British Journal of Pharmacology

DOI:10.1111/bph.12949
www.brjpharmacol.org

RESEARCH PAPER

Preventing leptin resistance by blocking angiotensin II AT_1 receptors in diet-induced obese rats

Helge Müller-Fielitz[1,2], Margot Lau[1], Cathleen Geißler[1], Lars Werner[1], Martina Winkler[1] and Walter Raasch[1,2]

[1]*Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Lübeck, Germany, and* [2]*DZHK (German Centre for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck, Lübeck, Germany*

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

Parts of the work presented in the article were presented at the 52nd Annual Meeting of the German Society of Experimental and Clinical Pharmacology and Toxicology (DGPT) in Frankfurt 2011 (Naunyn-Schmiedebergs Archives of Pharmacology, 383, Supp.1, 33).

Received
27 June 2014
Revised
3 September 2014
Accepted
18 September 2014

BACKGROUND AND PURPOSE

AT_1 receptor blockers (ARBs) represent an approach for treating metabolic syndrome due to their potency in reducing hypertension, body weight and onset of type 2 diabetes. The mechanism underlying ARB-induced weight loss is still unclear.

EXPERIMENTAL APPROACH

Leptin resistance tests (LRTs) in diet-induced obese or lean rats were conducted to determine whether telmisartan (8 $mg \cdot kg^{-1} \cdot day^{-1}$, 14 days) enhances leptin sensitivity. Phosphorylation of signal transducer and activator of transcription 3 (pSTAT3) staining was performed in hypothalami to determine leptin transport across the blood–brain barrier.

KEY RESULTS

Telmisartin reduced weight gain, food intake and plasma leptin but blood pressure remained unchanged. The 24 h profiles of plasma leptin after saline injections were similar in controls and telmisartan-treated rats, but after leptin injections were higher in controls and slightly lower in telmisartan-treated animals. After telmisartan, energy intake during LRT was lower in leptin- than in saline-pretreated rats, but remained unchanged in controls, irrespectively of whether rats received saline or leptin. Leptin minimized the gain in body weight during LRT in telmisartan-treated rats as compared with saline-treated animals. pSTAT3 staining was reduced in cafeteria diet-fed rats as compared with chow-fed rats but this was normalized by telmisartan. Telmisartin reduced hypothalamic mRNA levels of the orexigenic peptides melanin-concentrating hormone and prepro-orexin.

CONCLUSIONS AND IMPLICATIONS

Rats fed a cafeteria diet develop leptin resistance after 2 weeks. Leptin sensitivity was preserved by telmisartan treatment even in rats fed a cafeteria diet. This pleiotropic effect is not related to the hypotensive action of telmisartan.

Abbreviations

AC, arcuate nucleus; AgRP, agouti-related protein; AngII, angiotensin II; ARB, AT_1 receptor blocker; AT_1 receptor, angiotensin II type 1 receptor; BBB, blood–brain barrier; CART, cocaine- and amphetamine-regulated transcript; CD, cafeteria diet; C_{max}, maximal concentration; LHA, lateral hypothalamus; LRT, leptin resistance test; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; OB-Rb receptor, the long form of the leptin receptor; PFA, perifornical area; POMC, pro-opiomelanocortin; PPO, prepro-orexin; pSTAT3, phosphorylation of signal transducer and activator of transcription 3; SD rat, Sprague Dawley rat; TG, triglycerides; UCP-1, uncoupling protein 1

Tables of Links

TARGETS
GPCRs[a]
AT_1 receptor
Catalytic receptors[b]
Leptin receptor
Transporters[c]
Uncoupling protein 1

LIGANDS	
Agouti-related protein	Melanin-concentrating hormone
Angiotensin II	Neuropeptide Y
Bestatin	Orexin
Corticosterone	Telmisartan
Leptin	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c]Alexander *et al.*, 2013a,b,c).

Angiotensin II type 1 (AT_1) receptor blockers (ARBs) are well established in the therapy of high blood pressure and congestive heart failure. Moreover, ARBs have been demonstrated to reduce the incidence of type 2 diabetes (Scheen, 2004). In the past few years, evidence has accumulated that ARBs might also help reduce obesity. Prevention of weight gain was demonstrated in numerous experimental studies when ARB treatment was concurrently initiated with high-calorie feeding (Benson *et al.*, 2004; Schupp *et al.*, 2005; Sugimoto *et al.*, 2006; Zorad *et al.*, 2006; Zanchi *et al.*, 2007; He *et al.*, 2010; Müller-Fielitz *et al.*, 2011; 2012; Miesel *et al.*, 2012). Moreover, AT_1 blockade even promoted weight loss in animals with fully diet-induced obesity when ARBs were administered in a treatment setting (Souza-Mello *et al.*, 2010; Müller-Fielitz *et al.*, 2014). Such observations suggest that the treatment may be clinically relevant. Although many clinical trials have been performed on ARBs, only few have addressed their potencies in reducing obesity. Moreover, these trials were rather small and heterogeneous and differed in outcome (no effect or a decrease or increase in body weight) as well as in the type of ARB and dosage used (Lau and Raasch, 2012). However, in one prospective study including 14 200 patients, waist circumference was found to be reduced by the ARB irbesartan (Kintscher *et al.*, 2007).

We recently demonstrated that loss of body weight is not a general feature of AT_1 blockers as it was not observed after normal or moderately supranormal doses, but only after treatment with the highest doses (Müller-Fielitz *et al.*, 2011). The need for high drug doses to induce anti-obese effects may indicate that the underlying mechanism is CNS driven as the lipophilicity of ARBs is low, which limits blood–brain barrier (BBB) penetration (Michel *et al.*, 2013). Considering this aspect, we could claim that anti-obese effects have been observed in particular in studies in which telmisartan has been used (Benson *et al.*, 2004; Schupp *et al.*, 2005; Sugimoto *et al.*, 2006; Zorad *et al.*, 2006; Zanchi *et al.*, 2007; He *et al.*, 2010; Müller-Fielitz *et al.*, 2011; 2012; Miesel *et al.*, 2012) as telmisartan is more lipophilic than other ARBs (Michel *et al.*, 2013).

Results are also conflicting whether the ARB-induced loss in body weight is paralleled by a reduction in food intake (Zorad *et al.*, 2006; Müller-Fielitz *et al.*, 2011; 2012; Miesel *et al.*, 2012) or not (Benson *et al.*, 2004; Schupp *et al.*, 2005; Sugimoto *et al.*, 2006; Zanchi *et al.*, 2007; He *et al.*, 2010). Reports showing no effects are mainly based on findings merely demonstrating cumulative food intake over the entire observation time or final food intake at the end of the treatment period. In contrast, when food intake of rats was determined in detail, it was obvious that the energy intake fell below that of controls during the first weeks of ARB treatment, but gained or even exceeded control levels thereafter until the end of the study (Zorad *et al.*, 2006; Müller-Fielitz *et al.*, 2011; 2012; Miesel *et al.*, 2012). This time dependency of ARB treatment on food intake raises questions regarding the underlying mechanism. Because anti-obese effects of ARBs occur mainly after high dosing, an extensive drop in blood pressure may cause hypophagia. However, we and others have demonstrated that an ARB-induced loss of body weight is independent of the hypotensive potencies of the drugs (Zorad *et al.*, 2006; Müller-Fielitz *et al.*, 2012; 2014). Thus, we performed the current study using normotensive Sprague Dawley (SD) rats to avoid taking into account the possible effect of blood pressure-dependent mechanisms on outcome.

