Angiotensin II stimulates the reactivity of the pituitary-adrenal axis in leptin-resistant Zucker rats, thereby influencing the glucose utilization

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In the past decade, symptoms clustering the metabolic syndrome, such as hypertension, insulin resistance, obesity, and dyslipidemia, have all been associated with a dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis (8). In this regard, increased plasma levels of corticosterone and ACTH in the morning, stimulated expression of hypothalamic corticotropin-releasing hormone (CRH) mRNA as well as hippocampal mineralocorticoid receptor mRNA, and a decreased glucocorticoid negative feedback sensitivity all indicate a hyperreactivity of the HPA axis in diabetes (15).

Visceral fat accumulation is related to a progressive mal-function of the HPA axis with elevations of cortisol levels and failure of central feedback control by glucocorticoid receptors (7, 22). The peptide hormone leptin, which is produced by adipocytes, plays an important regulatory role in the body’s fat stores by inhibiting food intake and increasing energy expenditure. Leptin has inhibitory effects on the HPA axis, since the release of CRH from isolated hypothalamus is reduced, the stress- or fasting-induced stimulation of corticosterone or adrenocorticotropic hormone (ACTH) is attenuated, and both the secretion and synthesis of cortisol are directly blocked by leptin in adrenocortical cells (3, 9, 32). Additional evidence supporting the inhibitory actions of leptin on the HPA axis comes from experiments showing reciprocally behaving plasma concentrations of leptin and corticosterone (3). The relationship between adipose tissue and the HPA axis is bidirectional, since many studies revealed an increase in leptin after glucocorticoid stimulation (21, 37, 38, 43, 44, 47). Consequently, plasma leptin is diminished in adrenalectomized rats but returns to control levels when dexamethasone is supplemented (62). However, the inhibitory potency of leptin on HPA axis reactivity becomes inverted under conditions where leptin signaling is impaired. Mice with mutations in the leptin receptor (db/db mice) exhibit an obese phenotype that is indistinguishable from that of leptin-deficient ob/ob mice, and both mice strains exhibit hypercorticosteronemia (18, 27). Consistently with this, the fa/fa Zucker rat, which is a genetic animal model for leptin resistance due to a one-point mutation in the fatty allele that causes rats to have an amino acid substitution in the extracellular domain of the leptin receptor, is both obese and hyperphagic (16, 17). The decline in weight gain after adrenalectomy suggests a tight relationship between the HPA axis and obesity in the fa/fa Zucker rat (11, 23). Food deprivation induces a strong stress response in obese (OZR) but not in lean Zucker rats (LZR); this phenomenon is associated with an activation of hypothalamic CRH neurons in the paraventricular hypothalamic nucleus during food deprivation in obese but not in lean rats (65, 66). As such, the fatty Zucker rat is characterized by high circulating levels of corticosterone (29, 48), an increased stress sensitization, and a hyperexpression of CRH when faced with stressful experimental conditions (29, 48, 54, 69).

In addition to leptin, angiotensin (ANG) II has also been shown to influence HPA axis reactivity by enhancing the synthesis and secretion of CRH, ACTH, and corticosterone (33, 55, 63). In pituitary cells, stimulation of AT1A receptors increases CRH-mediated ACTH release (1, 59). In the hypothalamus, the HPA axis is AT1A receptor-dependently acti-

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ANG-induced PA axis hyperreactivity in leptin-resistant rats

Table 1. Influence of ANG on blood pressure as well as on endocrine and metabolic plasma parameters in obese and lean Zucker rats

<table>
<thead>
<tr>
<th></th>
<th>Obese Zucker</th>
<th>Lean Zucker</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>ANG II, pmol/l</td>
<td>5.7 ± 0.6</td>
<td>6.3 ± 1.5</td>
</tr>
<tr>
<td>ACTH, pg/ml</td>
<td>152 ± 26</td>
<td>107 ± 18</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>163 ± 25</td>
<td>175 ± 34</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>127 ± 8</td>
<td>116 ± 5</td>
</tr>
<tr>
<td>Insulin, pg/ml</td>
<td>22.4 ± 3.1</td>
<td>30.1 ± 4.7</td>
</tr>
<tr>
<td>Leptin, pg/ml</td>
<td>34.6 ± 1.4</td>
<td>32.4 ± 2.5</td>
</tr>
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Values are means ± SE and in μg/h; n = 8–10. ANG, angiotensin. *P < 0.05 vs. corresponding controls; †P < 0.05, obese vs. corresponding lean rats.

