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Morphology and Function of the Adrenal Zona Glomerulosa of Transgenic Rats TGR [mREN2] 27: Effects of Prolonged Sodium Restriction

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Heterozygous female transgenic rats for the murine Ren-2 gene (TGR) display a high blood pressure, together with a low kidney and high adrenal renin content. The effects of prolonged sodium restriction on the morphology and secretory activity of adrenal zona glomerulosa (ZG) of TGR and their age- and sex-matched Sprague–Dawley control rats (SDR) were investigated. Under basal conditions, TGR had a moderately hypertrophic ZG, that showed a significantly higher secretion of 18-hydroxylated (18OH) steroids: 18-hydroxy-11-deoxycorticosterone (18OH-DOC), 18-hydroxycorticosterone (18OH-B) and aldosterone (ALDO); ZG cells of TGR showed angiotensin II (AII)-binding site concentrations and ALDO secretory responses to AII similar to those of SDR ZG cells. Prolonged sodium restriction increased plasma ALDO level in both SDR and TGR, and significantly raised the volume of ZG. ZG hypertrophy was due to the increase in both the number and average volume of its parenchymal cells. The secretion of 18OH-steroids was markedly enhanced in both groups of rats; however, in TGR this rise was exclusively due to increases of 18OH-DOC and 18OH-B, while in SDR also ALDO production was enhanced. The yield of non-18OH-steroids was not affected. 11-Dehydrocorticosterone production was not changed in SDR, but doubled in TGR. ZG cells of sodium-restricted SDR and TGR displayed similar increases in their AII-binding site concentration and ALDO secretory response to AII. In conclusion, our present findings confirm that TGR possess a hypertrophic ZG and an elevated secretory capacity of 18OH-steroids, but show only slight differences in ZG and ZG-cell responses to prolonged sodium deprivation.

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INTRODUCTION

The hypertensive rat strain TGR [mREN2]27 (TGR) was developed by Mullins *et al.* [1] by the transfection of the murine Ren-2[d] gene into the genome of Sprague–Dawley rats (SDR). TGR are characterized by high levels of plasma prorenin, low levels of plasma and kidney renin, and overexpression of renin in extrarenal tissues, especially in the adrenal zona glomerulosa (ZG) [2–4]. Since hypertension in TGR is reversed by chronic medication with angiotensin-converting-enzyme inhibitors or angiotensin-II (AII)-receptor

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Abbreviations: AII, angiotensin II; ACTH, adrenocorticotropic hormone; ALDO, aldosterone; ANOVA, analysis of variance; B, corticosterone; BP, systolic blood pressure; DH-B, 11-dehydrocorticosterone; DOC, 11-deoxycorticosterone; PAC, plasma aldosterone concentration; PRA, plasma renin activity; PROG, progesterone; CYP11B$_2$, aldosterone synthase; RAS, renin–angiotensin system; SDR, Sprague–Dawley rats; SER, smooth endoplasmic reticulum; TGR, transgenic rats; ZG, zona glomerulosa; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 18OH-B, 18-hydroxycorticosterone; 18OH-DOC, 18-hydroxy-11-deoxycorticosterone.

antagonists [2, 5], it seems conceivable that the adrenal renin–angiotensin-system (RAS) may contribute to the pathogenesis of this form of hypertension. Convincing evidence indicates that intra-adrenal RAS plays a major role in the control of ZG secretory activity [6–8]. Accordingly, the plasma concentration and urinary excretion of mineralocorticoids are markedly increased during the development of hypertension in TGR [9], and *in situ* hybridization indicates that $CYP11B_2$ (aldosterone synthase, the enzyme which converts deoxycorticosterone to aldosterone) is overexpressed in the ZG of TGR compared with control SDR [10].

We have recently studied the morphology and function of the ZG of 10-week-old heterozygous female TGR [11]. The ZG displays a moderate hypertrophy, but ZG cells show no evidence of conspicuous ultrastructural differences compared with SDR, with the exception of a markedly higher content of lipid droplets. Dispersed ZG cells of TGR show a higher basal aldosterone (ALDO) production, but their response to AII is similar to that of SDR; AII-receptor density and affinity are not dissimilar in ZG cells of TGR and SDR. Thus, the basal hyperfunction of ZG of TGR does not appear to be coupled with an enhanced responsivity to its main secretagogue [12], at least in terms of ALDO secretion.