We also demonstrated that ARB-induced hypophagia is leptin dependent as a reduction in both food intake and in body weight was only observed in lean but not in obese Zucker rats with the long form of the leptin receptor (OB-Rb) deficiency (Müller-Fielitz *et al.*, 2011). Leptin is produced by white adipose tissue and has a key function in regulating energy intake and energy expenditure (Schwartz *et al.*, 2000). Circulating leptin enters the CNS in proportion to its plasma levels before it stimulates appropriate receptors that are expressed in those brain neurons involved in the complex network controlling energy intake. The arcuate nucleus (AC) secretes orexigenic substances such as neuropeptide Y (NPY) and agouti-related peptide (AgRP) as well as anorexigenic peptides such as pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). Downstream of the AC, the anorexigenic peptide corticotropin-releasing hormone is produced in the paraventricular nucleus (PVN) and the orexigenic substances orexins and melanin-

concentrating hormone (MCH) are released from the lateral hypothalamus (LHA) and perifornical area (PFA) (Schwartz *et al.*, 2000). In this context, we have demonstrated that MCH and prepro-orexin (PPO) expression was suppressed after AT_1 receptor blockade (Zorad *et al.*, 2006; Müller-Fielitz *et al.*, 2011). In line with these results, telmisartan has also been found to suppress food intake in mice via the melanocortin pathway (Noma *et al.*, 2011). Because food intake was suppressed by ARBs in particular during the first 3 weeks of drug treatment (Miesel *et al.*, 2012; Müller-Fielitz *et al.*, 2012), in the present study we aimed to investigate whether leptin sensitivity is improved in animals after a short-term treatment using a high dose of telmisartan. This assumption is based on our observation that both plasma leptin and energy intake are high in rats after a 2 week feeding period with cafeteria diet (CD), which indicates leptin resistance. In contrast, plasma leptin and energy intake were low in telmisartan-pretreated rats (Müller-Fielitz *et al.*, 2012) (Supporting Information Fig. S1). Here, we mainly investigated leptin sensitivity, taking a functional approach by measuring food intake and body weight after leptin exposure. Following binding to the OB-Rb receptor, leptin's anorexigenic action is mediated by different pathways involving activation of the so-called JAK2/STAT3 pathway (Morris and Rui, 2009). Thus, we additionally performed immunohistochemical staining experiments against phosphorylation of signal transducer and activator of transcription 3 (pSTAT3) to attribute function to signalling.

Methods

Animals

SD rats were obtained from Charles River (Sulzfeld, Germany). The total number of rats used in this study was 109. The study was conducted according to the National Institutes of Health guidelines for the care and use of laboratory animals. All animal care and experimental procedures were carried out with the ethical approval of the local regulatory authority (Ministerium für Energiewende, Landwirtschat, Umwelt und ländliche Räume des Bundeslandes Schleswig-Holstein). The animals were housed individually in cages (height × width × length: 200 × 220 × 250 mm) kept at room temperature with a 12/12 h dark (1400–0200 h)/light (0200–1400 h) cycle and received water *ad libitum*. All animal care and experimental procedures were in accordance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

In protocols 2–5, rats could choose freely between standard chow and a CD, which were both abundantly offered. The CD comprised six 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. Rats received only one kind of chocolate or cookie bar per day, these being switched daily in a regular manner (Miesel *et al.*, 2010). This kind of feeding is termed CD feeding in our study. 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 metabolic energy from the standard chow is 65% carbohydrates, 24% protein and 11% fat. The intake of chow and chocolate/cookie bars was monitored daily. Rats were habituated to research assistants and *vice versa* 2 weeks before drug treatment was initiated.

Study protocols

Protocol 1: Rats (approximately 150 g) only received standard chow. After 2 weeks, a leptin resistance test (LRT) was performed. Because others have demonstrated that, on suppressing food intake, leptin has a greater potency when administered by osmotic minipumps or by repeated s.c. infusions (Pelleymounter *et al.*, 1995; Halaas *et al.*, 1997; Nishiyama *et al.*, 1999; McAlister and Van Vugt, 2004; Wetzler *et al.*, 2004), we injected leptin (R&D Systems, Inc., Minneapolis, MN, USA, n = 10) at 0800, 1100, 1400 and 1700 h (each time 100 $\mu g \cdot kg^{-1}$ s.c.) and at 2000 h (200 $\mu g \cdot kg^{-1}$s.c.). The next day, rats were treated with leptin again at 0800 h (100 $\mu g \cdot kg^{-1}$ s.c.), 1100 h (100 $\mu g \cdot kg^{-1}$ s.c.) and 1400 h (200 $\mu g \cdot kg^{-1}$ s.c.). Controls received saline (n = 10). Blood for leptin measurements was drawn from a tail nick at 0800, 1100, 1400, 1700 and 2000 h, as well as at 0800 h on the second day. Body weight and food intake were determined.

Protocol 2: From day 0 until the end of the study, rats were allowed to choose freely between a CD and a standard diet, both were abundantly offered. In parallel with the CD, rats (n = 20) were treated once daily for 19 days by gavage with telmisartan (8 $mg \cdot kg^{-1}$body $wt \cdot day^{-1}$; Boehringer Ingelheim Pharmaceuticals, Inc., Ingelheim, Germany). Controls (n = 20) were given an identical volume of water (1 $\mu L \cdot g^{-1}$ body wt) (Miesel *et al.*, 2012; Müller-Fielitz *et al.*, 2012). Blood samples (200 µL) were taken to measure leptin levels before treatment and after 4 and 14 days. To determine leptin sensitivity, telmisartan-treated rats and controls received either leptin or saline (n = 10 each group) according to protocol 1 at days 17 and 18. At day 19, rats were additionally treated with leptin or saline at 0800 h (100 $\mu g \cdot kg^{-1}$ s.c.), 1100 h (100 $\mu g \cdot kg^{-1}$ s.c.) and 1400 h (200 $\mu g \cdot kg^{-1}$ s.c.). Within the first day of the leptin sensitivity test, blood samples were taken for measuring leptin plasma levels. Energy intake and body weight were determined in the animals and periodically monitored. Rats were killed at 1500 h and brown adipose tissue was removed to detect uncoupling protein 1 (UCP-1). To determine angiotensin II (AngII) levels, blood (1 mL) was collected in an inhibitor solution containing 12.1 mM EDTA and 20 µM bestatin (final concentration).

Protocol 3: According to protocol 2, CD-fed rats (n = 10 each group) were treated either once day with telmisartan (8 $mg \cdot kg^{-1}{}_{BW} \cdot day^{-1}$) or water (1 $\mu L \cdot g^{-1}{}_{BW}$) and received leptin at days 17–19. In contrast to protocol 2, neither leptin levels, energy intake nor body weight were monitored during leptin exposure. At day 19 rats were killed at 1500 h, and hypothalami were removed to determine (an-orexigenic peptide levels.

Protocol 4: According to protocol 2, CD-fed rats (n = 5) were treated once daily for 19 days by gavage with telmisartan (8 $mg \cdot kg^{-1}{}_{BW} \cdot day^{-1}$). Blood pressure and heart rate were determined by plethysmography before telmisartan treatment and at day 14 (Raasch *et al.*, 2002).

Protocol 5: CD-fed rats were treated with telmisartan (8 $mg \cdot kg^{-1}{}_{BW} \cdot day^{-1}$) or with vehicle ($n$ = 8 each group). An

additional group only received chow and was administered vehicle by gavage ($n = 8$). Blood samples were taken at day 15 from animals that had been deprived of food for 16 h. Then, all rats received one s.c. leptin injection (100 mg·kg^{-1}; R&D Systems, Inc.). Animals were killed 25 min later and transcardially perfused with PBS and 4% paraformaldehyde (PFA). Brains were removed for immunohistochemical analysis of pSTAT3.

Histology

For pSTAT3 staining, brains were immediately incubated after preparation in 4% PFA for 24 h and then in 30% sucrose solution for 7 days. For immunohistochemistry, serial coronal cryosections of the brain (20 µm in thickness) were fixed on Polysin slides (Thermo Scientific, Braunschweig, Germany). After being washed (PBS, three times) and incubated (twice) in methanol (–20°C), slides were permeabilized (i) by 0.3% glycerine (Roth, Karlsruhe, Germany) in PBS for 10 min and (ii) with 0.03% SDS (Roth). Both permeabilization steps were followed by the three times washing step with PBS. After the sections had been blocked with 3% BSA [in PBS Tween 20 (0.4%); Sigma, St. Louis, MO, USA] for 2 h at room temperature, the rabbit anti-pSTAT3 (signal transducer and activator of transcription) (Tyr^{705}) (D3A7)XP® antibody (1:500; A-9145, Lot 17, Cell Signaling, Danvers, MA, USA) was added for 48 h. The sections were washed (three times, 5 min) with PBS and incubated in darkness for 2 h at room temperature in anti-rabbit Alexa 488 (1/2000, A-11034, Lot 464519, Invitrogen, Carlsbad, Germany) to label pSTAT3 antibodies. Slides were co-labelled with DAPI (1/2000, Invitrogen). Sections were mounted with Mowiol (Roth) after they had been washed (three times) with PBS. Fluorescence signals were detected using the fluorescence microscope Leica DMI 6000B (ex. 488 nm, em. 519 nm). Representative pictures were taken with a digital colour camera. Pictures were analysed using Fiji (ImageJ 1.47q, Wayne Rasband National Institutes of Health, Bethesda, MD, USA). We counted the cells on 29–30 sections from animals on which the AC was seen. The investigator was blinded to the experimental group (Plum *et al.*, 2006).

