AT₁ Receptor Antagonist Telmisartan Administered Peripherally Inhibits Central Responses to Angiotensin II in Conscious Rats


Institute of Pharmacology, Christian-Albrechts University of Kiel, Kiel, Germany (P.G., S.W., A.J., J.C., T.U.); German Institute for High Blood Pressure Research, Heidelberg, Germany (P.G., J.C., T.U.); Boehringer Ingelheim, Biberach an der Riss, Germany (W.W., J.S.); and Department of Paediatrics, University of Erlangen-Nürnberg, Erlangen, Germany (W.R.)

Received December 21, 2000; accepted March 13, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

The effects of systemic treatment with the AT₁ receptor antagonist telmisartan on central effects of angiotensin II (Ang II), namely, increase in blood pressure, vasopressin release into the circulation, and drinking response, were investigated in conscious, normotensive rats. The central responses to i.c.v. Ang II (30 ng/kg) were measured at 0.5, 2, 4, and 24 h following acute i.v. or acute and chronic oral telmisartan application. At a dose of 10 mg/kg i.v., the drinking response to i.c.v. Ang II was completely blocked over 4 h, while the pressor response and the release of vasopressin in response to i.c.v. Ang II were blocked by 60 to 80%. The inhibition of the centrally mediated pressor and drinking response to Ang II was sustained over 24 h. The lower doses of telmisartan (0.3 and 1 mg/kg) significantly inhibited the Ang II-induced actions over 4 h. A consistent 24-h inhibition of the central responses to i.c.v. Ang II was obtained after acute and chronic oral treatment with 30 mg/kg telmisartan. Oral treatment with 1 and 3 mg/kg telmisartan produced a slight but inconsistent inhibition of the central actions of Ang II. Telmisartan concentrations measured in the cerebrospinal fluid following 8 days of consecutive daily oral treatment (1–30 mg/kg) ranged from 0.87 ± 0.27 ng/ml (1 mg/kg/day) to 46.5 ± 11.6 ng/ml (30 mg/kg/day). Our results demonstrate that, following peripheral administration, the AT₁ receptor antagonist telmisartan can penetrate the blood-brain barrier in a dose- and time-dependent manner to inhibit centrally mediated effects of Ang II.

The effector peptide of the renin-angiotensin system (RAS), angiotensin II (Ang II), is involved in the maintenance and regulation of salt and volume homeostasis and in the cardiovascular control by peripherally as well as centrally mediated effects. Ang II contributes to a number of pathophysiological events such as left ventricular hypertrophy, neointima formation, nephrosclerosis, and postinfarct remodeling by stimulation of AT₁ receptors in peripheral organs (Edling et al., 1995; Griendling et al., 1996). The existence of an independent brain RAS is well established and all components of the system have been localized in the brain (Unger et al., 1988; Phillips and Sumners, 1998). There is also evidence that the brain RAS substantially contributes to the development and maintenance of hypertension (Phillips and Sumners, 1998).

Activation of centrally located AT₁ receptors induces a release of vasopressin (AVP) and other pituitary hormones, drinking behavior, natriuresis, and an increase in blood pressure, and has been shown to impair performance in learning and memory paradigms in animals (Rettig et al., 1986; Unger et al., 1988; Phillips and Sumners, 1998). Therefore, the brain RAS can be an important target for inhibitors of the RAS such as angiotensin-converting enzyme inhibitors and AT₁ receptor antagonists. Inhibition of Ang II-mediated actions in the brain may not only contribute to antihypertensive actions of these inhibitors but may also enhance cognitive functions, alter autoregulation of cerebral blood flow, and limit the detrimental effects of cerebral ischemia and infarction (Barnes et al., 1990; Vraamark et al., 1995; Dai et al., 1999; Hirawa et al., 1999).

Blood-borne Ang II or Ang II injected i.c.v. interacts with AT₁ receptors in the subfornical organ or in the organum vasculosum laminae terminalis and initiates drinking and pressor responses. The latter comprises the release of AVP into the circulation and the sympathoadrenal activation (Johnson and Edwards, 1990; Saavedra, 1992; Phillips and Sumners, 1998). The subfornical organ and organum vasculosum laminae terminalis belong to the circumventricular organs, which are referred to as structures lacking the blood-brain barrier (Saunders et al., 1999). However, besides AT₁ receptors in the circumventricular organs, AT₁ receptors localized in the lamina terminalis, paraventricular and supraoptic nuclei, and in a number of brain stem nuclei, i.e., in areas inside the blood-brain-barrier, are involved in the generation of the cardiovascular responses to i.c.v. Ang II (Phillips and Sumners, 1998).

ABBREVIATIONS: RAS, renin-angiotensin system; Ang II, angiotensin II; AVP, vasopressin; CSF, cerebrospinal fluid; MAP, mean arterial blood pressure; ANOVA, analysis of variance.
Peripheral administration of AT₁ receptor antagonists can interact with AT₁ receptors in the circumventricular organs, or with AT₁ receptors localized inside the blood-brain barrier, providing that the antagonists cross the blood-brain barrier. We have demonstrated recently that systemically administered losartan at doses high enough to effectively block AT₁ receptors in the circumventricular organs failed to completely inhibit the central effects induced by i.c.v. Ang II (Culman et al., 1999). These findings clearly indicate that a complete inhibition of the Ang II-mediated actions requires an effective blockade of AT₁ receptors localized outside as well as inside the blood-brain barrier.

