Altered Renal Response Following an Amino Acid Infusion in Essential Hypertension

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To assess the clinical implications of protein load in essential hypertension, we measured renal plasma flow (RPF) and glomerular filtration rate (GFR) at 30-min intervals after initiating 10% amino acid infusion (6.7 mg/kg/min for 30 min) in 10 untreated patients with essential hypertension and 8 subjects with normotension. Amino acids provoked significant and equivalent renovasodilation without alteration of systemic hemodynamics in both hypertensive and normotensive groups (% change of RPF induced by amino acids: $+12.8 \pm 3.0$ % in hypertensive vs. $+15.3 \pm 4.4$ % in normotensive group). GFR also significantly increased in both groups, but the magnitude of this rise was lower in the hypertensive than in the normotensive group ($+8.7 \pm 3.2$ % vs. $+34.1 \pm 4.0$ %, $p < 0.01$). Consequently, filtration fraction did not change following the amino acid infusion in hypertensive patients (0.28 ± 0.01 to 0.27 ± 0.02 , ns), whereas it significantly increased in normotensive subjects (0.23 ± 0.02 to 0.27 ± 0.02 , $p < 0.01$). Amino acids also increased plasma glucagon to a comparable level (120.6 ± 14.5 pg/ml in hypertensive vs. 101.1 ± 14.8 pg/ml in normotensive group) in both groups, and this level was correlated with the degree of hyperfiltration induced by amino acids in the normotensive group ($r = 0.79$, $p < 0.05$). In conclusion, renal functional reserve estimated by the increase in GFR following the amino acid infusion was lower in the hypertensive than in the normotensive group. This abnormality found in essential hypertension might be related to enhanced responsiveness of efferent arterioles to the vasodilatory stimuli induced by amino acids. (*Hypertens Res* 1992; 15: 77-84)

Key Words: amino acid infusion, renal functional reserve, essential hypertension, glomerular hypertension

Recent experimental studies have shown that the transmission of systemic pressure to glomeruli (glomerular hypertension), rather than glomerular ischemia, could initiate and accelerate hypertensive renal damage in systemic hypertension (1). In spontaneously hypertensive rats (SHR) and deoxycorticosterone-salt hypertensive rats, heminephrectomy results in glomerular injury concomitant with an increase in glomerular pressure (2,3). On the other hand, lowering of glomerular pressure with angiotensin converting enzyme inhibitor prevents the progression of morphological changes, renal insufficiency and proteinuria in rats with glomerular hypertension (4,5).

In contrast to experimental models, it seems unlikely that glomerular hypertension occurs in human essential hypertension, since the effective afferent arteriolar constriction inhibits the elevation of glomerular pressure (6). This intrarenal vasoconstriction may also explain the normal histology or only minor abnormalities, such as tubular atrophy or interstitial fibrosis, noted in renal biopsies of hypertensive patients (7,8). However, some renal vasodilatory stimuli may allow transmission of systemic high pressure to the glomerular capillary network and potentially induce the hemodynamically mediated glomerular

injury in essential hypertension. In a clinical setting, one of the common renovasodilatory stimuli is protein load (9,10). Hence, to assess the clinical implications of protein load in essential hypertension, we compared the renal response to an amino acid infusion in patients with essential hypertension to that in subjects with normotension. In addition, attention was paid to the renal functional reserve estimated by the increase in glomerular filtration rate (GFR) following the amino acid infusion.

Method

Subjects

Ten untreated outpatients with mild essential hypertension and 8 subjects with normotension were entered into the study. Mean age and the male/female ratio were 46 ± 4 (26 to 65) years, 3/7 in the hypertensive and 39 ± 4 (22 to 57) years, 5/3 in the normotensive group. The diagnosis of hypertension was applied when the sitting diastolic pressure measured with a standard sphygmomanometer was more than 90 mmHg on three separate occasions during 4 weeks prior to the study (11). The hypertensive patients belonged to WHO stage I and had no clinical signs suggesting secondary hyperten-

sion. All of the normotensive subjects were judged to be healthy and not to have a family history of hypertension according to their medical histories and physiological examinations. This study was approved by the institutional ethics committee. Informed consent was obtained from each subject before taking part in the study.