Biochemical and molecular analysis

Plasma concentrations of AngII (IBL, Hamburg, Germany), leptin (Linco, Seaford, DE, USA) and corticosterone (MP Biomedicals, Eschwege, Germany) were determined by RIAs using commercial kits (Miesel *et al.*, 2010; 2012; Müller-Fielitz *et al.*, 2011; 2012). Hypothalamic mRNA levels of (an)orexigenic peptides and mRNA levels were measured as previously described (Miesel *et al.*, 2010). Triglycerides (TGs) were quantified in plasma using a Roche/Hitachi Modular P Chemistry Analyzer (Mannheim, Germany). UCP-1 protein levels were quantified in brown adipose tissue by immunoblot analysis using a specific antibody for UCP-1 (Santa Cruz Biotechnology, Heidelberg, Germany) (Miesel *et al.*, 2010).

Statistics

In order to quantify the total effect of changes in plasma concentrations of leptin in response to the leptin injections over the observation period, the AUCs were calculated for each individual animal. Data shown are expressed as means ± SEM. Statistical analysis was performed by one- or two-way ANOVA, followed by appropriate *post hoc* tests (Bonferroni's multiple comparison test). Correlation coefficients (two-tailed *P*-values) were computed according to Pearson, assuming a Gaussian distribution, using GraphPad Prism version 4 (GraphPad Software, Inc., La Jolla, CA, USA). Differences were considered to be statistically significant at an error level of $P < 0.05$. If Gaussian distribution was missed, the Wilcoxon signed-rank test was performed. When only two groups were being compared, a Student's *t*-test was conducted (protocol 4).

Results

Haemodynamics

SD rats were normotensive and neither systolic blood pressure nor heart rate was altered by treating rats with 8 mg·kg^{-1}·day^{-1} telmisartan for 14 days (protocol 4; Figure 1A and B). Others have also demonstrated that blood pressure of normotensive rats remained unchanged even after adminis-

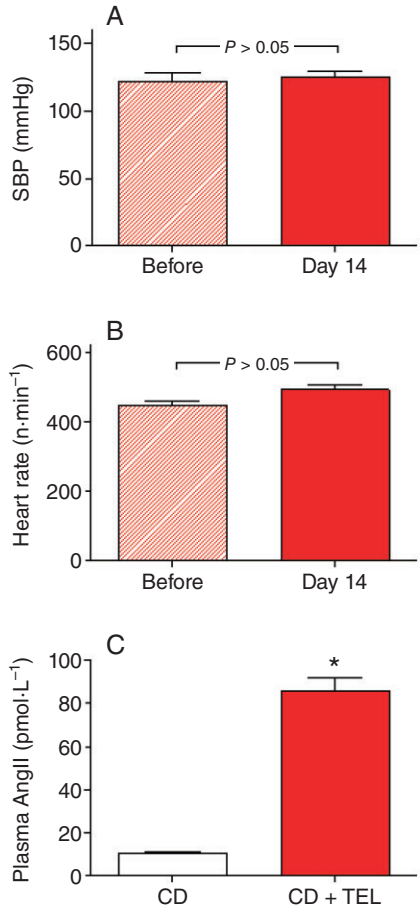

Figure 1

Influence of telmisartan treatment (8 mg·kg^{-1}·day^{-1}) on blood pressure (A) and heart rate (B) of normotensive CD-fed SD rats. Blood pressure and heart rate were determined before and at day 14 of treatment (means ± SEM, $n = 5$). Panel C depicts plasma AngII of CD-fed SD rats that were treated with telmisartan (TEL) or with vehicle. Means + SEM, $n = 26$, *$P < 0.05$ versus vehicle.

tering 8 mg·kg^{-1}·day^{-1} (Villa *et al.*, 2011). Plasma AngII increased after AT_1 receptor blockade (protocol 2), confirming the efficacy of the telmisartan treatment (Figure 1C).

Body weight and energy intake

When rats of protocol 2 were fed with CD, body weight increased in controls by more than 100 g within 14 days. The gain in body weight was diminished by telmisartan, becoming significant after 10 days of treatment (Figure 2A). Immediately after starting CD feeding, the energy intake increased in controls and telmisartan-treated rats, declining within the course of 2 weeks. From day 4 until the end of the study, telmisartan diminished the energy intake (Figure 2B). Thus, the total energy intake of telmisartan-treated animals was lower than that of controls, which can mainly be attributed to a reduced intake of CD. The intake of chow was even enhanced after telmisartan (Figure 2C). We observed a strong correlation between the gain in body weight and energy intake. The regression curve of the telmisartan-treated rats shifted leftwards compared with that representing the controls (Figure 2D). Because of CD feeding, plasma leptin increased time dependently by a factor of 4, but was attenuated by telmisartan (Figure 2E). A positive correlation between body weight and plasma leptin was observed in controls but not in telmisartan-treated rats (Figure 2F). Accordingly, plasma leptin was markedly increased by CD feeding but normalized to lean control levels by telmisartan in protocol 5 (Figure 3F). UCP-1 expression in brown adipose tissue, a surrogate parameter for energy expenditure, was similar in all groups (Supporting Information Fig. S2). In protocols 3 and 5, we were able to corroborate the potency of telmisartan to diminish food intake and body weight within a time period of 2 weeks (Table 1; Figure 3D and E). Furthermore, in protocol 4 we demonstrated reduced plasma levels of TG and corticosterone in telmisartan-treated rats (Table 1).

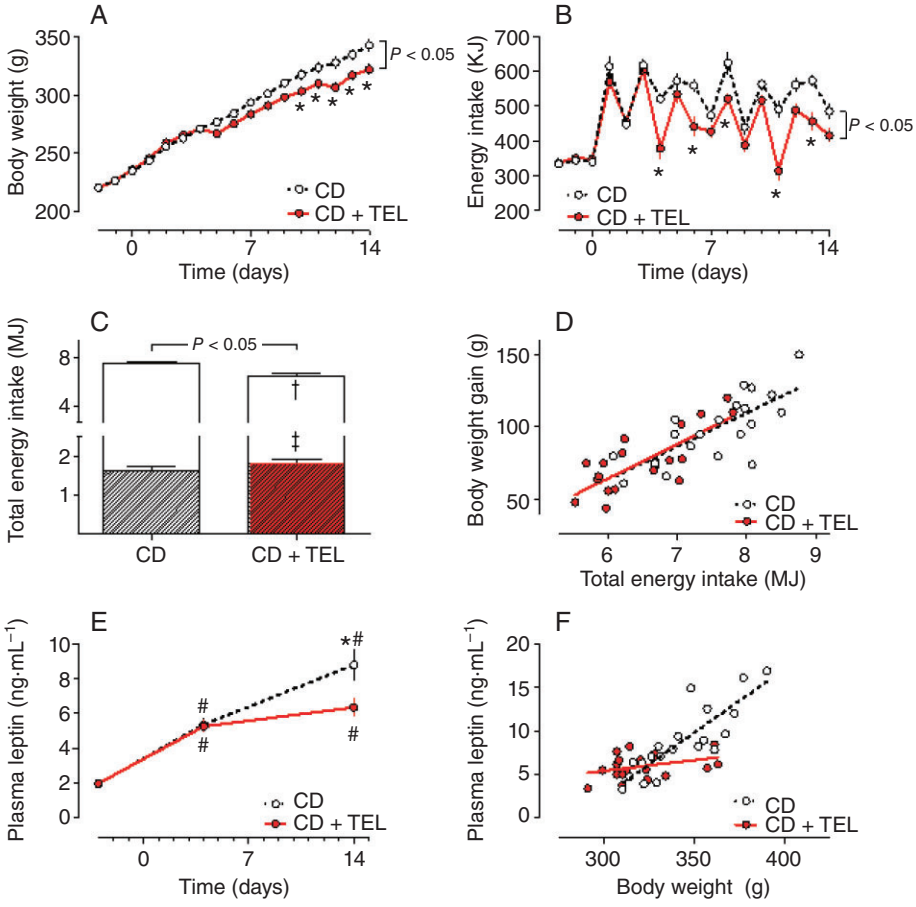

Figure 2

Influence of telmisartan (8 mg·kg^{-1}·day^{-1}) on body weight (A), energy intake (B) and plasma leptin (E). Controls were treated with water. Panel C depicts the total energy intake of telmisartan (TEL)- or vehicle-treated animals. The hatched bars indicate the intake of chow and the open bars the CD intake. Panel D shows the total energy intake positively correlated with gain in body weight in telmisartan- ($r = 0.769$, $P < 0.0001$) and vehicle-treated rats ($r = 0.742$, $P = 0.0002$). Panel F illustrates that body weight positively correlates with plasma leptin in CD-fed controls ($r = 0.847$, $P < 0.0001$, slope 0.146 ± 0.021) but not in telmisartan-treated rats ($r = 0.356$, $P = 0.134$, slope 0.025 ± 0.016). Means ± or + SEM, $n = 19$–20, *$P < 0.05$ versus vehicle; †$P < 0.05$ intake of CD of telmisartan versus that of CON; ‡$P < 0.05$ intake of chow of telmisartan versus that of CON; #$P < 0.05$ versus before treatment.

