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Functional evidence for the ability of angiotensin AT_1 receptor antagonists to cross the blood-brain barrier in rats

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Abstract

The angiotensin AT_1 receptor antagonists, losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole potassium salt), EXP3174 (2-*n*-butyl-4-chloro-1-[(2′(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid), GR117289 (1-[[3-bromo-2-[2-(1*H*-tetrazol-5-yl)phenyl]-5-benzofuranyl]methyl]-2-butyl-4-chloro-1*H*-imidazole-5-carboxylic acid) and LR-B/081 (methyl-2-[[4-butyl-2-methyl-6-oxo-5-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1(6*H*)-pyrimidinyl]methyl]-3-thiophenecarboxylate), given by intraperitoneal (i.p.) injection 15 min before intracerebroventricular administration of angiotensin II, inhibited drinking with the following order of potency: EXP3174 > GR117289 > losartan > LR-B/081. When 20 μmol/kg of each antagonist was i.p. injected 15 min, 4, 12 or 24 h before angiotensin II, EXP3174 and GR117289 inhibited water intake at each observation time, losartan at 4, 12 and 24 h, LR-B/081 only at 4 and 12 h. After per os administration of the same dose 4 or 12 h before angiotensin II, losartan reduced drinking at 4, but not at 12 h; LR-B/081 did not inhibit drinking either at 4 or 12 h. The present results suggest that EXP3174 and GR117289 cross the barrier readily. The effect of i.p. losartan on central angiotensin mechanisms is not prompt, suggesting that it may require conversion to EXP3174. LR-B/081 apparently crosses the barrier less readily than the other antagonists following both i.p. and per os administration.

Keywords: Angiotensin AT_1 receptor antagonist; Angiotensin II-induced drinking; Blood-brain barrier; Losartan; EXP3174; GR117289; LR-B/081

1. Introduction

Angiotensin II is known to play an important role in the physiopathological control of blood pressure (Doyle and Bearn, 1984; Unger et al., 1988), which can be related not only to peripheral effects, such as vasoconstriction, stimulation of aldosterone secretion and direct actions on the renal tubule, but also to central effects, such as increase of sympathetic nerve activity, stimulation of vasopressin release, synaptic inhibition of the baroreflex in the nucleus tractus solitarii, stimulation of water and salt intake (Epstein, 1990; Fitzsimons, 1979, 1986; Johnson and Edwards, 1990; Phillips, 1987; Unger et al., 1988).

Several studies have shown that angiotensin AT_1 receptors mediate both peripheral vasodilation and central effects of angiotensin II on body fluid regulation, including stimulation of water intake (Beresford and Fitzsimons, 1992; Dourish et al., 1992; Fregley and Rowland, 1991; Polidori et al., 1991, 1995; Rowland et al., 1992; Quadri et al., 1993; Sakai et al., 1994; Timmermans et al., 1993).

Angiotensin II may be also involved in the control of adrenocorticotropic hormone and reproductive hormones release, in cognitive processes, anxiety and analgesia (Barnes et al., 1990; Phillips, 1987; Wright and Harding, 1992; for review).

Therefore, it was of interest to know whether, and under which conditions, peripherally administered angiotensin receptor antagonists are able to cross the blood-brain barrier and to influence central angiotensin mechanisms.

While the dipsogenic effect of blood-borne angiotensin II appears to be mainly due to activation of angiotensin receptors within the subfornical organ, a circumventricular organ which lies outside the blood-brain barrier (Simpson et al., 1978; Simpson, 1981), this nucleus plays a minor

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role in the drinking response to intracerebroventricular (i.c.v.) injection of angiotensin II (Perfumi et al., 1986; Johnson and Edwards, 1990). Lesion studies suggest that drinking in response to i.c.v. angiotensin II may be primarily mediated by the median preoptic nucleus (Gardner and Stricker, 1985; Lind and Johnson, 1982; Johnson and Edwards, 1990). In keeping with these findings, a recent study showed that i.c.v. angiotensin II induces c-fos expression mainly in the median preoptic nucleus (McKinley et al., 1995). Since this nucleus lies inside the blood-brain barrier, drinking induced by i.c.v. angiotensin II can represent a valuable functional test to evaluate the ability of peripherally administered angiotensin receptor antagonists to cross the blood-brain barrier.