Numerous lines of evidence indicate that a low-sodium intake stimulates not only the kidney RAS (see [13] for review), but also the intra-adrenal RAS [14, 15], and in particular raises the number of AII-binding sites in the ZG cells [15, 16]. It therefore seemed worthwhile to compare the effects of prolonged sodium restriction on the morphology and function of the ZG of TGR and SDR.

EXPERIMENTAL

Animals and diets

Heterozygous female TGR (10 weeks of age, when hypertension is well established), and age- and sex-matched SDR were housed 4 per cage at $20 \pm 2^{\circ}C$ on a 12:12 h light–dark cycle. Groups of TGR and SDR were maintained on a sodium-deprived diet (<0.01 mEq Na^{+} g^{-1}; Laboratorio Dr Piccioni, Milan, Italy) with demineralized water as drinking fluid. Control TGR and SDR were fed normal rat/mouse chow and tap water. Animals were maintained on these specimens for 7 days. The systolic blood pressure (BP) was measured by tail sphygmomanometry (BP-Recorder; Basile, Comerio, Italy) 24 h before the sacrifice. The rats were decapitated between 10:00 and 11:00 a.m.; trunk blood was collected and frozen, and adrenal glands were promptly removed and freed of pericapsular fat.

Light and electron microscopy

The adrenal glands of control and sodium restricted TGR and SDR were weighed. The left adrenals were fixed in Bouin's solution, embedded in paraffin, and serially cut at 6–7 μm. Sliced pieces of the right glands were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, counterstained with uranyl acetate overnight, and then embedded in Epon.

Thick (0.5 μm) and thin sections (70 nm) were cut with an LKB Supernova ultramicrotome (Reichert-Jung, Wien, Austria) at the level of ZG. Thin sections were stained with lead hydroxide, and examined in a Hitachi H-300 electron microscope at a direct magnification of 7000 or 20,000.

For each rat, 3 plastic blocks containing ZG were cut and, at low magnification, 3 technically adequate series of thick and thin sections for each block were selected. Four light micrographs at a magnification of 2000 for each series of thick sections were obtained with a camera-connected Leitz microscope and stored in a Hantares-80 computer. From each series of thin sections, 8 electron micrographs at a final magnification of 21,000, and 4 electron micrographs at a final magnification of 60,000 were recorded.

Morphometry

Stage I: volume of ZG. With a magnification of 100 and a square lattice test system of type A [17], the volume density (Vv) of ZG was evaluated by 'differential point counting'. The measurement was performed on every fifth paraffin section of the gland. The volume of the adrenal gland was calculated from its weight (specific gravity, 1.039).

Stage II: size and number of ZG cells. The Vv values of nuclei and cytoplasm of parenchymal cells and stroma of ZG, as well as the numerical density (Na) of nuclear profiles, were estimated on the images at $2000\times$ of 0.5-μm-thick sections [17]. The edge effect in estimating Na was eliminated as previously described [18]. The number of nuclei of parenchymal cells per mm^3 of ZG (Nv) was estimated according to the formula of Weibel–Gomez [17]: $Nv = (K/\beta) \times (Na^{3/2}/Vv^{1/2})$. The size-distribution and shape coefficients K and β were calculated as previously detailed [18]. Since rat adrenocortical cells are mononucleated, Nv of nuclei corresponds to the number of cells per mm^3 of ZG. Subsequently, the average volume and number of ZG cells (as well as the volume of their nuclei) were computed. All this procedure was semiautomatically performed using specific software purchased from Studio Casti Imaging (Venice, Italy).

Stage III: stereology. On the electron micrographs at a final magnification of 21,000, the Vv values of mitochondria, lipid droplets and dense bodies were estimated by 'differential point counting'. On the electron micrographs at a final magnification of 60,000, the surface density (Sv) of mitochondrial cristae and smooth endoplasmic reticulum (SER) membranes was measured by the 'intersection method' [17], as detailed previously [18]. The absolute values per ZG cell of the

various organelles were obtained by multiplying Vv and Sv by the mean cell volume.

