Hypotonic saline infusion alters the renal response to amino acids in men

ALDO CLARIS-APPIANI,1 AMEDEA S. TIRELLI,1 GIANLUIGI ARDISSINO,1 VALERIA DACCO,1 EUGENIA MORETTO,2 CARLO CORBETTA,2 LAURA GUIDI,2 AND BAROUKH M. ASSAEL.1

1Department of Pediatrics, University of Milan, and 2Pharmacy and Laboratory of Istituti Clinici di Perfezionamento, I-20122 Milan, Italy

Claris-Appiani, Aldo, Amadea S. Tirelli, Gianluigi Ardissino, Valeria Dacco, Eugenia Moretto, Carlo Corbetta, Laura Guidi, and Baroukh M. Assael. Hypotonic saline infusion alters the renal response to amino acids in men. Am. J. Physiol. 276 (Renal Physiol. 45): F137–F142, 1999.—We investigated the effects of hypotonic saline-induced modifications of extracellular volume and sodium handling on the renal and metabolic response to amino acids (AA). Renal hemodynamics (Inutest, p-aminohippurate clearance), plasma AA, and glucagon levels, as well as urea and sodium excretion, were studied in seven adult volunteers infused for 2 h, on six separate occasions, according to the following protocols: 1) high-AA solution (300 mg·min⁻¹·1.73 m⁻²); 2) low-AA solution (150 mg·min⁻¹·1.73 m⁻²); 3) low AA + 2.00 mmol·1.73 m⁻² of 0.23% saline solution; 4) high AA + 0.23% saline; 5) high AA + 0.45% saline; and 6) 0.45% saline alone. The glomerular filtration rate (GFR) rise induced by the high-AA solution was similar to that induced by the low-AA solution (ΔGFR = +24 ± 6 and +20.2 ± 7 mL·min⁻¹·1.73 m⁻², respectively), whereas the plasma AA and glucagon levels and urea excretion rate increases were related to AA dose. The addition of 0.23% saline to the low-AA solution and of 0.45% saline to the high-AA solution blunted the renal hemodynamic response (ΔGFR = +6.6 ± 10.1 and +11.4 ± 8.3 mL·min⁻¹·1.73 m⁻², respectively) without modifying the pattern of plasma AA and glucagon levels and urea excretion observed with the AA infusion alone. Urinary sodium excretion increased from baseline with each protocol and rose even further when saline was added to AA. A negative correlation (r = −0.36, P < 0.05) was found between the changes from basal values in GFR and those in serum sodium concentration. These data suggest that AA-induced hyperfiltration might be blunted by hypotonic saline infusion, possibly through an acute modification of renal sodium handling and extracellular volume.

Study Design

Seven healthy male volunteers aged 22–25 yr were selected for the study. Clinical and laboratory investigations revealed no indication of any disease. Dietary records were obtained and urinary sodium and nitrogen excretion were determined for 3–4 days before each test to calculate average dietary intakes. No differences were observed among the estimated sodium and protein intakes of each period. The subjects underwent six experimental protocols in random order with intervals of at least 1 mo between each of them. All tests were performed in the morning after an overnight fast. Informed consent was obtained from each subject, and the study was approved by the institutional review board.

Study Design

Protocol common to all six studies. GFR and RPF were measured by the clearance of Polyfructosan S and p-aminohippurate (PAH), respectively. The test was started at 8:00 AM with priming doses of Polyfructosan S (Inutest, 25%; Laevosan-Gesellschaft, Linz, Austria) and PAH (5% PAH, Monico) administered through an antecubital vein at the rate of 1.1 mL/min for 1.5 min, followed by continuous infusions at rates calculated on the basis of the subject’s body weight and designed to reach and maintain plasma levels of 20 mg/dl for Inutest and 2 mg/dl for PAH (average rate of 0.11 mL/min for 25% Inutest and 13% PAH). The total amount of these solutions over the test ranged from 70 to 80 ml. Tap water was given orally within 20 min of starting the test (20 ml/kg body wt) and subsequently throughout the study to induce and maintain maximal water diuresis and urinary osmolality to <100 mosmol/kg. After a 90-min equilibration period, 30-min urine was collected by means of spontaneous voiding. Three blood samples were drawn at 7-min intervals during the 30-min collection period. The baseline urine and plasma collections were followed by an experimental period of 2 h according to one of the six following protocols (summarized in Table 1). During the last 20 min of each of the 2 h of infusion, urine was collected and two further blood samples were drawn.

