Regulatory responses to an oral D-glutamate load: formation of D-pyrrolidone carboxylic acid in humans

DOMINIC RAJ, MARYLN LANGFORD, STEPHAN KRUEGER, MARTIN SHELTON, AND TOMAS WELBOURNE

Departments of Medicine, Ophthalmology, Pediatrics, and Molecular and Cellular Physiology, Louisiana State University Health Science Center, Shreveport, Louisiana 71130

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Raj, Dominic, Maryln Langford, Stephan Krueger, Martin Shelton, and Tomas Welbourne. Regulatory responses to an oral D-glutamate load: formation of D-pyrrolidone carboxylic acid in humans. Am J Physiol Endocrinol Metab 280: E214–E220, 2001.—Previously published studies have shown D-glutamate to be the most potent natural inhibitor of glutathione synthesis known, yet how D-glutamate is handled in humans is unknown. Therefore, we administered an oral D-glutamate load to four healthy volunteers and monitored the plasma D-glutamate concentration and excretion over a 3-h postload period. Compared with time controls, the plasma D-glutamate concentration increased 10-fold in the 1st h and then reached a plateau over the remaining time course. In contrast, plasma D-pyrrolidone carboxylic acid concentration increased progressively throughout the 3-h time course to a level 10-fold higher than the D-glutamate plasma concentration. Excretion of D-glutamate progressively increased despite a constant filtered D-glutamate load rising from only 5 to 95% of the filtered amount. Excretion of D-pyrrolidone carboxylic acid increased with the rise in filtered load without significant reabsorption. The amount of D-pyrrolidone carboxylic acid excreted over the 3-h time course was 10 times the amount excreted as D-glutamate and accounted for almost 20% of the administered D-glutamate. These findings indicate that plasma D-glutamate concentration is tightly regulated through two mechanisms: 1) the transport into cells and metabolic conversion to D-pyrrolidone carboxylic acid and excretion, and 2) the enhancement of D-glutamate clearance by the kidneys.

L-glutamate; D-glutamate cyclotransferase; renal handling; creatinine clearance; D-, L-glutamine synthetase; D-glutamine; glutamate transport

D-Glutamate loads are normally presented to the intestine as the free amino acid present in certain foods e.g., soybeans (5, 10) and from the turnover of the intestinal tract microflora, whose cell walls contain significant D-glutamate (19). Unlike other D-amino acids, D-glutamate is not oxidized by the D-amino acid oxidases (24), and therefore this detoxification pathway is not available for handling D-glutamate. Free D-glutamate is found in mammalian tissue at surprisingly high levels, with D-glutamate accounting for 9% of the total glutamate present in liver (9). Noteworthy, significant accumulation of D-glutamate in the body fluids would impair a number of important physiological processes. For example, D-glutamate is the most potent natural inhibitor of glutathione synthesis known (21); in fact, the D-isomer is more reactive [inhibitory constant (K_i) = 0.8 mM] than the natural L-glutamate (K_i = 1.8 mM) with γ-glutamylcysteine synthetase (13). Given the liver intracellular glutamate concentration of ~10 mM (9), D-glutamate concentration would approach 1 mM and thus might play a modulating role in the intraorgan glutathione fluxes. Indeed, when D-glutamate at 1.6 mmol/kg was injected into rats intraperitoneally, renal glutathione content decreased 51% within an hour (21). D-glutamate may also act as a false neurotransmitter in the central nervous system (15) and may therefore contribute to excitotoxicity (18).

