

Transforming Growth Factor- β 1 Production Is Correlated With Genetically Determined ACE Expression in Congenic Rats

A Possible Link Between ACE Genotype and Diabetic Nephropathy

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Genetic background appears to modulate the development of diabetic vascular complications. In particular, polymorphisms in the ACE gene have been associated with diabetic nephropathy and, in some studies, macrovascular complications. However, the links between ACE gene polymorphism and factors implicated in diabetes complications remain unknown. The aim of this study was to determine whether the ACE genotype could modify factors, such as transforming growth factor (TGF)- β 1, involved in the complications of diabetes. For this purpose, congenic rats (L.BNAce10), differing from the LOU strain in only a small segment of chromosome 10 containing the ACE locus, were generated. These congenic rats have plasma ACE levels twice as high as the donor strain. Diabetes was induced in rats of both strains, and its effects on ACE and TGF- β 1 expressions were evaluated in lungs and kidneys. In lung, the main source of ACE production, ACE mRNA levels and activity were higher in L.BNAce10 rats than in LOU rats. Diabetes increased ACE lung expression in rats of both strains in a similar manner. TGF- β 1 expression was also higher in lungs of L.BNAce10 compared with LOU rats and was also increased by diabetes. Furthermore, a strong correlation was found between TGF- β 1 and ACE expressions. In renal arterioles, ACE and TGF- β mRNA expressions were higher in L.BNAce10 rats than LOU rats (both diabetic and nondiabetic). In these vessels, there was also a correlation between ACE and TGF- β 1 expressions. Urine TGF- β 1 concentration depended on the genotype and was further increased by diabetes. These results show that TGF- β 1 expression is correlated with ACE expression and suggest that this growth factor could be a link between ACE gene polymorphism and diabetic vascular complications. *Diabetes* 53: 1111–1118, 2004

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I/D, insertion/deletion; MAPK, mitogen-activated protein kinase; TGF, transforming growth factor.

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The high incidence of vascular complications in diabetes is not fully explained by hyperglycemia or by association with other known risk factors such as hypertension and dyslipidemia. Genetic background seems to contribute to the development of diabetic vascular complications, as suggested by familial studies (1). The genes involved in sensitization to diabetic micro- and macroangiopathy remain unknown, but those of the renin-angiotensin system are possible candidates. An association between the human ACE gene insertion/deletion (I/D) polymorphism and diabetic nephropathy has been found in several studies (2–4). Some, but not all, studies have shown associations between the I/D polymorphism and macrovascular complications (5–8).

The pathophysiological mechanisms relating the ACE genotype to vascular complications have not been established (9). Most studies have aimed to correlate plasma ACE levels with disease, but plasma ACE concentration is a biological phenotype of limited value. Indeed, it has been shown in transgenic mice that tissue-bound ACE concentrations rather than plasma levels are important in the control of blood pressure and renal function (10). It therefore appears necessary to develop animal models allowing the evaluation of interactions between ACE genotypes, tissue ACE concentrations, and diseases such as diabetes.

Hyperglycemia modifies the function of vascular cells by changing their production pattern of several factors, including enzymes, growth factors, adhesion molecules, and vasoactive and coagulation factors (11). These mediators profoundly impair the physiological remodeling of the vessel wall with alterations in extracellular matrix. Among growth factors implicated in vascular remodeling in diabetes, transforming growth factor (TGF)- β 1 appears to be a good candidate (12). TGF- β 1 is a potent regulator of extracellular matrix synthesis and has a regulatory role on cell growth (13). There is recent evidence of its importance in the pathogenesis of complications of diabetes, such as nephropathy and macrovascular disease (14,15). High glucose concentrations are known to increase TGF- β 1 expression (16), but angiotensin II could also

induce it (17). This effect of angiotensin II is further supported by studies showing that ACE inhibitors or AT1 receptor antagonists inhibit TGF- β 1 expression (18,19). However, data about a possible link between TGF- β 1 expression and ACE genotype are not available.

The aim of this study was to determine the influence of ACE genotype on factors, such as TGF- β 1, involved in diabetes complications. For this purpose, we have generated a strain of congenic rats differing from the recipient strain (LOU rats) in only a small part of chromosome 10, which contains the ACE gene. This introgressed segment originated from the BN strain, which presents plasma ACE levels that are double those of the recipient strain (20). These alterations are similar to those observed with the I/D ACE polymorphism in humans and offer the opportunity to evaluate the impact of ACE polymorphism on tissue gene regulation. These rats were thus used to assess tissue ACE expression and its consequences on the basal expression of TGF- β 1, as well as the influence of hyperglycemia on the expression of these two genes depending on the genetic background. ACE and TGF- β 1 expressions were evaluated in lungs (since pulmonary endothelium is the main source of ACE) and kidneys (in glomeruli and arterioles).

RESEARCH DESIGN AND METHODS

All studies involving rats were performed in accordance with the principles set forth in the Guide for the Care and Use of Laboratory (authorization no. 05577 from the French Ministry of Agriculture).