In the present study, four angiotensin AT_1 receptor antagonists were tested: 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole potassium salt (losartan; Carini and Duncia, 1988; Timmermans et al., 1993; for review); its active metabolite -2-*n*-butyl-4-chloro-1-[(2′(1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole-5-carboxylic acid (EXP3174; Wong et al., 1990b); 1-[[3-bromo-2-[2-(1*H*-tetrazol-5-yl)phenyl]-5-benzofuranyl]methyl]-2-butyl-4-chloro-1*H*-imidazole-5-carboxylic acid (GR117289; Dennes et al., 1993; Hilditch et al., 1994; Robertson et al., 1991); and methyl-2-[[4-butyl-2-methyl-6-oxo-5-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]-methyl]-1(6*H*)-pyrimidinyl]methyl]-3-thiophenecarboxylate (LR-B/081; Cirillo et al., 1995; Renzetti et al., 1995). Their ability to inhibit the dipsogenic effect of i.c.v. angiotensin II was evaluated following intraperitoneal (i.p.) injection to ensure high and prompt bioavailability of the administered drugs. Losartan and LR-B/081 were also tested following per os (p.o.) administration.

2. Materials and methods

2.1. Animals

All the experiments were carried out in male Wistar rats (Charles River, Calco, Italy), weighing 325–350 g. The rats were individually housed in stainless-steel cages in a room with a 12 : 12-h light : dark cycle, controlled temperature (20–21°C) and humidity (45–55%). They had free access to food pellets (4RF18; Mucedola, Settimo Milanese, Italy) and tap water.

2.2. Intracranial surgery

The animals were anesthetized by i.p. injection of 100–150 μl/100 g body weight (b.w.) of a solution containing ketamine (86.2 mg/ml) and acepromazine (1.3 mg/ml) and were injected with a prophylactic dose of gentamycin (10 mg/0.2 ml/rat, intramuscularly) before surgery. A stereotaxic instrument was used to reach the lateral ventricle and a guide cannula was implanted and cemented with dental acrylic cement to the skull, where three stainless-steel screws had been installed. The following coordinates were used for the guide cannula: AP = 1 mm behind the bregma, L = 2 mm from the sagittal suture, V = 2 mm from the surface of the skull. I.c.v. injections were made by means of a stainless-steel injector temporarily inserted into the guide cannula and protruding 2.5 mm beyond the cannula tip.

2.3. Drugs

LR-B/081 was a gift of Lusofarmaco (Milan, Italy); losartan and its active metabolite EXP3174 were gifts of DuPont Merck (Research and Development, Wilmington, DE, USA); GR117289 was a gift of Glaxo Group Research (Greenford, Middlesex, UK); [Ile^5]angiotensin II was purchased from Novabiochem-Inalco (Milan, Italy); carbachol was purcased from Sigma Italia (Milan, Italy).

The antagonists were dissolved as follows: 10 mg of each drug was dissolved in 0.19 ml of dimethyl sulphoxide and 1.81 ml of NaOH 0.01 N. The final solution was prepared by adding distilled water and bringing it to pH 7.8.

2.4. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on angiotensin II-induced drinking: dose-response relationship

Angiotensin II was given by pulse i.c.v. injection at the dose of 10 pmol/rat in a volume of 1 μl, 15 min after the i.p. injection of the angiotensin AT_1 receptor antagonist or of vehicle.

Groups of 6–10 animals for each dose of the four antagonists were employed. The rats were gently removed from their cages and i.p. injected. The doses used were 1, 5, 10 and 30 mg/kg for losartan; 1, 5 and 10 mg/kg for EXP3174; 1, 5 and 10 mg/kg for GR117289 and 1, 10 and 30 mg/kg for LR-B/081. The controls were injected with vehicle solution. Water intake was recorded at 15 and 30 min after i.c.v. injection of angiotensin II.

2.5. Effect i.c.v. injections of angiotensin AT_1 receptor antagonists on angiotensin II-induced drinking: dose-response relationship

Angiotensin II was given by pulse i.c.v. injection at the dose of 10 pmol/rat in a volume of 1 μl, 5 min after the i.c.v. injection of the angiotensin AT_1 receptor antagonist tested or of vehicle.

GR117289 was tested at the doses of 10, 25 and 100 pmol/rat, according to a between-subject design. The other antagonists were tested by i.c.v. injection, only at the dose that our previous study (Polidori et al., 1995) had shown to be the ID_{50} of the other 3 antagonist: 357 pmol (164.5 ng)/rat for losartan; 3.9 pmol (1.7 ng)/rat for EXP3174; 25.9 pmol (14.3 ng)/rat for LR-B/081.

