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Effects of systemic treatment with irbesartan and losartan on central responses to angiotensin II in conscious, normotensive rats

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Abstract

Angiotensin AT_1 receptor antagonists represent a novel class of cardiovascular drugs. In conscious, normotensive rats, irbesartan ((2-*n*-butyl-3-[(2′-(1*H*-tetrazol-5-yl)-biphenyl-4-yl) methyl]-1,3-diaza-spiro[4,4]non) and losartan ((2*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl) methyl] imidazol), two specific, high- affinity angiotensin AT_1 receptor antagonists administered intravenously (i.v.) at doses of 0.3, 1, 3 and 10 mg/kg body weight, or orally (p.o.) at doses of 1, 3, 10 and 30 mg/kg body weight, antagonized the pressor responses to i.v. angiotensin II (50 ng/kg body weight) in a dose-related manner and with similar potency. In the following sets of experiments, we tested the hypothesis that these angiotensin AT_1 receptor antagonists, when applied systemically, can inhibit the effects of angiotensin AT_1 receptor stimulation in the brain. Irbesartan and losartan were administered i.v. or p.o. at doses of 3, 10, 30 and 100 mg/kg body weight. The responses to 100 ng angiotensin II injected into the lateral brain ventricle (i.c.v.), namely blood pressure increase, vasopressin release into the circulation and drinking, were recorded for up to 3 h. While both angiotensin AT_1 receptor antagonists dose-dependently attenuated the pressor responses to central angiotensin AT_1 receptor stimulation to a similar degree (maximal inhibition, irbesartan: 62% i.v., 39% p.o.; losartan: 62% i.v., 46% p.o.; respectively), irbesartan was more effective with respect to the inhibition of vasopressin release (76% i.v., 65% p.o.) and drinking (63% i.v., 79% p.o.) than losartan (58% i.v., 33% p.o and 22% i.v., 56% p.o., respectively). We conclude that systemically administered angiotensin AT_1 receptor antagonists have access to central angiotensin receptors. The degree of central angiotensin AT_1 receptor blockade following peripheral application may vary between different representatives of this class of drugs.

Keywords: Angiotensin II; Irbesartan; Losartan; Angiotensin AT_1 receptor; Pressor response; Vasopressin release; Drinking behaviour; Brain; (Rat)

1. Introduction

Angiotensin II, the effector peptide of the renin–angiotensin system, exerts its effects by interacting with specific receptors. Two subtypes of angiotensin receptors, AT_1 and AT_2, have been cloned and pharmacologically characterised (Timmermans et al., 1993; Unger et al., 1996). Peripheral angiotensin AT_1 receptors mediate most of the known effects of angiotensin II on hemodynamics, cardiac and renal functions. Angiotensin peptides acting at peripheral angiotensin AT_1 receptors have been implicated in the pathogenesis of various cardiovascular diseases. Antihypertensive effects of the non-peptide angiotensin AT_1 receptor antagonists have been demonstrated in genetically and experimentally induced animal models of hypertension and in hypertensive patients (Wong et al., 1990b; Van den Meiracker et al., 1995; Griendling et al., 1996; Gillis and Markham, 1997). This class of drugs may also be beneficial in the treatment of congestive heart failure, cardiac hypertrophy, myocardial infarction and ventricular remodelling as well as restenosis after angioplasty (Griendling et al., 1996).

Besides the peripheral renin–angiotensin system, the existence of a brain renin–angiotensin system has been

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firmly established. All components of the renin–angiotensin system have been identified in the brain, and it has become evident that the brain can generate its own angiotensin peptides independently of the peripheral renin–angiotensin system (Unger et al., 1988; Steckelings et al., 1992). The brain renin–angiotensin system has been implicated in cardiovascular control, regulation of volume and electrolyte homeostasis, dipsogenic responses and endocrine functions (Unger et al., 1988; Saavedra, 1992). There is also evidence that the brain renin–angiotensin system may substantially contribute to the development and maintenance of hypertension. In spontaneously hypertensive rats, an animal model of genetic hypertension, biochemical, neurophysiological and molecular biological studies have yielded results pointing to an overactive renin–angiotensin system in the brain (Phillips and Kimura, 1988). Spontaneously hypertensive rats also show increased angiotensin II-binding activity in brain areas implicated in the cardiovascular control (Plunkett and Saavedra, 1985; Gutkind et al., 1988).

While the antihypertensive effects of angiotensin AT_1 receptor antagonists are mainly ascribed to the inhibition of angiotensin AT_1 receptors in the periphery, evidence has been provided that inhibition of central angiotensin AT_1 receptors may also contribute to the antihypertensive effects of these compounds (Gyurko et al., 1993). However, experiments aimed to investigate the penetration of the blood–brain barrier by losartan ((2*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl) methyl] imidazol), the first non-peptide angiotensin AT_1 receptor antagonist used for the treatment of hypertension, have yielded conflicting results so far (Song et al., 1991; Bui et al., 1992; Li et al., 1993).

Irbesartan ((2-*n*-butyl-3-[(2′-(1*H*-tetrazol-5-yl)-biphenyl-4-yl) methyl]-1,3-diaza-spiro[4,4]non) represents another potent and selective non-peptide angiotensin AT_1 receptor antagonist which inhibits the binding of ^{125}I-angiotensin II in rat liver membranes with an IC_{50} of 1.3 nM. In the same preparation, the affinity of irbesartan for angiotensin AT_1 binding sites exceeds that of losartan (IC_{50} = 14 nM). In conscious rats, irbesartan and losartan equipotently antagonized the pressor responses to intravenously (i.v.) injected angiotensin II in a dose-related manner. In conscious cynomolgus monkeys, irbesartan was at least 10-fold more potent than losartan (Cazaubon et al., 1993; Timmermans et al., 1993). Little is known as to what extent irbesartan, which appears to be more lipophilic than losartan (Cazaubon et al., 1993), is able to interact with central angiotensin AT_1 receptors after systemic treatment. In the present study, we compared the effects of irbesartan and losartan administered i.v. or orally with respect to their ability to affect the following angiotensin II effects mediated by central angiotensin AT_1 receptors: increase in mean arterial pressure, release of vasopressin from the posterior pituitary into the circulation, and drinking response.

2. Materials and methods

Male, normotensive Wistar rats weighing 300 to 350 g obtained from Charles River (Sulzfeld, Germany) were used. Rats were housed on a 12 h/12 h light dark cycle with free access to food and water.