How D-glutamate is removed from the body fluids is not clear. In the sole human study (16), radiolabeled D-[14C]glutamate was infused intravenously into a patient with multiple myeloma, and ~20% was recovered in the urine unchanged as D-glutamate after 3 h (16). Although taken as evidence for excretion without significant metabolic conversion, the low recovery suggests that D-glutamate could have undergone metabolic conversion. In the rat, 75% of orally administered D-[15N]glutamate was recovered in the urine as D-[15N]pyrrolidone-carboxylic acid after 24 h (17). Conversion of 14C-labeled D-glutamate to D-pyrrolidone-carboxylic acid was observed when administered orally or intravenously to rats (26), with >50% of the total administered radioactivity recovered in the urine after 24 h as D-pyrrolidone-carboxylic acid. The enzymatic activity of D-glutamate cyclotransferase, responsible for converting D-glutamate to D-pyrrolidone-carboxylic acid, was demonstrated to be highly expressed in mammalian kidney and livers (12); kidney and liver obtained from humans had a D-glutamate cyclotransferase activity approaching that of the rat (12). These findings point to a potential regulatory role for D-glutamate transport into cells and metabolic conversion to D-pyrrolidone-carboxylic acid in the handling of D-glutamate.

Address for reprint requests and other correspondence: T. C. Welbourne, Dept. of Molecular and Cellular Physiology, LSUHSC, Shreveport, LA 71130 (E-mail twelbo@lsumc.edu).


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How the kidneys handle D-glutamate has more recently been studied using microperfusion (6, 22) and extraction procedures (4). In the rat proximal convoluted tubule, luminal D-glutamate is neither taken up nor does it inhibit the avid uptake of L-glutamate (22). In contrast, both D-glutamate and L-glutamate are taken up in the proximal straight and loop segments (6). In extraction studies, the rat kidney removes almost 70% of the D-glutamate arterial load, indicating both luminal and antiluminal uptake sites for the D-glutamate (4); in addition, virtually all of the filtered D-glutamate was reabsorbed (4), indicating a significant tubular reabsorptive capacity apparently in the proximal straight and loop segments (6). Furthermore, the uptake of D-glutamate could be largely, if not entirely, accounted for as D-pyrrolidone-carboxylic acid formation in the functioning rat kidney (20). D-pyrrolidone-carboxylic acid, in turn, is filtered and excreted in contrast to the D-glutamate (20).

The purpose of the present study was to determine the response in humans to an oral D-glutamate load. Specifically we wished to know whether D-glutamate would be taken up from the intestine and appear in the systemic circulation. If so, then the handling of the D-isomer becomes subject to physiological regulation, potentially involving both transport into cells and metabolic conversion. Our second objective was to determine whether D-glutamate entering the plasma would be excreted as D-glutamate or metabolically converted to D-pyrrolidone-carboxylic acid. Finally, we wished to know how the D-glutamate and, if formed, D-pyrrolidone-carboxylic acid, would be handled by the kidneys.

The results to follow show that D-glutamate appears in the plasma after an oral load, confirming its uptake from the intestine. Whereas the rise in plasma D-glutamate plateaus, plasma D-pyrrolidone-carboxylic acid concentration rises to a level 10-fold higher than plasma D-glutamate. The kidneys effectively excrete the filtered D-pyrrolidone-carboxylic acid without reabsorption, whereas D-glutamate excretion progressively increases despite a constant filtered load.

MATERIALS AND METHODS

Studies were carried out on four healthy male volunteers. The authors were the volunteers and gave their consent to a protocol approved by the Louisiana State University Medical Center Institutional Review Board for Human Research. Vehicle, 500 ml water, vehicle plus 2 g (13.3 mmol) D-glutamate (D-Glu), or vehicle plus 2 g D-glutamine (D-Gln; Sigma, St. Louis, MO) were ingested within a 20-min period 1 h after a light breakfast (coffee, toast, and juice). This large dose of D-Glu was chosen to challenge the putative systems and to reveal the underlying mechanisms. Time controls and D-Glu or D-Gln loading were performed over 2 wk, one paired test per week. The D-Gln loading provided an index of renal D-Glu formation from this potential metabolite of D-Glu. After subjects emptied their bladders and an initial forearm venous blood sample was obtained (t = 0), either vehicle or vehicle plus D-Glu was ingested. Blood samples were then obtained at hourly intervals for 3 h (t = 1, 2, and 3), with urine collected at the end of each hourly period; water was consumed at a rate matching the urine volumes to maintain the state of hydration.

