Increased Adrenal Renin in Transgenic Hypertensive Rats, TGR(mREN2)27, and Its Regulation by cAMP, Angiotensin II, and Calcium

Jörg Peters, Klaus Münter, Michael Bader, Eberhard Hackenthal, John J. Mullins, and Detlev Ganten
Department of Pharmacology, University of Heidelberg, German Institute for High Blood Pressure Research, 6900 Heidelberg; and Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany

Abstract

The newly established rat strain TGR(mREN2)27 is a mono-genetic model in hypertension research. Microinjecting the mouse $Ren-2^d$ renin gene caused it to become a stable part of the genome. The rats are characterized by fulminant hypertension, low plasma active renin, suppressed kidney renin, high plasma inactive renin, and high extrarenal transgene expression, most prominently in the adrenal cortex. Additionally, they exhibit significantly enhanced excretion of corticosteroids.

Here we demonstrate that part of the plasma renin and most of the adrenal renin are transgene determined and that the adrenal renin is strongly activated. TGR(mREN2)27 adrenal cells may serve as a new tool to investigate the regulation and processing of $Ren-2^d$–derived renin and its significance in hypertension and steroid metabolism.

Adrenal renin in TGR(mREN2)27 is stimulated by 8-bromo-cAMP (8-Br-cAMP), angiotensin II (ANGII), and calcium. 8-Br-cAMP significantly stimulates active renin and prorenin release, as well as $Ren-2^d$ mRNA. Interestingly, within 60 min 8-Br-cAMP, ANGII, and calcimycin stimulate active renin, but not prorenin release. This indicates different intracellular pathways.

An activated adrenal renin-angiotensin system in TGR(mREN2)27 as well as the lack of negative feedback on renin secretion by ANGII may be of pathophysiological significance in this hypertensive model. (*J. Clin. Invest.* 1993. 742–747.) Key words: $ren-2^d$ • cells • secretion • hypertension • tissue renin-angiotensin system

Introduction

The endocrine renin-angiotensin system (RAS)[1] is a major focus in hypertension research. Its effector peptide angiotensin II (ANGII) is a potent regulator of blood pressure and electrolyte balance. The aspartyl protease renin cleaves angiotensinogen to form the decapeptide ANGI. ANGI is further hydrolized to ANGII by angiotensin-converting enzyme.

The juxtaglomerular cells of the kidney are the major source of plasma active renin. A low sodium concentration in the proximal tubulus, low renal arterial pressure, noradrenaline, and the sympathetic nerve activity are important stimulators of kidney renin secretion. ANGII, high renal arterial pressure and calcium influx, however, are known inhibitors of renal renin release (for review see [1]). The dynamic interactions of blood pressure and electrolyte homeostasis with kidney renin are thus strongly involved in many forms of hypertension.

Growing evidence for renin synthesis and for the local generation of ANGII in many extrarenal tissues (1–5) has recently focused attention on the existence of autocrine or paracrine RAS and their possible importance in primary hypertension. However, the function, regulations, and interactions between the plasma RAS and local paracrine RAS are far from being understood.

The existence of an adrenal RAS had already been proposed in 1967 by Ryan (6) and Ganten et al. (2, 7), who demonstrated the presence of iso-renin in rat and human adrenal gland and its stimulation by long-term sodium deficiency and hypophysectomy. The description of modified smooth muscle cells in the adrenal capsule with a similarity to kidney juxtaglomerular cells by Goormaghtigh and Handovsky (8), and the demonstration of renin secretory granules by Mizuno et al. (9), suggest the possibility of regulated secretion of adrenal renin. Little data, however, are available for studying acute regulation of adrenal renin release.

We have recently generated a new monogenetic model of hypertension, the transgenic hypertensive rat TGR(mREN2)27 (10). The entire mouse $Ren-2^d$ renin gene is integrated into their genome and they are characterized by fulminant hypertension and suppressed plasma renin concentration, as well as high plasma prorenin levels and high tissue $Ren-2^d$ mRNA content. The highest expression of the transgene was found in the adrenal cortex, and the adrenal gland was demonstrated as a major source of plasma prorenin. Furthermore, adrenalectomy resulted in a dramatic drop in blood pressure in TGR(mREN2)27 (10, 11). Conversely, kidney renin was strongly suppressed. Parallel to the development of hypertension, the 24-h urinary excretions of deoxycorticosterone, corticosterone, 18-OH-corticosterone, and aldosterone are significantly elevated, suggesting a role of an intraadrenal RAS in the regulation of adrenal steroid synthesis (12).

This study demonstrates that adrenal renin in TGR(mREN2)27 is strongly enhanced and its regulation by cAMP, ANGII, and calcium is characterized.

Address correspondence to Dr. Jörg Peters, Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, 6900 Heidelberg, Germany. Dr. Mullins's present address is AFRC Center for Genome Research, University of Edinburgh, West Mains Road, Edinburgh EH9 3JQ, United Kingdom.

1. *Abbreviations used in this paper:* ANGI, II; angiotensin I, II; 8-Br-cAMP, 8-bromo-cAMP; RAS, renin-angiotensin system; SD, Sprague-Dawley; TES, N-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid.

