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Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II[1]

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Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II.

Background. Most of the known actions of angiotensin II (Ang II) are mediated by the Ang II type 1 receptor (AGT_1R). A noncoding polymorphism of the AGT_1R gene has been described in which there is either an adenine (A) or cytosine (C) base at position 1166. The functional significance of this polymorphism is unknown, prompting us to examine the relationship between this polymorphism and the systemic and renal responses to AGT_1R blockade and subpressor Ang II infusion.

Methods. Sixty-six healthy Caucasian men and women, genotyped for the AGT_1R polymorphism by polymerase chain reaction, were chosen to form two homogeneous groups: AA and AC/CC. Renal hemodynamic function was assessed with inulin and para-aminohippurate clearance before and after AGT_1R receptor blockade with losartan and Ang II infusion.

Results. The mean values at baseline for glomerular filtration rate (GFR), renal plasma flow (ERPF), and renal blood flow (RBF) were significantly lower in the AC/CC group compared with the AA group. Losartan increased the GFR and decreased the mean arterial pressure (MAP) in the AC/CC group, but did not influence these parameters in the AA group. The aldosterone responses to losartan were blunted in the AA subgroup. During Ang II infusion, AC/CC subjects maintained GFR despite equivalent declines in RBF, suggesting an enhanced efferent arteriolar constrictive response.

Conclusions. Taken together, these results suggest that there is a relationship between the AGT_1R $A^{1166}\rightarrow C$ polymorphism and the humoral and renal hemodynamic responses to AGT_1R blockade and to Ang II infusion in the sodium-replete state, and that the C allele is associated with enhanced intrarenal and peripheral Ang II activity. Further studies are required to determine the genetic locus for this effect.

The activation of the renin-angiotensin system (RAS) and the subsequent generation of angiotensin II (Ang II) play important roles in both normal physiology and the progression of cardiac and renal disease [1–6]. Most of the known actions of Ang II are mediated by the Ang II type 1 receptor (AGT_1R) [7], including vascular contraction, pressor responses, proximal tubule sodium transport, and aldosterone secretion. The receptor is expressed on the surface of a variety of cell types, including vascular smooth muscle cells and myocardial cells, as well as vascular smooth muscle cells of the afferent and efferent arterioles, mesangial cells and proximal tubule cells of the kidney, and the glomerulosa cells of the adrenal gland [8].

Recently, a polymorphism of the AGT_1R gene has been described in which there is either an adenine (A) or cytosine (C) base at position 1166 in the 3′ untranslated region of the gene [9]. Epidemiological studies have revealed an association between this noncoding polymorphism and hypertension [9] and aortic stiffness in hypertensive patients [10]. There may also be a synergistic interaction between the AGT_1R polymorphism and the angiotensin-converting enzyme (ACE) insertion/deletion polymorphism on the risk of myocardial infarction [11]. In contrast, other case control studies have failed to establish significant associations between the AGT_1R polymorphism and endpoints such as diabetic microangiopathy [12].

Because the AGT_1R $A^{1166}\rightarrow C$ polymorphism is in an untranslated region of the gene, the functional significance of the polymorphism is unclear, although Amant et al have recently reported that methylergonovine induces more coronary artery vasoconstriction in patients with at least one copy of the C allele compared with patients homozygous for the A allele [13]. We first hypothesized that there would be an association between the AGT_1R polymorphism and renal and systemic hemodynamic function. This hypothesis was tested by comparing baseline function in normal healthy male and premenopausal female subjects ingesting a controlled sodium and protein diet and grouped according to the AGT_1R $A^{1166}\rightarrow C$ polymorphism. A sodium intake of approximately 200 mmol/day was chosen so that all subjects were sodium replete, thus avoiding RAS activation and ensuring a

[1] **See Editorial by Blantz, p. 2317**

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uniform response to our interventions. We further hypothesized that renal and systemic Ang II activity would be augmented in subjects with the C allele. We tested this hypothesis by comparing hemodynamic and humoral responses to AGT_1R blockade with losartan and with low-dose subpressor infusions of Ang II.

