Enhanced Glomerulopressin Production and Glomerular Filtration Rate by Amino Acid Infusion in Normal Humans (43174)

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Abstract. **Amino acid infusion induces a rise in glomerular filtration rate (GFR) in normal subjects, but the mechanism is as yet unknown. Glomerulopressin infused into the renal arteries of rats and dogs increases GFR. The aim of this study was to ascertain whether amino acid infusion raised glomerulopressin production and GFR. Accordingly, before renal arteriovenography, in 11 potential kidney donors, the caval catheter was introduced into the right hepatic vein and 60-ml blood samples were collected at the beginning and end of each experiment; six patients received amino acid infusion and five a saline infusion. Glomerulopressin in ultrafiltrates from hepatic vein plasma was measured by toad bioassay and GFR determined with diethylenetriamine pentaacetic acid-Tc^{99}. The amino acid-infused group showed significant glomerulopressin activity in ultrafiltrates, as well as a significant GFR increase, whereas in the control group no glomerulopressin activity was observed, and there was no change in GFR. These findings suggest that intravenous amino acid infusion stimulates glomerulopressin production, which may in turn induce an increase in GFR.** [P.S.E.B.M. 1991, Vol 196]

The ultrafiltrate of liver vein blood from toads (1), rabbits (2), and dogs (3) contains a hormone/factor named glomerulopressin (1) due to its enhancing effect on glomerular pressure in the toad. Ultrafiltrates containing glomerulopressin increase glomerular filtration rate (GFR) in rats (2) and dogs (4), and the infusion of purified glomerulopressin (5) also increases GFR in rats (6). Glomerulopressin is also produced by isolated perfused rat liver (7). Moreover, this hormone/factor is found in pancreatectomized dogs (3), in dogs infused intravenously with glucagon (8), in normal humans treated with glucagon, and in newly diagnosed Type I insulin-dependent diabetic patients (9).

Glomerulopressin is thermostable and has a molecular mass lower than 500 Da (1). Incubation with Pronase does not destroy its activity (1), and extractions of peptides with glacial acetic acid (10) do not decrease the activity of the ultrafiltrates on the glomerular pressure of the toad (11). Incubation of the ultrafiltrates with β-glucuronidase destroyed glomerulopressin activity. When β-glucuronidase was inhibited by boiled saccharate, a specific inhibitor of β-glucuronidase (12), the activity of glomerulopressin was not altered (3); these data suggest that glomerulopressin is not a peptide and that it may be a glucuronide. It has been obtained in a purified form by extraction with *n*-butanol and use of high-performance liquid chromatography with different elution systems (5).

As shown by several authors, intravenous amino acid infusion increases GFR in humans (13–17) and dogs (18). A protein meal ingestion also raises GFR levels in humans (19–21), dogs (22), and rats (23). So far, the mechanisms underlying amino acid-induced GFR enhancement are poorly understood. Brenner *et al.* (24) suggested that a humoral agent, possibly a hormone, may mediate the postprandial increase in renal hemodynamics after protein intake, whereas Alvestrand and Bergström (25) tentatively identified this humoral factor as glomerulopressin, a hypothesis supported by some authors (13–17, 19–21, 23, 26–28) but rejected by others (29, 30).

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The aim of this study was therefore to ascertain whether amino acid infusion not only increases GFR but also enhances hepatic glomerulopressin production in humans.

Materials and Methods

Eleven potential kidney donors, seven females and four males, with ages ranging from 22 to 42 years, were included in the study. The study protocol for kidney donors of the Sanatorio Mitre includes a renal arteriography, a venography, and the determination of functional renal reserve. Our experiments were based on the simultaneous realization of both studies.

Informed verbal consent was secured for the experiment, whose protocol was approved by the Research and Training Department at the Aeronautical Hospital Center. After physical examination and laboratory evaluation, all of the patients were found to be in good health.

Seven days before the study, volunteers were put on a normocaloric diet that included 50 g of protein/day. They were admitted to the hospital on the evening before the experiment, and received their last meal at 8:00 PM. Two hours before catheterization, they drank 10 ml of water/kg body wt; 1 hr before, 5 ml/kg; and thereafter, 180 ml every 30 min was ingested until the end of the experiment.