Plasma biochemical assays

Plasma renin activity (PRA) was assayed by RIA of angiotensin-I generated after incubation of plasma (ANG-I RIA kit; Peninsula Merseyside, England). ALDO was extracted and purified [11], and its concentration (PAC) was measured by RIA (ALDO-CTK2; IRE-Sorin, Vercelli, Italy). Intra- and interassay variations were: angiotensin-I, 6.0 and 8.7%; and ALDO, 5.6 and 7.1%, respectively.

Capsular-strip secretion

Capsular strips (ZG) from control and sodium-restricted SDR and TGR were put in Medium 199 (DIFCO, Detroit, MI) and potassium-free Krebs–Ringer bicarbonate buffer with 0.2% glucose (2:1, v/v), containing 5 mg ml^{-1} human serum albumin; the final K^+ concentration of the mixture was 5.35 mM. They were incubated in a shaking bath at 37°C for 90 min in an atmosphere of 95% O_2 and 5% CO_2. The concentrations of progesterone (PROG), 11-deoxycorticosterone (DOC), corticosterone (B), 18-hydroxy-11-deoxycorticosterone (18OH-DOC), 18-hydroxycorticosterone (18OH-B), ALDO and 11-dehydrocorticosterone (DH-B) in the incubation media were assayed by quantitative HPLC, as previously detailed [19].

Preparation of dispersed ZG cells

Twenty adrenal glands from control and sodium-restricted SDR and TGR were employed. Subcapsular tissue (ZG) was separated from the inner zones by squeezing. Isolated ZG cells were obtained by sequential collagenase digestion and mechanical dispersion, as described elsewhere [11]. Contamination with zona fasciculata cells was less than 5%, and viability of ZG cells was checked by the Trypan blue exclusion test and found to be higher than 90%. Dispersed cells were resuspended in Medium 199 containing 4.5 mM K^+ and 1% bovine serum albumin. Aliquots of cell suspensions (5×10^4 cells ml^{-1}) were incubated, in duplicate, in the presence of AII or ACTH (Peninsula), for 120 min at 37°C, in an atmosphere of 95% O_2 and 5% CO_2. Cell suspensions were then centrifuged at 2000 rpm, and supernatants were stored at −20 C. ALDO was assayed by specific RIA directly in the medium as described above (intra- and interassay variations 7.1 and 8.3%, respectively).

Determination of AII-receptor concentration

The subcapsular tissue of 10 adrenal glands from control and sodium-restricted SDR and TGR was minced into very small pieces, and homogenized by a Polytron homogenizer in 15 ml of 20 mM sodium bicarbonate solution. The homogenate was stirred on ice for 20 min, filtered through a nylon gauze, and then centrifuged at 30,000 *g* for 30 min. The pellet was resuspended and homogenized with a glass–teflon homogenizer in Tris–HCl-buffered medium (pH 7.4), containing 5 mM $MgCl_2$ and 2 mM EGTA [11]. The AII-receptor concentration in ZG-cell membranes (100–150 mg of protein) was assayed by incubation with 0.3 nM ^{125}I-AII (native hormone; Amersham, England) in the Tris-HCl-buffered medium containing 120 mM NaCl and 0.2% bovine serum albumin. Non-specific binding was determined in the presence of 1 mM cold AII. The incubation (45 min at 22°C) was stopped by adding cold phosphate-buffered solution. Bound radioactivity was separated by repeated centrifugations, and measured in a γ-counter. The receptor concentration was calculated by computer analysis with Ligand software.

Statistics

Data were averaged per experimental group, and SE was calculated. Statistical comparison was by ANOVA, followed by Duncan's Multiple Range Test.

RESULTS

Prolonged sodium restriction did not change BP in SDR, but it slightly decreased BP in the hypertensive TGR (−14%). PRA was not dissimilar in control SDR and TGR, while PAC was about double in TGR; sodium restriction induced a small increase in PRA and a larger increase in PAC in both SDR (31% and 3.7-fold) and TGR (49 and 92%) (Table 1).