The aim of this study was to investigate whether the liphophilic, nonpeptide AT₁ receptor antagonist telmisartan (Wienen et al., 2000) can penetrate the blood-brain barrier in sufficient amounts to block brain AT₁ receptors and to inhibit centrally mediated actions of Ang II. For this purpose, the time- and dose-dependent inhibitory actions of peripherally administered telmisartan on three well established central effects of Ang II, namely, the drinking response the pressor effects of Ang II, were studied in conscious normotensive rats. A further, more direct, evidence for the response and the release of AVP, were studied in conscious effects of Ang II, namely, the drinking response the pressor administered telmisartan on three well established central actions of Ang II. For this purpose, the sufficient amounts to block brain AT₁ receptors and to inhibit centrally mediated actions of Ang II. For this purpose, the drinking response to i.c.v.-injected Ang II was determined 0.5 and 4 h, and in the second group 2 h and 24 h following i.v. telmisartan.

Materials and Methods

Animals

Male, normotensive Wistar rats weighing 300 to 350 g obtained from Charles River (Sulzdorf, Germany) were used. Rats were housed on a 12-h light/dark cycle with free access to food (sniff R/M-H, 10 mm; sniff Spezialdiäten GmbH, Soest, Germany) and water. All experiments were carried out in conscious, freely moving rats.

Surgical Methods

All surgical procedures were performed in rats anesthetized with an intraperitoneal injection of chloralhydrate (400 mg/kg of body weight). For i.c.v. injection, chronic polyethylene cannulae (PP-20) were implanted into the left lateral brain ventricle using a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The coordinates for the i.c.v. cannula were: 0.6 mm caudal to the bregma, 1.3 mm lateral to the midline, and 5.0 mm vertical from the skull surface. The rats were then placed in individual plastic cages. Five days later, Ang II (30 ng/kg of body weight) was injected i.c.v. to verify the correct position of the i.c.v. cannula. Only those rats that responded with an immediate drinking were included in the study. For measurement of blood pressure or for blood withdrawal, a polyethylene catheter (PP-10 in PP-50) was inserted through the femoral artery into the abdominal aorta. In some groups, a polyethylene catheter (PP-50) was inserted into the femoral vein for intravenous drug administration. The arterial and venous catheters were filled with heparinized saline. Both catheters were exteriorized, sealed, and emerged at the nape of the neck. Experiments were conducted 24 to 48 h after femoral artery and vein cannulation.

General Procedures

Measurement of Drinking Response. Water intake was determined by weighing of water that the rat drank during a 20-min time period starting immediately after i.c.v. injection of Ang II (30 ng/kg of body weight). The drinking response to i.c.v.-injected Ang II was recorded at different time points (0.5, 2, 4, and 24 h) following i.v. injection or oral gavage application of telmisartan. Since the animals would drink about one-third of their normal daily water intake after one single injection of Ang II, it was not possible to measure the water intake at all four time points in one animal. Therefore, for each dose of telmisartan two groups of animals were used. In the first group, the drinking response to i.c.v. Ang II was determined 0.5 and 4 h, and in the second group 2 h and 24 h following i.v. telmisartan.

Measurement of Cardiovascular Responses. Measurements of mean arterial pressure (MAP) were performed via the arterial catheters using a pressure transducer (DFTX/Plus; Spectramed Inc., Oxnard, CA) connected to a pressure processor (Gould Inc., Valley View, OH) coupled to a Gould Brush recorder (Gould Series 2400; Gould Inc.). The analog output signals of MAP from the Gould Brush pressure computer were digitalized and then processed using a computer program. This program permits sampling of hemodynamic data from experimental animals directly onto a hard disk and subsequent analysis with an interactive and graphic program.

The femoral artery catheter was connected to the transducer. The experiments were started when the animals were resting and when basal MAP and heart rate were stable. Drugs were administered i.v. through the intravenous catheter connected to an extension catheter with syringe. When telmisartan was administered intragastrically, the femoral artery catheter was connected to the transducer after intragastric treatment. Ang II was dissolved in isotonic saline and injected as a bolus i.c.v. (30 ng/kg of body weight) before and at various time points (0.5, 2, 4, and 24 h) after i.v. or intragastric treatment with the antagonists or vehicle (controls).

Measurement of AVP Release. One milliliter of blood was withdrawn 90 s following i.c.v. injection of Ang II and the volume was immediately replaced by i.v. infusion of saline. The peak of AVP in plasma in response to i.c.v. Ang II lies within a time interval between the 1st and 3rd min after the i.c.v. injection of the peptide. Blood samples were collected in ice-cold Eppendorf tubes and centrifuged immediately at 3000g in a refrigerated centrifuge.

The release of AVP in response to i.c.v.-injected Ang II was recorded at different time points (0.5, 2, 4, and 24 h) following i.v. injection or oral application of telmisartan. For each dose of telmisartan, two groups of animals were used to avoid 1) inadequate loss of blood cells within a short time period, and 2) activation of the RAS and subsequent increase in basal plasma AVP concentration due to repeated blood sampling within a short time period. In the first group, blood for AVP determination was taken 0.5 and 4 h and in the second group 2- and 24-h following telmisartan.

Determination of AVP in Plasma. Plasma AVP was determined by radioimmunoassay as described elsewhere (Rascher et al., 1981). The antibody used cross-reacts with lysine vasopressin (25%) but not with oxytocin (<0.1%) or with vasotocin (0.7%). The intra-assay coefficient of variance is 7.6%, the interassay coefficient of variance is 12.2% (Rascher et al., 1981). The detection limit was 1.5 pg/ml.