Voted via a modulation of CRH gene expression during immobilization stress (33). The tight relationship between the HPA axis and the renin-angiotensin-aldosterone system (RAAS) is further confirmed by the findings that AT1 receptors are present in the organs of the HPA axis (12, 28, 34, 36, 41) and are also regulated during stress (2, 14, 40).

Very recently, we demonstrated (50) that HPA reactivity is reduced in hypertensive rats when treated with an AT1 receptor blocker and that this effect is also associated with a diminished glucose response. Since obese patients feature an impaired glucose homeostasis, leptin resistance, and a stimulated RAAS (24, 61, 67), we aimed in this study to investigate whether ANG-induced stimulation of the HPA axis is amplified under conditions of leptin resistance and whether an enhancement of plasma glucose also occurred. Since the pathophysiological stimulus of RAAS in patients is chronic rather than acute, we aimed to mimic such a condition in our experimental setup by applying a long-term ANG infusion. We determined the reaction of the HPA axis using an oral glucose tolerance test (OGTT) both in leptin-resistant OZR and their adequate lean controls (LZR). These rats were pretreated chronically with both a high and a low dose of ANG.

MATERIALS AND METHODS

Animals. Eight-week-old male OZR and LZR (Charles River Laboratories, Sulzfeld, Germany) were used. At the beginning of the study the body weight of the OZR (304 ± 9 g) was significantly higher than that of the LZR (190 ± 4 g). The study was conducted according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and authorized by the local regulatory authority (Ministerium für Landwirtschaft, Umwelt und ländliche Räume des Bundeslandes Schleswig-Holstein). The animals were kept at room temperature with a 12:12-h dark (2 PM–2 AM)-light (2 AM–2 PM) cycle. They received a standard diet and water ad libitum. Rats were habituated to research assistants and vice versa 3 wk before ANG treatment was initiated.

Study protocol. OZR and LZR (n = 8–10 each dosage) were treated for 4 wk with ANG using osmotic minipumps (2ML4, Alzet; release rate 0.9 and 9 μg ANG/h, respectively). The high dose of ANG was previously demonstrated to be blood pressure effective (46, 72). Immediately after the minipumps releasing 0.9 μg ANG/h were inserted, the ANG doses in LZR and OZR were ~68 and ~43 ng·kg body wt·min⁻¹, respectively. These doses declined until the end of the study to ~53–67 ng·kg⁻¹·min⁻¹ in lean or to ~33–42 ng·kg⁻¹·min⁻¹ in obese rats, depending on the increase in body weight. ANG doses in rats treated with 9 μg ANG/h were 10-fold higher. Controls received saline. Pumps were subcutaneously placed between the scapulae during pentobarbitone anesthesia (67 mg/kg, maximum dose was 20 mg absolute). On day 20 of ANG treatment, chronic polyethylene catheters were inserted during pentobarbitone anesthesia into the right femoral vein and artery. Catheters were tunneled 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.

Blood pressure was monitored via arterial catheters 1 day after catheterization between 9 and 10 AM. Values were recorded for 5 min and expressed as means within this time period (50). Afterward, EDTA-blood (0.5 ml) was withdrawn from the femoral artery to determine the plasma concentration of ACTH, corticosterone, glucose, and insulin.

