Original Article

Modulation of the Renal Response to ACE Inhibition by ACE Insertion/Deletion Polymorphism During Hyperglycemia in Normotensive, Normoalbuminuric Type 1 Diabetic Patients

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ACE inhibition protects kidney function, but ACE insertion/deletion (I/D) polymorphism affects renal prognosis in type 1 diabetic patients. ACE genotype may influence the renal benefits of ACE inhibition. We studied the impact of ACE I/D polymorphism on the renal hemodynamic changes induced by ACE inhibition in type 1 diabetes. We studied renal hemodynamics (glomerular filtration rate [GFR], effective renal plasma flow [ERPF], filtration fraction [GFR/ERPF], mean arterial pressure [MAP], and total renal resistances [MAP/ERPF]) repeatedly during normoglycemia and then hyperglycemia in 12 normotensive, normoalbuminuric type 1 diabetes and the II genotype (associated with nephroprotection) versus 22 age- and sex-matched subjects with the ACE D allele after three randomly allocated 2- to 6-week periods on placebo, 1.25 mg/day ramipril, and 5 mg/day ramipril in a double-blind, cross-over study. During normoglycemia, the hemodynamic changes induced by ramipril were similar in both genotypes. During hyperglycemia, the changes induced by ramipril were accentuated in the II genotype group and attenuated dose dependently in the D allele group (treatment-genotype interaction *P* values for ERPF, 0.018; MAP, 0.018; and total renal resistances, 0.055). These results provide a basis to different renal responses to ACE inhibition according to ACE genotype in type 1 diabetes.*Diabetes* 54:2961–2967, 2005

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ERPF, effective renal plasma flow; GFR, glomerular filtration rate; I/D, insertion/deletion; MAP, mean arterial pressure; TRR, total renal resistance; UAE, urinary albumin excretion.

The cardiovascular and vital prognoses of type 1 diabetic patients are conditioned by their renal status (1). The glomerular hypertension responsible for diabetic nephropathy results from an interaction between preglomerular capillary vasodilatation provoked by hyperglycemia and individual postglomerular resistances (2). These resistances depend on vasoconstriction due to angiotensin 2 (3). The intrarenal production of angiotensin 2 from angiotensin 1 depends on the availability of ACE. ACE activity can be modulated pharmacologically by ACE inhibitors and genetically by a 267-bp insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene (4). ACE inhibition can prevent or slow the progression of diabetic nephropathy (5–7). The ACE II genotype (the genotype associated with the lowest ACE levels) is associated with a reduced risk of diabetic nephropathy in type 1 diabetes (8–10).

The effect of ACE I/D polymorphism on the benefits obtained with ACE inhibition in terms of kidney function in patients with type 1 diabetes is therefore an important issue. In one study on proteinuric type 1 diabetic patients, the ACE DD genotype was found to be associated with a more rapid decrease in glomerular filtration rate (GFR) on captopril (11). In another study, on normo- or microalbuminuric, normotensive type 1 diabetic patients, the II genotype was associated with a more rapid decrease in urinary albumin excretion (UAE) on lisinopril (12). However, the mechanisms by which the ACE I/D polymorphism affects renal response to ACE inhibition remain unclear.

In this study, we investigated whether the renal hemodynamic changes induced by high glucose concentrations during ACE inhibition differ in type 1 diabetic patients with the ACE II genotype and in those with the ACE D allele. We studied renal hemodynamic responses to graded doses of ramipril (an ACE inhibitor) in normotensive, normoalbuminuric type 1 diabetic patients selected on the basis of ACE genotype. During this proof-of-concept study, we investigated renal hemodynamics in conditions of normo- and hyperglycemia, because hyperglycemia causes glomerular capillary hypertension, which leads to nephropathy (13), and because we could relate these changes to ACE I/D genotypes (14).