2.1. Surgical methods

Rats were anaesthetized with an intraperitoneal injection of chloralhydrate (400 mg/kg body weight). For intracerebroventricular (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, Calif, USA). 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, 25 ng angiotensin II were injected i.c.v. to verify the correct position of the i.c.v. cannula. Only those rats which responded with immediate drinking were included in the study. These rats were anaesthetized again, and a polyethylene catheter was inserted through the femoral artery into the abdominal aorta for measurement of blood pressure or for blood withdrawal. In some groups, a polyethylene catheter (PP 50) was inserted into the femoral vein for i.v. 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 48 h after femoral artery- and vein cannulation.

2.2. General procedures

2.2.1. Measurement of cardiovascular responses

All experiments were carried out in conscious, freely moving rats. The femoral artery catheter was connected to the transducer. The experiments were started when the animals were resting and when basal mean arterial pressure and heart rate were stable. Irbesartan or losartan were administered i.v. through the i.v. catheter connected to an extension catheter with syringe. When the angiotensin AT_1 receptor antagonists were administered intragastrically, the femoral artery catheter was connected to the transducer after intragastric treatment. Angiotensin II was dissolved in isotonic saline and injected as a bolus i.v. (50 ng/kg body weight) or i.c.v. (100 ng) before and at various time points after i.v. or intragastric treatment with the antagonists or vehicle (controls).

Measurements of mean arterial pressure were performed via the arterial catheters using a pressure transducer (DTX/Plus; Spectramed, Oxnard, CA) connected to a pressure processor (Gould, Valley View, OH) coupled to a

Gould Brush recorder (Gould Series 2400, Gould). The analogue output signal of mean arterial pressure from the Gould Brush pressure computer was digitalized and then processed using a computer programme. This programme permits sampling of hemodynamic data from experimental animals directly onto a hard disk and subsequent analysis with an interactive and graphic programme. The analysis of the mean arterial pressure changes has been described in detail recently (Culman et al., 1997).

Normotensive rats were used in the present study. Basal mean arterial pressure values in individual groups of rats varied between 96.4 ± 3.1 and 104.8 ± 4.1 mm Hg and did not differ significantly.

2.2.2. Blood collection for vasopressin determination in plasma

All experiments were carried out 48 h after intravascular surgery. Experiments were started when rats were resting in their home cages. Blood (1 ml) was withdrawn from the femoral artery catheter. On the day of experiment, a basal blood sample was collected in each rat. An equal volume of saline was slowly infused to replace the volume withdrawn. Two to 3 h later, rats were treated either i.v. or orally with irbesartan or losartan, while controls received vehicle. Each rat received only one single dose of one antagonist. Thirty minutes after the i.v. injection or 60 min after the oral treatment, angiotensin II (100 ng) was injected i.c.v., and blood (1 ml) was withdrawn 90 s thereafter. The volume was replaced with 1 ml of physiological saline. The peak of vasopressin in plasma in response to i.c.v. angiotensin II lies within a time interval between the 1st and 3rd min after the i.c.v. injection of the peptide (Hogarty et al., 1992).

2.2.3. Determination of vasopressin in plasma

Blood was collected in ice-cold Eppendorf tubes. Blood samples were centrifuged immediately at $3000 \times g$ in a refrigerated centrifuge. Plasma vasopressin 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 inter-assay coefficient of variance is 12.2% (Rascher et al., 1981). The detection limit was 1.5 pg ml.

2.2.4. Determination of drinking response

Thirty or 60 min prior to i.c.v. angiotensin II injection (100 ng), rats were treated i.v. or orally with vehicle (controls), or irbesartan and losartan. Water intake was determined by weighing of water which the rat drank during a 15-min time period starting immediately after the i.c.v. injection of angiotensin II.

2.3. Experimental protocols

2.3.1. Effect of i.v. treatment with irbesartan and losartan on pressor responses to angiotensin II injected i.v.

Rats ($n = 7–10$ per group) were treated i.v. with various doses of irbesartan and losartan: 0.3, 1, 3 and 10 mg/kg body weight. Controls ($n = 7$) received vehicle. Pressor responses induced by i.v.-injected angiotensin II (50 ng/kg body weight) were recorded before and 5, 15, 30 and 90 min after i.v. treatment with vehicle or with the angiotensin AT_1 receptor antagonists.

2.3.2. Effect of p.o. treatment with irbesartan and losartan on pressor responses to angiotensin II injected i.v.

Rats were treated p.o. with vehicle (controls, $n = 7$) or with irbesartan and losartan ($n = 6–7$ per group) at doses 1, 3, 10 and 30 mg/kg body weight. Pressor responses induced by i.v.-injected angiotensin II (50 mg/kg body weight) were recorded before and 15, 30, 60 and 90 min after p.o. treatment with vehicle or with the angiotensin AT_1 receptor antagonists.

2.3.3. Effect of i.v. treatment with irbesartan and losartan on pressor responses to angiotensin II injected i.c.v.

Rats ($n = 6–8$ per group) were treated i.v. with various doses of irbesartan or losartan: 3, 10, 30, 100 mg/kg body weight. Controls ($n = 6$) received vehicle. Angiotensin II (100 ng) was injected i.c.v. before and 60, 120 and 180 min after i.v. treatment with vehicle or the angiotensin AT_1 receptor antagonists, and the pressor responses were recorded. We observed in control, vehicle-treated rats, that the pressor response to the second i.c.v. angiotensin II injection was significantly smaller than that of the preceding one (most probably due to desensitization of angiotensin AT_1 receptors (Griendling et al., 1996)) when the time interval between the two consecutive i.c.v. angiotensin II injections was 30 min or shorter. Therefore, a separate experiment was carried out to investigate the effect of i.v. treatment with the angiotensin AT_1 receptor antagonists on the pressor response to i.c.v. angiotensin II injected 30 min thereafter. Rats ($n = 6–8$ per group) were treated i.v. with the above doses of irbesartan or losartan, controls ($n = 7$) received vehicle. Thirty minutes after the i.v. treatment, angiotensin II (100 ng) was injected i.c.v. in all groups of rats and the pressor responses to the peptide were recorded.

2.3.4. Effect of p.o. treatment with irbesartan and losartan on pressor responses to angiotensin II injected i.c.v.

The same protocol and the same doses of the angiotensin AT_1 receptor antagonists were employed as with the i.v. treatment. Seven to 10 rats were used in each group. Control rats ($n = 8$) were treated p.o. with vehicle. Again, in a separate experiment, the pressor responses to i.c.v. angiotensin II (100 ng) were recorded 30 min after

oral treatment of rats with vehicle ($n = 8$) or with 3, 10, 30 and 100 mg/kg body weight of irbesartan ($n = 7–8$ per group) or losartan ($n = 7–10$ per group).