CSF Sampling. Rats were anesthetized with chloralhydrate (400 mg/kg of body weight). CSF was collected by puncture of the cisterna magna. The atlanto-occipital membrane was exposed and a 27-gauge cannula connected to a polyethylene tubing (PE-20) was inserted into the cisterna magna. With a 1-ml syringe the CSF was withdrawn carefully until it flowed freely by gravity. CSF was collected in two to three fractions of 50 to 100 µl each in 0.5-ml tubes and kept on ice. Special care was taken to prevent blood contamination of the CSF samples. Each fraction was examined microscopically in a Neubauer chamber. All fractions contaminated with red blood cells were discarded. Blood-free fractions of each animal were pooled and centrifuged at 3000 rpm and 4°C for 2 min. Samples were stored at −20°C until assayed.

Determination of Telmisartan in CSF. The competitive enzyme-linked immunosorbent assay used polyclonal rabbit anti-telmisartan antibodies, which were modified with biotin. Biotinylated antibodies were immobilized on avidin-coated microrater plates.
Free telmisartan in the sample competed with a fixed amount of added horseradish peroxidase conjugates of telmisartan for the antigen binding sites on the platelet surface. Bound enzyme conjugate was detected photometrically after incubation of the plate wells with a chromogenic substrate. The enzyme-linked immunosorbent assay enabled the accurate and precise measurement of telmisartan in the range of 0.3 to 1000 ng/ml. Samples were diluted 10-fold with assay buffer prior to analysis. The assay calibration range was 0.03 to 100 ng/ml. Assay precision based on measurement of quality control samples was 6.5, 2.3, and 2.1% coefficient of variation at 0.3, 17.3, and 1000 ng/ml, respectively. The corresponding assay accuracy was within ±2.0 and 1.4%.

**Drugs.** The nonpeptide AT<sub>1</sub> receptor antagonist telmisartan [4-{[1,4-biphenyl]-2-carboxylic acid}] was supplied by Boehringer Ingelheim, Biberach an der Riss, Germany. Ang II was purchased from Sigma, Deisenhofen, Germany. Ang II for i.c.v. injection was dissolved in physiological saline. Physiological saline was used as vehicle solution instead of artificial cerebrospinal fluid since in previous experiments, Ang II dissolved either in saline or in artificial cerebrospinal fluid had yielded identical results. Ang II (30 ng/kg of body weight) was injected i.c.v. in a total volume of 1 μl and flushed with 4 μl of physiological saline. For oral application, telmisartan (30 mg/kg of body weight) was dissolved in 1 N NaOH solution and the pH was adjusted to 9.5. Telmisartan was applied by gavage in a volume of 3 ml/kg of body weight. For i.v. administration telmisartan (10 mg/kg of body weight) was solubilized in 1 N NaOH solution and the pH of the solution was adjusted to pH 9.5. In previous experiments, an NaOH solution (pH 9.5) alone injected i.v. was without any appreciable effects on cardiovascular parameters. The solutions were further diluted with isotonic saline to achieve doses of 3, 1, and 0.3 mg/kg telmisartan. Two vehicle solutions, NaOH (pH 9.5) and isotonic saline, were used for the experiments. Because both vehicle groups did not affect the i.c.v. Ang II-induced central actions, data for both groups were pooled. Vehicle and the angiotensin AT<sub>1</sub> receptor antagonist were injected slowly through the venous catheter in a volume of 3 ml/kg of body weight.

**Experimental Protocols**

**Effects of i.v. Applied Telmisartan on Centrally Mediated Actions of Ang II.** *Drinking response to i.c.v.-injected Ang II.* On the 1st day, the basal drinking response to i.c.v.-injected Ang II (30 ng/kg of body weight) was recorded over a 20-min period. On the 2nd day, telmisartan was applied i.v. at four different doses: 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg of body weight (n = 11). Control animals received vehicle (n = 13). The MAP responses to i.c.v.-injected Ang II (10 ng) were recorded at two different time points (4 and 24 h) following i.v. injection of telmisartan. The drinking response to i.c.v.-injected Ang II was again measured 0.5 and 4 h (group 1) and 2 and 24 h (group 2) following i.v. injection of telmisartan as described above.

**MAP response to i.c.v.-injected Ang II.** On the 1st day, the basal MAP response to i.c.v.-injected Ang II (30 ng/kg of body weight) was recorded. On the next day, telmisartan was applied i.v. at four different doses: 0.3 mg/kg, n = 11 (group 1) and n = 10 (group 2); 1 mg/kg, n = 10 (group 1) and n = 8 (group 2); 3 mg/kg, n = 9 (group 1) and n = 9 (group 2); and 10 mg/kg, n = 8 (group 1) and n = 8 (group 2). Control animals received vehicle, n = 9 (group 1) and n = 10 (group 2). The MAP responses to i.c.v.-injected Ang II was again measured 0.5 and 4 h (group 1) and 2 and 24 h (group 2) following i.v. injection of telmisartan.

