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Role of the renin-angiotensin system on the renal functional reserve in renal transplant recipients

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Role of the renin-angiotensin system on the renal functional reserve in renal transplant recipients. To determine the renal functional reserve in renal transplant recipients, we measured the glomerular filtration rate by inulin clearance and the renal plasma flow by PAH clearance before and during an amino acid infusion (Totamine, 6 to 8 mg/kg/min for 90 to 120 min) in 18 transplanted patients with stable renal function. To test the role of the renin-angiotensin system on the renal functional reserve, we performed a crossover placebo-controlled randomized trial of acute blockade of the renin-angiotensin system by injection of perindoprilat (2 mg i.v.), an inhibitor of angiotensin converting enzyme before amino acid infusion, each patient being studied twice at seven day intervals. Amino acid infusion induced a time-dependent increase in the glomerular filtration rate ($P = 0.04$), whether or not the renin-angiotensin system was blocked. Maximal increases were from 49.1 ± 4.1 to 58.9 ± 5.4, mean ± SE (18.5%), in control conditions and from 52.4 ± 5.6 to 62.1 ± 5.5 ml/min/1.73 m^2 (19.7%) after perindoprilat. The increase in glomerular filtration rate was less pronounced in patients taking cyclosporin A than in patients treated with steroid and azathioprine. Amino acid infusion also induced a significant and time-dependent increase (15.2 to 20.2%) in the renal plasma flow ($P < 0.01$) whether or not perindoprilat had been given. Furthermore, perindoprilat alone increased renal plasma flow by 13.6%, and this effect seemed additive with that of amino acids. Perindoprilat injection decreased filtration fraction (from 0.20 ± 0.01 to 0.19 ± 0.01). This parameter returned to basal values after amino acid infusion (0.20 ± 0.01). Amino acid infusion decreased renal vascular resistances as did perindoprilat alone. After perindoprilat administration, renal vascular resistances further decreased during amino acid infusion ($P = 0.03$). These results demonstrate that renal transplant recipients possess a renal functional reserve of which the mobilization is not inhibited in acute experiments by blockade of the renin-angiotensin system.

A long-term protein-rich diet has been shown to increase glomerular blood flow and mean glomerular capillary pressure, and to accelerate the appearance of glomerulosclerosis in various models of reduced renal mass [1]. In humans, short-term experiments demonstrated that an oral protein meal [2–4] or an amino acid (AA) infusion [5] increased renal blood flow (RBF) and glomerular filtration rate (GFR) and could be used to measure the renal functional reserve (RFR) defined as the difference between the maximal and the basal GFR values.

Renal transplantation in humans represents a typical model of prolonged reduction of renal mass. Although renal graft survival has been improved with the use of new immunosuppressive agents, some patients experience a progressive loss of renal function due to rejection, hypertension and/or progressive glomerular sclerosis [6]. The latter may be secundary to glomerular hypertension and hyperfiltration that accompany important reduction in renal mass [7]. In renal transplanted patients some studies have shown that the renal functional reserve can be recruited by protein loading [8–11]. However, despite normal or subnormal creatinine levels, some of these patients with systemic hypertension [11] or taking cyclosporin A [9] have been shown to have low or no renal functional reserve, suggesting that they had permanent hyperfiltration or renal vasoconstriction. During hyperfiltration induced by protein loading, there is an increase in afferent glomerular blood flow and in glomerular capillary pressure [12]. The exact mechanism of these changes remains unclear and could involve an alteration of the tubuloglomerular feedback [13] and/or changes in the secretion of prostaglandins [14], glucagon [14–17], growth hormone [18], or endothelial-derived relaxing factor [19]. Controversial results were reported concerning the role of the renin-angiotensin system. In the rat, a protein-rich diet increases plasma renin activity [20, 21] but decreases the vasopressive response to exogenous angiotensin II, probably through an increase in prostaglandin synthesis. In humans, activation of the renin-angiotensin axis by salt depletion prevents the mobilization of the renal functional reserve during AA infusion, and this inhibition is reversed by angiotensin converting enzyme inhibition [22]. These results suggest that angiotensin II inhibits the mobilization of the renal functional reserve. On the other hand, it has been shown in the rat that chronic administration of an angiotensin converting enzyme inhibitor inhibits the mobilization of the renal functional reserve [23] and prevents glomerular sclerosis in experimental models of renal mass reduction [7]. These results would rather suggest that angiotensin II plays a role in hyperfiltration and associated glomerular lesions.

