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RESEARCH PAPER

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

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BACKGROUND AND PURPOSE

Combination therapies are becoming increasingly important for the treatment of high blood pressure. Little is known about whether double blockade of angiotensin II (AT_1) receptors and angiotensin-converting enzyme (ACE) exert synergistic metabolic effects.

EXPERIMENTAL APPROACH

Spontaneously hypertensive rats were allowed to choose between palatable chocolate bars and standard chow and were simultaneously treated with the AT_1 blocker telmisartan (8 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$), the ACE inhibitor ramipril (4 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$) or a combination of the two (8 + 4 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$) for 12 weeks.

KEY RESULTS

Although food-dependent energy intake was increased by telmisartan and telmisartan + ramipril compared with ramipril or controls, body weight gain, abundance of fat and plasma leptin levels were decreased. Increased insulin levels in response to an oral glucose tolerance test were comparably attenuated by telmisartan and telmisartan + ramipril, but not by ramipril. During an insulin tolerance test, glucose utilization was equally as effectively improved by telmisartan and telmisartan + ramipril. In response to a stress test, ACTH, corticosterone and glucose increased in controls. These stress reactions were attenuated by telmisartan and telmisartan + ramipril.

CONCLUSIONS AND IMPLICATIONS

The combination of telmisartan + ramipril was no more efficacious in regulating body weight and glucose homeostasis than telmisartan alone. However, telmisartan was more effective than ramipril in improving metabolic parameters and in reducing body weight. The association between the decrease in stress responses and the diminished glucose levels after stress supports our hypothesis that the ability of telmisartan, as an AT_1 receptor blocker, to alleviate stress reactions may contribute to its hypoglycaemic actions.

Abbreviations

ACE, angiotensin-converting enzyme; ACEI, ACE inhibitors; ACTH, adrenocorticotropic hormone; AgRP, agouti-related protein; Ang, angiotensin; AT receptor, angiotensin II (AT) receptor; AUC, areas under the curve; BMI, body mass index; bw, body weight; CART, cocaine- and amphetamine-regulated transcript; CD, cafeteria diet; C_{max}, maximal concentration; CRH, corticotropin-releasing hormone; FST, forced swim test; HPA axis, hypothalamic–pituitary–adrenal axis; ITT, insulin tolerance test; MCH, melanin-concentrating hormone; MRT, magnetic resonance tomography; NPY, neuropeptide Y; OGTT, oral glucose tolerance test; POMC, pro-opiomelanocortin; PPO, prepro-orexin; qPCR, quantitative PCR; RAAS, renin–angiotensin–aldosterone system; RAM, ramipril; SBP, systolic blood pressure; SHR, spontaneously hypertensive rat; TEL, telmisartan

Introduction

In recent years, metabolic syndrome has evolved into a problem of epidemic proportions in Western countries and associates obesity with numerous other abnormalities, including alterations in glucose metabolism, dyslipidaemia and hypertension. Blocking the renin–angiotensin–aldosterone system (RAAS) represents one established approach for treating MetS (de Kloet *et al.*, 2010). AT_1 receptor blockers and ACE inhibitors (ACEI) are well known as first-line antihypertensive drugs. In the ONTARGET trial, the antihypertensive potencies of the ARB telmisartan (TEL) and the ACEI ramipril (RAM) were demonstrated to be comparable: the primary outcome of the study (death from cardiovascular causes, myocardial infarction, stroke or hospitalization for heart failure) was affected in a similar way (Yusuf *et al.*, 2008). As a secondary endpoint of the ONTARGET trial, TEL was found to be equally as effective as RAM in preventing type-2 diabetes (Yusuf *et al.*, 2008). This finding confirms a meta-analysis of 22 clinical trials including 143 153 participants showing that the onset of type-2 diabetes during hypertensive drug treatment is lowest with AT_1 receptor blockers and ACEI, followed by calcium channel blockers and placebo, β blockers and diuretics – ranked in that order (Elliott and Meyer, 2007). In addition to improving glucose homeostasis, AT_1 receptor blockers and ACEI have also been demonstrated to induce a loss in body weight in animal and human studies (McGrath *et al.*, 1990; Campbell *et al.*, 1995; Benson *et al.*, 2004; Carter *et al.*, 2004; Fogari *et al.*, 2005; Schupp *et al.*, 2005; Zorad *et al.*, 2006). However, these results seem to conflict with data on angiotensin II (AngII). AngII itself has been shown to diminish body weight by inducing lipolysis and by impairing thermogenesis due to its capacity to stimulate sympathetic activity (Engeli *et al.*, 2000; Cabassi *et al.*, 2005). It also decreases food intake (Brink *et al.*, 1996) by promoting the secretion of leptin from adipocytes (Skurk *et al.*, 2005).

In the present study, we have focused on the efficacy of TEL and RAM in affecting body weight and glucose utilization. The ONTARGET trial revealed no differences between TEL and RAM regarding the onset of new type-2 diabetes (Yusuf *et al.*, 2008). However, the improvement in insulin sensitivity after TEL has been attributed in particular to its potency in activating PPARγ. This action is AngII-independent and not observed with ACE inhibitors (Schupp *et al.*, 2004; 2005). Thus, we aimed to investigate whether the proposed TEL- or RAM-induced improvement in glucose utilization is related to PPARγ and to other mechanisms, in particular in reducing hypothalamic–pituitary–adrenal (HPA) axis activity. This endeavour was prompted by findings showing increased HPA axis activity in diabetes (Cameron *et al.*, 1984; Jöhren *et al.*, 2007), hypertension (Filipovsky *et al.*, 1996) and obesity (Masuzaki *et al.*, 2001). Consistent with the findings showing that AngII receptors are present in all organs of the HPA axis and that AngII increases HPA axis reactivity (Saavedra and Benicky, 2007), we recently demonstrated that glucose utilization is selectively impaired after AngII stimulation in leptin-resistant obese, but not in lean Zucker rats, as a result of a hyper-reactive HPA axis, thus confirming the functional importance of dysregulation in the HPA axis specifically in metabolic syndrome (Müller *et al.*, 2007).

Often, experimental research in metabolic syndrome is conducted in rats or mice presenting only some symptoms of metabolic syndrome or in genetically modified rodents exhibiting, for example, mutations within the leptin or leptin receptor. We performed our study by applying high-calorie dietary protocols to provoke obesity. Compared with control rats (only receiving standard diet), body weight, energy intake, abundance of abdominal fat, plasma levels of leptin, triglycerides, glucose and insulin were increased in spontaneously hypertensive rats (SHR) that were allowed to choose between a cafeteria diet and standard chow for 12 weeks. In addition, plasma levels of adiponectin decreased and glucose utilization had worsened after glucose challenge. Thus, this model of CD-fed SHR promotes leptin and insulin resistance, hypertension and obesity and was demonstrated to mimic the situation of patients suffering from metabolic syndrome (Miesel *et al.*, 2010). In the present study, rats received the same feeding regimen to provoke symptoms of metabolic syndrome. In parallel to the 12 week feeding period, rats were treated either with vehicle (controls) or with 8 $mg \cdot kg^{-1}$ TEL and 4 $mg \cdot kg^{-1}$ RAM, respectively, since a 1:2 ratio has been found to be equally effective in lowering blood pressure (Dupuis *et al.*, 2005), and only high doses of AT_1 receptor blockers have been demonstrated to reduce body weight (Müller-Fielitz *et al.*, 2011). We also investigated whether double blockade of ACE and AT_1 receptors is superior to effective single-drug treatments in reducing weight gain and improving glucose homeostasis.

Methods

Animals

Eight-week-old, male, spontaneously hypertensive rats (SHR/NCrI, Charles River, Sulzfeld, Germany) were used in the

study. All animal care and experimental procedures were in accordance with the NIH guidelines for the care and use of laboratory animals and were approved by the ethics committee of the local regulatory authority (Ministerium für Landwirtschaft, Umwelt und ländliche Räume des Bundeslandes Schleswig-Holstein). The animals were kept in pairs at room temperature with a 12 h/12 h dark (2:00 h–14:00 h)/light (14:00 h–2:00 h) cycle. Rats were randomly allocated into four groups and were habituated to research assistants and *vice versa* 3 weeks before drug treatment was initiated. Initial body weight (197 ± 1 g), systolic blood pressure (SBP: 160 ± 2 mmHg) and heart rate (HR: 396 ± 2 beats min^{-1}) did not differ among the four groups of SHR.