The volume of ZG was moderately but significantly higher in control TGR than in SDR (21%). This was coupled with a higher average number (8%) and greater mean volume (12%) of ZG cells: however, due to the considerable intra-group variability, these apparent differences were not significant ($0.1 > P > 0.05$) (Table 2). The ultrastructure of ZG cells of control

Table 1. Effects of sodium restriction on physical and biochemical parameters of SDR and TGR

	SDR		TGR	
	Normal diet	Low sodium diet	Normal diet	Low sodium diet
BP (mmHg)	115 ± 3	110 ± 4	235 ± 13^A	202 ± 9†
PRA (fmol ml^{-1} h^{-1})	8.8 ± 0.5	11.5 ± 0.6*	7.8 ± 0.7	11.6 ± 2.0†
PAC (pM)	560 ± 80	2090 ± 201*	1036 ± 131^A	1985 ± 221*

Data are means ± SE ($n = 10$). †$P < 0.05$ and *$P < 0.01$ from the respective control group; $^A P < 0.01$ from SDR controls

Table 2. Effects of sodium restriction on the morphometric parameters of ZG of SDR and TGR

	SDR		TGR	
	Normal diet	Low sodium diet	Normal diet	Low sodium diet
Volume of zona (mm^3)	3.62 ± 0.31	5.29 ± 0.34*	4.38 ± 0.33^a	6.27 ± 0.41*
Number of cells ($\times 10^3$)	4458 ± 443	5289 ± 508	4822 ± 279	5619 ± 404†
Volume of cells (μm^3)	650 ± 47	800 ± 61†	729 ± 32	894 ± 59
Volume of nuclei (μm^3)	103 ± 7	122 ± 7†	113 ± 6	131 ± 6†

Data are means ± SE ($n = 8$). †$P < 0.05$ and *$P < 0.01$ from the respective control group, $^aP < 0.05$ from SDR controls.

SDR and TGR paralleled that previously described [11]: compared with SDR, ZG cells of TGR do not display striking fine structural differences, with the exception of an abundance of lipid droplets (stereology indicated that the volume of the lipid-droplet compartment was 2.6-fold greater in TGR) (Table 3).

Prolonged sodium restriction caused similar morphometrically demonstrable effects in SDR and TGR (Tables 2 and 3). The volume of ZG was raised (43–46%), as well as the number and average volume of its parenchymal cells (17–19 and 23%, respectively). The volume of ZG-cell nuclei was also increased (16–18%). Sodium restriction-induced ZG-cell hypertrophy was associated with significant increases in the volume of the mitochondrial compartment (32%), and in the surface area per cell of mitochondrial cristae (35–38%) and SER membranes (31–33%). A marked rise in the volume of dense-body compartment was also observed in both SDR and TGR (52 and 78%). Conversely, the volume of the lipid-droplet compartment showed a net decrease (−36% in SDR, and −45% in TGR).

The secretion of 4-ene, 3-one steroids detected at 240 nm wavelength (hereinafter called post-pregnenolone secretion) of capsular strips of control SDR consisted of DOC (2.5%), B (48.5%), 18OH-DOC (6.4%), 18OH-B (12.1%), ALDO (6.9%) and DH-B (22.6%); the production of 18OH-steroids (18OH-DOC, 18OH-B and ALDO) and non-18OH-steroids (DOC and B) represented about 25.3 and 74.7%, respectively, of the total post-pregnenolone yield (Table 4). Total post-pregnenolone output by capsular strip was slightly higher in control TGR (35%), due to a significantly greater production of 18OH-steroids (18OH-DOC, 2.4-fold; 18OH-B, 2.2-fold; and ALDO, 3-fold), representing about 50% of the total post-pregnenolone yield. The secretion of non-18OH hormones was not different. while that of DH-B was markedly less (−60%) (Table 4).

Prolonged sodium restriction did not significantly affect total post-pregnenolone yield in either SDR or TGR, but it significantly enhanced mean values for the secretion of 18OH-steroids (18OH-DOC, 53% and 58%; 18OH-B, 43% and 80%; and ALDO, 95% and 35%), although it should be noted that the sodium restriction-induced increase in ALDO output was not significant in TGR. Sodium restriction did not change the secretion of non-18OH-steroids in either SDR or TGR, nor DH-B yield in SDR; however in TGR DH-B production more than doubled (Table 4).