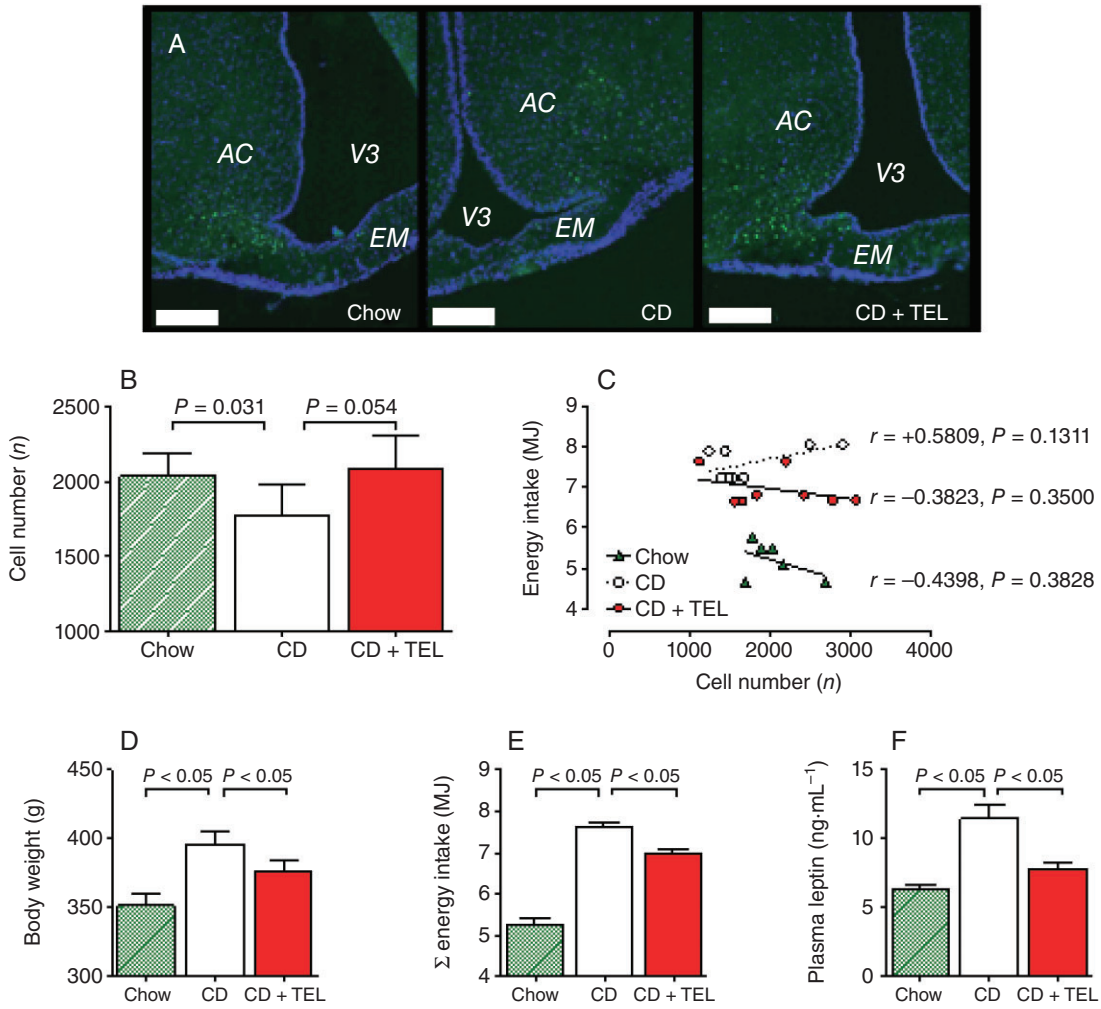

Figure 3

Hypothalamic pSTAT3 staining in CD-fed rats that were treated with telmisartan (8 mg·kg^{-1}·day^{-1}) and of CD- or chow-fed controls receiving vehicle (A; AC, arcuate nucleus; EM, eminentia mediana; V3, third ventricle); scale bar: 100 μm. Numbers of pSTAT3-positive cells were counted in 29–30 slices of each animal (B). Correlation between cell numbers and energy intake (C). Gain in body weight (D), energy intake (E) and plasma leptin (F) were reduced by telmisartan to levels of lean controls. Means ± or + SEM, n = 6–8. Statistical analysis was performed by Wilcoxon signed-rank test due to missing Gaussian distribution.

Table 1

Body weight, energy intake and plasma levels of TG and corticosterone of rats (protocol 3) after treatment with telmisartan (8 mg·kg^{-1}·day^{-1}) and simultaneous CD feeding compared with CD-fed rats only receiving vehicle

	CD	CD + TEL	*P*
Body weight (g)	341.3 ± 4.0	326.1 ± 5.9	0.050
Energy intake (MJ)	9.20 ± 0.14	8.31 ± 0.28	0.012
Plasma triglycerides (mmol·L^{-1})	0.61 ± 0.07	0.41 ± 0.05	0.048
Plasma corticosterone (mg·L^{-1})	94.4 ± 20.8	49.4 ± 9.6	0.037

Means ± SEM, n = 8–10.

Leptin sensitivity

To evaluate leptin's functional efficacy, energy intake and body weight upon exogenous leptin were first monitored in chow-fed SD rats. In response to repeated leptin injections, plasma concentrations of leptin clearly increased and remained beyond control levels for almost 24 h (Supporting Information Fig. S3A). Accordingly, food intake for the 48 h period (Supporting Information Fig. S3B) was diminished and gain in body weight lessened (Supporting Information Fig. S3C).

Secondly, to determine the influence of telmisartan on leptin sensitivity, telmisartan-treated rats and vehicle-treated controls (which both were fed with CD) received either leptin or saline. After saline injections, plasma leptin varied in telmisartan-treated rats between 6.2 ± 1.0 and 9.2 ± 1.2 ng·mL^{-1} and tended to be slightly lower than the corresponding minimal concentration (7.8 ± 0.9 ng·mL^{-1}) and maximal concentration (C_{max}; 12.3 ± 1.3 ng·mL^{-1}) values of the controls. Minimal values were found at approximately 0800 h and maximal values at approximately 2000 h, with no differences between controls and telmisartan-treated animals (Figure 4A). C_{max} (43.8 ± 3.4 vs. 36.7 ± 2.4 ng·mL^{-1}) were observed 5.7 ± 0.8 and 6.0 ± 0.7 h, respectively, after injecting leptin in controls and in telmisartan-treated rats and differed between the two groups (Figure 4B). Considering the AUCs of plasma leptin after leptin injections, leptin was tripled compared with controls receiving saline. AUC after telmisartan was decreased compared with controls (Figure 4C). Rats became leptin resistant as a result of CD feeding as the energy intake was similar between controls that received either leptin or saline. The energy intake of telmisartan-treated rats tended to be reduced during LRT even though they received only saline. However, after injecting leptin the energy intake was significantly lower than in controls receiving no