METHODS

Subjects

Sixty-six normal healthy males and females were recruited to participate in the study. Their mean age was 28 ± 1 years (range 20 to 35 years). Each subject underwent a detailed history and physical examination by a qualified internist. All were Caucasian, normotensive and nonobese, and nonsmokers and were on no medications. They were subdivided on the basis of the presence of the C allele, and thus, two homogeneous groups (AA, $N = 30$, and AC/CC, $N = 36$) were formed for these experiments. The study was performed with the approval of the University of Toronto Human Subjects Review Committee and with the informed written consent of each subject.

All subjects were counseled to adhere to a diet that maintained their normal caloric intake, their sodium intake to 200 mmol/day, and their protein intake to 1.5 to 2 g/kg/day for seven days prior to each study day. A 24-hour urine sample was obtained one day prior to each study for the measurement of sodium and urea excretion to assess compliance with the controlled diet. On the day of the testing, the volunteer subjects reported to the renal physiology laboratory. All studies were conducted at 8:30 a.m. after an overnight fast, with the subjects lying supine in a warm quiet room.

Study protocol

Part 1. On the first day of the protocol, an 18-gauge peripheral venous cannula was inserted into an antecubital vein for infusions of inulin and para-aminohippuric acid (PAH), and a second cannula was inserted in the contralateral arm for blood sampling. Each subject voided and then drank 800 ml water in the first 45 minutes to induce a water diuresis. Two hundred milliliters were ingested in each hour of the protocol to maintain an adequate urine output for collection of spontaneously voided samples. Hemodynamic parameters [mean arterial pressure (MAP), heart rate] were measured throughout the study by an automated sphygmomanometer (Dinamapp) and were recorded once in each half hour of the protocol. Renal hemodynamics were measured using inulin and PAH clearance techniques, as previously described [14]. Three timed urine collections of 20 minutes' duration each were then obtained by spontaneous voiding for the determination of baseline glomerular filtration rate (GFR) and renal plasma flow (ERPF). At the end of this period, losartan (Cozaar®; Merck, Sharpe, and Dohne, Hamburg, Germany) was administered at a subdepressor dose of 25 mg. During each hour for three hours, blood was collected for inulin and PAH, hematocrit (Hct), and aldosterone, and urine was collected for inulin, PAH, and sodium.

Part 2. On the second day of the protocol, a minimum of one week later, the subjects reported to the renal physiology laboratory, having again followed the sodium- and protein-controlled diet, and collected a 24-hour urine. As in part 1, subjects were studied while supine, in a warm quiet room, after an overnight fast. Inulin and PAH were infused, and three timed urine collections were obtained. At the end of this baseline period, blood samples were obtained for Hct, inulin, and PAH, and aldosterone. A solution of Ang II (Hypertensin®; 2.5 mg/vial; Ciba Geigy Canada, Ltd., Mississauga, Ontario, Canada) was prepared by dissolving the diluent in normal saline to produce a concentration of 0.5 mg/ml. Two hundred and fifty milliliters of normal saline were there added to 0.2 ml Ang II to produce a concentration of 400 ng/ml. Ang II was infused at a subpressor dose of 0.5 ng/kg/min for 30 minutes. Subjects remained supine except to void. Blood was collected once at the end of the infusion period for inulin and PAH, aldosterone, and Hct, and urine was collected for sodium, inulin, and PAH. MAP was also measured at the midpoint of each infusion. A further collection of both blood and urine was obtained at the end of the Ang II infusion after a 30-minute recovery period.

Sample collection and analytical methods

Blood samples collected for inulin and PAH determinations were immediately centrifuged at 3000 r.p.m. for 10 minutes at 4°C. Plasma was separated, placed on ice, and then stored at −70°C before the assay. Inulin concentrations in plasma and urine were measured by a modified method of Walser, Davidson, and Orloff [15], and the PAH concentration was measured by a spectrophotometric method according to Brun [16]. The mean of the final two clearance periods represent GFR and ERPF, expressed per 1.73 m^2. Filtration fraction (FF) represented the ratio of GFR to ERPF. Renal blood flow (RBF) was calculated by dividing the ERPF by (1 − Hct). Renal vascular resistance (RVR) was derived by dividing MAP by the RBF.

