An oral glutamine load enhances renal acid secretion and function\textsuperscript{1,2}

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ABSTRACT In a recent study, a small oral glutamine load acutely elevated plasma bicarbonate concentrations in healthy adults (Am J Nutr 1995;61:1058–61). The present study was designed to elucidate the renal mechanism underlying the base-generating response to L-glutamine. Accordingly, vehicle (489 mL diet soda) or vehicle plus 2 g L-glutamine (28 mg/kg body wt) was ingested and the gain in extracellular fluid volume bicarbonate was compared with renal acid elimination as either ammonium excretion or tubular acid secretion (titratable acid plus bicarbonate reabsorption). Vehicle alone, which contained 27 mmol acid, did not increase extracellular fluid volume bicarbonate over the 90-min period. In contrast, L-glutamine increased plasma bicarbonate concentration (from 25.4 ± 2 to 27.9 ± 1 mmol/L, \(P < 0.05\)) and extracellular fluid volume bicarbonate by an estimated 39 ± 10 mmol. When added to that required to neutralize the ingested acid, the combined total for new bicarbonate generated gave an estimated 66 ± 10 mmol. Surprisingly, ammonium excretion accounted for < 2% of this newly generated bicarbonate. However, acid secreted and excreted as net acid (5.2 ± 4.0 mmol/90 min) as well as that coupled to enhanced bicarbonate reabsorption (76 ± 20 mmol/90 min) readily accounted for the estimated base gain (81 ± 24 compared with 66 ± 10 mmol/90 min). Concomitant with enhanced renal acid secretion, the oral glutamine load elicited an increase in glomerular filtration rate. These results rule out a role for L-glutamine as a direct precursor of bicarbonate and instead point to an indirect role in accelerating acid secretion, apparently coupled to increased glomerular filtration rate. Am J Clin Nutr 1998;67:660–3.

KEY WORDS L-Glutamine load, plasma bicarbonate, ammonium excretion, renal acidification, glomerular filtration rate, growth hormone, men

INTRODUCTION

Glutamine is basogenic by merit of being metabolized within the functioning kidney to ammonium and bicarbonate (1). Secretion of ammonium (2) with release of the bicarbonate into the renal vein ensures net base formation (Figure 1A). Administration of a small oral glutamine load to healthy subjects ranging in age from 32 to 64 was shown previously to acutely elevate their circulating plasma bicarbonate (3), and, although ammonium excretion was not measured, this response was attributed to presumed renal glutamine metabolism and ammoniagenesis. The possibility also exists that glutamine acts indirectly via elevated circulating growth hormone, which activates glucose-dependent renal acid secretion (Figure 1B) and bicarbonate generation (4, 5). Because these two mechanisms for generating bicarbonate are fundamentally different, their expression in response to glutamine loading should be discernible in terms of either ammonium excretion or tubule acid secretion (titratable acid plus bicarbonate reabsorption). Therefore, studies were performed to assess the contribution of each mechanism to the newly bicarbonate generated in response to a small oral glutamine load.

METHODS

Studies were carried out exactly according to the previously published protocol (3) by performing time controls and glutamine-loading experiments on three healthy male volunteers (A, B, and C) aged 53, 44, and 18 y weighing 70, 120, and 50 kg, respectively. The authors were volunteers and gave their consent to a protocol approved by the Louisiana State University Medical Center Institutional Review Board for Human Research. Vehicle, 490 mL diet soda (Diet Coke; Coca-Cola Co, Atlanta), or vehicle plus 2 g L-glutamine or 2 g L-glycine was administered on alternate days. Glycine in place of glutamine served as a control for glutamine loading should be discernible in terms of either ammonium excretion or tubule acid secretion (titratable acid plus bicarbonate reabsorption). Therefore, studies were performed to assess the contribution of each mechanism to the newly bicarbonate generated in response to a small oral glutamine load.