Crossbreeding procedures. The basic breeding scheme was to insert the segment of chromosome 10 of the BN (B) rat, which contains the ACE gene, into the genetic background of the LOU (L) strain. BN rats were initially crossed with LOU rats and the resulting F1 rats crossed to produce F2 rats. Then, BNxLOU F2 rats homozygous for the BN allele (BB) at the ACE locus were backcrossed with LOU rats to produce the first backcross generation (BC1). These rats were then crossed to generate the first congenic progeny (C1). These two steps were repeated five times. A genotype-based selection was performed to select breeders for the third and fourth backcrosses. C2 rats homozygous (BB) for three markers located at the ACE locus (D10Mgh4, D10Mit1, and D10Wox17) were used to produce the BC3 generation. A more rigorous selection was further performed on C3 rats homozygous BB at the ACE locus: 23 polymorphic markers were used to genotype rats and two animals homozygous BB for the 3 markers at the ACE locus and almost completely homozygous LL for the other markers were used as breeders for the fourth backcross. A complete genotyping of C5 rats was carried out on animals homozygous BB for the ACE polymorphic marker (D10Mit1).

Genotyping of rats. Genomic DNA was prepared by phenol extraction from a 1-cm fragment of the tip of the tail. The (CA)_n repeat located at the 5' end of intron 13 of the rat ACE gene was genotyped as described previously (20). Genotyping of C2, C3, and C5 rats was performed by PCR amplification of total genomic DNA around a selection of microsatellite markers known to exhibit allele variations between BN and LOU strains, using the appropriate PCR primer pairs (http://www.well.ox.ac.uk/rat_mapping_resources) as previously described (21).

In vivo experiments. Diabetes was induced in rats by the injection of a high dose of streptozotocin (65 mg/kg) in a citrate buffer into the jugular vein. Once a week, animals were housed in metabolic cages and urine and blood samples were collected. Blood pressure was measured by the tail cuff method.

Four weeks after the induction of diabetes, rats were killed under pentobarbital anesthesia. Blood was sampled, and aorta, lung, kidney, and heart were quickly excised, rinsed, and frozen in liquid nitrogen for subsequent RNA or protein extractions.

Analytical techniques. Blood glucose concentration was measured using a glucose oxidase method. Plasma and urine creatinine were evaluated by an enzymatic method using a commercial kit (Sigma, St. Louis, MO). Plasma renin activity was determined through the measurement of angiotensin I generated in vitro (20).

Urinary albumin concentration was measured by immunonephelometry, using a rabbit anti-rat albumin antibody (22). Briefly, 150 μ l urine were incubated with 40 μ l anti-rat albumin antibody (ICN BioMedical, Aurora, OH),

and immunocomplexes were measured by an immunonephelometer. Results were calculated using a standard curve with rat albumin (Sigma).

Isolation of renal afferent arterioles and glomeruli. Renal afferent arterioles and glomeruli were isolated after perfusion with a magnetized iron oxide suspension (1% Fe₃O₄ in isotonic saline solution), excision, and decapsulation (23). Cortical tissue, dissected from the medulla, was minced with a razor blade and then filtered through sieves of 106 μ m. The material remaining on the top of the sieve was passed through an 18-gauge needle five times and separated with the aid of a magnet; this tissue is mainly the afferent arterioles, as checked with light microscopy.

Glomeruli, which went through the 106- μ m sieves, were recuperated on the top of a 75- μ m sieve. Both arterioles and glomeruli were lysed in Trizol (Life Technologies) for RNA extraction.

ACE and TGF- β 1 mRNA expressions. After reverse transcription with random priming, expressions of ACE and TGF- β 1 were evaluated by real-time RT-PCR assays using a Light-Cycler with the FastStart DNA Master SYBR Green Kit (Roche Diagnostics, Meylan, France). For quantification, a standard curve was generated with six different amounts of cDNA. Light-Cycler software was used for the analysis. Oligo primers for ACE mRNA amplification were 5'-TCCAGTTCAGTTCACGA-3' for the antisense and 5'-CTAGG AAGAGCAGCACCA-5' for the sense; for TGF- β 1, they were 5'-CTGGAA AGGGCTCAACACC-3' and 5'-GTAGACGATGGGCGAGTGGCT-3'. ACE and TGF- β 1 mRNA expressions were normalized to the housekeeping gene β -actin, amplified using the following primers: 5'-TGGAACTCTGTGGCATCCAT GAAAC-3' and 5'-TAAACGCAGCTCAGTAAACAGTCCG-3'.

ACE activity. Tissue samples were homogenized in 10 vol of cold 50 mmol/l Tris-HCl buffer, pH 7.4, using a Teflon-glass homogenizer. After centrifugation of the homogenate, the supernatant was recovered, sonicated, and then assayed for ACE activity. To evaluate the shedding of ACE, tissue explants were incubated in Dulbecco's modified Eagle's medium at 37°C for 24 h. ACE activity in plasma, tissue extracts, or conditioned media was measured using a fluorometric assay, as previously described (20).

Quantification of TGF- β 1. TGF- β 1 was measured in plasma, urine, conditioned media, and tissue extracts. Plasma samples were diluted 1/100, and urine was concentrated 10-fold using Nanosep centrifugal devices (cutoff 10 kDa; Pall Gelman Laboratory, Ann Arbor, MI). Active and total TGF- β 1 levels were determined before and after acid treatment, respectively, using an immunoassay system (Promega, Madison, WI).