Study Protocol

During the week before the study, all subjects were instructed to eat a normal diet (NaCl 10 g/day), avoiding very high or low sodium intake. Dietary intakes of sodium were also checked by a dietitian.

After fasting for 10h overnight, each subject was admitted to the hospital in the morning, and timed renal clearance was estimated in the following manner. After hydration with 500 ml of water, a priming dose of sodium thiosulfate (50mg/kg) and para-aminohippurate (8mg/kg) was injected through a venous catheter inserted into an antecubital vein, and subsequently a sustaining infusion of saline containing thiosulfate and para-aminohippurate was started at a constant rate of 1 ml/min to maintain plasma thiosulfate at 20mg/dl and plasma para-aminohippurate at 2 mg/dl. Thereafter, subjects were requested to urinate at 30-min intervals until the end of the study, and blood samples were obtained at the midpoint of each clearance period through another catheter inserted into the contralateral vein for the measurement of thiosulfate, para-aminohippurate and hematocrit. At urine collection, each subject ingested 200 ml of water to ensure a urinary flow of more than 6 ml/min. After one hour for equilibration, two baseline 30-min urine collections were made, and immediately a 10% amino acid solution was added to the perfusate at the rate of 6.7 mg/kg/min over 30 min. The duration and total dose of the amino acid infusion in our study were comparable to those described by Kopple and associates (12,13). From the start of the amino acid infusion, three more 30-min urine samples were also obtained. Before and at the end of the amino acid infusion, blood samples were taken for the measurement of plasma glucagon, plasma renin activity, plasma aldosterone concentration and plasma norepinephrine. During the procedure, the subjects were asked to maintain bed rest except at the time of urination. Blood pressure and pulse rate were recorded continuously using an Arteriosound automatic recording device (BP-103N, Nippon Colin Co., Ltd., Komaki, Japan).

Measurements

Thiosulfate and para-aminohippurate in plasma and urine were determined according to the methods described by Brun (14,15). Plasma glucagon, plasma renin activity, plasma aldosterone concentration and urinary albumin were measured by radioimmunoassay technique (16-18). Plasma norepinephrine was assessed according to a modification of the method of Anton and Sayre (19). The composition of the 10% amino acid solution we used (Amiparen: Otsuka Medical Company, Osaka, Japan) is presented in Table 1.

Table 1. Composition of 10% Amino Acid Solution

Amount (mg/dl)		Amount (mg/dl)	
L- Isoleucine	800	L- Histidine	500
L- Leucine	1400	L- Alanine	800
L- Lysine	1050	L- Cysteine	100
L- Methionine	390	L- Glutamic acid	100
L- Phenylalanine	700	L- Proline	500
L- Threonine	570	L- Tyrosine	50
L- Tryptophan	200	L- Serine	300
L- Valine	800	Amino acetate	590
L- Arginine	1050	L- Asparaginic acid	100

Calculations and Data Analysis

Clearance was calculated as the urinary concentration divided by the plasma concentration, multiplied by the urine volume in milliliters per minute, and was corrected for a 1.73m² body surface area. Thiosulfate clearance and para-aminohippurate clearance were considered to represent GFR and renal plasma flow (RPF), respectively. Filtration fraction (FF) was expressed as GFR divided by RPF. Renal vascular resistance (RVR) was obtained using the formula of Gomez: (mean blood pressure - 10) × 60 × 1328/renal blood flow, where the mean blood pressure was calculated as diastolic pressure plus one-third pulse pressure and renal blood flow was calculated as the para-aminohippurate clearance multiplied by 100/(100-Hct) (6).

All values are expressed as mean ± SEM. The baseline values for renal functional parameters were taken as the average of the two values prior to the amino acid infusion, and differences between the two groups were analyzed by Wilcoxon signed-rank test. Two-way analysis of variance was used to determine the significance of changes in repeated measurements, followed by partitioning analysis (20). Intragroup and intergroup differences in clinical and humoral parameters were evaluated by Student's *t*-test or χ^2 test. Correlations were calculated by linear regression analysis. The probability factor was considered significant if $p < 0.05$.