Studies were performed over 2 wk, one pair test per week, at exactly the same time (0800–1100) and 45 min after a light breakfast (toast, coffee, and juice). After subjects emptied their bladders and an initial forearm venous blood sample was obtained (\(t = 0\)), either vehicle (490 mL diet soda, pH = 3.8, containing 27 mmol phosphoric acid) or vehicle plus 2 g L-glutamine or 2 g L-glycine was administered on alternate days. Glycine in place of glutamine served as a control for the amino acid load. Studies were performed over 2 wk, one pair test per week, at exactly the same time (0800–1100) and 45 min after a light breakfast (toast, coffee, and juice). After subjects emptied their bladders and an initial forearm venous blood sample was obtained (\(t = 0\)), either vehicle (490 mL diet soda, pH = 3.8, containing 27 mmol phosphoric acid) or vehicle plus 2 g L-glutamine (Sigma, St Louis) was ingested over a 20-min period with blood sampled and urine collected after 90 min.

Plasma and urine samples were placed on ice and processed the same day. Plasma and urine bicarbonate were determined by microgasometry (6). Urine pH was measured on a Corning pH meter (CIBA Corp, Sudbury, United Kingdom) with titratable acid determined by back titrating urine to pH 7.4 with 0.2 mol NaOH/L. Urinary ammonium was determined by the phenol-
hypochlorite colorimetric method (7). Plasma and urinary creatinine were measured colorimetrically by using reagents from a commercial kit (Creatinine; Sigma). Endogenous creatinine clearance was used as an estimate of glomerular filtration rate (GFR); filtered bicarbonate was calculated from the GFR times plasma bicarbonate concentration and bicarbonate reabsorption was determined as the difference between filtered and excreted bicarbonate.

The results from each paired test (n = 3) are expressed as means ± SDs. Differences between vehicle and vehicle plus L-glutamine or L-glycine treatments were analyzed by paired Student’s t test. Where directional changes were predicted a priori, a one-tailed t test was used. The computer program Instat (Graphpad; San Diego) was used for the analyses.

RESULTS

Plasma bicarbonate concentrations before and 90 min after ingestion of the vehicle or vehicle plus 2 g L-glutamine for subjects A, B, and C are shown in Figure 2. With vehicle alone, plasma bicarbonate did not increase after 90 min (from 25.1 ± 1 to 24.9 ± 2 mmol/L). In marked contrast, L-glutamine elevated plasma bicarbonate (from 25.4 ± 2 to 27.9 ± 1 mmol/L, P < 0.05), similar to results obtained for the eight subjects of the previous study (3). Accordingly, after 90 min oral glutamine resulted in an increment in plasma bicarbonate concentration significantly greater than that of the time control (2.6 ± 1.3 compared with 0 ± 0.6 mmol/L, P < 0.05). Ammonium and titratable acid excretion are presented in Figure 3. Ammonium excretion increased 1.8-fold (from 17 ± 3 to 30 ± 11 μmol/min, P = 0.11), whereas titratable acid excretion was threefold higher (from 20 ± 5 to 60 ± 35 μmol/min, P = 0.08); neither, however, changed significantly. Urinary pH was reduced after oral L-glutamine compared with vehicle alone (5.71 ± 0.08 compared with 6.11 ± 0.15, P < 0.05).