Statistical analysis. Data are expressed as means \pm SE. Clinical and biological data of animals were compared between groups by two-way ANOVA during multiple regression analysis to assess differences according to ACE genotype (animal strain), glycemic status (diabetic or nondiabetic animals), and the interaction between the two parameters. The variations in plasma ACE activity with time were compared between groups by a repeated-measures ANOVA, with ACE genotype and glycemic status as cofactors. The correlation between ACE and TGF- β 1 values was tested by linear regression analysis. For all of these comparisons, data were log transformed when the normality of the distribution was rejected by the Shapiro-Wilk *W* test. Differences were considered significant when *P* < 0.05. Statistics were performed with JMP software (SAS Institute, Cary, NC).

RESULTS

Development of the congenic strain. A congenic strain (L.BNAce10) was produced through introgression of the segment of chromosome 10 containing the ACE locus from the BN strain into the recipient LOU strain. A genomewide screening of congenic rats was carried out on animals that were homozygous BB for the ACE polymorphic marker (D10Mit1) and for two closely linked polymorphic markers (D10Mgh4 and D10Mit7) (Fig. 1). Five other polymorphic markers localized on chromosome 10 and 46 markers dispersed over the other chromosomes were used for this purpose. The congenic rats were homozygous LL for the two markers, D10Mgh1 and D10Mgh14 (Fig. 1), localized on either side of the ACE locus and for all the other 49 markers. The congenic strain was maintained by brother-sister mating.

General characteristics of rats. Clinical data of rats are shown in Table 1. Fasting plasma glucose was similar in animals of the same glycemic status in both strains. Body weight was significantly decreased in diabetic animals of

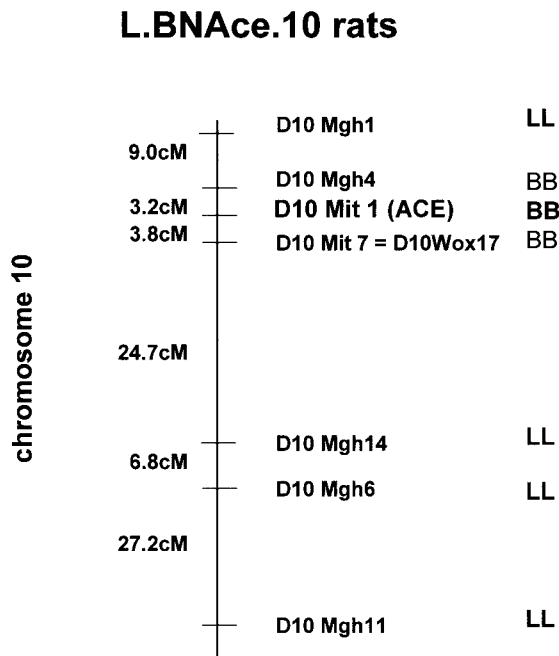


FIG. 1. Genetic map of the ACE locus on chromosome 10 of the congenic strain L.BNAce10.

both strains, with no strain-related differences observed. No differences in blood pressure were observed in the four groups of animals, but heart rate was significantly higher in diabetic rats of both strains.

ACE plasma levels in L.BNAce10 rats were twofold higher compared with LOU rats (189.2 ± 5.2 vs. 91.4 ± 2.7 $\text{nmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$) (Fig. 2). This difference is similar to that observed between LOU and BN rats, confirming that ACE expression in L.BNAce10 rats is comparable to that of the donor strain (20). Diabetes induced a small but significant increase in plasma ACE levels, whatever the genetic background (Fig. 2). This increase was observed 1 week after the onset of diabetes and became significantly different after the second week. ANOVA showed an effect of genotype ($P < 0.0001$), diabetes ($P < 0.01$), and time ($P < 0.001$).

Plasma renin activity was not different between LOU and L.BNAce10 rats but decreased with diabetes (Table 1). TGF- β 1 plasma concentrations were similar in both

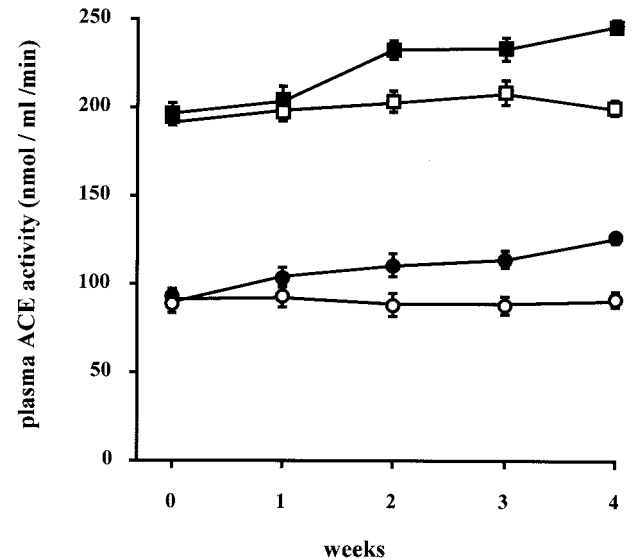


FIG. 2. Evolution of plasma ACE activity during diabetes in congenic L.BNAce10 rats compared with LOU rats. Groups are diabetic (filled symbols), nondiabetic (open symbols), LOU (circles), and L.BNAce.10 (squares) rats. $n =$ at least 10 rats per group. Repeated-measures ANOVA shows an effect of genotype ($P < 0.0001$), diabetes ($P < 0.01$), and time ($P < 0.001$), without interaction.

strains, and these values remained unchanged after 4 weeks of diabetes (Table 1).