Feeding and drug treatment

From day 0 until the end of the study after 12 weeks, SHR were allowed to choose freely between a cafeteria diet and a standard diet, which both were abundantly offered. The cafeteria diet comprised 10 various commercial chocolate and cookie bars with calorific content of 20.3 ± 0.5 $kJ \cdot g^{-1}$ and consisting of 60.1 ± 3.0% carbohydrates; 24.9.1 ± 2.2% fat, 6.5 ± 0.7% protein and 2.1 ± 0.4% fibre. The standard diet was the maintenance diet 1320 (Altromin, Lage, Germany) with a calorific value of 11.7 $kJ \cdot g^{-1}$, consisting of crude protein 19%, crude fat 4%, crude fibre 6%, crude ash 7.5% and nitrogen-free extracts 53%. The metabolizable energy from the standard chow was 65% carbohydrates, 24% protein and 11% fat. Rats received only one kind of chocolate or cookie bar per day, these being switched daily in a regular manner. In the following, the feeding regimen comprising standard diet and chocolate/cookie bars is indicated as 'CD diet' and the feeding regimen constituting only standard diet as 'chow'. In parallel to CD feeding, rats were treated by gavage with TEL (8 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$, n = 14), RAM (4 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$, n = 14) or the combination TEL+RAM (8 + 4 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$, n = 14). Drugs were generous gifts from Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT). Doses of TEL and RAM at a 2:1 ratio were given since this ratio was found to provoke a similar reduction in blood pressure (Dupuis *et al.*, 2005). Metabolic effects of TEL have been demonstrated especially when TEL was given at doses ≥5 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$ (Wienen *et al.*, 2001; Benson *et al.*, 2004; Sugimoto *et al.*, 2006; 2008; Kamari *et al.*, 2008; Younis *et al.*, 2010). All animals had free access to water. Controls were given an identical volume of water (1 mL kg_{bw}^{-1}, n = 13).

Test protocols

The body weights of the rats and their food and water intakes were monitored by daily weighing at 14:00 h, the beginning of the light cycle. At the end of the study, the abdominal girth and the body length were determined in sedated animals by a person unaware of the different treatments. Body mass index (BMI) was calculated, by considering body weight and body length. Leptin, adiponectin, ACTH, corticosterone, glucose and insulin were measured at regular intervals in blood samples taken from a tail nick between 8:00 and 9:00 h.

SBP and heart rate were determined before the feeding period and at the end of the study by plethysmography, as described elsewhere (Raasch *et al.*, 2002). Randomized measurements were performed only between 9:00 h and 13:00 h to avoid circadian variations. After 6 weeks, glucose and insulin levels were determined during an oral glucose tolerance test (OGTT; 1× g glucose·kg^{-1} body weight) in rats that had been deprived of food for 18 h. EDTA blood (80 µL) was withdrawn immediately before administration of glucose (by gavage) and after 12, 24, 36, 60, 90, 120 and 240 min in blood samples taken from a tail nick (Raasch *et al.*, 2006; Müller *et al.*, 2007; Miesel *et al.*, 2010). Two days later, the glucose levels were monitored during an insulin tolerance test (ITT, 0.5 IU insulin i.p./kg_{bw}) in SHR that had been deprived of food for 18 h. Glucose level was determined before and during a 45 min period in blood samples taken from a tail nick. Plasma levels of insulin were measured 15 min after it was administered (Miesel *et al.*, 2010).

After 11 weeks, the home cage activity of the rats was monitored for 2 days by using the InfraMot System (TSE, Bad Homburg, Germany). The InfraMot System uses passive infrared sensors mounted on the top of the cages. These sensors register the activity by sensing the body heat image. As such, movement of the rats could be detected under any lighting conditions and without habituation since the measurements were performed in the individual home cages.

Two days before the end of the study, rats were subjected to the forced swim test (FST). Before stress testing, blood samples were taken from a tail nick in order to establish the baseline conditions of the stress hormones. Then, 30 and 90 min after swimming (7 min) in a basin (diameter 35 cm, water depth 20 cm, water temperature 15°C), tail blood was taken again to determine ACTH, corticosterone and glucose levels (Müller *et al.*, 2010).

At the end of the study after the functional tests had been performed, fat distribution was determined in anaesthetized (pentobarbitone 75 $mg \cdot kg^{-1}$) SHR by using the MRT technique (Philips, Achieva, 1.5 Tesla with the use of an eight-channel SENSE knee coil, a transverse T1-weighted turbo spin echo sequence and an imaging matrix of 320 × 320 pixels). Images were recorded from the anus to the diaphragm (section thickness 2 mm; gap 0) (Miesel *et al.*, 2010).

Biochemical analyses

Plasma concentrations of adiponectin, insulin, leptin (all from Linco, St. Charles, MO), ACTH, corticosterone, aldosterone (all from MP Biomedicals, Eschwege, Germany) or angiotensin II (IBL, Hamburg, Germany) were determined by radioimmunoassay using commercial kits.

Blood glucose was determined using glucose sensors, which operated on the principle of amperometric measurement after enzymatic glucose oxidation (Ascensia® ELITE XL, Bayer, Leverkusen, Germany).

Hepatic glycogen content was determined as previously described (Miesel *et al.*, 2010). After the glycogen had been metabolized to glucose, the glucose was measured using a glucose oxidase/peroxidase assay (GAGO20®, Sigma, Munich, Germany).

RNA isolation and cDNA synthesis

Hypothalami were dissected according to Paxinos and Watson (1998). The brains were adjusted to –10°C, and coronal sections were made 0.26 mm (at the optic chiasm) and 4.8 mm posterior to the bregma. In order to cut apart the hypothalamus, the slice was turned on its posterior surface

and cut sagitally 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 preparing the pituitary RNA. Total RNA from the hypothalami was extracted on the ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems, Darmstadt, Germany). The total amount of RNA was determined using a RiboGreen RNA quantification assay (Invitrogen, Karlsruhe, Germany). First-strand cDNA was synthesized using oligo-$(dT)_{15}$ primer and AMV Reverse Transcriptase (Invitrogen). cDNA was stored at –20°C until PCR.

qPCR

mRNA steady-state levels of anorexigenic peptides [e.g. cocaine- and amphetamine-regulated transcript (CART), corticotropin-releasing hormone (CRH), pro-opiomelanocortin (POMC)], and orexigenic peptides [e.g. prepro-orexin (PPO), neuropeptide Y (NPY), melanin-concentrating hormone (MCH), and agouti-related protein (AgRP)] were quantified in the hypothalami of the rats. Quantitative measurements of mRNA were performed by qPCR 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 for AgRP, CART, MCH, NPY PPO, CRH and GAPDH have been published elsewhere (Raasch *et al.*, 2006; Miesel *et al.*, 2010). All primers were obtained from Invitrogen. No-template controls served as negative controls (Raasch *et al.*, 2006; Müller *et al.*, 2007; Miesel *et al.*, 2010). 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).

Calculations and statistics

Data shown are expressed as means ± SEM. To depict total abdominal fat, 3D images were calculated (iQ-3D software; http://www.k-pacs.de/) on the basis of the transverse T1-weighted turbo spin echo images. Representatively, the amount of fat in retroperitoneal fat pads and s.c. fat were determined by using the ImageJ software (http://rsbweb.nih.gov/ij/). Only intensity signals of >80 were considered to ensure that fat was being analysed. Using five sequential images that were located 3.0 cm rostral from the femoral head, the areas of retroperitoneal fat deposits were bilaterally determined by planimetry. Areas were multiplied by 0.2 cm (= section thickness). Values were summed to obtain the volume of the fat pad within a defined length of 1 cm. The amount of s.c. fat was also assessed as described above by analysing five sequential images that were located 1.0 cm rostral from the femoral head. The average of the left and right side is depicted in Figure 2.