AII increased ALDO secretion of dispersed ZG cells of both control SDR and TGR, with the output doubled at an agonist concentration of 10 nM. Sodium restriction increased basal ALDO secretion of isolated ZG cells in SDR (2-fold), but not in TGR. AII raised ALDO secretion of ZG cells of sodium-restricted SDR and TGR. with the response to the maximal effective concentration of AII similar in the two groups (3.6-fold in SDR, and 3.4-fold in TGR) (Fig. 1). ACTH (1 nM) evoked a marked increase in ALDO output of ZG cells of both SDR and TGR, both on a normal diet (12- and 5-fold) and on a low sodium intake (7- and 5-fold) (Fig. 2).

The AII-receptor density was not significantly higher in ZG-cell membranes from TGR than SDR,

Table 3. Effects of sodium restriction on the morphometric parameters of ZG cells of SDR and TGR

	SDR		TGR	
	Normal diet	Low sodium diet	Normal diet	Low sodium diet
Volume of mitochondrial compartment (μm^3/cell)	119 ± 12	154 ± 16†	134 ± 12	171 ± 18†
Surface area of mitochondrial cristae (μm^2/cell)	2173 ± 252	2927 ± 356†	2339 ± 212	3232 ± 408†
Surface area of SER (μm^2/cell)	3896 ± 448	5090 ± 538†	4224 ± 515	5618 ± 605†
Volume of lipid-droplet compartment (μm^3/cell)	57 ± 8	36 ± 6†	145 ± 26^A	80 ± 10*
Volume of dense-body compartment (μm^3/cell)	4.2 ± 1.1	6.4 ± 0.8†	6.2 ± 1.4	11.4 ± 2.2†

Data are means ± SE ($n = 8$). †$P < 0.05$ and *$P < 0.01$ from the respective control group, $^AP < 0.01$ from SDR control.

Table 4. Effects of sodium restriction on steroid secretion of capsular strips of SDR and TGR adrenals

	SDR		TGR	
(pmol mg $^{-1}$)	Normal diet	Low sodium diet	Normal diet	Low sodium diet
PROG	—	—	—	—
DOC	8 ± 1	7 ± 1	10 ± 1	9 ± 1
B	150 ± 21	128 ± 20	178 ± 23	158 ± 20
DH-B	68 ± 6	51 ± 7	28 ± 2^{A}	59 ± 6*
18OH-DOC	19 ± 3	29 ± 4†	46 ± 7^{A}	73 ± 8*
18OH-B	36 ± 3	52 ± 7†	80 ± 12^{A}	144 ± 21*
ALDO	21 ± 3	40 ± 7*	64 ± 9^{A}	86 ± 13
Non-18OH-steroids	226 ± 29	186 ± 27	208 ± 25	226 ± 41
18OH-steroids	76 ± 7	121 ± 17*	190 ± 27^{A}	303 ± 43†
Total post-pregnenolone output	302 ± 32	307 ± 43	406 ± 51^{a}	529 ± 70

Data are means ± SE (n = 8). †P < 0.05 and P < 0.01 from the respective control group, ^{a}P < 0.05 and ^{A}P < 0.01 from SDR controls.

and rose with sodium restriction in both SDR (48%) and TGR (63%) (Fig. 3).

DISCUSSION

According to our previous investigation [11], ZG of control TGR is hypertrophic and possesses a more elevated basal mineralocorticoid secretory capacity than that of SDR, although responsiveness to AII and AII-receptor concentrations are not significantly different in the two groups. The increased activity of intra-adrenal RAS in TGR may conceivably be the cause of this, inasmuch as AII is well known to enhance the growth of ZG [20, 21] and the expression of the enzymes of the late steps of ALDO synthesis (i.e. CYP11B_2) [22–24].

