Tubuloglomerular feedback in ACE-deficient mice

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1Departments of Physiology and Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109; 2Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27599; 3Guilford Medical Associates, Greensboro, North Carolina 27405; and 4National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892

Traynor, Timothy, Tianxin Yang, Yuning G. Huang, John H. Krege, Josie P. Briggs, Oliver Smithies, and Jürgen Schnermann. Tubuloglomerular feedback in ACE-deficient mice. Am. J. Physiol. 276 (Renal Physiol. 45): F751–F757, 1999.—In these experiments, we used a strain of angiotensin converting enzyme (ACE) germline null mutant mice, generated by J. H. Krege and co-workers (J. H. Krege, S. W. M. John, L. L. Langenbach, J. B. Hodglin, J. R. Hagaman, E. S. Bachman, J. C. ennet, D. A. O’Brien, and O. Smithies. Nature 375: 146–148, 1995), to examine the effect of chronicACE deficiency on the magnitude of tubuloglomerular feedback (TGF) responses. The genotype was determined by PCR on DNA extracted from the tail and was verified after each experiment by assessment of the blood pressure response to an injection of ANG I. To assess TGF responsiveness, we determined the change in stop-flow pressure (PSF) caused by increasing NaCl concentration at the macula densa by using micropuncture techniques. When loop of Henle flow rate was increased from 0 to 40 nl/min, PPSF fell from a mean of 42.3 ± 1.95 to 33.6 ± 2.09 mmHg (n = 6, P = 0.005) in wild-type mice (+/+); fell from 40.6 ± 2.35 to 38.6 ± 1.93 mmHg in heterozygous (+/−) mice (n = 7, P = 0.014); and did not change in homozygous ACE (−/−) mice (36.7 ± 2.02 mmHg vs. 36.4 ± 2.01 mmHg; n = 4, P = not significant (NS)). During an infusion of ANG II at a dose that did not significantly elevate blood pressure (70 ng·kg−1·min−1), TGF response magnitude (PSF + PSFAT) increased from 6.5 ± 1.4 to 9.8 ± 1.19 mmHg in +/+ (P = 0.006), from 1.14 ± 0.42 to 4.6 ± 1.3 mmHg in +/− (P = 0.016), and from 0.42 ± 0.25 to 4.02 ± 1.06 in −/− mice (P = 0.01). Absence of TGF responses in ACE null mutant mice and restoration of near-normal responses during an acute infusion of ANG II supports previous conclusions that ANG II is an essential component in the signal transmission pathway that links the macula densa with the glomerular vascular pole.

transgenic mouse; gene knockout; micropuncture; angiotensin converting enzyme; stop-flow pressure

AN INCREASE in NaCl concentration at the macula densa cells causes a prompt increase in the resistance of afferent arterioles and a subsequent decrease in glomerular capillary pressure and glomerular filtration rate (3). There is abundant evidence to indicate that the effect of NaCl on vascular tone is linked to Na-K-2Cl cotransport activity in macula densa cells and that an alteration in transport causes the formation of a paracrine mediator capable of interacting with the vascular smooth muscle cells of the afferent arterioles (3). Identification of this flow-dependent mediator is still incomplete, mainly because interference with the generation or action of a number of potential vasoactive compounds has been demonstrated to affect the tubulovascular response magnitude. Results from a variety of technical approaches lend support to the notion that the renin-angiotensin system is intimately involved in determining the magnitude of the vascular response.

Pharmacological inhibition of ANG II type 1 (AT1A) receptors and of converting enzyme has been shown to reduce the magnitude of the tubulovascular response (8, 16, 18, 22, 25, 28), whereas peritubular and systemic infusion of ANG II was found to enhance the vascular response magnitude (13, 19). The physiological implication of the dependence of feedback regulation on ANG II levels is that a change in the activity of the renin-angiotensin system may be responsible for the alteration in macula densa-dependent responses seen during changes in extracellular fluid volume (3, 21).

The generation of transgenic mice with null mutations in components of the renin-angiotensin system offers a new way to examine the role of ANG II in tubuloglomerular feedback (TGF) coupling. Previous results have shown that the effect of changes in macula densa NaCl concentration on afferent arteriolar tone is virtually abolished in mice with a knockout mutation in the AT1A receptor gene (24). Two laboratories have independently generated mice with a targeted disruption of the angiotensin converting enzyme (ACE) gene that interferes with the production of functional ACE enzymes of both the somatic and testicular isoforms (5, 10). ACE enzyme activity in plasma and tissues such as lung, kidney, heart, and brain, as well as ACE mRNA and protein in various tissues, was virtually undetectable in homozygous ACE-knockout mice (5, 26). The current experiments in one of these ACE-knockout strains were performed to examine whether interference with the renin-angiotensin system by an independent intervention results in the same alteration of the tubulovascular signaling pathway previously found in the AT1A receptor knockout animals (24). The second aim of these studies was to test whether an acute administration of ANG II can restore the ability of increases in distal NaCl concentration to cause afferent vasoconstriction.