In order to investigate the role of the renin-angiotensin axis in hyperfiltration induced by protein loading in renal transplant

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Table 1. Patient characteristics

Patient no.	Age *years*	Time since transplantation *months*	Antihypertensive treatment	Serum creatinine *μmol/liter*	Proteinuria *g/24 hr*	Treatment	Cyclosporin A
1	30	25	b,a,h	140	0.44	triple	yes
2	40	14	none	68	0	triple	yes
3	34	29	b	200	0.58	triple	yes
4	39	62	b	130	0	double	no
5	28	31	b	210	0	triple	yes
6	45	56	t,b	130	0.28	triple	yes
7	45	34	b,h	70	2.46	triple	yes
8	46	80	none	100	0.24	double	no
9	46	73	none	85	0	double	no
10	37	48	none	110	0	triple	yes
11	33	9	b	90	0.26	triple	yes
12	58	18	c	140	0.34	triple	yes
13	40	35	none	125	0.32	triple	yes
14	44	240	none	66	0.22	double	no
15	58	85	none	84	0.32	triple	yes
16	34	71	none	90	0	double	no
17	44	71	none	120	0.24	triple	yes
18	24	11	none	129	0	double	yes

Abbreviations are: a, diuretic; b, beta blocking agent; c, calcium channel inhibitor; h, hydralazine; t, thiazidic.

recipients, we performed a randomized crossover placebo-controlled trial of acute blockade of angiotensin converting enzyme during AA infusion. In these patients, the results show that ACE inhibition does not prevent, but even potentiates the increase in GFR induced by AA infusion.

Methods

Patients

Between July 1989 and June 1990, eighteen patients who had received a cadaveric renal transplant were included in the study, after they had signed an informed consent. The protocol was approved by the Ethical Committee of the Faculté de Médecine Saint Antoine (Paris, France). The main data concerning the patients are given in Table 1. Criteria for patients included a stable renal function for at least two months before entry with a plasma creatinine level lower than or equal to 210 μmol/liter and who were not treated by an ACE inhibitor. Renal arterial stenosis was eliminated on clinical and echographic-Doppler examinations. Immunosuppressive therapy (usually triple therapy) was unchanged during the entire study, and no patient received nonsteroidal anti-inflammatory agents. All received 100 mg/day aspirin as a routinely administered anti-platelet agent. Patients were asked not to modify their salt and protein diet during the study to reduce individual variations. This was controlled by measurements of sodium and urea urinary excretion. Urinary excretions of sodium were not different between the first and second periods (17.9 ± 5.8 vs. 23.8 ± 7.1 mmol/mmol creatinine, respectively, $P > 0.05$). Similarly, there was no significant difference in urinary excretion of urea between the first and second period (46.7 ± 5.8 vs. 59.5 ± 6.7 mmol/mmol creatinine respectively, $P > 0.05$).

Study design

This was a crossover double-blind study. Each patient was studied twice (2 periods, P1 and P2), at one week intervals and each acted as his own control. Patients received an AA infusion with or without previous acute blockade of the renin-angiotensin system by intravenous injection of sodium perindoprilat, an angiotensin converting enzyme inhibitor [24]. Patients were randomized to receive, in a double-blind trial, either placebo for the first period and perindoprilat for the second period or vice-versa. Each period consisted of three successive phases (control, placebo or perindoprilat, AA infusion) and each phase was divided into three time intervals of 30 minutes.

Before starting the first phase of each period, patients had been fasting for at least 12 hours except that they took their immunosuppressive treatment including cyclosporin A, and an hydric charge of 200 ml distilled water at 8 a.m. Antihypertensive treatment was stopped the day before the study. Patients remained supine except to void. At 9 a.m. an intravenous catheter was inserted in each arm for perfusion on one side and blood sampling on the other. After basal urine and blood samples were taken, inulin (150 mg/liter of extracellular water estimated to 20% of body wt) and paraaminohippuric acid (15 mg/liter of diffusion volume of PAH estimated to 40% of body wt) were given as loading doses followed by a continuous infusion calculated to obtain constant plasma levels of inulin and PAH. After the first phase of 3 × 30 minutes, used to measure basal values of glomerular filtration rate (GFR) and renal plasma flow (RPF), placebo or perindoprilat (2 mg) were injected in a two-minute i.v. infusion, and GFR and RPF were further measured during a second phase of 3 × 30 minutes. Then, the third phase was started consisting of an AA infusion (Totamine, 6 to 8 mg/kg body wt/min) maintained for 90 to 120 minutes. The first five patients included in the protocol received Totamine at a rate of 8 mg/kg body wt/min for 90 minutes. Since two of them experienced nausea and vomiting in relation with the AA infusion, the rate of infusion was decreased to 6 mg/kg/min for 120 minutes in the 13 remaining patients. Totamine composition is given in Table 2.

