Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 3975–3980, March 1999
Medical Sciences

Blood pressure reduction and diabetes insipidus in transgenic rats deficient in brain angiotensinogen

MARTINA SCHINKE*†, OVIDIU BALTATU*†, MANFRED BÖHM*†, JÖRG PETERS‡, WOLFGANG RASCHER§, GIAMPIERO BRICCA*¶, ANDREA LIPPOLDT*, DETLEV GANTEN*‖, AND MICHAEL BADER*,**††

*Max-Delbrück-Center for Molecular Medicine (MDC), Robert-Rössle-Strasse 10, D-13092 Berlin-Buch, Germany; ‡Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, D-69120 Heidelberg, Germany; §Department of General Pediatrics and Neonatology, University Clinic Giessen, Germany; ¶Institut National de la Santé et de la Recherche Médicale U331, 22 avenue du Doyen Lépine, 69675 Bron, France; ‖Department of Clinical Pharmacology, University Clinic Benjamin Franklin, Free University Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany; and **Transgenics in Berlin-Buch GmbH, Robert-Rössle-Strasse 10, D-13125 Berlin, Germany

Communicated by Alexander G. Bearn, American Philosophical Society, Philadelphia, PA, January 15, 1999 (received for review September 14, 1998)

ABSTRACT **Angiotensin produced systemically or locally in tissues such as the brain plays an important role in the regulation of blood pressure and in the development of hypertension. We have established transgenic rats [TGR(ASrAOGEN)] expressing an antisense RNA against angiotensinogen mRNA specifically in the brain. In these animals, the brain angiotensinogen level is reduced by more than 90% and the drinking response to intracerebroventricular renin infusions is decreased markedly compared with control rats. Blood pressure of transgenic rats is lowered by 8 mmHg (1 mmHg = 133 Pa) compared with control rats. Crossbreeding of TGR(ASrAOGEN) with a hypertensive transgenic rat strain exhibiting elevated angiotensin II levels in tissues results in a marked attenuation of the hypertensive phenotype. Moreover, TGR(ASrAOGEN) exhibit a diabetes insipidus-like syndrome producing an increased amount of urine with decreased osmolarity. The observed reduction in plasma vasopressin by 35% may mediate these phenotypes of TGR(ASrAOGEN). This new animal model presenting long-term and tissue-specific down-regulation of angiotensinogen corroborates the functional significance of local angiotensin production in the brain for the central regulation of blood pressure and for the pathogenesis of hypertension.**

Hypertension is a leading risk factor for cardiovascular diseases, and the molecular dissection of its complex genetic causes is a great challenge. As for most physiological processes, the brain also seems to play a dominant role in the regulation of blood pressure and the pathogenesis of hypertension (1). Already more than 50 years ago, centrally acting drugs have been introduced as effective therapeutics for this disease (2). One of the hormone systems crucially involved in the central control of blood pressure is the renin-angiotensin system (RAS) (1, 3). The genotype of angiotensinogen (AOGEN), the only precursor of the effector peptide angiotensin II, correlates with blood pressure in genetically engineered mice (4–6) and hypertensive patients (7, 8). Mice with an AOGEN gene dose increased by transgenesis (4) or gene titration (5) exhibit enhanced blood pressure; animals with zero (6) or only one (5) AOGEN allele are hypotensive. In humans, certain AOGEN alleles are associated with higher plasma AOGEN levels and increased blood pressure (7, 8).

AOGEN not only is synthesized and secreted into the blood stream by the liver but also is produced locally in several organs, including the brain (9), representing the basis of tissue-based RAS. Because of the blood–brain barrier precluding circulating angiotensin II from accessing most of its central receptors, the brain RAS acts independently from the systemic RAS on blood pressure regulation by influencing the secretion of arginine-vasopressin (AVP) and adrenocorticotropic hormone and modulating the baroreceptor reflex and the sympathetic output (1, 9). However, despite high local production, the function and the significance of AOGEN and angiotensin II in the brain are only partially understood (9, 10). An important role of central AOGEN is supported by increased concentrations of AOGEN and angiotensin peptides in the brain of transgenic and spontaneously hypertensive rats, which, at the same time, exhibit normal plasma levels of these molecules (10–13). Furthermore, intracerebroventricular (i.c.v.) injections of drugs inhibiting angiotensin formation and action or of antisense (AS) oligonucleotides against AOGEN and the angiotensin II receptor AT1 did efficiently lower blood pressure in hypertensive rats and mice (14–17). However, the mechanical perturbation induced by the injections itself causes unspecific side effects, including an activation of the brain RAS and the release of AVP (18). Moreover, i.c.v. injections of AS oligonucleotides elicit only transient reductions of gene expression. To avoid these pitfalls, we chose a genetic approach to silence the AOGEN gene tissue-specifically. To suppress AOGEN exclusively in astrocytes, which represent the main source of brain AOGEN (19), we generated transgenic rat lines expressing an AS RNA targeted against the 5′ region of the AOGEN mRNA. The inhibitory effect of this AS RNA on AOGEN expression previously has been demonstrated in cell culture (20). Because AOGEN in the brain is colocalized with the intermediate filament glial fibrillary acidic protein (GFAP) (21), the GFAP promoter was chosen to drive AS RNA expression. The newly generated transgenic rats exhibit more than 90% reduced AOGEN levels in the brain and a significantly lowered blood pressure and underscore the relevance of the local production of angiotensin II in the brain for cardiovascular homeostasis.