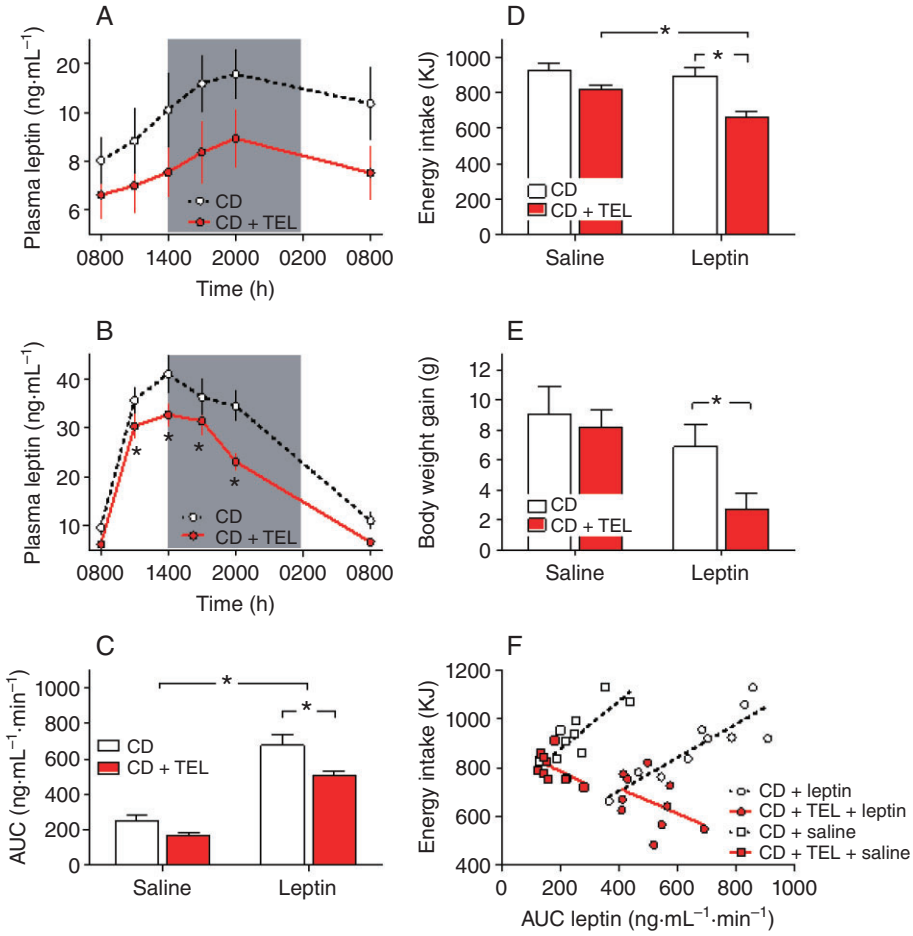

Figure 4

Telmisartan improves leptin sensitivity. CD-fed rats were treated with telmisartan (8 mg·kg^{-1}·day^{-1}) or water, and leptin sensitivity was functionally determined by injecting (s.c.) leptin or saline. Plasma concentrations of leptin were determined within the first 24 h period of the LRT. Leptin varied, time- dependently, in rats only receiving saline, irrespectively of whether rats were pretreated with telmisartan or vehicle (A). This circadian rhythm was overruled when rats received leptin (B). Two-way ANOVA indicates higher plasma leptin levels after leptin injections (C). The cumulative energy intake (D) and the gain in body weight (E) were determined during the 48 h duration of the LRT. AUC of plasma leptin positively correlated with energy intake (F), independently of whether rats received leptin (r = 0.8697, P = 0.001) or saline (r = 0.7541, P = 0.011). When rats were treated with telmisartan, correlation tended to be negative both after leptin (r = –0.4415, P > 0.05) and saline injections (r = –0.4915, P > 0.05). Means ± or + SEM, n = 10, *P < 0.05.

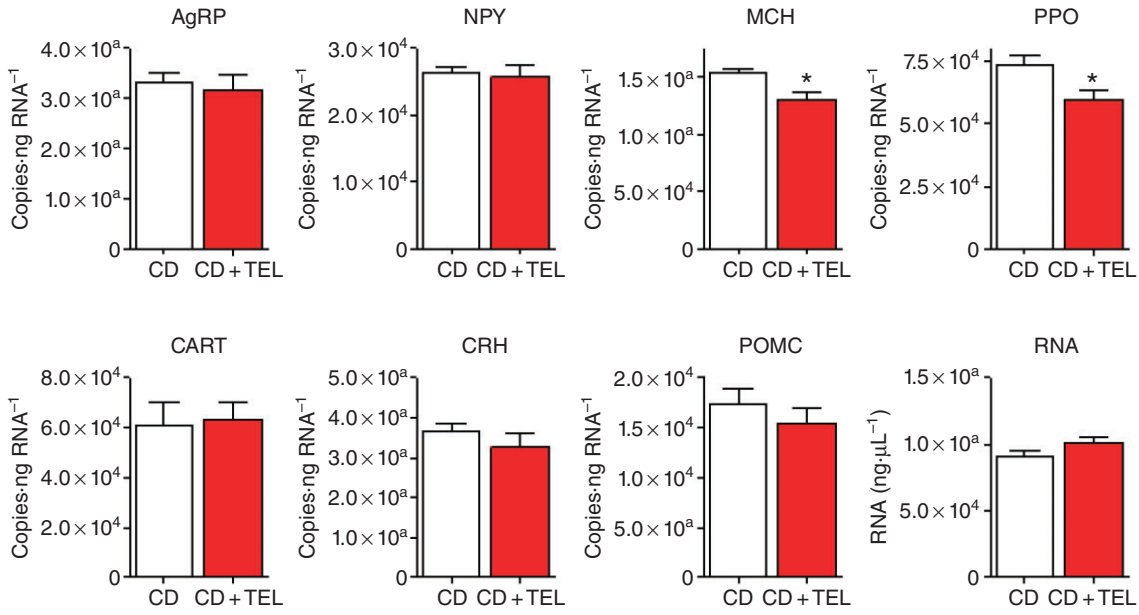

Figure 5

mRNA steady–state levels of the orexigenic peptides PPO, NPY, MCH, AgRP and the anorexigenic peptides CART, corticotropin-releasing hormone (CRH) and POMC in the hypothalami of rats that were treated either with telmisartan (8 mg·kg^{-1}·day^{-1}) or vehicle (= controls) during LRT. RNA levels were similar between the two groups. Hypothalami were prepared 1 h after the last leptin injection (for detailed information, see 'Methods, protocol 3'), means + SEM, $n = 10$, *$P < 0.05$ versus CON.

telmisartan and in rats that had been treated with telmisartan but received saline instead of leptin (Figure 4D). The reduction in energy intake was mainly attributed to a decrease in CD consumption (867 ± 56 vs. 579 ± 43 kJ per 2 days, $P < 0.05$), while the intake of chow was similar (155 ± 11 vs. 163 ± 16 kJ). The gain in body weight in the LRT of telmisartan/leptin-treated rats was <3 g and markedly less than in the other groups (Figure 4E). The AUC of plasma leptin levels correlated positively with the energy intake in controls, independently of whether rats received leptin or saline, which strongly supports leptin resistance in these rats (Figure 3F). In contrast, this correlation was negative in telmisartan-treated rats, irrespectively of whether saline or leptin was administered, indicating that leptin sensitivity is retained (Figure 4F). Steady–state mRNA levels of the (an-)orexigenic peptides were determined in rats immediately after LRT was completed. MCH and PPO were lower in the hypothalami of telmisartan-treated rats than in controls, but all the other values remained unchanged (Figure 5).

To thirdly verify telmisartan's influence on leptin sensitivity, immunohistochemical analyses were performed in chow- and CD-fed animals as well as in CD-fed and telmisartan-treated rats by immunostaining of pSTAT3 in the hypothalami of the animals. pSTAT3-positive cells in the AC were higher after injecting leptin than after saline (Supporting Information Fig. S4). When rats were fed with CD, cell numbers of pSTAT3 were reduced in AC compared with chow-fed controls. telmisartan treatment tended ($P = 0.054$) to normalize pSTAT3-positive cell numbers despite CD feeding (Figure 3B). No correlation was observed between pSTAT3 cell number and energy intake, which might be due to the low number of animals involved in this part of the study (Figure 3C). Although these levels did not reach statistical significance, it may be speculated that the positive Pearson r of CD-fed rats supports leptin resistance of these rats whereas negative Pearson r might hint at leptin sensitivity in chow-fed controls and telmisartan-treated rats.