A week later, the second study was performed (period 2) in the same patient. The same protocol was used except for

Table 2. Amino acid composition of totamine

Amino acid	mg/100 ml
L-Isoleucine	556.6
L-Leucine	800
L-Lysine	480
L-Methionine	300
L-Phenylalanine	666.6
L-Threonine	293
L-Tryptophane	146.6
L-Valine	605
L-Arginine	800
L-Histidine	250
L-Alanine	533
L-Aspartate	266.6
L-Cysteine	133.2
L-Glutamate	267
L-Glycine	933.2
L-Ornithine	238
L-Proline	533.2
L-Serine	266.6
L-Tyrosine	40

placebo or perindoprilat injection depending on the randomization.

Measurements

Sodium and potassium concentrations were measured by flame photometry (Il Meter 243), protein concentration by colorimetry (Technicon RAXT), osmolality by freezing point (Fiske Osmometer) and inulin and PAH concentrations by colorimetry (Technicon AA II). Plasma renin activity was determined by RIA (RIANEN 022); aldosterone and ANF were measured according to Pham Huu Trung et al [25] and Pruzczynski et al, respectively [26]. cGMP and glucagon were measured by RIA using commercial kits from Amersham and Pharmacia, respectively. ACE activity was measured by colorimetry [27]. Normal values in our laboratory are 18.4 ± 1.1 nmol/min/ml (mean ± SE).

Inulin clearance and PAH clearance were calculated according to the UV/P formula. Creatinine plasma levels were determined at the beginning and at 30 minute intervals during each phase, as were serum and urine electrolytes and glycemia. Plasma renin activity, aldosterone, ANF, and glucagon were measured at the end of each phase of each period. Mean arterial pressure (MAP) was calculated by the formula: diastolic pressure + 1/3 differential pressure. Systolic and diastolic pressures were measured with a sphygmomanometer each 30 minutes during each phase.

Calculations

GFR (inulin clearance) and RPF (PAH clearance) were normalized to 1.73 m^2 of body surface area. Filtration fraction (FF) was calculated by the ratio GFR/RPF. Renal vascular resistances (RVR) were estimated by the ratio MAP/RBF where MAP is the mean arterial pressure and RBF is the renal blood flow, calculated as RPF/(1 − Hematocrit).

Statistical analysis

Results are presented as mean ± SEM. Mean plasma and urine concentrations of electrolytes and hormones of the different phases were compared by two way analysis of variance or by paired *t*-test. Linear regression analysis was used to study the correlation between two quantitative values. Crossover three-way analysis of variance was used to test the effect of sequence (A factor representing the order of treatment administration for the first and second period: placebo then perindoprilat or the opposite), treatment (B factor for placebo or perindoprilat) and time during each phase of each period (C factor) for the renal functional parameters [28]. If there was no statistically significant interaction for A × B × C and A × C as well as no significant effect of A, B × C interaction was considered. If B × C interaction was significant, indicating a role of perindoprilat compared to placebo on the time-course of the parameter studied, complementary analyses were performed for each treatment and each time. If the treatment effect was significant, then the differences between values at different times for each treatment were evaluated by Neuman Keuls test. If B × C interaction was not significant, the treatment effect and the time effect were described and only the evolution of the parameter under placebo conditions from both periods was considered to evaluate the effect of time. If time effect was significant, comparison between values were performed by the Neuman Keuls method.

On the other hand, if A × B × C or A × C or A effect were statistically significant, then successive analyses of variance were performed to compare the different phases. If an interaction was still found, then the first period only was used for a two way analysis of variance followed, in case of significant time effect, by the Neuman Keuls test to compare values at different times.