One day later, CRH tests were performed as described previously (50). In brief, 3 h before the tests were started, arterial catheters were extended by ~4 cm to avoid stress reactions during blood withdrawal. CRH (10 μg/kg iv) was injected 4 h before the light cycle. During

Fig. 1. Comparison of the pituitary-adrenal responses in obese (■) and lean (□) Zucker rats to a challenge with exogenous corticotropin-releasing hormone (CRH; 10 μg/kg body wt iv). Both ACTH (A) and corticosterone (B) increased after CRH injections and returned to baseline values during the observation period. *P < 0.05, obese vs. lean Zucker rat. Means ± SE (n = 8–9).
CRH tests the work benches were illuminated by very dim illumination to allow manipulations on rats while maintaining virtual darkness. Thirty and 15 min before CRH injections, 120 µl EDTA-blood was acquired from arterial catheters as well as within the 4-h duration of the CRH test for monitoring ACTH and corticosterone. Glucose levels in response to CRH were examined due to the potency of glucocorticoids to increase plasma glucose. To avoid hemorrhage-induced alterations, platelets and erythrocytes were reconstituted and returned to each animal.

One day after the CRH test, glucose, insulin, and corticosterone were determined during an OGTT (1 g glucose/kg body wt) in rats that had been deprived of food for 18 h. EDTA-blood (80 µl) was withdrawn immediately before glucose application (by gavage) as well as after 10, 20, 30, 60, 90, 120, and 240 min.

One day later rats were killed by decapitation, and the organs were removed for biochemical analysis. For determining ANG, blood (2 ml) was collected into an inhibitor solution containing 12.1 mM EDTA and 20 µM bestatin (final concentration).

**Biochemical analysis: radioimmunoassays.** Plasma concentrations of ACTH, corticosterone (MP Biomedicals), ANG (IBL), insulin, or leptin (Linco) were determined by radioimmunoassays using commercial kits. Assays were performed as recommended by the manufacturer, except that a reduced sample volume was employed (ACTH 50 µl, corticosterone 50 µl of a 1:200 dilution, insulin 50 µl, leptin 50 µl, ANG 250 µl) (50).

Blood glucose was determined using glucose sensors that detected on the basis of amperometric measurements made after enzymatic glucose oxidation (Ascensia ELITE XL; Bayer).

mRNA levels of AT1A receptor or the ACTH receptor [melanocortin-2 receptor (MC2 receptor)] were quantified in organs of the HPA axis, whereas those of GLUT1 or GLUT4 were assessed in skeletal muscle or liver. The hypothalamus was prepared from whole brain and stored in a freezer, ensuring that the tissue did not defrost. Total RNA was isolated from organs. Isolation of genomic DNA was avoided by thorough treatment with DNase I. cDNA was synthesized with standard kits (Reverse Transcription System; Promega, Mannheim, Germany). Quantitative measurements of mRNA were performed by quantitative PCR with the cycle threshold method, using SYBR Green I as a fluorescent dye on the GeneAmp 7000 sequence detection system (PerkinElmer Applied Biosystems, Weiterstadt, Germany) and cDNA-specific primers (MC2 receptor: sense 5'-CAGT-GCCATTTCGACA-3', antisense 5'-AAGCTGCCACGAGCCTTGAGAT-3'). Primers for AT1A receptors have been published elsewhere (49). All primers were obtained from Invitrogen (Karlsruhe, Germany). Product purity was confirmed by dissociation curve analysis and agarose gel electrophoresis in the presence of ethidium bromide. No-template controls served as negative controls (35). Expression values were normalized to total RNA content (13).

**Statistics.** Data shown are expressed as means ± SE. To quantify the total effect over the observation time in response to CRH or

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**Fig. 2.** Influence of chronic angiotensin (ANG) treatment (○, 0 µg/h; ▲, 0.9 µg/h; ●, 9.0 µg/h) on plasma concentrations of ACTH (A and B), corticosterone (C and D), and glucose (E and F) in obese Zucker rats (left) and lean Zucker rats (right) in the CRH test (10 µg/kg body wt iv). Means ± SE (n = 8–9).
glucose regarding changes in plasma concentrations of corticosterone, ACTH, insulin, or glucose, the area under the curves (AUCs) were calculated for each individual animal on the basis of their Δ-values. Maximal concentration (Cmax) and time point of maximal concentration (Tmax) were read off individually from the curves. If necessary, the slopes of the logarithmically linearized curves were assessed by curve fitting within the range between the peak and the basal values.

