L-dopa, dopamine, glycerol, kidney, renal failure, sodium.

Introduction
It is well known that prior oral loading with sodium chloride protects the rat from the development of acute renal failure after injection of glycerol [1] or mercuric chloride [2]. The reason for this remains obscure. It was originally thought to be due to suppression of the renin-angiotensin system, but there is now a great deal of evidence suggesting that if the renin-angiotensin system is involved at all, it is not the sole factor [3-5].

Sodium chloride loading results in an increase in urine dopamine excretion in both the rat and man [6]. Preliminary experiments (unpublished) suggested to us that urine dopamine, already high, increased further, after glycerol, in rats on a high salt diet, but fell further, from low values, in rats on a low salt diet. It seemed possible, therefore, that endogenous dopamine might play a role in the prevention of the syndrome of acute renal failure in the rat and that manipulation of endogenous dopamine might be beneficial. There is evidence that exogenous dopamine will ameliorate the development of the acute renal failure which follows clamping of the renal artery [7].

γ-Glutamyl derivatives of amino acids and peptides are actively taken up by the kidney [8]. In the brush border of cells of the proximal tubules, the γ-glutamyl moiety is removed by γ-glutamyltransferase (EC 2.3.2.2), leaving the free amino acid. Wilk [9] has shown that γ-glutamyl L-3,4-dihydroxyphenylalanine (γ-glutamyl L-dopa) accumulates in the kidney and that the free L-dopa is then decarboxylated to dopamine by aromatic L-amino acid decarboxylase (EC 4.1.1.28). Administration of γ-glutamyl L-dopa will therefore increase endogenous renal production of dopamine. This provides a suitable model with which to test the hypothesis that dopamine can protect against the development of acute renal failure in the glycerol model in the rat. It has the advantage that dopamine is produced locally within the kidney, where it may exert its specific effect; whereas infused dopamine is more likely to have a generalized effect which can be complicated by α- and β-adrenoceptor agonist activity.
Methods

Experiment 1

Eighteen male Wistar rats were placed singly in Techniplast metabolism cages. They were randomized in three groups of six: group A, to receive γ-glutamyl L-dopa and glycerol; group B, to receive γ-glutamyl L-dopa and water; group C, to receive sodium chloride solution (154 mmol/l: saline) and glycerol. They were provided with free access to Oxoid 41B powdered diet and deionized water. The rats were handled daily and recordings made of their food and fluid intake and weight gain. After they had acclimatized to their cages (10 days), as shown by a steady weight gain and constant fluid intake, their urine was collected daily into sufficient hydrochloric acid (3 mol/l) to keep the pH of the urine below 3 and prevent oxidation of dopamine. Urine was collected for 3 days (the run-in period). On the fourth day, group A rats were each given a subcutaneous injection of 53.6 mg of γ-glutamyl L-dopa dissolved in 1 ml of saline, followed 30 min later by a subcutaneous injection of 7 ml of 50% glycerol/kg. After a further 6 h a further 53.6 mg of γ-glutamyl L-dopa/kg was injected. Group B rats received 53.6 mg of γ-glutamyl L-dopa/kg, dissolved in 1 ml of saline, followed by 7 ml of distilled water/kg, and after 6 h a further 53.6 mg of γ-glutamyl L-dopa/kg, dissolved in 1 ml of saline, was injected. Group C rats were injected with 1 ml of saline, followed 30 min later by 7 ml of 50% glycerol/kg and a further injection of saline 6 h later. Urine was collected throughout day 4 and for the next 4 days.

Experiment 2

A further group of 18 male Wistar rats was randomized to three groups of six. They received similar treatments to their corresponding groups in experiment 1. These rats were, however, anesthetized with sodium pentobarbitone (60 mg/kg) intraperitoneally 48 h after the glycerol injection and 2 ml of blood was withdrawn by cardiac puncture into heparinized syringes. The plasma was separated after centrifugation and stored at –20°C.