Urinary albumin excretion was low in nondiabetic animals with no difference between strains (Table 1). Diabetes increased these levels in both strains, but to a greater extent in L.BNAce10 rats. ANOVA showed a significant effect of diabetes ($P < 0.05$), but not of genotype, probably due to the high variability of values.

ACE and TGF- β in lung. Since pulmonary endothelium represents the main source of ACE, the expression of ACE in lungs was studied in diabetic and nondiabetic rats of both strains. TGF- β 1 expression was evaluated in the same lung samples. The results are presented in Figs. 3 and 4 and summarized in Table 2. ACE mRNA level in lungs of L.BNAce10 rats was almost twice as high as that of LOU rats, a difference similar to that described between LOU and BN rats (20). Diabetes increased ACE mRNA levels in both strains (Fig. 3A). Similarly, TGF- β 1 mRNA levels in lungs of L.BNAce10 rats were higher than in LOU

TABLE 1
Characteristics of rats

	Lou		L.BNAce10		<i>P</i> values		
	Control	Diabetes	Control	Diabetes	Genotype	Diabetes	Interaction
<i>n</i>	12	17	12	16			
Body weight (g)	272 \pm 8	199 \pm 8	276 \pm 9	205 \pm 10	NS	<0.0001	NS
FPG (mmol/l)	5.18 \pm 0.21	23.32 \pm 1.54	4.67 \pm 0.29	23.89 \pm 1.57	NS	<0.0001	NS
Blood pressure (mmHg)	148 \pm 3	150 \pm 3	150 \pm 4	157 \pm 6	NS	NS	NS
Heart rate (bpm)	347 \pm 14	380 \pm 12	369 \pm 13	400 \pm 9	NS	0.02	NS
Plasma values							
Plasma renin activity	31.9 \pm 1.7	18.6 \pm 3	27.1 \pm 1.8	17.7 \pm 2	NS	<0.0001	NS
Active TGF- β 1 (ng/ml)	16.5 \pm 0.7	15.6 \pm 0.9	19.3 \pm 2.13	18.1 \pm 1.68	NS	NS	NS
Total TGF- β 1 (ng/ml)	186.5 \pm 24.7	177.9 \pm 19.8	185.5 \pm 24.5	191.4 \pm 22.1	NS	NS	NS
Urine values							
Albuminuria (mg/ μ mol creatinine)	3.74 \pm 0.74	5.61 \pm 1.11	3.34 \pm 0.32	12.11 \pm 4.12	NS	<0.05	NS

Data are means \pm SE. FPG, fasting plasma glucose.

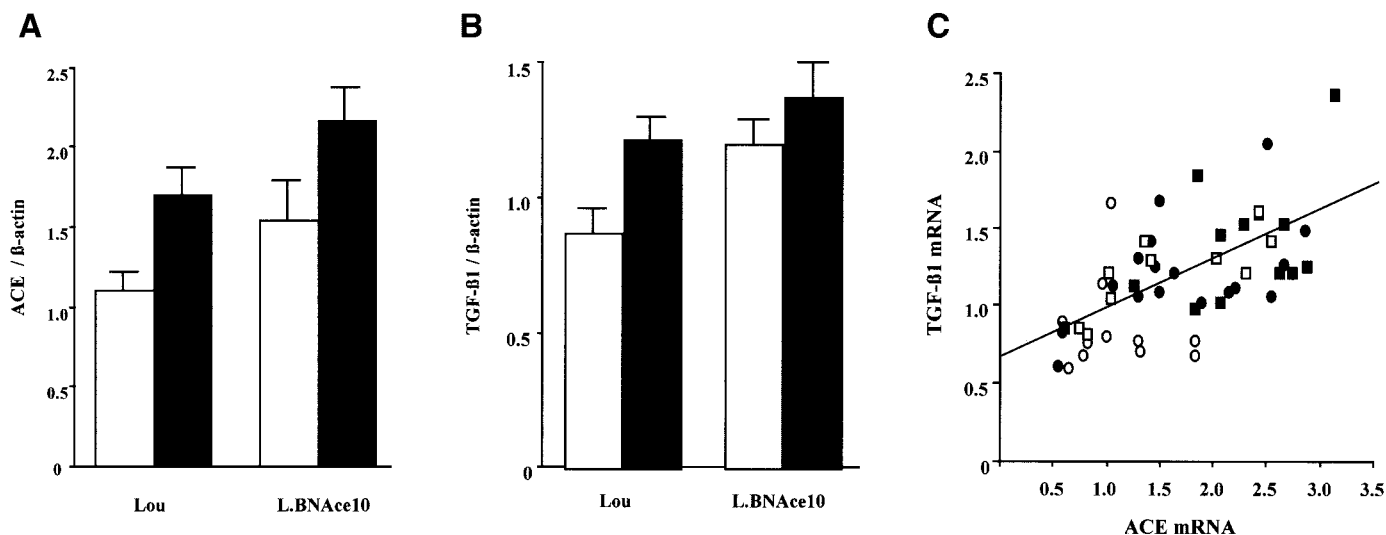


FIG. 3. ACE (A) and TGF- β 1 (B) mRNA levels in lungs of diabetic (■) and nondiabetic (□) LOU and L.BNAce10 rats, assessed by real-time RT-PCR. ANOVA shows that ACE mRNA and TGF- β 1 mRNA levels increased with genotype and diabetes. C: Correlation between ACE and TGF- β 1 mRNA levels in diabetic (filled symbols), nondiabetic (open symbols), LOU (circles), and L.BNAce10 (squares) rats.