2.3.5. Effect of i.v. treatment with irbesartan and losartan on i.c.v. angiotensin II-induced vasopressin release into the circulation

Rats were treated i.v. with 30 mg/kg body weight of irbesartan ($n = 10$) or losartan ($n = 11$) followed, 30 min later, by an i.c.v. injection of angiotensin II (100 ng). The control group ($n = 9$) was treated i.v. with vehicle 30 min prior to the i.c.v. angiotensin II injection. Ninety seconds after the i.c.v. peptide injection, blood (1 ml) was withdrawn for vasopressin determination. The volume removed was immediately replaced by physiological saline into the femoral vein. Separate groups of rats were treated i.v. either with vehicle (physiological saline) ($n = 12$) or irbesartan ($n = 8$) and losartan ($n = 6$), at a dose of 30 mg/kg body weight. Thirty minutes later, blood was withdrawn without prior i.c.v. injection of angiotensin II. These groups of rats were included to reveal possible effects of irbesartan or losartan on basal vasopressin release.

2.3.6. Effect of p.o. treatment with irbesartan and losartan on i.c.v. angiotensin II-induced vasopressin release into the circulation

In another series of experiments, rats were treated p.o. either with vehicle (controls, $n = 10$) or with 100 mg/kg body weight of irbesartan ($n = 12$) or losartan ($n = 12$), 60 min prior to the i.c.v. injection of angiotensin II (100 ng). Blood for vasopressin determination was withdrawn 90 s after the i.c.v. injection of the peptide. Again, separate groups of rats were treated orally either with vehicle ($n = 8$) or irbesartan ($n = 7$) and losartan ($n = 8$) (100 mg/kg body weight of each antagonist). Sixty minutes later, blood samples were collected without prior i.c.v. angiotensin II injection.

2.3.7. Effect of i.v. and p.o. treatment with irbesartan and losartan on i.c.v. angiotensin II-induced drinking response

The same protocol and the same doses of the angiotensin AT_1 receptor antagonists were used as in the previous experimental settings. Angiotensin II (100 ng) was injected i.c.v. 30 min post i.v. treatment with either vehicle or irbesartan and losartan (30 mg/kg body weight) ($n = 7$ in each group), or 60 min post p.o. administration of vehicle or the angiotensin AT_1 receptor antagonists (100 mg/kg body weight) ($n = 9$ in each group). Water intake was determined during a 15-min time period starting immediately after the i.c.v. angiotensin II injection.

2.4. Drugs

The non-peptide angiotensin AT_1 receptor antagonist, irbesartan, was a gift from Dr. C. Cazaubon, Sanofi Recherche, Montpellier, France. The non-peptide angiotensin AT_1 receptor antagonist, losartan, was a gift from Dr. R. Smith, DuPont-Merck, Wilmington, DE, USA. Angiotensin II was purchased from Sigma, Deisenhofen, Germany. Angiotensin II for i.v. injections was dissolved in physiological saline (50 ng angiotensin II/100 μl) and injected as a bolus at a dose of 50 ng/kg body weight. Angiotensin II for i.c.v. injections was also dissolved in physiological saline. Physiological saline was used as vehicle solution instead of artificial cerebrospinal fluid since in previous experiments, angiotensin II dissolved either in saline or in artificial cerebrospinal fluid had yielded identical results. Angiotensin II (100 ng) was injected i.c.v. in a total volume of 1 μl and flushed with 4 μl of physiological saline. Irbesartan for p.o. administration was dissolved in water by neutralization with a stoichiometric equivalent of KOH. Losartan was dissolved in water (pH value of the solution was 7.8–8.3). The antagonists were given by gavage in a volume 2.5 ml/kg body weight. For i.v. administration, irbesartan was solubilized in saline by neutralization with a stoichiometric equivalent of L-arginine (Cazaubon et al., 1993). To obtain a clear solution, a minimal volume of 5 M of KOH solution was added. The final pH of the irbesartan solution was 9.0–9.5. The vehicle solution contained the same concentration of L-arginine in physiological saline. The pH value of the vehicle solution was adjusted to 9.5. Losartan was dissolved in physiological saline (pH value of the solution was 7.8–8.3). In previous experiments, saline alone (pH 8.0) injected i.v. was without any appreciable effects on cardiovascular parameters. Vehicle and the angiotensin AT_1 receptor antagonists were injected slowly through the venous catheter in a volume of 1 ml/kg body weight. Because the molecular weights of irbesartan and losartan differ only slightly, nearly equimolar doses of the antagonists were administered.

2.5. Statistics

Results are presented as the means ± S.E.M. To evaluate whether the effects of irbesartan and losartan administered i.v. or orally at the same dose on mean arterial pressure responses to i.v. or i.c.v. angiotensin II differ over the time, the repeated measures of analysis of variance with two independent groups of subjects were used (Ludbrook, 1994). Because statistically significant differences were not detected ($P > 0.05$), no further pairwise comparisons were performed. The effects on mean arterial pressure responses to i.c.v. angiotensin II of irbesartan and losartan administered i.v. or orally 30 min prior to i.c.v. angiotensin II were analysed by one-way analysis of variance (ANOVA) followed by Dunnet test for pairwise comparisons between the control (vehicle-treated) group and other groups (Ludbrook, 1991). Pairwise comparisons between groups of rats treated with the same dose of

irbesartan and losartan were carried out with Tukey test (Ludbrook, 1991). The effects of i.v. or oral treatment with irbesartan and losartan on plasma vasopressin levels and drinking behaviour in response to i.c.v. angiotensin II were analysed by ANOVA followed by a post-hoc Bonferroni test (Ludbrook, 1994). Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effect of i.v. treatment with irbesartan and losartan on pressor responses to angiotensin II injected i.v.

Angiotensin II (50 ng/kg body weight) injected i.v. 5, 15, 30, 60 and 90 min post i.v. treatment with vehicle elicited consistent increases in the mean arterial pressure of about 50 mm Hg. Both angiotensin AT_1 receptor antagonists inhibited the pressor response to i.v. angiotensin II in a dose-dependent manner (Fig. 1). Losartan and irbesartan injected 5, 15, 30, 60 and 90 min prior to i.v. angiotensin II equipotently attenuated the pressor responses to the peptide (Fig. 1).