RESEARCH DESIGN AND METHODS

The participants were selected from type 1 diabetic patients attending the Diabetic Clinic of Angers University Hospital (France). The inclusion criteria were age of <50 years; men or women with mechanical contraception; onset of type 1 diabetes before the age of 35 years; normal blood pressure (<140/80 mmHg); normal UAE (<30 mg/24 h); no proliferative retinopathy; no chronic disease other than type 1 diabetes; and no treatment other than insulin. We asked patients to consent to genotyping for ACE I/D polymorphism. Those selected as case subjects or control subjects were then asked to participate in the study described below. For ethical reasons, none of the patients were informed of their own ACE genotype. The study protocol was reviewed by the local ethics committee, and all patients gave written informed consent.

The patients selected as case subjects had the II genotype. Each case subject was matched with two control subjects carrying the D allele for age (±5 years), sex, and diabetes duration (±5 years), because several cross-sectional studies (15–17) and follow-up studies (8–10) have shown that the ACE D allele has a dominant effect on the risk for nephropathy.

We compared two doses of ramipril, a low dose (1.25 mg/day) and the usual dose (5 mg/day), with placebo in a randomized, double-blind, cross-over experiment. Study drugs were given once per day in the morning, for treatment periods of 2–6 weeks, separated by 4-week washout periods. The doses of ramipril used were chosen based on previous studies (18,19). The durations of the minimal treatment period (2 weeks) and of the washout period (4 weeks) were selected based on previous studies, to ensure steady ACE inhibition and the absence of carry-over effects (20). At the end of each treatment period, we investigated renal hemodynamics in the patients and the changes in renal hemodynamics induced by hyperglycemia, as described below.

Determinations. The protocol used for renal hemodynamic studies has been described elsewhere (14). Briefly, participants were admitted to the center for clinical investigations the evening before the study. They were asked to collect their urine during the 24 h before admission and to bring it to the hospital with them for urinary sodium and albumin measurements. Patients ate their evening meal at the hospital, and their regular dose of insulin was administered subcutaneously. They received an intravenous infusion of regular insulin overnight, according to a published algorithm (21). At 7 A.M., blood was sampled from patients in the supine position for the determination of serum ACE activity, plasma active renin and aldosterone levels, and HbA_{1c} (A1C). They were then given tablets corresponding to the placebo or ramipril and asked to drink tap water regularly thereafter to obtain forced diuresis of ~10–15 ml/min. Once diuresis was established, a primed intravenous infusion of ^{125}I-iodothalamate and ^{131}I-labeled hippurate was initiated. This infusion was continued throughout testing. Thus, the tracer clearances were calculated from their urine concentrations divided by their plasma concentrations, multiplied by urine flow. There were six consecutive 30-min clearance periods. During the first three periods, the morning insulin infusion rate was kept constant, such that plasma glucose concentration remained close to normal (~5 mmol/l, "normoglycemia" period). We then infused exogenous glucose for 10 min to achieve high blood glucose levels, while keeping the insulin infusion rate constant, for the remaining three 30-min clearance periods (~15 mmol/l, "hyperglycemia" period).

The renal clearance of ^{125}I-iodothalamate was assumed to be equal to GFR and that of ^{131}I-labeled hippurate to effective renal plasma flow (ERPF). The ERPF value was not corrected for renal hippurate extraction, because it has been reported that ACE inhibition does not affect renal extraction coefficients in a given individual (22). Mean arterial pressure (MAP) was measured automatically (Dinamap, Critikon, FL) every 5 min, and values were averaged for each clearance period. We calculated filtration fraction by dividing GFR by ERPF. Total renal resistances (TRRs) were calculated by dividing MAP by ERPF. The renal hemodynamics values for the normoglycemia period were calculated as a mean of the values for the second and third clearance periods, and those of the hyperglycemia period were calculated as a mean of the values for the fifth and sixth clearance periods (14). In our laboratory, the intraindividual coefficient of variations were 5 ± 1, 7 ± 1, and 2 ± 1% for GFR, ERPF, and MAP, respectively (obtained from nine type 1 diabetic subjects).

Serum ACE activities were measured enzymatically (23), plasma active renin was determined by immunoradiometry (24), plasma aldosterone concentration by radioimmunoassay (25), plasma glucose concentration by enzymatic assay, and A1C levels by high-performance liquid chromatography (Diamat; Bio-Rad, Ivry-sur-Seine, France). Urinary albumin and sodium concentrations were measured by nephelometry (26) and flame spectrometry, respectively.