The serum sodium concentration was measured by an ion-selective electrode method and urine sodium by a flame photometry method. Aldosterone was measured by radioimmunoassay, using the Coat-A-Count system. Serum ACE activity was measured by a spectrophotometric method using an ACE kinetic test kit (Buhlmann Laboratories AG, Schonembuch, Switzerland). Briefly, serum samples and calibrators (20 μl) were allowed to react with a synthetic peptide (200 μl) in a 96-well

Table 1. Baseline values

Parameter	Genotype AA ($N = 30$)	Genotype AC/CC ($N = 36$)	P value
Male/female	22/8	28/8	NS
Age *years*	28 ± 1	27 ± 1	NS
BMI *kg/m²*	24 ± 1	25 ± 1	NS
U_{Na} *mmol/day*	228 ± 30	197 ± 14	NS
U_{urea} *mmol/day*	359 ± 27	389 ± 25	NS
Serum ACE *U/liter*	39 ± 4	41 ± 3	NS
Ang II *pg/ml*	12 ± 2	11 ± 2	NS
PRA *ng Ang I/liter/second*	0.28 ± 0.04	0.30 ± 0.03	NS
MAP *mm Hg*	88 ± 2	87 ± 2	NS
Hct	0.406 ± 0.009	0.406 ± 0.008	NS

Abbreviations are: BMI, body mass index; U_{Na}, 24-hour urine sodium excretion; U_{urea}, 24-hour urine urea excretion; ACE, angiotensin-converting enzyme; Ang II, angiotensin II; PRA, plasma renin activity; MAP, mean arterial pressure; Hct, hematocrit.

Table 2. Baseline renal hemodynamic and excretory values

Parameter	Genotype AA ($N = 30$)	Genotype AC/CC ($N = 36$)	P value
GFR *ml/min/1.73 m²*	113 ± 4	105 ± 3	0.04
ERPF *ml/min/1.73 m²*	642 ± 26	535 ± 28	0.04
RBF *ml/min/1.73 m²*	1103 ± 37	924 ± 39	0.04
FF	0.20 ± 0.01	0.17 ± 0.005	0.01
RVR *mm Hg/liter/min*	78 ± 3	90 ± 5	0.01
$U_{Na}V$ *μmol/min*	383 ± 31	346 ± 44	NS

Abbreviations are: GFR, glomerular filtration rate; ERPF, renal plasma flow; RBF, renal blood flow; FF, filtration fraction; RVR, renal vascular resistance; $U_{Na}V$, urinary sodium excretion.

microplate. The enzyme kinetics at 37°C was followed by the decrease in absorbance at 340 nm for 15 minutes with a Bio-Tek microplate reader (model #Ceres UV 900 Hdi). Blood for determination of Ang II was collected into prechilled tubes containing ethylenediaminetetraacetic acid (EDTA) and angiotensinase inhibitor (0.1 ml Bestatin solution; Buhlmann Laboratories). Samples were analyzed using a competitive radioimmunoassay kit supplied by Buhlmann Laboratories AG.

Polymerase chain reaction. Genomic DNA was extracted from peripheral blood leukocytes as previously described [14, 17]. The DNA was resuspended in 10 mM Tris-HCl, 0.2 mM sodium EDTA, pH 7.5, and the concentration was determined by spectrophotometry. To determine the AGT_1R genotype of the subjects, 0.1 μg of genomic DNA was subjected to polymerase chain reaction (PCR) amplification [17]. The reaction mixture (20 μl) contained 50 mM KCl, 10 mM Tris, pH 8.3, 1.5 mM $MgCl_2$, 1 μmol of each primer, 200 μmol of deoxy-ATP, GTP, CTP, TTP, gelatin 200 μg/ml, and 1 U of Taq polymerase. Thirty cycles of PCR were performed: 1 minute at 95°C, 1 minute at 55°C, and 1.5 minutes at 72°C. The primers were 5′GCACCATGTTTTGAGGTT 3′ and 5′CGACTACTGCTTAGCATA 3′. This PCR amplification yields a 546 bp product. The amplicon was subjected to overnight incubation with DdeI at 37°C, and the digestion products were separated by electrophoresis on 1.5% agarose gels containing ethidium bromide. The ^{1166}C AGT_1R allele contains a recognition site for the restriction endonuclease, DdeI, so that digestion of the PCR product with DdeI yields 435 bp and iii bp fragments. The ^{1166}A AGT_1R allele does not contain a recognition site for the restriction endonuclease, DdeI, so that the 546 bp amplicon remains unaltered after incubation with DdeI [18, 19].