Plasma and urine samples were placed on ice immediately and processed for same day analysis. Total glutamate concentration of plasma and urine was determined on aliquots of protein-cleared samples by HPLC as previously described (20). Recovery of L-Glu (50 nmol) added to 1 ml plasma was 103 ± 4% (n = 3). Recovery of L-Glu was chosen to challenge the putative systems and to reveal the underlying mechanisms. Time controls and D-Glu or vehicle plus 2 g D-glutamine (D-Gln; Sigma, St. Louis, MO) were ingested within a 20-min period 1 h after a light breakfast (coffee, toast, and juice). This large dose of D-Glu was chosen to challenge the putative systems and to reveal the underlying mechanisms. Time controls and D-Glu or D-Gln loading were performed over 2 wk, one paired test per week. The D-Gln loading provided an index of renal D-Glu formation from this potential metabolite of D-Glu. After subjects emptied their bladders and an initial forearm venous blood sample was obtained (t = 0), either vehicle or vehicle plus D-Glu was ingested. Blood samples were then obtained at hourly intervals for 3 h (t = 1, 2, and 3), with urine collected at the end of each hourly period; water was consumed at a rate matching the urine volumes to maintain the state of hydration.

Plasma and urine samples were placed on ice immediately and processed for same day analysis. Total glutamate concentration of plasma and urine was determined on aliquots of protein-cleared samples by HPLC as previously described (20). Replicate analysis of 100 μM L-Glu standards was made with a coefficient of variation of 1.6% (n = 3). Recovery of L-Glu (50 nmol) added to 1 ml plasma was 103 ± 4% (n = 3). To determine the D-Glu concentration, 15 μl of untreated plasma and urine samples or standards were incubated at 37°C with 0.02 U of L-glutamate oxidase (1 unit converts 1 μmol L-Glu/min at 30°C, Sigma) for 2 h in 10 mM phosphate buffer, pH 7.4. The samples and standards were then processed as described above and analyzed for glutamate by HPLC (Fig. 1). Figure 1, A and B, shows the complete re-

![Fig. 1. The enzyme L-glutamate oxidase (−E or +E) effectively removes L-glutamate from plasma and urine samples. A and B show that 50 nmol/ml of L-glutamate (L-Glu; A) are completely oxidized after 2 h (B). C and D show that 20 nmol/ml of D-glutamate (D-Glu) are resistant to the oxidase. E and F show that plasma glutamate (E) contains oxidase-resistant D-Glu (F). G and H show that urine glutamate (G) contains mostly D-Glu 3 h after the D-Glu load. Glutamate and homoserine (internal standard) had retention times of 6.8 and 13.5 min, respectively.](Image)
The plasma concentrations of D-Glu and D-PCA before and for 3 h after the oral D-Glu load are shown in Fig. 2. The corresponding time control is shown in the inset; note the difference in y-axis scales. The circulating D-Glu concentration increases 10-fold (14 ± 1 vs. 1.1 ± 0.1 μM for the time control, P < 0.0001) over the 1st h and then plateaus at this concentration for the remainder of the 3-h time course (14 ± 1 and 12 ± 1 μM at 2 and 3 h, all P < 0.01 vs. time controls). The plasma D-PCA concentration before the 18 ± 7 μM load, Fig. 2, rose 5-, 13-, and 18-fold above the time controls at 1, 2 and 3 h postload (70 ± 14 vs. 14 ± 3, 176 ± 24 vs. 14 ± 5, and 182 ± 21 vs. 10 ± 1 μM, respectively, all P < 0.005 vs. time controls). Whereas the plasma D-Glu concentration increases to ~14 μM and plateaus, the increase in plasma D-PCA concentration is far greater and progressive.