Statistical analysis. Results are presented as means ± 1 SD or median (range). Because no previous study was available for power calculation, we used previously observed differences in renal hemodynamics during hyperglycemia between II and non-II patients (14). Taking an α-risk of 5% and a β-risk of 90%, we would need 12 case subjects to show a difference in TRR. For technical reasons, assessments were not completed in two patients with the D allele. We therefore present results for 12 case subjects with the II genotype and 22 control subjects. We used nonparametric tests to analyze the global effects of genotype (Mann-Whitney *U* test), of ramipril dose (Kruskal-Wallis), and of their interaction (Mantel-Haenszel test). We separately tested, within each genotype, the dose dependence of the effect of ramipril (Kruskal-Wallis), and, within each ramipril dose, the genotype effect (Mann-Whitney *U* test).

TABLE 1
Clinical and biological characteristics of the study participants, at baseline

Genotype (*n*)	II (12)	non-II (22)	*P*
Age (years)	30 (7)	31 (6)	NS
Sex (men/women)	6/6	11/11	NS
Diabetes duration (years)	12.0 ± 9.3	14.4 ± 5.9	NS
BMI (kg/m^2)	23.6 ± 2.6	23.6 ± 3.5	NS
A1C (%)	8.3 ± 1.1	8.7 ± 1.0	NS
SBP (mmHg)	123 ± 14	118 ± 10	NS
DBP (mmHg)	66 ± 9	66 ± 7	NS

Data are means ± SD. DBP, diastolic blood pressure; SBP, systolic blood pressure.

RESULTS

Baseline characteristics of the participants. The participants' characteristics are given in Table 1. No differences according to genotype were observed. Baseline BMIs and A1C levels were similar at the start of each treatment period and at the time of the renal hemodynamics study (data not shown). The urinary sodium excretions were 149 ± 35 for the II participants vs. 162 ± 36 mmol/24 h for the non-II ones at baseline (NS); they remained not different with 1.25 mg/day ramipril (146 ± 65 vs. 165 ± 40 mmol/24 h) and 5 mg/day ramipril (161 ± 50 vs. 163 ± 35 mmol/24 h). However, UAE values were lower for II participants than for D allele carriers at baseline: 3 mg/24 h (range 2–4) vs. 6 (2–23); $P = 0.0052$. These UAE values were reduced to 3 mg/24 h (2–24) in the II patients and to 3 mg/24 h (2–19) in the non-II patients by treatment with 1.25 mg/day ramipril. UAE values of 3 mg/24 h (2–6) were recorded in the II participants on 5 mg/day ramipril versus 3 mg/24 h (2–71) in the D allele carriers. No order effect was observed in this cross-over study for these results or for any of the variables analyzed below.

Changes in serum ACE activity and plasma active renin and aldosterone levels (Table 2). Baseline serum ACE activities were lower in the II participants than in the others. Ramipril treatment had a strong effect on plasma ACE concentration, regardless of genotype. This effect was observed with the lower dose of ramipril in all groups. Plasma active renin levels were similar in both groups at baseline. They rose very significantly on ramipril treatment ($P < 0.0001$); plasma active renin levels were slightly lower in II patients than in patients with the D allele for treatment with 5 mg/day ramipril. Plasma aldosterone levels were similar in all groups; they were not affected by treatment, genotype, or treatment × genotype interaction.

Kidney function during renal hemodynamics study (Table 3)

Changes induced by treatments during normoglycemia (Table 3, left panel). At baseline, kidney function during normoglycemia was similar in the two groups. GFR was not affected by genotype, treatment dose, or their interaction. ERPF increased slightly with ramipril dose, independently of genotype. The filtration fraction de-

TABLE 2
Changes in serum ACE activity, plasma active renin, and aldosterone levels according to ACE I/D genotype, treatment dose, and interaction