Results

Clinical Characteristics

The hypertensive group was slightly older than the normotensive group, but the difference was not statistically significant. There were more females in the hypertensive than in the normotensive group, but the sex distribution did not differ significantly between the two groups. Sitting blood pressure was significantly higher in the hypertensive ($157 \pm 8/96 \pm 3$ mmHg) than in the normotensive group ($118 \pm 7/81 \pm 5$ mmHg) ($p < 0.01/p < 0.01$), but height and body weight did not differ between the two groups (159.1 ± 4.0 cm, 59.1 ± 3.0 kg in hypertensive vs. 168.1 ± 2.7 cm, 66.0 ± 3.9 kg in normotensive group). Urinary albumin was less than 1.0 μ g/ml in the baseline urine collections in both groups. There were no differences in baseline blood urea nitrogen and urinary urea nitrogen between the hypertensive

(13.7 ± 1.3 mg/dl/ 0.54 ± 0.06 g/h) and normotensive group (14.0 ± 1.7 mg/dl/ 0.48 ± 0.06 g/h), implying that dietary intakes of protein were similar in the two groups.

Effects of Amino Acids on Systemic and Renal Hemodynamics

Figure 1 shows the serial changes in systemic and renal hemodynamics in each group. At baseline, RPF did not differ between the two groups, although mean blood pressure was significantly higher in the hypertensive than in the normotensive group ($p < 0.05$). The baseline GFR was slightly

higher in the hypertensive group (119 ± 4 ml/min/1.73 sqm) than in the normotensive group (109 ± 7 ml/min/1.73 sqm). However, the difference was not statistically significant. Increased GFR in hypertension has also been reported by several other authors (21, 22). After starting the amino acid infusion, mean blood pressure and pulse rate did not change in either group. RPF and GFR significantly increased (RPF: 471 ± 20 ml/min/1.73 sqm in hypertensives vs. 521 ± 36 ml/min/1.73 sqm in normotensives, ns; GFR: 129 ± 4 ml/min/1.73 sqm in hypertensives vs. 138 ± 14 ml/min/1.73 sqm in normotensives, ns) during the infusion period

Fig. 1. The effects of amino acids on systemic and renal hemodynamics in subjects with essential hypertension and normotension. ●, essential hypertensives; ○, normotensives; Baseline, clearance period at the baseline period; AA, clearance period during the amino acid infusion; Post I, post II, the first and second clearance period after the end of the amino acid infusion. * $p < 0.05$, when compared to the baseline value. + $p < 0.05$, when compared to normotensives.

and thereafter returned to the basal value in the two groups. In some instances (3 patients with essential hypertension and 3 subjects with normotension), however, the maximal increases of RPF occurred during the first or second clearance period

after the end of the amino acid infusion. Thus, there were considerable variations in the time course of renal hemodynamic responses to amino acids in the two groups, as found also by Slomowitz (12). Because the renal effect of amino acids is

Fig. 2. Renal hemodynamics at the period of maximal response in renal plasma flow to amino acids in subjects with essential hypertension and normotension. ▨, essential hypertensives; □, normotensives; Baseline, clearance period at the baseline period; Max, clearance period at the maximal response in renal plasma flow. * $p < 0.05$, when compared to normotensives.

mainly vasodilation (9,10), the maximal response to amino acids is considered to occur at the time of maximal rise of RPF following the amino acid infusion. Therefore, statistical comparisons using Student's *t* test were carried out between the baseline levels and the maximal values obtained after starting the amino acid infusion (Fig. 2).