2.6. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on angiotensin II-induced drinking: time course

Angiotensin II was given at the dose of 10 pmol/rat at various times (15 min, 4, 12 or 24 h) after the i.p. injection of 20 μmol/kg b.w. (about 10 mg/kg) of each angiotensin AT_1 receptor antagonist, in 1 ml/kg of vehicle. Previous experiments had shown that this dose of losartan and LR-B/081, given by p.o. administration, is able to evoke a marked antihypertensive effect (Cirillo et al., 1995). Water intake was measured 15 and 30 min following angiotensin II injection. The experiment was carried out according to a between-subject design.

2.7. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on carbachol-induced drinking

In order to evaluate the behavioural selectivity of the inhibitory effect observed on angiotensin II-induced drinking, the same doses of LR-B/081, losartan, EXP3174 or GR117289 were also tested on drinking induced by i.c.v. carbachol. Carbachol was given by pulse i.c.v. injection at the dose of 300 ng/rat in 1 μl of isotonic saline. Carbachol-induced water intake was measured 15 and 30 min later. The experiment was carried out according to a between-subject design.

2.8. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on the pressor response to intravenous (i.v.) angiotensin II

Rats were premedicated with i.p. fentanyl (0.024 mg/kg) plus fluanisone (1.2 mg/kg) (Hypnorm) and anesthetized with an i.v. injection of sodium pentobarbitone (30–35 mg/kg). Polyethylene catheters were inserted into the left carotid artery and right jugular vein and exteriorized behind the head through a single-channel swivel (U. Danuso, Milan, Italy). The carotid catheter was connected to a Transpac II transducer (Abbott, Campoverde, Italy) connected to a 8805 D preamplifier (Hewlett-Packard, Milan, Italy); blood pressure and heart rate were recorded by a 7758 D polygraph (Hewlett-Packard). Values of the variables were recorded with an IDAS BM 9000 (Biomedica Mangoni, Pisa, Italy) coupled to a Compaq 386/20e computer.

18 h after surgery, following an overnight fast with water ad libitum, a submaximal pressor dose of angiotensin II (0.105 nmol/kg) was given by i.v. injection to conscious rats three times at 20-min intervals to establish the baseline response. Then both vehicle and one of the angiotensin AT_1 receptor antagonists to be tested were i.p. administered at the dose of 20 μmol/kg (about 10 mg/kg), in 1 ml/kg of vehicle. Bolus injections of angiotensin II were given 15 min, 4, 12 and 24 h after the i.p. injection of the angiotensin AT_1 receptor antagonist (i.e. at the same times as in the above experiment).

2.9. Effect of p.o. administration of losartan or LR-B / 081 on angiotensin II-induced drinking

Losartan, LR-B/081 or their vehicle were administered p.o., by means of a PE100 tubing. Angiotensin II was given at the dose of 10 pmol/rat 4 or 12 h after the administration of vehicle or of 20 μmol/kg of losartan or LR-B/081, in 1 ml/kg of vehicle. Water intake was measured 15 and 30 min after angiotensin II injection. Two groups of animals were used, one for LR-B/081 and the other for losartan. Treatment with vehicle or the antagonist was given in the reverse order, at interval of 7 days from the previous experimental session.

2.10. Verification of i.c.v. cannula placement

Before the experiments, behavioural verification of i.c.v. cannula placement was done by evaluating the drinking response to i.c.v. injection of angiotensin II, 10 ng/rat. Only animals showing a drinking response of at least 7 ml/rat were included in the experimental groups.

After completion of experiments, 1 μl of black India ink was i.c.v. injected just before the rat was killed and ink diffusion into the ventricular space was evaluated.

2.11. Statistics

Data are presented as the means ± S.E.M. Statistical analysis of data was performed by 'split-plot' multifactorial analysis of variance. Only data obtained following p.o. administration of losartan and LR-B/081 were analyzed by multifactorial analysis of variance 'repeated measures', since the experiment was carried out according to a within-subject design. Planned pairwise comparisons were carried out by means of *t*-tests. Statistical significance was set at $P < 0.05$.

The ID_{50} of the antagonists was determined according to Snedecor and Cochran (1967).

3. Results

3.1. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on angiotensin II-induced drinking: dose-response relationship

In response to i.c.v. angiotensin II, 10 pmol/rat, the controls drank 9.1 ± 1.5 ml/rat of water in 15 min and 11.6 ± 1.6 ml/rat in 30 min.