Methods

Animals. The transgenic rats TGR(mREN2)27 were housed and bred in our laboratory (10). Heterozygous TGR(mREN2)27 were obtained by cross-breeding female homozygous TGR(mREN2)27 with male Sprague Dawley (SD) rats. SD rats were purchased from the Zentralinstitut für Versuchstierkunde, Hannover, FRG. Unless otherwise stated, homozygous rats were treated daily with captopril 10 mg/kg body wt in their drinking water. This was done to prevent organ damage, which may modify organ function. The treatment was discontinued for at least 7 d before the experiment. Animals had free access to tap water and were fed the standard laboratory diet of Altromin®. They were housed under alternating 12-h light and dark cycles at a constant temperature between 20°C and 22°C.

Adrenal homogenates. Adrenals were trimmed of fat, minced, washed three times with 0.9% NaCl, and frozen in dry ice/methanol. They were homogenized in 0.1 M N-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid (TES) pH 7.2, containing 0.1 mM PMSF and 15 mM EDTA, freeze-thawed three times, and centrifuged at 15,000 g at 4°C for 30 min. The supernatants were stored at −80°C until analysis of renin activity was carried out. For the determination of renin release, each adrenal was cut into eight slices, which were washed three times in medium 199, containing 10 mM Hepes, pH 7.2, and 0.5% BSA (No. A-7906; Sigma Chemical Co., St. Louis, MO), and incubated in 1 ml of fresh medium at 37°C in a 5% CO_2 atmosphere. The medium was stored at −80°C until analysis.

Preparation of cell suspensions. Adult female rats weighing 200–300 g were decapitated under ether anesthesia and the adrenals were removed, trimmed of fat, and washed in cold medium (see above). Adrenal cell suspensions were prepared by digesting minced adrenal slices three times with collagenase (2 mg/ml) and DNAseI (0.2 mg/ml) in medium 199/2% BSA per 10 mM of Hepes at 37°C in a 5% CO_2 atmosphere for 3 × 15 min. During each interval, the cells were dispersed, and undigested tissue was sedimented out. The supernatant of the first 15 min interval was discarded, the supernatants of the second and third incubations were kept on ice, pooled, filtered, and washed three times with fresh medium. The cell number and viability were determined by trypan blue exclusion in a Neubauer haemocytometer (LHD, Heidelberg, Germany). Cell viability was between 80 and 90% and did not decrease during the experiments. After 30 min of preincubation, the medium was discarded, followed by replacement and collection of the medium after the times indicated in the figure legends, the first interval always being untreated basal release to control the preconditions. For the determination of the intracellular renin content, cells were washed three times after collagenase/DNAse digestion. Then the pellet was dissolved in 0.1 M TES, pH 7.2, containing 0.1 mM PMSF and 15 mM of EDTA, and freeze-thawed three times in dry ice/methanol, centrifuged at 15,000 g, 4°C, for 30 min and the supernatants stored at −80°C until analysis.

Angiotensin II amide (Hypertensin) was purchased from Ciba (Zurich, Switzerland) and dissolved in 5 mM NaH_2PO_4. Final dilutions were done in the incubation medium. 8-bromo-cAMP (8-Br-cAMP) and A23187 (Sigma, (Nos. B7880 and C7522, respectively; Sigma Chemical Co.) were dissolved in DMSO, and further diluted with the incubation medium.

Renin determinations. Plasma was collected as EDTA plasma, obtained from the retroorbital plexus after light ether anesthesia. Plasma active and inactive renin concentrations were determined according to a modification of the method described by Glorioso et al. (13). In brief, to activate inactive renin 20 µl of plasma was incubated with 40 µl of trypsin (400 U/ml, dissolved in TES-buffer: 0.1 M TES, pH 7.2, 0.01% neomycin, 10 mM EDTA). Samples were incubated on ice for 10 min, and the reaction was stopped by adding 40 µl of soybean trypsin inhibitor (600 U/ml in TES-buffer). To determine inactive renin concentrations in adrenal homogenates and incubates, the concentrations of trypsin and soybean trypsin inhibitor were reduced to 50 U/ml and 100 U/ml, respectively. For the measurement of active renin, 80 µl of TES-buffer was added without trypsin.

The pretreated samples were incubated with lyophilized renin substrate isolated from nephrectomized rat plasma (final concentration: 80 mg/ml, 0.11% 2,3-dimercapto-1-propanol, 1.15 mg/ml 8-hydroxychinolin in TES-buffer). The reaction was stopped with RIA buffer (0.1 M Tris acetate, pH 7.4), (*a*) immediately before the incubation and (*b*) 1–3 h after incubation at 37°C. Generated ANGI was measured by radioimmunoassay (14, 15).

Specific immunoprecipitation of mouse renin. To determine the contribution of mouse renin to the sum of mouse and rat renin, the samples were incubated for 2 h at 4°C with a monoclonal antirenin antibody (No. 120), which we found to be specific for murine renin with < 3% cross-reactivity to purified rat renin. The antibody was kindly provided by Dr. Celio, Institut d'Histologie et Embryologie générale, Université Pérolles, Fribourg, Switzerland. The incubation buffer was 0.1 M Tris-acetate buffer, pH 8.1. Incubation with antibody-free buffer served as a control. Antibody/renin complexes were precipitated with formalin-fixed *Staphylococcus aureus* protein A (Immunoprecipitin, No. 9321 SB; Bethesda Research Laboratories, Gaithersburg, MD). The renin activities were determined before the procedure and in the supernatant after immunoprecipitation.