Statistical analysis

Subjects were segregated into subgroups on the basis of the presence or absence of the C allele (AA vs. AC/CC). Data are presented as mean ± SEM. A between-group comparison of all parameters at baseline was made using nonparametric methods (Wilcoxin rank sum test). Differences in within-subject and between-group responses to losartan and Ang II were determined by two-way repeated-measures analysis of variance (ANOVA) and Bonferroni correction. All statistical analyses were performed using the statistical package SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

Subject characteristics

Sixty-six normal subjects were segregated into groups on the basis of the presence or absence of the C allele of the AGT_1R gene. Thirty subjects displayed the AA genotype. Twenty-nine subjects displayed the AC genotype, and seven subjects displayed the CC genotype. Therefore, 36 subjects comprised the second group carrying the C allele. As can be seen from Table 1, no significant differences existed in proportion of females, age, body mass index, urine sodium excretion, urine urea excretion, serum ACE and Ang II levels, plasma renin activity, MAP, or Hct.

Baseline hemodynamic, humoral and renal excretory function

As shown in Table 2, the AC/CC subgroup exhibited significantly lower baseline values for GFR, ERPF, and RBF, and significantly higher values for FF and RVR. Baseline values for MAP did not differ between subgroups nor did the urine sodium excretion ($U_{Na}V$). Aldosterone, although numerically increased in the AC/CC genotype subgroup, did not differ significantly (316 ± 44 vs. 188 ± 24 pmol/liter, P = NS).

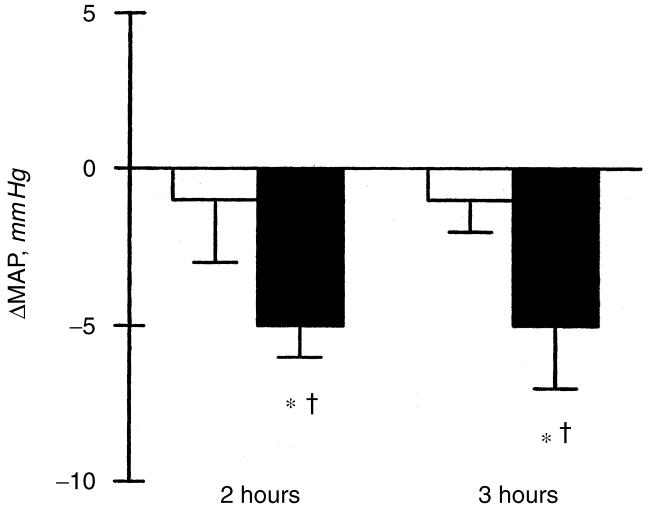

Fig. 1. Response of mean arterial pressure (ΔMAP) to losartan at two and three hours after administration in the AA genotype subgroup (□) and the AC/CC genotype subgroup (■). *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. response of AA genotype subgroup.

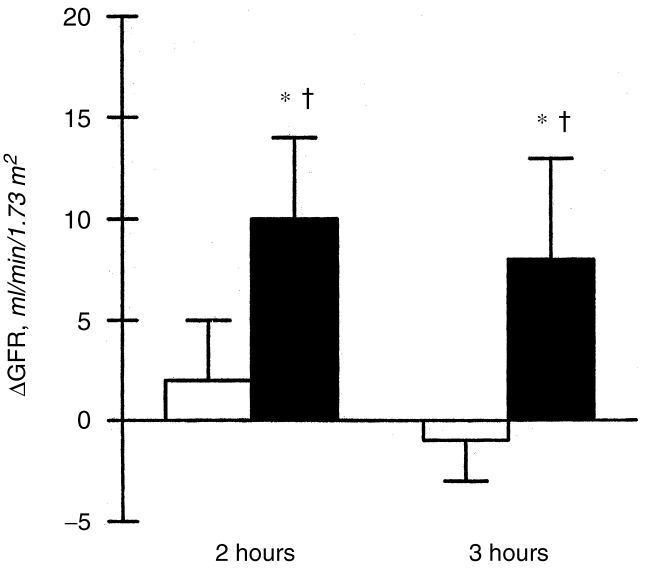

Fig. 2. Response of glomerular filtration rate (ΔGFR) to losartan at two and three hours after administration in the AA genotype subgroup (□) and the AC/CC genotype subgroup (■). *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. response of AA genotype subgroup.