The magnitude of maximal response in RPF to amino acids was equivalent in the two groups ($+12.8 \pm 3.0\%$ in hypertensive vs. $+15.3 \pm 4.4\%$ in normotensive group). On the other hand, both the maximal GFR and % increase of GFR from baseline were significantly lower in the hypertensive (129 ± 4 ml/min/1.73 sqm, $+8.7 \pm 3.2\%$) than in the normotensive group (146 ± 11 ml/min/1.73 sqm, $+34.1 \pm 4.0\%$) ($p < 0.05$, $p < 0.01$). Consequently, FF remained unchanged following the amino acid infusion in hypertensive patients (0.28 ± 0.01 to 0.27 ± 0.02 , ns), whereas it significantly increased in normotensive subjects (0.23 ± 0.02 to 0.27 ± 0.02 , $p < 0.05$). Significant and equivalent decreases of RVR were also observed after the amino acid infusion in the two groups (Fig. 2). In addition, the % increase of GFR induced by amino acids did not differ between the three male and the seven female subjects ($9.4 \pm 2.9\%$ vs. $8.4 \pm 4.5\%$ respectively) with essential hypertension. Thus, it is unlikely that the observed abnormality in essential hypertension can be explained on the basis of sex-related differences between essential hypertensives and normotensives.

Effects of Amino Acids on Neurohumoral Parameters

At baseline, there were no differences in neurohum-

oral parameters between the two groups (Table 2). After the amino acid infusion, plasma glucagon significantly increased to a similar level in both groups. On the other hand, amino acids did not alter plasma renin activity, plasma aldosterone concentration and plasma norepinephrine in either group. Figure 3 shows the relationship between the plasma level of glucagon after the amino acid infusion and the % increase of GFR induced by amino acids in the two groups. There was a significant relationship between these two factors in the normotensive group ($r = 0.79$, $p < 0.05$), but not in the hypertensive group ($r = -0.02$, ns).

Discussion

In healthy human subjects, oral protein intake or the infusion of amino acids produce significant elevations of RPF and GFR mainly through vasodilation of afferent arterioles (9,10). Recently, such glomerular hyperperfusion and hypertension have been highlighted as risk factors responsible for the progressive deterioration of renal function in chronic renal disease (23). However, the clinical implications of protein load remain to be elucidated in essential hypertension.

In the present study, amino acids provoked significant and equivalent renovasodilation without altering systemic hemodynamics in both the hypertensive and normotensive group. On the other hand, GFR significantly increased after the amino acid infusion in both groups, but the maximal increment of GFR was lower in the hypertensive than in the normotensive group. Consequently, FF did not change in hypertensive patients following the amino acid infu-

Table 2. The Effects of Amino Acids on Plasma Glucagon, Plasma Renin Activity, Plasma Aldosterone, and Plasma Norepinephrine in Subjects with Essential Hypertension and Normotension

	Baseline	30 min after the initiation of amino acid infusion	Change
plasma glucagon (pg/ml)			
essential hypertensives	76.5 ± 13.4	$120.6 \pm 14.5^*$	44.1 ± 6.6
normotensives	70.7 ± 12.1	$101.1 \pm 14.8^*$	30.4 ± 5.5
plasma renin activity (ng/ml/hr)			
essential hypertensives	1.23 ± 0.59	0.83 ± 0.38	-0.40 ± 0.22
normotensives	0.59 ± 0.18	0.43 ± 0.09	-0.17 ± 0.10
plasma aldosterone (pg/ml)			
essential hypertensives	47.4 ± 4.3	40.8 ± 4.0	-6.6 ± 2.8
normotensives	57.1 ± 15.4	44.8 ± 5.9	-12.3 ± 11.0
plasma norepinephrine (pg/ml)			
essential hypertensives	201.5 ± 24.4	171.0 ± 12.2	-30.5 ± 21.6
normotensives	205.0 ± 57.0	142.3 ± 24.0	-62.7 ± 65.2

Statistical comparison between baseline value and the value after amino acids was performed using paired *t* test, and comparison between hypertensives and normotensives was made using unpaired *t* test. Values are mean $\pm$ SEM. * $p < 0.01$, when compared to baseline value using paired *t* test.