Results

Hemodynamic parameters

As shown in Figure 1, AA infusion induced a 19.7% increase in GFR in control conditions and a 18.5% increase in GFR in perindoprilat conditions. This effect was time-dependent ($P = 0.04$). Mean GFR rose from 49.1 ± 4.11 to the maximal value of 58.8 ± 5.44 ml/min/1.73 m^2 ($P \leq 0.05$) which was observed after 60 to 90 minutes of AA infusion under placebo conditions ($P = 0.006$). GFR rose from 52.4 ± 5.6 to 62.1 ± 5.5 ml/min/1.73 m^2 in perindoprilat-pretreated patients, the maximal value being observed after 60 minutes of AA infusion. There was a significant linear correlation between absolute increment of GFR and the baseline GFR ($y = -23.1 + 0.651\ x$, $r = 0.547$, $P < 0.025$, where y is increment of GFR and x baseline GFR), indicating that the lower was baseline GFR, the lower was the AA induced increase in GFR. When expressed as a percentage of baseline GFR, the increment in GFR was not correlated with GFR ($r = 0.19$, $P > 0.25$). Perindoprilat *per se* did not significantly change GFR in these patients. AA infusion induced a significant increase in RPF whether or not patients had received perindoprilat (Fig. 1). Three-way analysis of variance indicated that this effect was time-dependent ($P = 0.008$) and that perindoprilat by itself also increased significantly RPF ($P = 0.004$). A 15.2% increase in RPF was observed under placebo conditions, from 244.2 ± 19.1 to 281.3 ± 29.3 ml/min/1.73 m^2 at 90 minutes of AA infusion. After perindoprilat administration, a 13.6% increase in RPF was found, from 239.1 ± 20.7 to 271.6 ± 21.8 ml/min/1.73 m^2 during the next 90 minutes, and a further increase, 20.2%

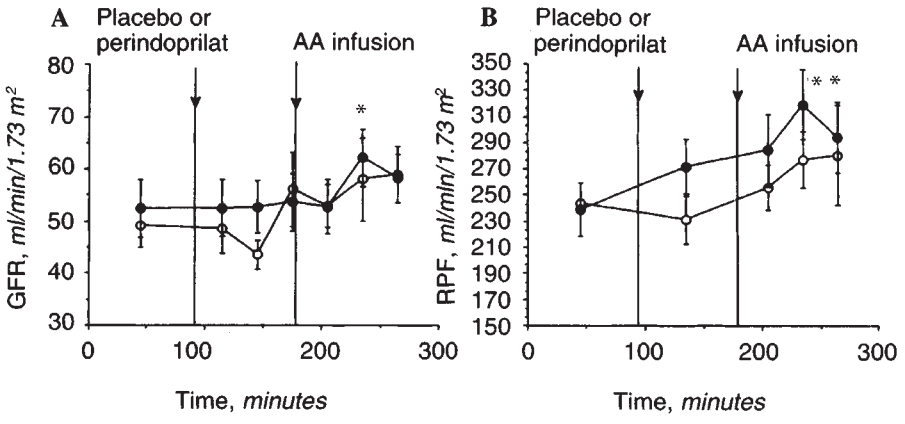

Fig. 1. *Time-course of glomerular filtration rate (A) and of renal plasma flow (B) in renal transplanted patients during an aminoacid infusion with (●) or without (○) pretreatment by an angiotensin converting enzyme inhibitor, perindoprilat.* Means ± SEM are represented. *$P < 0.05$, and **$P < 0.01$ compared to respective basal value, according to the three-way analysis of variance described in the **Methods**.

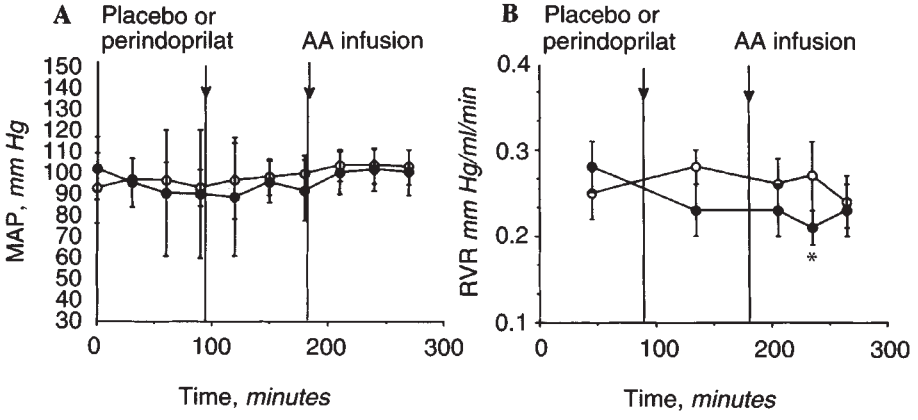

Fig. 2. *Time-course of mean arterial blood plasma pressure (A) and of renal vascular resistances (B) during an amino acid infusion in renal transplanted patients with (●) or without (○) pretreatment by an angiotensin converting enzyme inhibitor, perindoprilat.* Means ± SEM are represented. *$P < 0.05$ compared to respective basal value, according to the three-way analysis of variance described in the **Methods**.

increment after AA infusion to a maximal value of 319.9 ± 27.1 ml/min/1.73 m^2, was also found at 60 minutes of AA infusion.