Analysis of RNA from adrenal cells. Cytosolic RNA was prepared according to the method described by Wilkinson (16). The RNAse protection assay was performed using 1 µg of total RNA and a mouse *Ren-2^d*-specific [^{32}P]UTP-labeled antisense RNA, previously described (10). A [^{32}P]UTP-labeled antisense rat β-actin mRNA was used as the endogeneous control.

Results

We recently demonstrated that the major site of *Ren-2^d* gene expression in TGR(mREN2)27 is the adrenal cortex (10, 17). To examine the intraadrenal RAS, we addressed the question of whether adrenal renin activity is enhanced in TGR (mREN2)27, due to the presence of mouse renin protein.

Fig. 1 shows renin activity with and without trypsin activation in plasma, adrenal homogenates, and supernatants of adrenal slice incubations. In plasma, the active renin level is significantly lower in TGR(mREN2)27 than in SD controls (Fig. 1 *A;* bar *1* vs. *3*), while prorenin is elevated 20-fold in TGR(mREN2)27 (Fig. 1 *D;* column *1* vs. *3*). In adrenal homogenates both active renin and prorenin are strongly elevated in TGR(mREN2)27 as compared with controls (Fig. 1, *B* and *E;* bar *1* vs. *3*).

Additionally, prorenin as well as active renin could be detected in the incubate of transgenic adrenal slices, indicating their release. Renin release from adrenal slices of SD rats was almost undetectable. To evaluate the contribution of mouse renin to determined renin activities, we measured the renin activities before and after immunoprecipitation with a monoclonal antibody capable of specifically recognizing mouse, but not rat, renin.

As shown in Fig. 1, the immunoprecipitation with a mouse renin-specific antibody (No. 120) had no effect on the renin activities measured in SD rats (Fig. 1, *A–F*, bar *1* vs. *2*), but significantly reduced renin activities in plasma, adrenal homogenates, and incubates of TGR(mREN2)27 (Fig. 1, *A–F*, bar *3* vs. *4*). In TGR(mREN2)27, renin activities in adrenal homogenates and incubates, as well as plasma prorenin, were almost completely blocked by the antibody to < 10% of the original values (Fig. 1, *B–F*, bar *3* vs. *4*).

The plasma active renin level in transgenic rats was decreased by about two-thirds (Fig. 1 *A,* bar *3* vs. *4*), indicating that the majority of plasma renin activity is derived from mouse renin, while a significant part still represents endo-

A Plasma

B Adrenal Homogenate

C Adrenal Supernatant

Anti - Mouse - Renin Antibody

D Plasma

E Adrenal Homogenate

F Adrenal Supernatant
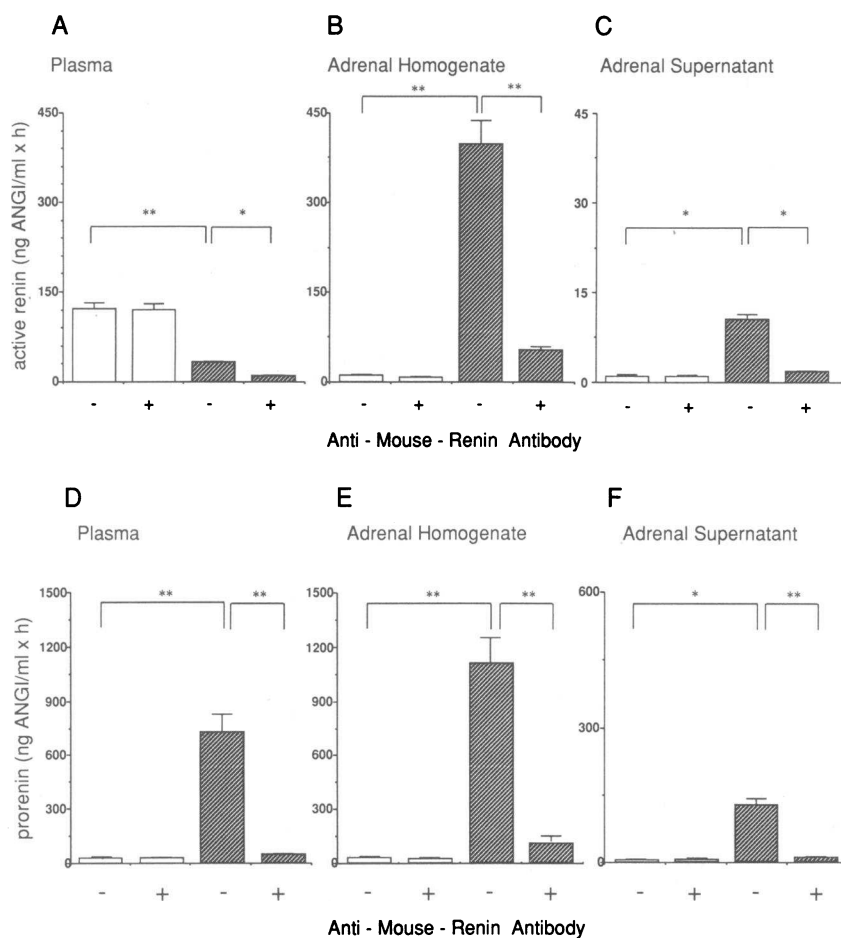
Anti - Mouse - Renin Antibody