Discussion

We have demonstrated here that untreated controls became obese and hyperphagic and developed leptin resistance. In contrast, telmisartan preserved leptin sensitivity in rats despite CD feeding. Because of the sensitization towards leptin, telmisartan decreased food intake and body weight in parallel with reduced pSTAT3 staining and lower mRNA levels of MCH and PPO.

Telmisartan attenuated the increase in body weight and decreased food intake in rats over the course of treatment (Figures 2 and 3; Table 1). A temporary reduction in food intake was also observed in chow-fed Wistar Kyoto rats that were treated with candesartan and in CD-fed spontaneously hypertensive rats after telmisartan treatment (Zorad *et al.*, 2006; Miesel *et al.*, 2012; Müller-Fielitz *et al.*, 2012). We addressed the question of whether telmisartan-induced hypophagia might be caused by preventing peripheral leptin resistance from developing. According to our previous findings (Miesel *et al.*, 2012; Müller-Fielitz *et al.*, 2012), SD rats were thought to develop leptin resistance within 2 weeks as energy intake was increased despite enhanced leptin levels (Figure 4). A major component of this peripheral resistance is likely an impaired transport of leptin across the BBB via a permeable leptin transporter (Banks *et al.*, 1999; Banks and

Farrell, 2003). The short form of the leptin receptor was found to be highly expressed at the BBB and to act as a unidirectional and saturable transporter (Banks *et al.*, 1996; Boado *et al.*, 1998; Burguera *et al.*, 2000). Saturation of leptin transport across the BBB is thought to be relevant in obesity (Banks *et al.*, 1996; Banks and Farrell, 2003; Burguera *et al.*, 2000) because obese humans exhibit increased leptin serum levels, but similar levels of leptin in the CSF in comparison with those in non-obese subjects (Caro *et al.*, 1996). Serum TGs impair the ability of the BBB to transport leptin (Banks *et al.*, 2004). In contrast, central leptin resistance specifies an impaired ability of leptin to induce a response, which might be due to fewer leptin receptors and diminished leptin signalling (Scarpace *et al.*, 2001; Sahu and Metlakunta, 2005). In line with these results, we have demonstrated here (Table 1) and elsewhere that (i) CD feeding caused not only leptin resistance but also increased serum TG (Miesel *et al.*, 2010; Müller-Fielitz *et al.*, 2014) and (ii) that telmisartan normalized body weight and TG to lean control levels (Figure 3; Müller-Fielitz *et al.*, 2014). Thus, the question arises whether telmisartan's potency to reduce body weight gain, leptin and TG is only coincidental or whether these effects may be causally related. Hence, it seemed justified to administer exogenous leptin peripherally, not intracerebroventricularly, to investigate whether telmisartan prevents peripheral leptin resistance. LRT revealed that leptin sensitivity is still preserved after 2 weeks of telmisartan although rats had the freedom to consume the palatable and high-calorie CD. This became particularly apparent as the energy intake in response to leptin was lower in telmisartan-treated rats. Indeed, leptin levels were slightly lower after leptin injections in these animals than in the controls (which might be due to the body weight-related leptin dosage), and thus we have more likely underestimated than overestimated the observed effects. In addition, the positive correlation between leptin and energy intake that was observed in controls corroborates leptin resistance and this was inverted towards a negative correlation in telmisartan-treated rats (Figure 4F). This correlation analysis strongly reconfirms at the functional level the ability of telmisartan to maintain leptin sensitivity. We further conclude that leptin still crosses the BBB as we and others have demonstrated that telmisartan reduced TG in humans and animals (Kamari *et al.*, 2008; Fogari *et al.*, 2009; Müller-Fielitz *et al.*, 2014). Although we did not directly examine BBB transport of leptin, for example, by measuring radioactivity in the brain after applying radioactively labelled leptin (Banks *et al.*, 2004), we still have evidence that this assumption is true.

In accordance with others (Perello *et al.*, 2010; de Lartigue *et al.*, 2011), we have shown here that pSTAT3 staining upon exogenous leptin was lower in diet-induced obese rats than in lean rats, strongly indicating the onset of leptin resistance in these animals. In contrast, pSTAT3 staining tended to be normalized after telmisartan. This may fit to previous observations of others who demonstrated that anti-obese strategies such as exercise (Patterson *et al.*, 2009) or drug treatment (Roth *et al.*, 2008) diminished the number of pSTAT3-positive cells. After leptin stimulates OB-Rb receptors and the JAK2/STAT3-dependent pathway, the anorexigenic peptides POMC and CART are expected to be up-regulated whereas the orexigenic peptides AgRP and NPY should be down-regulated (Schwartz *et al.*, 2000). Thus, we aimed to determine mRNA expression of these (an)orexigenic peptides immediately after LRT. None of these peptides were found to be altered in our study, which is in contrast to the data of others who found that food intake of mice was reduced via the melanocortin pathway (Noma *et al.*, 2011). However, the orexigenic peptides MCH and PPO encoding for the orexins were down-regulated in hypothalami of telmisartan-treated rats. It is not yet clear why we have not found any regulation of first-order neuronal signalling in the AC (NPY and POMC neurons), whereas second-order neuronal signalling was diminished in LHA (MCH) and PVN (PPO). We previously observed that MCH expression was reduced in the hypothalami of candesartan-treated rats whereas other first-order peptides were not altered (Müller-Fielitz *et al.*, 2011). Both MCH and the orexins were assumed to reduce food intake by affecting reward, thereby alleviating obesity (Cason *et al.*, 2010; Chung *et al.*, 2011). Thus, it may be speculated that reward also contributes to the anti-obese potency of telmisartan in our experiments as the proportion of palatable high-calorie food was decreased by telmisartan (Figure 2C); furthermore, others have found that alcohol consumption was diminished in mice lacking AT_1 receptor and in transgenic rats with reduced AngII levels exclusively in the CNS, which was suggested to be mediated via dopamine (Maul *et al.*, 2005). The ARB losartan inhibited nicotine-evoked dopamine and norepinephrine release from striatal and hypothalamic slices, which additionally confirms that the central RAS is involved in regulating reward via the dopaminergic system (Narayanaswami *et al.*, 2013). Moreover, serotonin (5-HT) may also be involved in AngII-regulated reward as the expression of the 5-HT(2C) receptor and 5-HT transporter was higher in hypothalami of transgenic rats with a brain-specific angiotensinogen deficiency; in addition, the anorectic 5-HT reuptake inhibitor, 5-HT releaser and 5-HT(2C) receptor agonist fenfluramine reduced food intake more potently in transgenic rats than in wild-type controls (Voigt *et al.*, 2008).

We have clearly shown that telmisartan improved leptin sensitivity and we speculated that leptin transport across the BBB is improved; however, the underlying mechanism for this still remains a matter of debate. Inconsistent findings have been published on the ability of AngII to influence BBB function. On the one hand, AngII infusion increased BBB permeability via AT_1 receptors and the ARB olmesartan markedly reduced BBB microvessel permeability in an experimental model, showing substantial leakage from BBB microvessels (Vital *et al.*, 2010; Pelisch *et al.*, 2011). In agreement with these results, epinephrine increased microvascular Evans blue-albumin efflux to brain in diabetic hypertensive rats whereas candesartan attenuated permeability to brain tissue (Awad, 2006). Only considering these findings, it seems unlikely that ARBs increase BBB leakage, thus increasing availability of central leptin. On the other hand, permeability of BBB endothelial cell monolayers was decreased in the presence of AngII in an AT_1 receptor-dependent manner by influencing the rearrangement of specific multiprotein tight junction proteins to lipid rafts, a phenomenon necessary to promote BBB integrity (Wosik *et al.*, 2007). Moreover, leptin specifically enters central tissue via leptin transporters located in the choroid plexus (Chodobski and Szmydynger-Chodobska, 2001). AngII was shown to decrease blood flow in choroidal blood vessels through interactions with the sym-

pathetic nervous system and the NO synthetic pathways (Chodobski and Szmydynger-Chodobska, 2001) and AT_1 receptors are located within the choroid plexus (Jöhren and Saavedra, 1996). Hence, blocking AT_1 receptors may improve choroidal blood flow, thus increasing the penetration of leptin into the hypothalamus. Moreover, we can also speculate that the inhibitory action of glucocorticoids on leptin may contribute to the observed increase in leptin sensitivity after telmisartan. In rodents, obesity is often accompanied by hypercorticism, overresponsiveness of the HPA axis or increased 24 h urinary corticosterone output (Cunningham *et al.*, 1986; Guillaume-Gentil *et al.*, 1990). Circulating leptin is under adrenal glucocorticoid control as adrenalectomy decreases circulating leptin concentrations, and corticosterone replacement restores it to levels observed in sham-operated controls (Spinedi and Gaillard, 1998). Considering the facts that AT_1 receptors are located in hypothalamus, pituitary gland and adrenals and the reactivity of the HPA axis was increased after AngII but decreased after AT_1 receptor blockade (Raasch *et al.*, 2006; Müller *et al.*, 2007; Miesel *et al.*, 2012; Müller-Fielitz and Raasch, 2013), inhibiting HPA axis reactivity by blocking AT_1 receptors may contribute to the increase in leptin sensitivity observed after administering telmisartan. The reduced corticosterone levels demonstrated in this study seem to support this hypothesis.