Calculation of FF and two-way analysis of variance of the results indicated that there was no significant changes of FF during AA infusion whether patients had received placebo or perindoprilat. However, perindoprilat by itself induced a small decrease in FF from 0.20 ± 0.01 to 0.19 ± 0.01% which was reversed by AA infusion inducing a time-dependent significant variation of FF in this case ($P = 0.016$).

As shown in Figure 2, mean arterial blood pressure did not change significantly during the study whether or not the patients received perindoprilat. In contrast, the renal vascular resistances (RVR) were significantly modified according to treatment and time. AA infusion induced a decrease in RVR that was more pronounced when patients had received perindoprilat. Perindoprilat alone lowered RVR from 0.28 ± 0.03 to 0.23 ± 0.03 mm Hg/ml/min/1.73 m^2 ($P = 0.055$). The treatment effect was significant after 60 minutes of AA infusion ($P = 0.031$).

Hormonal and hydroelectrolytic variations

Analysis of variance indicated that there was a slight but significant decrease in protein plasma level during AA infusion whether or not patients had received perindoprilat (Table 3, $P < 0.001$). Perindoprilat alone had no effect on plasma osmolality.

Plasma renin activity was slightly but not significantly decreased by AA infusion under placebo conditions whereas perindoprilat increased PRA before AA infusion. Similarly and as expected, perindoprilat induced a rapid and profound decrease of serum ACE activity ($P < 0.001$), and AA infusion induced a small decrease of ACE activity under placebo conditions.

Aldosterone plasma levels were slightly and not significantly increased by AA infusion, but were significantly lower after perindoprilat compared to placebo conditions ($P < 0.014$).

Before AA infusion, plasma ANF levels tended to decrease with time whether or not the patients had received perindoprilat. AA infusion induced a slight but not significant increase in ANF levels under placebo conditions. A significant increase in ANF levels was observed during AA infusion when patients received perindoprilat ($P < 0.034$). No significant variation of urinary excretion of cGMP was observed. Plasma glucagon levels were significantly ($P < 0.001$) increased by AA infusion, whether or not patients had received perindoprilat.

A posteriori, we compared patients who took cyclosporin A to those who did not. As shown in Figure 3, patients who received cyclosporin A ($N = 13$) had a lower GFR than those who did not ($N = 5$). In both groups AA infusion induced a rise in GFR, although this rise was less pronounced in patients receiving cyclosporin A. We also compared hypertensive patients ($N = 8$) who were treated by antihypertensive drugs other than converting enzyme inhibitors, and normotensive patients ($N = 10$). As shown in Figure 3, AA infusion induced a rise in GFR in both groups of patients after placebo or perindoprilat injection.

Table 3. Laboratory measurements in renal transplanted patients during amino acid infusion with or without pretreatment with an angiotensin converting enzyme inhibitor, perindoprilat