Statistical analysis was performed by one- or two-way analysis of variance (ANOVA) followed by appropriate post hoc tests (Bonferroni’s multiple comparison test). Wilcoxon signed-rank test was used when variances differed between the groups. Student’s t-test was applied for statistically analyzing only two groups. Differences were considered to be statistically significant at an error level of \( P < 0.05 \).

RESULTS

Baseline parameters. Based on their lean-to-resistant resistance, the body weight of saline-treated OZR greatly exceeded that of the LZR (451 ± 10 vs. 269 ± 8 g, \( P < 0.05 \)). In addition, OZR exhibited a significant rise in plasma leptin and developed a pronounced insulin resistance that was reflected by an increase in insulin and only a moderate enhancement of glucose that just exceeded the normal range (Table 1). Neither low nor high ANG affected plasma levels of glucose, insulin, or leptin (Table 1). Baseline levels of circulating ANG were lower in ANG-affected plasma levels of glucose, insulin, or leptin (Table 1). Baseline levels of circulating ANG were lower in saline-treated OZR compared with identically treated LZR. Mean arterial blood pressures were similar between saline-treated OZR and saline-treated LZR (112 ± 4 mmHg) and OZR (109 ± 3 mmHg). The high-dose ANG selectively enhanced circulating ANG levels in LZR and OZR to a similar extent. Blood pressure increased in LZR (144 ± 10 mmHg) and OZR (192 ± 8 mmHg) as a result of this increase in plasma ANG. Blood pressure remained unaffected in both strains after low-dose ANG.

Influence of ANG on the reactivity of the HPA axis and glucose homeostasis depending on leptin resistance in the CRH test. Baseline concentrations of ACTH and corticosterone remained unaltered by ANG treatment irrespective of dose and rat strain (Table 1). In saline-treated LZR and OZR, plasma ACTH sharply increased (5-fold) in response to CRH and returned to baseline levels within the observation periods. Although Cmax (611 ± 40 vs. 573 ± 40 pg/ml) and Tmax (53 ± 9 vs. 70 ± 9 min) did not differ between saline-treated LZR and OZR, the two-way ANOVA indicated a significant difference for the interaction between time and strain that was most likely due to the differences in the late phase of the CRH test (Fig. 1A). As a consequence of the ACTH release, corticosterone levels time-dependently increased in a manner that did not differ regarding Tmax (53 ± 9 vs. 70 ± 9 min) and Cmax (313 ± 34 vs. 372 ± 35 pg/ml). However, corticosterone response to CRH of OZR and LZR (\( P = 0.039 \)), as well as the interaction between time and strain (\( P = 0.0005 \)), was found to be significantly different by two-way ANOVA (Fig. 1B).

Neither high nor low ANG affected ACTH release after CRH in LZR or OZR (Figs. 2, A and B). After high ANG, the corticosterone release was selectively enhanced in OZR, reflected by the increase in Cmax (376 ± 33 vs. 514 ± 32 pg/ml, \( P < 0.05 \); Fig. 2, C and D) and AUC (Fig. 3A). Low ANG was ineffective.

In response to CRH, glucose levels were moderately enhanced after 15–30 min in saline-treated OZR and LZR and returned to baseline levels thereafter (Fig. 2, E and F). In parallel to corticosterone, plasma glucose was regulated in response to CRH, meaning that 1) the CRH-induced increase in plasma glucose was enlarged in parallel to corticosterone in high ANG-treated OZR (Fig. 2, E and F, and Fig. 3B), 2) the ratio of the AUC regarding corticosterone (AUCCort) and glucose (AUCGluc; B) a time-delayed (130 ± 13 min) glucose peak could be observed selectively in the high ANG-treated OZR in addition to a rapid glucose response to CRH within the first 10–20 min, and 3) this time-delayed glucose release occurred ~30 min later than the corresponding peak concentrations of corticosterone (95 ± 12 min).

