British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.01953.x
www.brjpharmacol.org

RESEARCH PAPER

Blood pressure response to angiotensin II is enhanced in obese Zucker rats and is attributed to an aldosterone-dependent mechanism

Helge Müller-Fielitz[1], Margot Lau[1], Olaf Jöhren[1], Florian Stellmacher[2], Markus Schwaninger[1] and Walter Raasch[1]

[1]*Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Germany,* [2]*Leibniz Center for Medicine and Biosciences, Borstel, Germany*

Correspondence
Walter Raasch, Institute of Experimental and Clinical Pharmacology and Toxicology, University at Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: raasch@medinf.mu-luebeck.de

This study was supported by a grant (N11-2004) of the Dean of the Medical Faculty of the University of Lübeck.

Keywords
obesity; hypertension; adrenal gland; aldosterone; angiotensin II; aldosterone synthase; AT_{1B} receptor

Received
15 September 2011
Revised
10 February 2012
Accepted
11 March 2012

BACKGROUND AND PURPOSE

Plasma aldosterone levels correlate positively with obesity, suggesting a link between the hypertension associated with obesity and increased mineralocorticoid levels. We tested the hypothesis that aldosterone is involved in the BP response to angiotensin II (AngII) in obese rats.

EXPERIMENTAL APPROACH

Lean (LZR) and obese (OZR) Zucker rats were treated with AngII (9 $\mu g \cdot h^{-1}$; 4 weeks), and BP and plasma AngII and aldosterone were determined.

KEY RESULTS

Chronic AngII increased the BP in OZR markedly more so than in LZR. Plasma AngII levels in LZR and OZR were similar after AngII treatment. The AngII stimulated a rise in plasma aldosterone that was sixfold more in OZR than in LZR. The thickness of the zona glomerulosa of the adrenal glands was selectively increased by AngII in OZR. Adrenal mRNA levels of CYP11B2 aldosterone synthase and the AT_{1B} receptor were selectively increased in AngII-treated OZR. The BP response to chronic AngII stimulation was diminished in OZR after adrenalectomy when plasma aldosterone was absent. Acute bolus injections of AngII did not increase the BP response or aldosterone release in OZR.

CONCLUSIONS AND IMPLICATIONS

The AngII-induced BP response is enhanced in obesity and this is associated with a specific increase in circulating aldosterone. Due to the AngII-induced growth of the zona glomerulosa in OZR, the AT_{1B} receptors and aldosterone synthase may be selectively enhanced in obesity under concomitant AngII stimulation, increasing the adrenal synthesis of aldosterone. Our results confirm functionally that aldosterone plays a major role in obesity-related hypertension.

Abbreviations

adx, adrenalectomy; AGT, angiotensinogen; AngII, angiotensin II; AT_1 receptor, angiotensin II type 1 receptor; AT_2 receptor, angiotensin II type 2 receptor; AUC, area under the curve; C_{max}, maximal concentration; CON, controls; DIO, diet-induced obesity; ko, knock out; LVW, left ventricular weight; LZR, lean Zucker rat; MR, mineralocorticoid receptor; NKCC2, Na-K-2Cl cotransporter; OZR, obese Zucker rat; qPCR, quantitative PCR; RAAS, renin-angiotensin-aldosterone system; SBP, systolic BP; StAR, steroidogenic acute regulatory protein; ZG, zona glomerulosa

Obesity, which has become a problem of epidemic proportions in Western countries, is frequently accompanied by hypertension and an increased incidence of cardiovascular mortality. Data from the Framingham Offspring Study suggest that 65–75% of the risk for hypertension can be attributed to an excess of abdominal fat mass (Garrison *et al.*, 1987). The mechanisms by which fat mass leads to hypertension are not fully known. Several central and peripheral abnormalities that can explain the development or maintenance of high arterial pressure in obesity have been identified, among these are hormonal systems and also the sympathetic nervous system (Rahmouni *et al.*, 2005). In the last decade, it has become apparent that adipose tissue is a prolific organ that secretes several immunomodulators and bioactive molecules, which have been implicated in promoting obesity hypertension (Hutley and Prins, 2005). Historically, activation of the renin–angiotensin–aldosterone system (RAAS) has been established as a major determinant of BP, and there is evidence that the RAAS may constitute an important link between obesity and hypertension. With respect to obesity, the RAAS has been shown to be activated in fat tissue of rats and human patients (Giacchetti *et al.*, 2002; Boustany *et al.*, 2004; Engeli *et al.*, 2005). In addition, obesity has been found to be associated with an up-regulation of ACE in adipocytes (Bloem *et al.*, 1995; Cooper *et al.*, 1998; Karlsson *et al.*, 1998), as well as with increased levels of circulating angiotensin II (AngII) (Harte *et al.*, 2005). When body weight is reduced in obese women, plasma levels of renin, AGT, and aldosterone and plasma ACE activity are normalized, a phenomenon that is also associated with a reduction in BP (Engeli *et al.*, 2005).

Aldosterone, another key player in the RAAS, also correlates positively with obesity (Lamounier-Zepter *et al.*, 2005; Krug and Ehrhart-Bornstein, 2008), suggesting a link between obesity-associated hypertension and increased mineralocorticoid levels. Indeed, a recent study in African-Americans verified that BP correlates positively with plasma aldosterone levels, and the latter correlates significantly with waist circumference, total cholesterol, triglycerides, insulin and the insulin-resistance index (Kidambi *et al.*, 2007). From a mechanistic point of view, AngII has been shown to regulate the secretion of aldosterone from adrenal glands in an autocrine/paracrine manner; it not only stimulates aldosterone release in human adrenocarcinoma cells, but AngII is also produced in these cells (Hilbers *et al.*, 1999). Adipocyte secretory products also increase aldosterone release (Ehrhart-Bornstein *et al.*, 2003; 2004), but this process seems to be independent of adipose tissue AngII as AT_1 receptor blockade did not diminish aldosterone secretion induced by fat cell-conditioned medium (Ehrhart-Bornstein *et al.*, 2003). However, the Ehrhart–Bornstein group demonstrated that adipocytes not only secrete mineralocorticoid-stimulating factors but also sensitize adrenocortical cells to AngII. In addition, they found that this sensitization of adrenocortical cells to stimulation by AngII is possibly mediated via ERK1/2-dependent up-regulation of steroidogenic acute regulatory protein (StAR) activity (Krug *et al.*, 2007). Indeed, in a clinical study it was established that AngII-mediated aldosterone secretion is higher in obese than in lean patients, confirming these experimental findings (Bentley-Lewis *et al.*, 2007). This indicates that the adrenal glands of overweight individuals are hypersensitive.

As outlined above, AngII and aldosterone are both increased in obese individuals; moreover, blood aldosterone is higher in overweight subjects after AngII infusion (Bentley-Lewis *et al.*, 2007). This indicates a sensitization of the adrenals to AngII. This conclusion is also supported by findings showing an increased corticosterone response during stress in obese rats after chronic AngII (Müller *et al.*, 2007). Since aldosteronogenesis in obesity has been demonstrated to be caused not only by AngII but also by other adipocyte-derived factors, such as oxidized fatty acids (Goodfriend *et al.*, 2004), and it is still not known whether AngII-induced increase in BP is also indirectly associated with the hypertensive potency of aldosterone, we investigated the contribution of aldosterone to the hypertensive response induced by chronic AngII administration to obese rats. We tested our hypothesis using Zucker rats, which serve as a genetic animal model for leptin resistance. Zucker rats become obese, hyperphagic and hyperleptinaemic (OZR) due to a homozygous point mutation in the leptin receptor. Zucker rats with only a heterozygous mutation remain lean (LZR). The simple fact that Zucker rats are obese but not inevitably hypertensive means that this strain can serve as an experimental animal model to study whether the BP response to chronic AngII is enhanced during obesity and to determine the involvement of aldosterone in this response. In addition, the involvement of aldosterone in obesity-induced hypertension was investigated in a rat model of diet-induced obesity (DIO).

Methods

Animals

Male obese (OZR) and lean (LZR) Zucker rats and spontaneous hypertensive rats (SHR) (Charles River, Sulzfeld, Germany) were used. The study was conducted according to the National Institutes of Health guidelines for the care and use of laboratory animals. All animal care and experimental procedures were carried out with the ethics approval of the local regulatory authority (Ministerium für Landwirtschaft, Umwelt und ländliche Räume des Bundeslandes Schleswig-Holstein). The animals were kept at room temperature with a 12 h/12 h dark (14 h–2 h)/light (2 h–14 h) cycle, which allowed us to perform experiments in the active phase of the animals. Rats were habituated to laboratory conditions and the light/dark cycles before the start of the experiments. LZR and OZR received a standard diet (1320, Altromin, Germany) and water *ad libitum*. The results of all studies involving animals are reported in accordance with the ARRIVE guidelines (Kilkenny *et al.*, 2010;McGrath *et al.*, 2010).