In order to quantify the total effect over the observation period in response to OGTT or ITT regarding changes in plasma concentrations of glucose and insulin, the areas under the curves (AUC) were calculated for each individual animal on the basis of their Δ values. Accordingly, the maximal increases (C_{max}) in glucose or insulin were also calculated by considering the Δ values. Half-life of glucose utilization after insulin challenge was determined by linear regression analysis of log values.

The correlation coefficient (two-tailed *P*-value) was computed for each group according to Pearson, considering Gaussian distribution by using GraphPad Prism, Version 4 (GraphPad Software, Inc., San Diego, CA, USA).

Statistical analysis was performed by one-way ANOVA followed by appropriate *post hoc* tests (Bonferroni or Dunnett). Wilcoxon Signed Rank Test was used when variances differed between groups. A two-way ANOVA was performed, followed by Bonferroni's *post hoc* test for multiple comparisons, to examine the effects of two variables. Differences were considered to be statistically significant at $P < 0.05$.

Results

Body weight, energy intake and home cage activity

Metabolic and feeding behaviour was investigated in SHR that were fed with a CD and simultaneously treated with TEL, RAM or a combination of the two. The gain in body weight was markedly diminished by TEL and TEL + RAM, but less so by RAM. The decrease in body weight was mainly related to a reduced growth in girth and only partially to a reduced growth in length (Table 1, Figure 1). After treatment with TEL, RAM or the combination, the left ventricular weight was

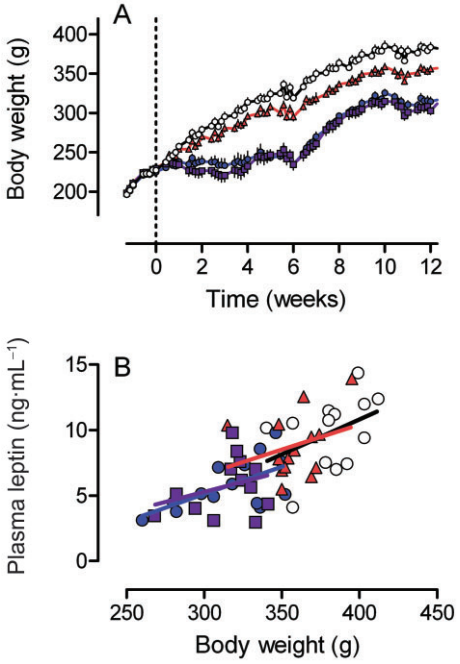

Figure 1

Influence of telmisartan (8 mg·kg_{bw}^{-1}·day^{-1}) (blue symbols), ramipril (4 mg·kg_{bw}^{-1}·day^{-1}) (red symbols) and telmisartan + ramipril (8 + 4 mg·kg_{bw}^{-1}·day^{-1})(purple symbols) on body weight. Controls (open symbols) received water. (A) Development of body weight within the treatment period. (B) An overall correlation between plasma leptin and body weight may be assumed. However, correlation analysis for each group did not indicate any group-specific correlation. Means ± SEM, n = 12–14.

Table 1

Growth of CD-fed SHR after treatment (12 weeks) with telmisartan (TEL; 8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (RAM; 4 mg·kg$_{bw}^{-1}$·day^{-1}), telmisartan + ramipril (TEL + RAM; 8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}) or water (CON)

	CON	TEL	RAM	TEL + RAM
BMI (kg·m^{-2})	8.10 ± 0.16	7.34 ± 0.14*	7.80 ± 0.16†	7.11 ± 0.17*
Abdominal girth (cm)	18.6 ± 0.2	17.1 ± 0.2*	17.9 ± 0.2†	16.5 ± 0.2*
Weight gain (g in 12 weeks)	155 ± 4	90 ± 4*	126 ± 4*†	78 ± 5*
Body length (cm)	21.8 ± 0.1	20.9 ± 0.1	21.5 ± 0.2	20.9 ± 0.3
Femur length (mm)	36.7 ± 0.1	35.5 ± 0.1*	36.4 ± 0.1†	35.6 ± 0.1*
Liver (g)	11.5 ± 0.2	9.9 ± 0.4*	11.4 ± 0.4	8.9 ± 0.2*
Kidney (g)	1.12 ± 0.02	1.08 ± 0.03	1.14 ± 0.02	1.08 ± 0.02
Adrenal gland (mg)	22.4 ± 0.6	23.7 ± 0.6	22.3 ± 0.6	23.2 ± 0.5
Left ventricular index (mg·g$_{bw}^{-1}$)	2.98 ± 0.09	2.01 ± 0.09*	2.23 ± 0.09*†	2.07 ± 0.11*

Means ± SEM, n = 12–14; *P < 0.05 versus CON, †P < 0.05 versus. TEL or TEL + RAM.

decreased, indicating more a significant reduction in blood pressure than a general retardation of organ growth because the weights of kidneys and adrenal glands remained unchanged (Table 1). In representative 3D-MRT images, the decrease in total fat mass could be visualized particularly in TEL- and TEL + RAM-treated rats (Figure 2A). The visceral fat mass was diminished by TEL, RAM and TEL+RAM (Figure 2B). In contrast, the s.c. fat mass was only reduced equally effectively in TEL- and TEL + RAM-treated rats (Figure 2B). Good correlations between body weights and the s.c. and visceral fat masses were observed only in CD-fed SHR. Due to diminished fat mass and probably to the small number of animals in each group, no correlation could be observed in TEL-, RAM- and TEL + RAM-treated animals (Figure 2B). Initial plasma concentrations of adiponectin (not shown) and leptin (Figure 3) were similar between groups. At the end of the study, plasma adiponectin was enhanced beyond control levels (5.6 ± 0.4 μg·mL^{-1}) after TEL (6.9 ± 0.4 μg·mL^{-1}, P = 0.027) and TEL + RAM (7.2 ± 0.2 μg·mL^{-1}, P = 0.001) but remained unchanged after RAM (4.6 ± 0.2, P > 0.05). Plasma leptin increased over time. This increase was partially prevented for at least 7 weeks by TEL and TEL + RAM. After 9 weeks, plasma leptin in TEL- and TEL + RAM-treated rats exceeded initial values (Figure 3A). The initial energy intake was similar among all groups (Figure 3B). When SHR had the freedom to choose between CD and chow, the total energy intake was enhanced although the intake of chow was concurrently diminished (Figure 3D). The weekly energy intake (related to body weight) decreased over time in controls (r = –0.970, P < 0.0001) and RAM-treated SHR (r = –0.922, P = 0.0004; Figure 3B). When rats were treated with TEL or TEL + RAM, energy intake initially dropped below control levels before they exceeded them after 5 weeks, an effect which persisted until the end of the study (Figure 3B). In parallel with body weight-related values, the absolute energy intake was initially diminished by TEL and TEL + RAM but did not differ from that in controls later on (Figure S1). As such, the total energy intake (related to body weight) was slightly increased compared with control by TEL (9.2%) and TEL + RAM (8.8%), an effect that could mainly be attributed to the increase in chow intake, while CD intake was unchanged (Figure 3D). The energy intake of RAM-treated SHR was indeed increased after offering CD, but it was lower than control during the first 3 weeks (Figure 3B). Thus, the total energy intake was diminished (8.1%) and this must have been due to the reduced CD intake as the intake of chow was concurrently enhanced (Figure 3D). In response to stress, the energy intake and in particular the CD intake was increased in control and RAM-treated rats. In contrast, CD intake was diminished when rats were treated with TEL or TEL + RAM (Figure S2B). Controls and TEL-, RAM- and TEL + RAM-treated rats seemed to be leptin-resistant since plasma leptin positively correlated with food intake (Figure 3C) (Friedman and Halaas, 1998). However, in contrast to control and RAM-treated rats, only a low correlation between leptin and food intake was found in TEL- and TEL + RAM-treated rats. Although an overall correlation between plasma leptin and body weight may be assumed, correlation analysis for each group did not indicate any group-specific correlation between body weight and plasma leptin (Figure 1B). In parallel to food intake, hypothalamic mRNA levels of orexigenic peptides were almost identical between controls and TEL-, RAM- or TEL + TAM-treated rats. Only AgRP-mRNA was increased after TEL. Anorexigenic peptides were unchanged (Table 2). Depending on the circadian rhythm, home cage activity varied in CD-fed SHR; these rats were more active during the dark period than during the light period (Figure 4A). This circadian rhythm remained preserved in the treated groups and total activity of TEL-, RAM- and TEL + RAM-treated animals did not differ from that of control rats (Figure 4B).