Figure 1. Plasma and adrenal renin in TGR(mREN2)27. Blood and adrenal glands were collected from adult homozygous TGR(mREN2)27 and from age-matched SD rats. One adrenal gland was either homogenized in 1 ml of buffer or incubated in 1 ml of medium for a period of 1 h. *Open bars,* SD; *hatched bars,* TGR(mREN2)27; −, supernatant after treatment with buffer and protein A; +, supernatant after treatment with AB 120 and protein A. (*A–C*) Active renin; (*D–E*) Prorenin. Mann-Whitney U test for unpaired variables: $n = 4$; $*P < 0.05$; $**P < 0.01$.

geneous rat renin. Thus, we have demonstrated that the adrenal gland of TGR(mREN2)27 contains functionally active mouse renin derived from the $Ren-2^d$ transgene and that this adrenal renin activity is strongly enhanced as compared with SD rats.

To examine the pattern of renin release from adrenal cells of TGR(mREN2)27 in more detail and to study its regulation, we analyzed adrenal renin secretion in vitro. While the renin release from adrenal cell suspensions of SD rats (Table I) and also Wistar Kyoto rats (data not shown) was below the detection limit, adrenal cells of TGR(mREN2)27 secreted considerable amounts of active renin and prorenin (Table I). Most of the intracellular content of prorenin was released into the me-

Table I. Renin Content and Renin Release from Adrenal Cells of TGR(mREN2)27

	Renin release (ng ANGI/h) per 1 mio cells/h		Renin content (ng ANGI/ h) per 1 mio cells	
	Active renin	Prorenin	Active renin	Prorenin
Sprague Dawley	−0	0	3.4±0.5	0.75±0.2
TGR(mREN2)27	1.5±0.3	6±0.75	25±3.8*	10±2.2*

Adrenal cells were prepared from adult female TGR(mREN2)27 and from age matched SD rats. They were incubated at a concentration of 250,000 cells/ml for a period of 90 min. $*P < 0.05$, TGR vs. SD; values represent mean±SEM of four samples per group.

dium after 1 h of incubation. However, only ∼ 6% of the intracellular content of active renin was released during the same period of time.

To demonstrate the local synthesis of renin in adrenal cells of TGR(mREN2)27 we next examined the effect of translation inhibition by cycloheximide. As shown in Fig. 2 *B,* cycloheximide significantly inhibits the release of prorenin to 20% of that of untreated cells and the release of active renin to 65% (Fig. 2 *A*). The release of renin was not affected by cycloheximide during the first 90 min of incubation. In addition, it was more effective in inhibiting prorenin release than active renin release.

Using this system of adrenal cell suspensions in vitro, we were able to study the regulation of renin release from adrenal cells of TGR(mREN2)27. As shown in Fig. 3 *A,* the release of active renin was significantly stimulated by 8-Br-cAMP for 16 h compared with unstimulated transgenic adrenal cells. The prorenin release was significantly elevated by 8-Br-cAMP even after 6 h of stimulation (Fig. 3 *B*). This was accompanied by a significant rise in $Ren-2^d$ mRNA as detected by a $Ren2^d$-specific RNAse protection assay (Fig. 3 *C*). Thus, the release of active renin and prorenin from transgenic adrenal cells is stimulated by 8-Br-cAMP. This is in part due to enhanced de novo synthesis, as we conclude from the parallel increases of prorenin and $Ren-2^d$ mRNA under the same conditions.

We also focused on the major component of the negative feedback loop controlling renin release from the kidney, angiotensin II. Our question was: does the negative feedback mechanism also exist in adrenal cells of TGR(mREN2)27? Surpris-
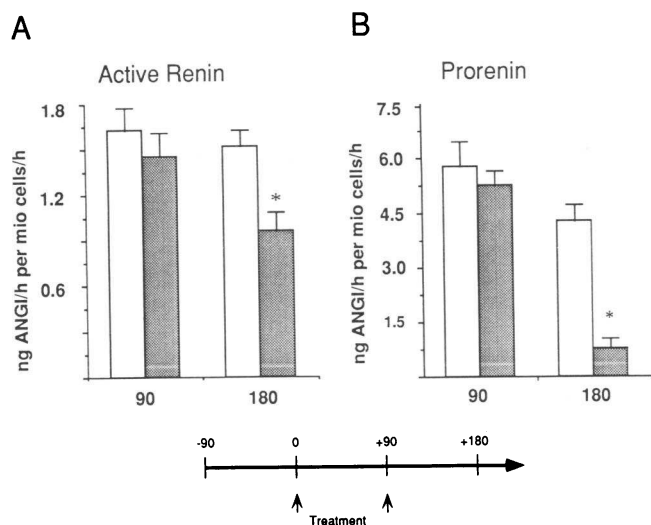
Figure 2. Effect of cycloheximide on renin release from adrenal cell suspensions of TGR(mREN2)27. Cell suspensions of adrenal glands of adult female TGR(mREN2)27 were incubated at a cell concentration of 250,000 cells/ml for 90-min periods. (Open bars) Untreated cells; (hatched bars) cycloheximide 10 μg/ml. (A) Active renin released; (B) prorenin released. Mann-Whitney U test for unpaired variables and matched pairs: $n = 5$; *$P < 0.01$.