Fig. 3. Relationship between plasma level of glucagon after the infusion of amino acids and % change of glomerular filtration rate induced by amino acids in subjects with essential hypertension and normotension. ●, essential hypertensives; ○, normotensives.

sion, whereas it significantly increased in normotensive subjects. The mechanism underlying these renal hemodynamic effects was not fully elucidated by the present study. However, it is interesting to speculate on possible events affecting intrarenal hemodynamics. Assuming that glomerular permeability is constant during and after the amino acid infusion, GFR and FF are hemodynamically determined by intraglomerular pressure, which in turn is dependent upon the balance between constriction of the afferent and efferent arterioles (6). Because total RVR, which represents the summation of afferent and efferent arteriolar resistance, decreased by a similar degree in both groups, the altered glomerular response to amino acids found in the hypertensive group may be related to enhanced responsiveness of efferent arterioles to the vasodilatory stimuli induced by amino acids. This vasodilatory response of efferent arterioles to amino acids is similar to that to calcium antagonists. Frohlich and associates reported that the efferent arteriole is more sensitive than the afferent arteriole to the vasodilatory effect of diltiazem in essential hypertension (24). Furthermore, this property of the efferent arteriole assumed in essential hypertension has considerable clinical significance, because enhancement of efferent arteriolar dilatation might block the excessive increase in glomerular pressure induced by amino acids (25). In uncomplicated patients with essential hypertension, therefore, it seems unlikely that protein load alone can produce hemodynamically mediated glomerular injury. This speculation is supported by experimental evidence that the mobilization of dietary protein does not influence the course

of renal injury in SHR (26). However, attention must be paid to the cited mechanism concerning intrarenal hemodynamics, because GFR is regulated by glomerular permeability, mesangial cell contraction and tubuloglomerular feedback, independent of glomerular pressure. Further investigations using micropuncture or clearance data of macromolecules are needed to clarify the intrarenal mechanisms leading to the observed changes in GFR and RPF.

The rise of GFR after acute protein load is considered to represent the renal functional reserve, assuming that the pre-existing elevation of glomerular pressure and flow will prevent any further rise of GFR (27). Actually, a decrease in this reserve capacity has been reported in patients with single kidney or glomerular disease (28, 29). In the present study, the renal functional reserve estimated by the increase in GFR following amino acid infusion, was lower in the hypertensive than in the normotensive group. This observation is consistent with the result obtained by Lostio (30). Because the hypertensive patients in this study had normal renal function with no albuminuria, the decrease in renal functional reserve might be due to an early (subclinical) renal abnormality in essential hypertension, as assessed by Grunfeld (31). In addition, our study confirmed that this renal abnormality found in hypertensive patients might be associated with the altered response of the renal microcirculation to amino acids.

Because amino acids do not increase GFR in isolated perfused kidney, several circulating mediators such as glucagon, growth hormone and atrial natriuretic factor are postulated to be candidates re-

sponsible for the renal response following amino acid infusion (9, 10). Our data showed that amino acids produced significant and similar increases in plasma glucagon without any significant changes in the renin angiotensin aldosterone system and sympathetic activity in both the hypertensive and normotensive group. In addition, the plasma level of glucagon after the infusion was correlated with the degree of hyperfiltration induced by amino acids in the normotensive subjects. It has been reported that amino acids stimulate the endogenous secretion of glucagon, that the infusion of glucagon increases RPF and GFR, and that prostaglandins are necessary for these effects in man (13,32). Moreover, somatostatin, a widely known inhibitor of glucagon secretion, inhibits both renal hyperfiltration and elevation of glucagon after amino acid infusion (33). However, somatostatin inhibits the secretion of other putative mediators such as growth hormone as well as other hormones including insulin, so that the specificity of glucagon blockade in preventing hyperfiltration is uncertain (34). Changes in the intrarenal renin angiotensin system could also contribute to the observed responses, although amino acids did not change plasma renin activity and plasma aldosterone concentration. Nevertheless, it seems reasonable to conclude that the changes in renal hemodynamics following the amino acid infusion are mediated, at least in part, by the release of glucagon from the pancreas.

In summary, renal functional reserve estimated by the increase in GFR following the amino acid infusion, was lower in the hypertensive than in normotensive group. This abnormality found in essential hypertension might be related to enhanced responsiveness of efferent arterioles to the vasodilatory stimuli induced by amino acids.

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