**Results**

**Effect of Intravenously Applied Telmisartan on Drinking Response to i.c.v.-Injected Ang II.** Ang II (30 ng/kg of body weight) injected i.c.v. elicited a prompt drinking behavior, which was determined over a 20-min period. Pretreatment of rats with telmisartan (i.v.) at various doses significantly reduced the drinking response to i.c.v. Ang II in a dose- and time-dependent manner (Fig. 1). Statistical analysis was performed in two groups of rats. Group 1 (F = 15.62, p < 0.0001): basal (N.S.); 0.5 h (F = 25.55, p < 0.0001); 4 h (F = 12.55, p < 0.0001). Group 2 (F = 11.01, p < 0.0001): basal (N.S.); 2 h (F = 30.77, p < 0.0001); 24 h (F = 4.6, p < 0.005). The drinking response to i.c.v. Ang II was nearly completely blocked over 4 h following i.v. injection of telmisartan at a dose of 10 mg/kg of body weight. The inhibition was sustained with the highest dose of telmisartan (10 mg/kg...
Fig. 1. Effect of telmisartan administered intravenously at various doses on drinking response induced by Ang II (30 ng/kg of body weight) injected i.c.v. before and at various time points post antagonist treatment. ●, vehicle; ○, 0.3 mg/kg; △, 1 mg/kg; ▲, 3 mg/kg; □, 10 mg/kg. B, basal water intake in response to i.c.v. Ang II 1 day before i.v. drug application (arrow). *p < 0.05 versus vehicle-treated animals.

Effect of Intravenously Applied Telmisartan on MAP Response to i.c.v.-Injected Ang II. Ang II (30 ng/kg of body weight) injected i.c.v. elicited an immediate and sustained increase in MAP of about 25 to 30 mm Hg. Pretreatment of the rats with telmisartan (i.v.) at various doses significantly reduced the MAP response to i.c.v. Ang II in a dose- and time-dependent manner (Fig. 1). The lower doses of telmisartan (0.3 and 1 mg/kg of body weight) significantly inhibited the Ang II-induced drinking response over 4 h, but had no effect after 24 h.

Acute and Chronic Effects of Orally Applied Telmisartan on MAP Response to i.v.-Injected Ang II. Ang II (30 ng/kg of body weight) injected i.v. elicited an immediate, short-lasting increase in MAP of about 35 to 40 mm Hg. Pretreatment of the rats with telmisartan (0.3–10 mg/kg of body weight i.v.) markedly reduced the MAP response to i.v. Ang II 4 and 24 h after drug application (Fig. 2). Telmisartan injected i.v. at the highest dose of 10 mg/kg of body weight inhibited the MAP response to i.v. Ang II by 60 to 70% over a time interval of 4 h. The lowest dose of telmisartan (0.3 mg/kg of body weight) slightly inhibited the Ang II-induced MAP responses 4 h after drug intake by 35% and 25%, respectively. The higher doses of telmisartan (3 and 10 mg/kg of body weight) partially inhibited the MAP response to i.v. Ang II by 37 and 42% even 24 h after drug application (Fig. 2).

Acute and Chronic Effects of Orally Applied Telmisartan on Drinking Response to i.c.v.-Injected Ang II. Acute pretreatment of rats with telmisartan orally at various doses significantly reduced the drinking response to i.c.v. Ang II in a dose- and time-dependent manner. Statistical analysis was performed in two groups of rats. Group 1 (F = 2.85, p < 0.036): basal (N.S.); 0.5 h (F = 11.32, p < 0.0001); 4 h (F = 2.68, p < 0.048). Group 2 (F = 2.73, p < 0.040): basal (N.S.); 2 h (F = 4.50, p < 0.004); 24 h (N.S.) (Fig. 3). The AVP release in response to i.c.v. Ang II was nearly completely blocked over 2 h and still inhibited 4 h following i.v. injection of telmisartan at a dose of 10 mg/kg of body weight (Fig. 3). The lowest dose of telmisartan (0.3 mg/kg of body weight) had only slight effects on Ang II-induced release of AVP 0.5 h following i.v. drug injection.

Effect of Intravenously Applied Telmisartan on MAP Response to i.v.-Injected Ang II. Ang II (30 ng/kg of body weight) injected i.v. elicited an immediate, short-lasting increase in MAP of about 35 to 40 mm Hg. Pretreatment of the rats with telmisartan (0.3–10 mg/kg of body weight i.v.) markedly reduced the MAP response to i.v. Ang II 4 and 24 h after drug application (F = 29.82, p < 0.0001): basal (N.S.); 4 h (F = 91.42, p < 0.0001); 24 h (F = 23.48, p < 0.0001) (Fig. 4). The inhibition of the MAP response to i.v. Ang II was very effective and long-lasting following i.v. application of higher doses of 3 and 10 mg/kg of body weight (88 and 84% inhibition 4 h after drug intake and 77 and 65% 24 h after drug intake, respectively) (Fig. 4). The lowest dose of telmisartan (0.3 mg/kg of body weight) markedly inhibited the Ang II-induced MAP responses 4 h after drug intake by 65%, but was only slightly effective 24 h after drug intake (30% inhibition) (Fig. 4).