Before renal arteriovenographic recording, the caval catheter was introduced into the upper right hepatic vein for blood sample collection, with placement of catheters controlled by radioscopy alone. Accurate urine collection was ensured by means of an indwelling bladder catheter.

After placement of catheters, subjects rested for 1 hr. Experiments were conducted from 10:00 AM to 3:00 PM, a period displaying the smallest circadian variations in GFR (31).

GFR was measured with diethylenetriamine pentaacetic acid-Tc^{99} (National Atomic Energy Commission, Argentina). A 39.6-μCi/kg bolus was injected as a priming dose, followed at once by a 20 μCi/1.73 m^2/min maintenance infusion. After 60 min for equilibration, the bladder was emptied and 30-min urine samples were collected. Aortic arterial blood samples were drawn at the midpoint of each urine collection. Five volunteers infused with saline made up the control group. After a control period, the saline infusion in the other six volunteers was replaced by an amino acid solution (amino acids Abbott 10%, see Table I) supplemented with 20 ml of NaCl, 20 g/dl, so that the NaCl concentration was similar to that of the saline infusion. Both infusions were delivered at the rate of 3 ml/min/1.73 m^2.

During the following days, subjects were monitored by means of urine and blood cultures, which proved negative throughout. During the first and sixth periods, 60 ml of blood were drawn from the hepatic vein, immediately centrifuged at 4°C, and plasma ultrafiltered through Diaflo YC-05 membranes under N_2 pressure. The samples were kept at −20°C until glomerulopressin activity could be determined. YC-05 Diaflo membranes allow the passage of substances having a molecular mass lower than 500 Da, retaining those substances like glucagon, growth hormones, and kinins, among others, with a higher molecular mass. Amino acid concentrations of plasma ultrafiltrates from the first and sixth periods were determined according to the method of Rosen (32).

Table I. Amino Acid Content (mg/100 ml) of "Amino Acid Abbott 10%" Solution

Essential amino acids	
L-Isoleucine	720
L-Leucine	940
L-Lysine (as salt acetate)	720
L-Methionine	400
L-Phenylalanine	440
L-Threonine	520
L-Tryptophan	160
L-Valine	800
Nonessential amino acids	
L-Alanine	1280
L-Arginine	980
L-Histidine	300
L-Proline	860
L-Serine	420
L-Tyrosine	44
Glycine (amino acetic acid, USP)	1280

Toad Bioassay. Pressure of the occluded ureter in the toad equals the pressure in Bowman's capsule and responds rapidly and accurately to its variations (33). Therefore, it was regarded as an index of glomerular pressure.

Mildly dehydrated male *Bufo arenarum* Hensel weighing 140–160 g were used. After anesthesia with 20% urethane, 1% of their body weight was injected into a lymphatic sac. For infusion, a PE 10 cannula was placed above the renal arteries in the aorta, then the latter ligated caudally to the former. A PE 50 cannula was then placed in one ureter and connected to a Gould P 23 ID transducer to record pressure in a 7 D Grass polygraph. Ultrafiltrates were infused at a rate of 0.013 ml/min with a Harvard pump.

Incubation with β-Glucuronidase. For this purpose, 0.104 mg of β-glucuronidase was added to 3.0 ml of ultrafiltrate and incubated for 1 hr at 38°C, (pH 4.5–5.0), then ultrafiltrated through Diaflo YC-05 membranes to separate the enzyme.

Statistical Analysis. Data are expressed as means ± SE. Statistical significance was evaluated by means of analysis of variance and Dunnett's test for multiple comparisions.

Results

GFR showed no significant change in the saline-infused group, but in the amino acid-infused group, mean GFR values increased during the first 30 min after starting infusion and were significantly elevated after a 120-min infusion (Fig. 1).