MATERIALS AND METHODS

Generation of the Transgenic Rats TGR(ASrAOGEN). The first 200 bp at the 5′ end of AOGEN cDNA containing exon 1 and parts of exon 2, the translational start site, and the coding region for angiotensin II were cloned in reverse orientation into the vector Rc/CMV (Invitrogen). The second intron of the rabbit β-globin gene was integrated into the *Stu*I site of the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

Abbreviations: AOGEN, angiotensinogen; AS, antisense; AVP, arginine-vasopressin; GFAP, glial fibrillary acidic protein; i.c.v., intracerebroventricular; RAS, renin-angiotensin system; SD, Sprague–Dawley.

†M.S., O.B., and M.B. contributed equally to this work.
††To whom reprint requests should be addressed at the * address. e-mail: mbader@mdc-berlin.de.

AS sequence, and the human GFAP promoter (22) was inserted 5′ to it. The *Bgl*II/*Bam*HI fragment of the vector was used for the production of transgenic rats as described previously (23). The identification of transgenic animals in the offspring was achieved by Southern blotting (23) with *Pvu*II-digested total DNA from tail biopsies and a ^{32}P-labeled DNA fragment containing the whole AS sequence as probe.

Detection of AS RNA and AOGEN mRNA Expression. Northern blot and *in situ* hybridization was performed as described previously (18, 23) by using as probe for AS RNA the AS cRNA ^{32}P- and ^{35}S-labeled, respectively. AOGEN mRNA was detected by *in situ* hybridization using a previously described, ^{35}S-labeled probe (4) and was densitometrically quantified by using an interactive image analysis system (IBAS) (Kontron, Zurich).

AOGEN Protein Quantification. Rats were killed under anesthesia by cardiac perfusion with 0.9% ice-cold saline, and the brain was immediately transferred into ice-cold DMEM (GIBCO/BRL). After dissection, the tissue was homogenized in ice-cold buffer (10 mM Tris·HCl, pH 7.8/3 mM EDTA/0.25 M sucrose/10^{-4} M phenylmethylsulfonyl fluoride) with a Teflon homogenizer and the protein was extracted by differential centrifugation at $100 \times g$, $800 \times g$, $5{,}000 \times g$, and $15{,}000 \times g$. AOGEN was detected in the 800–$15{,}000 \times g$ fractions by ELISA according to Klett *et al.* (24). This assay does not distinguish between AOGEN and desangiotensin I AOGEN. Plasma-AOGEN concentration was measured by a radioimmunoassay that quantifies the amount of angiotensin I liberated by an excess of renin as described (25).

Intracerebroventricular Renin Injections. A stainless-steel guide cannula (25 gauge) was implanted into the right lateral cerebroventricle under anesthesia and fixed with dental cement (Bisico, Bichfeld, Germany). It was positioned at the following coordinates with respect to bregma: 1-mm lateral, 0.9-mm posterior, and 4.5-mm ventral and was closed by a stainless-steel stylet. The correct position of the guide cannula was confirmed after the experiments by sectioning the brain tissue. The animals were allowed to recover for 5 days before the infusion of renin. For the injections, a 29-gauge injection cannula was placed through the guide cannula to a depth of 0.5 mm beyond the tip of the guide cannula. The injection cannula was connected to a polyethylene tube, which was filled with the solution and attached to a Hamilton microsyringe (10 μl). Porcine renin (Sigma) was administered through the injection cannula over a period of 30 sec in a volume of 5 μl beginning with the lowest concentration and leaving 5-day intervals between the injections. In control experiments, the renin preparation was inactivated by boiling, which led to a total loss of its dipsogenic activity.

Measurement of Blood Pressure, Electrolytes, and Plasma AVP. In conscious freely moving rats, systolic and diastolic blood pressure were recorded by a telemetric pressure transducer implanted in the aorta as described (26). For electrolyte measurement, rats were put in metabolic cages and urine was collected for 3 days. Plasma was collected by retroorbital puncture. Electrolytes and osmolarity in plasma and urine were determined by a flame photometer and an osmometer, respectively. Plasma AVP was measured by using a specific radioimmunoassay (27). The recovery from synthetic AVP (2–8 pg/ml) added to AVP-free plasma from Brattleboro rats was 64.4% ± 6.8%. The intraassay coefficient of variance was 10.3%, and the interassay coefficient of variance was 13.1%. All values were corrected for incomplete recovery. The detection limit was 1.2 pg/ml.

RESULTS

Generation of Transgenic Rat Strains. The construct depicted in Fig. 1*A* was used to generate transgenic rats. The intron of the rabbit β-globin gene was included in the AS sequence because it has been shown that introns increase the efficiency of transgene expression (28) and, as we have demonstrated recently, this insert did not affect the activity of the AS RNA in rat hepatoma cells (20). By Southern blot analysis, animals 1, 599, and 680 were identified to carry the transgene (Fig. 1*B*).