rats, and diabetes increased this expression in both strains (Fig. 3B). The increases induced by diabetes in ACE and TGF- β 1 mRNA levels were similar in both strains. Intergroup regression analysis (Fig. 3C) showed a correlation between ACE and TGF- β 1 mRNA levels (log-transformed data, $r = 0.644$, $P < 0.0001$). Analysis within each group showed a correlation for all groups (L.BNAce10 rats: diabetic $r = 0.592$, $P < 0.05$, nondiabetic $r = 0.847$, $P < 0.05$; LOU rats: diabetic $r = 0.669$, $P < 0.01$) except for nondiabetic LOU rats.

The tissue-bound quantities of both proteins, ACE and TGF- β 1, were evaluated in lung extracts. Diabetes increased ACE activity and TGF- β 1 concentration in both strains (Figs. 4A and B and Table 2). As for TGF- β 1 mRNA values, the protein concentration was dependent on the genotype.

To evaluate the release of ACE and TGF- β 1, samples of lung tissue were incubated in culture medium for 24 h and ACE activity and total TGF- β 1 concentration were measured in these conditioned media. ACE activity in conditioned media was higher in L.BNAce10 rats than in LOU rats, and diabetes increased this activity (Fig. 4C). In these conditioned media, TGF- β 1 concentration was very low (data not shown), suggesting that most of the TGF- β 1 produced remained tissue bound. This result could explain the absence of any difference in TGF- β 1 plasma concentrations between L.BNAce10 and LOU rats.

TGF- β 1 concentration in tissue extracts was correlated with both tissue ACE activity (log-transformed data, $r = 0.702$, $P < 0.0001$) and ACE activity in conditioned media (log-transformed data, $r = 0.629$, $P < 0.0001$) (Fig. 4D and E, respectively). Regression analysis within each group showed a correlation between TGF- β 1 concentration and tissue ACE activity for all groups (L.BNAce10 rats: diabetic $r = 0.616$, $P < 0.05$, nondiabetic $r = 0.568$, $P = 0.08$; LOU rats: diabetic $r = 0.528$, $P < 0.05$) except for nondiabetic LOU rats.

ACE and TGF- β 1 in arterioles and glomeruli. The expression of the ACE gene in arterioles and glomeruli from L.BNAce10 and LOU rats was also evaluated by

real-time RT-PCR (Table 2 and Fig. 5). As observed in pulmonary tissue, ACE mRNA level in arterioles was dependent on both the genotype at the *Ace* locus and the presence of diabetes (Fig. 5A). Similarly, TGF- β 1 mRNA levels were higher in arterioles of L.BNAce10 rats than in LOU rats. Diabetes enhanced these levels (Fig. 5B), with similar increases in both strains. Intergroup regression analysis (Fig. 5C) showed a correlation between ACE and TGF- β 1 mRNA levels (log-transformed data: $r = 0.667$, $P < 0.0001$). Analysis within each group showed a correlation for all four groups (log-transformed data: L.BNAce10 rats: diabetic $r = 0.786$, $P < 0.05$, nondiabetic $r = 0.851$, $P < 0.01$; LOU rats: diabetic $r = 0.707$, $P < 0.05$, nondiabetic $r = 0.889$, $P < 0.05$).

Contrasting with results in arterioles, the ACE mRNA level in glomeruli did not change in either rat strain, in the presence or absence of diabetes (Table 2). This result could be explained by the low expression level of ACE in the glomeruli. Similar results were observed with TGF- β 1 expression in this tissue (Table 2). Immunohistochemical analysis of the kidney using TGF- β 1 antibodies showed a very low level of glomerular staining compared with tubules in all groups of rats; no difference was observed in diabetic rats (data not shown).

Urine TGF- β 1. Unlike plasma levels, TGF- β 1 concentration in urine was higher in L.BNAce10 rats than in LOU rats ($P < 0.05$), and a considerable increase was observed with diabetes ($P < 0.001$) (Fig. 6). The diabetes-induced TGF- β 1 increase was higher in L.BNAce10 rats than in LOU rats, although the interaction between genotype and diabetes did not reach statistical significance ($P = 0.07$).

DISCUSSION

In this study, we have developed a congenic rat strain differing from the LOU strain only in the locus on chromosome 10, containing the ACE gene, which originates from the BN strain. Since the genetic difference between this congenic strain (L.BNAce10 rats) and the recipient strain (LOU rats) is limited to the introgressed segment (the

