The Effect of Intravenous Infusion of L-Arginine, Glycine and D-Lysine on Urinary Calcium Excretion in the Rat

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Abstract Standard renal clearance techniques were used to compare the effects of intravenous infusions of L-arginine, D-lysine and glycine on urinary calcium excretion in the rat. A significant calciuric response was evident following the infusion of all three amino acids in all the animals. The maximal effect was evident in rats receiving L-arginine. The mechanism for the increased urinary calcium excretion in rats infused with L-arginine and D-lysine appeared more due to a decreased fractional reabsorption of this cation as no significant changes in the glomerular filtration rate (GFR) were evident in these two groups. The calciuria in rats receiving glycine appears due to increased filtered load secondary to the increased GFR, suggesting that the mechanism for calciuria evident following protein ingestion or amino acid infusion may vary and may be dependent upon the amino acid ingested or infused.

Key words: L-arginine, glycine, D-lysine, urinary calcium.

Protein intake has long been known to influence renal function. In addition to increasing the glomerular filtration rate (GFR) and renal hemodynamics, protein intake has also been shown to increase urinary excretion of calcium [1, 2]. The mechanism involved in the alteration of renal handling of this cation following protein ingestion is not well understood. Besides, it is also uncertain if all dietary proteins share the same potency to alter renal excretion of this cation as alterations in other renal parameters have been shown to depend on the type of dietary protein [3].

It has been suggested that following protein intake, the increase in plasma amino acid concentration in some way determines the changes in renal function [4, 5]. Although the effects of protein intake on renal function have been shown to be reproducible by amino acid infusion [6, 7], it is uncertain if the calciuric response evident following amino acid infusion is the same with all the amino acids when they are administered individually. Moreover, renal responses have also been shown to vary with the route of administration of the amino acids [8]. This study
therefore examines the effects of acute intravenous infusion of \( l \)-arginine, glycine and \( d \)-lysine on urinary calcium and magnesium excretion in the rat.

**METHODS**

*Animals.* Experiments were performed on male Sprague-Dawley rats weighing 220–250 g. Rats had free access to food until the day before the experiment, and to water until the experiment began.

*Renal studies.* Animals were anesthetized with an intraperitoneal injection of Intralval sodium (5-ethyl-5-(1-methyl-butyl)-2-thiobarbituric acid; 100 mg/kg body weight) and placed on a thermostatically heated operating table. Catheters were then placed in the left jugular vein (for infusion), carotid artery (for blood pressure monitoring and blood sampling) and urinary bladder (suprapubically, for collecting urine). A tracheostomy was performed to ensure a clear airway. All animals received a priming dose of 0.3 ml 0.9% saline containing 6 \( \mu \)Ci \( [\text{H}] \)-inulin followed by a continuous infusion at 200 \( \mu \)l/min of 0.9% saline containing 1 \( \mu \)Ci/ml \( [\text{H}] \)-inulin. After 2 h, the infusion was reduced to 150 \( \mu \)l/min and maintained thus for 4 h. In our laboratory, we have found that an infusion rate of 150 \( \mu \)l/min provides a very stable diuresis. The first 3 h of infusion served for rapid volume expansion and equilibration. During this period, all animals received 0.9% saline. The final 3 h of infusion was designated as the experimental period. During this time, control animals (group 1: \( n = 14 \)) received 0.9% saline throughout. In the experimental animals (groups 2, 3 and 4), however, the saline was replaced either by an infusion of 5% \( l \)-arginine (\( n = 14 \)) or 5% glycine (\( n = 11 \)) or 5% \( d \)-lysine (\( n = 6 \)) in 0.9% saline respectively for a 30-min period commencing 1 h into the experimental period. In all groups, 30-min urine collections were commenced at the end of the third hour and blood samples (50 \( \mu l \)) obtained from the carotid artery every hour for the determination of \( [\text{H}] \)-inulin.

*Analysis.* \( [\text{H}] \)-Inulin in plasma and urine was assessed by liquid scintillation counting using a phase combining system (Beckman). Urine samples were analyzed for sodium and potassium by flame photometry (Corning model 450), for calcium and magnesium by fluorometry (Biomerieux) and for osmolality by freezing point depression (Roebling osmometer).

All data are presented as mean \( \pm \) SEM. Statistical analysis between groups was performed using Student’s \( t \)-test for unpaired samples. A paired \( r \) test was used for any within-group analysis. Levels of significance in the figures are indicated by the symbols \( * p < 0.05 \), \( ** p < 0.01 \), \( *** p < 0.001 \).