Influence of ANG on the reactivity of the HPA axis and glucose homeostasis depending on leptin resistance in the glucose challenge test. Compared with baseline levels before an OGTT, plasma concentrations of glucose and insulin became decreased after fasting by approximately one-third and two-thirds, respectively, irrespective of rat strain or ANG dose (Fig. 4, A and B).

**Fig. 3.** Influence of chronic ANG treatment [0 µg/h (open bars), 0.9 µg/h (hatched bars), 9 µg/h (filled bars)] on the cumulative plasma concentrations of corticosterone ([depicted as area under the curve (AUC) of the corresponding concentration-time curves; see Fig. 2] of corticosterone (A) and glucose (B) in obese and lean Zucker rats after CRH stimulation. C: ratio between the AUC of the corticosterone (AUCCort) and glucose curves (AUCCort/ B). Means ± SE (n = 8–9). *\( P < 0.05 \) vs. ANG, 0 µg/h. ns, Not significant.
After glucose exposure, plasma glucose increased to a similar extent in saline-treated LZR and OZR and returned to baseline levels within the 4-h observation period (Fig. 4, A and B) without differing with regard to maximal increase (89 ± 4 vs. 87 ± 7 mg/dl), T_max (11 ± 1 vs. 15 ± 1 min), or the AUC (7.8 ± 0.8 vs. 10.1 ± 2.1 g·dl⁻¹·min⁻¹). However, the recovery to baseline levels occurred earlier in LZR than it did in OZR, which was reflected by a different slope (7.2 ± 0.8 vs. 3.9 ± 1.1 mg·dl⁻¹·min⁻¹, P < 0.05), thus revealing the insulin resistance of OZR. This conclusion was further confirmed by the pronounced increase in glucose-stimulated insulin release (20.2 ± 3.1 vs. 4.3 ± 0.5 ng/ml, P < 0.05; Fig. 4, C and D).

Only high ANG increased plasma glucose in OZR compared with the corresponding controls, indicated by the doubling of the maximal increase (87 ± 7 vs. 145 ± 12 mg/dl, P < 0.05) and the AUC (21.5 ± 5.1 vs. 8.5 ± 1.3 g·dl⁻¹·min⁻¹, P < 0.05), whereas T_max remained unaffected (Fig. 4, A and B). Plasma insulin tended to be decreased by high ANG in OZR after glucose exposure (Fig. 4C). In LZR, high ANG slightly enhanced plasma glucose after glucose exposure, but with a lower potency compared with the OZR. The T_max of plasma glucose was doubled (11 ± 1 vs. 23 ± 3 min, P < 0.05), but maximal glucose levels remained unaffected (Fig. 4B). The insulin secretion was halved (4.3 ± 0.5 vs. 2.2 ± 0.9 ng/ml, P < 0.05) by high ANG at 10 min after a glucose stimulus (Fig. 4, C and D).

Following a glucose stimulus, plasma corticosterone was affected in parallel to glucose (Fig. 4, E and F). Although the AUCs of the corresponding curves (28.3 ± 6.0 vs. 31.8 ± 3.9 μg·ml⁻¹·min⁻¹) and maximal corticosterone release (203 ± 46 vs. 295 ± 44 ng/ml) were similar in saline-treated OZR and LZR, the OZR demonstrated a different kinetic with regard to corticosterone release, since T_max was enhanced (43 ± 6 vs. 23 ± 3 min, P < 0.05) and the return to baseline levels was delayed (90 vs. 120 min). Corticosterone plasma levels were enhanced by high ANG in OZR but not in LZR (Fig. 4, E and F). Compared with controls, glucose, insulin, or corticosterone was affected neither in OZR nor in LZR by low ANG.