Study protocol

The 8-week-old OZR (348 ± 9 g) and LZR (218 ± 4 g; $n = 9$ each dosage) were chronically treated with AngII (Sigma, Germany) using osmotic minipumps (2ML4, Alzet®, Charles River Laboratories, Sulzfeld, Germany). We performed experiments not only using a hypertensive AngII dose (release rate 9 μg AngII h^{-1}), but also using a low dose (release rate 0.9 μg AngII h^{-1}) that was previously reported not to affect BP (Simon *et al.*, 1998), since organ-specific actions of AngII have been demonstrated to occur independently of BP

(Simon *et al.*, 1998). OZR and LZR were treated with the same doses of AngII as the blood volume was demonstrated to be similar between the two strains (Schreihofer *et al.*, 2005). Controls received saline. Pumps were placed s.c. between the scapulae during pentobarbitone anaesthesia (67 mg·kg^{-1}, maximum dose was 20 mg absolute). The respiration was monitored) and the eyelid (by gently raising the upper eyelid with a cotton stick) and nociceptive reflexes (toe-pinch method) were tested before the start of the surgical procedure. On day 20 of AngII treatment, chronic polyethylene catheters were inserted during pentobarbitone anaesthesia into the right femoral vein and artery. Catheters were tunnelled under the back skin, exteriorized in the region of the cervical vertebra, and fixed at the skin. Thereafter, rats were housed individually in cages (height × width × length: 20 × 22 × 25 cm) until the end of the study. Intake of chow and water was monitored during this period. BP was monitored 2 days later via arterial catheters between 9 h and 10 h. Values were recorded for 5 min and expressed as means within this time period (Raasch *et al.*, 2006). At the end of the study, rats were killed by decapitation, and the organs were removed for biochemical analysis. To determine AngII, 2 mL blood was collected in an inhibitor solution containing 12.1 mM EDTA and 20 µM bestatine (final concentration). To determine aldosterone, corticosterone, leptin and insulin levels, EDTA blood was sampled and plasma was prepared.

In a second set of experiments, the effects if AngII on BP responses were determined only in obese Zucker rats that were either adrenalectomized (adx) during pentobarbitone anaesthesia or sham operated. The initial body weight (329 g) was similar in all groups. Corticosterone was substituted by inserting pellets (s.c.; containing 100 mg corticosterone for release over 21 days; IRA, Sarasota, FL, USA). Sham animals received placebo pellets. Corticosterone and placebo pellets were replaced by new ones after 19 days under a short period of ether anaesthesia to maintain constant corticosterone plasma levels for 4 weeks. For ether anaesthesia, 2 ml diethylether was dropped into a 5 L beaker that was lined with filter tissue and the rats were kept in this beaker for approx. 10 s. Respiration, lid and nociceptive reflexes were tested as described above before the start of the surgical procedure. In adx rats, drinking water was replaced by saline. Osmotic minipumps (2ML4, Alzet) were concurrently inserted for AngII (9 µg AngII h^{-1}) or saline treatment. BP was determined after 4 weeks by the tail cuff method (Raasch *et al.*, 2004). To verify the complete removal of the adrenals, rats were stressed by a 10-min forced swim test (water temperature 15°C, water depth 0.25 m, diameter of the basin 0.5 m) and corticosterone was determined in blood samples taken from a tail nick before and after this stress test.

Pithed rat preparation

The BP and aldosterone responses to AngII were additionally studied in an acute setting in LZR and OZR (body weight 363 ± 9 g vs. 601 ± 7 g, $P < 0.05$). To exclude an impaired baroreflex response as the cause of alterations in the BP response to AngII, which has been demonstrated in OZR (Barringer and Bunag, 1989), we performed our experiments in a 'pithed rat model', particularly since vascular reactivity to AngII has also been suggested to be increased in OZR (Alonso-Galicia *et al.*, 1996). Rats were pithed at the beginning of the light cycle as previously described (Raasch *et al.*, 2002). Briefly, the animals were anaesthetized with ether and artificially respirated. The medulla and thoracolumbar portions of the spinal cord were destroyed using a steel pithing rod. Catheters were placed into a carotid artery and both femoral veins. Both vagal nerves were severed. BP was measured via the carotid catheter. Animals were pretreated with tubocurarine (3 mg·kg^{-1}, i.v.), propranolol (1 mg·kg^{-1}, i.v.) and atropine (2 mg·kg^{-1}, i.p.). After surgery, the pithed rats were allowed to recover for approximately 1 h until their BPs and heart rates were constant. As the OZR and LZR had similar blood volumes (Schreihofer *et al.*, 2005), the amount of AngII (3.8 µg, injection volume 380 µL) injected i.v. within 30 s was not related to body weight. Aldosterone levels were determined in 200 µL blood drawn from the femoral veins before and 5, 10, 15, 10 and 30 min after the AngII injection.

Aldosterone in diet-induced obese rats

Aldosterone levels were also investigated in a non-genetic obese rat model. Here, rats (n = 12 in each group) were fed with a high-calorie cafeteria diet (various chocolate/cookie bars), which was abundantly offered, whereas the controls received only chow (Miesel *et al.*, 2010; 2011). After 3 months of feeding, we determined plasma concentrations of lipids (triglycerides, cholesterol, free fatty acids and free glycerol), leptin, insulin, AngII and aldosterone, as well as mRNA levels of AT_{1B} receptors and cytochrome P450 family 11, subfamily B, polypeptide 2 (CYP11B2). In an additional group of rats (n = 9 in each group) their survival was measured. At regular intervals, BP and heart rate were determined by plethysmography (Raasch *et al.*, 2004), and blood was taken from a tail nick to measure leptin, glucose and insulin levels.

Biochemical analysis

Plasma concentrations of aldosterone (07–108202, MP Biomedicals, Eschwege, Germany), corticosterone (07–120103, MP Biomedicals), AngII (ED29051, IBL, Hamburg, Germany), insulin (RI-13 K, Linco, St. Charles, MO, USA), and leptin (RL-83 K, Linco) were determined by radioimmunoassays (RIAs). Assays were performed as recommended by the manufacturer. The coefficient of variance of the intra-assay variability for the AngII RIA was 2.6% and for the aldosterone RIA 5.7%. Levels of plasma lipids were determined using a labour analyser.

Morphometric analysis

One adrenal gland from each rat was fixed in 4% formol for more than 24 h. Serial transverse paraffin sections were cut at a thickness of 2 µm and then stained with haematoxylin and eosin for light microscopy. Only equatorial slices of the adrenals were included in the morphometric analysis of the zona glomerulosa (ZG), which was performed by using a Zeiss microscope (magnification ×150). At least three sections of each slice were examined in each adrenal gland. The thickness of the ZG was determined by measuring the distance between the capsule and the zona fasciculata in a straight line in a blinded manner.

RNA isolation and cDNA synthesis

Total RNA from whole adrenal glands was extracted on the ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosys-

tems). The amount of total RNA was determined using a RiboGreen RNA quantification assay (Invitrogen, Germany). First-strand cDNA was synthesized using oligo-$(dT)_{15}$ primer and AMV reverse transcriptase (Invitrogen). Contamination with genomic DNA was avoided by thorough treatment with DNase I. cDNA was stored at –20°C until PCR.

qPCR

mRNA steady-state levels of AT_{1A}, AT_{1B}, and AT_2 receptors and of CYP11B2 were quantified by qPCR using SYBR green I as a fluorescent dye on the GeneAmp 7000 sequence detection system (Perkin-Elmer Applied Biosystems, Germany) and cDNA-specific primers (CYP11B2: sense 5′-ATC CTT TCA GCT GCA AGT CGG-3′, antisense 5′-CTT TGG AAT TTG GCA CAC ACA-3′; StAR: sense 5′- TTG CTG GCC CAC TTT TCT GT-3′, antisense, 5′- CGC GTT CCA TGT TGT TCT GTT-3′). Primers for AT_{1A}, AT_{1B} and AT_2 receptors were as published previously (Raasch *et al.*, 2004). All primers were obtained from Invitrogen (Karlsruhe, Germany). Product purity was confirmed by dissociation curve and agarose gel analysis. No-template controls served as negative controls (Jöhren *et al.*, 2001). Copy number calculations were based on the cycle threshold method by using serial dilutions of known amounts of specific cDNA fragments to generate standard curves. Expression values were normalized to the amount of total RNA/sample (Bustin, 2002).

Statistics

Data shown are expressed as means ± SEM. Statistical analysis was performed by two-way ANOVA, followed by appropriate *post hoc* tests (Bonferroni's multiple comparison test). Wilcoxon signed-rank test was used when variances differed between the groups. For pairwise comparisons, Student's *t*-test was employed. Correlation analyses were carried out by using Pearson's product-moment correlation test. $P < 0.05$ indicated a significant difference. Survival was tested by use of a logrank test.

Results

Effects of chronically administered AngII in OZR and LZR

None of the parameters (e.g. BP response and aldosterone biosynthesis) was observed to be altered by the low dose of AngII; these results are not depicted for reasons of clarity. Leptin resistance in OZR was demonstrated since body weight was distinctively higher than in LZR, and plasma leptin levels exceeded those of LZR. AngII did not affect plasma leptin levels (Table 1). The daily food and water intake of OZR was twice that of LZR. AngII lowered intake of chow selectively in OZR, but enhanced thirst in both strains (Table 1). Plasma concentrations of sodium and potassium were similar in all the groups.

BP did not differ between LZR and OZR when they were treated with saline. The BP response to AngII in OZR amounted to 83 mmHg, but was considerably lower (32 mmHg) in LZR (Figure 1A). The enhanced hypertensive response to AngII was functionally relevant in OZR since the left ventricular weight (LVW) was higher than in saline-treated OZR (Figure 1B).