Acute and Chronic Effects of Orally Applied Telmisartan on Drinking Response to i.v.-Injected Ang II. Acute pretreatment of rats with telmisartan orally at various doses significantly reduced the drinking response to i.v. Ang II in a dose- and time-dependent manner. Statistical analysis was performed in two groups of rats. Group 1 (F = 4.36, p < 0.006): basal (N.S.); 0.5 h (F = 4.65, p < 0.005); 4 h (F = 4.37, p < 0.006). Group 2 (F = 3.23, p < 0.022): basal (N.S.); 2 h (F = 5.61, p < 0.001); 24 h (F = 3.15, p < 0.024) (Fig. 5). The onset of action of telmisartan was delayed reach-
Effect of telmisartan administered intravenously at various doses on mean arterial pressure increase (ΔMAP) induced by Ang II (30 ng/kg of body weight) injected i.v. before (B) and at various time points post antagonist treatment. ○, vehicle; ●, 0.3 mg/kg; △, 1 mg/kg; ▲, 3 mg/kg; □, 30 mg/kg. Arrow indicates the time point of i.v. drug injection. *p < 0.05 versus vehicle-treated animals.

Fig. 4. Effect of telmisartan administered intravenously at various doses on mean arterial pressure increase (ΔMAP) induced by Ang II (30 ng/kg of body weight) injected i.v. before (B) and at various time points post antagonist treatment. ○, vehicle; ●, 0.3 mg/kg; △, 1 mg/kg; ▲, 3 mg/kg; □, 30 mg/kg. Arrow indicates the time point of i.v. drug injection. *p < 0.05 versus vehicle-treated animals.

Fig. 5. Effect of telmisartan administered orally at various doses on drinking response induced by Ang II (30 ng/kg of body weight) injected i.c.v. before and at various time points post acute antagonist treatment. ○, vehicle; ●, 1 mg/kg; △, 3 mg/kg; ▲, 10 mg/kg; □, 30 mg/kg. B, basal water intake in response to i.c.v. Ang II 1 day before oral drug application (arrow). *p < 0.05 versus vehicle-treated animals.

Chronic pretreatment of rats with telmisartan revealed a similar dose- and time-dependent inhibition of drinking in response to i.c.v. Ang II compared with acute pretreatment (Fig. 6). Group 1 (F = 6.01, p < 0.001); basal (N.S.); 0.5 h (F = 5.95, p < 0.001); 4 h (F = 6.48, p < 0.001). Group 2 (F = 3.26, p < 0.026); basal (N.S.); 2 h (F = 6.57, p < 0.001); 24 h (F = 2.69, p < 0.05). Inhibition of the drinking response to i.c.v. Ang II was nearly complete (80–100%) following chronic oral application of telmisartan at a dose of 30 mg/kg of body weight. Oral treatment with the lower doses of 3 and 10 mg/kg of body weight led to a continuous 50 to 80% inhibition of the drinking response to i.c.v. Ang II (Fig. 6). Chronic application of the lowest dose of 1 mg/kg of body weight telmisartan significantly inhibited the drinking response to i.c.v. Ang II by 47% 2 h after the last drug application, but not at other time points (Fig. 6).

Fig. 6. Effect of telmisartan administered orally for 1 week at various doses on drinking response induced by Ang II (30 ng/kg of body weight) injected i.c.v. at various time points following the last antagonist treatment (arrow). ○, vehicle; ●, 1 mg/kg; △, 3 mg/kg; ▲, 10 mg/kg; □, 30 mg/kg. *p < 0.05 versus vehicle-treated animals.

Acute and Chronic Effects of Orally Applied Telmisartan on MAP Response to i.c.v.-Injected Ang II. Acute pretreatment of rats with telmisartan at the highest oral dose of 30 mg/kg of body weight inhibited the MAP response to i.c.v. Ang II by 60 to 70% over a time interval of 4 h (Fig. 7). The lower oral doses of 1 and 3 mg/kg of body weight did not significantly inhibit the MAP response to i.c.v. Ang II at any time point (Fig. 7). At 24 h after drug intake, the Ang II-induced MAP response was inhibited by 30% following oral application of 30 mg/kg telmisartan but not at lower doses (Fig. 7) (F = 9.48, p < 0.0001); basal (N.S.); 0.5 h (F = 6.27, p < 0.001); 2 h (F = 6.31, p < 0.001); 4 h (F = 8.73, p < 0.0001); 24 h (F = 2.65, p < 0.05).

Chronic pretreatment of rats with telmisartan revealed a dose- and time-dependent inhibition of the MAP response to i.c.v. Ang II (Fig. 8) (F = 11.14, p < 0.0001); basal (N.S.); 0.5 h (F = 8.72, p < 0.0001); 2 h (F = 9.12, p < 0.0001); 4 h (F = 8.39, p < 0.0001); 24 h (F = 5.00, p < 0.003). At a dose of 30 mg/kg of body weight telmisartan the MAP responses to i.c.v. Ang II were markedly blocked by 60 to 75% over 4 h following the last drug intake and remained inhibited 24 h following the last drug intake (54% inhibition). The lowest dose of 1 mg/kg of body weight telmisartan did not significantly inhibit the MAP response to i.c.v. Ang II at any time point (Fig. 8).

Fig. 7. Effect of telmisartan administered orally at various doses on mean arterial pressure increase (ΔMAP) induced by Ang II (30 ng/kg of body weight) injected i.c.v. before and at various time points post acute antagonist treatment. ○, vehicle; ●, 1 mg/kg; △, 3 mg/kg; ▲, 10 mg/kg; □, 30 mg/kg. B, basal MAP response to i.c.v. Ang II 1 day before oral drug application (arrow). *p < 0.05 versus vehicle-treated animals.

