Stress sensitivity is increased in transgenic rats with low brain angiotensinogen

Helge Müller1, Juliane Kröger1, Olaf Jöhren1, Silke Szymczak2, Michael Bader3, Peter Dominiak1 and Walter Raasch1

1Institute of Experimental and Clinical Pharmacology and Toxicology and 2Institute of Medical Biometry and Statistics, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany
3Max-Delbrück-Center for Molecular Medicine (MDC), Berlin-Buch, 13125 Berlin, Germany
(Correspondence should be addressed to W Raasch; Email: raasch@medinf.mu-luebeck.de)

Abstract

AT1 blockers attenuate hypothalamo–pituitary–adrenal (HPA) axis reactivity in hypertension independently of their potency to lower blood pressure. A reduced pituitary sensitivity to CRH and a downregulation of hypothalamic CRH expression have been suggested to influence HPA axis activity during chronic AT1 blockade. This study was aimed at confirming the role of central angiotensin II in regulating HPA reactivity by using the transgenic rat TGR(ASrAOGEN), a model featuring low levels of brain angiotensinogen. Different stress tests were performed to determine HPA reactivity in TGR(ASrAOGEN) and appropriate controls. In TGR(ASrAOGEN), blood pressure was diminished compared to controls. The corticosterone response to a CRH or ACTH challenge and a forced swim test was more distinct in TGR(ASrAOGEN) than it was in controls and occurred independently of a concurrent enhancement in ACTH. Using quantitative real-time PCR, we found increased mRNA levels of melanocortin 2 (Mc2r) and AT2 receptors (Agtr2) in the adrenals of TGR(ASrAOGEN), whereas mRNA levels of Cnr1, Pomc, and AT1 receptors (Agtr1) remained unchanged in hypothalami and pituitary glands. Since stress responses were increased rather than attenuated in TGR(ASrAOGEN), we conclude that the reduced HPA reactivity during AT1 blockade could not be mimicked in a specific transgenic rat model featuring a centrally inactivated renin–angiotensin–aldosterone system. The ACTH independency of the enhanced corticosterone release during CRH test and the enhanced corticosterone response to ACTH rather indicates an adrenal mechanism. The upregulation of adrenal MC2 and AT2 receptors seems to be involved in the stimulated facilitation of adrenal corticosterone release for effectuating the stimulated stress responses.

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Introduction

Over the last few years, it has been established that angiotensin II (Ang II) AT receptors (AGTR as listed in the MGI Database) are present in the organs of the hypothalamo-pituitary–adrenal (HPA) axis (Burson et al. 1994, Gasc et al. 1994, Llorens-Cortes et al. 1994, Jöhren et al. 1995, Jöhren & Saavedra 1996), that they are regulated during stress (Castren & Saavedra 1988, Aguilera et al. 1995, Leong et al. 2002), and that Ang II influences HPA axis reactivity by enhancing the synthesis and secretion of CRH, ACTH, and glucocorticoids (Rivier & Vale 1983, Abou-Samra et al. 1986, Schoenenberg et al. 1987, Sumitomo et al. 1991, Naville et al. 1993, Jezova et al. 1998). This crosstalk between the renin–angiotensin–aldosterone system (RAAS) and the HPA axis is functionally relevant for hypertension. In the transgenic rat TGR(mREN2)27 (which develops a fulminant hypertension as a result of the integrated mouse Ren-2d renin gene into its genome), the urinary excretion of corticosterone is increased during the developmental phase of hypertension, the adrenal response to ACTH is stimulated (Sander et al. 1992), and hypertension can be prevented when HPA reactivity is suppressed by dexamethasone (Djavidani et al. 1995). In spontaneously hypertensive rats (SHR), the Ang II-induced release of ACTH and corticosterone is stimulated compared to normotensive WKY rats, an observation that has been linked to a higher expression of AT1A receptors (Agtr1a) in the pituitary gland (Jöhren et al. 2003). From a therapeutic viewpoint, chronic AT1 receptor blockade diminishes HPA reactivity in rats in a blood pressure-independent manner (Seltzer et al. 2004, Raasch et al. 2006). Reductions in HPA reactivity induced by AT1 blockade have been suggested to mediate metabolic benefits, since on the one hand the HPA axis is hyperreactive in diabetes (Chan et al. 2002, 2003, Jöhren et al. 2007) and on the other hand glucose utilization is enhanced after AT1 blockade through a concurrent alteration in HPA reactivity (Uresin et al. 2004, Raasch et al. 2006, Müller et al. 2007).
Even though a functional impact of the interaction between the RAAS and the HPA axis has been demonstrated for hypertension, whether the reactivity of the HPA axis in response to peripherally applied AT_1 blockers or Ang II reflects a central (hypothalamic or pituitary) and/or a peripheral (adrenal) mechanism has not been sufficiently addressed. Both a reduced pituitary sensitivity to CRH and a downregulation of hypothalamic CRH expression have been discussed as reducing HPA axis activity during chronic AT_1 blockade or Ang II. We aimed to answer this question in the present study by using transgenic rats with low brain angiotensinogen (TGR(ASrAOGEN)) (Schinke et al. 1999). In TGR(ASrAOGEN), angiotensinogen protein concentration was reduced to 10% in medulla, pons, hypothalamus, thalamus, and cerebellum compared to controls, while angiotensinogen plasma concentrations and plasma renin activity were similar. This transgenic rat lacking in brain Ang II induced a 6–8 mmHg decrease in blood pressure (Schinke et al. 1999). The hypotension is comparable to the efficacy of AT_1 blockers at antagonizing an Ang II-induced increase in blood pressure when administered i.v. (Oliveira et al. 1996, Camara & Osborn 1998). Since the RAAS activity has been demonstrated to be impaired in the brain specifically, these transgenic rats may corroborate the functional significance of local Ang II production in the brain for centrally regulating the reactivity of the HPA axis.