METHODS

Experiments were performed in a strain of ACE germline null mutant mice that has been generated as described by...
Krege et al. (10). All animals used in this study [wild-type ACE (+/+), heterozygous ACE (+/-), and homozygous ACE (−/−)] were derived from a heterozygous breeder pair from the original North Carolina colony. At weaning, animals were ear-tagged and a short piece of the tail was clipped off. Genomic DNA was extracted from the tail samples with a standard procedure involving digestion with proteinase K and purification of DNA with ethanol. We determined the genotype of each DNA sample by testing for the absence of wild-type or modified ACE sequence using PCR. To identify the presence of the wild-type gene, ACE gene-specific primers upstream and downstream of the wild-type ACE sequence using PCR. To identify and purification of DNA with ethanol. We determined the ear-tagged and a short piece of the tail was clipped off.

![Image](https://via.placeholder.com/150)

A Fig. 1. PCR genotyping of angiotensin converting enzyme (ACE) mutant mice. A and B: bars (exons) and lines (introns) indicate schematically the position of PCR products on ACE and neomycin resistance genes (Neo’), respectively. C: example of genotyping gels, with −/− mice being positive for Neo’ and negative for wild-type product, and +/+ mice being positive for wild-type and negative for Neo’. E 14 and E 15, exon 14 and 15.

RESULTS

Experiments were performed in six +/+ mice, seven +/+ mice, and four −/− mice. Average body and kidney weights, respectively, were 27.7 ± 0.6 g and 168 ± 3.8 mg in wild-type, 27.6 ± 2 g and 149 ± 14.7 mg in heterozygous mice, and 30.2 ± 0.95 g and 173 ± 12.8 mg in homozygous mice, values not significantly different between genotypes. Mean arterial blood pressure of the anesthetized mice was 100 ± 3.4 mmHg in wild-type (78.5–110 mmHg), 90.5 ± 2.9 mmHg in heterozygous (83–102 mmHg), and 79.5 ± 5.9 mmHg in homozygous mice (73–97 mmHg; P = 0.057 compared with wild-type). To determine whether elevated bradykinin levels may contribute to the low blood pressure in ACE-knockout mice, two ACE −/− and two ACE +/+ mice were injected intravenously with 10 µg of the bradykinin B2-receptor antagonist Hoe-140 immediately after cannulation of the femoral vein. No sustained blood pressure effects were observed in Hoe-140-pretreated mice. Blood pressure responses to exogenous bradykinin were totally absent in ACE −/− mice, but responses were similar in ACE +/+ mice, confirming that ACE is essential for the response to bradykinin.
ANG I were similar between ACE +/+ and ACE +/− mice but were markedly blunted in ACE −/− animals, providing an independent verification of the ACE-null genotype (Fig. 2). Blood pressure responses to ANG II were not significantly different between ACE +/+ , +/−, and −/− animals (Fig. 2).

In response to an increase in loop of Henle flow rate from 0 to 40 nl/min, PSF fell from a mean of 42.3 ± 1.95 to 33.6 ± 2.09 mmHg (mice/tubules = 6/23; P = 0.005) in wild-type mice and from 40.6 ± 2.35 to 38.6 ± 1.93 mmHg in ACE +/+ mice (Fig. 3). Blood pressure responses to ANG II infusion tended to be somewhat lower than those measured during control in wild-type mice, the difference did not achieve statistical significance, suggesting that angiotensin infusion restored responsiveness to near normal levels. During ANG II infusion, PSF, in the absence of loop perfusion, averaged 46.3 ± 2.2 mmHg in +/+ mice, 39.8 ± 2.2 mmHg in +/− mice, and 41.7 ± 2.07 mmHg in −/− mice, values not significantly different from each other (as determined by ANOVA). Mean arterial blood pressure during ANG II infusion averaged 101 ± 5.6 mmHg in +/+ mice (vs. 99.2 ± 5.6 mmHg before the infusion), 89.5 ± 3.1 mmHg in +/− mice (vs. 88.4 ± 3.3 mmHg before ANG II), and 78.7 ± 2.8 mmHg in −/− mice (vs. 77.1 ± 2.8 mmHg before ANG II). Blood pressures were not significantly elevated by ANG II in any of the three groups of mice.