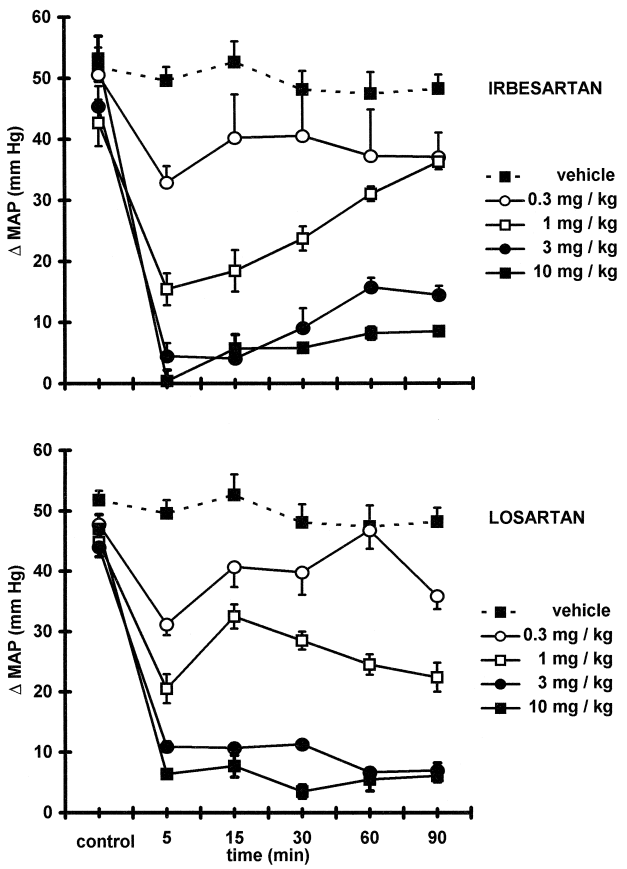

Fig. 1. Effect of irbesartan and losartan administered intravenously at various doses on mean arterial pressure increase (ΔMAP) (mm Hg) induced by angiotensin II (50 ng/kg body weight) injected intravenously before and at various time points post-antagonist treatment.

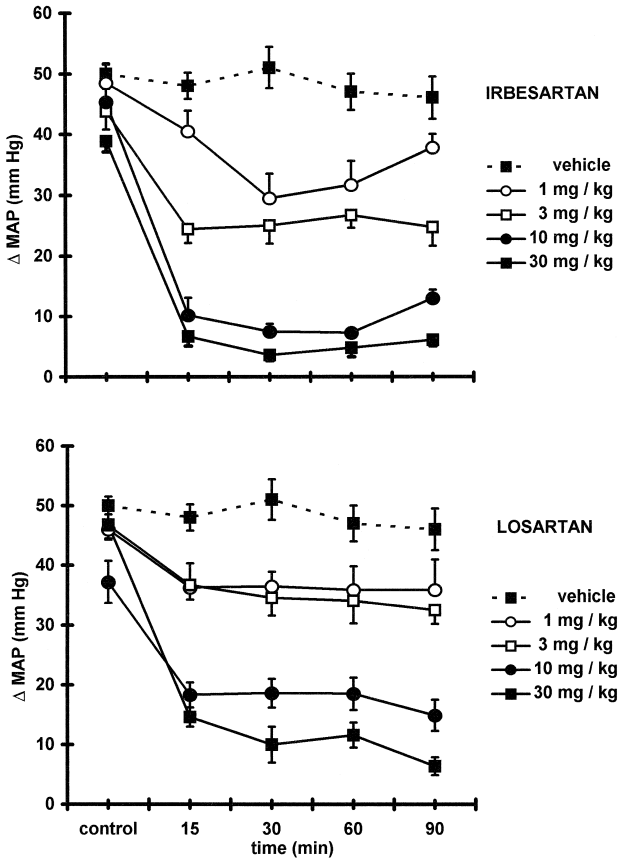

Fig. 2. Effect of irbesartan and losartan administered orally at various doses on mean arterial pressure increase (ΔMAP) (mm Hg) induced by angiotensin II (50 ng/kg body weight) injected intravenously before and at various time points post-antagonist treatment.

3.2. Effect of p.o. treatment with irbesartan and losartan on pressor responses to angiotensin II injected i.v.

Both antagonists administered p.o. at various doses inhibited dose-dependently and equipotently the pressor response to i.v. angiotensin II (Fig. 2).

3.3. Effect of i.v. treatment with irbesartan and losartan on pressor responses to angiotensin II injected i.c.v.

Angiotensin II (100 ng) injected i.c.v. elicited an immediate increase in mean arterial pressure of about 30 mm Hg. There were significant effects on pressor responses to angiotensin II injected i.c.v. 30 min after i.v. treatment of rats with various doses of irbesartan and losartan ($F(8,56) = 12.69$; $P < 0.001$). Irbesartan and losartan at the dose of 3 mg/kg injected i.v. 30 min prior to i.c.v. angiotensin II did not affect the pressor response to the peptide (Table 1). At the dose of 10 mg/kg, only irbesartan significantly reduced the mean arterial pressure response to i.c.v. angiotensin II ($P < 0.05$). Both antagonists administered at doses of 30 and 100 mg/kg were equally effective in inhibiting the i.c.v. angiotensin II-induced mean arterial

Table 1
Effect of irbesartan and losartan administered intravenously (i.v.) or orally (p.o.) on mean arterial pressure increase (ΔMAP) (mm Hg) induced by angiotensin II (100 ng) injected intracerebroventricularly

Pretreatment (antagonist)	Dose (mg/kg)	ΔMAP (mm Hg)			
		Mode of antagonist administration			
		i.v.	*n*	p.o.	*n*
Vehicle	–	29.9±0.7	7	30.9±1.0	8
Irbesartan	3	23.6±2.0	8	27.5±1.9	8
Irbesartan	10	22.4±2.8[a]	6	24.0±3.1	7
Irbesartan	30	16.1±1.5[c]	7	25.0±1.8	7
Irbesartan	100	11.3±2.8[c]	7	13.9±1.7[c]	7
Losartan	3	27.8±1.9	8	23.2±1.8[a]	10
Losartan	10	23.6±1.6	8	26.9±2.9	7
Losartan	30	22.6±1.4[a]	7	22.3±1.1[b]	10
Losartan	100	11.2±1.5[c]	7	19.0±1.9[c]	8

Values represent the means±S.E.M. of (*n*) rats. Irbesartan and losartan were injected i.v. 30 min, or administered p.o. 60 min prior to i.c.v. angiotensin II.
[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, statistical comparison to the appropriate vehicle-treated group, calculated with one-way ANOVA followed by a post-hoc Dunnet test. No statistical differences were found between groups treated with the same dose of irbesartan and losartan (one-way ANOVA, followed by a post-hoc Tukey test).

pressure increases ($P < 0.001$) (Table 1). Both antagonists injected i.v. 60, 120 and 180 min prior to i.c.v. angiotensin II equipotently inhibited the pressor response to the peptide (Fig. 3).