Variable	Ramipril (daily dose)	II (n = 12)	non-II (n = 22)	P	
				Genotype effect	Global genotype effect
ACE activity (units/l)	Placebo	18 ± 7	26 ± 10	0.0077	
	1.25 mg	7 ± 2	10 ± 7	0.1301	0.1349
	5 mg	5 ± 2	6 ± 3	NS	
Treatment effect (P)		<0.0001	<0.0001		
Global treatment effect			<0.0001		NS*
Active renin (pg/ml)	Placebo	7.5 ± 3.3	9.0 ± 3.9	NS	
	1.25 mg	15.1 ± 9.0	17.6 ± 10.1	NS	0.0812
	5 mg	16.3 ± 11.6	26.2 ± 14.8	0.0335	
Treatment effect (P)		0.0131	<0.0001		
Global treatment effect			<0.0001		NS*
Aldosterone (pg/ml)	Placebo	311 ± 167	253 ± 136	NS	
	1.25 mg	319 ± 178	278 ± 178	NS	NS
	5 mg	290 ± 176	318 ± 268	NS	
Treatment effect (P)		NS	NS		
Global treatment effect			NS		NS*

Data are means ± SD. NS, $P > 0.20$. P values are global effects of genotype (Mann-Whitney U test), of ramipril doses (Kruskal-Wallis), and of their interaction (Mantel-Haenszel). P values within each genotype: treatment effect (Kruskal-Wallis). P values within each ramipril dose are genotype effect (Mann-Whitney U test). *P values for interaction.

creased (P = 0.0130) with increasing ramipril dose, with no effect of genotype. There was no interaction between genotype and treatment. MAP was reduced by treatment, but not significantly so. Baseline TRR values did not differ according to genotype. TRR decreased significantly on ramipril treatment (P = 0.030); this response was genotype independent.

Hyperglycemia-induced changes according to treatment and genotype (Table 3, right panel and Fig. 1). Changes in GFR during hyperglycemia were not significantly affected by treatment dose or genotype, and no genotype-treatment interaction was observed. Conversely, the increase in ERPF during hyperglycemia seen in the II participants was potentiated by ramipril, whereas it was attenuated in the non-II participants (P = 0.018 for treatment-genotype interaction). No significant change in filtration fraction was seen during hyperglycemia, regardless of treatment, genotype, or their interaction.

MAP decreased in the II participants, but not in the non-II participants, during hyperglycemia. This difference in MAP response was exacerbated by increasing the dose of ramipril (P = 0.0342 for the genotype effect, P = 0.0749 for the treatment effect, P = 0.018 for treatment-genotype interaction). Hyperglycemia provoked a decrease in TRR in the II participants, which was potentiated by ramipril dose; this was not the case in the non-II participants (P = 0.1058 for the genotype effect, NS for the treatment effect, and P = 0.055 for the genotype-treatment interaction).

DISCUSSION

This study shows that the beneficial effects of ACE inhibition on the renal circulation of type 1 diabetes subjects depend on ACE I/D polymorphism, during hyperglycemic surges. After increases in blood glucose levels, individuals with the ACE II genotype increased their ERPF and decreased MAP and TRR (variables indicating beneficial changes in intraglomerular hydraulic pressure [2]) to a greater extent than those carrying the ACE D allele. These findings provide a rationale to explain the smaller long-term renal benefits of ACE inhibition in type 1 diabetic patients with the ID or DD ACE genotypes than in those with the II genotype (11,12). In humans, it is not possible to measure intraglomerular pressures directly. However, changes in renal hemodynamics can be estimated by assessing changes in ERPF and MAP and by calculating TRR.

The maximum dose of ramipril used in this study was 5 mg/day. The ATLANTIS (Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke) study demonstrated a dose-dependent effect of ramipril on kidney function in normotensive type 1 diabetic subjects with microalbuminuria, using the same doses as used here (19). The GISEN (Gruppo Italiano di Studi Epidemiologici in Nefrologia) study also demonstrated a nephroprotective effect of 5 mg/day ramipril (27). A dose of 10 mg/day ramipril has also been shown to have a cardioprotective effect (28,29). The renal hemodynamic effects of 10 mg/day ramipril were not assessed. However, we found that 1.25 mg/day ramipril decreased albuminuria without affecting cardiovascular outcome (30), suggesting that the renal effects of ramipril may begin at lower doses than the cardiovascular effects (30).