Alterations in glucose utilization

Initial plasma concentrations of glucose and insulin were similar in all groups. After 12 weeks, fasting glucose was not affected by RAM but was increased by TEL and TEL + RAM, while fasting insulin was not altered by any treatment regimen (Table 3). The glucagon levels were lowest in control, almost doubled in TEL- and TEL + RAM-treated SHR, and marginally increased in RAM-treated rats (Table 3). None of the drug treatments altered hepatic glycogen.

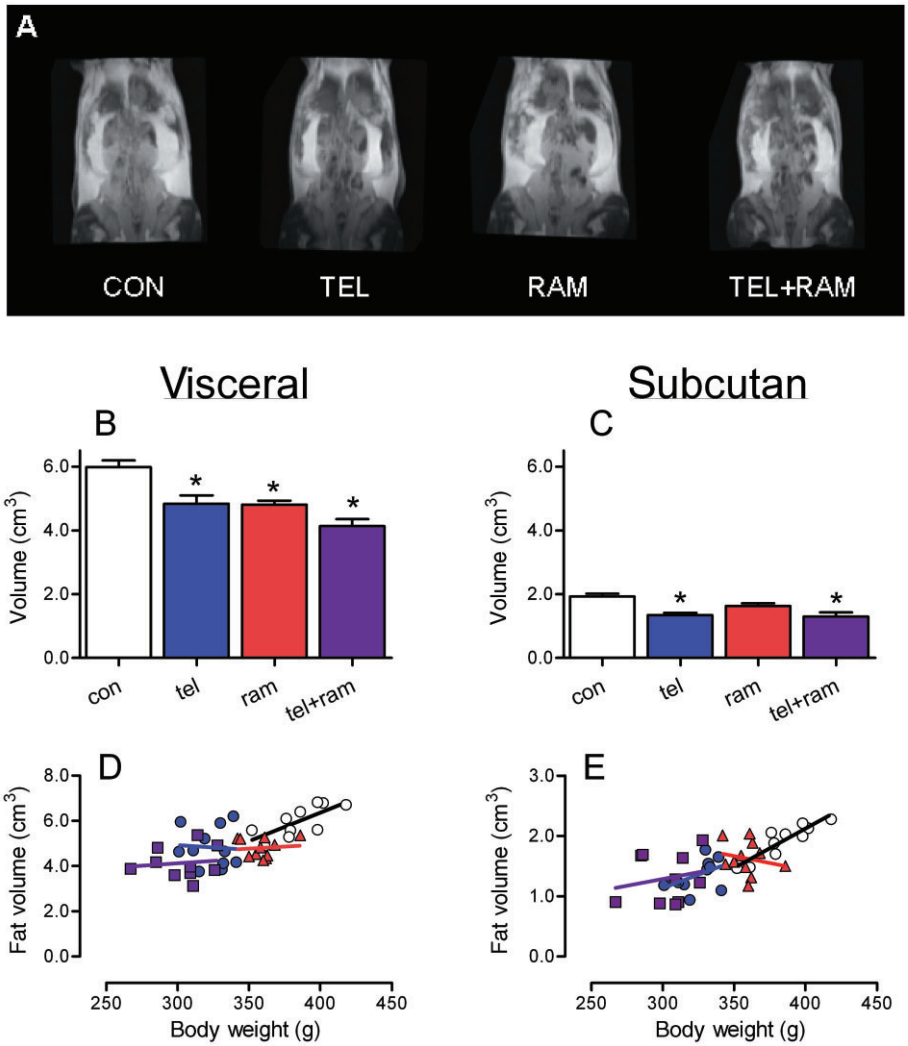

Figure 2

Distribution of the visceral and s.c. fat in CD-fed SHR after treatment with telmisartan (8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (4 mg·kg$_{bw}^{-1}$·day^{-1}), telmisartan + ramipril (8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}) or water.(A) Exemplary 3D images obtained by transverse T1-weighted turbo spin echo MRT. The abundance of visceral (B) and s.c. fat (C) depots was quantified by planimetry (see Methods). Visceral fat (Pearson $r = 0.734$; $P = 0.016$; D) and s.c. fat (Pearson $r = 0.899$; $P < 0.0001$; E) correlated with body weights only in controls (CON), but not in rats treated with TEL, RAM or TEL + RAM. Means ± SEM, $n = 10–11$; *$P < 0.05$ versus controls.

Alterations in glucose metabolism were further identified by performing an OGTT and ITT in fasting rats. Regarding the glucose response towards the OGTT, neither TEL, RAM nor TEL + RAM altered either C_{max} or the AUCs (Figure 5). However, insulin secretion was affected since the AUCs of corresponding plasma insulin time curves were selectively reduced by TEL and TEL + RAM (Figure 5). Accordingly, C_{max} was halved by TEL (2.8 ± 0.4 ng·mL^{-1}, $P < 0.05$) and TEL + RAM (2.7 ± 0.4 ng·mL^{-1}, $P < 0.05$) compared to controls (5.4 ± 0.7 ng·mL^{-1}), but was unaffected by RAM (4.7 ± 0.4 ng·mL^{-1}, $P > 0.05$). Compared to controls, glucose utilization in response to an insulin challenge was found to be faster in TEL and TEL + RAM-treated rats since both the minimal glucose concentrations and the plasma half-life of glucose were diminished by TEL and TEL + RAM (Figure 6), although the circulating levels of insulin did not differ among the groups 15 min after administration (control: 6.6 ± 0.5 ng·mL^{-1}; TEL: 7.3 ± 0.6 ng·mL^{-1}; RAM: 5.7 ± 0.6 ng·mL^{-1}; TEL + RAM: 7.5 ± 0.6 ng·mL^{-1}).

Influence of drug treatment on HPA axis activity

AngII plasma levels of TEL-treated rats were 10-fold higher than in controls, reflecting the effective blockade of AT_1 receptors with TEL. The increase in AngII was attenuated in rats that had been treated with TEL + RAM. Plasma AngII was not altered in RAM-treated rats (Table 3). Aldosterone was reduced in TEL- or TEL + RAM-treated SHR compared with CD-fed SHR that were treated with RAM or water (Table 3). Neither baseline concentrations of the stress hormones ACTH and corticosterone nor adrenal weight differed among the groups at the end of the study (Table 3, Figure S2A). Although