The corresponding excretion of D-Glu and D-PCA over the 3-h time course is shown in Fig. 3. The corresponding time controls are shown in the inset; note the difference in the y-axis scales. The D-Glu excretion is 33-fold greater (P < 0.005) than the time control for the 1st h (198 ± 33 vs. 6 ± 4 nmol/min) and then increases to 130- and 351-fold higher (650 ± 147 vs. 5 ± 3 and 700 ± 124 vs. 2 ± 1 nmol D-Glu/min) than their respective control and D-glutamate load activities (6° 1° 1° 3° M for the time control, P < 0.0001) over the 1st h and then plateaus, the increase in plasma D-PCA concentration is far greater and progressive.

RESULTS

The plasma concentrations of D-Glu and D-PCA before and for 3 h after the oral D-Glu load are shown in Fig. 2. The corresponding time control is shown in the inset; note the difference in y-axis scales. The circulating D-Glu concentration increases 10-fold (14 ± 1 vs. 1.1 ± 0.1 μM for the time control, P < 0.0001) over the 1st h and then plateaus at this concentration for the remainder of the 3-h time course (14 ± 1 and 12 ± 1 μM at 2 and 3 h, all P < 0.01 vs. time controls). The plasma D-PCA concentration before the 18 ± 7 μM load, Fig. 2, rose 5-, 13-, and 18-fold above the time controls at 1, 2 and 3 h postload (70 ± 14 vs. 14 ± 3, 176 ± 24 vs. 14 ± 5, and 182 ± 21 vs. 10 ± 1 μM, respectively, all P < 0.005 vs. time controls). Whereas the plasma D-Glu concentration increases to ~14 μM and plateaus, the increase in plasma D-PCA concentration is far greater and progressive.

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FIG. 2. Plasma D-Glu and D-pyrrolidone-carboxylic acid (D-PCA) concentrations before and for 3 h after a D-Glu load. Results are means ± SE from 4 subjects. The increase in both D-Glu and D-PCA concentrations at 1, 2, and 3 h was significant compared with the time 0 by ANOVA, P < 0.001. Inset: time-control value for 4 subjects; note the difference in y-axis scales.

Fig. 3. Excretion of D-Glu and D-PCA before and for 3 h after an oral D-Glu load. Results are means ± SE from 4 subjects. The increase in D-Glu excretion at 1, 2, and 3 h was significant compared with time 0 by ANOVA, P < 0.001. Inset: time-control value for 4 subjects; note the difference in y-axis scales.
The kidneys handle the filtered D-Glu and D-PCA as well as L-Glu shown in Fig. 4 and Table 1. Figure 4 shows the FE of L- and D-Glu as well as D-PCA before and for 3 h after the D-Glu load. Results are means ± SE from 4 subjects. Time-control FE values were 1.8 ± 0.5, 1.8 ± 0.4, 1 ± 0.3, and 1.5 ± 0.6% for L-Glu and 2 ± 0.6, 5 ± 5, 4 ± 3, and 1 ± 1% for D-Glu.

Table 1. Filtered and excreted glutamate isomers and D-PCA after an oral D-Glu load