**RESULTS**

Figure 1 presents data for urine output. No significant differences were evident in urine flow between the four groups before the administration of the amino acids. Infusion of amino acids resulted in significant diuresis in all three groups. (\( p <

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0.001). Urine flows during the infusion of amino acids in groups 2, 3 and 4 were 215, 137 and 166%, respectively, of the control. Urine flow in rats receiving arginine was significantly higher than the other amino acid-infused groups (p < 0.01). Urine flow, however, returned to control levels in all groups after discontinuation of the amino acid infusion.

GFR data are presented in Fig. 2. No significant changes were evident in GFR during the experimental phase in the control animals. Increased GFR was only evident in rats infused with glycine and was also slightly higher than that in the other groups during the corresponding periods (p < 0.05; paired samples). The difference was, however, not significant statistically.

Osmolar clearance was significantly higher during the infusion of amino acids (Fig. 3; p < 0.01). In addition, osmolar clearance in rats receiving arginine was significantly higher than in the other two amino acid groups.
Figure 3 presents the data for osmolar output. Urinary sodium excretion increased following the infusion of amino acids but it was only significantly higher during the infusion of L-arginine and D-lysine ($p < 0.05$).

Urinary calcium excretion in the four groups is presented in Fig. 5. Amino acid infusion resulted in increases in urinary calcium excretion in all groups. However statistically significant increases were only evident in the rats receiving L-arginine and D-lysine ($p < 0.05$). Urinary calcium excretion in rats receiving
arginine and lysine was 276 and 214%, respectively, when compared to the corresponding period in the controls. The increase in calcium excretion following amino acid infusions was significantly greater in rats receiving arginine ($p < 0.05$). Urinary calcium excretion in glycine-infused animals was slightly but not significantly higher than that in controls. It was nevertheless significantly increased from that during the two previous control periods ($p < 0.05$; paired samples).

Fractional fluid reabsorption is presented in Fig. 6. Fractional fluid reabsorption decreased significantly in all animals during the infusion of amino acids, and the decrease was highest in animals receiving L-arginine. Fractional fluid reabsorption remained significantly lowered in rats receiving glycine even after the discontinuation of glycine infusion.

Urinary magnesium excretion increased significantly in animals receiving
arginine (Fig. 7; $p < 0.05$) but it was not significantly different from the controls in the other two amino acid groups.

DISCUSSION

The effects of protein intake have been suggested to be of important pathogenic consequences. In addition to increasing urinary calcium excretion, changes in renal function and hemodynamics associated with high protein intakes might also be responsible for the progressive decline in renal function and glomerular sclerosis that characterizes chronic renal failure [5]. A retrospective study over a period of 10 years revealed an average decline of 6.3% in creatinine clearance in patients on total parenteral nutrition [9]. Dietary supplementation of L-arginine on the other hand has been shown to ameliorate the progression of renal disease in rats with subtotal nephrectomy [10]. Numerous epidemiological studies have also linked high protein intake to a higher incidence of calcium oxalate urolithiasis, and hypercalciuria is a well known risk factor in urinary tract stones. This study was designed to investigate the acute effects of amino acids, when infused individually, on urinary calcium excretion in the rat. We begin here by investigating the effects of intravenous infusion of L-arginine, glycine and D-lysine on urinary calcium excretion. The amount of amino acids administered intravenously were within the $LD_{50}$ doses in the rat [11]. Plasma or urinary amino acid concentrations were however not determined in this study. The infusates were hypertonic and their osmolalities were 658, 884, 515 mOsm/kg H$_2$O for arginine, glycine and lysine respectively (5% w/v of amino acid in saline). The reason for choosing 5% solutions was because their anticipated osmolalities were within the osmolalities of glucose solutions we had infused before.

Urinary calcium excretion was significantly higher during the periods of amino acid administration when compared to the controls. Urinary calcium excretion