**Material and Methods**

**Animals**

Eight-week-old transgenic rats deficient in brain angiotensinogen TGR(ASrAOGEN) were obtained from the Max-Delbrück-Center for Molecular Medicine (MDC), Berlin-Buch, Germany. Age–matched Hannover Sprague–Dawley rats (also from MDC) served as controls. The study was conducted according to the NIH guidelines for the care and use of laboratory animals, and was 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 h/12 h darkness (1400–0200 h)/light (0200–1400 h) cycle. They received a standard diet and water *ad libitum*.

**Study protocol**

**Protocol 1** At the age of 17 weeks, chronic polyethylene catheters were inserted during pentobarbital anesthesia into the right femoral vein and artery. Catheters were tunneled under the back skin, exteriorized on 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 2 days after catheterization at between 0900 and 1000 h in conscious rats. Values were recorded for 5 min. Blood pressure and heart rate were averaged over two time periods (30 s each) when conditions were stable. One day later, CRH tests were performed (Raasch et al. 2006, Müller et al. 2007). During the CRH tests, rats had free access to water and food. Three hours before starting the tests, arterial catheters were extended by ~4 cm to avoid stress reactions during blood withdrawal. CRH (10 μg/kg, i.v.) was injected 4 h before the light cycle. During the CRH tests, the lights at working benches were kept as dim as possible to maintain the darkness period. Before CRH injections and at regular intervals over a 4–h time period, 50 μl blood was withdrawn for analysis of corticosterone and ACTH. To avoid hemorrhage-induced alterations, platelets and erythrocytes were reconstituted and returned to each animal.

**Protocol 2** A second set of age-matched rats undertook the forced swim test (FST). Before stress testing, blood samples were taken from a tail nick in order to distinguish the baseline conditions of the stress hormones. Thirty minutes after swimming (10 min) in a basin (diameter 35 cm, water depth 20 cm, and water temperature 15 °C), tail blood was taken again for the determination of corticosterone and ACTH.

**Protocol 3** In a third set of animals (17 weeks), an ACTH test was performed 3 days after catheter insertions (see protocol 1). Before ACTH injections (2 μg/kg, i.v.) and at regular intervals within a 4–h time period, 30 μl blood was withdrawn. During ACTH tests, the lights at the working benches were kept as dim as possible.

**Protocol 4** Organs and blood for the biochemical/molecular analysis were obtained from age-matched animals that were not included in any functional tests. Rats of this group were housed and handled similar to the rats of the other protocols. These rats had not been catheterized to avoid any surgery stress. Thus, surgery–induced alterations in baseline ACTH and corticosterone could be eliminated and MC2 receptor, CRH and AT receptors reflect native expression in organs of the HPA axis in these rats.