Circulating AngII levels were similar in LZR and OZR treated with saline and were comparably enhanced by AngII in both strains (Figure 2A). Plasma aldosterone increased in response to AngII treatment (Figure 2B), whereas corticosterone remained unaffected (LZR: 188 ± 28 vs. 243 ± 33 ng·mL^{-1}, $P > 0.05$; OZR 163 ± 25 vs. 223 ± 30 ng·mL^{-1}, $P > 0.05$). However, aldosterone levels in OZR were three times those in LZR (Figure 2B). Only in AngII-treated OZR were the plasma levels of AngII and aldosterone found to correlate in a significant manner (Figure 2C).

The thickness of the ZG did not differ between saline-treated LZR and OZR, but was increased by AngII in OZR compared with LZR (Figure 3). Steady-state mRNA levels of CYP11B2 in the adrenals of saline-treated LZR and OZR were similar. However, after AngII, CYP11B2 mRNA was significantly higher in OZR, but remained unaffected in LZR

Table 1

Influence of chronic AngII (9 µg·h^{-1}) treatment on body weight and food intake as well as on circulating leptin, sodium and potassium in lean (LZR_{AngII}) and obese (OZR_{AngII}) Zucker rats

	LZR_{CON}	LZR_{AngII}	OZR_{CON}	OZR_{AngII}	
Body weight (g)	290 ± 8	248 ± 7†	466 ± 10	390 ± 20‡	*
Daily food intake (g)	21 ± 1	22 ± 1	40 ± 2	34 ± 3‡	*
Daily Na^+ intake (mg)	42 ± 2	44 ± 2	80 ± 4	68 ± 6‡	*
Daily water intake (g)	26 ± 1	37 ± 3†	48 ± 4	52 ± 4‡	*
Plasma leptin (pg·mL^{-1})	2.7 ± 0.2	1.7 ± 0.3	34.6 ± 1.4	32.6 ± 2.6	*
Plasma Na^+ (mmol L^{-1})	154 ± 1	153 ± 1	154 ± 1	152 ± 1	
Plasma K^+ (mmol L^{-1})	5.6 ± 0.3	5.2 ± 0.2	5.3 ± 0.2	5.3 ± 0.3	

Lean (LZR_{CON}) and obese controls (OZR_{CON}) were treated with saline. Means ± SEM, $n = 9$.
*Indicates group differences ($P < 0.05$) between LZR and OZR.
$^{\dagger}P < 0.05$ vs. LZR_{CON}.
$^{\ddagger}P < 0.05$ vs. OZR_{CON}

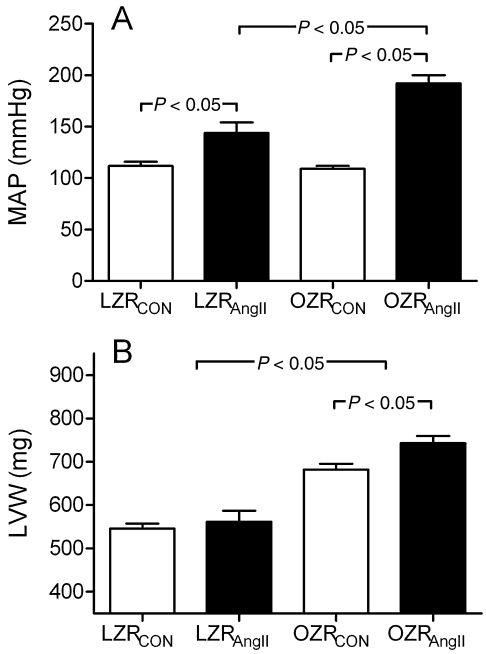

Figure 1

Influence of chronic AngII treatment (9 μg·h^{-1}) on mean arterial pressure (MAP; A) and left ventricular weight (LVW, B) of LZR and OZR. Controls received saline. Results depicted are means ± SEM (n = 9).

(Figure 4A). CYP11B2 mRNA levels correlated with circulating aldosterone concentration in a positive manner, selectively in OZR, independently of whether rats were treated with AngII or saline (Figure 4B). After AngII, steady-state mRNA levels of StAR only tended to be increased in OZR and only tended to correlate with plasma aldosterone (Figure 4B and D). Steady-state mRNA levels of adrenal AT_{1A} and AT_{1B} receptors were similar in saline-treated LZR and OZR. AngII selectively enhanced the mRNA levels of both AT_{1A} and AT_{1B} receptors in OZR (Figure 5). However, the magnitude of the up-regulation of the AT_{1B} receptor mRNA exceeded that of the AT_{1A} receptor by a factor of 6. AT_{1B} receptor mRNA levels correlated with the aldosterone plasma concentrations in OZR treated with the high-dose AngII (Figure 5D). The AT_{1A} receptor mRNA levels were decreased in LZR after the high-dose AngII (Figure 5A). In contrast, adrenal AT_2 receptor mRNA levels were not affected by genotype or by AngII (Figure 5).

Effects of chronically administered AngII in adx OZR

To verify the significance of aldosterone for the AngII-stimulated increase in BP in obesity, analogous experiments were performed in adx and sham OZR. In adx OZR, corticosterone was substituted by pellets and drinking water was replaced by saline. Gain in body weight and food intake was reduced by adrenalectomy, and AngII treatment further altered these two parameters (Table 2). Plasma levels of sodium and chloride were not affected by either adrenalectomy or AngII administration (Table 2), indicating that liquid

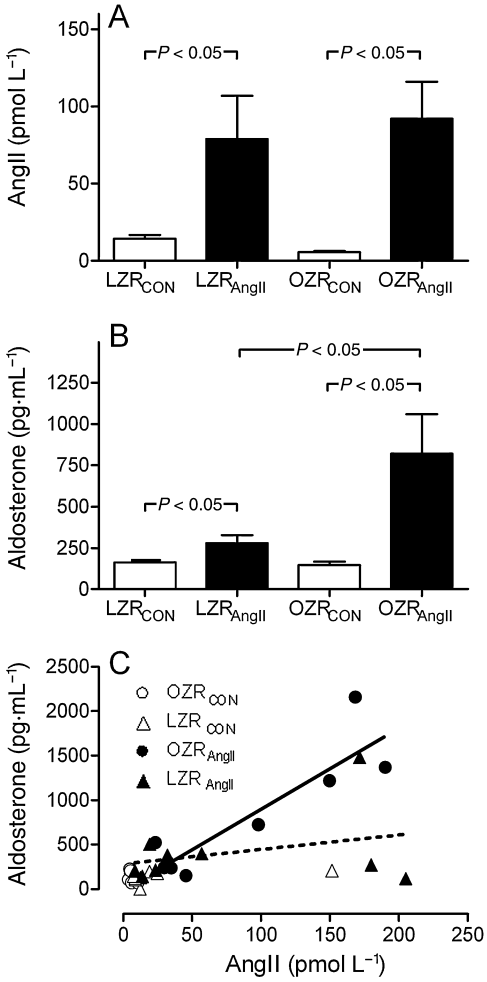

Figure 2

Influence of chronic AngII treatment (9 μg·h^{-1}) on plasma concentrations of AngII (A) and aldosterone (B) in LZR and OZR. Controls received saline. (C) Correlation between AngII and aldosterone in LZR and OZR. Aldosterone concentration correlated with AngII only in OZR (solid line; r = 0.8864, P = 0.0032) and in LZR (dotted line) that were treated with AngII. Results depicted are means ± SEM (n = 9).

intake was effectively substituted by saline. Baseline levels of plasma corticosterone did not differ between the various groups, irrespective of whether the OZR were adrenalectomized or not. This indicates that the corticosterone substitution was properly adjusted to the baseline levels of the sham-operated rats (Figure 6). The efficacy of the adrenalectomy was verified by an abolished corticosterone response to stress in the adx OZR compared with the sham-operated OZR (Figure 6). After the stress test, corticosterone plasma levels were increased by a factor of 3 in sham OZR, an effect that was not seen in adx OZR. The BP in saline-treated rats was similar in the adx and sham OZR. AngII induced an increase in BP in both adx and sham OZR. However, this effect was not as great in adx OZR as in sham OZR (–20 mmHg, Figure 7A). In parallel, the AngII-induced left ventricular growth of adx OZR was less than that of sham OZR (Figure 7B). In response to AngII, the plasma concentrations of aldosterone increased