In summary, we have demonstrated that telmisartan preserves leptin sensitivity despite CD feeding, whereas CD feeding promotes leptin resistance and obesity in controls. This effect occurs independently of its efficacy in lowering blood pressure using high doses of telmisartan. Thus, telmisartan may be very valuable for treating patients presenting with all the cardinal symptoms of metabolic syndrome. The limitation of this therapeutic approach is that dosages >80 mg telmisartan in humans represent an off-label use and systematic studies addressing safety aspects by considering such high doses of 160–320 mg have not been published yet. Thus, the relevance of our experiments for humans needs to be investigated in future trials.

Acknowledgements

The authors would like to thank Mrs. Ines Stölting for expert technical assistance. This study was supported by the Excellent Funding of the Dean of the Medical Faculty of the University of Lübeck.

Author contributions

H. M.-F., M. L., C. G., L. W. and M. P. performed the research. W. R. and H. M.-F. designed the research study. W. R., M. L. and C. G. analysed the data. W. R. wrote the paper. H. M.-F. and M. L. contributed equally.

Conflict of interest

No conflict of interests.

References

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic receptors. Br J Pharmacol 170: 1676–1705.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013c). The Concise Guide to PHARMACOLOGY 2013/14: Transporters. Br J Pharmacol 170: 1706–1796.

Awad AS (2006). Role of AT1 receptors in permeability of the blood–brain barrier in diabetic hypertensive rats. Vascul Pharmacol 45: 141–147.

Banks WA, Farrell CL (2003). Impaired transport of leptin across the blood–brain barrier in obesity is acquired and reversible. Am J Physiol Endocrinol Metab 285: E10–E15.

Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM (1996). Leptin enters the brain by a saturable system independent of insulin. Peptides 17: 305–311.

Banks WA, DiPalma CR, Farrell CL (1999). Impaired transport of leptin across the blood–brain barrier in obesity. Peptides 20: 1341–1345.

Banks WA, Coon AB, Robinson SM, Moinuddin A, Shultz JM, Nakaoke R *et al.* (2004). Triglycerides induce leptin resistance at the blood–brain barrier. Diabetes 53: 1253–1260.

Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M *et al.* (2004). Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. Hypertension 43: 993–1002.

Boado RJ, Golden PL, Levin N, Pardridge WM (1998). Up-regulation of blood–brain barrier short-form leptin receptor gene products in rats fed a high fat diet. J Neurochem 71: 1761–1764.

Burguera B, Couce ME, Curran GL, Jensen MD, Lloyd RV, Cleary MP *et al.* (2000). Obesity is associated with a decreased leptin transport across the blood–brain barrier in rats. Diabetes 49: 1219–1223.

Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH *et al.* (1996). Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet 348: 159–161.

Cason AM, Smith RJ, Tahsili-Fahadan P, Moorman DE, Sartor GC, Aston-Jones G (2010). Role of orexin/hypocretin in reward-seeking and addiction: implications for obesity. Physiol Behav 100: 419–428.

Chodobski A, Szmydynger-Chodobska J (2001). Choroid plexus: target for polypeptides and site of their synthesis. Microsc Res Tech 52: 65–82.

Chung S, Parks GS, Lee C, Civelli O (2011). Recent updates on the melanin-concentrating hormone (MCH) and its receptor system: lessons from MCH1R antagonists. J Mol Neurosci 43: 115–121.

Cunningham JJ, Calles-Escandon J, Garrido F, Carr DB, Bode HH (1986). Hypercorticosteronuria and diminished pituitary responsiveness to corticotropin-releasing factor in obese Zucker rats. Endocrinology 118: 98–101.

Fogari R, Zoppi A, Ferrari I, Mugellini A, Preti P, Lazzari P *et al.* (2009). Comparative effects of telmisartan and eprosartan on

insulin sensitivity in the treatment of overweight hypertensive patients. Horm Metab Res 41: 893–898.

Guillaume-Gentil C, Rohner-Jeanrenaud F, Abramo F, Bestetti GE, Rossi GL, Jeanrenaud B (1990). Abnormal regulation of the hypothalamo-pituitary-adrenal axis in the genetically obese fa/fa rat. Endocrinology 126: 1873–1879.

Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM (1997). Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci U S A 94: 8878–8883.

He H, Yang D, Ma L, Luo Z, Ma S, Feng X *et al.* (2010). Telmisartan prevents weight gain and obesity through activation of peroxisome proliferator-activated receptor-{delta}-dependent pathways. Hypertension 55: 869–879.

Jöhren O, Saavedra JM (1996). Expression of AT1A and AT1B angiotensin II receptor messenger RNA in forebrain of 2-wk-old rats. Am J Physiol 271: E104–E112.

Kamari Y, Harari A, Shaish A, Peleg E, Sharabi Y, Harats D *et al.* (2008). Effect of telmisartan, angiotensin II receptor antagonist, on metabolic profile in fructose-induced hypertensive, hyperinsulinemic, hyperlipidemic rats. Hypertens Res 31: 135–140.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Kintscher U, Bramlage P, Paar WD, Thoenes M, Unger T (2007). Irbesartan for the treatment of hypertension in patients with the metabolic syndrome: a sub analysis of the Treat to Target post authorization survey. Prospective observational, two armed study in 14,200 patients. Cardiovasc Diabetol 6: 12.

de Lartigue G, Barbier de la Serre C, Espero E, Lee J, Raybould HE (2011). Diet-induced obesity leads to the development of leptin resistance in vagal afferent neurons. Am J Physiol Endocrinol Metab 301: E187–E195.

Lau M, Raasch W (2012). The influence of the renin angiotensin II aldosteron system on energy intake and body weight. Adipositas 6: 110–117.

Maul B, Krause W, Pankow K, Becker M, Gembardt F, Alenina N *et al.* (2005). Central angiotensin II controls alcohol consumption via its AT1 receptor. FASEB J 19: 1474–1481.

McAlister ED, Van Vugt DA (2004). Effect of leptin administration versus re-feeding on hypothalamic neuropeptide gene expression in fasted male rats. Can J Physiol Pharmacol 82: 1128–1134.

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

Michel MC, Foster C, Brunner HR, Liu L (2013). A systematic comparison of the properties of clinically used angiotensin II type 1 receptor antagonists. Pharmacol Rev 65: 809–848.

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

Miesel A, Müller-Fielitz H, Jöhren O, Vogt FM, Raasch W (2012). Double blockade of angiotensin II (AT(1))-receptors and ACE does not improve weight gain and glucose homeostasis better than single-drug treatments in obese rats. Br J Pharmacol 165: 2721–2735.

Morris DL, Rui L (2009). Recent advances in understanding leptin signaling and leptin resistance. Am J Physiol Endocrinol Metab 297: E1247–E1259.

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

Müller-Fielitz H, Raasch W (2013). Angiotensin II impairs glucose utilization in obese Zucker rats by increasing HPA activity via an adrenal-dependent mechanism. Horm Metab Res 45: 173–180.

Müller-Fielitz H, Markert A, Wittmershaus C, Pahlke F, Jöhren O, Raasch W (2011). Weight loss and hypophagia after high-dose AT(1)-blockade is only observed after high dosing and depends on regular leptin signalling but not blood pressure. Naunyn Schmiedebergs Arch Pharmacol 383: 373–384.