Two transgenic lines were analyzed in detail. Heterozygous animals of the lines derived from animal 1 [TGR(ASrAOGEN)1] and animal 680 [TGR(ASrAOGEN)680] were characterized with regard to the expression pattern of the AS RNA and pathophysiological alterations. In TGR(ASrAOGEN)1, the transgene is transmitted exclusively to male offspring, indicating that it is most likely integrated into the Y chromosome. Transgene-positive rats of both gender were obtained from the other lines, proving the existence of different integration sites. The pathophysiological alterations are common for all lines and, therefore, are caused by the expression of AS RNA rather than by insertional mutations. The AS RNA was highly expressed in the brain of all lines (Fig. 1*C*), and the expression pattern was similar to that of the endogenous GFAP promoter (Fig. 1*D*). In addition, low amounts of AS RNA also were found in peripheral organs, e.g., liver, heart, and adrenal gland (Fig. 1*C*). Nonradioactive *in situ* hybridization experiments showed that expression in these organs was restricted to endothelial cells, which have not yet been reported to express GFAP in the rat (data not shown).

AS RNA Reduces Brain AOGEN but Not AOGEN mRNA. Quantification of the *in situ* hybridization signals revealed no difference in AOGEN mRNA levels between transgenic and Sprague–Dawley (SD) rats in all brain areas examined (Fig. 2*A*). In contrast, for AOGEN-protein concentrations in the brain as determined by ELISAs a significant reduction to approximately 10% of controls was observed in medulla/pons, hypothalamus/thalamus, and cerebellum (Fig. 2*B*). Regions with low AOGEN and AS RNA expression, such as the cerebral hemispheres, showed no change in AOGEN concentration. Thus, the expression level of AS RNA and the amount of target protein are inversely correlated in different brain regions. Significantly, plasma concentrations of AOGEN were unchanged in TGR(ASrAOGEN) in comparison with SD animals (Fig. 2*B*).

Reduced Drinking Response to Intracerebroventricular Renin in Transgenic Rats. The transgenic rats develop normally and do not show any higher mortality or morbidity than control animals.

Brain AOGEN function was evaluated by comparing drinking of heterozygous TGR(ASrAOGEN) with that of SD control animals. The drinking response after i.c.v. infusion of renin was markedly attenuated in transgenic rats down to 39% ($P < 0.01$) of controls (Fig. 3). These results confirm the biochemical and functional down-regulation of brain AOGEN in TGR(ASrAOGEN).

Reduced Blood Pressure in Transgenic Rats. Systolic and diastolic blood pressure measured in conscious, freely moving animals by telemetry was lowered significantly in TGR(ASrAOGEN) compared with nontransgenic controls by 8.3 ± 2.3 mmHg ($P < 0.01$, Fig. 4*A*).

To further examine the significance of brain AOGEN concentrations in hypertension, we crossbred TGR(ASrAOGEN) with the hypertensive transgenic rat TGR(mREN2)27 (23). These rats were considered particularly suitable for the study of the role of the brain RAS in the hypertensive process because they develop hypertension with suppressed plasma renin, but increased renin production in several tissues including the brain (23, 29). In these animals, the levels of angiotensin peptides are increased severalfold in the hypothalamus and in the medulla oblongata, representing important regions for cardiovascular control (1, 11). The resulting new double-transgenic line TGR(ASrAOGEN/mREN2) harbors both the mouse renin as well as the ASrAOGEN transgene. Expression