**Biochemical analysis**

Plasma concentrations of ACTH, corticosterone, and aldosterone were determined by RIAs using commercial kits (all from MP Biomedicals, Eschwege, Germany). Assays were performed as recommended by the manufacturers (Raasch et al. 2006, Müller et al. 2007). Norepinephrine and epinephrine were measured in plasma that was obtained one day after catheter insertion by HPLC and electrochemically as previously described (Raasch et al. 2004a, 2005).

**RNA isolation and cDNA synthesis**

Hypothalami were dissected according to Paxinos & Watson (1998). The brains were adapted to −10 °C, and coronal cuts were made 0.26 mm (at the optic chiasm) and 4.8 mm posterior to the bregma. To cut apart the hypothalamus, the
slice was turned on its posterior surface and cut sagittally 2-6 mm lateral to the midline directly before the amygdala and horizontally 7-4 mm under the cortical surface. The neurointermediate lobes were not removed before preparation of pituitary RNA. Hypothalami and pituitary and total adrenal glands were extracted on the ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems) according to the manufacturer’s instructions (Jöhren et al. 2003). Total RNA 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 AT1A (Agtr1a), AT1B (Agtr1b), AT2 (Agtr2), and ACTH receptors (melanocortin 2 receptors = MC2 receptor; Mc22), as well as of pro-opiomelanocortin (Pomc) and Cth, were quantified in organs of the HPA axis. Quantitative measurements of mRNA were performed by qPCR with the cycle threshold method using SYBR green I as a fluorescent dye on the GeneAmp 7000 sequence detection system (Perkin-Elmer Applied Biosystems, Weiterstadt, Germany), and cDNA-specific primers. Primers for AT1A, AT1B, AT2 receptors, MC2 receptor, POMC and CRH have been published elsewhere (Raasch et al. 2004b, 2006, Müller et al. 2007). All primers were obtained from Invitrogen. Product specificity was confirmed by dissociation curve analysis and agarose gel electrophoresis. No template controls served as negative controls (Jöhren et al. 2001). Expression values were normalized to total RNA content (Bustin 2002).

Statistical analysis

Data shown are expressed as means ± s.e.m. To quantify the total effect over the observation time in response to CRH regarding changes in plasma concentrations of corticosterone and ACTH, the area under the curves (AUC) was calculated for each individual animal on the basis of their delta values. Correlation analysis was carried out by using Pearson’s product moment correlation. For pairwise comparisons, Student’s t-test was employed, and differences in MC2 receptor mRNA were also analyzed by the median quantitative mutual information score (QMIS; Tsalenko et al. 2006). QMIS is powerful specifically when study groups exhibit different skewness or kurtosis. A two-way ANOVA followed by Bonferroni’s post-hoc test for multiple comparisons was performed to examine the effects of two variables. Differences were considered to be statistically significant at \( P<0.05 \).

Results

Blood pressure was slightly decreased in TGR(ASrAOGEN) compared to controls, whereas heart rate and left ventricular weight were not affected (Table 1). Weight of adrenals was similar between the TGR(ASrAOGEN) and the controls (31.9 ± 2.1 vs 27.1 ± 1.1 mg; \( P=0.1011 \)). Baseline levels of ACTH, corticosterone, and aldosterone did not differ between the two rat strains (Table 2). Moreover, controls and TGR(ASrAOGEN) featured similar plasma levels of norepinephrine and epinephrine (Table 2). Both norepinephrine and epinephrine exceeded the normal range, which might be due to the withdrawal modality.