<table>
<thead>
<tr>
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<th>Filtered</th>
<th>Excreted</th>
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<td>2 h</td>
<td>3 h</td>
<td>0 h</td>
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<td>L-Glu</td>
<td>Controls</td>
<td>4.27 ± 0.28</td>
<td>0.06 ± 0.01</td>
<td>4.40 ± 2.04</td>
<td>0.04 ± 0.02</td>
<td>4.28 ± 1.47</td>
<td>0.04 ± 0.02</td>
<td>4.83 ± 1.47</td>
<td>0.05 ± 0.02</td>
<td>1.98 ± 0.06</td>
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<td>D-Glu-1</td>
<td>4.13 ± 0.09</td>
<td>0.02 ± 0.02</td>
<td>3.29 ± 0.06</td>
<td>0.09 ± 0.03</td>
<td>2.16 ± 0.05</td>
<td>0.32 ± 0.28</td>
<td>1.98 ± 0.06</td>
<td>0.28 ± 0.12</td>
<td>1.10 ± 0.35</td>
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<td>D-Glu-2</td>
<td>0.27 ± 0.05</td>
<td>0.003 ± 0.001</td>
<td>0.13 ± 0.03</td>
<td>0.006 ± 0.004</td>
<td>0.13 ± 0.02</td>
<td>0.004 ± 0.005</td>
<td>0.23 ± 0.03</td>
<td>0.002 ± 0.001</td>
<td>0.89 ± 0.25</td>
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<td>D-Glu-3</td>
<td>0.29 ± 0.07</td>
<td>0.009 ± 0.003</td>
<td>0.69 ± 0.18</td>
<td>0.20 ± 0.03</td>
<td>1.10 ± 0.35</td>
<td>0.65 ± 0.14</td>
<td>1.00 ± 0.10</td>
<td>0.98 ± 0.08</td>
<td>11.14 ± 3.35</td>
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<td>D-Glu-4</td>
<td>0.84 ± 0.20</td>
<td>0.96 ± 0.25</td>
<td>1.23 ± 0.36</td>
<td>1.38 ± 0.36</td>
<td>1.25 ± 0.27</td>
<td>1.20 ± 0.21</td>
<td>11.14 ± 3.35</td>
<td>11.44 ± 2.36</td>
<td>11.14 ± 3.35</td>
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<td>D-Glu-5</td>
<td>1.23 ± 0.35</td>
<td>0.89 ± 0.16</td>
<td>2.93 ± 0.68</td>
<td>2.76 ± 0.65</td>
<td>7.18 ± 1.37</td>
<td>6.99 ± 0.92</td>
<td>11.14 ± 3.35</td>
<td>11.44 ± 2.36</td>
<td>11.14 ± 3.35</td>
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<td>Gln</td>
<td>Controls</td>
<td>58 ± 12</td>
<td>0.80 ± 0.30</td>
<td>58 ± 6</td>
<td>0.50 ± 0.10</td>
<td>47 ± 8</td>
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<td>55 ± 4</td>
<td>0.50 ± 0.20</td>
<td>44 ± 9</td>
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<td>D-Glu-1</td>
<td>70 ± 12</td>
<td>0.60 ± 0.20</td>
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<td>0.40 ± 0.10</td>
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Values are means ± SE in μmol/min from 4 subjects before (controls) and after 13.3 mmol D-glutamate (D-Glu) load (D-Glu-1). Filtered and excreted metabolites were calculated as described in METHODS. L-Glu, L-glutamate; Gln, glutamine; D-PCA, D-pyrridilone carboxylic acid. Significant difference after D-Glu load: *P < 0.05; †P < 0.005.
plasma (517 ± 37, 509 ± 42, 458 ± 21, and 535 ± 76 units/ml plasma, respectively) nor that in urine (69 ± 1, 46 ± 1, 46 ± 6, and 76 ± 12 × 10³ U/period) increased over the 3 h after the D-Glu load.

**DISCUSSION**

The biological significance of D-glutamate uptake and conversion to D-pyrrolidone-carboxylic acid and excretion is suggested by the presence of the small, but detectable, plasma D-glutamate (Fig. 2) and the considerable excretion of D-pyrrolidone-carboxylic acid (Fig. 3), even in the absence of the exogenous D-glutamate load. Based on the minute excretion rate (Table 1) of D-pyrrolidone-carboxylic acid, and extrapolated to 24 h, the daily D-glutamate load approximates 0.5–1 mmol/day. In addition, a large exogenous D-glutamate load greatly enhances the D-pyrrolidone-carboxylic acid formation, consistent with a precursor-product relationship and the demonstration in rats that 15N-labeled D-glutamate appears in the urine as D-pyrrolidone-carboxylic acid (17). There has been one other report of a detectable D-glutamate concentration in normal human plasma by use of chiral chromatography (3), but this determination encompasses plasma D-glutamine, which, according to the authors, is hydrolyzed to D-glutamate under their conditions. Whether D-glutamine is normally present in plasma, and how much, remains to be determined. Thus the present paper is the first report of D-glutamate in human plasma and the first demonstration that D-pyrrolidone-carboxylic acid formation is indeed a significant, if not the sole, detoxification pathway for D-glutamate in humans.