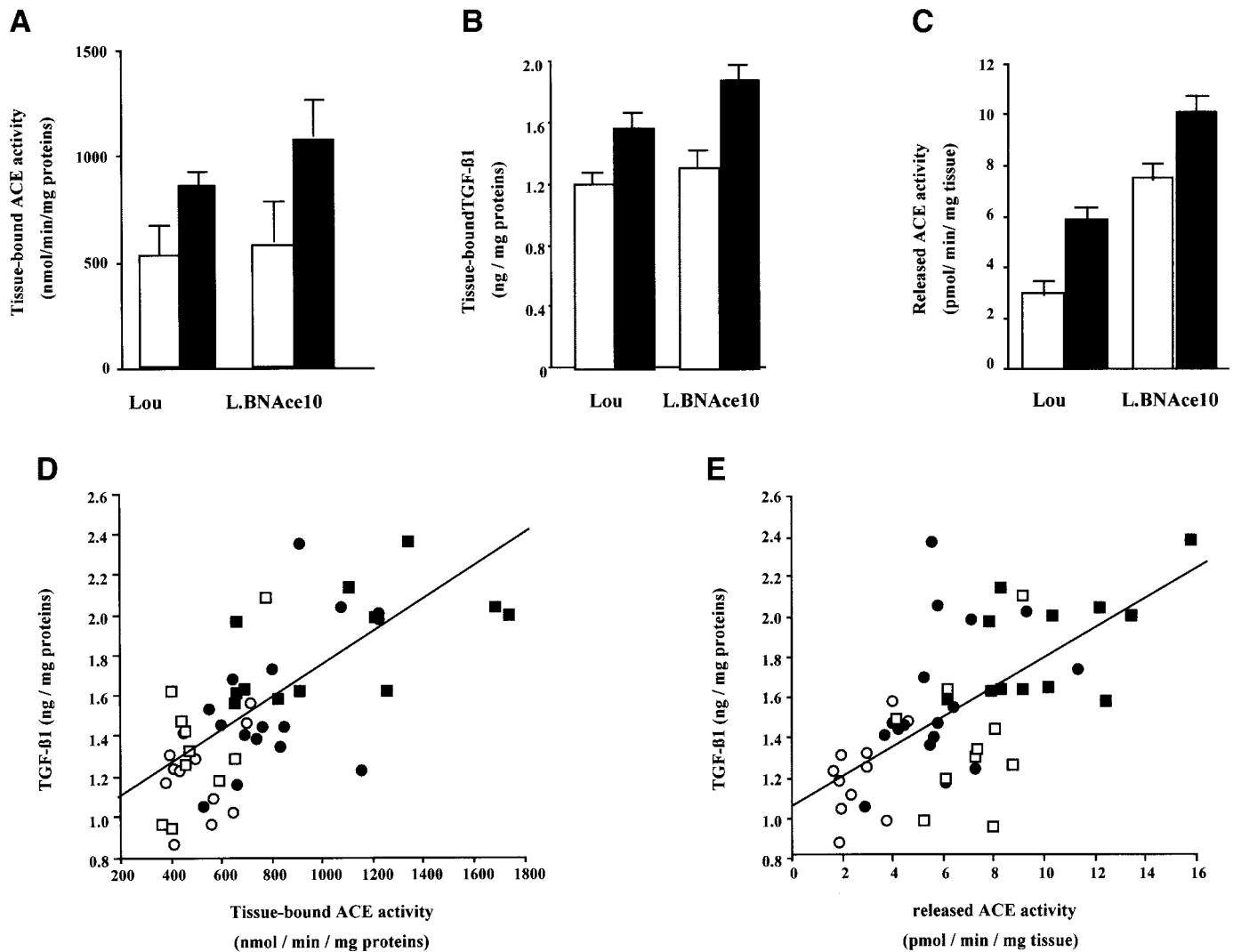


FIG. 4. Tissue-bound and -released ACE activities and TGF- β 1 concentrations in lungs. *A* and *B*: Total ACE activity and TGF- β 1 concentration in lung extracts. ANOVA shows an effect of genotype and diabetes, with no interaction between them. *C*: ACE activity released by lung, evaluated by incubating lung tissue in culture medium and measuring the ACE activity in the conditioned media. ANOVA shows an effect of genotype and diabetes without interaction. *D* and *E*: Correlation between tissue-bound (*D*) or -released (*E*) ACE activity and TGF- β 1 concentrations in diabetic (filled symbols), nondiabetic (open symbols), LOU (circles), and L.BNAce.10 (squares) rats.

region of the ACE gene), this model provides a powerful tool to investigate the functional disturbances associated with ACE genotype. Plasma ACE levels in L.BNAce10 rats were identical to those of BN rats and twofold higher than those of the LOU rats, showing that the genotype at the ACE locus accounts for a large part of the total plasma ACE levels. These results are similar to those observed in humans, in which the I/D polymorphism is a major determinant of circulating ACE levels (24).

Plasma ACE levels are increased in patients with diabetes (25,26). This increase is independent of the genotype, as shown in humans (2) or in mice having several copies of the ACE gene (27). In the present study, we confirm that diabetes enhanced plasma ACE activity within a short period of time (2 weeks), independently of the genotype at the *Ace* locus. In addition, we demonstrate that lung ACE mRNA level and ACE shedding from pulmonary endothelium depend on the *Ace* genotype and that diabetes enhances both, suggesting that the greater plasma ACE levels related to genotype and/or diabetes are due to a

higher production rather than to a decrease in blood clearance.

Despite differences in plasma ACE levels, blood pressure values were similar in L.BNAce10 and LOU rats. However, the increase in tissue ACE levels enhances angiotensin II concentrations, which could regulate gene expression locally. TGF- β 1 could be one of the affected genes, since angiotensin II stimulates TGF- β 1 in vitro (17) and ACE inhibitors decrease TGF- β 1 in vivo (18,28). This is confirmed by our results showing a higher expression of TGF- β 1 in L.BNAce10 rats than in LOU rats. Moreover, a strong correlation was found, not only between ACE and TGF- β 1 mRNA levels, but also between ACE activity and TGF- β 1 protein levels. These correlations suggest that the ACE gene polymorphism accounts in part for the variation in TGF- β 1 expression, since these rats differ only with respect to a segment of rat chromosome 10, whereas chromosome 1, containing the TGF- β 1 gene, is identical in both strains.