Our HPLC findings indicate that ZG cells of control TGR display an increased production not only of ALDO, but of the entire spectrum of 18OH-steroids, in keeping with the current belief that rat ZG CYP11B_2 possesses both 11β-hydroxylase and 18-hydroxylase/18-dehydrogenase activities (thus being able to convert DOC to ALDO). However, it must be noted that the raised yield of 18OH-DOC is rather surprising, inasmuch as CYP11B_2 has only a minor effect on the conversion of DOC to 18OH-DOC (see [25, 26] for review). Our data also show that under basal conditions ZG cells of TGR produce markedly less DH-B than SDR. 11β-Hydroxysteroid dehydrogenase (11β-HSD) is an enzyme that rapidly converts B to inactive DH-B, thus modulating the access of physiological glucocorticoids to their peripheral receptors [27]. Though being maximally expressed in kidney and liver (see [28] for review), 11β-HSD is also present in steroidogenic tissues (see [29] for review). Hence, the relative hypoactivity of 11β-HSD in capsular tissue of TGR adrenals may enhance the availability of intermediate products that can be converted to mineralocorticoids by hyperexpressed CYP11B_2. In this connection it must be recalled that a relative deficiency of 11β-HSD activity has been reported to occur in human patients with essential hypertension [30] and in the hypertensive Bianchi–Milan rats [31].

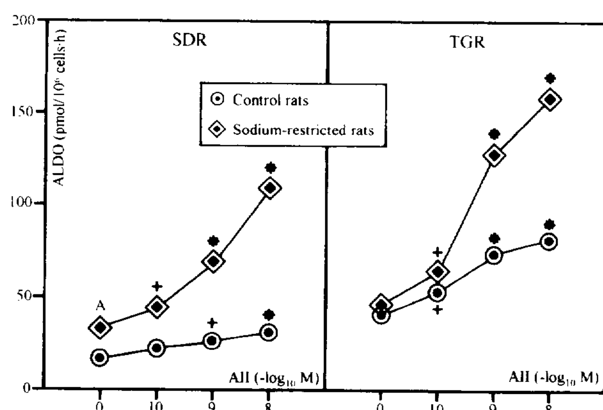

Fig. 1. Effect of AII on ALDO secretion of dispersed ZG cells of SDR and TGR kept under normal or sodium-deprived diet. Data are means (n = 6) and SE are not shown. †P < 0.05 and *P < 0.01 from the respective basal (0) group; ^{A}P < 0.01 from the respective basal control group.

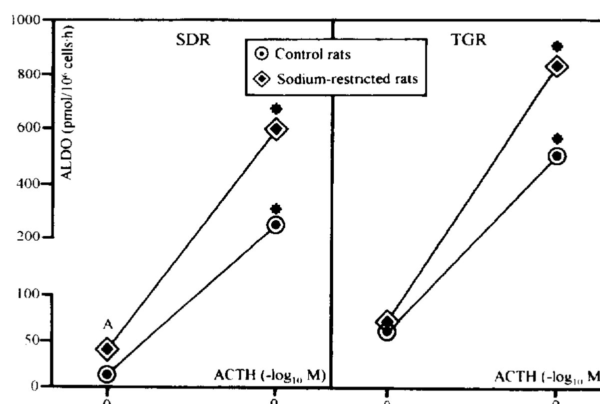

Fig. 2. Effect of ACTH on ALDO secretion of dispersed ZG cells of SDR and TGR kept under normal or sodium-deprived diet. Data are means (n = 6) and SER are not shown. *P < 0.01 from the respective basal (0) group; ^{A}P < 0.01 from the respective basal control group.

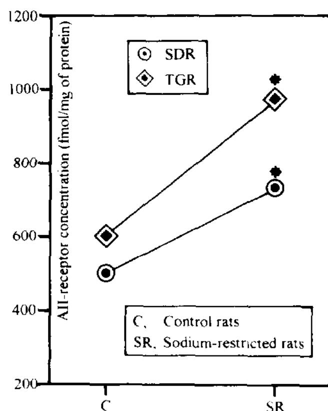

Fig. 3. Effect of prolonged sodium restriction on AII-receptor density in ZG cells of SDR and TGR. Data are means ($n = 6$) and SE are not shown. *$P < 0.01$ from the respective control group; $^{A}P < 0.01$ from control SDR.

As previously pointed out (see Introduction), prolonged sodium restriction enhances RAS activity both in the kidney and adrenals. Our findings indicate that a sodium-deprived diet induces a small rise in PRA not only in SDR, but also in TGR, where kidney renin expression is very low: this strongly suggests that, according to Tokita *et al.* [4], peripheral tissues (especially the adrenal glands) are the main source of circulating renin in TGR.