Figure 4. Long-term effect of ANGII on renin release from adrenal cell suspensions of adult female TGR(mREN2)27. Cell suspensions of adrenal glands of adult female TGR(mREN2)27 were incubated at a cell concentration of 750,000 cells/ml and the supernatant was exchanged every 90 min. Mann-Whitney U test for matched pairs (basal vs. stimulated) and for unpaired variables (treated vs. control): $n = 5$; *$P < 0.05$. (A) Active renin released; (B) prorenin released. (Open bars) Basal release; (hatched bars) 3 h after stimulation.

ingly, as shown in Fig. 4, ANGII has the opposite effect in adrenal cells of TGR(mREN2)27. During 3 h of incubation, ANGII significantly stimulates active renin release from transgenic adrenal cells, with a maximum three- to fourfold increase, when compared with its basal release (Fig. 4 A, $P < 0.05$). Importantly, with ANGII, prorenin release remained unaffected or only slightly increased during the experiment (Fig. 4 B, $P > 0.1$). The regulation of prorenin and active renin release seem to be dissociated.

To date, studies on acute regulation of renin release from adrenal cells are not available, due to the detection limit
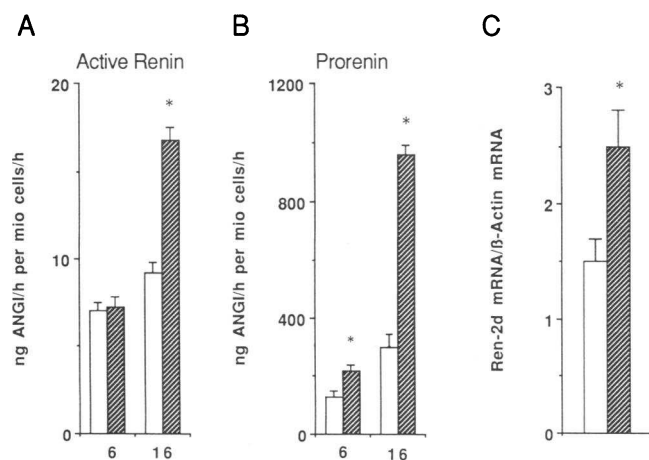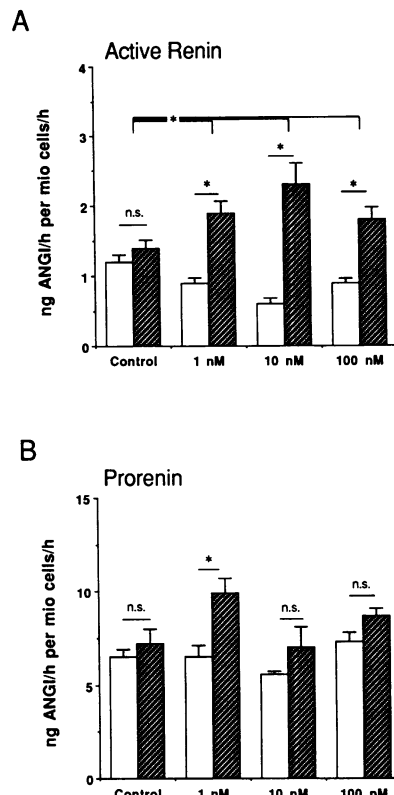

Figure 3. Long-term effect of 8-Br-cAMP on renin in adrenal cell suspensions of TGR(mREN2)27. Cell suspensions of adrenal glands of adult female TGR(mREN2)27 were incubated at a cell concentration of 150,000 cells/ml. (Open bars) Transgenic control cells; (hatched bars) transgenic cells treated with 5 mM 8-Br-cAMP. (A) Active renin released; (B) Prorenin released; (C) Ren2^d mRNA/β-actin mRNA after 6 h of stimulation. Mann-Whitney U test for unpaired variables: $n = 5$; *$P < 0.05$.

for renin activity after short incubation periods. The overexpression of transgenic renin in adrenal glands of TGR(mREN2)27, offers the opportunity to study those phenomena in TGR(mREN2)27. The above findings suggest the existence of a regulated secretory pathway for active renin release in adrenal cells of TGR(mREN2)27. Therefore, we next examined the acute effects of 8-Br-cAMP, ANGII, and the calcium ionophore A23187.

As demonstrated in Fig. 5, A–C, active renin release is significantly stimulated by all three drugs within 60 min of incubation (5 mM 8-Br-cAMP: 166±20%, $P < 0.05$; 1 nM ANGII: 118±10%, $P > 0.05$; 10 nM ANGII: 162±20%, $P < 0.05$; 100 nM ANGII: 155±10%, $P < 0.05$; 1 μM A23187: 173±12%, $P < 0.05$). The effect of ANGII was dose dependent, reaching maximal stimulation with 10 nM ANGII. In the same experiments prorenin release was not significantly affected.