Fig. 8. Effect of telmisartan administered orally at various doses on mean arterial pressure increase (ΔMAP) induced by Ang II (30 ng/kg of body weight) injected i.c.v. before and at various time points post acute antagonist treatment. ○, vehicle; ●, 1 mg/kg; △, 3 mg/kg; ▲, 10 mg/kg; □, 30 mg/kg. B, basal MAP response to i.c.v. Ang II 1 day before oral drug application (arrow). *p < 0.05 versus vehicle-treated animals.
Acute and Chronic Effects of Orally Applied Telmisartan on AVP Release in Response to i.c.v.-Injected Ang II. Acute pretreatment of rats with telmisartan orally at various doses significantly reduced the AVP release in response to i.c.v. Ang II in a dose- and time-dependent manner (Fig. 9). Analysis of Variance: 0.5 h ($F = 2.64, p < 0.048$); 2 h ($F = 4.03, p < 0.011$); 4 h ($F = 6.363, p < 0.001$) and 24 h ($F = 4.09, p < 0.008$). The AVP release in response to i.c.v. Ang II was markedly suppressed over 24 h following oral application of telmisartan at doses of 10 and 30 mg/kg of body weight. The lowest dose of 1 mg/kg of body weight did not significantly inhibit the AVP release in response to i.c.v. Ang II at any time-point (Fig. 9). The AVP release in response to i.c.v. Ang II was continuously blocked over 24 h following chronic pretreatment of rats with telmisartan at a dose of 30 mg/kg of body weight (Fig. 10). Analysis of variance: 0.5 h ($F = 2.66, p < 0.049$); 2 h ($F = 5.85, p < 0.001$); 4 h ($F = 3.99, p < 0.01$); and 24 h ($F = 2.63, p < 0.05$). The lower doses of 3 and 10 mg/kg of body weight significantly inhibited the release of AVP by 71 and 55% and by 40 and 68% at 0.5 and 24 h following the last drug application. The lowest dose of 1 mg/kg of body weight did not significantly affect the AVP release in response to i.c.v. Ang II at either time point. (Fig. 10).

Acute and Chronic Effects of Orally Applied Telmisartan on AVP Release in Response to i.c.v.-Injected Ang II. Acute pretreatment of rats with telmisartan orally at various doses significantly reduced the AVP release in response to i.c.v. Ang II in a dose- and time-dependent manner (Fig. 9). Analysis of Variance: 0.5 h ($F = 2.64, p < 0.048$); 2 h ($F = 4.03, p < 0.011$); 4 h ($F = 6.363, p < 0.001$) and 24 h ($F = 4.09, p < 0.008$). The AVP release in response to i.c.v. Ang II was markedly suppressed over 24 h following oral application of telmisartan at doses of 10 and 30 mg/kg of body weight. The lowest dose of 1 mg/kg of body weight did not significantly inhibit the AVP release in response to i.c.v. Ang II at any time-point (Fig. 9). The AVP release in response to i.c.v. Ang II was continuously blocked over 24 h following chronic pretreatment of rats with telmisartan at a dose of 30 mg/kg of body weight (Fig. 10). Analysis of variance: 0.5 h ($F = 2.66, p < 0.049$); 2 h ($F = 5.85, p < 0.001$); 4 h ($F = 3.99, p < 0.01$); and 24 h ($F = 2.63, p < 0.05$). The lower doses of 3 and 10 mg/kg of body weight significantly inhibited the release of AVP by 71 and 55% and by 40 and 68% at 0.5 and 24 h following the last drug application. The lowest dose of 1 mg/kg of body weight did not significantly affect the AVP release in response to i.c.v. Ang II at either time point. (Fig. 10).

Effect of Orally Applied Telmisartan on CSF Drug Concentration. The concentrations of telmisartan in the CSF following 8 days of oral treatment at various doses are shown in Fig. 11. Four hours following the last application of the drug, telmisartan was readily detectable in the CSF reaching concentrations of $0.87 \pm 0.27$ ng/ml (approximately 1.7 nmol/l) up to $46.5 \pm 11.6$ ng/ml (approximately 91 nmol/l) following oral treatment with 1 and 30 mg/kg of body weight per day of telmisartan, respectively (Fig. 11).

Discussion

The AT$_1$ receptor antagonist telmisartan administered peripherally, attenuated dose and time dependently centrally mediated actions of Ang II, namely, the pressor response, AVP release, and drinking response induced by i.c.v.-applied Ang II. Furthermore, the dose-dependent increase in telmisartan concentration in the CSF following chronic oral treatment is an additional indicator for a central penetration of the drug.

An overactive renin-angiotensin system in the brain has been suggested to be involved in the development and maintenance of hypertension in spontaneously hypertensive rats.

![Fig. 8.](image8.png) Effect of telmisartan administered orally for 1 week at various doses on mean arterial pressure increase (ΔMAP) induced by Ang II (30 ng/kg of body weight) injected i.c.v. at various time points following the last antagonist treatment (arrow). ○, vehicle; ●, 1 mg/kg; △, 3 mg/kg; ▲, 10 mg/kg; □, 30 mg/kg. *p < 0.05 versus vehicle-treated animals.

![Fig. 9.](image9.png) Effect of telmisartan administered orally at various doses on AVP release (pg/ml plasma) induced by Ang II (30 ng/kg of body weight) injected i.c.v. before and at various time points post acute antagonist treatment. ○, vehicle; ●, 1 mg/kg; △, 3 mg/kg; ▲, 10 mg/kg; □, 30 mg/kg. B, basal release of AVP in response to i.c.v. Ang II 1 day before oral drug application (arrow). *p < 0.05 versus vehicle-treated animals.