In response to CRH stimulation, serum levels of ACTH and corticosterone sharply increased and returned to baseline levels within 4 h. The plasma profiles of ACTH after CRH stimulation did not differ between both rat strains (Fig. 1A). Despite similar ACTH profiles, the CRH-induced corticosterone response was enlarged in TGR(ASrAOGEN). The maximal concentration (Cmax; 362 ± 17 vs 437 ± 21 ng/ml; \( P=0.0158 \)) was increased, but tmax (70 ± 8 vs 60 ± 7 min; \( P=0.3589 \)) remained unchanged in TGR(ASrAOGEN) compared with the controls (Fig. 1B). The observation that the corticosterone response to CRH was enlarged in TGR(ASrAOGEN) independently of a concurrent ACTH increase was pointed out by plotting individual AUCs of corticosterone (\( \text{AUC}_{\text{corticosterone}} \)) and ACTH (\( \text{AUC}_{\text{ACTH}} \)) respectively. The regression analysis of corresponding AUCs verified a positive correlation between the ACTH and corticosterone response to CRH in TGR(ASrAOGEN) and controls (Fig. 1C). However, the regression line relating to TGR(ASrAOGEN) was shifted upwards (Fig. 1C) indicating an improved sensitivity towards ACTH. Accordingly, the mean AUCcorticosterone was significantly increased in TGR(ASrAOGEN) (34 535 ± 3047 vs 22 209 ± 3911 pg/ml per min, \( P=0.0202 \)), whereas the mean AUCACTH was similar between TGR(ASrAOGEN) and controls (Fig. 1C).

### Table 1 Hemodynamic parameters of TGR(ASrAOGEN) and controls. Means ± s.e.m. (n=13)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>TGR(ASrAOGEN)</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124 ± 2</td>
<td>118 ± 2</td>
<td>0.028</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>370 ± 10</td>
<td>370 ± 6</td>
<td>0.969</td>
</tr>
<tr>
<td>Left ventricular weight (mg)</td>
<td>1067 ± 31</td>
<td>1034 ± 25</td>
<td>0.429</td>
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### Table 2 Endocrine parameters of TGR(ASrAOGEN) rats and controls. Means ± s.e.m. (n=8–13)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>TGR(ASrAOGEN)</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Plasma ACTH (pg/ml)</td>
<td>110 ± 15</td>
<td>91 ± 6</td>
<td>0.148</td>
</tr>
<tr>
<td>Plasma corticosterone (ng/ml)</td>
<td>152 ± 34</td>
<td>135 ± 20</td>
<td>0.329</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/ml)</td>
<td>191 ± 32</td>
<td>1091 ± 34</td>
<td>0.096</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>1039 ± 96</td>
<td>844 ± 96</td>
<td>0.164</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>654 ± 95</td>
<td>512 ± 75</td>
<td>0.281</td>
</tr>
</tbody>
</table>
After FST, the plasma levels of ACTH and corticosterone increased in TGR(ASrAOGEN) and controls to a similar extent compared to the CRH test (Fig. 2A and B). The corticosterone response was enlarged in TGR(ASrAOGEN), which was not again associated with a concurrent increase in ACTH. This was emphasized by the graphical analysis of the correlations between ACTH and corticosterone values. Consistently with the CRH test, the regression line representing the TGR(ASrAOGEN) was shifted upwards (Fig. 2C).

In order to determine the impact of the adrenals on the sensitization of the HPA axis in TGR(ASrAOGEN), an ACTH test was performed. Baseline corticosterone levels did not differ between both rat strains (129±5 vs 100 ±14 ng/ml; P=0.088). In response to ACTH, increase in corticosterone was slightly heightened in TGR(ASrAOGEN) compared to controls (Fig. 3).

Figure 1 Plasma profiles of ACTH (A) and corticosterone (B) in response to CRH (10 µg/kg i.v.) in TGR(ASrAOGEN) (●) and controls (○). The increase in corticosterone response was statistically evaluated by two-way ANOVA. (C) Correlation between CRH-induced alterations of cumulative plasma concentrations of ACTH and corticosterone (depicted as AUC of the corresponding concentration–time curves; see Fig. 1) in TGR(ASrAOGEN) (●, solid line, Pearson R=0.6447, P=0.0118) and controls (○, dotted line, Pearson R=0.5572, P=0.0471). Means ±S.E.M. (n=10–12).