	Treatment	Basal values	After treatment	After amino acid infusion
Plasma				
Sodium *mmol/liter*	Placebo	138.5 ± 1.8	138.3 ± 2	136.1 ± 1.5[c]
	Perindoprilat	139 ± 2.2	138.8 ± 2.1	136.6 ± 2[c]
Potassium *mmol/liter*	Placebo	4.2 ± 0.4	4.2 ± 0.4	4.4 ± 0.6[a]
	Perindoprilat	4.2 ± 0.4	4.2 ± 0.4	4.4 ± 0.4[a]
Proteins *g/liter*	Placebo	63.5 ± 1.4	64.2 ± 1.5	62.1 ± 1.4[b]
	Perindoprilat	63 ± 1.4	63.4 ± 1.4	61.7 ± 1.4[b]
Osmolality *mOsm/kg*	Placebo	292.1 ± 1.6	291.5 ± 1.8	296.3 ± 1.7[b]
	Perindoprilat	291.8 ± 1.3	291.7 ± 1.2	295.2 ± 1.4[b]
PRA *pg/ml/hr*	Placebo	1488 ± 1265	1426 ± 342	1224 ± 640
	Perindoprilat	1198 ± 740	2138 ± 1611	1939 ± 1424
Aldosterone *pg/ml*	Placebo	68.6 ± 17.4	108.9 ± 26.7	146.3 ± 34.6
	Perindoprilat	72.1 ± 22.7	37.8 ± 5.9	40.7 ± 7.6[d]
ANF *pg/ml*	Placebo	63.8 ± 11.1	51.1 ± 8.3	69 ± 13.2
	Perindoprilat	68 ± 10.4	48.1 ± 6.7[a]	59.8 ± 9.6
ACE *nmol/min/ml*	Placebo	15.8 ± 1.3	15.8 ± 1.2	14.4 ± 1.2
	Perindoprilat	15.8 ± 1.5	0.7 ± 0.2[b]	1.7 ± 0.2[bd]
Glucagon *μg/100 ml*	Placebo	143 ± 23	118 ± 15	346 ± 47[c]
	Perindoprilat	142 ± 26	128 ± 24	342 ± 44[c]
Urine				
cGMP *pmol/min*	Placebo	682 ± 148	504 ± 62	636 ± 76
	Perindoprilat	550 ± 67	462 ± 56	693 ± 73

Data are mean ± SEM. Mean values at each phase were compared by paired *t*-test.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ compared to respective basal values
[d] $P < 0.05$ compared to placebo

Discussion

This study demonstrates that in renal transplant recipients with normal or subnormal renal function, a renal functional reserve can be acutely mobilized by AA infusion. Acute blockade of the renin-angiotensin system by perindoprilat injection does not inhibit the effect of AA infusion and even tends to increase GFR and RPF.

Effect of AA infusion

In renal transplant recipients with subnormal plasma creatinine levels, we found that AA infusion was able to induce a 15 to 20% increase in mean GFR and mean RPF. Furthermore, the absolute increase in GFR was positively correlated with the baseline GFR, but the percentage of increase was not statistically different. Similar observations have been reported in patients with normal kidney function, glomerulonephritis, unilateral nephrectomy or large reduction in renal mass by Zuccala et al [29]. However, in contrast with these authors who reported a 40% increase in GFR, we found only a 20% increase in GFR in our patients. A low response to protein loading is usually attributed to chronic hyperfiltration and glomerular hypertension, both conditions which may lead to glomerular sclerosis [1] and which may exist in some transplanted patients. It has been shown that the response to AA may be decreased in the presence of renal parenchymal injury [30, 31], systemic hypertension [11], high protein diet [31] or cyclosporin A treatment [9], conditions that are also frequently observed in transplanted patients. Biochicchio et al found a significant but transient and blunted increase in GFR after a high protein meal in a selected group of hypertensive renal transplant recipients compared to normal subjects [11]. These authors suggested that systemic hypertension found in their patients may have induced increased glomerular pressure and hyperfiltration precluding further increase in GFR during protein load. Such an effect may also explain in part the low percentage of GFR increase after AA infusion that we observed. However, it has to be outlined that, in contrast to the study by these authors, our patients had no hypertension during the study and that this may explain why we did not find differences in AA effects between chronic normotensive and hypertensive patients.

Although we did not measure plasma AA concentration, it is likely that AA infusion induces a greater increase in AA concentration and has a more pronounced effect on GFR than oral protein load [5, 13–15]. Furthermore, our hypertensive patients were treated until the day before AA infusion and had normal blood pressure during the study period. This may explain why we did not find significant differences in the response to AA infusion between normotensive and hypertensive patients (Fig. 3). Although the magnitude of increase in GFR was lower in patients taking cyclosporin A than in those who received conventional immunosuppression, we found that AA infusion was able to increase mean GFR and RPF in both these groups of patients. This is in agreement with a previous study which showed that a high protein meal increased blood flow to transplanted human kidneys, even under cyclosporin A treatment [10]. In contrast, Nunley et al [9] failed to demonstrate an increase in GFR and in RPF in renal transplanted patients taking cyclosporin A after an oral protein load.