Losartan, at doses of 1–30 mg/kg, reduced the water intake induced by i.c.v. angiotensin II (Fig. 1). The analysis of variance revealed a statistically significant treatment effect [$F(4,29) = 4.13$; $P < 0.01$], time effect [$F(1,29) = 28.59$; $P < 0.001$] and treatment-time interaction [$F(4,29) = 10.46$; $P < 0.001$]. Pairwise comparisons showed that the water intake in losartan-treated rats was significantly

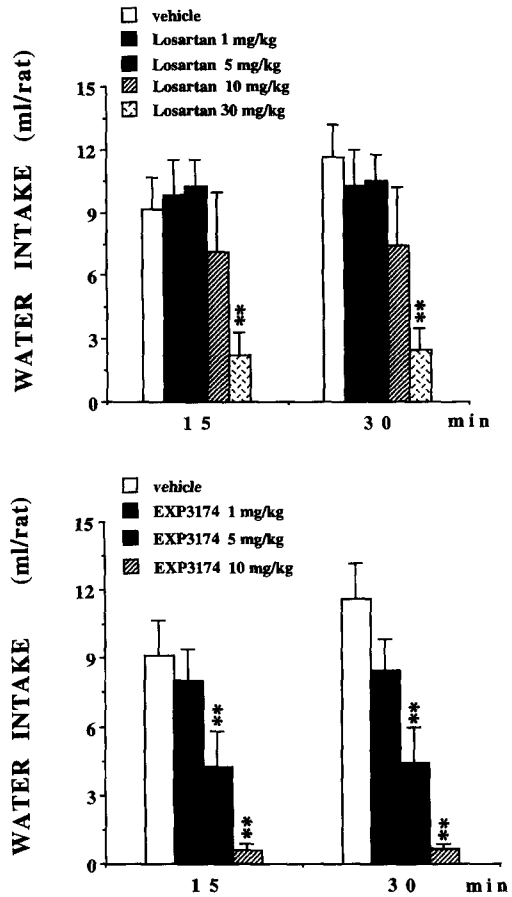

Fig. 1. Cumulative water intake over 15 and 30 min after i.c.v. injection of angiotensin II, 10 pmol/rat, in animals i.p. pretreated with vehicle (controls) or with different doses of losartan (upper panel) or EXP3174 (lower panel). Values are means ± S.E.M. of 6–10 values. Difference from controls: * $P < 0.05$; ** $P < 0.01$; where not indicated difference from controls was not statistically significant.

less than that of the controls only at the dose of 30 mg/kg, both at 15 and 30 min after angiotensin II injection. At this dose the % inhibition of drinking was 75.4 and 78.9% at 15 and 30 min, respectively. The ID_{50} of losartan, at 15 min after angiotensin II injection, was 23.01 (C.L. 9.73–53.7) mg/kg, i.e. 49.9 (C.L. 21.1–116.4) μmol/kg.

EXP3174, at doses of 1–10 mg/kg, also markedly reduced the water intake induced by angiotensin II (Fig. 1). The analysis of variance revealed a statistically significant treatment effect [$F(3,25) = 13.73$; $P < 0.001$], time effect [$F(1,25) = 27.58$; $P < 0.001$] and treatment-time interaction [$F(3,25) = 14.73$; $P < 0.0001$]. Pairwise comparisons revealed that the water intake in EXP3174-treated rats was significantly less than that of the controls in response to 5 and 10 mg/kg, both at 15 and 30 min after angiotensin II injection. Following 10 mg/kg, the percent inhibition of drinking was 93.2 and 94.5% at 15 and 30 min, respectively. The ID_{50} of EXP 3174, at 15 min after angiotensin II injection, was 2.92 (C.L. 1.50–4.52) mg/kg, i.e. 6.7 (C.L. 3.4–10.3) μmol/kg.

The results concerning GR117289 are shown in Fig. 2. The analysis of variance showed a statistically significant treatment effect [$F(3,25) = 7.97$; $P < 0.001$], time effect [$F(1,25) = 34.05$; $P < 0.001$] and treatment-time interaction [$F(3,25) = 10.03$; $P < 0.001$]. Planned pairwise comparisons revealed that the water intake in GR117289-treated rats was significantly lower than that of controls in response to 10 mg/kg, both at 15 and 30 min after angiotensin II injection. The ID_{50} of GR117289, at 15 min after angiotensin II injection, was 7.01 (C.L. 4.52–14.06) mg/kg, i.e. 12.0 (C.L. 7–24) μmol/kg.