The accumulation of the D-pyrrolidone-carboxylic acid in plasma after the oral administration of D-glutamate (Fig. 2) and the 13-fold increase in D-pyrrolidone-carboxylic acid excretion (Fig. 3) are consistent with the absorption of D-glutamate from the intestine and conversion via D-glutamate cyclotransferase (12). The cumulative excretion of D-pyrrolidone-carboxylic acid over this 3-h time course accounted for ~20% of the administered D-glutamate. If the excretion rate had continued at the 3-h rate, virtually all of the load would have been excreted in ≤15 h. The fact that a significant fraction appears as D-pyrrolidone-carboxylic acid confirms that humans utilize the same pathway as that present in the rat to detoxify this potentially harmful metabolite.

The rise in plasma D-glutamate during the postload period demonstrates that the D-glutamate is taken up into the blood and reaches the systemic circulation. Rather than continue to rise, the blood concentration plateaus, despite continuous absorption (based on the D-pyrrolidone-carboxylic acid formation). In contrast to the plasma D-glutamate concentration that is defended at 14 μM, the plasma D-pyrrolidone-carboxylic acid concentration progressively increases to levels that are more than fivefold higher than the D-glutamate plasma concentration in the 1st h. It may be noteworthy that this plasma concentration of D-pyrrolidone-carboxylic acid (70 μM) corresponds to, or is slightly higher than, that reported circulating in the plasma of patients with chronic renal failure [total pyrrolidone-carboxylic acid = 86 μM, with 33 μM as the D-isomer (14)].

Although the detoxification of D-glutamate requires at a minimum a transporter capable of delivering D-glutamate into cells expressing D-glutamate cyclotransferase activity and functioning kidneys for effective clearance of the D-pyrrolidone-carboxylic acid, there may be other pathways involved in the transit of D-glutamate. In our previous studies in the rat with intravenously infused D-glutamate, the combination of renal extraction and conversion to D-pyrrolidone-carboxylic acid was the major organ-metabolic pathway (4, 20). In contrast, an oral D-glutamate load would be delivered to the liver, which expresses both D-glutamate cyclotransferase activity (12) and D-,L-glutamine synthetase (11). The D-pyrrolidone-carboxylic acid formed in the liver is effectively cleared through the kidney, as demonstrated in Fig. 4. On the other hand, D-glutamate incorporated into D-glutamine by hepatic D-,L-glutamine synthetase (11) would present another problem, because it can be readily hydrolyzed to D-glutamate at specific sites expressing GGt activity. In
the present study this was demonstrated for the kidneys by the large excretion of D-glutamate after an oral D-glutamine load (Fig. 5). If D-glutamine was indeed formed after the D-glutamate load, then intraluminal hydrolysis might explain the increase in D-glutamate’s FE (Fig. 4). In addition, the antiluminal D-glutamine hydrolysis (25) and D-glutamate uptake (20), coupled to renal D-glutamate cyclotransferase activity (20), would result in release of D-pyrrolidone-carboxylic acid into the renal vein. Consequently, any D-pyrrolidone-carboxylic acid formed in the kidney from D-glutamine would join that produced by the liver for renal filtration and excretion. Regardless of the relative contributions of the liver and kidneys, over the 3 h ~20% of the D-glutamate load could be accounted for as D-pyrrolidone-carboxylic acid and <5% as D-glutamate.