Acid secreted and neutralized in reabsorbing filtered bicarbonate is depicted in Figure 4. The amount of filtered bicarbonate increased 32% with the oral glutamine load (from 2474 ± 1000 to 3253 ± 1178 μmol/min, P < 0.01) as the result of an elevated GFR (from 98 ± 31 to 121 ± 36 mL/min, P < 0.02) and higher plasma bicarbonate concentrations (Figure 2); excreted bicarbonate decreased 75% (from 8.2 ± 1 to 2 ± 1 μmol/min, P < 0.01) despite the increased filtered bicarbonate. Consequently, the amount of bicarbonate reabsorbed clearly increased (from 2466 ± 1000 to 3251 ± 1178 μmol/min, P < 0.01) indicative of enhanced tubular acid secretion. A comparison between the estimated renal base generated as a consequence of net acid excreted, (ammonium plus titratable acid minus bicarbonate excreted) versus the L-glutamine–induced increment in tubular acid secretion is presented in Table 1. The estimated renal base generated reflects the amount needed to neutralize the 27 mmol acid present in the vehicle plus that required to increase extracellular bicarbonate 2.6 ± 1.3 mmol/L or 39 ± 10 mmol for a total of 66 ± 10 mmol over 90 min. Against this, the kidneys generate < 2% from ammoniagenesis (30–17 = 13 μmol NH₄⁺/min × 90 min = 1.2 mmol HCO₃⁻ generated from ammonium excretion/66 mmol base required × 100 = 1.8% of that generated). Increased net acid excretion, titratable acid plus ammonium minus bicarbonate excretion, accounts for < 8% of the estimated bicarbonate generation. On the other hand, acid secreted in reabsorbing the augmented filtered bicarbonate with glutamine treatment easily accounted for the required amount (66 required compared with 76 mmol generated from enhanced bicarbonate reabsorption). Note that the combined net acid excretion and acid secreted to reabsorb the increased filtered bicarbonate more than accounted for the bicarbonate generated (81 ± 24 compared with 66 ± 10 mmol). In contrast with glutamine, 2 g glycine dissolved in the acid vehicle did not increase plasma bicarbonate concentration (1.3 ± 0.4 compared with 2.6 ± 0.8 mmol/L, P < 0.05, n = 3) or the total base generated (–6 ± 18 compared with 81 ± 24 mmol/90 min, P < 0.007). Thus, the elevation in alkaline reserves may be fairly attributed to an effect of glutamine, although clearly not through the ammoniagenic pathway (Figure 1A).

DISCUSSION

A small oral glutamine load is able to significantly elevate the extracellular plasma bicarbonate concentration in a population of healthy subjects with an average age of 45 y (3). This is particu-
larly significant because plasma bicarbonate concentration spontaneously declines with age, being strikingly apparent after the age of 50 y (8). Interestingly, this decline in plasma bicarbonate correlates with the decrease in GFR with aging (9). In the present study, the effectiveness of oral glutamine in elevating plasma bicarbonate was associated with an acute increase in GFR, suggesting a relationship between these two processes.

Restoration of normal plasma bicarbonate concentration in the present study as well as in the earlier study of eight subjects (3) required considerable bicarbonate generation. The amount of new bicarbonate required was conservatively estimated at 66 mmol, based on equilibration throughout the extracellular fluid; this may be an underestimation if the volume of distribution actually exceeds the extracellular volume. In either case, this large amount of new bicarbonate required, ≥66 mmol over the 90-min time course, should have been associated with a large ammoniagenic response and acid excretion nearly equivalent to the base generated. In this regard, the results were clear cut: ammonium excretion increased only marginally and accounted for < 2% of the base requirement; net acid excretion, the sum of ammonium plus titratable acid minus bicarbonate excretion, accounted for < 8%. On the other hand, acid secretion was enhanced and, furthermore, readily accounted for and even exceeded the conservatively estimated base requirement (Table 1).