Figure 2 Plasma concentrations of ACTH (A) and corticosterone (B) before and after TGR(ASrAOGEN) (closed bars) and controls (open bars) were stressed by a forced swim test (FST, 10 min 15 °C). The stress response and the increase in corticosterone after FST were statistically evaluated by two-way ANOVA. In (C), the correlation between ACTH and corticosterone after FST in TGR(ASrAOGEN) (●, solid line, Pearson R=0.8771, P=0.0048) and controls (○, dotted line, Pearson R=0.7777, P=0.0115) is depicted. Means ±S.E.M. (n=7–8).
The steady-state mRNA levels of \textit{Crh} and \textit{Pomc} in the hypothalami and pituitary glands were similar between both strains (Fig. 4). The steady-state mRNA levels of MC2 receptors were increased by one-third in the adrenals of TGR(ASrAOGEN) (Fig. 5). Finally, while the mRNA levels of AT\textsubscript{1A} and AT\textsubscript{1B} receptors in the organs of the HPA axis did not differ between the two rat strains (Tables 3 and 4), the adrenal mRNA levels of AT\textsubscript{2} receptors were doubled in TGR(ASrAOGEN) (Fig. 5).

**Discussion**

Recently, we have shown that inhibition of AT\textsubscript{1} receptors attenuates HPA axis reactivity. A reduced pituitary sensitivity to CRH and a downregulation of hypothalamic CRH expression were speculated to underlie these effects (Raasch et al. 2006). Following on from this, in this study, we aimed to confirm the relevancy of Ang II-dependent central mechanisms for regulating HPA activity by using TGR(ASrAOGEN) that lacked brain Ang II (Schinke et al. 1999). Consistent with many other studies, a slight hypotension was observed in the TGR(ASrAOGEN), which served as the fundamental characteristic of this transgenic rat in most of the studies (Baltatu et al. 2000, 2001, Wang et al. 2004, Campos et al. 2006).

At first sight, TGR(ASrAOGEN) and controls do not seem to differ regarding stress parameters, since baseline levels of ACTH, corticosterone, aldosterone, norepinephrine, epinephrine, and adrenal weights were all similar. Even though morphometric examinations were not carried out, we speculate here that zonal differentiation was not altered in the adrenal cortex of TGR(ASrAOGEN), since no changes or hypertrophy in response to Ang II were observed in the zona fasciculata or the zona glomerulosa (Ogishima et al. 1992, McEwan et al. 1999, Aguilar et al. 2004). In accordance with these findings, baseline levels of stress hormones also remained unaffected in SHR after chronic AT\textsubscript{1} blockade (Raasch et al. 2006). In contrast to our expectations and the observation that HPA reactivity is increased by Ang II and reduced by AT\textsubscript{1} blockade (Raasch et al. 2006, Müller et al. 2007), the CRH-induced corticosterone response in TGR(ASrAOGEN) was by no means diminished, but in fact increased. In order to confirm our results, a second stress test was performed. Accordant to the CRH challenge, the corticosterone plasma levels raised to the same extent after FST, and the stress-induced increase in corticosterone was enlarged in TGR(ASrAOGEN). The stimulated corticosterone response in TGR(ASrAOGEN) was not associated with a concurrent increase in ACTH in the CRH test or FST. As such, a combined hypothalamic and pituitary related mechanism seems rather unlikely. This conclusion is further supported by similar \textit{Crh} and \textit{Pomc} mRNA levels in the hypothalamus and pituitary glands of TGR(ASrAOGEN). However, i.c.v. or peripheral administrations of AT\textsubscript{1} blockers were found to alleviate the increases in hypothalamic \textit{Crh} mRNA or pituitary ACTH in response to stress (Jezova et al. 1998, Raasch et al. 2006). While this inhibiting effect was observed in animals that were exposed to stress (Jezova et al. 1998, Raasch et al. 2006), the \textit{Crh} and \textit{Pomc} mRNA levels presented here reflect native expression in the organs of rats, thus accounting for the differences between our and the cited studies.
MC2 receptor may account for the stimulated corticosterone response to stress in TGR(AsrAOGEN). In order to confirm this mechanism functionally, the adrenal responsiveness to ACTH was investigated. In accordance with our hypothesis, plasma corticosterone in response to ACTH was slightly enhanced in TGR(AsrAOGEN) compared to controls.