Besides ACE gene expression, TGF- β 1 expression was

TABLE 2
Effect of genotype and diabetes on ACE and TGF- β 1 expressions in lung and kidney

	Lou		L.BNAce10		P values		
	Control	Diabetes	Control	Diabetes	Genotype	Diabetes	Interaction
Lung mRNA							
<i>n</i>	11	17	12	12			
ACE	1.10 \pm 0.13	1.70 \pm 0.17	1.52 \pm 0.23	2.17 \pm 0.21	0.009	0.004	NS
TGF- β 1	0.87 \pm 0.09	1.22 \pm 0.08	1.20 \pm 0.09	1.38 \pm 0.12	0.006	0.018	NS
Lung proteins							
<i>n</i>	12	17	12	12			
Tissue-bound ACE activity (nmol \cdot min ⁻¹ \cdot mg protein ⁻¹)	528.9 \pm 30	801.5 \pm 58	505.6 \pm 35	1055 \pm 112	NS	<0.0001	NS
Tissue-bound TGF- β 1 (ng/mg protein)	1.21 \pm 0.06	1.57 \pm 0.09	1.37 \pm 0.11	1.88 \pm 0.09	0.015	<0.0001	NS
Released ACE activity (pmol \cdot min ⁻¹ \cdot mg tissue ⁻¹)	2.95 \pm 0.29	5.83 \pm 0.5	7.47 \pm 0.54	10.11 \pm 0.81	<0.0001	<0.0001	NS
Kidney mRNA							
<i>n</i>	5	11	9	8			
Arterioles ACE	1.85 \pm 0.29	3.19 \pm 0.44	2.91 \pm 0.40	4.11 \pm 0.34	0.021	0.004	NS
Arterioles TGF- β 1	1.47 \pm 0.23	2.07 \pm 0.18	2.53 \pm 0.59	4.90 \pm 1.05	0.007	0.047	NS
Glomeruli ACE	0.43 \pm 0.02	0.57 \pm 0.05	0.36 \pm 0.03	0.43 \pm 0.03	NS	NS	NS
Glomeruli TGF- β 1	0.11 \pm 0.01	0.15 \pm 0.04	0.10 \pm 0.01	0.13 \pm 0.01	NS	NS	NS

Data are means \pm SE.

also modified by diabetes. However, no interaction was found between the *Ace* genotype and diabetes. This lack of interaction between the ACE gene polymorphism and diabetes in inducing TGF- β 1 gene expression could be explained by the mechanisms implicated in the regulation of this expression. It has been shown that increased protein kinase C activity in the diabetic state leads to activation of the mitogen-activated protein kinase (MAPK) cascades such as extracellular signal-regulated kinase and p38 MAPK (29). Moreover, a recent study has demonstrated that angiotensin II shares this mechanism with hyperglycemia in inducing TGF- β 1 synthesis: both stimulate TGF- β 1 gene expression through the same protein kinase C- and p38 MAPK-dependent pathways and the same regulatory elements of the TGF- β 1 promoter (30).

In view of studies suggesting an association between

ACE gene polymorphism and diabetic nephropathy, as well as the role of TGF- β 1 in the development of this complication, the changes in ACE and TGF- β 1 expressions in the kidney were further investigated. We evaluated the expression of these genes in glomeruli and arterioles, as well as the concentration of TGF- β 1 in urine, which may reflect tubular expression (31). As observed in lung, ACE and TGF- β 1 expressions in arterioles were higher in L.BNAce10 rats than in LOU rats. Diabetes increased both expressions without interaction with the genotype. ACE or TGF- β 1 expressions in glomeruli were low and not modified either with genotype or with diabetes. It has been reported that ACE expression is low in glomeruli (32); however, data concerning glomerular TGF- β 1 levels are more controversial. Diabetes has been shown to increase TGF- β 1 levels in glomeruli in humans and in several