3.4. Effect of p.o. treatment with irbesartan or losartan on pressor responses to angiotensin II injected i.c.v.

Both angiotensin AT_1 receptor antagonists administered orally at various doses 30 min prior to angiotensin II attenuated the i.c.v. angiotensin II-induced increases in mean arterial pressure ($F(8,64) = 6.13$; $P < 0.001$). Losartan administered at the dose of 30 mg/kg significantly reduced the pressor response to i.c.v. angiotensin II ($P < 0.01$); irbesartan administered at the same dose was without effect. At the dose of 100 mg/kg, both antagonists were equally effective in reducing the increases in mean arterial pressure induced by i.c.v. angiotensin II ($P < 0.001$) (Table 1). Both antagonists administered orally, 60, 120 and 180 min prior to i.c.v. angiotensin II, equipotently inhibited the pressor responses to the peptide (Fig. 4).

3.5. Effect of i.v. treatment with irbesartan and losartan on i.c.v. angiotensin II-induced vasopressin release into the circulation

Both antagonists injected i.v. at the dose of 30 mg/kg, 30 min prior to i.c.v. angiotensin II, significantly inhibited

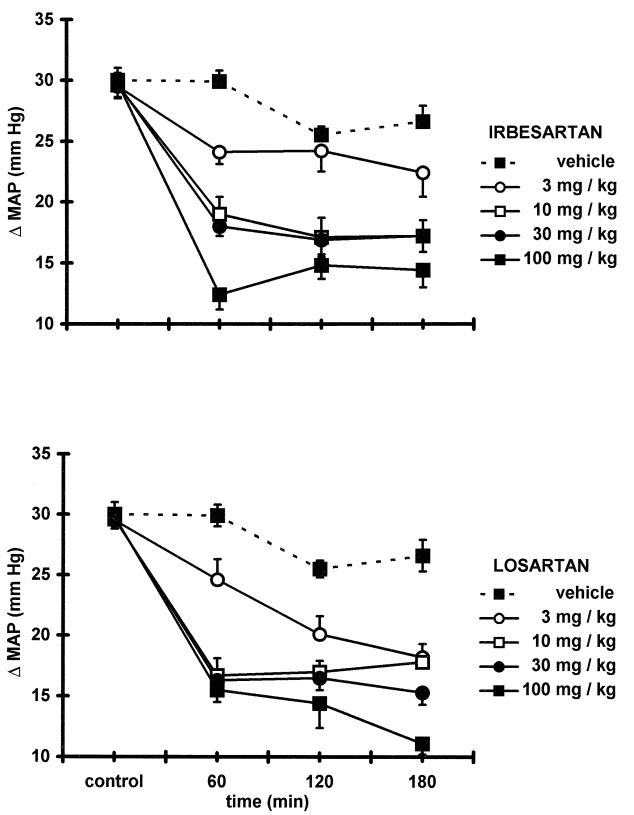

Fig. 3. Effect of irbesartan and losartan injected intravenously at various doses on mean arterial pressure increase (ΔMAP) (mm Hg) induced by angiotensin II (100 ng) injected intracerebroventricularly before and 60, 120 and 180 min after intravenous treatment with the angiotensin AT_1 receptor antagonists.

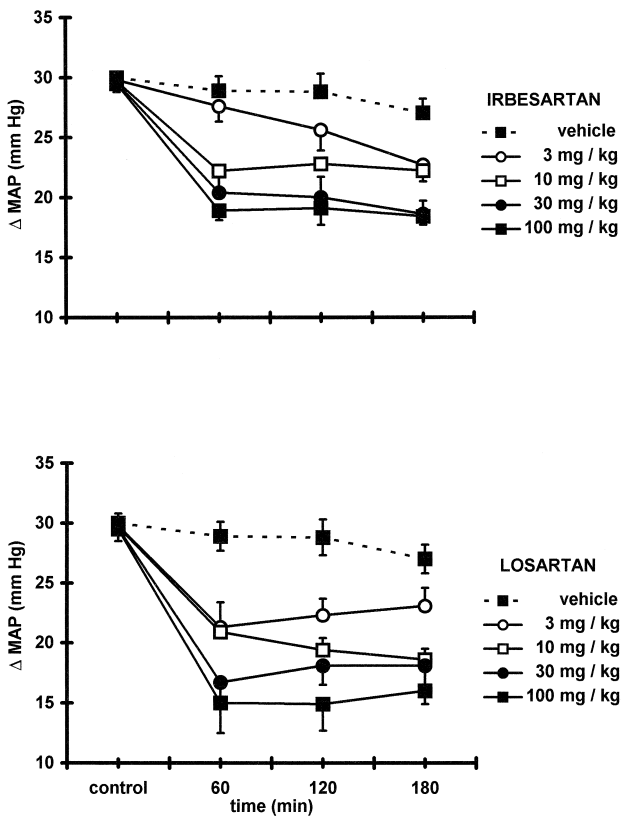

Fig. 4. Effect of irbesartan and losartan administered orally at various doses on mean arterial pressure increase (ΔMAP) (mm Hg) induced by angiotensin II (100 ng) injected intracerebroventricularly before and 60, 120 and 180 min after oral treatment with the angiotensin AT_1 receptor antagonists.