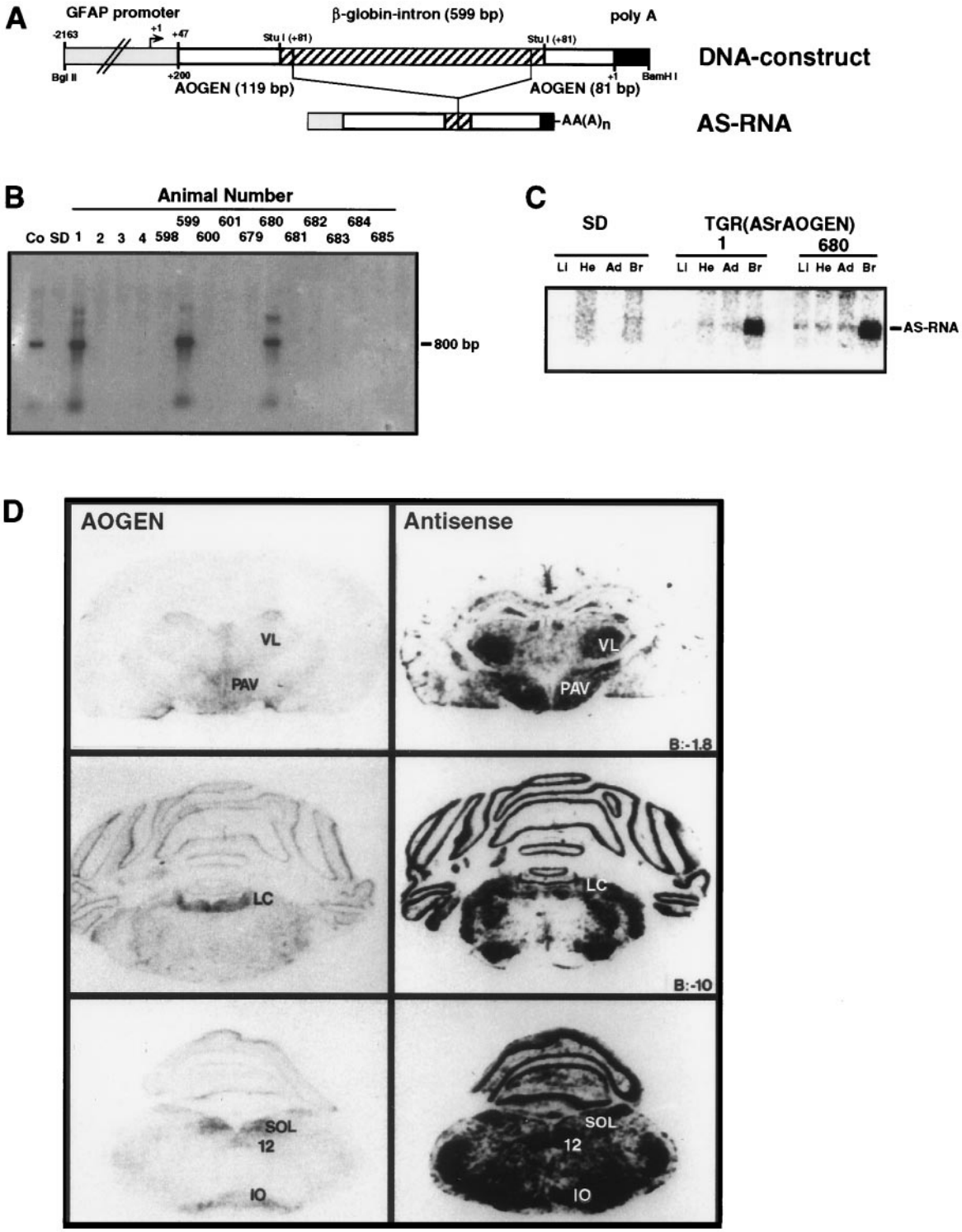

FIG. 1. Generation of TGR(ASrAOGEN). (*A*) DNA construct used for the production of transgenic rats and the resulting structure of the AS RNA. β-globin intron (striped box), second intron with flanking exonic sequences of the rabbit β-globin gene; poly(A), polyadenylation signal of the bovine growth hormone gene. (*B*) Identification of transgenic rats by Southern blot analysis. Ten micrograms of genomic DNA of each animal (SD rat) and, as control, 1 ng of plasmid DNA containing the transgene (Co) were digested with *Pvu*II and analyzed by Southern blotting. (*C*) Detection of AS RNA expression by Northern blot analysis in organs of two lines of TGR(ASrAOGEN) and SD rats. Li, liver; Ki, kidney; He, heart; Ad, adrenal; Br, brain. (*D*) Localization of AS RNA and AOGEN mRNA in the brain of TGR(ASrAOGEN)1. AOGEN mRNA (*Left*) and AS RNA (*Right*) were detected in coronal sections of three different brain areas by *in situ* hybridization. Sol, nucleus of the solitary tract; 12, hypoglossal nucleus; IO, inferior olive; VL, ventrolateral thalamic nucleus; PAV, paraventricular hypothalamic nucleus; LC, locus coeruleus; B, bregma.

of the AS RNA against AOGEN in the glial cells of these animals also led to a 90% reduction of the AOGEN content in the brain without influencing circulating AOGEN concentrations (data not shown). As a consequence, blood pressure was reduced by about 25 mmHg ($P < 0.05$) at the age of 16 weeks compared with rats harboring only the mouse-renin transgene (Fig. 4*B*).

Diabetes Insipidus-Like Syndrome in Transgenic Rats. TGR(ASrAOGEN) were analyzed for water intake and uri-