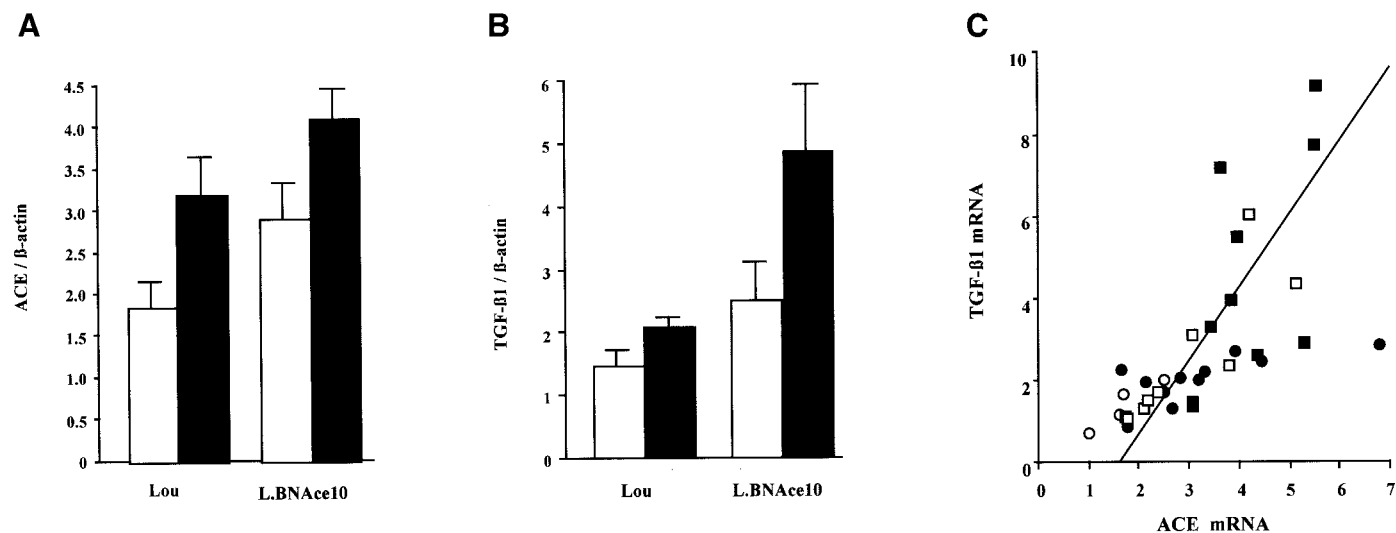


FIG. 5. ACE (A) and TGF- β 1 (B) mRNA levels in renal arterioles of diabetic (■) and nondiabetic (□) LOU and L.BNAce10 rats, as assessed by real-time RT-PCR. ANOVA shows an effect of genotype and diabetes on ACE and TGF- β 1 mRNA levels. C: Correlation between ACE and TGF- β 1 mRNA levels in renal arterioles of diabetic (filled symbols), nondiabetic (open symbols), LOU (circles), and L.BNAce10 (squares) rats.

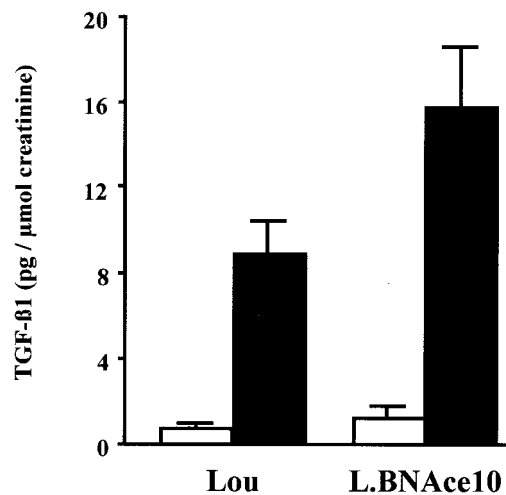


FIG. 6. Urinary TGF- β 1 concentration in urine of diabetic (■) and nondiabetic (□) LOU and L.BNAce10 rats. ANOVA shows an effect of genotype and diabetes.

models of experimental diabetes (33,34), but in a streptozocin model of diabetes in rats, the immunoreactivity of TGF- β 1 in glomeruli decreased slightly during the acute phase and returned to control levels 30 days after induction of diabetes (35). Further studies at different stages of diabetes in our experimental model will be helpful to understand the role of hyperglycemia and ACE genotype in the control of glomerular TGF- β 1 levels.

Urinary TGF- β 1 concentration was slightly but significantly higher in L.BNAce10 rats compared with LOU rats, and diabetes induced a dramatic increase in this concentration without significant interaction with the *Ace* genotype. Urinary TGF- β 1 could come from glomerular filtration but probably mostly reflects production by renal tubules. This is supported by immunohistochemical analysis showing important staining with TGF- β 1 antibodies in tubular epithelial cells (data not shown). These results confirm the role of ACE in the control of TGF- β 1 levels, as has been suggested by studies showing that ACE inhibitors decrease TGF- β 1 levels (18,19).

The pathophysiological consequences of the changes in ACE expression in the kidney could involve both hemodynamics and remodeling of the extracellular matrix (36). First, the increase in ACE in arterioles may enhance local concentrations of angiotensin II, leading to an increase in intraglomerular pressure, one of the major mechanisms in the progression of renal disease (37,38). Second, the local increase in angiotensin II will enhance TGF- β 1 expression, leading to an overexpression of collagen genes and subsequent interstitial fibrosis. This interstitial fibrosis is correlated with renal dysfunction, as documented in several studies, although the relative contributions of glomerular versus tubulointerstitial changes to disease progression remain uncertain (18,39).

In view of our results, TGF- β 1 may be a link between ACE gene polymorphism and diabetic nephropathy. However, it may not be the only one. Several factors implicated in the vascular complications of diabetes, such as endothelins, thromboxanes, vascular endothelial growth factor, or cell adhesion molecules, have been shown to be regulated by both hyperglycemia and angiotensin II. Further studies will be necessary to evaluate whether these or

other factors are influenced by ACE gene polymorphism. The congenic rats developed in this study provide an interesting model to elucidate mechanisms related to the genetic determinants of ACE leading to diabetes complications. Such information is important, not only for the further understanding of the observed associations, but also for applying such insights on the role of genetic factors to the development of improved strategies for disease intervention.

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