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increased significantly in all the amino acid groups (Fig. 5). The increase in calcium excretion was also significantly higher in rats receiving L-arginine when compared to the other two amino acid groups. Precisely how the infusions of amino acids increase urinary calcium excretion is uncertain. An increased filtered load or decreased tubular reabsorption can both result in increased urinary excretion of this cation. The ultrafiltrable fraction of calcium was not determined in this study and it is uncertain if amino acid infusion alters the filtrable fraction of calcium. Renal plasma flow and GFR have both been shown to increase following the ingestion of protein [12] or the infusion of amino acids [7]. Plasma glucagon, growth hormone and other neurohypophyseal secretions, which have been shown to increase following protein intake or amino acid infusion, are hypothesized to be involved in this hyperfiltration process [5, 10, 13, 14]. Other studies however suggest a possible direct effect of amino acids on renal hemodynamics [15, 16]. Whilst the precise mechanism by which amino acids increase GFR is not fully understood, an increase in GFR nevertheless can lead to an increased filtered load and this could consequently, though not always because of glomerular tubular balance, lead to an increased urinary excretion of this cation. Despite increased urinary calcium excretion in all the groups following amino acid administration, increased GFR was only evident in rats receiving glycine (p < 0.05; paired samples). The overall GFR was, however, not different between the four groups when their corresponding periods were compared (Fig. 2) suggesting that whilst the increase in urinary calcium excretion in rats receiving glycine could, to an extent, be attributed to an increased filtered load this does not appear to be the case following the infusion of L-arginine or D-lysine where increased urinary calcium excretion was evident in the absence of any significant increases in GFR. Serum calcium during the infusion of amino acids was not determined and it is unlikely that serum calcium or more importantly the filtrable fraction will have changed significantly to change the filtered load of this cation. It therefore appears that the increased urinary calcium excretion evident in rats receiving L-arginine and D-lysine and to some extent glycine may be due to decreased tubular reabsorption of this cation. The mechanism(s) responsible for the decreased reabsorption of calcium during the infusion of amino acids is unclear. Increased urine output was evident in all groups receiving amino acids (Fig. 1). Fractional fluid reabsorption was significantly decreased in all the rats receiving amino acids (Fig. 6). Urinary osmolar output was also significantly increased in all the groups, suggesting an overall increase in solute output (Fig. 3). Urinary amino acid concentration was not estimated. It is possible that the elevated serum concentrations of amino acids following their intravenous administration results in their increased filtration where the filtered load greatly exceeds the amino acid reabsorptive capacity of the renal tubules resulting in osmotic diuresis. The osmotic phenomenon could explain the rises in calcium excretions. This could similarly explain the rise in urinary sodium excretion. There was a significant correlation between urine flow and osmolar output and between these two parameters and urinary calcium and sodium excre-
tions \(p < 0.001\). Increases in urinary excretions of calcium and sodium were also the greatest in arginine-treated rats where the diuresis was also the greatest. Interestingly, increased urinary magnesium excretion was also only evident in rats receiving arginine (Fig. 7). Magnesium excretion in rats receiving glycine and D-lysine was not different from the controls. The reason for this discrepancy is not apparent but it may be due either to the level of diuresis or a selective inhibition of tubular reabsorption of this cation. In view of the significant correlations between urine flow and urinary calcium output, it appears that the increased excretion of calcium evident in this study may be consequent to a decreased tubular reabsorption, secondary perhaps to the osmotic diuresis. It is, however, also possible that some of the effects may be due to a direct effect of the amino acids on renal cation handling. We had earlier demonstrated a direct effect of glucose on renal tubular handling [17] and L-lysine has been suggested to modulate cytosolic calcium and stimulate calcium extrusion from the cell [18]. It is however uncertain if a similar effect is evident with D-lysine. We are currently comparing the effects of infusion of the two lysine isomers on renal function and early indications are that infusion of L-lysine decreases GFR whilst increasing urinary calcium excretion (unpublished data).

So far, it appears that when arginine, glycine and D-lysine are individually administered intravenously to the rat there follows a significant increase in urinary calcium and sodium excretion. The increase in the excretion of these ions appears to be due to their decreased tubular reabsorption. Diuresis occurred at the concentrations of amino acids used and seems primarily due to decreased fractional reabsorption of fluid secondary perhaps to increased solute output as evidenced by the raised osmolar output. The level of diuresis and solute output however varied with the amino acids used. It was greatest with arginine. GFR increased only with glycine. It is uncertain as to why increases in GFR were not seen in all the amino acid groups. This observation is also in contrast to a recent report where intravenous infusion of arginine in the rat resulted in significant increases in RPF and GFR [19]. Arginine infusion in dogs has been shown to increase GFR in this species [20]. On the other hand, the magnitude of the effect of arginine administration in humans depended on the route of administration. Large increases in GFR were, however, only evident when arginine was administered orally [8]. The reason for the lack of increase in GFR following the intravenous infusion of arginine is unclear. It is possible the absolute amount of amino acids administered varied as in the study of Chen et al. L-Arginine was administered at a rate of 0.71 mmol/100 g/min whereas, in this study, the infusion was approximately 0.1 mmol/100 g/min. It is also possible that the lack of an obvious effect of arginine and D-lysine on GFR in this study may be due to toxicity, particularly with D-lysine. The precise mechanism for the glycine-induced increase in glomerular filtration is unclear although adenosine, dopamine and the renin-angiotensin system [21–23] have all been proposed to be involved in this mechanism.

In summary, it appears that intravenous infusion of amino acids results in
significant increases in urinary calcium excretion but the magnitude or the type of
effect may depend on the amino acid used. The increased urinary calcium excretion
may be due either to increased GFR or more significantly due to decreased tubular
reabsorption of this cation, secondary perhaps to an osmotic effect. The present
results do not rule out a possible direct effect of the amino acids on renal calcium
handling. The predominant mechanism responsible for calciuresis may, however,
depend on the amino acid used.

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