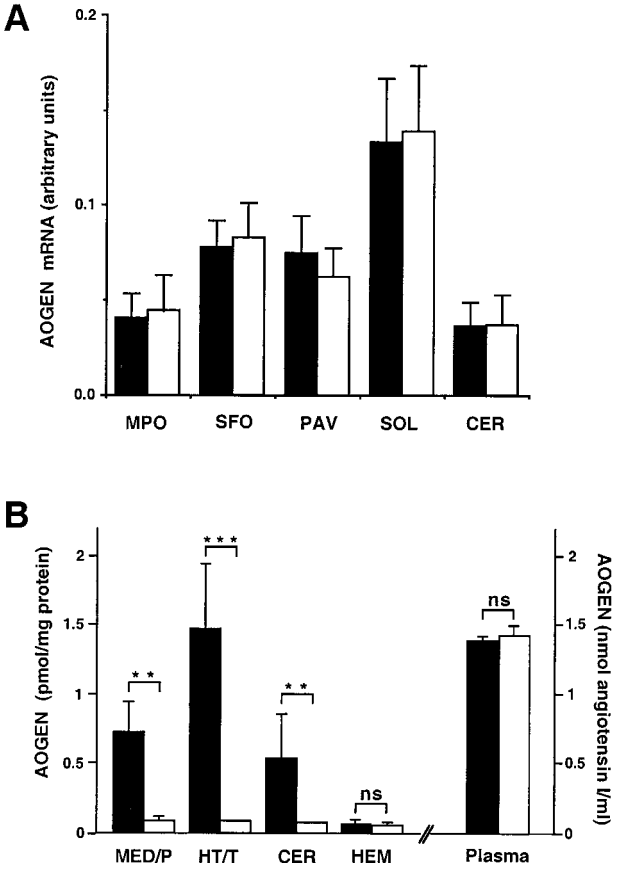

FIG. 2. AOGEN mRNA (*A*) and protein (*B*) concentrations in TGR(ASrAOGEN)1. (*A*) AOGEN mRNA in median preoptic nucleus (MPO), subfornical organ (SFO), hypothalamic paraventricular nucleus (PAV), nucleus of the solitary tract (SOL), and cerebellum (CER) was densitometrically quantified on autoradiographs of *in situ* hybridization experiments (see Fig. 1*D*) using an IBAS. No significant difference was observed between TGR(ASrAOGEN)1 (open bars) and SD rats (solid bars). (*B*) Brain-AOGEN content was measured in protein extracts of medulla/pons (MED/P), hypothalamus/thalamus (HT/T), cerebellum (CER), and cerebral hemispheres (HEM) in each of eight SD rats (solid bars) and TGR(ASrAOGEN)1 (open bars). Plasma-AOGEN concentration was measured by a radioimmunoassay that quantifies the amount of angiotensin I liberated by an excess of renin. One of two independent experiments is shown. Values are means ± SE. Significance of differences from SD rats were determined by Mann–Whitney *U* test (**, $P < 0.01$; ***, $P < 0.001$; ns, not significant).

nary output by using metabolic cages. Volume, osmolarity, and sodium concentration of urine were measured. In addition, plasma levels of AVP were determined.

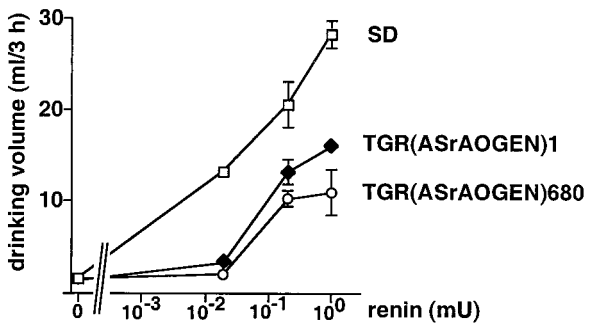

FIG. 3. Drinking volume after i.c.v. renin infusions. The water intake was determined for 3 h after the infusion of the indicated amounts of porcine renin. Values are means ± SE. For the control value (0), 1 milliunit of renin was boiled before infusion.

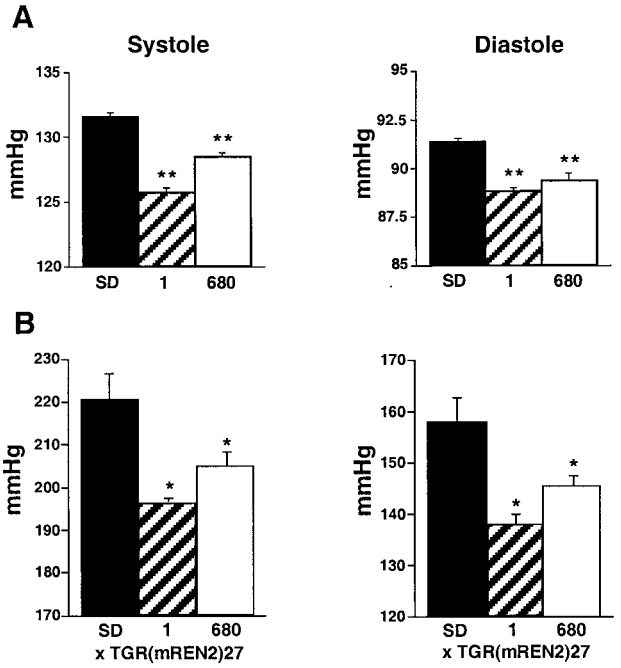

FIG. 4. Blood pressure of TGR(ASrAOGEN) (*A*) and TGR(ASrAOGEN/mREN2) (*B*). Systolic (*Left*) and diastolic (*Right*) blood pressure was recorded by a telemetric pressure transducer implanted into the aorta. Each group [TGR(ASrAOGEN)1, TGR(ASrAOGEN)680, and SD rats as well as the same lines crossbred with TGR(mREN2)27] consisted of eight males at 16 weeks of age. Values are mean arterial pressures of a continuously measured 2-day interval ± SE. **, Significantly different ($P < 0.01$) from SD rats; *, significantly different ($P < 0.05$) from TGR(mREN2)27 as determined by Mann–Whitney *U* test.

TGR(ASrAOGEN) have significantly higher urinary volumes, which correlates with significantly lower osmolarities and Na^+ concentrations in urine, keeping total natriuresis constant (Fig. 5). However, plasma osmolarity, Na^+, and K^+ concentrations are unchanged (Table 1). Primary polydipsia as a cause for the observed polyuria was excluded by depriving the animals of water for 24 h, after which they still excreted a higher amount of urine with a lower osmolarity compared with SD rats (data not shown). High urinary volume and low osmolarity are symptoms associated with central diabetes insipidus caused by AVP deficiency. Therefore, plasma AVP concentrations were measured by a specific radioimmunoassay. TGR(ASrAOGEN) exhibit significantly lower plasma AVP (−34.7 ± 3.5%) in comparison with SD rats (Fig. 6).

DISCUSSION

We have generated transgenic rats TGR(ASrAOGEN), which express an AS RNA directed against the AOGEN-mRNA and driven by an astrocyte-specific promoter. Expression of the AS RNA mainly was restricted to the brain; however, small amounts also could be detected in endothelial cells of several other organs. Because the GFAP promoter used was of human origin, this unwanted site of expression possibly reflects species-specific differences or missing regulatory sequences (30, 31). AOGEN mRNA is not present in endothelial cells (32); therefore, an inhibitory influence of the AS RNA on the activity of the RAS in peripheral tissues or in the plasma is unlikely and there is no evidence for this from our data showing normal plasma levels of AOGEN in the transgenic rats.