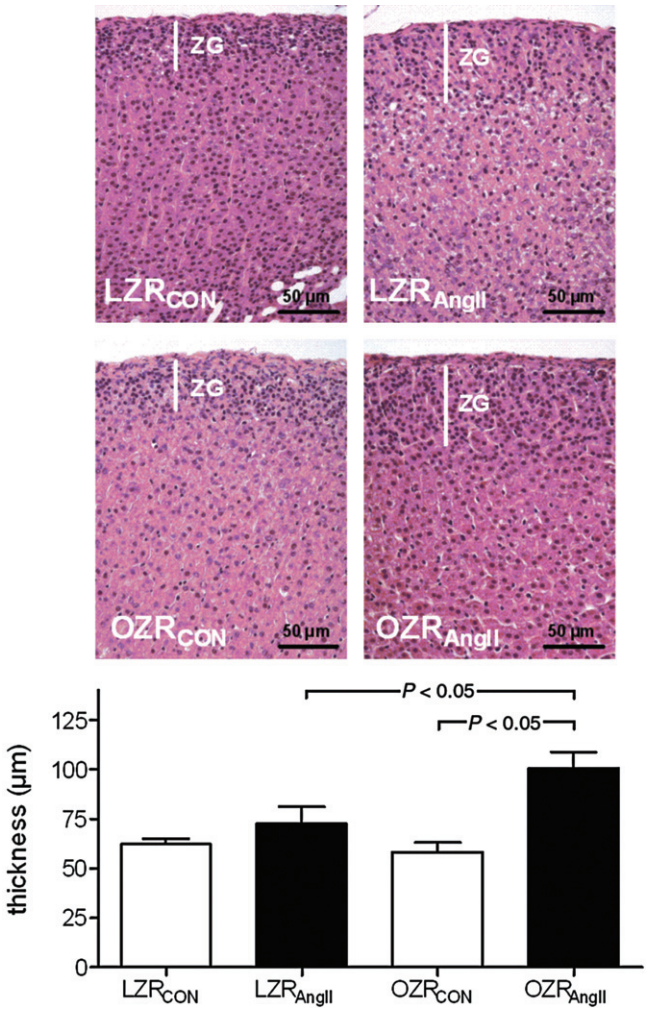

Figure 3

Morphometric analyis of the ZG of adrenal glands of lean and obese Zucker rats (OZR) that were treated with AngII (9 µg·h^{-1}; LZR$_{AngII}$; OZR$_{AngII}$) or saline (LZR$_{CON}$; OZR$_{CON}$). Results in histogram are depicted as means ± SEM (n = 9).

in sham OZR by a factor of 30. In adx OZR, aldosterone levels were lower than in saline-treated sham OZR, irrespectively of whether they were stimulated with AngII (Figure 7C).

Effects of acute AngII on BP and aldosterone levels in OZR and LZR

To determine whether the aldosterone response to AngII is differentially regulated in LZR and OZR after acute AngII administration, we performed additional experiments in pithed OZR. The BP did not differ initially between LZR and OZR. The AngII-induced increase in systolic BP was the same in LZR and OZR (136 ± 3 vs. 144 ± 2 mmHg; P > 0.05), functionally indicating similar circulating levels of AngII. Moreover, the aldosterone response to AngII was comparable between the two strains, since neither C_{max} (273 ± 26 vs. 245 ± 24 pg·mL^{-1}) nor AUC (3.35 ± 0.57 vs. 3.01 ± 0.30 ng·mL^{-1} x min) differed between LZR and OZR (Figure 8); however, baseline aldoseterone concentrations were slightly higher in the obese animals (+128 ± 27 pg·mL^{-1}; P < 0.05).

Aldosterone in DIO rats

To assess the relevance of our findings in OZR, we further assessed the aldosterone levels and AngII effects in an obesity model that is not caused by genetic alterations. Body weight was clearly increased in rats after CD feeding (Figures S1, S2). Abdominal fat mass was increased since girth was also greater in CD-fed than in chow-fed rats (Figure S2). Accordingly, plasma lipids, leptin, and insulin levels were increased in these rats (Figures S1, S2). Due to CD feeding, adrenal gland weights were also enhanced (Figure S2). Plasma concentrations of AngII and aldosterone were higher in DIO rats than in lean controls. Body weight positively correlated with plasma AngII, as well as with plasma aldosterone (Figure S3). mRNA levels of CYP11B2 mRNA, but not of AT_{1B} receptors, were increased in the adrenals of DIO rats. Plasma aldosterone positively correlated with adrenal mRNA levels of CYP11B2 (Figure S4). BP and heart rate were time-dependently increased in both groups. The BP of DIO rats transiently exceeded that of chow-fed controls, which may be due to the fact that maximal BP levels seem to be reached after ~160 days. The heart rate of DIO rats was almost higher than controls (Figure S1). Due to haemodynamic and metabolic impairments, the survival of DIO rats was reduced but this did not reach significance levels in logrank testing; this may be related to the small number of animals studied (Figure S1).

Discussion

The goal of the study was to determine whether the hypertensive reaction to chronic AngII administration is enhanced in obesity and, if so, whether this effect is associated with an aldosterone-dependent mechanism. The key findings were: (i) the BP response to chronic but not to acute AngII was enhanced in OZR; (ii) the thickness of the ZG of the adrenal glands, plasma aldosterone levels, adrenal mRNA levels of CYP11B2 and the AT_{1B} receptor were selectively increased in chronically AngII-treated OZR as compared with LZR even though AngII plasma levels were similar; and (iii) the BP response to chronic AngII stimulation was diminished in OZR after adrenalectomy. These findings clearly indicate that the higher plasma levels of aldosterone in obese than in lean rats after AngII stimulation can be attributed more to an enhanced biosynthesis of aldosterone and less to other factors, for example reduced degradation and/or tissue-specific resistance to aldosterone.

BP response to AngII

The hypertensive response to chronic AngII was considerably enhanced, by ~50 mmHg, in OZR. As a consequence, the left ventricular weight was selectively increased in OZR after chronic AngII treatment, which clearly indicates cardiac damage. Chronic treatment with AngII increased plasma AngII levels similarly in both strains. Plasma AngII levels of ~80 pmol·L^{-1}, as found in this study, have been reported to characterize an activated RAAS in heart failure (Pedersen

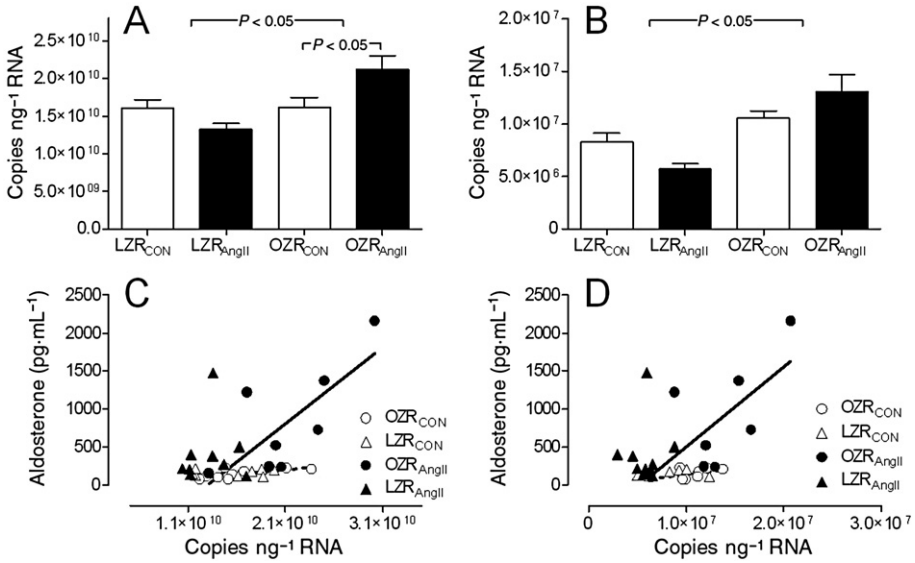

Figure 4

Steady-state mRNA levels of CYP11B2 (A) and StAR (C) in the adrenals of lean and obese Zucker rats that were treated with AngII (9 μg·h^{-1}; LZR_{AngII}; OZR_{AngII}) or saline (LZR_{CON}; OZR_{CON}). (B) Correlation between CYP11B2 mRNA and aldosterone in LZR and OZR. Aldosterone levels only correlated with adrenal CYP11B2 mRNA in OZR_{AngII} (solid line; $r = 0.7476$, $P = 0.033$) and OZR_{CON} (solid line; $r = 0.7030$, $P = 0.0346$), but not in lean Zucker rats, independently of whether they received AngII or saline. (D) Correlation between STAR mRNA and aldosterone levels in LZR and OZR. Aldosterone only tended to correlate with adrenal StAR mRNA in obese AngII-treated Zucker rats ($r = 0.6766$, $P = 0.065$). Results depicted are means ± SEM ($n = 9$).

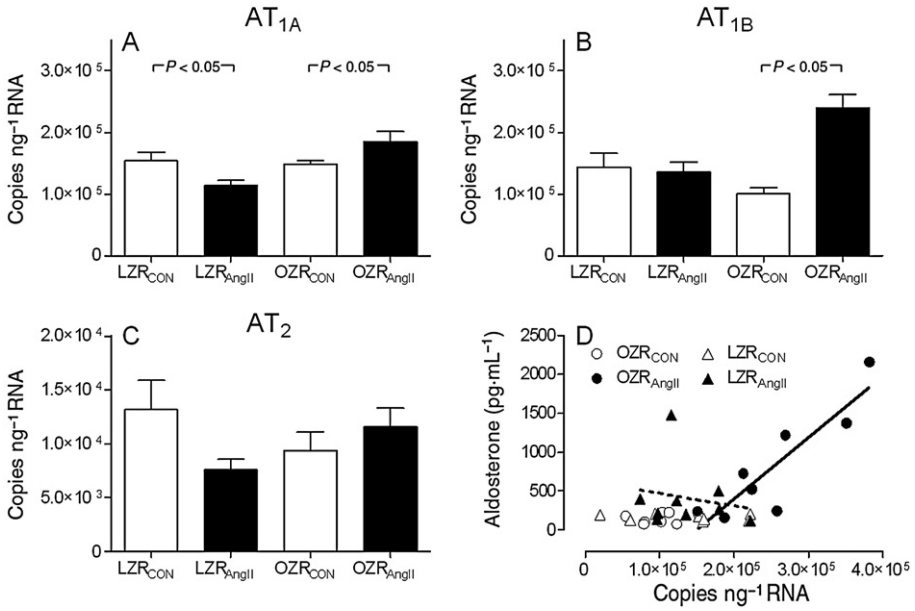

Figure 5

Steady-state levels of mRNA of AT_{1A} (A), AT_{1B} (B) and AT_2 receptors (C) in the adrenals of lean and obese Zucker rats that were treated with AngII (9 μg·h^{-1}; LZR_{AngII}; OZR_{AngII}) or saline (LZR_{CON}; OZR_{CON}). (D) Correlation between AT_{1B} receptor mRNA and aldosterone levels in LZR and OZR. Aldosterone only correlated with adrenal AT_{1B} receptor mRNA in OZR_{AngII} (solid line; $r = 0.8881$, $P = 0.0034$) but not in LZR_{AngII} (dotted line). Results depicted are means ± SEM ($n = 9$).