In the control group, toad bioassay of hepatic vein ultrafiltrates after a 150-min saline infusion failed to show glomerulopressin activity. Ultrafiltrates obtained during the control period from amino acid-infused subjects likewise proved inactive, but ultrafiltrates obtained after a 150-min amino acid infusion induced a significant response on the toad bioassay. Incubation with β-glucuronidase entirely inhibited activity in ultrafiltrates obtained after 150 min of amino acid infusion (Table II).

Total amino acid concentration in ultrafiltrates from the amino acid-infused group increased from 1.80 ± 0.35 to 3.60 ± 0.37 μmol/ml ($P < 0.025$) after 150-min infusion.

Discussion

In toads, the main tubular reabsorption functions are carried out by distal tubules, which are irrigated by the peritubular capillary network following the efferent arteriole. They lack a Henle loop, presenting in its place a very short intermediate segment with minimal resistance to intratubular flow (34). Their fractional tubular functions are low and they cannot concentrate urine above the tonicity of their corporal fluids (35).

Glomeruli may be directly visualized in toads and are readily accesible to micropuncture. Uranga (33) carried out micropuncture in Bowman's capsule and recorded its pressure simultaneously with that in the occluded ureter. In 153 toads, the pressure in Bowman's capsule and in the occluded ureter were similar; the difference between both pressures was 0.00 ± 0.26 mm Hg. Variations in intracapsular pressure were at once followed by similar variations in ureter pressure. On stabilizing the closed system, such pressures remained constant. In these stop-flow conditions, there is neither glomerular filtration nor tubular reabsorption, so that the pressure recorded by the system is equivalent to glomerular capillary pressure minus plasmatic oncotic pressure. As the arterial infusion of 0.013 ml/min of ultrafiltrate does not significantly alter plasma oncotic pressure, the glomerular pressure increase observed in the occluded ureter (ΔGPI) represents an increase in glomerular capillary pressure.

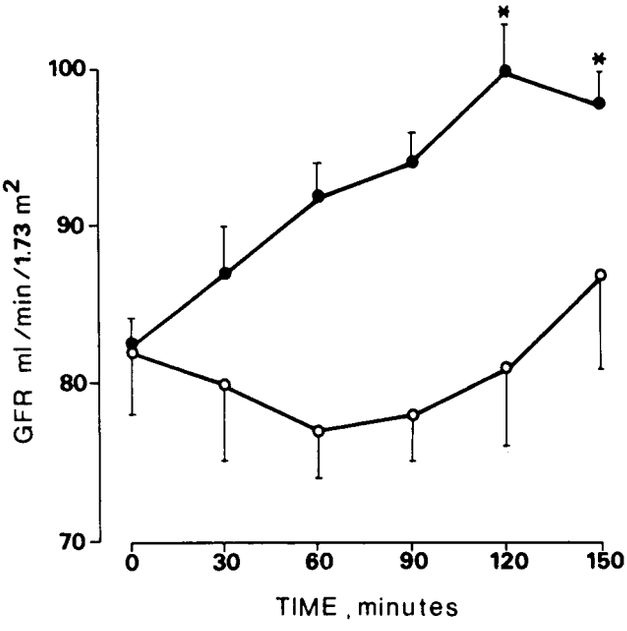

Figure 1. Effect of amino acid infusion (closed circles) or saline infusion (open circles) on GFR; *$P < 0.05$.

Table II. Changes in Glomerular Pressure (ΔGPI) of Toads Produced by the Different Ultrafiltrates

Ultrafiltrate	n[a]	Glomerular pressure (mm Hg)	P
Control group after 150 min of saline infusion	18	1 ± 0.50[b]	NS
Control period of amino acid-infused group	18	0 ± 0.23	NS
After 150 min of amino acid infusion	18	5 ± 0.78	0.001
After 150 min of amino acid infusion, incubated with β-glucuronidase	10	0 ± 0	NS

[a] n, Number of toads in each group.
[b] Data are mean values ± SE.

By means of microphotographic studies, individual variations in afferent arteriole diameters were demonstrated, which allowed the vasodilator effect of glomerulopressin to be observed (36).