Hemodynamic, humoral, and renal excretory response to losartan

The GFR response to losartan differed significantly between subgroups, with the AA group remaining stable (113 ± 4 ml/min/1.73 m^2 at baseline, 114 ± 6 at 2 hours and 112 ± 6 at 3 hours post-dose, P = NS vs. baseline) and the AC/CC group exhibiting a pronounced increase in GFR from 105 ± 3 ml/min/1.73 m^2 at baseline to 116 ± 5 at two hours and 112 ± 9 at three hours (P = 0.001 vs. baseline, P = 0.03 vs. response of AA group). The response of MAP also differed significantly between genotype subgroups. The AA subgroup did not experience a significant decline in arterial pressure (83 ± 2 mm Hg at baseline, 82 ± 2 at 2 hours, and 83 ± 3 at 3 hours postdose, P = NS vs. baseline), whereas the AC/CC subgroup experienced a pressure decline that, although only mild in magnitude, was statistically significant (85 ± 1 mm Hg at baseline, 80 ± 1 at 2 hours, and 80 ± 2 at 3 hours, P = 0.02 vs. baseline, P = 0.04 vs. response of AA subgroup).

No significant differences were discernible between subgroups in the ERPF response in that the AA subgroup value was 642 ± 26 ml/min/1.73 m^2 at baseline, 629 ± 27 at two hours, and 635 ± 38 at three hours (P = NS vs. baseline), and the AC/CC subgroup value was 535 ± 28 ml/min/1.73 m^2 at baseline, 577 ± 41 at two hours, and 593 ± 33 at three hours (P = NS vs. baseline, P = NS vs. response of AA subgroup). In parallel with the observations on ERPF, no significant differences were discernible between subgroups in the RBF response. The AA subgroup value was 1103 ± 37 ml/min/1.73 m^2 at baseline, 1064 ± 39 at two hours, and 1082 ± 72 at three hours (P = NS vs. baseline), and the AC/CC subgroup value was 924 ± 39 ml/min/1.73 m^2 at baseline, 981 ± 71 at two hours, and 1000 ± 60 at three hours (P = NS vs. baseline, P = NS vs. response of AA subgroup).

No significant differences were noted between subgroups in the FF response in that the AA subgroup value was 0.17 ± 0.005 at baseline, 0.18 ± 0.01 at two hours, and 0.17 ± 0.01 at three hours (P = NS vs. baseline), and the AC/CC subgroup value was 0.20 ± 0.01 at baseline, 0.20 ± 0.01 at two hours, and 0.19 ± 0.01 at three hours (P = NS vs. baseline, P = NS vs. response of AA subgroup). Similar to the FF response, no significant differences were found between subgroups in the RVR response in that the AA subgroup value was 78 ± 3 mm Hg/liter/min at baseline, 79 ± 3 at two hours, and 81 ± 5 at three hours (P = NS vs. baseline), and the AC/CC subgroup value was 90 ± 5 mm Hg/liter/min at baseline, 94 ± 7 at two hours, and 88 ± 7 at three hours (P = NS vs. baseline, P = NS vs. response of AA subgroup).

There was a significant difference between groups in the aldosterone response to losartan. The AA subgroup value was 188 ± 24 pmol/liter at baseline, 131 ± 17 at two hours, and 108 ± 14 at three hours (P = NS vs. baseline) compared with the AC/CC group, which exhibited a significant decline from 316 ± 44 pmol/liter at baseline to 152 ± 11 at two hours and 128 ± 15 at three hours (P = 0.001 vs. baseline, P = 0.03 vs. response of AA subgroup). As expected, urinary sodium excretion rates were similar in both genotype subgroups. No significant differences were found between subgroups in the $U_{Na}V$ response, in that the AA subgroup value was