Analytical methods

Dopamine was extracted from the urine samples at pH 8.3 (5 ml of urine) by using 500 mg of alumina previously treated with 5 ml of disodium ethylene diaminetetra-acetic acid (0.2 mol/l) and eluted with 3 ml of acetic acid (0.2 mol/l) [6, 10]. The efficiency of extraction was determined by incorporation of an internal standard of tritiated dopamine in urine, which was counted for radioactivity before and after extraction. Dopamine was measured by a radioenzymatic method [11]. An aliquot of the extracted sample was incubated with S-[3H]adenosylmethionine and rat liver catechol methyltransferase (EC 2.1.1.6) in the presence of magnesium ions and dithiothreitol. Conversion of free L-dopa into dopamine by L-aromatic amino acid decarboxylase was inhibited by the addition of benzylxoxamine. Catechol methyltransferase was prepared from rat livers by a modification of the method described by Axelrod & Tomchick [12]. The methylated catecholamines were separated from unused S-[3H]adenosylmethionine by partitioning into ether as a tetraphenylboron complex. The individual methylated catecholamines were separated by thin-layer chromatography and the radioactive 3-methoxytyramine was measured by liquid scintillation counting. The results were corrected for efficiency of extraction with alumina; the mean recovery was 67% (±7% SD). Interassay variation was 14%, intra-assay variation 4.5%. Urine and plasma creatinine was measured by a colorimetric method based on the Jaffe reaction [13]. Urinary sodium was measured on the Nova 1 sodium/potassium analyser, after buffering the urine back to pH 7.

Statistical methods

Data collected for the first 3 days of the study were compared with those from the last 5 days by analysis of variance. All analyses were carried out after logarithmic conversions of the data to base 10. The analysis was performed by a repeated measures analysis of variance with one trial factor (time) and one grouping factor (treatment) and six replications. The statistical package BMDP 2V [14] was used, together with a purpose-written programme to extract the relevant sums of squares for the situation when the time × group interaction was significant. Where multiple tests were performed after the initial analysis of variance, the Akuman Newman–Keuls method was used [15].

In the second experiment the plasma creatinine values were initially compared by the Kruskal-Wallis one-way analysis of variance. Because of the small numbers in the groups involved, the critical range test is only capable of showing a difference between extreme groups. As an alternative, selected pairs of groups were compared by Wilcoxon's two-sample test. Because multiple comparisons were made only a P value less than or equal to 0.01 was considered to be significant.
Results

All rats given glycerol demonstrated haemoglobinuria, some within 20 min of injection. On the day of injection of glycerol, the rats appeared unwell and ate less. They had recovered by the next day and there were no deaths in any group. For the three groups of rats in the first experiment, body weight, sodium intake, urine volume, urine sodium and urine creatinine are shown in Fig. 1. There was a significant group x time interaction for body weight, sodium intake, urinary volume and urinary dopamine ($P < 0.001$) and also urine sodium excretion ($P < 0.01$). For urinary creatinine excretion the group x time interaction was not significant, but there was a significant time effect ($P < 0.05$),

FIG. 1. Mean (±SEM) body weight, urine volume, sodium intake (●●●), urinary sodium excretion (●●●●), urine dopamine excretion and urinary creatinine for the three groups of rats, for 3 days before treatments and 5 days afterwards.
which must have been due to the fall in creatinine excretion in group C, since there was virtually no change in groups A and B.

In groups A and B receiving γ-glutamyl L-dopa there was an increase in dopamine excretion of the order of 4000-fold on day 4. However, because of the large standard error involved, urinary dopamine of neither group A nor group B individually was significantly greater than that of group C, but taken overall there was a significant difference between the groups (P<0.001). When the data for dopamine excretion on day 4 were considered separately and analysed by Wilcoxon's two-sample test, then groups A and B were significantly different from C (P<0.01). On days 5, 6 and 7 dopamine excretion of both groups A and B was significantly greater than that of group C (P<0.01), but by day 8 there was no significant difference between groups.

Although the pattern of weight gain was different in the three groups, there was only a significant difference between the groups overall on days 7 and 8 (P<0.05). There was no significant difference between individual pairs of groups. Urine volume increased significantly in groups A and C (P<0.001 for both), but there was no change in group B. Although the effect of treatment group on days 4 and 5 was significant (P<0.001 and P<0.05 respectively), a statistical difference in urine volume could not be shown between individual groups. It is clear from Fig. 1, however, that the polyuria after glycerol was much reduced in group A compared with that in group C.