Discussion

Transgenic rats TGR(mREN2)27, having integrated the mouse Ren-2^d renin gene, develop fulminant hypertension with systolic blood pressure ranging from 200 to 280 mmHg. We consider TGR(mREN2)27 as a model of "low plasma renin hypertension." We further hypothesize that the overexpression of the renin gene in certain tissues results in stimulated paracrine RAS, like in the brain, the blood vessels, the adrenal gland, or the heart. This may then lead to hypertension development in TGR(mREN2)27 and also to the observed downregulation of kidney renin. The existence of autocrine or paracrine RAS is supported by a number of recent findings (1, 4, 5).

Several lines of evidence indicate that hypertension in TGR(mREN2)27 is indeed determined by the transgene activity and is not a result of insertional mutagenesis. First, in sev-
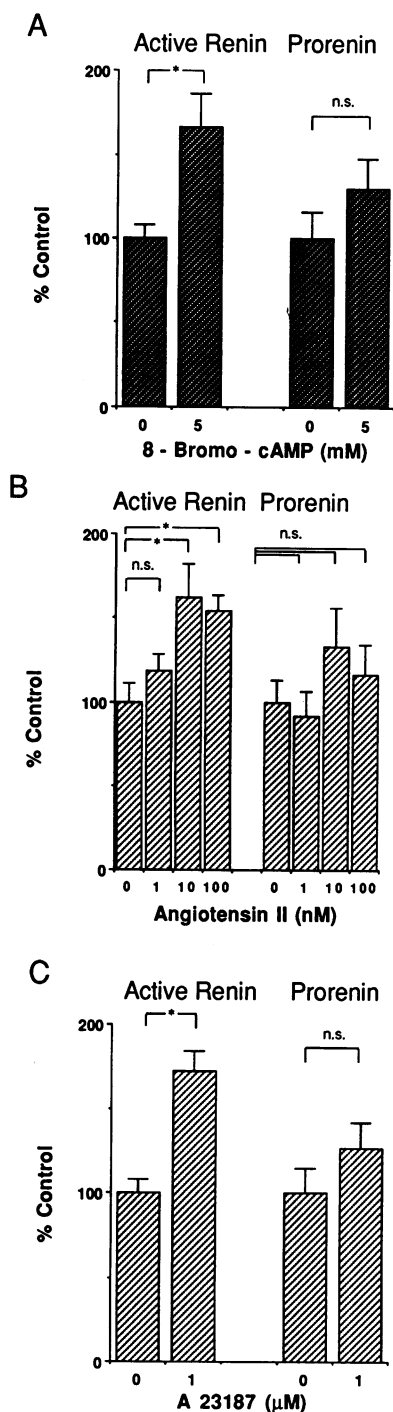
A

Active Renin   Prorenin

B

Active Renin   Prorenin

Figure 5. Short-term effect of 8-Br-cAMP, AN-GII, and A23187 on renin release from adrenal cell suspensions of TGR(mREN2)27. Cell suspensions of adrenal glands of adult female TGR(mREN2)27 were incubated at a concentration of 1 million cells/ml. The supernatants were collected 60 min after stimulation with the various substances. Mann-Whitney U test for unpaired variables (treated vs. control): $n = 5$; *P < 0.05. (*A*) 8-Bromo-cAMP; (*B*) ANGII; (*C*) A23187.

C

Active Renin   Prorenin

eral independent experiments hypertensive transgenic rat strains were generated with the same transgene integrated at different sites. Second, the hypertension cosegregates completely with the presence of the transgene. Third, the hypertension responds with great sensitivity to converting enzyme inhibitors and ANGII antagonists. These drugs interfere with the system to which the genetic manipulation was directed. Fourth, mouse $Ren-2^d$-specific transcripts were detected in the same tissues as in $Ren-2^d$ mice, indicating correct expression of the gene.

Here we demonstrate that the adrenal gland of TGR (mREN2)27 contains, synthesizes, and secretes high amounts of mouse renin originating from transcription and translation

of the transgene. Renin activity is highly increased in adrenal cells and adrenal tissue of TGR(mREN2)27, when compared with SD or Wistar Kyoto rats. The inhibitory effect of cycloheximide on renin release confirms that mouse renin is indeed synthesized locally.

To demonstrate that the transgene has retained its regulatory properties and to gain insight into the dynamic regulation of adrenal renin of TGR(mREN2)27, we analyzed the long-term and short-term effects of 8-Br-cAMP, ANGII, and calcimycin (A23187) on renin release from transgenic adrenal cells.

Recently, Nakamura et al. (18) and Burt et al. (19) proposed a cAMP responsible element in the 5' flanking sequence of the $Ren-2^d$ gene and demonstrated its function. However, the stimulatory effect of cAMP on $Ren-2^d$ transcription appears to be cell-type-specific and depends on the composition of *trans*-acting factors (20). Also, an acute stimulation of renin secretion by cAMP was demonstrated by several models, but this effect is dependent on the cell type (21). The $Ren-2^d$ transgene of TGR(mREN2)27 should have retained its regulatory properties, since the fragment we used to establish this strain contains large parts of its 5'- and 3'-flanking sequences, including the cAMP responsible element.