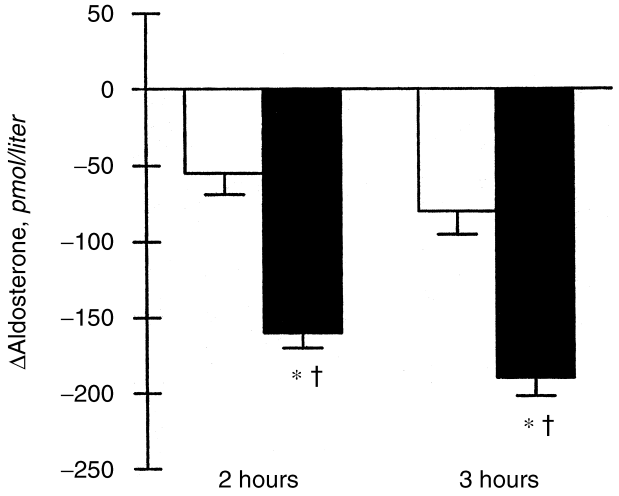

Fig. 3. Response of aldosterone (Δaldosterone) to losartan at two and three hours after administration in the AA genotype subgroup (□) and the AC/CC genotype subgroup (■). *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. response of AA genotype subgroup.

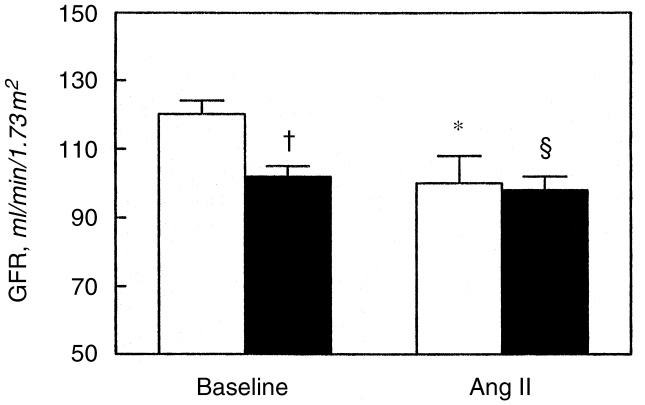

Fig. 4. Glomerular filtration rate (GFR) at baseline and during angiotensin II infusion at 0.5 ng/kg/min in the AA genotype subgroup (□) and the AC/CC genotype subgroup (■). *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. baseline value in AA genotype subgroup; §$P < 0.05$ vs. response of AA genotype subgroup.

383 ± 31 μmol/min at baseline, 316 ± 32 at two hours, and 359 ± 40 at three hours (P = NS vs. baseline), and the AC/CC subgroup value was 391 ± 35 μmol/min at baseline, 315 ± 29 at two hours, and 305 ± 27 at three hours (P = NS vs. baseline, P = NS vs. response of AA subgroup). The MAP, GFR, and aldosterone responses to losartan are illustrated in Figures 1–3.

Hemodynamic, humoral, and renal excretory response to angiotensin II

The GFR response to Ang II differed significantly between subgroups (Fig. 4), with the AA group declining significantly from 120 ± 4 ml/min/1.73 m^2 at baseline to 100 ± 8 by the end of the infusion period (P = 0.001 vs. baseline) and the AC/CC group remaining stable (102 ± 3 to 98 ± 4 ml/min/1.73 m^2, P = NS, vs. baseline, P = 0.03 vs. response of AA group). The response of MAP did not differ significantly between genotype subgroups. The AA subgroup did not experience an increase in arterial pressure (86 ± 2 mm Hg at baseline and 88 ± 2 during the Ang II infusion, P = NS) nor did the AC/CC subgroup (88 ± 2 mm Hg at baseline and 89 ± 2 during the Ang II infusion, P = NS).

No significant differences were discernible between subgroups in the ERPF response in that the AA subgroup value was 646 ± 34 ml/min/1.73 m^2 at baseline and 477 ± 35 during the infusion, P = 0.004 vs. baseline), and the AC/CC subgroup value was 570 ± 24 ml/min/1.73 m^2 at baseline and 448 ± 18 during the infusion (P = 0.001 vs. baseline, P = NS vs. response of AA subgroup). In parallel with the observations on ERPF, no significant differences were discernible between subgroups in the RBF response in that the AA subgroup value was 1082 ± 58 ml/min/1.73 m^2 at baseline and 779 ± 60 during the infusion (P = 0.002 vs. baseline), and the AC/CC subgroup value was 1001 ± 41 ml/min/1.73 m^2 at baseline and 754 ± 32 during the infusion (P = 0.01 vs. baseline, P = NS vs. response of AA subgroup).