The reasons for these discrepancies remain unclear but may be related to the differences in plasma AA concentrations after oral protein intake and i.v. AA infusion. We found a single report of AA infusion in transplanted patients, which demonstrated a significant increase in both GFR (22%) and RPF (19%) in renal transplanted patients not taking cyclosporin A, but low

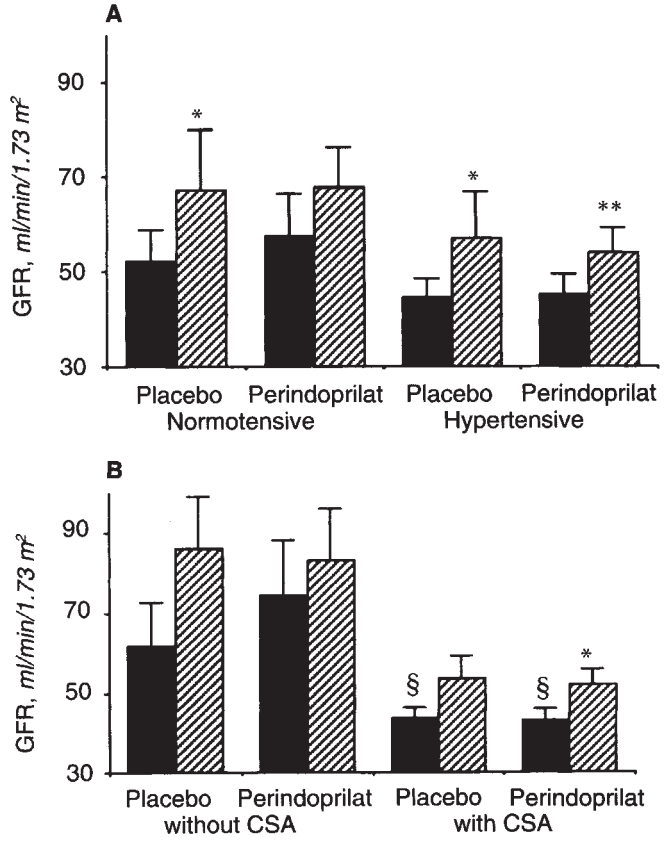

Fig. 3. *Effect of hypertension (A) and of cyclosporin A treatment (B) on the mobilization of the renal functional reserve by an amino acid infusion in renal transplanted patients pretreated or not with an angiotensin converting enzyme inhibitor, perindoprilat.* Symbols are: (■) before and (▨) after AA infusion. Means ± SEM are represented. *$P < 0.05$, **$P < 0.01$ compared to respective basal values, §$P < 0.025$ compared to values of patients without cyclosporin A, by unpaired *t*-test.

or no response in patients treated by cyclosporin A [32]. However, compared to our study protocol, these authors used a lower rate of AA infusion, lasting for two hours and in their study the increase in GFR was observed 30 to 90 minutes after the end of AA infusion.

The mechanisms of increase in GFR and RPF during oral or i.v. protein loading have been extensively studied and are related to a decrease in RVR with afferent and efferent arteriolar vasodilation. This explains the absence of significant changes in filtration fraction that we observed and that has been reported previously in nontransplanted patients [3]. Mediators of this vasodilation are still debated, and glucagon [14–17], prostaglandins [14] and more recently nitric oxide [19] have been incriminated. Conversely, activation of the renin-angiotensin axis seems to have an inhibitory effect on the mobilization of the renal functional reserve [22]. Interestingly, renal innervation is not required to observe an increase in blood flow after a high protein meal as it has been reported in patients shortly after renal transplantation [10].

As expected and previously reported in nontransplanted patients, we found that AA perfusion induced a significant increase in plasma glucagon level [14–17] and little variations in PRA, plasma ANF or urinary excretion of cGMP. In normal volunteers, meals with high or low protein content were not found to modify plasma ANF levels suggesting that this hormone does not play a major role in the protein-induced hyperfiltration [33]. In our study, plasma ANF remained unchanged during the three phases of the placebo period suggesting that AA infusion did not induce fluid overload which could have been responsible for the increase in GFR and RPF. The absence of increase in urinary cGMP excretion can also be explained by the constant ANF plasma levels. However, urinary cGMP was not well correlated with ANF variations in patients with renal failure [34]. In normal volunteers receiving AA infusion, no variation of urinary cGMP was observed [15] but these results do not exclude an increased glomerular generation of cGMP. A role for EDRF which increases guanylate cyclase activity as a potential mediator of AA-induced hyperfiltration has been suggested. Controversial results exist concerning the role of other vasodilatory mediators such as prostaglandins [14, 20, 22]. We did not measure these compounds. It remains possible that steroids may have inhibited and cyclosporin A stimulated prostaglandin synthesis in our patients as reported by others [35]. Recently, Wada, Don and Schambelan [16] did not observe an increased urinary excretion of PGE_2 and 6-keto-$PGF1\alpha$ during AA infusion in humans confirming the observations of Hirschberg et al [14]. However, the latter authors reported a significant decrease in AA-induced hyperfiltration in patients taking indomethacin or ibuprofen, suggesting that prostaglandins are indeed involved in this phenomenon. A possible explanation for this discrepancy may be that urinary excretion of prostaglandins is not well related to glomerular or vascular prostaglandin synthesis.