Ramipril had a more pronounced hypotensive effect during hyperglycemia in the participants with the II genotype than in the others. This is consistent with previous findings concerning kidney function and blood pressure in type 1 diabetic subjects selected on the basis of ACE genotype, studied during hyperglycemia (31), and during infusions of angiotensins 1 and 2 (32). During normoglycemia, ramipril had a dose-dependent effect on renal hemodynamics that did not differ with genotype. This is consistent with the previously reported lack of effect of the ACE I/D polymorphism on the hypotensive response to renin inhibition in healthy volunteers (33).

In the II genotype participants, hyperglycemia enhanced the decrease in intraglomerular pressure due to ACE inhibition in a dose-dependent fashion. The opposite effect was seen in participants carrying the ACE D allele: renal circulatory changes due to ACE inhibition were strictly similar to those in the II participants during normoglyce-

TABLE 3
Renal circulatory changes induced by ramipril doses, according to ACE genotypes in normoglycemia and hyperglycemia

Variable	Ramipril (daily dose)	Normoglycemia			Global genotype effect*	Hyperglycemia-induced changes			Global genotype effect*
		II (n = 12)	non-II (n = 22)	Genotype effect*		II (n = 12)	non-II (n = 22)	Genotype effect*	
GFR (ml/min per 1.73 m^2)	Placebo	144 ± 23	144 ± 19	NS		3.4 ± 19.0	2.5 ± 13.3	NS	
	1.25 mg	148 ± 18	153 ± 21	NS	NS	−0.4 ± 14.0	−5.1 ± 10.3	NS	NS
	5 mg	146 ± 15	150 ± 15	NS		4.0 ± 19.4	−2.8 ± 14.0	NS	
Treatment effect*		NS	NS			NS	0.0886		
Global treatment effect*		NS			NS†		0.1367		0.1830†
ERPF (ml/min per 1.73 m^2)	Placebo	627 ± 126	622 ± 107	NS		17.4 ± 137.9	3.1 ± 66.7	NS	
	1.25 mg	656 ± 96	656 ± 105	NS	NS	21.8 ± 102.2	−3.2 ± 65.4	NS	NS
	5 mg	675 ± 91	691 ± 98	NS		41.1 ± 96.4	−30.6 ± 80.6	0.0436	
Treatment effect*		NS	0.1630			NS	0.1601		
Global treatment effect*		0.1219			NS†	NS			0.018†
FF ($\times 10^{-2}$)	Placebo	23.3 ± 2.7	23.4 ± 2.3	NS		0.13 ± 2.00	0.18 ± 1.49	NS	
	1.25 mg	22.7 ± 2.9	23.4 ± 1.8	NS	NS	−0.83 ± 2.60	−0.49 ± 1.43	NS	NS
	5 mg	21.7 ± 2.1	21.9 ± 2.4	NS		−0.59 ± 1.38	0.53 ± 1.70	0.0774	
Treatment effect*		NS	0.0158			NS	0.1533		
Global treatment effect*		0.0130			NS†		NS		NS†
MAP (mmHg)	Placebo	87 ± 12	84 ± 8	NS		0.67 ± 3.85	1.72 ± 3.83	NS	
	1.25 mg	85 ± 10	82 ± 7	NS	0.1201	−0.70 ± 3.03	0.27 ± 4.78	NS	0.0342
	5 mg	85 ± 10	80 ± 7	NS		−2.74 ± 3.37	0.18 ± 4.67	0.0192	
Treatment effect*		NS	NS			0.1428	NS		
Global treatment effect*		0.1380			NS†		0.0749		0.018†
TRR (mmHg · ml^{-1} · min^{-1} per 1.73 m^2)	Placebo	0.144 ± 0.039	0.141 ± 0.037	NS		−4.25 ± 34.54	−0.32 ± 16.88	NS	
	1.25 mg	0.132 ± 0.023	0.128 ± 0.026	NS	NS	−4.40 ± 18.35	+3.13 ± 15.26	NS	0.1058
	5 mg	0.128 ± 0.028	0.118 ± 0.021	NS		−10.88 ± 15.85	+5.30 ± 11.68	0.0028	
Treatment effect*		NS	0.0412			0.0985	NS		
Global treatment effect*		0.030			NS†		NS		0.055†

Same presentation as Table 2. †P values for interaction. *Left panel*, values during normoglycemia; *right panel*, changes induced by hyperglycemia. FF, filtration fraction.