No significant differences were noted between subgroups in the FF response in that the AA subgroup value was 0.19 ± 0.01 at baseline and 0.21 ± 0.01 during the infusion (P = 0.04 vs. baseline), and the AC/CC subgroup value was 0.18 ± 0.01 at baseline and 0.22 ± 0.01 during the infusion (P = 0.005 vs. baseline, P = NS vs. response of the AA subgroup). Similar to the FF response, no significant differences were found between subgroups in the RVR, the $U_{Na}V$, or the aldosterone responses to Ang II infusion.

DISCUSSION

This study was designed first to test the hypothesis that genetic factors influence renal hemodynamic function. Specifically, we hypothesized that a polymorphism of the AGT_1R gene would be associated with lower values for GFR in normal subjects. We chose to study the AGT_1R $A^{1166}\rightarrow C$ polymorphism of the AGT_1R gene because Bonnardeaux et al showed that the C allele was increased in frequency in a case control study of hypertensive patients [9]. Subsequent studies by this group have related the C allele to aortic stiffness [10] and increased risk of myocardial infarction [11].

In order to test our hypothesis, we genotyped subjects for the AGT_1R $A^{1166}\rightarrow C$ polymorphism and then divided the subjects onto two groups based on the presence of the C allele (AA and AC/CC). We then compared baseline renal and peripheral hemodynamic function between the groups. In order to control for confounding variables, all subjects were counseled by a research dietitian and

were prescribed a controlled diet. Because Du et al have reported that renal AGT_1R mRNA levels are increased by a low-sodium diet [20], subjects adhered to a 200 mmol sodium diet [14]. Urinary sodium excretion rates were measured and found to be similar between the two groups. Because protein intake can influence the activity of the RAS [21] as well as increase GFR [22, 23], the subjects were also prescribed a diet containing 1.5 to 2.0 g of protein per kg body wt. A 24-hour urine collection, obtained prior to study, confirmed similar urinary urea excretion rates in the two groups.

Our first major observation was that baseline values for renal hemodynamic function differed between groups. Subjects in the AC/CC group exhibited significantly lower mean values for GFR, ERPF, and RBF compared with subjects homozygous for the A allele. Values for MAP did not differ between the groups so that lower values for ERPF and RBF in the AC/CC group reflected increased RVR. Myers, Deen, and Brenner were the first to show that infusion of Ang II into normal rats lowers GFR [24], whereas more recently, Allon, Pasque, and Rodriguez have reported that the ACE inhibitor captopril increases the GFR in normal human subjects [25]. Taken together, these studies suggest that intrarenal Ang II activity is an important determinant of intrarenal vascular resistance in normal subjects. Thus, our observation of differences in renal hemodynamic function in patients segregated on the basis of AGT_1R polymorphism could have been due to an increase in intrarenal Ang II activity in the AC/CC subjects.

Our study, therefore, was also designed to test the hypothesis that intrarenal Ang II activity was increased in the AC/CC group compared with the AA group. To test this hypothesis, the renal and peripheral hemodynamic response to AGT_1R blockade with losartan was compared in the two groups. Our second major observation was that subjects in the AC/CC group exhibited significant decreases in MAP and increases in GFR after AGT_1R blockade. In contrast, values for GFR and MAP were unchanged after losartan administration in subjects homozygous for the A allele. The response to losartan in the AC/CC group occurred even though all of the subjects were sodium replete and the studies were performed supine, both of which are conditions expected to blunt the hemodynamic response to AGT_1R blockade [26]. These studies support our hypothesis that intrarenal Ang II activity is increased in the AC/CC group.