How oral l-glutamine acts to bring about this response was not resolved by this study, but one real possibility is by elevating the circulatory growth hormone (3). Growth hormone has been shown to acutely activate tubular acid secretion while enhancing bicarbonate reabsorption in the intact functioning rat kidney (4). In addition, growth hormone elicited an acute increase in GFR whereas amiloride blocked both the increase in acidification as well as GFR (4), suggesting that Na\(^+\)-H\(^+\) exchange activation may be common to both responses. In humans, exogenous growth hormone elevated plasma bicarbonate in chronically acid-loaded subjects, a response associated with a significant increase in GFR and tubular acid secretion (10), not unlike the acute response observed in the present study as well as that observed previously (4). Thus, both effects associated with oral l-glutamine, enhanced acid secretion and the rise in filtration rate, could reflect growth hormone and IGF-I actions in these nephron segments. In addition, conversion of glucose to lactic acid occurs at these sites (14) and, with secretion of acid moiety (Figure 1B), lactate oxidation yields net bicarbonate for release into the renal vein. Note that both acid secretion and bicarbonate generation in response to growth hormone require the presence of glucose (5, 13). Consequently, oral l-glutamine may prove effective in amelio-

Receptors for growth hormone and IGF-I are present along the thick ascending limb of Henle’s loop (12). Activation of the thick ascending limb or the late proximal tubule’s Na\(^+\)-H\(^+\) exchanger by growth hormone (13) or IGF-I (11) would effectively acidify the urine at a site downstream from proximal ammonium secretion as well as reduce the filtered load to the macula densa; a reduction in delivered sodium bicarbonate should in turn enhance glomerular filtration. Consequently, a displacement of bound IGF-I and free IGF-I activation of the loop Na\(^+\)-H\(^+\) exchanger could certainly explain the rapid acidification response observed in the present study as well as that observed previously (4). Thus, both effects associated with oral l-glutamine, enhanced acid secretion and the rise in filtration rate, could reflect growth hormone and IGF-I actions in these nephron segments. In addition, conversion of glucose to lactic acid occurs at these sites (14) and, with secretion of acid moiety (Figure 1B), lactate oxidation yields net bicarbonate for release into the renal vein. Note that both acid secretion and bicarbonate generation in response to growth hormone require the presence of glucose (5, 13). Consequently, oral l-glutamine may prove effective in amelio-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Estimated bicarbonate balance in the extracellular compartment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>mmol</td>
</tr>
<tr>
<td>Required</td>
<td></td>
</tr>
<tr>
<td>Vehicle acid</td>
<td>27</td>
</tr>
<tr>
<td>Bicarbonate elevation</td>
<td>39 ± 10</td>
</tr>
<tr>
<td>Total</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>Generated after oral l-glutamine</td>
<td></td>
</tr>
<tr>
<td>Net acid</td>
<td>5.2 ± 4.0</td>
</tr>
<tr>
<td>Bicarbonate reabsorption</td>
<td>76 ± 20</td>
</tr>
<tr>
<td>Total</td>
<td>81 ± 24</td>
</tr>
</tbody>
</table>

* Estimated for extracellular fluid volume equivalent to 20% of body weight of subjects A, B, and C.

1 | Required to titrate 489 mL of diet soda (Diet Coke; Coca-Cola Co, Atlanta) to pH 7.4.

2 | Required to increase bicarbonate concentration from 25.0 to 27.6 mmol/L in the extracellular fluid.

3 | Net acid excreted over that of the time control during the 90 min after oral l-glutamine.

4 | Bicarbonate reabsorption over that of the time control during the 90 min after oral l-glutamine.
rating the age-dependent decline in renal function (9) and alkaline reserves (8) that parallels the reduction in growth hormone secretion (15), particularly in subjects aged ≥ 50 y.

Glutamine has also been proposed as a supplement in the treatment of catabolic illness, in part to meet the demands of associated metabolic acidosis (16). Indeed, in patients subjected to surgical stress, muscle glutamine depletion served as an index for differentiating survivors from nonsurvivors (17). Because metabolic acidosis and associated enhanced glutamine flow to the kidneys for base generation would limit glutamine availability for the small intestine and possibly compromise its barrier function, glutamine could well become conditionally essential under precisely these conditions (18). In this light, providing a glutamine supplement should not only enhance renal function, as suggested in the present study, but may also prevent muscle glutamine depletion (19) and consequently aid in the maintenance of gut barrier integrity.

REFERENCES