Table 2

Influence of AngII (9 μg·h^{-1}) treatment for 21 days on baseline parameters of sham (shamOZR$_{AngII}$) or adrenalectomized (adxOZR$_{AngII}$) obese Zucker rats

	shamOZR$_{CON}$	shamOZR$_{AngII}$	adxOZR$_{CON}$	adxOZR$_{AngII}$	
Gain in body weight (g)	120 ± 6	45 ± 14†	79 ± 7†	23 ± 10‡	
Food intake at day 17 (g)	41 ± 1	27 ± 2†	34 ± 2†	26 ± 3‡	
Na^+ intake at day 17 (mg)	82 ± 2	54 ± 4†	68 ± 4†	52 ± 5‡	
Plasma Na^+ (mmol L^{-1})	146 ± 1	143 ± 2	143 ± 1	142 ± 1	
Plasma K^+ (mmol L^{-1})	6.9 ± 0.1	6.6 ± 0.3	7.7 ± 0.1	8.1 ± 0.2	*
Plasma Cl^- (mmol L^{-1})	101 ± 1	97 ± 2	99 ± 1	99 ± 1	

Sham (shamOZR$_{CON}$) and adrenalectomized controls (adxOZR$_{CON}$) were treated with saline. Means ± SEM, $n = 9$.
*Indicates group differences ($P < 0.05$) between sham and adrenalectomized OZR.
$^{\dagger}P < 0.05$ vs. shamOZR$_{CON}$.
$^{\ddagger}P < 0.05$ vs. adxOZR$_{CON}$.

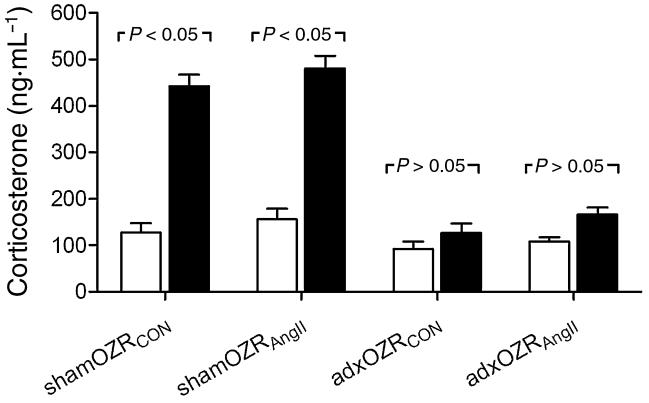

Figure 6

Plasma levels of corticosterone before (open columns) and after the forced swim test (solid columns) in sham-operated and adrenalectomized (adx) obese Zucker rats in the presence of AngII (shamOZR$_{AngII}$, adxOZR$_{AngII}$). Controls received saline (shamOZR$_{CON}$, adxOZR$_{CON}$). Adx rats received corticosterone via pellets. Corticosterone increased in shams but not in adx obese Zucker rats in response to stress. Results depicted are means ± SEM (n = 9–15).

et al., 1986), indicating that our dose regimen was pathophysiologically relevant. The increased BP response to AngII in OZR also supports previous data showing that the fall in BP after AT_1-blockade was significantly greater in OZR than in LZR (Alonso-Galicia *et al.*, 1996).

The increased pressure response to AngII in OZR raises a question concerning the underlying mechanisms. Alonso-Galicia *et al.* reported that BP responses to exogenous bolus injections of AngII were higher in OZR than in LZR and postulated an increased vascular sensitivity of OZR (Alonso-Galicia *et al.*, 1996). This conclusion was mainly based on their results showing more of an enhanced duration of pressure response than an increase in peak BP. Here we demonstrated that changes in peak BP after acute AngII injections did not differ between the strains. It has also been suggested that renal mechanisms are responsible for the increased sensitivity to AngII in OZR; these include proximal tubule sodium pump activity (Shah and Hussain, 2006), activity of the sodium-hydrogen exchanger (Becker *et al.*, 2003) and natriuresis (Hakam and Hussain, 2005). Since BP has been shown to be selectively increased in OZR, but not LZR after high doses of NaCl (Riazi *et al.*, 2006), we need to consider whether our finding regarding an enhanced pressure response to AngII in OZR might be related to an enhanced intake of sodium. One finding in favour of this idea is that food intake and consequently intake of sodium was indeed increased in AngII-treated OZR as compared to AngII-treated LZR (Table 1). However, other findings argue against this hypothesis: (i) Riazi *et al.* observed that sodium plasma concentrations increased in OZR in parallel with a high salt diet (Riazi *et al.*, 2006), whereas we found no differences in plasma levels of Na^+ between Ang-treated OZR and LZR; (ii) high-dose NaCl feeding according to Riazi means a 200-fold increase in daily sodium intake. In contrast, sodium intake was marginally increased in AngII-treated OZR by a factor of 1.5; and (iii) neither intake nor plasma levels of sodium were decreased in AngII-treated adx OZR as compared with sham OZR, although BP was reduced in these animals (Table 2). Thus, it is unlikely that an increase in sodium intake would account for the enhanced BP response to AngII selectively seen in OZR. Secondly, leptin may be involved in regulating BP in obesity. AngII promotes leptin production in human adipocytes (Skurk *et al.*, 2005), and chronic leptin increases BP (Shek *et al.*, 1998). However, leptin does not seem to be involved in the increase BP response to AngII as OZR were leptin resistant, and, plasma leptin was not increased by AngII in either the OZR or LZR. Thirdly, it is possible that the hypertensive action of AngII can be attributed to an insulin-dependent mechanism since insulin infusion increases BP (Shek *et al.*, 1998; Song *et al.*, 2006; Kobayashi *et al.*, 2008), and plasma insulin is higher in OZR than in LZR (Müller *et al.*, 2007). However, insulin levels declined somewhat after chronic AngII in OZR (Müller *et al.*, 2007) and were only transiently increased in DIO rats (Figure S1), leading us to speculate that insulin does not play a major role in our settings.

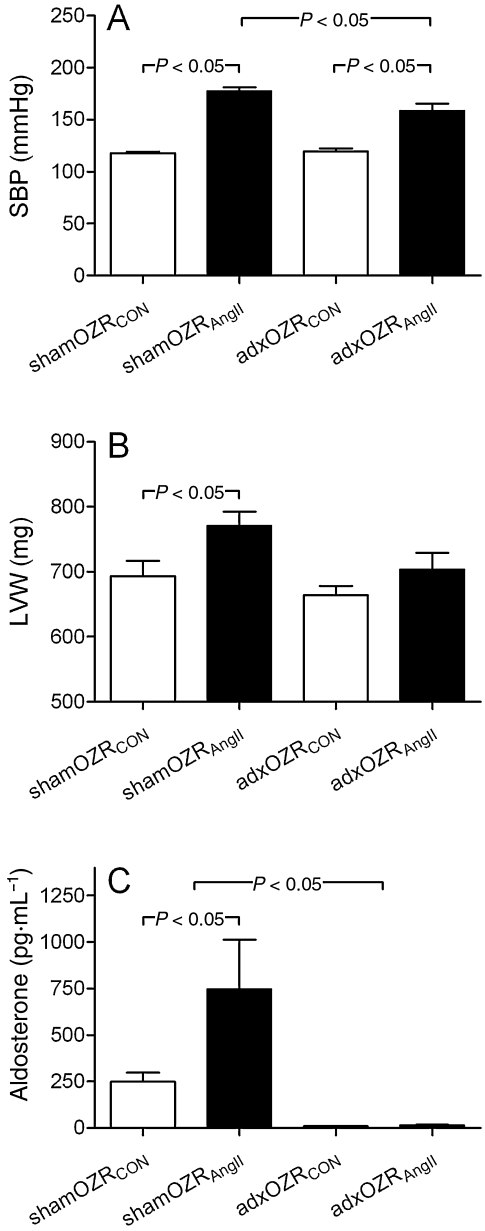

Figure 7

Influence of chronic AngII treatment (9 μg·h^{-1}) on SBP (A), LVW (B) and plasma aldosterone concentration (C) of obese Zucker rats after adrenalectomy or sham operation (adxOZR$_{AngII}$, shamOZR$_{AngII}$). Controls received saline (shamOZR$_{CON}$, adxOZR$_{CON}$). Results are depicted as means ± SEM (n = 9–15).