THE INFUSION OF AMINO ACIDS (AA) INCREASES GLOMERULAR FILTRATION RATE (GFR) AND RENAL PLASMA FLOW (RPF) (5, 14, 18, 21). Some lines of evidence suggest that an intermediate step in the splanchnic region, characterized by stimulation of gluconeogenesis, ureagenesis, and glucagon secretion, is necessary for the renal response to AA to take place (6, 8, 9, 11, 18). However, there is a growing body of information indicating that intrarenal mechanisms related to extracellular volume control, renal sodium handling, and tubuloglomerular feedback (TGF) are important in the genesis of AA-induced hyperfiltration: a low-sodium diet preceding AA infusion prevents the glomerular response (19); AA-induced hyperfiltration is associated with increased proximal sodium reabsorption in vivo (7, 16) and in vitro (2, 21); and a protein meal fails to induce a rise in GFR in dogs with impaired proximal tubular function due to experimental Fanconi’s syndrome or to administration of some diuretics (22, 23).

In this study, in a first set of experiments, we investigated the renal and metabolic response to a low and a high dose of AA (compared with the effects of a saline infusion alone). Then, in a second set, we studied the acute modifications of this response when a low- and a high-sodium solution were infused with the AA. The results showed that hypotonic saline infusion inhibited AA-induced hyperfiltration.

METHODS

Seven healthy male volunteers aged 22–25 yr were selected for the study. Clinical and laboratory investigations revealed no indication of any disease. Dietary records were obtained and urinary sodium and nitrogen excretion were determined for 3–4 days before each test to calculate average dietary intakes. No differences were observed among the estimated sodium and protein intakes of each period. The subjects underwent six experimental protocols in random order with intervals of at least 1 mo between each of them. All tests were performed in the morning after an overnight fast. Informed consent was obtained from each subject, and the study was approved by the institutional review board.
Body weight and systolic and diastolic blood pressures were measured before and at the end of each test. Protocol 1: High AA. After the baseline period, a 2-h intravenous infusion of AA (10% Freamine III, Baxter) was administered at the rate of 3 ml·min⁻¹·1.73 m², corresponding to 300 mg·min⁻¹·1.73 m² (2,550 µmol·min⁻¹·1.73 m²) of AA.

Protocol 2: Low AA. This protocol was identical to protocol 1 except that the concentration of the AA solution was 5%, so that AA was infused at the rate of 150 mg·min⁻¹·1.73 m² (1,275 µmol·min⁻¹·1.73 m²).

Protocol 3: Low AA, 0.23% (low) saline concentration. This protocol was identical to protocol 2 except that a solution of sodium chloride was infused for 2 h together with the 5% AA (150 mg·min⁻¹·1.73 m²).

Protocol 4: High AA, 0.23% (low) saline concentration. This protocol was identical to protocol 1 except that a solution of 2,000 ml·1.73 m² (17 ml·min⁻¹·1.73 m²) of 40 mmol/l (0.23%) sodium chloride was infused for 2 h together with the 10% AA (300 mg·min⁻¹·1.73 m²).

Protocol 5: High AA, 0.45% (high) saline concentration. This protocol was identical to protocol 1 except that a solution of 2,000 ml·1.73 m² (17 ml·min⁻¹·1.73 m²) of 80 mmol/l (0.45%) sodium chloride was infused for 2 h together with the 10% AA (300 mg·min⁻¹·1.73 m²).

Protocol 6: 0.45% saline alone. An 80 mmol/l (0.45%) solution of sodium chloride alone was infused for 2 h at the rate of 20 ml·min⁻¹·1.73 m².

Analysis, Calculations and Statistics

We calculated Inun test and PAH clearances on the basis of their urinary concentrations at the collection times and the means of the plasma concentrations measured in each sample drawn during each collection period. Hematocrit (Hct), plasma urea, electrolytes, glucagon, and AA were measured in the last sample drawn during each period; urinary urea, osmolality, and electrolytes were measured during each urine collection period. All determinations were performed in duplicate.

The blood samples for the determination of Inun test, PAH, sodium, and osmolality were collected in lithium-heparin, those for sodium determinations in sodium-heparin, and those for glucagon determinations in EDTA + Trasyrol.

Plasma and urinary Inun test and PAH were measured as previously described (7), AA was measured by ion-exchange chromatography, glucagon was measured by radioimmunoassay (RIA-Mat; Byk Gulden), and urinary urea was measured by standard automated laboratory procedures using Hitachi 737 equipment. Plasma and urinary electrolytes were analyzed by flame photometry. Plasma and urinary osmolality were measured using a Fiske osmometer (freezing-point technique).

Filteration fraction was calculated as GFR/RPF, and fractional Na excretion (FE Na) was calculated as Unaf/PNa × Pinulin/Uinulin, where Unaf/PNa is the ratio of urinary to plasma sodium concentration, and Pinulin/Uinulin is the ratio of plasma to urinary inulin concentration.