There are reports about AOGEN synthesis in neurons (reviewed in ref. 33), which should not be affected by the glia-specific transgene expression. However, the extent of the

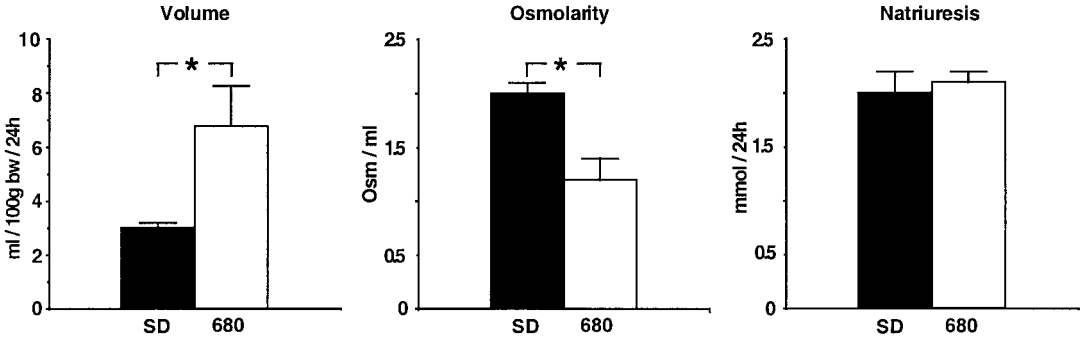

FIG. 5. Urinary volume, Na^+ concentration, and natriuresis in TGR(ASrAOGEN). Twenty-four-hour urine was collected in TGR(ASrAOGEN) 680 ($n = 6$; open bars) and SD rats ($n = 6$; solid bars) and analyzed for osmolarity by an osmometer and for Na^+ concentration by flame photometry. Values are means ± SE. *, Significantly different ($P < 0.05$) from SD rats as determined by Mann–Whitney *U* test.

inhibitory effect of the AS RNA on AOGEN protein synthesis excludes a major contribution of neuron-derived AOGEN to the brain RAS.

The extent of the inhibition nearly eliminating AOGEN in the brain was unexpectedly high given the previously published results with AS RNA in other transgenic animals (34–36). Probably more than 10-fold excess of AS RNA (Fig. 1*D*) compared with the target mRNA in our model is responsible for this efficiency approaching values of inhibition otherwise reached only by tissue-specific gene targeting in mice by the use of the Cre-loxP system (36, 37).

Several mechanisms underlying the inhibitory action of the AS RNA on the target mRNA have been postulated. These include an obliteration of mRNA synthesis or processing by binding to the target pre-mRNA, blockade of ribosomes and the translation process, or unwinding and subsequent degradation of the AS RNA/target mRNA duplex by double-stranded RNA-editing enzymes (36, 38–41). Because brain AOGEN mRNA levels are unchanged (Fig. 2*A*), it is most likely that the observed inhibition of AOGEN gene expression is a result of a hybrid arrest of translation of the mRNA and not of an effect on mRNA synthesis or degradation.

The observed reduction of plasma AVP most probably is responsible for the diabetes insipidus-like syndrome with high amounts of diluted urine observed in TGR(ASrAOGEN). It is well established that exogenously applied angiotensin II stimulates AVP release from the neurohypophysis (9). Our results indicate that the central RAS also tonically controls AVP secretion, and a reduction in basal concentrations of angiotensin II already decreases the liberation of AVP into the circulation or its *de novo* synthesis and has functional consequences on fluid and electrolyte homeostasis.

The basal blood pressure of the transgenic rats was reduced to a similar degree as in mice after reducing the copy number of the AOGEN gene by 1 (5). The gene-targeted mice, however, do not allow the discrimination between liver-derived AOGEN and AOGEN synthesized locally by other tissues. Therefore, that the partial elimination of brain-AOGEN synthesis and the total ablation of one AOGEN allele results in a comparable blood pressure reduction underscores the importance of centrally synthesized AOGEN for the maintenance of normotension. Further studies will clarify which mediator of the central angiotensin II effects is acting in the periphery to reduce blood pressure. Most likely, the lowered concentration of AVP in plasma plays a major role in the hypotensive effect of the transgenic AS RNA. At least the pressor response to i.c.v.-injected angiotensin II as well as to transgenically overproduced peptide in hypertensive mice carrying the human renin and AOGEN genes is mediated, in part, by AVP because in both cases it could be reduced by i.v. application of a V1 receptor antagonist (17, 42). However, reductions in sympathetic output as well as other mechanisms modulated by angiotensin II in the brain may be also involved (9).