The increase in BP may be attributed to an enhanced aldosterone response

Aldosterone positively correlates with obesity (Lamounier-Zepter *et al.*, 2005; 2006; Krug and Ehrhart-Bornstein, 2008). Here we partially confirmed such a relationship in both DIO rats and in those Zucker rats that were used in the acute but not in the chronic studies. Differences in age might account for this disparity. In addition to the higher obesity-related baseline aldosterone levels, we observed that the AngII-induced rise in plasma aldosterone was sixfold higher in OZR

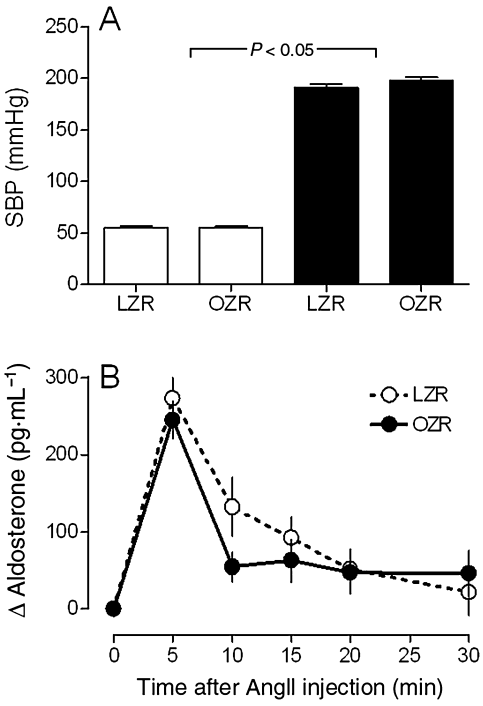

Figure 8

Influence of acute AngII on SBP and plasma aldosterone in LZR and OZR. (A) Baseline SBP (open columns) and maximal pressure response after acute AngII (3.8 μg abs.) bolus injections (solid columns). (B) Time-dependent alterations in plasma aldosterone in LZR and OZR in response to acute AngII (3.8 μg abs.) bolus injections. Results are depicted as means ± SEM (n = 9–10).

than in LZR in spite of the equivalent plasma AngII concentrations. In line with this finding, urinary aldosterone excretion and AngII-stimulated aldosterone have been demonstrated to be higher in overweight than in lean, normotensive adults (Bentley-Lewis *et al.*, 2007). Thus, the stronger hypertensive reaction to AngII in OZR may be related to aldosterone since aldosterone is known to increase BP.

We demonstrated the growth of the ZG of the adrenal glands of rats that were treated with AngII was increased in the present study. This is in agreement with others who also showed growth responses to AngII in glomerulosa cells *in vivo* and *in vitro* (Tian *et al.*, 1995; McEwan *et al.*, 1999). Growth may be due to hypertrophia and hyperplasia since in bovine adrenal glomerulosa cells, both mechanisms were demonstrated to contribute to the growth response to AngII in a time-dependent manner (Tian *et al.*, 1995). ZG growth in the LZR was quite low, but it was significant in the OZR. Hence, we conclude that the increase in plasma aldosterone was due to the growth of the ZG induced by the chronic administration of AngII. The relationship between chronic AngII stimulation and its functional consequences on ZG growth, plasma aldosterone and BP was further strengthened by our observation that neither the BP response nor aldosterone levels were enhanced in pithed OZR when AngII was injected only once via a bolus injection. In line with our results, differential ZG growth and plasma aldosterone levels in response to chronic AngII were also found to be increased in genetically hyper-

tensive Lyon rats compared with the normotensive Lyon controls, also revealing strain differences (Aguilar *et al.*, 2004).

AngII stimulates the synthesis of aldosterone within the ZG of the adrenal cortex and its secretion in an AT_1 receptor-dependent manner (Brown *et al.*, 1979; Hilbers *et al.*, 1999; Ye *et al.*, 2003; Bassett *et al.*, 2004). In rodents, the AT_{1A} receptors are involved in regulating glucocorticoid synthesis, whereas both the AT_{1A} and the AT_{1B} receptors are important for mineralocorticoid biosynthesis (Naruse *et al.*, 1998). Consistent with others (Ishihata *et al.*, 1998), we found that adrenal AT_{1A} mRNA was down-regulated in LZR in response to the high-dose AngII. In contrast, AT_{1A} mRNA was slightly and AT_{1B} mRNA markedly enhanced in the adrenal glands of OZR treated with high-dose AngII. The increase in adrenal AT_{1B} mRNA could be attributed to the ZG growth since this AT_1 receptor subtype is highly expressed in the adrenal gland and exclusively in the aldosterone-producing ZG (Gasc *et al.*, 1994; Jöhren *et al.*, 2003). The close correlation between plasma aldosterone levels and adrenal AT_{1B} mRNA supports the hypothesis that the elevation in plasma aldosterone can be attributed to the up-regulation of AT_{1B} receptors in obese individuals when stimulated with AngII. This conclusion does not by any means conflict with findings of normal BP and plasma aldosterone values in AT_{1B}-/- mice since AT_{1A} receptors have been reported to take over the role of AT_{1B} receptors in these ko mice (Chen *et al.*, 1997). It has been shown that stimulation of AT_2 receptors does not increase the release of aldosterone from ZG cells (Belloni *et al.*, 1998). However, AngII infusion upregulates the expression of AT_2 receptors in the ZG, (Aguilar *et al.*, 2004), allowing us to speculate as to whether an AT_2 receptor-dependent mechanism may be involved in the increased plasma aldosterone. In this context, AT_2 receptors stimulate NO-dependent dilatation of adrenal cortical arteries that may enhance the AngII delivery to the ZG and the secretion of aldosterone (Gauthier *et al.*, 2005). On the other hand, blocking the AT_2 receptor with PD123319 did not affect the ability of AngII to stimulate aldosterone (Moritz *et al.*, 1999). Since we did not observe any differences in adrenal AT_2 receptors in our various conditions, we suggest that the increase in aldosterone after AngII stimulation in OZR is not mediated via AT_2 receptors.

The capacity of the adrenal gland to produce aldosterone is controlled by the regulated transcription of CYP11B2, the gene that encodes aldosterone synthase. Aldosterone synthase catalyses the final step in aldosterone biosynthesis and is stimulated by AngII *in vitro* and *in vivo* (Bird *et al.*, 1993; Peters *et al.*, 1998; Ye *et al.*, 2003). In OZR treated with AngII, the adrenal CYP11B2 mRNA levels were enhanced and this may be attributed to the growth in the ZG, since aldosterone synthase is expressed only within the ZG of the adrenal cortex (Bassett *et al.*, 2004). Hyperplasia of the ZG and a correlation between CYP11B2 expression and aldosterone biosynthesis and function were recently demonstrated in patients with primary aldosteronism (Boulkroun *et al.*, 2010), thus confirming the morphological alterations in the adrenals and the functional effect of aldosterone found in our study. The stimulation of adrenocortical steroidogenesis also involves the up-regulation of StAR, a key factor in mediating the transfer of cholesterol from the outer to the inner mitochondrial membrane (Stocco, 2001). We found in the present study that AngII tended to enhance adrenal StAR mRNA selectively in OZR and may also have stimulated aldosterone synthesis. The StAR mRNA data may be underestimated because of the high amount of StAR in the zona fasciculata. The zona fasciculata does not seem to be changed in our conditions since corticosterone levels were unaffected by AngII. Thus, we confirmed *in vivo* the *in vitro* findings of Krug *et al*. showing that AngII-mediated aldosterone secretion was enhanced by pre-incubating adrenocortical cells with adipocyte secretory products and that StAR expression was upregulated in parallel (Krug *et al.*, 2007).

The aldosterone-mediated increase in BP is mainly related to its effects on renal sodium re-absorption. In this context, the Na-K-2Cl cotransporter (NKCC2) and the epithelial sodium channel (EnaC) are increased in the renal cortex of OZR, resulting in an increase in plasma sodium (Riazi *et al.*, 2006; Resch *et al.*, 2010). We determined plasma sodium concentrations as a crude surrogate parameter to assess renal aldosterone function. However, plasma sodium concentrations were not altered in OZR after AngII administration, even though the aldosterone level was enhanced. With all due caution, other mechanisms that are independent of renal tubular sodium transport stimulation may be involved in the aldosterone-stimulated increased BP response.