Regulation of AT receptors and MC2 receptors. In general, the steady-state mRNA levels of AT₁A receptors are higher in...
organs of the HPA axis of OZR compared with LZR. ANG had no influence on AT1A receptor regulation in the hypothalamus and pituitary gland. In the adrenals, AT1A receptor mRNA of OZR was increased (+25%) but reduced to a similar extent in LZR (Fig. 5) by high ANG. mRNA levels for the AT2 receptor were clearly reduced in all organs of the HPA axis compared with the AT1A receptor but were similar between LZR and OZR rats and were not influenced by ANG (data not shown). In contrast to AT1 receptors, steady-state levels of MC2 mRNA were higher in the adrenal glands of the saline-treated LZR compared with the OZR. In response to high ANG, MC2 receptor expression was downregulated in the LZR but remained unchanged in the OZR (Fig. 6).

**DISCUSSION**

The main subject of the present study was to test the hypothesis that the activation of the HPA axis by ANG is specifically potentiated under conditions of leptin resistance. Since leptin resistance is probably the most common cause of liporegulatory failure and metabolic syndrome in patients (67), the hypothesis put forward in this study was tested by using OZR. Leptin resistance was demonstrated in OZR by the dramatic increase in body weight, leptin, and insulin, whereas plasma glucose levels were only just outside the high normal range (Table 1). Baseline levels of corticosterone and ACTH did not differ between saline-treated OZR and LZR, which contrasts with the results of two studies that showed a hypercorticosteronemia (29, 48). However, there is a consensus that elevation of stress hormones occurs in the morning rather than the afternoon in these rats (69), and we measured baseline levels in the evening. Consistent with Plotsky et al. (48), a hyperreactivity of the PA axis in OZR was confirmed in our study, since ACTH and corticosterone plasma levels were enlarged in response to CRH stimulation, especially during the late phase of the test (Fig. 1).

After chronic ANG treatment (independent of dosing), baseline levels of stress hormones were just as little affected in LZR and OZR as they were after chronic AT1 blockade in hypertensive rats (50). However, the stimulatory potency of ANG on the PA axis could be clearly assessed in CRH tests (Fig. 2). Only high ANG selectively increased the corticosterone responsiveness to CRH in OZR, an effect that occurred independently of an extended ACTH response. In contrast, neither ACTH nor corticosterone was increased during a CRH test after low ANG.

A special role for the adrenals in regulating HPA reactivity in OZR was indicated by the observation that the ANG-induced increase in corticosterone after CRH was not paralleled by a simultaneous elevation in ACTH. We asked the question of whether the ANG-mediated sensitization of the HPA axis may have been due to a modified expression pattern of ANG receptors within the HPA axis. It has been shown (2, 14, 40) that the expression of AT1 and AT2 receptors is modulated during stress. AT1A receptor mRNA was upregulated in the hypothalamus and pituitary of OZR (Fig. 5). Cytokines (e.g., IL-6) may be involved in the upregulation of AT1 receptor in OZR since IL-6 was found to be increased in OZR (71), which also overexpressed AT1 receptors (70).
addition, mRNA levels of the MC2 receptor, which is the major regulator of ACTH-mediated corticosterone biosynthesis in the adrenal glands (10), were lower in this organ (Fig. 6). This counterregulation between AT1A and MC2 receptors may result in a balanced corticosterone response to CRH in vehicle-treated OZR compared with LZR. The importance of AT1A and MC2 receptors in regulating HPA reactivity in response to ANG is emphasized by the selective increase of AT1A receptors in high ANG-treated OZR. In addition, MC2 receptor was up- (in tendency) rather than downregulated in response to ANG in high ANG-treated OZR, an observation that is consistent with the parallel increased expression pattern of AT1 and ACTH receptors in adrenocortical tumors (60). Our findings showing that the adrenal mRNA levels of AT1A receptors are enhanced in OZR in response to ANG are consistent with most mRNA and protein analytical studies showing that AT1 receptor expression in the adrenal gland is upregulated by its ligand (30, 31). Conversely, the mRNA levels of both the AT1A and the MC2 receptor were reduced in the adrenals of LZR, which may explain the ineffectiveness of high ANG in these animals to stimulate PA reactivity in a manner similar to that observed in obese rats. Although this explanation sounds reasonable, it conflicts with previous findings (39) showing that ANG positively correlates with MC2 receptor mRNA levels. These discrepancies may be attributable to tissue and/or species differences. AT2 receptors might not in fact be involved in the differentiated responses to ANG in OZR and LZR, since expression is similar between both strains and not altered by ANG.