The exposure to very elevated concentrations of AII during sodium restriction enhances the growth of ZG, which is due not only to cell hyperplasia, but also to cell hypertrophy [20]. ZG-cell hypertrophy occurring in SDR and TGR is coupled with significant increases in the volume of the mitochondrial compartment and SER. These morphologic data accord well with the considerable rise of PAC, and of basal and agonist-stimulated mineralocorticoid production by ZG cells *in vitro*. The enzymes of the ALDO synthetic pathway are located in both mitochondria and SER (see [21, 29] for references), and the changes in the surface area per cell of mitochondrial cristae and SER tubules are tightly coupled with corresponding changes in the activity per cell of some of these enzymes [21, 32]. The increased utilization of cholesterol in mineralocorticoid synthesis occurring in sodium-restricted rats, coupled with a presumably normal uptake of cholesterol from serum lipoproteins, may well account for the marked decrease in the volume of the lipid-droplet compartment. In fact, it is commonly agreed that cholesterol and its esters are stored in adrenocortical lipid droplets (see [21] for review), and that lipoprotein uptake by adrenocortical cells is a receptor-mediated process mainly (if not exclusively) controlled by ACTH (see [33] for review). Taken together our present findings are in keeping with the many lines of evidence that sodium restriction (and potassium repletion) strikingly raise gene expression of the enzymes of the late pathway of mineralocorticoid synthesis [23, 24,34–36]. Parenthetically, enhanced mRNA transcription fits well with the increased nuclear volume observed in ZG cells of sodium-restricted SDR and TGR (see [21] for review). As expected, the higher responsiveness to AII of ZG cells of sodium-deprived SDR and TGR compared with that of the respective controls also reflects the marked increase in the number of AII receptors.

Histochemical evidence indicates that intra-adrenal renin is mainly located in the ZG [37, 38], but these light-microscopic studies do not allow us to ascertain whether renin is contained in stromal (connective or endothelial) or parenchymal cells. Rat ZG cells possess the organelles involved in the synthesis of secretory proteins (i.e. rough-endoplasmic-reticulum profiles and Golgi apparatus) (see [21] for review), and it has been clearly demonstrated that isolated ZG cells are able to release AI and AII in the superfusion medium [8]. Moreover, Kon *et al.* [39] reported immuno-electronmicroscopical findings indicating that renin is located in dense granules of fetal mouse ZG cells, and a significant increase in the number of dense bodies has been observed in ZG cells of sodium-restricted rats [40]. Unexpectedly, we have not previously shown any morphological counterpart of the elevated intra-adrenal renin content in control TGR [11]. However, after prolonged sodium restriction a stereologically-demonstrable increase in the number of lysosome-like dense bodies occurs in TGR cells. Whether or not this finding could be the expression of the sodium restriction-enhanced renin synthesis in ZG cells remains to be investigated, possibly by immunohistochemical methods.

In conclusion, our present investigation does not show marked differences in the ZG-cell response of SDR and TGR to prolonged sodium restriction and the consequent enhanced activity of kidney and peripheral RAS. ZG cells of both groups of rats undergo considerable hypertrophy and their mineralocorticoid secretory activity is significantly increased, which is also associated with a net up-regulation of their AII receptors. However, there are some differences that require further discussion and investigation. (i) Sodium restriction induces a moderate decrease in BP in hypertensive TGR but not in SDR. (ii) Sodium deprivation raises basal ALDO secretion of ZG cells of SDR, but not of TGR. Nevertheless, in TGR the overall production of 18OH-steroids is markedly increased, due to the enhanced yields of 18OH-DOC and 18OH-B. Though compelling evidence indicates that $CYP11B_2$ transforms B to ALDO (see above), it appears that in TGR sodium restriction differentially regulates the 18-hydroxylation and 18-dehydrogenation activities. (iii) Sodium restriction does not affect DH-B production by ZG of SDR, but markedly enhances it in TGR, in which basal activity of 11β-HSD appears to be depressed. It seems that sodium restriction is able to

stimulate the activity of capsular 11β-HSD in TGR (but not in SDR), thus reducing the availability of intermediate products and preventing an exceedingly high mineralocorticoid secretion. It remains to be elucidated whether the adrenal and kidney RAS affect adrenal 11β-HSD differently.

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