Mineralocorticoid receptor (MR) blockade has been repeatedly demonstrated to attenuate the BP response to exogenous AngII when plasma aldosterone was enhanced (Neves *et al.*, 2003; 2005) or to normalize BP in transgenic (mRen2)27 rats that display elevated levels of AngII and aldosterone (Habibi *et al.*, 2011). This provides evidence that the AngII-induced increase in BP is partially mediated by aldosterone. Thus, one approach to further confirm the involvement of aldosterone in the AngII-stimulated increase in BP in obesity would be to conduct experiments in the presence of eplerenone or spironolactone. Such a strategy would be particularly valuable in view of the findings of de Paula and colleagues showing that aldosterone antagonism markedly attenuated the rise in BP of obese dogs that were fed a high-fat diet (de Paula *et al.*, 2004). However, it has also been found that spironolactone and eplerenone do not to reduce the AngII-stimulated BP response (Cassis *et al.*, 2005; Zhao *et al.*, 2006); this may be related to the dose given not being high enough (Habibi *et al.*, 2011). Moreover, a recently published study shows a new CNS mechanism for AngII-induced hypertension that is activated through an aldosterone-dependent neuromodulatory pathway (Huang *et al.*, 2010). Blocking this pathway would require direct i.c.v. administration of eplerenone, as it is unlikely that sufficient central eplerenone concentrations can be achieved by peripheral administration. Considering the difficulties regarding effective dosages and central penetrations of MR antagonists, as an alternative we used the model of adx rats to indicate the involvement of aldosterone in AngII-induced hypertension. We observed that the increase in BP after chronic AngII stimulation was markedly diminished in adx OZR, in which plasma aldosterone levels could scarcely be detected, as compared with sham OZR. Thus, this result clearly supports the partial involvement of aldosterone in the hypertension associated with obesity.

In summary, we demonstrated, using Zucker rats that serve as a genetically based model of obesity, that there is a

close relationship between obesity and an increased BP response to chronic AngII, which is at least partially mediated by a mechanism involving aldosterone. We conclude that aldosterone may play a crucial role in obesity-induced hypertension, since we also showed that BP was at least transiently increased in DIO obese rats, and this was paralleled by an increase in plasma concentrations of AngII and aldosterone, as well as of adrenal CYP11B2 mRNA levels.

Acknowledgements

The authors would like to thank Mrs A. Kaiser and Mrs Ines Stölting for expert technical assistance. This study was supported by two grants from the Dean of the Medical Faculty of the University of Lübeck (N11-2004 and grant of 'Exzellenzmedizin' program).

Conflicts of interest

The authors have nothing to disclose.

References

Aguilar F, Lo M, Claustrat B, Saez JM, Sassard J, Li JY (2004). Hypersensitivity of the adrenal cortex to trophic and secretory effects of angiotensin II in Lyon genetically-hypertensive rats. Hypertension 43: 87–93.

Alonso-Galicia M, Brands MW, Zappe DH, Hall JE (1996). Hypertension in obese Zucker rats. Role of angiotensin II and adrenergic activity. Hypertension 28: 1047–1054.

Barringer DL, Bunag RD (1989). Uneven blunting of chronotropic baroreflexes in obese Zucker rats. Am J Physiol 256: H417–H421.

Bassett MH, White PC, Rainey WE (2004). The regulation of aldosterone synthase expression. Mol Cell Endocrinol 217: 67–74.

Becker M, Umrani D, Lokhandwala MF, Hussain T (2003). Increased renal angiotensin II AT1 receptor function in obese Zucker rat. Clin Exp Hypertens 25: 35–47.

Belloni AS, Andreis PG, Macchi V, Gottardo G, Malendowicz LK, Nussdorfer GG (1998). Distribution and functional significance of angiotensin-II AT1- and AT2-receptor subtypes in the rat adrenal gland. Endocr Res 24: 1–15.

Bentley-Lewis R, Adler GK, Perlstein T, Seely EW, Hopkins PN, Williams GH *et al.* (2007). Body mass index predicts aldosterone production in normotensive adults on a high-salt diet. J Clin Endocrinol Metab 92: 4472–4475.

Bird IM, Hanley NA, Word RA, Mathis JM, McCarthy JL, Mason JI *et al.* (1993). Human NCI-H295 adrenocortical carcinoma cells: a model for angiotensin-II-responsive aldosterone secretion. Endocrinology 133: 1555–1561.

Bloem LJ, Manatunga AK, Tewksbury DA, Pratt JH (1995). The serum angiotensinogen concentration and variants of the angiotensinogen gene in white and black children. J Clin Invest 95: 948–953.

Boulkroun S, Samson-Couterie B, Dzib JF, Lefebvre H, Louiset E, Amar L *et al.* (2010). Adrenal cortex remodeling and functional zona glomerulosa hyperplasia in primary aldosteronism. Hypertension 56: 885–892.

Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA (2004). Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. Am J Physiol Regul Integr Comp Physiol 287: R943–R949.

Brown JJ, Casals-Stenzel J, Cumming AM, Davies DL, Fraser R, Lever AF *et al.* (1979). Angiotensin II, aldosterone and arterial pressure: a quantitative approach. Arthur C. Corcoran Memorial Lecture. Hypertension 1: 159–179.

Bustin SA (2002). Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J Mol Endocrinol 29: 23–39.

Cassis LA, Helton MJ, Howatt DA, King VL, Daugherty A (2005). Aldosterone does not mediate angiotensin II-induced atherosclerosis and abdominal aortic aneurysms. Br J Pharmacol 144: 443–448.

Chen X, Li W, Yoshida H, Tsuchida S, Nishimura H, Takemoto F *et al.* (1997). Targeting deletion of angiotensin type 1B receptor gene in the mouse. Am J Physiol 272: F299–F304.

Cooper R, Forrester T, Ogunbiyi O, Muffinda J (1998). Angiotensinogen levels and obesity in four black populations. ICSHIB Investigators. J Hypertens 16: 571–575.

Ehrhart-Bornstein M, Lamounier-Zepter V, Schraven A, Langenbach J, Willenberg HS, Barthel A *et al.* (2003). Human adipocytes secrete mineralocorticoid-releasing factors. Proc Natl Acad Sci U S A 100: 14211–14216.

Ehrhart-Bornstein M, Arakelyan K, Krug AW, Scherbaum WA, Bornstein SR (2004). Fat cells may be the obesity-hypertension link: human adipogenic factors stimulate aldosterone secretion from adrenocortical cells. Endocr Res 30: 865–870.

Engeli S, Bohnke J, Gorzelniak K, Janke J, Schling P, Bader M *et al.* (2005). Weight loss and the renin-angiotensin-aldosterone system. Hypertension 45: 356–362.

Garrison RJ, Kannel WB, Stokes J III, Castelli WP (1987). Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. Prev Med 16: 235–251.

Gasc JM, Shanmugam S, Sibony M, Corvol P (1994). Tissue-specific expression of type 1 angiotensin II receptor subtypes. An in situ hybridization study. Hypertension 24: 531–537.

Gauthier KM, Zhang DX, Edwards EM, Holmes B, Campbell WB (2005). Angiotensin II dilates bovine adrenal cortical arterioles: role of endothelial nitric oxide. Endocrinology 146: 3319–3324.

Giacchetti G, Faloia E, Mariniello B, Sardu C, Gatti C, Camilloni MA *et al.* (2002). Overexpression of the renin-angiotensin system in human visceral adipose tissue in normal and overweight subjects. Am J Hypertens 15: 381–388.

Goodfriend TL, Ball DL, Egan BM, Campbell WB, Nithipatikom K (2004). Epoxy-keto derivative of linoleic acid stimulates aldosterone secretion. Hypertension 43: 358–363.

Habibi J, DeMarco VG, Ma L, Pulakat L, Rainey WE, Whaley-Connell AT *et al.* (2011). Mineralocorticoid receptor blockade improves diastolic function independent of blood pressure reduction in a transgenic model of RAAS overexpression. Am J Physiol Heart Circ Physiol 300: H1484–H1491.

Hakam AC, Hussain T (2005). Renal angiotensin II type-2 receptors are upregulated and mediate the candesartan-induced natriuresis/diuresis in obese Zucker rats. Hypertension 45: 270–275.

Harte A, McTernan P, Chetty R, Coppack S, Katz J, Smith S *et al.* (2005). Insulin-mediated upregulation of the renin angiotensin system in human subcutaneous adipocytes is reduced by rosiglitazone. Circulation 111: 1954–1961.

Hilbers U, Peters J, Bornstein SR, Correa FM, Jöhren O, Saavedra JM *et al.* (1999). Local renin-angiotensin system is involved in K+-induced aldosterone secretion from human adrenocortical NCI-H295 cells. Hypertension 33: 1025–1030.

Huang BS, Ahmadi S, Ahmad M, White RA, Leenen FH (2010). Central neuronal activation and pressor responses induced by circulating ANG II: role of the brain aldosterone-'ouabain' pathway. Am J Physiol Heart Circ Physiol 299: H422–H430.

Hutley L, Prins JB (2005). Fat as an endocrine organ: relationship to the metabolic syndrome. Am J Med Sci 330: 280–289.

Ishihata A, Uno S, Guo DF, Katano Y, Inagami T (1998). Inhibition of the expression of the gene for the angiotensin AT1 receptor by angiotensin II in the rat adrenal gland. Eur J Pharmacol 350: 129–139.

Jöhren O, Neidert SJ, Kummer M, Dendorfer A, Dominiak P (2001). Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. Endocrinology 142: 3324–3331.

Jöhren O, Golsch C, Dendorfer A, Qadri F, Häuser W, Dominiak P (2003). Differential expression of AT1 receptors in the pituitary and adrenal gland of SHR and WKY. Hypertension 41: 984–990.

Karlsson C, Lindell K, Ottosson M, Sjostrom L, Carlsson B, Carlsson LM (1998). Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II. J Clin Endocrinol Metab 83: 3925–3929.