Whether other mechanisms besides the upregulation of MC2 receptor may be involved in the increased stress response in TGR(AsrAOGEN) is discussed in the following:

Ang II stimulates glucocorticoid release by enhancing the CRH-mediated ACTH release (Gaillard et al. 1981, 1985, Abou-Samra et al. 1986, Schoenenberg et al. 1987) or by stimulating adrenal glucocorticoid production (Naville et al. 1993). We recently demonstrated that Ang II increased the corticosterone responsiveness to CRH, an effect that was associated with an upregulation of the AT1A receptor (Müller et al. 2007). This poses the question whether AT1 receptors are enhanced in the organs of the HPA axis of TGR(AsrAOGEN), and whether this causes the observed stress sensitization. We found no alterations in the mRNA levels of the AT1 receptor, indicating that the hypersensitization to stress is minor compared to alterations in the AT1 receptor. A limitation of our study was that we determined mRNA levels rather than protein or AT1 binding, even considering that AT1 receptor binding was significantly higher inside various regions of the blood–brain barrier of TGR(AsrAOGEN) compared to controls (Monti et al. 2001). While hypothalamic AT1 receptors are less stimulated in TGR(AsrAOGEN) by locally generated Ang II, it should be questioned whether peripherally generated Ang II causes the enhanced stress response. The following points tend to argue against an impact of peripherally arising Ang II: 1) if circulating Ang II is really involved in stress sensitization via hypothalamic AT1 receptors, the ACTH response should also be enhanced; however, in our study, the potentiation of corticosterone response occurred ACTH independently; 2) even considering that glucocorticoids are potent activators of angiotensinogen gene expression (Brasier & Li 1996), Ang II does not penetrate the blood–brain barrier (Schelling et al. 1976) and 3) Ang II may indeed act upon AT1 receptors in the circumventricular organs, but AT1 receptor binding was clearly shown to be decreased in these areas (Monti et al. 2001). It is certainly true that the increase in AT2 receptor mRNA in the adrenals of TGR(AsrAOGEN) may also have been related to the stimulated stress response of the transgenic rats, since another study on bovine fasciculata cells showed

The ACTH independency of simulated corticosterone responses to stress indicates an adrenal mechanism. We asked whether the adrenal MC2 receptor expression is increased, since this subtype of MC receptor preferentially promotes the ACTH-induced corticosterone response (Chhajlani et al. 1993, Liakos et al. 1998). In human adrenal fasciculata cells, Ang II and ACTH stimulated the expression of the MC2 receptor (Lebrethon et al. 1994, Penhoat et al. 1994, 1995, Naville et al. 2001, Blondet et al. 2002). In bovine adrenal fasciculata cells, Ang II was hypothesized to induce a steroidogenic desensitization (Penhoat et al. 1995), justifying our speculation that the MC2 receptor is increased in TGR(AsrAOGEN). Indeed, steady-state levels of MC2 receptor mRNA were increased in TGR(AsrAOGEN). It therefore seems conceivable that an upregulation of the

Table 3  Steady-state levels of mRNA of AT1A receptors in organs of the hypothalamo-pituitary–adrenal axis of TGR(AsrAOGEN) and controls. Means±s.e.m. (n=7–10).

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<td>Hypothalami</td>
<td>4.2±0.5×10⁴</td>
<td>4.7±0.6×10⁵</td>
</tr>
<tr>
<td>Pituitary glands</td>
<td>6.1±0.7×10⁴</td>
<td>8.1±0.6×10⁵</td>
</tr>
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<td>Adrenal glands</td>
<td>5.5±0.8×10⁴</td>
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Table 4  Steady-state levels of mRNA of AT1B receptors in organs of the hypothalamo-pituitary–adrenal axis of TGR(AsrAOGEN) and controls. Means±s.e.m. (n=7–10).

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that cortisol production was stimulated through AT₂ receptors, and thus independently of an AT₁-mediated mechanism (Defaye et al. 1995).

CRH is a key hormone in the integrated response to stress, which stimulates sympathetic outflow in addition to regulating pituitary ACTH release. Sympathoexcitatory responses to air-jet stress were found to be similar between controls and TGR(ASrAOGEN) and controls, and between Wistar rats receiving i.c.v. infusions of losartan or vehicle (Zhang et al. 1999, Wang et al. 2004). This indicates that the brain RAAS plays only a small role in the control of sympathetic function during stress. Our findings that hypothalamic CRH expression as well as baseline epinephrine did not differ between controls and TGR(ASrAOGEN) may strengthen this idea, even though we are aware that we have not determined changes during stress reactions. Peripheral RAAS certainly does influence sympathoexcitatory responses to stress, since adrenal biosynthesis and secretion of catecholamines are reduced after AT₁ blockade (Armando et al. 2001).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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