Previous studies have investigated the role of the AGT_1R $A^{1166}\rightarrow C$ polymorphism in the hemodynamic response to antihypertensive medications, and in accord with our work, Benetos et al found that the ACE inhibitor perindopril has a greater effect on aortic stiffness in individuals with the C allele [27]. The mechanism responsible for the association between the polymorphism and the hemodynamic response to RAS blockade, as in our study, remains unknown. The $A^{1166}\rightarrow C$ polymorphism is in the 3′ untranslated region of the AGT_1R gene and is therefore not a coding polymorphism that influences ligand binding or receptor signaling [9]. It is possible that the polymorphism is in linkage disequilibrium with a functional polymorphism of the gene promoter, as has recently been discovered for the M235T angiotensinogen polymorphism [28], or alternatively, elements in the 3′ untranslated region that influence transcript stability [29]. Finally, it is also possible that the AGT_1R $A^{1166}\rightarrow C$ polymorphism is a marker for a nearby gene. Any of these mechanisms could account for the observed responses, but they remain hypothetical.

Our findings regarding the aldosterone responses to the experimental maneuvers deserve comment. Similar to other studies [30], we were able to demonstrate a reduction in plasma aldosterone concentrations after losartan administration in our subjects, a response that was independent of genotype. In another study, Hopkins et al examined the relationship between M235T genotype and aldosterone plasma concentrations in hypertensive and normotensive volunteers on a controlled sodium diet and were unable to discern a genotypic effect [31]. Despite the fact that we demonstrated a numeric difference between genotype subgroups in aldosterone values, our data do not demonstrate a significant relationship between AGT_1R $A^{1166}\rightarrow C$ polymorphism and baseline plasma concentrations of aldosterone. It is apparent, however, that the response to losartan administration was more pronounced in those with the C allele, and these results support our hypothesis that the AGT_1R C allele is related to increased Ang II activity. It is interesting to note that we did not discern a losartan-mediated increase in sodium excretion in the AC/CC group, even though there was a rise in GFR and decline in aldosterone. Many factors affect sodium excretion, and in this case, it is possible that such an increase was countered by the decline in arterial pressure, but this cannot be proven from this protocol.

The infusion studies were performed in order to determine if there was an association between the AGT_1R $A^{1166}\rightarrow C$ polymorphism and the renal and systemic hemodynamic response to the receptor ligand, Ang II. Each group of patients exhibited declines in RBF and increases in RVR in response to Ang II, but subjects in the AC/CC group maintained GFR despite declines in RBF. Although the result was not significantly different between groups, there appeared to be a more pronounced numerical increase in the FF in the AC/CC subgroup. The mechanism responsible for the maintenance of GFR in the AC/CC group was not determined, but an attractive hypothesis is that pharmacological Ang II led to a greater increase in efferent arteriolar resistance in the AC/CC group. Additional mechanisms may be proposed. For example, the regulation of receptor

expression by dietary sodium intake may have differed between the two groups [32, 33]. There may have been differences in Ang II-mediated proximal tubular sodium reabsorption between the two groups, which could have resulted in differences in tubular pressure. Alternatively, differences in the ultrafiltration coefficient (K_f) response to Ang II may explain our results. These putative mechanisms cannot be proven by this protocol, and, therefore, must remain speculative. Finally we should also note that our studies were performed with synthetic Ang II (Hypertensin®; Ciba Geigy: 1-L-asparaginyl-5-L-valyl angiotensin octapeptide). Studies in rats suggest that unlike native Ang II, this preparation has a greater renal vascular than peripheral effect, in that low doses that do not increase arterial pressure may still have a significant renal constrictive effect [34].

In summary, we have demonstrated that there is an association between renal hemodynamic function and the AGT_1R A^{1166}→C polymorphism in normal subjects. Specifically, the C allele is associated with lower values for GFR, ERPF, and RBF. Our studies show that there is an association between the C allele and the hemodynamic response to losartan treatment. Receptor blockade lowered blood pressure and increased GFR only in subjects with the C allele. We have also demonstrated that the C allele is associated with a greater FF response to Ang II, presumably secondary to an augmented efferent arteriolar resistance. We interpret these findings to be consistent with the hypothesis that the C allele of the AGT_1R A^{1166}→C polymorphism is related to augmented Ang II activity. Further studies are necessary to determine the genetic locus responsible for this effect.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin converting enzyme; AGT_1R, angiotensin II type 1 receptor; Ang II, angiotensin II; ERPF, renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; Hct, hematocrit; K_f, ultrafiltration coefficient; MAP, mean arterial pressure; PAH, para-aminohippuric acid; PCR, polymerase chain reaction; RAS, renin-angiotensin system; RBF, renal blood flow; RVR, renal vascular resistance.

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