Data are reported as group means ± SD. Differences within each protocol were determined comparing data at baseline and 1 and 2 h of treatment; differences between protocols were analyzed as changes from baseline of variables at 1 and 2 h (Δ: value at 1 h minus baseline and at 2 h minus baseline). The statistical significance of differences was evaluated by analysis of variance for randomized blocks for repeated measures, followed by Duncan’s multiple range test. Correlations between variables were performed using linear multiple regression analysis.

RESULTS

The two protocols (protocols 1 and 2) in which AA solutions were infused without sodium did not cause variations in body weight, blood pressure, or Hct, whereas there was a significant increase in weight and a fall in Hct at the end of protocols 5 and 6 (high sodium content). A significant reduction in Hct was found at the end of protocols 3 and 4 (low sodium content). Blood
pressure, plasma sodium, and plasma osmolality never changed (Table 1).

Renal and Metabolic Effects of High-AA Infusion and of 0.45% Saline Solution: Protocols 1 and 6

As shown in Table 2, high-AA infusion (protocol 1) induced a significant increase in GFR and RPF; with saline (protocol 6), neither GFR nor RPF changed. High-AA infusion caused a significant increase in plasma AA, plasma glucagon levels, and the urinary urea excretion rate at 1 h, followed by a further significant rise at 2 h (Table 3). Diuresis, sodium excretion rate and FE\textsubscript{Na} were significantly modified only at 2 h (Table 4). On the contrary, saline solution did not change the above metabolic indexes (Table 3) but induced a brisk increase in diuresis, sodium excretion, and FE\textsubscript{Na} at 1 h and a further significant rise at 2 h of the infusion (Table 4).

### AA Dose-Related and Saline Infusion-Related Effects on Renal and Metabolic Response to AA: Protocols 2–5

Overall analysis and results of the data obtained with protocols 2–5 are shown in Tables 2–4 and in Figs. 1 and 2. The low-AA infusion (protocol 2) induced a GFR and RPF increase only at 2 h (Table 2), but the greatest GFR increase (between 1 and 2 h) was not significantly different from that obtained with the high-AA infusion (+20.2 ± 7 and +24 ± 6 ml·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}, respectively) (Fig. 1). The low AA dose induced a significantly (P < 0.05) smaller increase than the high AA dose in plasma AA levels (+2,524 ± 705 vs. +4,596 ± 819 µmol/l), plasma glucagon levels (+58 ± 20 vs. +123 ± 101 pg/ml), and the urea excretion rate (+9.8 ± 2.5 vs. +17.4 ± 3.7 mg·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}) (Fig. 2).

The addition of a 40 mmol/l (0.23%) sodium solution to the low AA dose (protocol 3) blunted the renal hemodynamic response (the greatest GFR increase, 6.6 ± 10.1 ml·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}) (Table 2; Fig. 1); the rise in plasma AA and plasma glucagon was significantly greater than in protocols 1 and 2 (Fig. 1). When the 0.23% sodium solution was infused together with the high AA dose (protocol 4), the glomerular response was not affected (the greatest GFR change, 11.4 ± 8.3 ml·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}) and the RPF rise (Table 2; Fig. 1) without modifying the pattern of the metabolic indexes observed with protocol 4 (Table 3).

### Table 2. Renal hemodynamic response to protocols 1–6

<table>
<thead>
<tr>
<th>Protocol</th>
<th>GFR, ml·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}</th>
<th>RPF, ml·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>102 ± 9</td>
<td>488 ± 59</td>
</tr>
<tr>
<td>1 h</td>
<td>126 ± 14*</td>
<td>485 ± 49</td>
</tr>
<tr>
<td>2 h</td>
<td>122 ± 15*</td>
<td>563 ± 37*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. basal. **P < 0.01 vs. basal. RPF, renal plasma flow; FF, filtration fraction.

### Table 3. Metabolic response to study protocols 1–6

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Plasma AA, µmol/l</th>
<th>Plasma glucagon, pg/ml</th>
<th>U\textsubscript{urea}, mg·min\textsuperscript{-1}·1.73 m\textsuperscript{-2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>2,677 ± 320</td>
<td>2,823 ± 400</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>1 h</td>
<td>5,956 ± 506*</td>
<td>4,575 ± 200*</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>2 h</td>
<td>7,667 ± 691†</td>
<td>5,397 ± 857*</td>
<td>21 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. basal. **P < 0.01 vs. basal. U\textsubscript{urea}, urinary urea excretion rate.
Diuresis increased with all of the protocols except protocol 2, and the increase was already observed at 1 h with protocols 4 and 5. Urinary sodium excretion (UNaV) and FENa showed an increase from baseline at 2 h with protocols 2 and 3, whereas with protocols 4 and 5 the increase was already significant at 1 h. With all of the protocols, the increase in UNaV at 2 h was significantly greater than that observed at 1 h. Protocol 5 produced the greatest increase in UNaV of all the protocols, at both 1 and 2 h (Table 4).