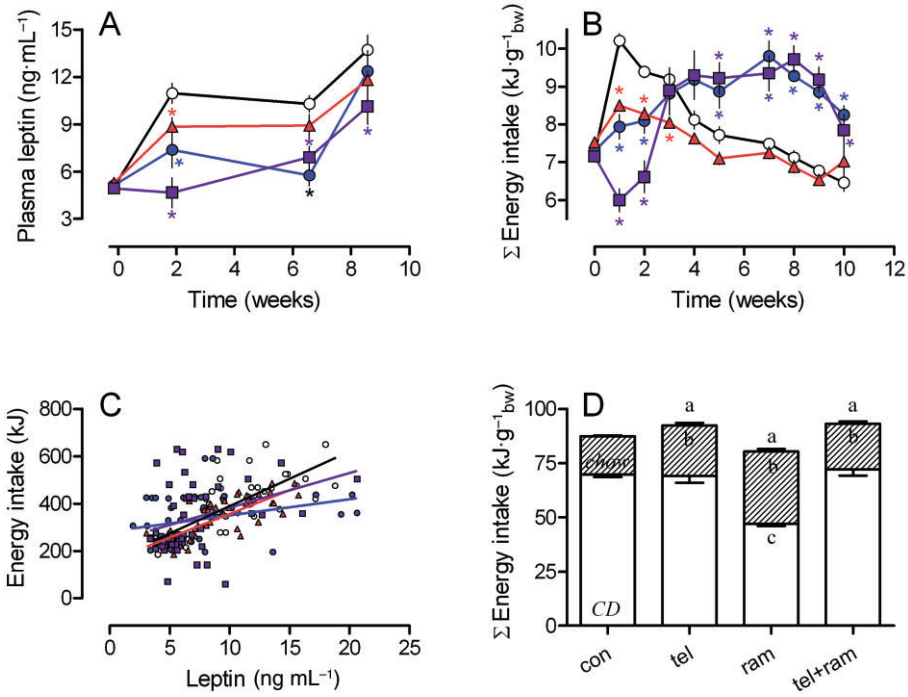

Figure 3

Plasma leptin and energy intake of CD-fed SHR that were treated with telmisartan (8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (4 mg·kg$_{bw}^{-1}$·day^{-1}), telmisartan + ramipril (8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}) or water (controls), respectively. (A) Alterations in plasma leptin within the treatment period. (B) Weekly energy intake related to body weight. (C) Correlation between plasma leptin at day 0, 46 and 60 and the corresponding energy intake (not related to body weight); CON: $r = 0.766$, $P < 0.0001$; TEL: $r = 0.309$, $P = 0.0368$; RAM: $r = 0.795$ $P < 0.0001$, TEL + RAM: $r = 0.330$, $P = 0.029$. (D) Cumulative energy intake related to body weight. The hatched bars represent the proportion fed with chow, and the open bars the proportion fed CD; means ± SEM, $n = 12–14$; *$P < 0.05$ versus control; (a) total energy intake (chow + CD) $P < 0.05$ versus control; (b) chow intake $P < 0.05$ versus control; (c) CD intake $P < 0.05$ versus control. For key to symbols used see legend of Figure 1.

Table 2

Hypothalamic mRNA steady state levels (copies/ng mRNA) of the orexigenic PPO, NPY, MCH, AgRP and the anorexigenic peptides CART and CRH

	CON	TEL	RAM	TEL + RAM
MCH	$4.7 \pm 0.5 \times 10^6$	$4.9 \pm 0.4 \times 10^6$	$4.1 \pm 0.2 \times 10^6$	$3.8 \pm 0.2 \times 10^{6*}$
AgRP	$1.7 \pm 0.2 \times 10^{6*}$	$2.1 \pm 0.2 \times 10^{6*}$	$2.0 \pm 0.2 \times 10^6$	$1.6 \pm 0.1 \times 10^{6*}$
CRH	$9.3 \pm 0.3 \times 10^5$	$9.7 \pm 0.6 \times 10^5$	$8.5 \pm 0.4 \times 10^5$	$8.7 \pm 0.5 \times 10^5$
NPY	$2.1 \pm 0.2 \times 10^{5*}$	$2.4 \pm 0.2 \times 10^{5*}$	$2.2 \pm 0.2 \times 10^{5*}$	$2.2 \pm 0.2 \times 10^{5*}$
PPO	$1.5 \pm 0.1 \times 10^{6*}$	$1.5 \pm 0.1 \times 10^{6*}$	$1.4 \pm 0.1 \times 10^{6*}$	$1.3 \pm 0.1 \times 10^{6*}$
CART	$9.2 \pm 0.9 \times 10^{5*}$	$9.5 \pm 0.7 \times 10^7$	$8.4 \pm 0.5 \times 10^{5*}$	$7.6 \pm 0.5 \times 10^{5*}$

Means ± SEM, $n = 12–14$. *$P < 0.05$ versus CON.

an increase in ACTH was observed 30 min after stress in TEL-, RAM- and TEL + RAM-treated rats, this increase was diminished compared to controls. ACTH returned to initial values in all groups after 90 min (Figure 7). No differences in corticosterone were observed between any of the groups at 30 min, but the corticosterone response was attenuated at 90 min by TEL, RAM and TEL + RAM. In these animals, corticosterone returned to almost the same levels seen before stress (Figure 7). In control rats, plasma glucose was increased 30 min after stress but declined below baseline levels after 90 min, an effect that is assumed to be related to the energy consumed while swimming. The stress-induced increase in glucose was inhibited only in TEL- and TEL + RAM-treated rats (Figure 7).

Influence of drug treatment on blood pressure and heart rate

Compared with initial values of 160 mmHg, SBP increased in controls (28 ± 6 mmHg) during the treatment period to final values of 188 mmHg. Compared with controls, SBP was

Table 3

Endocrine and metabolic parameters of CD-fed SHR after 12 week treatment with water (CON), telmisartan (TEL; 8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (RAM; 4 mg·kg$_{bw}^{-1}$·day^{-1}) or a combination of telmisartan + ramipril (TEL + RAM; 8 + 4 mg·kg$_{bw}^{-1}$·day^{-1})

	CON	TEL	RAM	TEL + RAM
Fasting glucose (mg·L^{-1})	3.33 ± 0.06	4.11 ± 0.11*	3.28 ± 0.06	3.77 ± 0.11*
Fasting insulin (ng·mL^{-1})	1.21 ± 0.11	0.91 ± 0.12	0.89 ± 0.08	0.87 ± 0.07
Plasma glucagon (pg·mL^{-1})	110 ± 6	212 ± 19*	136 ± 6*	185 ± 613*
Hepatic glycogen (mg·g$_{ww}^{-1}$)	32.8 ± 3.3	33.7 ± 2.6	35.4 ± 2.4	34.9 ± 2.9
Plasma AngII (pmol·L^{-1})	22 ± 2	207 ± 15*	33 ± 3	60 ± 5*
Plasma aldosterone (pg·mL^{-1})	125 ± 16	53 ± 6*	127 ± 26	52 ± 10*
Plasma ACTH (pg·mL^{-1})	241 ± 20	185 ± 14	210 ± 22	196 ± 17
Plasma corticosterone (ng·mL^{-1})	103 ± 19	103 ± 14	70 ± 10	86 ± 13

Means ± SEM, n = 12–14; *P < 0.05 versus CON.

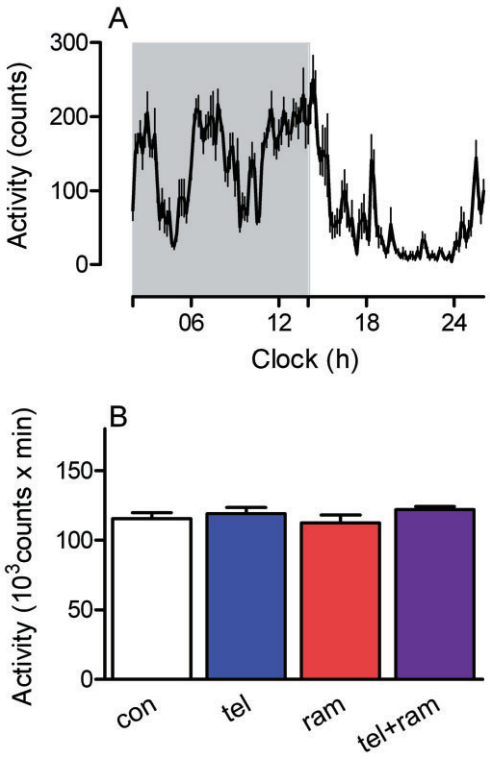

Figure 4

(A) Circadian home cage activity of CD-fed SHR (con) after 11 weeks of feeding. (B) Influence of telmisartan (tel; 8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (ram; 4 mg·kg$_{bw}^{-1}$·day^{-1}) and a combination of the two (tel + ram; 8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}) on the integrative home cage activity within 48 h compared with controls. Means ± SEM, n = 12–14.

reduced by 84 mmHg in TEL-treated and by 85 mmHg in TEL + RAM-treated rats after the 12 week treatment period. After RAM, SBP was reduced to high normal values (142 ± 3 mmHg). HR increased in controls to 445 ± 11 beats min^{-1} but remained unaffected compared with initial values after TEL (414 ± 9 beats·min^{-1}), RAM (404 ± 6 beats·min^{-1}) and TEL + RAM (394 ± 11 beats·min^{-1}).