Table 1. plasma osmolarity and electrolytes

	SD	TGR(ASrAOGEN)
Osmolarity, osmol/liter	0.305 ± 0.001	0.303 ± 0.001
Na^+, mmol/liter	142 ± 1.5	143 ± 0.7
K^+, mmol/liter	4.57 ± 0.13	4.45 ± 0.22

The blood pressure effect of the AS RNA is more pronounced on the genetic background of TGR(mREN2)27 rats. These animals contain increased angiotensin II levels especially in the brain (11). The enhanced activity of the brain RAS has been suggested to contribute substantially to the hypertension in this model because anesthesia with chloralose-urethane as well as i.c.v. injection of angiotensin II-specific mAbs reduced drastically the blood pressure to nearly normotensive levels (43, 44). Our results corroborate these studies, indicating that TGR(mREN2)27 represents, at least in part, a neurogenic model of hypertension. On the basis of reductions in blood pressure of spontaneously hypertensive rats and hypertensive transgenic mice elicited by i.c.v. injections of RAS-specific AS oligonucleotides or AT1-receptor antagonists, respectively, other authors also have postulated a major contribution of the local brain RAS to the development of hypertension in these models (15, 17). The blood pressure effects were more pronounced in these acute experiments than in our double-transgenic rats. This may be a result of long-term compensatory mechanisms that may have been activated during ontogeny of TGR(ASrAOGEN/mREN2) and that are not active in the acute experiments. Taking these results together,

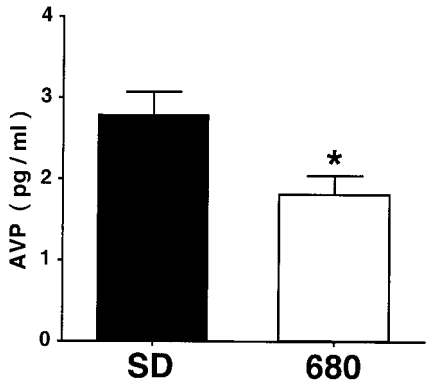

FIG. 6. Plasma AVP in TGR(ASrAOGEN). AVP was measured in plasma of TGR(ASrAOGEN) 680($n = 5$; open bars) and SD rats ($n = 5$; solid bars) by a specific radioimmunoassay. Values are means ± SE. *, Significantly different ($P < 0.05$) from SD rats as determined by Mann–Whitney *U* test.

the RAS in the brain seems to be a relevant factor in the etiology of hypertension, at least in animals.

TGR(ASrAOGEN) is the first transgenic animal model for the study of the role of a local RAS in a tissue. Our results demonstrate that brain AOGEN is involved in the basal regulation of blood pressure and in the manifestation of renin-dependent hypertension in TGR(mREN2)27. TGR(ASrAOGEN) will allow the study of the relevance of central AOGEN for other brain functions suggested to be targets of brain angiotensin, such as the regulation of baroreceptor reflex and sympathetic tone, the release of vasopressin, and the control of neurosecretion and behavior (9, 45).

Importantly, the methodology of gene inhibition by AS RNA expression used in this study is suitable for detecting tissue-specific functions of genes and, in contrast to gene-targeting methods, also can be applied to species other than the mouse. With the GFAP promoter it will be helpful to gain insight into the functionality and neuromodulatory actions of glial cells (46, 47). In concert with other methods, this technique of tissue-specific gene silencing (36) may be used to dissect the genetic basis of hypertension and other polygenic disorders in a systematic manner.

We thank M. Brenner for the GFAP promoter and gratefully acknowledge the critical review of the manuscript by C. Talsness and the technical help of A. Böttger and J. Zimmer. This work was supported by grants from the Deutsche Forschungsgemeinschaft to M.B. and J.P. and by the Transgeneur Program of the European Union. G.B. was supported by fellowships of the Alexander von Humboldt Stiftung and the Fondation pour la Recherche Médicale.