Kidambi S, Kotchen JM, Grim CE, Raff H, Mao J, Singh RJ *et al.* (2007). Association of adrenal steroids with hypertension and the metabolic syndrome in blacks. Hypertension 49: 704–711.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010) NC3Rs Reporting Guidelines Working Group.Br J Pharmacol 160:1577–1579.

Kobayashi T, Nogami T, Taguchi K, Matsumoto T, Kamata K (2008). Diabetic state, high plasma insulin and angiotensin II combine to augment endothelin-1-induced vasoconstriction via ETA receptors and ERK. Br J Pharmacol 155: 974–983.

Krug AW, Ehrhart-Bornstein M (2008). Aldosterone and metabolic syndrome: is increased aldosterone in metabolic syndrome patients an additional risk factor? Hypertension 51: 1252–1258.

Krug AW, Vleugels K, Schinner S, Lamounier-Zepter V, Ziegler CG, Bornstein SR *et al.* (2007). Human adipocytes induce an ERK1/2 MAP kinases-mediated upregulation of steroidogenic acute regulatory protein (StAR) and an angiotensin II-sensitization in human adrenocortical cells. Int J Obes (Lond) 31: 1605–1616.

Lamounier-Zepter V, Ehrhart-Bornstein M, Bornstein SR (2005). Mineralocorticoid-stimulating activity of adipose tissue. Best Pract Res Clin Endocrinol Metab 19: 567–575.

Lamounier-Zepter V, Rotthoff T, Ansurudeen I, Kopprasch S, Scherbaum WA, Ehrhart-Bornstein M *et al.* (2006). Increased aldosterone/renin quotient in obese hypertensive women: a novel role for low-density lipoproteins? Horm Metab Res 38: 471–475.

McEwan PE, Vinson GP, Kenyon CJ (1999). Control of adrenal cell proliferation by AT1 receptors in response to angiotensin II and low-sodium diet. Am J Physiol 276: E303–E309.

McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Miesel A, Müller H, Thermann M, Heidbreder M, Dominiak P, Raasch W (2010). Overfeeding-induced obesity in spontaneously hypertensive rats: an animal model of the human metabolic syndrome. Ann Nutr Metab 56: 127–142.

Miesel A, Müller-Fielitz H, Jöhren O, Vogt FM, Raasch W (2011). Double blockade of angiotensin II (AT(1))-receptors and ACE does not improve weight gain and glucose homeostasis better than single-drug treatments in obese rats. Br J Pharmacol 165: 2721–2735.

Moritz KM, Boon WC, Wintour EM (1999). Aldosterone secretion by the mid-gestation ovine fetus: role of the AT2 receptor. Mol Cell Endocrinol 157: 153–160.

Müller H, Schweitzer N, Jöhren O, Dominiak P, Raasch W (2007). Angiotensin II stimulates the reactivity of the pituitary-adrenal axis in leptin-resistant Zucker rats, thereby influencing the glucose utilization. Am J Physiol Endocrinol Metab 293: E802–E810.

Naruse M, Tanabe A, Sugaya T, Naruse K, Yoshimoto T, Seki T *et al.* (1998). Deferential roles of angiotensin receptor subtypes in adrenocortical function in mice. Life Sci 63: 1593–1598.

Neves MF, Virdis A, Schiffrin EL (2003). Resistance artery mechanics and composition in angiotensin II-infused rats: effects of aldosterone antagonism. J Hypertens 21: 189–198.

Neves MF, Amiri F, Virdis A, Diep QN, Schiffrin EL (2005). Role of aldosterone in angiotensin II-induced cardiac and aortic inflammation, fibrosis, and hypertrophy. Can J Physiol Pharmacol 83: 999–1006.

de Paula RB, da Silva AA, Hall JE (2004). Aldosterone antagonism attenuates obesity-induced hypertension and glomerular hyperfiltration. Hypertension 43: 41–47.

Pedersen EB, Danielsen H, Jensen T, Madsen M, Sorensen SS, Thomsen OO (1986). Angiotensin II, aldosterone and arginine vasopressin in plasma in congestive heart failure. Eur J Clin Invest 16: 56–60.

Peters B, Clausmeyer S, Obermuller N, Woyth A, Kranzlin B, Gretz N *et al.* (1998). Specific regulation of StAR expression in the rat adrenal zona glomerulosa. An in situ hybridization study. J Histochem Cytochem 46: 1215–1221.

Raasch W, Schäfer U, Qadri F, Dominiak P (2002). Agmatine, an endogenous ligand at imidazoline binding sites, does not antagonize the clonidine-mediated blood pressure reaction. Br J Pharmacol 135: 663–672.

Raasch W, Jöhren O, Schwartz S, Gieselberg A, Dominiak P (2004). Combined blockade of AT1-receptors and ACE synergistically potentiates antihypertensive effects in SHR. J Hypertens 22: 611–618.

Raasch W, Wittmershaus C, Dendorfer A, Voges I, Pahlke F, Dodt C *et al.* (2006). Angiotensin II inhibition reduces stress sensitivity of hypothalamo-pituitary-adrenal axis in spontaneously hypertensive rats. Endocrinology 147: 3539–3546.

Rahmouni K, Correia ML, Haynes WG, Mark AL (2005). Obesity-associated hypertension: new insights into mechanisms. Hypertension 45: 9–14.

Resch M, Bergler T, Fredersdorf S, Griese DP, Weil J, Kreuzer P *et al.* (2010). Hyperaldosteronism and altered expression of an SGK1-dependent sodium transporter in ZDF rats leads to salt dependence of blood pressure. Hypertens Res 33: 1082–1088.

Riazi S, Khan O, Hu X, Ecelbarger CA (2006). Aldosterone infusion with high-NaCl diet increases blood pressure in obese but not lean Zucker rats. Am J Physiol Renal Physiol 291: F597–F605.

Schreihofer AM, Hair CD, Stepp DW (2005). Reduced plasma volume and mesenteric vascular reactivity in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 288: R253–R261.

Shah S, Hussain T (2006). Enhanced angiotensin II-induced activation of Na+, K+-ATPase in the proximal tubules of obese Zucker rats. Clin Exp Hypertens 28: 29–40.

Shek EW, Brands MW, Hall JE (1998). Chronic leptin infusion increases arterial pressure. Hypertension 31: 409–414.

Simon G, Illyes G, Csiky B (1998). Structural vascular changes in hypertension: role of angiotensin II, dietary sodium supplementation, blood pressure, and time. Hypertension 32: 654–660.

Skurk T, van Harmelen V, Blum WF, Hauner H (2005). Angiotensin II promotes leptin production in cultured human fat cells by an ERK1/2-dependent pathway. Obes Res 13: 969–973.

Song J, Hu X, Riazi S, Tiwari S, Wade JB, Ecelbarger CA (2006). Regulation of blood pressure, the epithelial sodium channel (ENaC), and other key renal sodium transporters by chronic insulin infusion in rats. Am J Physiol Renal Physiol 290: F1055–F1064.

Stocco DM (2001). StAR protein and the regulation of steroid hormone biosynthesis. Annu Rev Physiol 63: 193–213.

Tian Y, Balla T, Baukal AJ, Catt KJ (1995). Growth responses to angiotensin II in bovine adrenal glomerulosa cells. Am J Physiol 268: E135–E144.

Ye P, Kenyon CJ, MacKenzie SM, Seckl JR, Fraser R, Connell JM *et al.* (2003). Regulation of aldosterone synthase gene expression in the rat adrenal gland and central nervous system by sodium and angiotensin II. Endocrinology 144: 3321–3328.

Zhao W, Ahokas RA, Weber KT, Sun Y (2006). ANG II-induced cardiac molecular and cellular events: role of aldosterone. Am J Physiol Heart Circ Physiol 291: H336–H343.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Body weight, plasma concentrations of leptin, glucose and insulin, and systolic blood pressure (SBP) and heart rate were increased in SHR that were fed with a high-calorie cafeteria diet (●, solid line) as compared with chow-fed controls (○, dashed line). 2-ANOVA indicated a significant difference regarding diet for all parameters. *$P < 0.05$ vs. CON (Boferroni *post hoc* testing). Survival tended to be decreased in DIO rats without reaching the significance level ($P = 0.2057$, logrank test). Means ± SEM, $n = 9$.

Figure S2 Body weight, abdominal girth and adrenal wet weight, as well as plasma concentrations of leptin, insulin and lipids (TG: triglycerides, FFA: free fatty acids and FGly: free glycerol) of SHR that were either fed with chow (CON) or a high-calorie cafeteria diet (DIO) for 3 months. Means ± SEM, $n = 11–12$.

Figure S3 (A) Plasma concentrations of Ang II and aldosterone were higher in obese SHR that were fed a high-calorie cafeteria diet (●) than in lean controls that received only chow. (○). (B) Body weight positively correlated with plasma levels of Ang II (B, Pearson $r = 0.4217$, $P = 0.036$) and aldosterone (C, Pearson $r = 0.4610$, $P = 0.027$). Means ± SEM, $n = 11–12$.

Figure S4 mRNA levels of AT_{1B} receptors (A) and CYP11B2 (B) in adrenals of obese SHR that were fed a high-calorie cafeteria diet (●) compared with lean controls that received only chow. (○). (C) mRNA levels of CYP11B2 positively correlated with plasma levels of aldosterone (Pearson $r = 0.4703$, $P = 0.024$). Means ± SEM, $n = 11–12$.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.