Discussion

Our study provides several important findings. Firstly, TEL therapy lowered body weight despite higher food intake, higher insulin sensitivity and suppression of ACTH and corticosterone release after stress. After RAM therapy, only a small reduction in BMI was observed even though food intake was diminished; it had no effect on insulin sensitivity but did depress HPA reactivity after stress. The combination TEL + RAM was no more efficacious in improving metabolic parameters and reducing body weight than TEL alone.

Anti-obese effects

Weight gain was slightly reduced after RAM and seems to have been due to a lower intake of CD (especially during the first 3 weeks), whereas the intake of chow was doubled. Others have also found a slight reduction in food intake after chronic blockade of ACE without specifying differences in food ingredients (Russell *et al.*, 2004; Weisinger *et al.*, 2009; Velkoska *et al.*, 2010). After TEL and particularly TEL + RAM, body weight, weight gain and visceral and s.c. fat depots were diminished, confirming previous findings in diet-induced obese rats and mice (Benson *et al.*, 2004; Schupp *et al.*, 2005; Sugimoto *et al.*, 2006; Zanchi *et al.*, 2007). We detected a time-dependent effect of TEL on energy intake, namely, a reduction during the first 2 weeks but an increase thereafter. Only in one study has a temporary reduction in food intake also been observed; this was in chow-fed Wistar Kyoto rats treated with candesartan (Zorad *et al.*, 2006). In all other studies presenting data on food intake, the cumulative food intake remained almost unchanged (Benson *et al.*, 2004; Schupp *et al.*, 2005; Sugimoto *et al.*, 2006; Zanchi *et al.*, 2007; He *et al.*, 2010). The efficacy of TEL in reducing visceral fat mass may be relevant for patients since an increase in visceral fat is related to hypertension, dyslipidaemia and an impaired metabolic pattern but also serves as an independent predictor

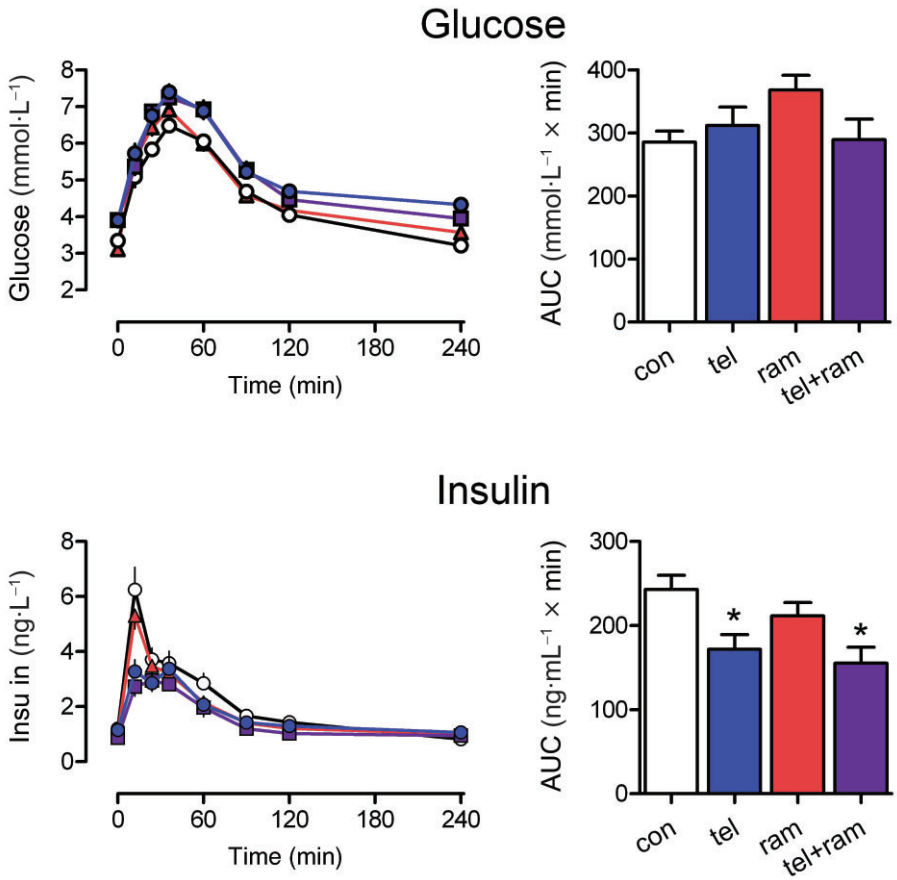

Figure 5

Plasma concentrations of glucose and insulin in response to an oral glucose tolerance test in CD-fed SHR, that were treated with water, telmisartan (8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (4 mg·kg$_{bw}^{-1}$·day^{-1}) or telmisartan + ramipril (8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}). AUCs were calculated for each individual animal on the basis of their Δ values. Means ± SEM, n = 12–14, *P < 0.05 versus controls.

of mortality in men (Kuk *et al.*, 2006). In contrast to our observations, Li *et al.* (2006) reported that 10 mg·kg$_{bw}^{-1}$·d^{-1} TEL for 4 weeks failed to reduce body weight when SHR were fed standard chow, which indicates that AT_1 blockade prevents the intake of a high-calorie diet. Since the body weight of rats decreased after TEL and TEL + RAM treatment, but energy intake was not reduced in parallel, we also tested whether energy expenditure was increased because TEL treatment has been shown to increase caloric expenditure (Araki *et al.*, 2006; Sugimoto *et al.*, 2006). We assessed energy expenditure by measuring locomotor activity. In contrast to our expectations, locomotor activity of TEL-, RAM- or TEL + RAM-treated rats did not differ from that in control rats. Others have also found locomotor activity levels to be unchanged by TEL (Sugimoto *et al.*, 2006), suggesting that simply measuring locomotor activity may not be sufficient for assessing energy expenditure and that the TEL-induced increase in energy expenditure involves more than just a stimulation of energy metabolism secondary to increased physical activity.

In the following, mechanistic aspects regarding weight loss are discussed with respect to potential dependencies on (i) leptin, (ii) HPA activity and (iii) PPARγ, as well as taking into account (iv) other potential mechanisms:

(i): TEL and other ARB failed to reduce the body weight of obese Zucker rats (Kajioka *et al.*, 2007; Madala, *et al.*, 2008; Munoz *et al.*, 2009; Sebekova *et al.*, 2009), which has been suggested to be related to a malfunction in leptin signalling in these animals (Müller-Fielitz *et al.*, 2011). In our experiments, the body weight-related energy intake was time-dependently altered by TEL and TEL + RAM. Energy intake fell below controls during the first 2 weeks but exceeded control levels thereafter until the end of the study. The initial hypophagia after TEL may have been due to intact leptin signalling since plasma leptin increased in these animals, although levels remained below control. Plasma AngII after TEL was considerably increased, which is probably important for the increase in plasma leptin. Indeed, AngII promotes the secretion of leptin from adipocytes, but this AngII action is mediated via AT_1 receptors (Skurk *et al.*, 2005) and AT_1 receptors were blocked by TEL in our study. Similar to AngII, the AngII metabolites AngIII and AngIV also stimulate leptin secretion (Skurk *et al.*, 2005). Whereas AngIII acts via the AT_1-receptor, the AT_4-receptor was found to be a non-AT_1/non-AT_2-binding site for AngIV (Albiston *et al.*, 2001). Since not only AngII but also AngIV is markedly increased in hypertensive patients after AT_1 receptor blockade (Shibasaki *et al.*,