![Fig. 10.](image10.png) Effect of telmisartan administered orally at various doses for 1 week on AVP release (pg/ml plasma) induced by Ang II (30 ng/kg of body weight) injected i.c.v. at various time points following the last antagonist treatment (arrow). ○, vehicle; ●, 1 mg/kg; △, 3 mg/kg; ▲, 10 mg/kg; □, 30 mg/kg. *p < 0.05 versus vehicle-treated animals.

![Fig. 11.](image11.png) Concentration of the AT$_1$ receptor antagonist telmisartan in the cerebrospinal fluid following 8 days of oral treatment at doses of 1 to 30 mg/kg. CSF was collected 4 h after the last drug intake. *p < 0.05 versus vehicle-treated animals.
Inhibition of brain AT$_1$ receptor expression by means of antisense oligonucleotides reduced hypertension in spontaneously hypertensive rats, demonstrating the involvement of brain AT$_1$ receptors in maintaining increased blood pressure in this model of genetic hypertension (Gyurko et al., 1993). Besides the blood pressure control, the brain RAS contributes to the regulation of autonomic nervous system activity as well as to the regulation of a number of physiological processes, including electrolyte homeostasis, secretion of pituitary hormones, water intake, and cognitive processes (Unger et al., 1988; Saavedra, 1992; Hirawa et al., 1999). Therefore, long-term treatment of patients suffering from essential hypertension with AT$_1$ receptor antagonists that can penetrate the blood-brain barrier might affect a number of physiological functions mediated by these receptors, including the central cardiovascular regulation.

However, results concerning the central effects of systemically administered losartan or other nonpeptide AT$_1$ receptor antagonists are contradictory. Losartan administered orally was shown not to interact with those brain AT$_1$ receptors that were inhibited when the antagonist was injected i.c.v. (Wong et al., 1990). Similarly, Bui et al. (1992) demonstrated that chronic oral treatment with losartan did not affect the dipsogenic and pressor responses induced by i.c.v. Ang II. These data suggest that losartan cannot cross the blood-brain barrier in sufficient amounts to block central AT$_1$ receptors. However, Bui et al. (1992) used only one single dose of losartan (3 mg/kg of body weight). Higher doses of 10 or 30 mg/kg of body weight of losartan given orally, which are usually required to adequately reduce blood pressure in hypertensive rats (Gohlke et al., 1996), were not investigated. Other studies clearly demonstrated inhibitory effects of peripherally administered losartan or its active metabolite EXP 3174 on responses mediated by brain Ang II (Li et al., 1993; Polidori et al., 1996; Culman et al., 1999). Furthermore, intravenously administered losartan at doses of 1 to 10 mg/kg of body weight inhibited binding to AT$_1$ receptors in brain structures inside the blood-brain barrier as assessed by in vitro autoradiography (Song et al., 1991; Zhuo et al., 1994). In a previous study, irbesartan and losartan administered systemically at various doses 30 min to 3 h prior to i.c.v. Ang II equipotentily inhibited the pressor responses to the peptide. However, high doses of the antagonists (30–100 mg/kg of body weight i.v.) had to be used to achieve satisfactory inhibition of centrally mediated actions of Ang II. Moreover, even at the highest doses of losartan and irbesartan used, the inhibition of the central Ang II effects was never complete (Culman et al., 1999). The central effects of losartan and irbesartan shown by Culman et al. (1999) can be directly compared with the effects of telmisartan reported in the present study. Both studies were performed in the same laboratory and under comparable experimental conditions. Polidori et al. (1996) reported that p.o.-administered losartan (20 μmol/kg of body weight; approximately 10 mg/kg of body weight) reduced drinking when given 4 h, but not 12 h prior to i.c.v. Ang II. Similarly, unpublished data from our group suggest that following acute i.v. treatment irbesartan can inhibit the central effects of Ang II only for few hours. In contrast, we demonstrate in the present study that much lower doses of telmisartan (0.3–10 mg/kg of body weight i.v. or 1–30 mg/kg of body weight p.o.) can effectively inhibit centrally mediated Ang II effects. The higher doses of 10 mg/kg i.v. and 30 mg/kg p.o. of the antagonist produced a nearly complete and sustained blockade of the peptide-mediated actions. Most importantly, the central effects of telmisartan can be observed at doses that have to be used to reduce blood pressure in hypertensive rats. For example, in a study by Wagner et al. (1998), 10 mg/kg of body weight of telmisartan p.o. reduced blood pressure in stroke-prone spontaneously hypertensive rats to a similar extent as 20 mg/kg of body weight losartan or 50 mg/kg of body weight captopril. Furthermore, in spontaneously hypertensive rats with streptozotocin-induced diabetes mellitus, oral doses of 3 and 10 mg/kg telmisartan had to be used to adequately lower blood pressure (Wienen et al., 2001). The results of the present study reveal a slightly stronger inhibition of peripherally mediated compared with centrally mediated pressor responses to Ang II following acute i.v. application of telmisartan. These differences are likely to be a result of different peak concentrations of the antagonist in plasma and CSF following oral treatment. Plasma peak concentrations of telmisartan reached 43.5 ng/ml following oral treatment of rats with 1 mg/kg (Wienen et al., 2000), thus being 40 to 50 times higher than telmisartan concentrations in CSF detected in the present study. Telmisartan concentrations in the CSF following 8 days of oral treatment with the lowest and highest dose (1 and 30 mg/kg of body weight per day) ranged from 0.9 ng/ml to 46.5 ng/ml, which corresponds to 2 to 100 nmol/l. In view of the low protein content in the CSF, when compared with plasma (CSF/plasma ratio of 0.004) (Rapoport, 1976), one can expect that lower amounts of telmisartan are bound to protein, which would then result in higher concentrations of free telmisartan that can effectively interact with its receptors. Binding studies performed in membrane preparations derived from rat lung in the presence of 0.2% bovine albumin revealed a high affinity of telmisartan to the AT$_1$ receptor with a $K_I$ value of 3.7 nmol/l (Wienen et al., 1993). Thus, the CSF concentrations of telmisartan measured in the present study appear to be sufficient to exert an effective central blockade of AT$_1$ receptors.