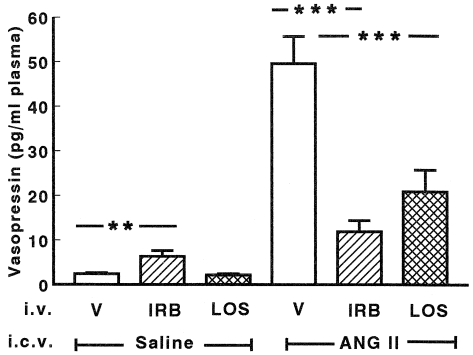

Fig. 5. Effect of intravenous (i.v.) treatment with irbesartan (IRB) and losartan (LOS) (30 mg/kg body weight) on vasopressin release (pg/ml plasma) induced by intracerebroventricularly (i.c.v.) injected angiotensin II (ANG II) (100 ng). The angiotensin AT_1 receptor antagonists were injected i.v. 30 min prior to i.c.v. angiotensin II. $**P < 0.01$; statistical comparison to the vehicle- (V) treated group; $***P < 0.001$, statistical comparison to the vehicle-treated, angiotensin II-injected group, calculated with one-way ANOVA followed by a post-hoc Bonferroni test.

Table 2
Effect of irbesartan and losartan administered intravenously (i.v.) or orally (p.o.) on water intake in response to angiotensin II (100 ng) injected intracerebroventricularly

Pretreatment (antagonist)	Dose (mg/kg)	Mode of administration	*n*	Water intake (ml)
Vehicle	–	i.v.	7	6.2 ± 0.4
Irbesartan	30	i.v.	7	2.3 ± 0.5^{a}
Losartan	30	i.v.	7	4.8 ± 0.7^{b}
Vehicle	–	p.o.	9	6.4 ± 0.6
Irbesartan	100	p.o.	9	1.3 ± 0.2^{a}
Losartan	100	p.o.	9	$2.8 \pm 0.1^{a,b}$

Values represent the water intake for a 15-min time period starting immediately after i.c.v. angiotensin II injection, and are indicated by means ± S.E.M. of (*n*) rats. Irbesartan and losartan were injected i.v. 30 min, or administered p.o. 60 min prior to i.c.v. angiotensin II.
[a] $P < 0.001$ statistical comparison to the appropriate vehicle-treated group.
[b] $P < 0.05$, statistical comparison between the irbesartan-treated and losartan-treated groups. Statistical differences were analysed with one-way ANOVA followed by a post-hoc Bonferroni test.

the release of vasopressin from the posterior pituitary ($F(5,50) = 24.84$; $P < 0.001$). Although irbesartan tended to inhibit the vasopressin release more effectively than losartan, the difference failed to reach statistical significance (Fig. 5). Thirty minutes after i.v. injection, irbesartan by itself increased the level of circulating vasopressin. ($P < 0.01$) (Fig. 5).

3.6. Effect of p.o. treatment with irbesartan and losartan on i.c.v. angiotensin II-induced vasopressin release into the circulation

Irbesartan and losartan administered orally at the dose of 100 mg/kg did not affect basal plasma vasopressin levels. Irbesartan (100 mg/kg) administered orally, 60 min prior to i.c.v. angiotensin II, significantly reduced the vasopressin release induced by the peptide, losartan was without effect ($F(5,51) = 19.98$; $P < 0.001$) (Fig. 6).

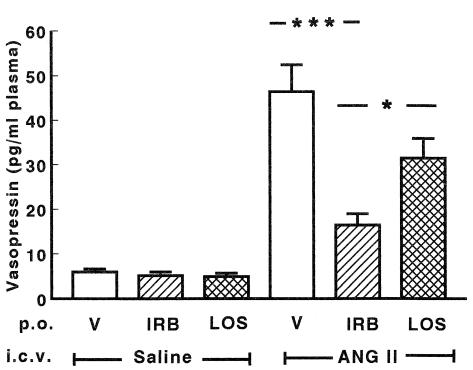

Fig. 6. Effect of oral treatment with irbesartan (IRB) and losartan (LOS) (100 mg/kg body weight) on vasopressin release (pg/ml plasma) induced by intracerebroventricularly (i.c.v.) injected angiotensin II (ANG II) (100 ng). The angiotensin AT_1 receptor antagonists were administered orally 60 min prior to i.c.v. angiotensin II. $***P < 0.01$, statistical comparison to the vehicle- (V) treated, angiotensin II-injected group; $*P < 0.05$, statistical comparison between the irbesartan-treated and losartan-treated groups, calculated with one-way ANOVA followed by a post-hoc Bonferroni test.

3.7. Effects of i.v. and p.o. treatment with irbesartan and losartan on i.c.v. angiotensin II-induced drinking response

When the angiotensin AT_1 receptor antagonists were administered i.v at the dose of 30 mg/kg, 30 min prior to i.c.v. angiotensin II, only irbesartan significantly attenuated the drinking response induced by the peptide ($F(2,18) = 14.49$; $P < 0.001$) (Table 2). Both antagonists given p.o. at the dose of 100 mg/kg reduced the drinking response induced by angiotensin II injected i.c.v. 60 min thereafter ($F(2,24) = 50.01$; $P < 0.001$). In both experimental settings, irbesartan inhibited the i.c.v. angiotensin II-induced drinking more effectively than losartan ($P < 0.05$) (Table 2).

4. Discussion

The selective, non-peptide angiotensin AT_1 receptor antagonists, irbesartan and losartan, administered systemically attenuated the pressor response elicited by i.v. angiotensin II as well as the pressor response, vasopressin release and drinking induced by i.c.v. angiotensin II. Generally, 30 and 60 min after systemic administration, irbesartan was more effective than losartan in inhibiting the vasopressin release and water intake in response to centrally injected angiotensin II.

Irbesartan administered i.v. or p.o. dose-dependently attenuated the pressor responses to i.v. injected angiotensin II. This observation is in line with data published by Cazaubon et al. (1993). Although the affinity of irbesartan to angiotensin AT_1 binding sites exceeds that of losartan

by about 10-fold (Timmermans et al., 1993), both antagonists administered systemically displayed similar potencies with respect to the inhibition of pressor responses induced by i.v. angiotensin II bolus injections. Losartan generates an active metabolite, EXP 3174 (2-*n*-butyl-4-chloro-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazol-5-carboxylic acid), which is about 10-fold more potent than losartan in rats (Wong et al., 1990c). Since EXP 3174 appears rapidly in plasma after systemic losartan treatment (Csajka et al., 1997), and assuming approximately equal oral bioavailability of irbesartan and losartan, it is not surprising that both antagonists displayed similar potencies to inhibit angiotensin II effects at later time points after systemic administration. However, both antagonists were also equipotent in inhibiting the pressor responses to angiotensin II when the peptide was injected i.v. 5 or 15 min post-systemic treatment with the antagonists. EXP 3174 cannot substantially contribute to the inhibitory effects of losartan at earlier time points. In view of the higher affinity of irbesartan to angiotensin AT_1 receptors, other factors such as distribution volumes of these angiotensin AT_1 receptor antagonists should be taken into consideration while explaining the equal inhibitory efficacies of both antagonists at early time points after systemic treatment.