Our results demonstrate that in adrenal cells of TGR (mREN2)27, 8-Br-cAMP significantly increases the $Ren-2^d$ mRNA content and that this is paralleled by an increased prorenin release 6 h after the addition of the component. Active renin release is also enhanced by 8-Br-cAMP, but there was a latency of several hours as compared with prorenin stimulation. The effect is almost identical to the findings by Pratt et al. (22) on human renin, Nakamaru et al. (23) on mouse $Ren-2^d$ in AtT20 cells, and Krieger et al. (20). Since the time necessary for packaging and maturation of storage granules is thought to be ~ 16 h (24), our results suggest regulated secretion of active renin from adrenal cells of TGR(mREN2)27.

Further results presented in this study confirm this possibility: cycloheximide only slightly suppressed active renin release but strongly suppressed prorenin release. This effect is not present in the first 90 min of incubation, as would be expected for an exclusively constitutive secretion. The lack of suppression of renin release during the first 90 min of incubation may be due to the fact that proteins synthetized before inhibition of translation by cycloheximide can still be released during this incubation period. Thus the effect of cycloheximide is masked. In addition, we cannot completely exclude the possibility of partial costorage of prorenin with active renin. The concept of dissociation of prorenin and active renin secretion in the adrenal gland is further supported by the finding that only a small portion of the active renin present intracellularly is released per hour, while almost all of the cellular prorenin is released during the same time. Furthermore, acute active renin and prorenin release seem to be dissociated when the cells are stimulated with 8-Br-cAMP, ANGII, or calcimycin. All three substances stimulate active renin, but not prorenin release within 60 min of treatment. These findings in adrenal glands of TGR (mREN2)27 support the concept of differentially regulated pathways of secretion for prorenin and active renin in adrenal cells, as was described also for the kidney, mouse submandibular gland, and in AtT20 cells (21, 25–27).

The mechanisms of renin regulation and secretion in the adrenal gland remain far from being clear. Mizuno et al. (9) isolated renin-containing secretory granules from the rat adrenal cortex and demonstrated that their number is increased by

nephrectomy, a maneuver that is known to increase the adrenal renin content. They, and Yamaguchi et al. (28), also suggested a stimulatory long-term effect of ANGII on renin secretion from rat adrenal cortical primary cell cultures. However, due to the low abundance of renin in the rat adrenal cortex, the analysis of short-term regulation is limited.

The adrenal gland was shown to be a major source of plasma prorenin in TGR(mREN2)27, whereas the kidney renin production is strongly suppressed (10, 11). The detection of high amounts of renin secreted from transgenic adrenal cells confirms this finding. We therefore asked whether or not adrenal renin is under regulatory control similar to kidney renin. 8-Br-cAMP, which is known to stimulate renin release from the kidney, also stimulates renin release from primary cells of TGR(mREN2)27 adrenal glands. ANGII and increased intracellular free calcium are well-known inhibitors of renal renin release and are important in negative feedback regulations of blood pressure (1). Both factors do not suppress adrenal renin release in TGR(mREN2)27 but instead significantly stimulate active renin release eliciting a quick response. Since ANGII is known to elevate intracellular free calcium, the effect of ANGII may be mediated via calcium.

The stimulatory effect of ANGII on active renin release may be an epiphenomenon of the transgenic model but could also represent an endogeneous mechanism. The findings of Mizuno et al. and Yamaguchi et al. (9, 28) speak in favor of the second possibility. Most likely, the specific apparatus of the cell type (e.g., endocrine cells, juxtaglomerular cells, submandibular gland cells) define in part the pattern of renin regulation. In any case, the positive feedback loop between renin release and ANGII may be important for the regulation of adrenal steroid metabolism, plasma renin, and in the pathophysiology of hypertension, at least in TGR(mREN2)27. This remains to be investigated.

Acknowledgments

References

1. Hackenthal, E., M. Paul, D. Ganten, and R. Taugner. 1990. Morphology, physiology, and molecular biology of renin secretion. *Physiol. Rev.* 70:1067–1116.

2. Ganten, D., J. S. Hutchinson, P. Schelling, U. Ganten, and H. Fischer. 1976. The iso-renin angiotensin systems in extrarenal tissue. *Clin. Exp. Pharmacol. Physiol.* 3:103–126.

3. Ganten, D., K. Hermann, T. Unger, and R. E. Lang. 1983. The tissue renin-angiotensin system: focus on brain angiotensin, adrenal gland and arterial wall. *Clin. Exp. Hypertens. Part A Theory Pract.* 5:1099–1118.

4. Jin, M., M. J. Wilhelm, R. E. Lang, T. Unger, K. Lindpaintner, and D. Ganten. 1988. Endogenous tissue renin-angiotensin systems. From molecular biology to therapy. *Am. J. Med.* 84(Suppl. 3a):28–36.

5. Ganten, D., J. Peters, and K. Lindpaintner. 1989. Pathophysiology and

6. Ryan, J. W. 1967. Renin-like enzyme in the adrenal gland. *Science (Wash. DC).* 158:1589–1590.

7. Ganten, D., U. Ganten, S. Kubo, P. Granger, W. Nowaczynski, R. Boucher, and J. Genest. 1974. Influence of sodium, potassium and pituitary hormones on iso-renin in rat adrenal gland. *Am. J. Physiol.* 227:224–229.