AT$_1$ receptors in brain structures that are located inside the blood-brain barrier, namely, the paraventricular and supraoptic nuclei mediate the release of AVP in response to i.c.v.-injected Ang II as demonstrated by microinjection studies. In these studies, losartan microinjected into the paraventricular or supraoptic nuclei attenuated the release of AVP in response to i.c.v. Ang II (Veltmar et al., 1992; Qadri et al., 1993). The higher potency of telmisartan to inhibit the i.c.v. Ang II-induced effects compared with losartan or irbesartan might therefore be linked to a more efficient inhibition of AT$_1$ receptors in brain structures that are located inside the blood-brain barrier.

Compared with losartan and irbesartan the apparent higher capability of telmisartan to penetrate into the brain tissue might be explained by the hydrophobic/hydrophilic properties of the drugs. Telmisartan is more lipophilic than losartan and irbesartan with a distribution ratio for octanol/water of approximately log D: +1.9 measured at physiological pH (Peter Morsing, personal communication) compared with log D at pH 7.4 of +0.8 for losartan and of +1.0 for irbesartan and exceeds the lipophilicity of the active compound of losartan, EXP 3174 (log D at pH 7.4: −1.6) by 4 orders of magnitude (Morsing et al., 1999). In another study,
this difference in lipophilicity between telmisartan and EXP3174 was even more dramatic (log $P = +3.2$ versus log $P = -2.45$) (Wienen et al., 2000). However, lipophilicity may not be the only predictor for the central penetration of these drugs. In line with this suggestion is the finding that EXP 3174 can cross the blood-brain barrier more effectively than losartan (Polidori et al., 1996) despite a lower distribution ratio for octanol/water as mentioned above. Furthermore, an inhibition of AT$_1$ receptor binding in several brain areas has been demonstrated following subcutaneous infusion of a more hydrophilic AT$_1$ receptor antagonist candesartan (Nishimura et al., 2000). Other mechanisms such as active transport of the drugs through the blood-brain barrier might be considered to explain this phenomenon.

AT$_1$ receptor antagonists specifically block the effects of Ang II on the AT$_1$ receptor and redirect the effects of Ang II to the unopposed AT$_2$ receptor. The AT$_2$ receptor is present in fetal tissues, including the central nervous system and is reexpressed during certain pathological conditions such as infarction and wound healing (De Gasparo et al., 2000). The AT$_2$ receptor is involved in neuronal cell differentiation (Laflamme et al., 1996; Meffert et al., 1996) and nerve regeneration processes (Lucius et al., 1998), and stimulation of these receptors can inhibit proliferation (Unger et al., 1996; De Gasparo et al., 2000). Therefore, stimulation of the AT$_2$ receptor as an indirect result of AT$_1$ receptor antagonism may contribute to the overall effects of AT$_1$ receptor antagonists during pathophysiological conditions such as cerebral ischemia and myocardial infarction.

In a recent study, we demonstrated in normotensive rats that blockade of central AT$_1$ receptors induced by i.c.v. infusion of the AT$_1$ receptor antagonist irbesartan for 5 days prior to the occlusion of the middle cerebral artery exerted neuroprotective effects in ischemic neuronal tissue and improved recovery from brain ischemia (Daí et al., 1999). In addition, the overexpression of c-Fos and c-Jun proteins in the brain ipsilateral to the injury, which positively correlated with the degree of neurological deficits following focal brain ischemia, was markedly reduced by central pretreatment with the AT$_1$ receptor antagonist. Central AT$_2$ receptors contribute to the beneficial effects of AT$_1$ receptor antagonists on neurological outcome following cerebral ischemia (J. Culman, W.-J. Dai, P. Gohlke, A. Blume, and T. Unger, submitted). However, peripheral pretreatment with irbesartan was without effect, because the drug was probably not able to completely and long-lasting block central AT$_1$ receptors (Polidori et al., 1998; Funk et al., 2000). Whether orally applied AT$_1$ receptor antagonists, which can cross the blood-brain barrier more readily than irbesartan, would exert beneficial effects in this animal model of focal brain ischemia is investigated at the present time.

In conclusion, our results demonstrate that the AT$_1$ receptor antagonist telmisartan can penetrate the blood-brain barrier to inhibit centrally mediated effects of Ang II following peripheral administration.

References


Address correspondence to: Dr. Peter Gohlke, Institute of Pharmacology, Christian-Albrechts-University of Kiel, Hospitalstrasse 4, 24105 Kiel, Germany. E-mail: peter.gohlke@pharmakologie.uni-kiel.de