1. Oparil, S., Chen, Y.-F., Berecek, K. H., Calhoun, D. A. & Wyss, J. M. (1995) in *Hypertension: Pathophysiology, Diagnosis, and Management,* eds. Laragh, J. H. & Brenner, B. M. (Raven, New York), pp. 713–740.
2. Freis, E. D. (1995) in *Hypertension: Pathophysiology, Diagnosis, and Management*, eds. Laragh, J. H. & Brenner, B. M. (Raven, New York), pp. 2741–2751.
3. Reid, I. A., Morris, B. J. & Ganong, W. F. (1978) *Annu. Rev. Physiol.* **40,** 377–410.
4. Kimura, S., Mullins, J. J., Bunnemann, B., Metzger, R., Hilgenfeldt, U., Zimmermann, F., Jacob, H., Fuxe, K., Ganten, D. & Kaling, M. (1992) *EMBO J.* **11,** 821–827.
5. Kim, H.-S., Krege, J. H., Kluckman, K. D., Hagaman, J. R., Hodgin, J. B., Best, C. F., Jennette, J. C., Coffman, T. M., Maeda, N. & Smithies, O. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2735–2739.
6. Tanimoto, K., Sugiyama, F., Goto, Y., Ishida, J., Takimoto, E., Yagami, K., Fukamizu, A. & Murakami, K. (1994) *J. Biol. Chem.* **269,** 31334–31337.
7. Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y. V., Lifton, R. P., Williams, C. S., Charru, A., Hunt, S. C., Hopkins, P. N., Williams, R. R., Lalouel, J. & Corvol, P. (1992) *Cell* **71,** 169–180.
8. Caulfield, M., Lavender, P., Farrall, M., Munroe, P., Lawson, M., Turner, P. & Clark, A. J. (1994) *N. Engl. J. Med.* **330,** 1629–1633.
9. Phillips, M. I. (1992) *Annu. Rev. Physiol.* **49,** 413–435.
10. Ganten, D., Hermann, K., Bayer, C., Unger, T. & Lang, R. E. (1983) *Science* **221,** 869–871.
11. Senanayake, P., Moriguchi, A., Kumagai, H., Ganten, D., Ferrario, C. M. & Brosnihan, K. B. (1994) *Peptides* **15,** 919–926.
12. Printz, M. P. & Healy, D. P. (1983) *Clin. Exp. Hypertens. [A]* **5,** 1037–1046.
13. Lee, M. A., Böhm, M., Paul, M., Bader, M., Ganten, U. & Ganten, D. (1996) *Am. J. Physiol.* **270,** E919–E929.
14. Unger, T., Kaufmann-Bühler, I., Schölkens, B. & Ganten, D. (1981) *Eur. J. Pharmacol.* **70,** 467–478.
15. Phillips, M. I., Wielbo, D. & Gyurko, R. (1994) *Kidney Int.* **46,** 1554–1556.
16. Lu, D. & Raizada, M. K. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2914–2918.
17. Davisson, R. L., Yang, G., Beltz, T. G., Cassell, M. D., Johnson, A. K. & Sigmund, C. D. (1998) *Circ. Res.* **83,** 1047–1058.
18. Lippoldt, A., Bunnemann, B., Ueki, A., Rosen, L., Cintra, A., Hasselrot, U., Metzger, R., Hilgenfeldt, U., Brosnihan, B., Ganten, D. & Fuxe, K. (1994) *Brain Res.* **668,** 144–159.
19. Milsted, A., Barna, B. P., Ransohoff, R. M., Brosnihan, K. B. & Ferrario, C. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5720–5723.
20. Schinke, M., Böhm, M., Bricca, G., Ganten, D. & Bader, M. (1996) *Hypertension* **27,** 508–513.
21. Deschepper, C. F., Bouhnik, J. & Ganong, W. F. (1986) *Brain Res.* **374,** 195–198.
22. Brenner, M., Kisseberth, W. C., Su, Y., Besnard, F. & Messing, A. (1994) *J. Neurosci.* **14,** 1030–1037.
23. Mullins, J. J., Peters, J. & Ganten, D. (1990) *Nature (London)* **344,** 541–544.
24. Klett, C., Nobiling, R., Gierschik, P. & Hackenthal, E. (1993) *J. Biol. Chem.* **268,** 25095–25107.
25. Hermann, K., Ganten, D., Unger, T., Bayer, C. & Lang, R. E. (1988) *Clin. Chem.* **34,** 1046–1051.
26. Lemmer, B., Mattes, A., Böhm, M. & Ganten, D. (1993) *Hypertension* **22,** 97–101.
27. Rascher, W., Lang, R. E., Unger, T., Ganten, D. & Gross, F. (1982) *Am. J. Physiol.* **242,** H496–H499.
28. Brinster, R. L., Allen, J. M., Behringer, R. R., Gelinas, R. E. & Palmiter, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 836–840.
29. Bader, M., Zhao, Y., Sander, M., Lee, M., Bachmann, J., Böhm, M., Djavidani, B., Peters, J., Mullins, J. J. & Ganten, D. (1992) *Hypertension* **19,** 681–686.
30. Sarkar, S. & Cowan, N. J. (1991) *J. Neurochem.* **57,** 675–684.
31. Kaneko, R. & Sueoka, N. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4698–4702.
32. Cassis, L. A., Saye, J. & Peach, M. J. (1988) *Hypertension* **11,** 591–596.
33. Sernia, C., Zeng, T., Kerr, D. & Wyse, B. (1997) *Front. Neuroendocrinol.* **18,** 174–208.
34. Munir, M. I., Rossiter, B. J. F. & Caskey, C. T. (1990) *Somat. Cell Mol. Genet.* **16,** 383–394.
35. Pepin, M.-C., Pothier, F. & Barden, N. (1992) *Nature (London)* **355,** 725–728.
36. Porter, A. (1998) *Trends Genet.* **14,** 73–79.
37. Rajewsky, K., Gu, H., Kühn, R., Betz, U. A., Müller, W., Roes, J. & Schwenk, F. (1996) *J. Clin. Invest.* **98,** 600–603.
38. Helene, C. & Toulme, J.-J. (1990) *Biochim. Biophys. Acta* **1049,** 99–125.
39. Nishikura, K. (1992) *Ann. N. Y. Acad. Sci.* **660,** 240–250.
40. Ch'ng, J. L., Mulligan, R. C., Schimmel, P. & Holmes, E. W. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 10006–10010.
41. Munroe, S. H. (1988) *EMBO J.* **7,** 2523–2532.
42. Unger, T., Rascher, W., Schuster, C., Pavlovitch, R., Schömig, A., Dietz, R. & Ganten, D. (1981) *Eur. J. Pharmacol.* **71,** 33–42.
43. Moriguchi, A., Tallant, E. A., Matsumura, K., Reilly, T. M., Walton, H., Ganten, D. & Ferrario, C. M. (1995) *Hypertension* **25,** 1260–1265.
44. Diz, D. I., Westwood, B., Bosch, S. M., Ganten, D. & Ferrario, C. (1998) *Hypertension* **31,** 473–479.
45. Wright, J. W. & Harding, J. W. (1992) *Brain Res. Rev.* **17,** 227–262.
46. Smith, S. J. (1994) *Curr. Biol.* **4,** 807–810.
47. Shao, Y. & McCarthy, K. D. (1994) *Glia* **11,** 147–155.