Sodium intake was significantly reduced in groups A and C after glycerol (P<0.01) but there was no change in group B. Group A's sodium intake was significantly lower than that of group B for only 2 days, day 4 (P<0.01) and day 5 (P<0.05), whereas group C had a significantly lower intake for 4 days, days 4, 5 and 6 (P<0.01) and day 7 (P<0.05). The sodium intake of group A was significantly greater than that of group C on days 6 (P<0.05) and 7 (P<0.01). The transient fall in sodium excretion in group A was not significant. There was a significant natriuresis in groups B (P<0.01) and C (P<0.05).

For the second experiment the plasma creatinine concentrations in individual rats of each group are shown in Fig. 2. For comparison the plasma creatinine values of 12 untreated rats, kept in metabolism cages for 7 days, are included. There is a clear difference in plasma creatinine between rats receiving glycerol alone and animals given γ-glutamyl L-dopa in addition (P=0.01).

Discussion

The dose of glycerol (7 ml/kg) used in this experiment was similar to that used in our previous work. Although this dose is smaller than that commonly used (10 ml/kg), it was chosen because of its reproducibility in producing renal damage with minimal constitutional upset in the rats. The rise in plasma creatinine, fall in urine creatinine excretion and polyuria confirm that this model is effective in producing a significant, though mild degree of renal failure. Histological studies confirm that significant renal damage ensues with this dose of glycerol [16].

The subcutaneous injection of γ-glutamyl L-dopa caused a 4000-fold increase in dopamine excretion on day 4; thus 14–20% of the γ-glutamyl L-dopa was excreted as free dopamine. As some dopamine is excreted as a conjugate [17], the total amount of γ-glutamyl L-dopa converted into dopamine must be in excess of this. An increase in dopamine excretion was maintained for 3 more days. That dopamine excretion can be increased in this way lends support to our theory that dopamine is produced by renal tubular cells after uptake of L-dopa from the circulation. γ-Glutamyl L-dopa, given alone, had no definite toxic effects on the rats; their rate of growth was maintained and their food intake remained constant. Infusion of dopamine down the renal artery in low concentrations results in a natriuresis, and an increase in glomerular filtration rate [18]. In this experiment,
in group B, there was a significant natriuresis after \( \gamma \)-glutamyl L-dopa, probably due to its conversion into dopamine. The days of maximum dopamine and sodium excretion, however, do not correspond; this could be due to the very large amounts of dopamine present on day 4, which may have exerted some \( \alpha \)-agonist activity, resulting in renal vasoconstriction. Creatinine excretion did not change significantly in this group. There was also a non-significant increase in urine volume. Subcutaneous injection of \( \gamma \)-glutamyl L-dopa therefore produces physiological changes similar to those after dopamine infusion into a renal artery, though spread over a longer period.

A protective effect of \( \gamma \)-glutamyl L-dopa is demonstrated by comparison of groups A and C. In the second experiment plasma creatinine was lower in rats given \( \gamma \)-glutamyl L-dopa, and, in the first experiment, there was only a transient fall in creatinine excretion and polyuria was less severe. Moreover, group A rats recovered their appetite and growth pattern more quickly. The effect on sodium excretion is interesting. When glycerol alone was given a natriuresis was seen, but when \( \gamma \)-glutamyl L-dopa, which itself causes a natriuresis, and glycerol were given together, there was a non-significant fall in sodium excretion which paralleled sodium intake.

Histological studies support the concept of a renal protective effect of \( \gamma \)-glutamyl L-dopa [16]. After its conversion into dopamine, \( \gamma \)-glutamyl L-dopa may protect the kidney by renal vaso-dilatation, which counteracts the vasoconstriction occurring after glycerol injection [19]. The evidence presented here supports the hypothesis that dopamine protects against renal failure. The quantity of dopamine produced by the rats in this experiment was far in excess of that produced after salt loading. If increased dopamine production is responsible for the protective effect of salt, further experiments will be necessary to prove this. \( \gamma \)-Glutamyl L-dopa may prove to be a useful therapeutic agent both in patients at risk of developing acute renal failure and in those receiving nephotoxic drugs.

Acknowledgments

We thank Mr H. J. Hopkins and staff of the Animal Laboratory Services at the Worsley Medical School of the University of Leeds and Miss A. Calderwood for typing the manuscript. We are particularly grateful to Professor S. Wilk for several generous gifts of \( \gamma \)-glutamyl L-dopa. I.F.C. is funded by a grant from the Yorkshire Regional Health Authority.

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