Role of the renin-angiotensin system

We also studied the role of the renin-angiotensin system in renal hemodynamics of renal transplanted patients. We found that acute blockade of RAS by perindoprilat injection, which was confirmed by the striking decrease in serum ACE activity and the subsequent increase in PRA, induced no variation of systemic blood pressure but a significant renal vasodilation (decreased RVR) and an increase in RPF. This suggests that there is an intrarenal basal activation of RAS in transplanted patients. Since GFR remained unchanged, the calculated FF was decreased. These hemodynamic variations were previously studied in experimental models, and it has been demonstrated that acute blockade of angiotensin II receptors induced an increase in the diameter of interlobular arteries and glomerular efferent arterioles [36]. Our results also demonstrate that AA-induced hyperfiltration is observed whether or not RAS has been blocked and that RPF which is increased by RAS blockade is further increased by AA infusion. These results suggest a permissive effect of RAS blockade on hyperfiltration induced by AA infusion. Activation of RAS by salt restriction prevents AA-induced renal hemodynamic effects in normal volunteers [22] indicating that high levels of renal angiotensin II may inhibit AA-induced vasodilation. Furthermore, when salt-depleted patients were treated with captopril, the hemodynamic effects of AA infusion were restored [22]. Dopamine combined with AA infusion has also been shown to induce an increase in GFR in salt-depleted normal volunteers indicating that RFR can

be demonstrated if appropriate antagonists of angiotensin II-induced vasoconstriction are used [37]. Concordant results were reported by Slomowitz, Hirschberg and Kopple [38], who found that in diabetic patients with normal renal function, AA-infusion induced a greater increase in GFR and RPF after pretreatment by captopril than before. These authors failed to demonstrate similar results in normal volunteers, and suggested that renal angiotensin II might be increased in diabetic patients or that captopril might have enhanced the AA-induced rise in RPF and GFR by other mechanisms, such as increasing tissue kinins, PGE_2 or other vasoregulatory compounds. We cannot exclude that these mechanisms were also involved in our transplanted patients receiving perindoprilat and AA infusion.

It has to be noted that we did not find a significant variation in PRA during AA infusion, and this is in agreement with short-term studies, using either AA infusion or protein-rich meal that have been reported [15, 16]. Conversely in long-term studies, it has been shown that a protein-rich diet may increase PRA in rats and decrease the pressor response to angiotensin II [20, 21]. This decrease has been related to secondary enhancement in prostaglandin synthesis. More recently, an increase in renin mRNA in renal cortex of rats fed a high-protein diet has been reported [39]. Long-term increase in renal angiotensin II may have deleterious effect on the permselectivity of the glomerular basement membrane and the glomerular structure.

Long-term treatment with ACE inhibitors also gave different results. In a rat experimental model, Corman et al [23] demonstrated that post-prandial increase in GFR was inhibited by chronic treatment with perindoprilat, an effect that might be related to the lower blood pressure of treated rats. On the other hand, Biochicchio et al reported that in renal transplant recipients who had hypertension, a significant but low increase in GFR could be induced by an acute protein intake [11]. After one to four months of treatment with an ACE inhibitor, fosinopril, baseline mean arterial pressure was decreased, as were GFR and proteinuria. Furthermore, an increase in GFR after protein load was observed [11]. These long-term hemodynamic effects of ACE inhibition are thus quite different from those that we and others have observed in acute experiments.

In conclusion, our study demonstrates that in kidney-transplanted patients AA infusion is able to increase GFR and RPF. Acute blockade of the renin-angiotensin system does not prevent this effect but even increases renal vasodilation and hyperfiltration induced by AA infusion. The short-term effects of AA and ACE inhibition on GFR may be quite different from that of long-term protein-rich diet and treatment with ACE inhibitors.

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