**DISCUSSION**

Previous studies from our laboratory have shown that TGF responses are essentially absent in mice homozygous for a null mutation in the AT1A receptor gene (24). The present experiments in ACE transgenic mice complement these previous studies by showing that both homozygosity and heterozygosity for the disrupted ACE gene are associated with a marked reduction in the efficiency of macula densa-mediated feedback control of vascular resistance. The observation that interference with the normal operation of the renin-angiotensin system by two independent genetic interventions causes the same change in the physiological endpoint studied is strong evidence for ANG II being an integral and necessary component of the TGF pathway. It is unlikely that genetic background effects, which would be expected to be different between animals of the AT1A and the ACE mutant strains, are responsible for the strong and systematic influence of functional ANG II deficiency in the juxtaglomerular
signaling pathway. Abolition of TGF-mediated effects of luminal NaCl concentration on glomerular hemodynamics in mice homozygous for the disruption in the ACE gene is paralleled by undetectable levels of ACE activities in plasma and by tissue ACE activities in lung, kidney, heart, and brain that approach zero (5, 10, 26). In the same tissues, ACE mRNA expression was found to be extremely low (26). Virtual absence of plasma and tissue ACE activity is supported at the functional level by the almost complete absence of blood pressure responses to intravenously injected ANG I in both the present study and previous observations (26).

Even though only a small number of homozygous ACE mutant animals were studied successfully, we believe that our conclusion of complete TGF blockade in these animals is justified. Results from 16 nephrons in four mice were remarkably homogeneous, with average responses in the four mice being between 0 and 0.3 mmHg. Statistical analysis in this small group of animals is, therefore, not skewed by abnormal responses in one of the mice. Micropuncture experiments in homozygous ACE mutants proved difficult for two main reasons. Consistent with previous observations in conscious mice, most of the −/− animals we attempted to study had blood pressures lower than 65–70 mmHg (5, 10, 11, 26). The reliable use of the P<br>Fig. 3. Original recordings of stop-flow pressure (P<sub>sf</sub>; top tracing in each panel) and arterial blood pressure (AP; bottom tracing in each panel) during changes in loop of Henle perfusion rate in ACE +/+ (A), +/− (B), and −/− (C) mice. During time periods marked by black bars, tubules were perfused at a rate of 40 nl/min. ANG II infusion periods are indicated by lines.
with plasma ACE activity and plasma ANG II levels.

responsiveness does not appear to correlate tightly plasma ANG II concentration to low levels (4, 9), TGF

during ACE inhibition were tested in most of the studies referred to above and were found to be largely

did not reach 100% (22). It is likely that the doses

even though loop of Henle flow elevation still caused a significant reduction of P_{SF}, the inhibition of TGF responses in the heterozygous ACE mice appeared greater than that previously observed in mice heterozygous for the AT_{1A} knockout mutation (24). In agreement with earlier observations, blood pressure levels in the +/− animals were not significantly different from those of +/+ animals, even though they tended to be slightly lower (5, 11, 26). Thus heterozygous animals appear to be able to generate sufficient amounts of ANG II to fulfill its role in sustaining blood pressure. Largely normal blood pressure responses to exogenous ANG I in heterozygous animals support the notion of a well-maintained ACE enzymatic activity in plasma and/or endothelial cells. Because blood pressure sustenance may be primarily dependent on plasma ACE, whereas TGF support may mainly come from kidney tissue ACE, it is conceivable that this difference in susceptibility to an ACE reduction in the heterozygous animals may signify a greater suppression of tissue ACE than plasma ACE. However, available data do not strongly support this possibility. Plasma ACE activity has been found to change in a linear fashion when ACE copy number was reduced to 1 or increased to 3 by gene knockout or gene duplication strategies, respectively (11). In agreement with this finding, ACE plasma activity was reduced by ~50% in the heterozygous ACE animals (5, 10, 26). Similarly, kidney tissue ACE activity, kidney ACE mRNA, and kidney ACE protein expression were also reduced to ~40–60% of control in ACE mutant heterozygotes (5, 26). It appears that, despite identical reductions in ACE activity, ANG II levels in renal tissue are affected to an extent that limits TGF responsiveness, whereas plasma ANG II is sufficient to support blood pressure. In fact, there is previous evi-

Fig. 4. Mean change in P_{SF} in response to an increase in loop of Henle perfusion rate, from 0 to 40 nL/min, in ACE +/+ , +/− , and −/− mice before and during infusion of ANG II in subpressor doses. *P < 0.05, **P < 0.01, control vs. ANG II in +/+ , +/− , and −/− mice; ††P < 0.01, control in +/+ vs. control in other 2 groups.

the possibility that the absence of feedback-induced vasomotion is, in part, related to the structural abnormalities that have been observed in the renal vasculature (5, 7). Restoration of TGF responses during ANG II infusion to values not significantly different from control would suggest that afferent arterioles have maintained at least some capacity to regulate their tone.