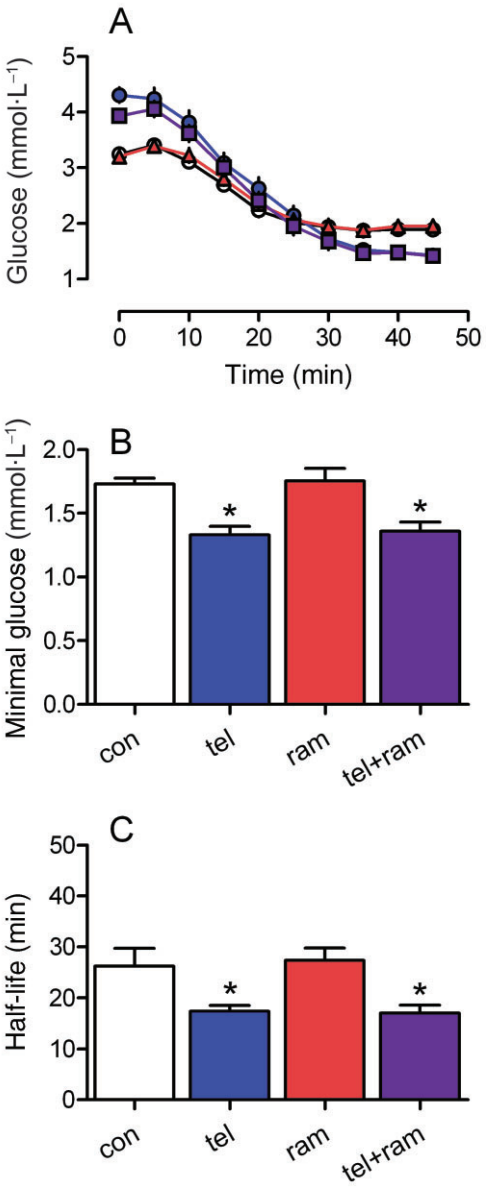

Figure 6

Influence of telmisartan (8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (4 mg·kg$_{bw}^{-1}$·day^{-1}) or telmisartan + ramipril (8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}) on blood glucose in response to an insulin tolerance test (0.5 IU insulin i.p./kg body weight). Controls were treated with water. (A) Glucose development after insulin injection. (B) Minimum glucose values. (C) Half-life was determined by linear regression analysis of log values. Means ± SEM, n = 12–14 *P < 0.05 versus CON.

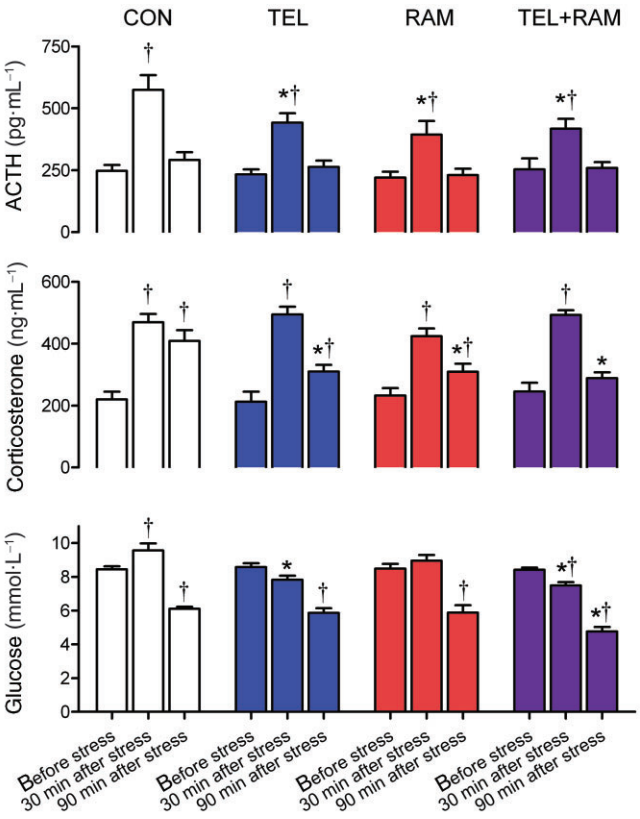

Figure 7

Plasma concentrations of ACTH, corticosterone and glucose in response to a stress test (forced swim test) in CD-fed SHR treated with water, TEL, RAM or TEL + RAM. Means ± SEM, n = 12–14 *P < 0.05 versus CON; †P < 0.05 versus before stress.

1999), and AT_4 receptors are also present in adipocytes (Weiland and Verspohl, 2008), the increase in plasma leptin might be due to AngIV. However, this explanation may not apply for TEL + RAM and needs to be further clarified since plasma leptin did not increase beyond initial values and food intake was in fact lower during the first 2 weeks in the TEL + RAM group compared to TEL alone. The time-dependent increase in food intake after TEL and TEL+RAM may have been due to the development of leptin resistance, which became evident despite their lean phenotype and their reduced plasma levels after 2, 7 and 9 weeks. The likelihood that TEL- and TEL + RAM-treated rats are indeed leptin-resistant is based on our observations that (1) the energy intake correlated with plasma leptin in a positive manner; (2) the cumulative energy intake of these rats was actually increased compared to controls; and (3) the expression of hypothalamic orexigenic peptides after 12 weeks did not differ from controls.

(ii): Glucocorticoids have a major effect on food intake (Dallman *et al.*, 2004). We demonstrated here that stress responses were decreased by TEL, RAM and TEL + RAM; thus, it seems valid to speculate that suppressing HPA activity may help promote weight loss. According to the hypothesis of Dallman *et al.* (2004), food intake is increased to compensate for stress, we observed in particular an increase in CD intake in control rats after stress (Figure S2B). Our findings that CD intake was reduced in TEL- and TEL + RAM-treated animals may support the hypothesis that decreased HPA reactivity induces anti-obese effects. However, food intake was not reduced in response to stress in RAM-treated SHR, even though stress-induced release of ACTH and corticosterone was diminished. Moreover, we observed a reduced food intake, particularly during the first 3 weeks of drug treatment, a time at which corticosterone levels were not diminished

(Figure S2A). These findings suggest that HPA activity is not crucially involved in the constant loss of body weight during the 12 week drug treatment period.

(iii): We demonstrated that adiponectin increased after TEL and TEL + RAM but not after RAM, indicating that PPARγ is activated in response to TEL. However, we can definitely exclude the notion that the reduction in body weight is due to the potential of TEL to stimulate PPARγ. Notably, PPARγ agonists such as thiazolidinedione have typically been demonstrated in rats and humans to increase body weight, food intake, fat quantity and adipocyte size (de Souza *et al.*, 2001; Larsen *et al.*, 2003).

(iv): Since body weight was also decreased by TEL in normotensive Sprague–Dawley rats after chronic treatment with high drug doses (Benson *et al.*, 2004; Thermann *et al.*, 2011), we believe that reducing blood pressure *per se* is not crucial for inducing weight loss after TEL and RAM. Thus, we assume that the low antihypertensive potency of RAM compared with TEL – as seen in this study – is not of great significance, and that differences in body weight between TEL and RAM occur independently of their antihypertensive effects. As a further possible mechanism, others have found that TEL prevents weight gain independently of food intake via an activation of PPARδ-dependent pathways (He *et al.*, 2010). However, we do not have the data to support this idea. Moreover, we cannot exclude the possibility that the reduction in body weight may be related to gastrointestinal side effects that include nausea, vomiting, dyspepsia, abdominal pain, diarrhoea and taste disturbance, as described in humans. However, we did observe that the consistency and colour of faeces were unaltered in drug-treated rats. In addition, based on the observation that locomotion was not affected by drug regimens as well as the finding that food intake increased at least after 3 weeks, it seems rather unlikely that nausea, vomiting, dyspepsia and abdominal pain would play a dominant role in the anti-obese effects particularly of TEL.