Since the hyperreactivity of the HPA axis has been shown to be associated with diabetes (15), we asked whether the synergistic effects of leptin resistance and ANG stimulation on the reactivity of the HPA axis are associated with changes in glucose utilization. Baseline glucose remained unchanged by ANG in LZR and OZR (Table 1). This is in contrast to studies revealing an AT1-dependent hyperglycemic response to acute ANG (19, 42, 45, 51). It can be assumed that ANG increases glucose when given acutely, but when given chronically it becomes ineffective. Although adipocytokines other than leptin may be involved in the metabolic effects of OZR, hyperglycemia as a result of ANG-induced PA stimulation could be revealed in the CRH tests since 1) both corticosterone and glucose were selectively increased by high ANG in OZR (Figs. 2 and 3), 2) the ratios between the AUC of corticosterone and the AUC of glucose were equal in all groups independent of the ANG dose and rat strain (Fig. 3), and 3) a second delayed increase in plasma glucose occurred in response to CRH when corticosterone still remained at a high level (Fig. 2). Thus, our observations are in accordance with previous findings (50, 68), which showed that the increases in both corticosterone and glucose were diminished following AT1 blockade after immobilization stress or CRH administration.

To further confirm the relevance of an impaired glucose utilization in OZR after ANG stimulation, glucose challenge tests that focused particularly on alterations in corticosterone that occurred in parallel to glucose changes were performed. A hyperglycemic effect of chronic ANG was indeed observed during OGTT. Although this effect occurred to a small extent in LZR and was attributed to a decrease in insulin release, it was much more pronounced in OZR (Fig. 4). In contrast to LZR, the glucose levels stayed high and did not return to baseline levels over the whole observation period during the OGTT. This effect is probably less due to an insignificant reduction of insulin and is much more due to the isochronic corticosterone release, which was markedly and selectively increased in OZR by high ANG. Our results are consistent with studies demonstrating 1) enhanced cortisol levels in diabetic patients (20, 56), 2) positive correlations between blood glucose and cortisol during an OGTT (52, 53), 3) hyperglycemia in healthy volunteers after ANG during an OGTT (26), and 4) attenuated glucose responses in diabetic Zucker rats under AT1 blockade during an OGTT (64).

Considering that plasma glucose was high in the CRH test and OGTT, whereas corticosterone remained elevated, one might hypothesize that this effect is due to enhanced gluconeogenesis, since glucocorticoids are known to play a permissive role in the regulation of gluconeogenesis (25). In addition, we can also assume that the increase in glucose is due to a diminished insulin release, since glucocorticoids directly suppress insulin secretion from pancreatic β-cells (4–6). This effect could be observed in LZR during OGTT but was less distinctive in OZR, probably as a result of natural biological variation (Fig. 4).

In summary, we were able to demonstrate that the PA axis is synergistically activated by ANG and leptin resistance and that hyperreactivity is as a result paralleled by an impaired glucose utilization. We hypothesize that this interaction may become particularly relevant in patients suffering from metabolic syndrome and obesity, where leptin resistance induces a hypersensitization of the HPA axis. Therefore, we speculate that a blockage of the RAAS and thereby a decrease in the reactivity of the HPA axis represents one mechanism to explain the reduced incidence of type 2 diabetes (57). Alternatively, other mechanisms identified to be involved include the recruitment and differentiation of adipocytes, insulin sensitivity, skeletal blood flow, hepatic gluconeogenesis, or peroxisome proliferator-activated receptor-γ activity (58).

GRANTS

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REFERENCES

9. Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M, Scherbaum WA. Evidence for a novel peripheral action of leptin as a...