Our present results are in general agreement with several previous studies showing that acute administration of a number of different ACE inhibitors, including captopril, enalapril, teprotide, and CGS-14824, caused a consistent attenuation of TGF responsiveness (8, 18, 22, 25, 28). Nevertheless, the degree of TGF inhibition in all of these studies was more modest, with TGF responses being reduced to only 25–50% of control.

Even with very high doses of captopril, TGF inhibition did not reach 100% (22). It is likely that the doses utilized in most cases reduced plasma ACE activity to very low levels for the duration of the experiment (4, 9, 12). For example, a single oral dose of 10 mg/kg enalapril has been shown to reduce plasma ACE activity in rats to undetectable levels for longer than 24 h (12). Furthermore, blood pressure responses to ANG II during ACE inhibition were tested in most of the studies referred to above and were found to be largely abolished (8, 18, 25, 28). Because acute and complete ACE inhibition is usually paralleled by a reduction of plasma ANG II concentration to low levels (4, 9), TGF responsiveness does not appear to correlate tightly with plasma ACE activity and plasma ANG II levels.
dence to show that the decrease in ANG II levels after the administration of low doses of converting enzyme inhibitors is markedly greater in kidney tissue than in plasma (4, 6). For example, a dose of the ACE inhibitor perindopril, which caused a 50% reduction in ACE activity, did not affect plasma ANG II while it reduced renal tissue ANG II to 40% of control (4). Measurements of ANG II levels in plasma and renal tissue of heterozygous ACE-knockout mice are required to determine if ANG II generation in the kidney is reduced in disproportion to plasma ANG II. Because the appearance of the kidneys in +/- mice was indistinguishable from that of +/+ animals, the reduced TGF activity does not seem to be explainable by changes in renal vascular structure.

The major additional finding in these experiments is the observation that administration of exogenous ANG II caused a restoration of TGF responsiveness to 71% of control in heterozygous and to 62% of control in homozygous ACE mutant mice. Even though TGF responses in the mutant mice during ANG II infusion were not significantly different from those observed in control mice before angiotensin infusion, it appears that full restoration of TGF responsiveness was not achieved. Because arterial blood pressure did not significantly change during the angiotensin infusion period, restoration of TGF responses does not appear to be explained by the hypertensive action of ANG II. Our observations are consistent with previous studies showing that infusion of ANG II in rats treated acutely with enalapril or captopril tended to restore TGF responses without reaching 100% normalization (8, 17). Furthermore, in volume-expanded rats, in which TGF responses were markedly reduced in parallel to a reduction in plasma ANG II concentration, infusion of ANG II caused a restoration of TGF responses to levels normally seen in hydropenic rats (21). It was concluded from these and other results that ANG II sensitizes the TGF target vessels to the constrictor effect of the flow-dependent TGF-mediating agent (3, 17, 21). Although in the current studies, the effect of angiotensin was highly significant and consistent, it is noteworthy that full normalization of TGF responses was not achieved. The reason for the tendency of an acute ANG II infusion to not fully correct the response deficit associated with acute or chronic ACE deficiency is unclear. Based on previous determinations of the relation between ANG II infusion rate and plasma ANG II, it seems unlikely that response restoration was limited by insufficiently high plasma ANG II levels (15, 21). Furthermore, the rate of infusion in the present study, as well as in previous studies, was higher than needed to restore TGF responses in the volume-expanded rats (21). These data suggest that the TGF-sensitizing effect of ANG II is only in part dependent on plasma ANG II and needs to be complemented from an extravascular source. In fact, there is strong evidence to suggest that marked differences exist in ANG II levels between the plasma and tissue compartments in the kidney and, most likely, between different regions of the kidney as well (14). The inability to quantitatively restore the functional consequences of chronic ANG II depletion with an acute and short-term infusion may be a consequence of this highly compartmentalized distribution of the renin-angiotensin system in the kidney. It is conceivable that, despite restoration of plasma ANG II concentrations, normalization of peptide levels was not achieved at the site where ANG II interacts with the TGF system, presumably in the juxtaglomerular interstitium.

In summary, marked blunting of TGF responses in mice homozygous or heterozygous for a null mutation of the ACE gene, and restoration of responsiveness in these animals by ANG II, supports the notion that the renin-angiotensin system is a necessary component of macula densa control of renal vascular tone.

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