8. Goormaghtigh, N., and H. Handovsky. 1938. *Arch. Pathol.* 26:1144.

9. Mizuno, K., L. H. Hoffman, J. C. McKenzie, and T. Inagami. 1988. Presence of renin secretory granules in rat adrenal gland and stimulation of renin secretion by angiotensin II but not by adrenocorticotropin. *J. Clin. Invest.* 82:1007–1016.

10. Mullins, J. J., J. Peters, and D. Ganten. 1990. Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. *Nature (Lond.).* 344:541–544.

11. Bachmann, S., J. Peters, E. Engler, D. Ganten, and J. Mullins. 1992. Transgenic rats carrying the mouse renin gene: morphological characterization of a low renin hypertension model. *Kidney Int.* 41:24–36.

12. Sander, M., M. Bader, B. Djavidani, C. Maser-Gluth, P. Vecsei, J. Mullins, D. Ganten, and J. Peters. 1992. The role of the adrenal gland in the hypertensive transgenic rats TGR(mREN2)27. *Endocrinology.* 131:807–814.

13. Glorioso, N., P. Madeddu, P. Dessi'-Fulgheri, G. Fois, F. Meloni, F. Bandiera, G. Tonolo, and A. Rappelli. 1983. Trypsin-activatable inactive renin in rat plasma. *Clin. Sci. (Lond.).* 64:137–140.

14. Schelling, P., U. Ganten, G. Sponer, T. Unger, and D. Ganten. 1980. Components of the renin-angiotensin system in the cerebrospinal fluid of rats and dogs with special consideration of the origin and the fate of angiotensin II. *Neuroendocrinology.* 31:297–308.

15. Hermann, K., D. Ganten, T. Unger, C. Bayer, and R. E. Lang. 1988. Measurement and characterization of angiotensin peptides in plasma. *Clin. Chem.* 34:1046–1051.

16. Wilkinson, M. 1988. RNA isolation: a mini-prep method. *Nucleic Acids Res.* 16:10933.

17. Peters, J., M. Bader, D. Ganten, and J. Mullins. 1991. Tissue distribution of ren-2 expression in transgenic rats. *In* Genetic Approaches to Coronary Heart Disease and Hypertension. K. Berg, V. Bulyzhenkov, Y. Christen, and P. Corvol, editors. Springer-Verlag, Berlin. 74–80.

18. Nakamura, N., D. W. Burt, M. Paul, and V. J. Dzau. 1989. Negative control elements and cAMP responsive sequences in the tissue-specific expression of mouse renin genes. *Proc. Natl. Acad. Sci. USA.* 86:56–59.

19. Burt, D. W., N. Nakamura, P. Kelley, and V. J. Dzau. 1989. Identification of negative and positive regulatory elements in the human renin gene. *J. Biol. Chem.* 264:7357–7362.

20. Krieger, J. E., M. Paul, R. E. Pratt, W. M. Philbrick, K. W. Gross, and V. J. Dzau. 1990. cAMP stimulates secretion of prorenin via increased transcription rate of renin gene in transfected cells. *J. Hypertens.* 8(Suppl. 3):S68. (Abstr.)

21. Paul, M., N. Nakamura, R. E. Pratt, D. W. Burt, and V. J. Dzau. 1992. Cell dependent posttranslational processing and secretion of recombinant mouse renin-2. *Am. J. Physiol.* 262:E224–E229.

22. Pratt, R. E., J. A. Flynn, P. M. Hobart, M. Paul, and V. J. Dzau. 1988. Different secretory pathways of renin from mouse cells transfected with the human renin gene. *J. Biol. Chem.* 263:3137–3141.

23. Nakamura, N., D. W. Burt, M. Paul, R. E. Pratt, and V. J. Dzau. 1988. Regulation of renin gene expression: evidence for negative and cAMP responsive elements. *Clin. Res.* 36:553A. (Abstr.)

24. Pratt, R. E., J. E. Carleton, T. P. Roth, and V. J. Dzau. 1988. Evidence for two cellular pathways of renin secretion by the mouse submandibular gland. *Endocrinology.* 123:1721–1727.

25. Pratt, R. E., J. E. Carleton, J. P. Richie, C. Heusser, and V. J. Dzau. 1987. Human renin biosynthesis and secretion in normal and ischemic kidneys. *Proc. Natl. Acad. Sci. USA.* 84:7837–7840.

26. Taugner, R., S.-J. Kim, K. Murakami, and R. Waldherr. 1987. The fate of prorenin during granulopoiesis in epithelioid cells immunocytochemical experiments with antisera against renin and different portions of the renin prosegment. *Histochemistry.* 86:249–253.

27. Pratt, R. E., A. J. Ouellette, and V. J. Dzau. 1983. Biosynthesis of renin: multiplicity of active and intermediate forms. *Proc. Natl. Acad. Sci. USA.* 80:6809–6813.

28. Yamaguchi, T., R. Franco-Saenz, and P. J. Mulrow. 1992. Effect of angiotensin II on renin production by rat adrenal glomerulosa cells in culture. *Hypertension (Dallas).* 19:263–269.

molecular biology of tissue renin. *In* Current Advances in ACE Inhibition. G. A. MacGregor and P. S. Sever, editors. Churchill Livingstone, London. 25–34.