The study described in this article confirms the observation of several authors that amino acid infusion increases GFR in humans. Some authors have attributed this effect to direct amino acid action on the kidney, but amino acid infusion into the renal artery of the dog failed to raise RBF or GFR significantly (30).

Woods *et al.* (29) found that after exclusion of the liver from the circulation by ligating all hepatic vessels and establishing a hepato-portal-femoral venous shunt, intraportal infusion of amino acids elevated renal hemodynamics by a similar amount as intravenous amino acid infusion in normal dogs. Yet, surgical stress suffered by hepatic vessel ligation resulted in a marked fluid loss, a decrease in arterial pressure, and a 35 ml/min decrease in baseline RBF. Although amino acid did elevate RBF in vessel-ligated dogs when compared with the compromised baseline blood flow, the rise in

RBF restores it to preligated values; in GFR there are no data on preligation values. There was no control group of hepatic ligated dogs infused with intraportal saline at 6–7 ml/min to verify whether this same increase may be due to restoration of extracellular volume.

It has been suggested that after ingestion of a meat meal or amino acid infusion, a factor of unknown origin is released into the general circulation that mediates RBF and GFR increase (24). Growth hormone has been postulated as this mediator (37), but the aminoacid infusion produces a strong renal vasodilation even when performed in patients with surgically induced panhypopituitarism (38).

Protein administration stimulates glucagon production by the pancreas, with a subsequent increase in GFR (39). Peripheral infusion of glucagon doses capable of inducing plasmatic concentrations similar to those obtained by amino acid infusion raise RPF and GFR levels significantly (17).

The simultaneous infusion of amino acids and somatostatin, which (among many other effects) blocks amino acid-induced glucagon release, prevents the amino acid-mediated increase in GFR and RBF (15).

Therefore, it has been suggested that glucagon may be the factor mediating the amino acid effect on glomerular hemodynamics (17). On the other hand, Bergström *et al.* (40) found that, after protein ingestion, plasma glucagon levels only increased after a significant rise in GFR. However, other authors have shown that, following amino acid stimulation of pancreatic glucagon production, glucagon concentration in the portal vein increases before that of peripheral veins and remains significantly higher (41).

Uranga *et al.* (4) and Premen (26) have found that intrarenal glucagon infusion fails to raise GFR, and that equal doses infused in the portal vein lead to a significant increase in RBF and GFR, so that the effect of glucagon on GFR is not attributable to direct action on the kidney, but is mediated by the liver.

Del Castillo *et al.* (8) demonstrated in dogs that intraportal glucagon administration enhances glomerulopressin production by the liver. Likewise, it was shown that diverse glucagon concentrations acutely increase glomerulopressin production in isolated rat liver (7). In humans, parenteral glucagon administration raises glomerulopressin activity in peripheral venous blood (9), therefore if there is a removal by the lung it is not enough to hinder a large increase of glomerulopressin activity in peripheral blood. Moreover, it has already been shown that blood ultrafiltrates from hepatic effluents of toads (1), rabbits (2), and dogs (3), which enhance glomerular pressure in toads, induce a significant increase in inulin clearance in rats (2) and dogs (4), on being infused by the intraarterial renal route. Intrarenal infusion of purified glomerulopressin (5) raises GFR levels in rats (6).

It has been demonstrated that incubation of the hepatic ultrafiltrate with β-glucuronidase inhibits glomerulopressin activity. However, when β-glucuronidase is pretreated with a specific inhibitor, glomerulopressin activity remains unaltered (3).

The ultrafiltrates from the amino acid-infused groups when incubated with β-glucuronidase, which inactivates glomerulopressin without altering amino acid concentration, were inactive on the toad bioassay, suggesting that the increase in the toad glomerular pressure is due to glomerulopressin and not to an increase in amino acid concentration.

Taken together, β-glucuronidase inhibition and the low molecular mass (below 500 Da) suggest that the effect of ultrafiltrates on toad bioassay could be attributed to glomerulopressin. It may therefore be suggested that amino acid infusion raises glomerulopressin production by the liver and that observed increases in GFR may be due to glomerulopressin.

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