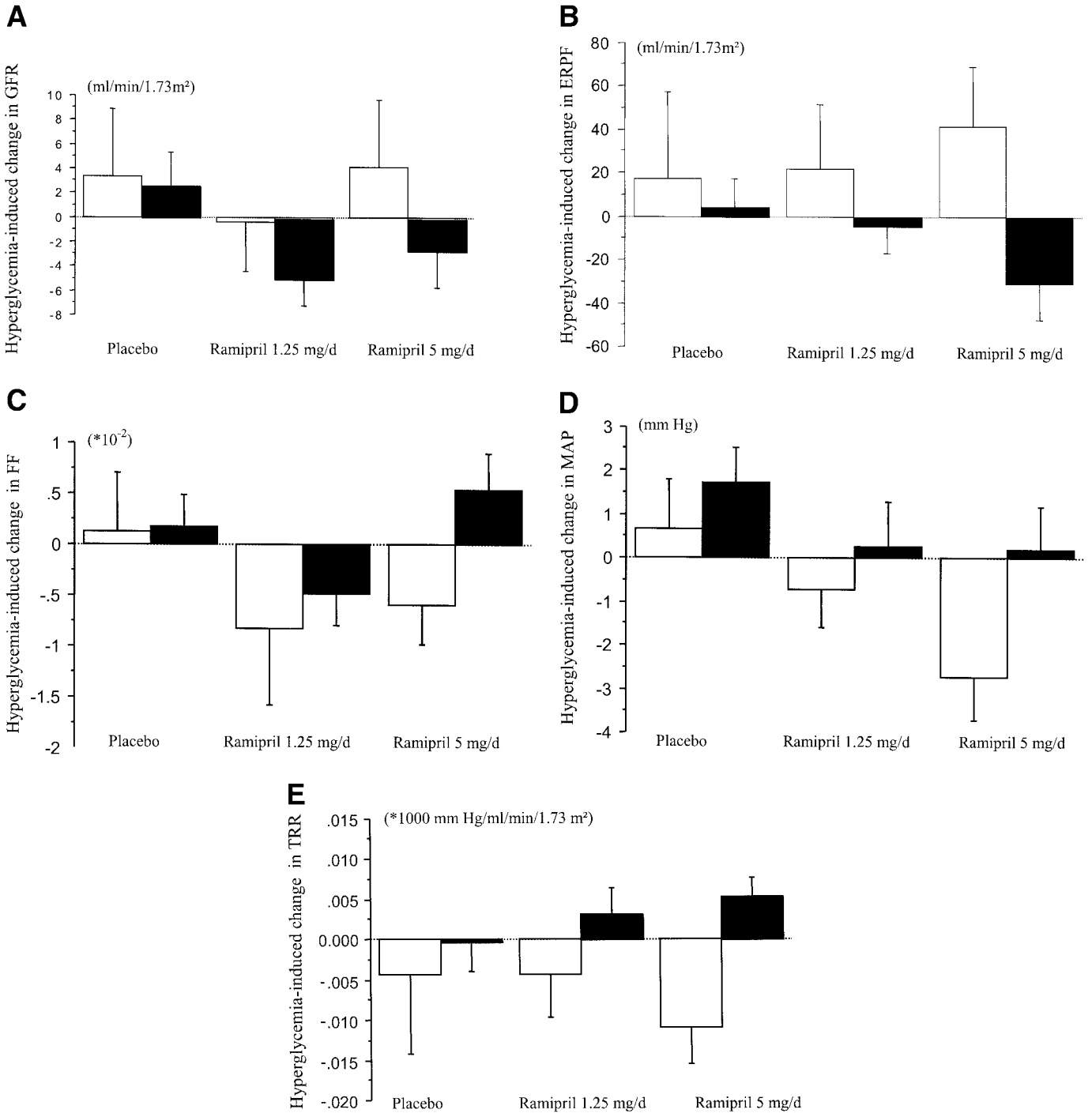

FIG. 1. Hyperglycemia-induced changes in renal hemodynamic variables, according to ACE I/D polymorphism. Data are means, and error bars are SE. □, II patients; ■, non-II patients. *Genotype effect (Mann-Whitney *U* test); **Treatment effect (Kruskal-Wallis); ***treatment-gene interaction effect (Mantel-Haenszel interaction). *A*: GFR (*P = NS; **P = 0.1367; ***P = 0.183). *B*: ERPF (*P = NS; **P = NS; ***P = 0.018). *C*: Filtration fraction (FF) (*P = NS; **P = NS; ***P = NS). *D*: MAP (*P = 0.0344; **P = 0.075; ***P = 0.018). *E*: TRRs (*P = 0.106; **P = NS; ***P = 0.055).

mia, but these beneficial changes were attenuated by increases in plasma glucose levels. How hyperglycemia interferes with the renal response to ACE inhibition must be discussed. There is evidence to suggest that the renin-angiotensin system is highly active during hyperglycemia in the renal circulation of subjects with type 1 diabetes (34,35). We and others (14,31,32) have demonstrated that these renal hemodynamic changes are greater in subjects carrying the ACE D allele than in those with the II genotype. Intraglomerular capillary hydraulic pressure, the main determinant of renal failure (36), is largely conditioned by interaction between the vasodilatory effects of nitric oxide (NO) and the vasoconstrictive effects of angiotensin 2. However, the vasoconstrictive effect of angiotensin 2 on the arterioles efferent from glomeruli is detectable even at very low angiotensin 2 concentrations in experimental conditions (3,37), whereas the NO-dependent vasodilatation of the arterioles afferent to the glomer-

uli is amplified by local angiotensin 2 levels in a dose-dependent manner (37). This phenomenon may account for the impact of the ACE D allele on renal circulation in type 1 diabetes (14,31,32). The well-known endothelial dysfunction observed in people with diabetes is primarily explained by a low capacity for NO-dependent vasodilatation (38), as a direct consequence of hyperglycemia (39). However, chronic ACE inhibition restores NO-mediated endothelial function in patients with type 1 diabetes (40). Thus, the beneficial effect of ACE inhibition on intraglomerular hydraulic