Angiotensin II, besides being a circulating hormone, is known to act in the brain as a neurotransmitter or neuromodulator and has been traditionally linked to central blood pressure control and the regulation of autonomic nervous system activity. Besides blood pressure control, the brain renin–angiotensin system is involved in the regulation of a number of physiological processes including electrolyte balance, secretion of pituitary hormones and water intake (Unger et al., 1988; Saavedra, 1992). Angiotensin AT_1 receptor antagonists administered peripherally may cross the blood–brain barrier and interact with angiotensin AT_1 receptors in brain areas inside the blood–brain barrier. Results concerning the central effects of systemically administered losartan or other non-peptide angiotensin AT_1 receptor antagonists are equivocal, and it has not yet been unambiguously established whether peripherally administered angiotensin AT_1 receptor antagonists have an access to angiotensin AT_1 receptors inside the blood–brain barrier. Losartan administered orally was shown not to interact with those brain angiotensin AT_1 receptors which were inhibited when the antagonist was injected i.c.v. (Wong et al., 1990a). Similarly, Bui et al. (1992) demonstrated that chronic oral treatment with losartan (3 mg/kg body weight) did not affect the dipsogenic and pressor responses induced by i.c.v. angiotensin II. On the other hand, data demonstrating inhibitory effects of peripherally administered losartan or of its active metabolite, EXP 3174, on responses mediated by brain angiotensin II have been reported (Li et al., 1993; Polidori et al., 1996). Peripherally administered losartan was also capable to inhibit dose-dependently angiotensin AT_1 receptors in brain structures inside the blood–brain barrier (Song et al., 1991; Zhuo et al., 1994). An insurmountable, non-peptide angiotensin AT_1 receptor antagonist, LR-B/081, inhibited dose-dependently and more effectively than losartan or EXP 3174 drinking induced by central injection of angiotensin II (Polidori et al., 1995).

In the present study, we studied the effects of systemic treatment of rats with irbesartan and losartan on the pressor response, release of vasopressin from the posterior pituitary and drinking response induced by i.c.v. angiotensin II. When injected into the lateral brain ventricle, angiotensin II primarily interacts with angiotensin AT_1 receptors localized in the circumventricular organs, the subfornical organ and the organum vasculosum laminae terminalis. These brain structures lack the blood–brain barrier due to the presence of fenestrated capillary endothelium, and circulating angiotensin AT_1 receptor antagonists can interact with angiotensin AT_1 receptors in the interstitium of the circumventricular organs. Neurons in the circumventricular organs are connected with other regions in the brain linked to central blood pressure control and the regulation of body fluid homeostasis (McKinley et al., 1990).

Angiotensin II is a potent pressor agent when injected into then brain ventricles or microinjected into distinct brain areas (Unger et al., 1988). The pressor responses to i.c.v. angiotensin II are in the initial phase mediated by secretion of vasopressin from the posterior pituitary into the circulation (Unger et al., 1985). Neurons localized in the subfornical organ and organum vasculosum laminae terminalis project directly or indirectly, via an interposed synapse in the median preoptic nucleus, to the magnocellular part of the paraventricular nucleus and to the supraoptic nucleus, where vasopressin-synthetizing neurons are localized (Lind et al., 1985; Oldfield et al., 1991). All these regions express predominantly angiotensin AT_1 receptors (Obermüller et al., 1991; Song et al., 1992; Lenkei et al., 1998). It is generally accepted that the pressor responses to i.c.v.-injected angiotensin II are mediated by angiotensin AT_1 receptors (Höhle et al., 1995). The contribution of the sympathetic activation to the pressor response induced by centrally administered angiotensin II is still a matter of controversy (Unger et al., 1988). Recently, the presence of sympathoexcitatory angiotensin AT_1 receptors in the rostral ventrolateral medulla has been postulated (Head, 1996).

In the present study, angiotensin AT_1 receptors in the circumventricular organs were most effectively inhibited after systemic treatment with both angiotensin AT_1 receptor antagonists, but blockade of angiotensin AT_1 receptors in cardiovascular centres throughout the brain was most probably responsible for the attenuation of the pressor responses to i.c.v. angiotensin II. Our data demonstrating that both antagonists administered i.v. or orally can interact with angiotensin AT_1 receptors in the brain are in line with the findings of others (Li et al., 1993; Zhuo et al., 1994; Polidori et al., 1996). Irbesartan and losartan admin-

istered systemically at various doses 30 min prior to i.c.v. angiotensin II equipotently inhibited the pressor responses to the peptide. Also at the later time points, losartan and irbesartan exhibited similar potencies in reducing the pressor responses produced by angiotensin II. Several facts should be considered to explain this observation. Irbesartan is more lipophilic than losartan and might, therefore, better penetrate into the brain after systemic administration. Moreover, its affinity to the angiotensin AT_1 receptor is about 10-fold higher than that of losartan (Cazaubon et al., 1993). On the other hand, losartan generates an active metabolite, EXP 3174, which appears rapidly in plasma after systemic losartan treatment (Csajka et al., 1997) and has about 10–20-fold higher affinity to the angiotensin AT_1 receptor than losartan (Timmermans et al., 1993). As EXP 3174 has been reported to cross the blood–brain barrier more effectively than losartan (Polidori et al., 1996) and its affinity to the angiotensin AT_1 receptor is comparable to that of irbesartan, the generation of EXP 3174 from losartan in the periphery can explain the similar potencies of losartan and irbesartan to inhibit the angiotensin II-induced pressor responses.