Müller-Fielitz H, Landolt J, Heidbreder M, Werth S, Vogt FM, Jöhren O *et al.* (2012). Improved insulin sensitivity after long-term treatment with AT1 blockers is not associated with PPARγ target gene regulation. Endocrinology 153: 1103–1115.

Müller-Fielitz H, Hübel N, Mildner M, Vogt FM, Barkhausen J, Raasch W (2014). Chronic blockade of angiotensin AT1 receptors improves cardinal symptoms of metabolic syndrome in diet-induced obesity in rats. Br J Pharmacol 171: 746–760.

Narayanaswami V, Somkuwar SS, Horton DB, Cassis LA, Dwoskin LP (2013). Angiotensin AT1 and AT2 receptor antagonists modulate nicotine-evoked [(3)H]dopamine and [(3)H]norepinephrine release. Biochem Pharmacol 86: 656–665.

Nishiyama M, Makino S, Asaba K, Hashimoto K (1999). Leptin effects on the expression of type-2 CRH receptor mRNA in the ventromedial hypothalamus in the rat. J Neuroendocrinol 11: 307–314.

Noma K, Toshinaia K, Koshinaka K, Nakazato M (2011). Telmisartan suppresses food intake in mice via the melanocortin pathway. Obes Res Clin Pract 5: e79–e156.

Patterson CM, Bouret SG, Dunn-Meynell AA, Levin BE (2009). Three weeks of postweaning exercise in DIO rats produces prolonged increases in central leptin sensitivity and signaling. Am J Physiol Regul Integr Comp Physiol 296: R537–R548.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.*; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucleic Acids Res 42 (Database Issue): D1098–106.

Pelisch N, Hosomi N, Ueno M, Nakano D, Hitomi H, Mogi M *et al.* (2011). Blockade of AT1 receptors protects the blood–brain barrier and improves cognition in Dahl salt-sensitive hypertensive rats. Am J Hypertens 24: 362–368.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T *et al.* (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269: 540–543.

Perello M, Cakir I, Cyr NE, Romero A, Stuart RC, Chiappini F *et al.* (2010). Maintenance of the thyroid axis during diet-induced obesity in rodents is controlled at the central level. Am J Physiol Endocrinol Metab 299: E976–E989.

Plum L, Ma X, Hampel B, Balthasar N, Coppari R, Munzberg H *et al.* (2006). Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet-sensitive obesity. J Clin Invest 116: 1886–1901.

Raasch W, Bartels T, Schwartz C, Häuser W, Rütten H, Dominiak P (2002). Regression of ventricular and vascular hypertrophy: are there differences between structurally different angiotensin-converting enzyme inhibitors? J Hypertens 20: 2495–2504.

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

Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE *et al*. (2008). Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc Natl Acad Sci U S A 105: 7257–7262.

Sahu A, Metlakunta AS (2005). Hypothalamic phosphatidylinositol 3-kinase-phosphodiesterase 3B-cyclic AMP pathway of leptin signalling is impaired following chronic central leptin infusion. J Neuroendocrinol 17: 720–726.

Scarpace PJ, Matheny M, Tumer N (2001). Hypothalamic leptin resistance is associated with impaired leptin signal transduction in aged obese rats. Neuroscience 104: 1111–1117.

Scheen AJ (2004). Renin-angiotensin system inhibition prevents type 2 diabetes mellitus. Part 1. A meta-analysis of randomised clinical trials. Diabetes Metab 30: 487–496.

Schupp M, Clemenz M, Gineste R, Witt H, Janke J, Helleboid S *et al*. (2005). Molecular characterization of new selective peroxisome proliferator-activated receptor gamma modulators with angiotensin receptor blocking activity. Diabetes 54: 3442–3452.

Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG (2000). Central nervous system control of food intake. Nature 404: 661–671.

Souza-Mello V, Gregorio BM, Cardoso-de-Lemos FS, de Carvalho L, Aguila MB, Mandarim-de-Lacerda CA (2010). Comparative effects of telmisartan, sitagliptin and metformin alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. Clin Sci (Lond) 119: 239–250.

Spinedi E, Gaillard RC (1998). A regulatory loop between the hypothalamo-pituitary-adrenal (HPA) axis and circulating leptin: a physiological role of ACTH. Endocrinology 139: 4016–4020.

Sugimoto K, Qi NR, Kazdova L, Pravenec M, Ogihara T, Kurtz TW (2006). Telmisartan but not valsartan increases caloric expenditure and protects against weight gain and hepatic steatosis. Hypertension 47: 1003–1009.

Villa L, Boor P, Konieczny A, Kunter U, van Roeyen CR, Denecke B *et al*. (2011). Effects and mechanisms of angiotensin II receptor blockade with telmisartan in a normotensive model of mesangioproliferative nephritis. Nephrol Dial Transplant 26: 3131–3143.

Vital SA, Terao S, Nagai M, Granger DN (2010). Mechanisms underlying the cerebral microvascular responses to angiotensin II-induced hypertension. Microcirculation 17: 641–649.

Voigt JP, Raasch W, Hortnagl H, Bader M, Fink H, Jöhren O (2008). Changes in the brain serotonin satiety system in transgenic rats lacking brain angiotensinogen. J Neuroendocrinol 20: 182–187.

Wetzler S, Dumaz V, Goubern M, Tome D, Larue-Achagiotis C (2004). Intraperitoneal leptin modifies macronutrient choice in self-selecting rats. Physiol Behav 83: 65–72.

Wosik K, Cayrol R, Dodelet-Devillers A, Berthelet F, Bernard M, Moumdjian R *et al*. (2007). Angiotensin II controls occludin function and is required for blood brain barrier maintenance: relevance to multiple sclerosis. J Neurosci 27: 9032–9042.

Zanchi A, Dulloo AG, Perregaux C, Montani JP, Burnier M (2007). Telmisartan prevents the glitazone-induced weight gain without interfering with its insulin-sensitizing properties. Am J Physiol Endocrinol Metab 293: E91–E95.

Zorad S, Dou JT, Benicky J, Hutanu D, Tybitanclova K, Zhou J *et al*. (2006). Long-term angiotensin II AT1 receptor inhibition produces adipose tissue hypotrophy accompanied by increased expression of adiponectin and PPARgamma. Eur J Pharmacol 552: 112–122.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

http://dx.doi.org/10.1111/bph.12949

Figure S1 Correlation analysis between plasma leptin levels and energy intake of rats before and 14 days after drug treatment (A). A clear upward shift was observed in CONobese in contrast to the downward shift of CONlean or telmisartan (TEL)-treated rats. The data in this figure originate from a study that was recently published by Müller-Fielitz *et al*. (2012). However, only mean values of plasma leptin and energy intake and no detail analysis reflecting their interdependency have been presented in this paper. In analogy to the feeding regime of the presented study, spontaneous hypertensive rats could freely choose between chow and a high-calorie and palatable cafeteria diet (CD) consisting of various cookies and chocolate bars (CONobese). Lean controls received only chow (CONlean). One group of CD-fed rats was treated with telmisartan (TEL, 8 $mg \cdot kg^{-1} \cdot day^{-1}$). Before any CD feeding and drug treatment, plasma leptin values of all rats negatively correlated with energy intake, which confirms leptin sensitivity of rats at this time (B). Already after 2 weeks CD-fed animals seem to develop leptin resistance as energy intake is high despite of high plasma leptin (D). Values particularly correlate in CD-fed controls after 2 weeks. This positive correlation was not observed in telmisartan-treated rats despite their CD feeding (E) and certainly not in CONlean (C), thus indicating that leptin sensitivity is still retained. Means ± SEM, n = 12–14.

Figure S2 Expression of UCP-1 in brown adipose tissue. Rats were treated with telmisartan (8 $mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle. Representative immunoblots and densitometric analyses demonstrate that UCP-1 is not influenced by leptin and telmisartan. Means ± SEM, n = 10.

Figure S3 Changes in plasma leptin (A), energy intake (B) and body weight (C) in chow-fed Sprague Dawley rats after injections of leptin or saline. The injection regime for leptin is given in the methods (protocol 1). Means ± SEM, n = 10, $^*P < 0.05$ versus saline.

Figure S4 Hypothalamic pSTAT3 staining in a male chow-fed Sprague Dawley rat after injections of (A) saline or (B) leptin (100 $mg \cdot kg^{-1}$). AC, arcuate nucleus; EM, eminentia mediana; V3, third ventricle; scale bar: 100 µm.