pressure, through the vasodilatation of the arterioles efferent from glomeruli, may be counterbalanced in type 1 diabetic patients by an increase in the NO-dependent vasodilatation of the arterioles afferent to glomeruli. In this case, people with type 1 diabetes and the ACE D allele would not benefit fully from high doses of ACE inhibitors during periods of hyperglycemia. Conversely, those with the ACE II genotype would not be adversely affected, because their renal circulation parameters have already been shown to be insensitive to hyperglycemia (14). Finally, ACE inhibition increases the level of bradykinin, a stimulator of NO release, in addition to suppression of angiotensin 2 production (41). Unfortunately, changes in angiotensin 2 and bradykinin concentrations within the renal circulation cannot be measured in humans, and the changes in serum ACE activities induced by low or high doses of ACE inhibitors do not reflect the actual changes occurring within the vascular wall (42).

We believe our findings to be of clinical relevance. ACE inhibitors are currently the only evidence-based treatment for the prevention or treatment of diabetic nephropathy in patients with type 1 diabetes (5–7). Intensified insulin treatment is currently recommended for most type 1 diabetic patients (43), and this is supported by the Diabetes Control and Complications Trial (DCCT) and its follow-up studies (44,45). Nonetheless, most individuals with type 1 diabetes remain hyperglycemic most of the time (44). The lower efficiency of ACE inhibition in hyperglycemia than in normoglycemia in type 1 diabetic patients with a high genetic risk for kidney disease is therefore important for patient care. Some investigators have suggested that angiotensin 2 subtype 1 receptor antagonists could be used to prevent or to treat diabetic nephropathy in type 1 diabetic patients, in conditions in which ACE inhibition is not feasible (43). Interestingly, the anti-albuminuric effect of these drugs is independent of ACE I/D polymorphism (46). However, angiotensin 2 subtype 1 receptor antagonists have not been demonstrated to block renal disease in type 1 diabetes.

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