Blood pressure changes in response to i.c.v. angiotensin II may not represent the most appropriate parameter to assess the efficacy of peripherally administered angiotensin AT_1 receptor antagonists to inhibit the central effects of angiotensin II. The vasopressin-induced rise in mean arterial pressure is a firmly established pressor mechanism of centrally administered angiotensin II in the rat (Unger et al., 1988). However, a number of factors, such as the activity of the baroreceptor bulbospinal neurons, or the baseline level of arterial blood pressure, may directly or indirectly influence the magnitude of the mean arterial pressure response induced by stimulation of central angiotensin AT_1 receptors. Therefore, the measurement of plasma vasopressin levels following i.c.v. angiotensin II injection may provide a more appropriate direct information on the ability of the angiotensin AT_1 receptor antagonists to interact with central angiotensin AT_1 receptors. As mentioned above, the circumventricular organs of the lamina terminalis, the subfornical organ and the organum vasculosum laminae terminalis, represent the target structures through which angiotensin II injected i.c.v. increases the excitability of the vasopressin neurons in the magnocellular part of the paraventricular nucleus and in the supraoptic nucleus. Besides angiotensin AT_1 receptors localized in the circumventricular organs and in the lamina terminalis, angiotensin AT_1 receptors in the paraventricular and supraoptic nuclei were demonstrated to mediate the vasopressin release induced by stimulation of periventricular angiotensin AT_1 receptors, as losartan microinjected into the paraventricular or supraoptic nuclei attenuated the release of vasopressin in response to i.c.v. angiotensin II (Veltmar et al., 1992; Qadri et al., 1993). In the present study, vasopressin levels in plasma were determined in rats pretreated with irbesartan or losartan i.v. 30, or orally 60 min prior to i.c.v. angiotensin II injection. By extrapolating the results from human studies, it can be assumed that plasma concentration of both antagonists reaches peak values at these time points (Csajka et al., 1997; Gillis and Markham, 1997). When injected i.v., irbesartan tended to inhibit more effectively than losartan the i.c.v. angiotensin II-induced vasopressin release. When administered orally, irbesartan was clearly more effective than losartan which failed to affect the increase in vasopressin levels in plasma induced by i.c.v. angiotensin II. Providing that plasma concentrations of both antagonists were high enough to completely inhibit the angiotensin AT_1 receptors in the circumventricular organs, the additional inhibitory effects of orally administered irbesartan on angiotensin II-induced vasopressin release was most probably achieved by a more effective inhibition of angiotensin AT_1 receptors in the median preoptic, paraventricular and supraoptic nuclei, i.e., areas inside the blood–brain barrier. These findings suggest that systemically administered irbesartan inhibits central angiotensin AT_1 receptors more effectively than losartan. The slight, but significant increase in basal levels of vasopressin observed after i.v. injection of irbesartan is most probably caused by an activation of vasopressin-synthetizing neurons in the paraventricular and supraoptic nuclei. We have observed in another set of experiments, that irbesartan injected i.v. at a dose of 100 mg/kg body weight stimulated the expression of c-Fos, an indicator of neural activation, in the paraventricular and supraoptic nuclei but not in the subfornical organ and the organum vasculosum laminae terminalis. As vehicle alone increased only slightly the number of c-Fos-positive neurons, (pH of the vehicle was about 9.0), irbesartan seems to be substantially responsible for the activation of vasopressin-synthetizing neurons by an unknown mechanism.

The neurons in the periventricular tissue of the anteroventral third ventricular region comprising the organum vasculosum laminae terminalis, the ventral portion of the median preoptic nucleus and the preoptic periventricular nucleus, are intimately linked to the regulation of drinking behaviour. Lesions in this area produce chronic impairments in the drinking response to several types of dipsogenic stimuli (Johnson and Edwards, 1990). Of all structures belonging to the anteroventral third ventricular region, only the organum vasculosum laminae terminalis lacks the blood–brain barrier. Another region closely associated with dipsogenic responses is the subfornical organ (Terrel et al., 1991; Fitts, 1994). All these brain regions express predominantly angiotensin receptors belonging to the AT_1 subtype (Obermüller et al., 1991; Song et al., 1992; Lenkei et al., 1998). Neurons localized in the organum vasculosum laminae terminalis and most probably also in the subfornical organ, which express angiotensin AT_1 receptors, represent a site of the action of i.c.v.-injected angiotensin II to induce drinking. We have observed that irbesartan administered i.v. or p.o. inhibited the i.c.v angiotensin II-induced drinking more effectively than

losartan. Since the organum vasculosum laminae terminalis and the subfornical organ lack the blood–brain barrier, both circulating angiotensin AT_1 receptor antagonists could easily interact with these receptors. The higher potency of irbesartan to inhibit the i.c.v. angiotensin II-induced drinking might, therefore, be related to a more efficient inhibition of angiotensin AT_1 receptors within the anteroventral third ventricular region which are situated inside the blood–brain barrier. As lesions of the median preoptic nucleus in the rat were shown to impair drinking induced by systemic treatment with hypertonic saline or angiotensin II and to produce deficits in vasopressin release (Mangiapane et al., 1983; Gardiner and Stricker, 1985), angiotensin AT_1 receptors in this particular region might represent the site of an additional inhibitory action of irbesartan resulting in the more pronounced attenuation of water intake in response to i.c.v. angiotensin II. Recently, Polidori et al. (1998) have reported on effects of peripheral treatment with irbesartan and losartan on drinking response to i.c.v. angiotensin II. In contrast to the present findings, irbesartan administered orally at various time points prior to i.c.v. angiotensin II was less effective than losartan to inhibit drinking responses induced by the peptide. The authors conclude that following acute intragastric administration, losartan influences more effectively than irbesartan central effects mediated by angiotensin II. The discrepancies between these two studies indicate that the rate of the penetration of peripherally administered angiotensin AT_1 receptor antagonists into the brain might depend on several, unknown factors and that additional, more detailed studies must be performed on this important and clinically relevant issue.

We have demonstrated that systemically administered irbesartan and losartan have access to angiotensin AT_1 receptors in the brain. At early time points, 30 and 60 min after systemic treatment, irbesartan appears to penetrate more effectively than losartan into the brain to block the biological actions of angiotensin II.

Although a number of studies pointed to the relevance of an overactive renin–angiotensin system in the brain in the development and maintenance of hypertension in spontaneously hypertensive rats (Phillips and Kimura, 1988; Unger et al., 1988), acute or chronic inhibition of central angiotensin AT_1 receptors with losartan and CV-11974 failed to lower the arterial blood pressure in conscious, adult spontaneously hypertensive rats (DePasquale et al., 1992; Bunting and Widdop, 1995). However, antisense inhibition of angiotensin AT_1 receptors mRNA in the brain reduced hypertension in spontaneously hypertensive rats, demonstrating the involvement of angiotensin AT_1 receptors in maintaining increased blood pressure in this model of genetic hypertension (Gyurko et al., 1993). Long- term treatment of essential hypertension with lipophilic angiotensin AT_1 receptor antagonists might result in a chronic blockade of these receptors in the brain which, in turn, would affect a number of physiological functions mediated by these receptors, including the central cardiovascular regulation.

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