How the kidney handles D-glutamate was another objective of our study. In the time controls, <5% of the filtered D-glutamate was excreted, consistent with previous studies in the rat demonstrating that filtered D-glutamate is largely, if not entirely, reabsorbed (4). Although the anionic amino acid transport system X_Ac displays a stereospecific anomaly for D-aspartate (8), the affinity of X_AG subtypes for D-glutamate is far below that for L-glutamate (1). Nevertheless, there appears to be a transporter present in the kidney capable of high-affinity D-glutamate transport, possibly X_Ac, that accounts for the reabsorption; it is also possible that this transporter is inhibited by D-pyrrolidone-carboxylic acid. After the D-glutamate load, there was an apparent progressive diminution of reabsorption from 95, 60, and 32, to only 5% of the filtered D-glutamate. Because the amount filtered did not change, the reduced reabsorption does not reflect saturation of this carrier and points to either a reduction in the reabsorption of the filtered D-glutamate or de novo formation of D-glutamate within the kidney (25). These observations may explain why some studies observed filtration and excretion without reabsorption (2, 16), whereas others found a significant renal reabsorptive capacity (4, 6).

Suppressing reabsorption of D-glutamate could account for the decreasing reabsorption over the course of the study. For example, if D-pyrrolidone-carboxylic acid inhibits glutamate transport via the high-affinity transporter, then the progressively increasing D-pyrrolidone-carboxylic acid concentration (mM concentrations in the urine) in the tubule lumen could become more effective in inhibiting glutamate reabsorption; this would explain the progressive increase in FE with time, as actually observed in Fig. 4. Curiously, there are no studies as to whether D-pyrrolidone-carboxylic acid can inhibit glutamate transport. However, it has been shown that micromolar concentrations of the L-isomer of pyrrolidone-carboxylic acid competitively inhibit the high-affinity uptake of radiolabeled L-glutamate into rat striatal synaptosomes and, surprisingly, as effectively as L-glutamate (7). If the D-pyrrolidone-carboxylic acid has a similar, or, more likely, lesser reactivity with one or more glutamate transporters, then inhibition of glutamate uptake could occur and might explain reductions in both D- and L-glutamate reabsorption. Further studies into whether D-pyrrolidone-carboxylic acid can inhibit either D- or L-glutamate transport would seem warranted.

The reduction in apparent reabsorption might also reflect the intraluminal hydrolysis of D-glutamate, leading to the formation of D-glutamate, as previously demonstrated in the functioning rat kidney (25). Because D-glutamine is a substrate for GGT’s glutaminase activity (23, 25), the formation of D-glutamate as the result of filtered D-glutamine hydrolysis might also contribute to the rising D-glutamate excretion. Regardless of the actual mechanism(s), the rise in D-glutamate excretion is obviously another mechanism for eliminating D-glutamate and maintaining a low plasma concentration.

The fact that plasma D-glutamate concentration is so tightly regulated after an oral D-glutamate load points to an intricate regulatory mechanism for maintaining the normal low plasma concentration. This regulation is more significant because the D-glutamate is not metabolized by D-amino acid oxidase (2, 24), as are other D-amino acids. The present study shows that this regulation involves both D-glutamate transport and metabolism and enhanced renal clearance. The availability of an active metabolic pathway(s), coupled to a high-affinity transporter, clearly would provide a tighter regulation of the plasma D-glutamate concentration. A second mechanism serving to prevent the rise in plasma D-glutamate is the enhanced renal clearance, which although quantitatively less significant than conversion to D-pyrrolidone-carboxylic acid, would nevertheless contribute at the critical time that the metabolic disposal reaches a maximum. This of course might be fortuitous, or might be by design if the tubular interactions proposed actually occur. Nevertheless, together the two regulatory mechanisms would be reinforcing and guarantee effective removal of the D-glutamate, thereby defending the low plasma D-glutamate concentrations.

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