Improvement of insulin resistance

We recently demonstrated that rats became insulin resistant when they are fed a high calorie CD (Miesel *et al.*, 2010). While we observed that fasting glucose actually exceeded control levels after TEL and TEL + RAM, and that fasting insulin was similar to controls, others have observed that fasting glucose after TEL is reduced or at least unchanged in diabetic patients and rats. Moreover, lower or unchanged fasting insulin after TEL has also been described (Sugimoto *et al.*, 2006; Derosa *et al.*, 2007; Mori *et al.*, 2007; Shimabukuro *et al.*, 2007; Usui *et al.*, 2007; Younis *et al.*, 2007; Zanchi *et al.*, 2007). We speculate that the increase in plasma glucose in our study was in fact related to glucagon, which was found to be doubled, particularly in the TEL- and TEL + RAM-treated rats. In order to further clarify whether the rats were insulin-sensitive after the drug treatments, glucose and insulin tolerance tests were performed. Consistent with previous findings (Li *et al.*, 2006; Shimabukuro *et al.*, 2007), glucose responses to glucose challenges were not altered by TEL or TEL + RAM. In contrast, other studies have found that TEL induces a decrease in glucose levels during an OGTT (Schupp *et al.*, 2005; Vitale *et al.*, 2005; Nagel *et al.*, 2006; Zanchi *et al.*, 2007; Sanchez *et al.*, 2008; Rong *et al.*, 2009). Neither the TEL doses used (5 vs. 8 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$) nor the treatment duration (4 vs. 6 weeks) can account for the differences between these results and those of our study, but rat strain (diet-induced SHR vs. obese Zucker rat) probably does. TEL has been shown to increase insulin secretion after a glucose challenge in several studies and it was concluded that beta-cell function had improved (Nagel *et al.*, 2006; Zanchi *et al.*, 2007). In contrast, we showed that insulin was decreased by TEL and TEL + RAM, confirming, on the one hand, observations in patients with metabolic syndrome (Shimabukuro *et al.*, 2007) and indicating, on the other hand, that insulin sensitivity rather than insulin secretion is increased. The conclusion that glucose utilization in the insulin tolerance test is improved after TEL is further strengthened by our findings and those of others (Schupp *et al.*, 2005; Shimabukuro *et al.*, 2007; Rong *et al.*, 2009). In this regard, irbesartan has been shown to improve insulin signalling in obese rats via the insulin receptor/insulin receptor substrate-1/phosphatidylinositol 3 kinase/Akt pathway (Munoz *et al.*, 2009). Conflicting data have been published regarding the question of whether RAM improves insulin sensitivity (Ko *et al.*, 2004; Russell *et al.*, 2004) or not (Ludvik *et al.*, 1991; Sanchez *et al.*, 2008), and our results tend to support the negative findings since neither glucose nor insulin were improved in response to OGTT or the insulin tolerance test.

The PPARγ agonistic action of TEL may account for it being superior to RAM as regards beneficial effects on insulin sensitivity (Benson *et al.*, 2004; Schupp *et al.*, 2004; 2005). In many studies (including the present one), an increase in adiponectin served as a positive surrogate parameter of TEL for PPARγ activation (Clasen *et al.*, 2005; Derosa *et al.*, 2007; Mori *et al.*, 2007; Shimabukuro *et al.*, 2007; Younis *et al.*, 2010). However, TEL has also been found to have no effect on adiponectin levels in rats and patients, even though it reduced the body weight and increased insulin sensitivity in the individuals studied in these trials (Benndorf *et al.*, 2006; Nagel *et al.*, 2006; Usui *et al.*, 2007; Kamari *et al.*, 2008). Thus, TEL may regulate glucose homeostasis by mechanisms that are independent of PPARγ activation.

As a possible PPARγ-independent mechanism, we investigated HPA axis activity after AT_1 blockade. HPA hyperreactivity has been verified in rats and patients with diabetes (Cameron *et al.*, 1984; Jöhren *et al.*, 2007) and AT_1 receptors identified as regulators of stress reactions (Aguilera *et al.*, 1995). Moreover, the AngII-stimulated hyper-reactivity in the HPA axis was found to account for the reduction in glucose utilization in obese Zucker rats (Müller *et al.*, 2007), revealing a functionally relevant crosstalk between AngII, the HPA axis and metabolic functions. Here we demonstrated, in rats with a diet-induced metabolic syndrome, that baseline concentrations of stress hormones and weights of adrenal glands were unchanged in response to any drug treatment. In all groups, we observed a stress-induced increase in ACTH and corticosterone after 30 min. However, the early ACTH response was diminished by all drug regimens. In agreement with the literature (Müller *et al.*, 2007; 2010), we demonstrated in controls that corticosterone remained elevated for 90 min, although ACTH had already returned to baseline levels. In contrast to controls, the corticosterone response was diminished by TEL or RAM or even normalized by TEL + RAM after

90 min. This confirms our findings, showing that HPA reactivity was reduced after both AT_1 blockade and after ACE inhibition in hypertension (Raasch *et al.*, 2006). The simultaneous decrease in ACTH indicates a pituitary mechanism, which is in line with previous reports describing the facilitating actions of AngII on ACTH release in response to corticosterone releasing hormone in pituitary cells of different animals (Abou-Samra *et al.*, 1986; Keller-Wood *et al.*, 1986). The proposed TEL action requires that peripherally administered TEL penetrates the blood–brain barrier. This was verified by measuring TEL concentrations in the cerebrospinal fluid and indirectly by the ability of TEL, administered i.v. or p.o., to antagonize the central effects of AngII (Gohlke *et al.*, 2001). In addition to the proposed involvement of the pituitary glands, we previously demonstrated that the adrenals of obese rats were sensitized to AngII, since the stimulation of corticosterone occurred in an ACTH-independent manner and adrenal AT_{1A} mRNA was concurrently up-regulated (Müller *et al.*, 2007). Although adrenal AT_{1A} mRNA levels were found to be unchanged in SHR treated, chronically, with candesartan (Raasch *et al.*, 2006), we cannot exclude with certainty the possible involvement of an adrenal mechanism in the reduced corticosterone response after RAAS inhibitors in this study: firstly, the dose of candesartan was lower than that of TEL used in the present study and, secondly, the negative findings regarding adrenal AT_1 receptor expression after candesartan derive from chow and not CD-fed SHR. In the control group in our study, hyperglycaemia developed 30 min after stress, which could be prevented only by TEL and TEL+RAM, but not by RAM alone. We therefore suppose that the ability of TEL, as an AT_1 receptor blocker, to alleviate stress reactions may contribute to its hypoglycaemic actions. This assumption would be in line with our previous findings and those of others showing a decrease in both HPA activity and in blood glucose after stress (Uresin *et al.*, 2004; Raasch *et al.*, 2006; Pavlatou *et al.*, 2008). However, if a functional link between depression of the HPA axis and improvement in glucose utilization exists, it remains unclear as to why RAM did not affect glucose under stress, although an increase in stress hormones was attenuated.

It is also possible that TEL-induced PPARδ activation (He *et al.*, 2010) participates in the improved glucose utilization seen in our experiments since Ye *et al.* (2011) recently reported that PPARδ agonists improve glucose tolerance in high-fat (HF) fed mice. However, an exacerbated insulin resistance was observed when HF fed rats were treated with PPARδ agonists, which was assumed to be related to different effects on lipid metabolism and insulin sensitivity in these two species.

In summary, using a rat model of the human metabolic syndrome we demonstrated that the anti-obese and metabolic potency of TEL exceeded that of RAM. The combined blockade of ACE and AT_1-receptors did not further increase the efficacy of TEL regarding weight gain and glucose metabolism. Although there is accumulating evidence for weight loss during therapy with AT_1 receptor blockers or ACEI, the limitation of these findings is that they were gathered mainly in rodents. Published data showing anti-obese effects in patients are scarce. Thus, more studies should be performed in patients with metabolic syndrome to elucidate the effects of AT_1 receptor blockers on body weight and to investigate the mechanisms found in experimental studies.

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Conflicts of interest

WR received grant support from Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT).

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Supporting information

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

Figure S1 Absolute weekly energy intake of SHR. SHR were allowed to freely choose between a cafeteria diet (various commercial chocolate and cookie bars which were daily changed) and a standard chow. Rats were simultaneously treated for 12 weeks with telmisartan (8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (4 mg·kg$_{bw}^{-1}$·day^{-1}), telmisartan + ramipril (8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}) or water (controls) respectively. Means ± SEM, n = 12–14; *P < 0.05 versus CON.

Figure S2 (A) Baseline plasma concentrations of corticosterone did not differ during the treatment time of 12 weeks in rats that were treated with telmisartan (8 mg·kg$_{bw}^{-1}$·day^{-1}), ramipril (4 mg·kg$_{bw}^{-1}$·day^{-1}), telmisartan + ramipril (8 + 4 mg·kg$_{bw}^{-1}$·day^{-1}) or water (controls). (B) Energy intake of rats one day before and 1 day after the stress test. The open bars represent the intake of chow, and the grey shaded bars represent the CD intake. Means ± SEM, n = 12–14; *P < 0.05 versus. before stress.

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