A negative correlation (r = –0.38, P < 0.05) between the change from basal values in GFR (ΔGFR) and in sodium excretion rate (ΔUNaV), was found with high-AA infusion administered with different amounts of sodium (protocols 1, 4, and 5) (Fig. 3).

**DISCUSSION**

The most important finding of this study was that hypotonic saline infusion reduced the renal hemodynamic effects of AA. Furthermore, the renal response to AA was not affected with a higher AA dose and was blunted when hypotonic saline of higher concentration was added to the high-AA infusion.

Although an increase in renal hemodynamics following AA infusion is a well-established physiological phenomenon (5, 7, 14, 18, 24), the precise mechanism underlying this response is still under discussion. The demonstration that direct infusion of three nonessential AA (serine, alanine, and proline) in the renal artery of dogs did not affect renal hemodynamics (18), whereas the GFR increased after systemic infusion of these AA, and the close relation between AA-induced hyperfiltration and the rise of some indexes of splanchnic metabolism such as the plasma glucagon/insulin ratio and ureagenesis (9) might suggest that an intermediate splanchnic step is necessary to induce hyperfiltration in vivo. These splanchnic factors, stimulated by hyperaminoacidemia, interact with an ultimate intrarenal pathway to induce AA-dependent hyperfiltration. The finding that hyperfiltration may be induced by AA in the isolated perfused kidney of the rat demonstrates the importance that an intrarenal system may have in the genesis of this phenomenon (2, 13). In the theoretical model recently proposed by Woods (24), such a mechanism has been identified with TGF and is consistent with most (although not all; see Ref. 4) of the literature data; AA-induced variations of sodium reabsorption proximally to the juxtaglomerular apparatus lead to modulation of TGF activity and GFR. This hypothesized role of TGF is supported by a number of observations: AA-induced hyperfiltration is associated with increased proximal sodium reabsorption in vitro (21) and in vivo (7, 16), and the renal response of conscious dogs to a protein meal is inhibited when the reabsorption ability of the proximal tubule is impaired [as in experimental maleic acid-induced Fanconi syndrome (22) or after the administration of diuretics such as acetazolamide that act selectively on the proximal tubule (23)]. Furthermore, it has been shown that TGF activity is modified by dietary protein (20). It has been suggested that glucagon (12), hepatic cAMP (1), and the
action of vasopressin on urea recycling (3) could modify NaCl concentration in the diluting segment and in the fluid reaching the macula densa, and thus vary TGF activity.

In the present study, we showed that the increase in GFR and RPF was associated with rises of 60% in plasma AA concentration and of 25–30% in plasma glucagon and urinary urea excretion; further increases, as observed with the higher AA dose, were not associated with any greater stimulation of renal hemodynamics but with a faster response (as early as 1 h). When hypotonic saline was present in the infusion, higher plasma AA and glucagon levels and urinary urea excretion were necessary to stimulate glomerular function. These levels in turn were ineffective when the sodium content of the solution was higher. Therefore, the acute modification of the extracellular volume and renal sodium handling induced by the hypotonic saline infusion seemed to modify the responsiveness of the intrarenal mechanism to the metabolic signal.

Other studies have demonstrated the role of extracellular volume on the mechanism leading to AA-induced hyperfiltration. Ruilope et al. (19) have shown that a volume contraction caused by sodium depletion inhibits AA-induced vasodilation, probably through an excess of vasoconstrictor factors as angiotensin II. Hadj-Aissa et al. (15) observed that protein-induced hyperfiltration is partially blunted by a high water intake, possibly by a direct or indirect effect on urine concentrating activity. Finally, Woods and Cobb (25) found that infusing isotonic saline before and during the administration of glycine prevented the GFR rise in the rat.

The reduced responsiveness of the intrarenal mechanism to the metabolic signal observed by us with the hypotonic saline infusion could be due to an increase in sodium delivery to the distal segment of the nephron secondary to the saline-induced reduction in proximal sodium reabsorption (10) and the consequent insensitivity of the TGF (17). The significant negative correlation that we found between AA-induced GFR variations and modifications of sodium excretion caused by saline infusion is in line with this hypothesis.

In conclusion, the addition of hypotonic saline to the AA infusion reduced the response of renal hemodynamics to the metabolic stimuli produced by AA infusion, possibly through an increase in urinary sodium excretion. These conclusions, derived from an experimental situation characterized by maximal diuresis (and then far from physiological levels), may not be generalized without reservation to the more normal conditions.

We thank A. M. Green for revision of the English text. This study received financial support from the Associazione per il Bambino Nefropatico.

Address for reprint requests: A. Claris-Appiani, Clinica Pediatrica De Marchi, Via Commenda 9, I-20122 Milan, Italy.

Received 31 December 1997; accepted in final form 30 September 1998.
REFERENCES