LR-B/081, i.p. injected at doses of 1–30 mg/kg, reduced the water intake induced by the i.c.v. injection of angiotensin II (Fig. 2). The analysis of variance revealed a statistically significant treatment effect [$F(3,28) = 2.95$; $P < 0.05$], time effect [$F(1,28) = 37.90$; $P < 0.001$] and treatment-time interaction [$F(3,28) = 13.55$; $P < 0.001$]. Planned pairwise comparisons showed that the water intake in LR-B/081-treated rats was significantly less than that of the controls only in response to 30 mg/kg. After this dose the percent inhibition of angiotensin II-induced drinking was 35.8 and 45.5% at 15 and 30 min, respec-

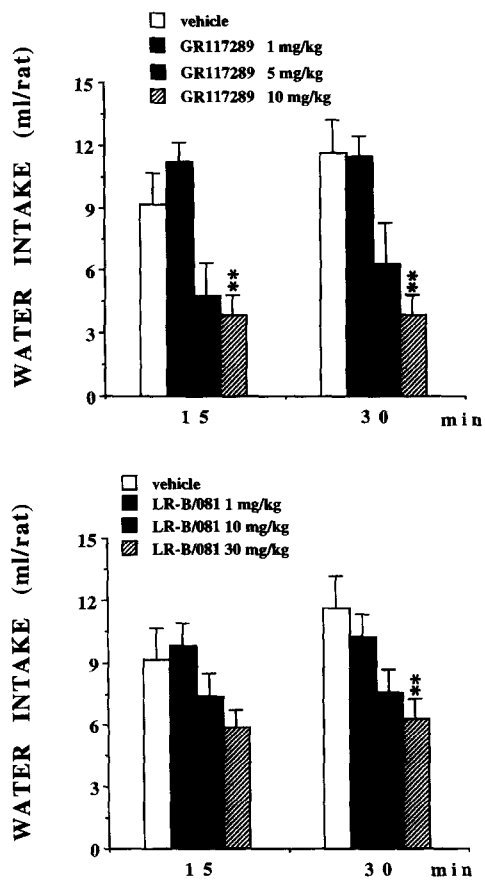

Fig. 2. Cumulative water intake over 15 and 30 min after i.c.v. injection of angiotensin II, 10 pmol/rat, in animals i.p. pretreated with vehicle (controls) or with different doses of GR117289 (upper panel) or LR-B/081 (lower panel). Values are means ± S.E.M. of 6–10 values. Difference from controls: * $P < 0.05$; ** $P < 0.01$; where not indicated difference from controls was not statistically significant.

tively, after angiotensin II injection. Since inhibition less than 50% does not allow determination of an accurate ID_{50}, six other rats were treated with a higher dose, 50 mg/kg. Following this very high dose of LR-B/081 most of the animals were rather immobile during the whole observation period. Only two animals moved to reach the drinking spout and drank copiously (6.2 and 18.5 ml of water, respectively, in 15 min). The behaviour of treated animals, together with the large variability in the drinking response, suggested strongly that results obtained at this high dose cannot be regarded as the expression of a selective effect on the drinking mechanisms. Accordingly, the 50-mg/kg data were not included in the statistical analysis and were not used to determine the ID_{50}. Therefore, the ID_{50} of LR-B/081, at 15 min after angiotensin II injection, can only be indicated as > 30 mg/kg, i.e. > 54.0 μmol/kg.

3.2. Effect of i.c.v. injections of angiotensin AT_1 receptor antagonists on angiotensin II-induced drinking; dose-response relationship

Following its i.c.v. injection, GR117289 markedly inhibited angiotensin II-induced drinking at doses of 10–100 pmol/rat. The analysis of variance revealed a highly significant treatment effect [$F(3,21) = 23.30$; $P < 0.0001$] and time effect [$F(1,21) = 10.36$; $P < 0.01$], without a significant time-treatment interaction. The ID_{50} of GR117289, at 15 min after angiotensin II injection, was 25.5 (C.L. 17.05–38.12) pmol/rat.

The other 3 angiotensin AT_1 receptor antagonists, tested at the i.c.v. ID_{50} determined in our previous study (Polidori et al., 1995), produced a mean drinking inhibition between 44 and 60%, thus confirming these values.

In Table 1, the i.c.v. ID_{50}, the i.p. ID_{50} as well the ratio i.p./i.c.v. ID_{50} of each antagonist are reported.

3.3. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on angiotensin II-induced drinking: time course

Losartan, 20 μmol/kg, significantly reduced the angiotensin II-induced water intake. The analysis of variance revealed a statistically significant treatment effect [$F(1,65) = 27.12$; $P < 0.0001$], but no significant effect due to the time at which the antagonist was administered [$F(3,65) = 0.42$; $P > 0.05$], nor a significant time-treatment interaction. A significant reduction in water intake was observed at 4, 12 and 24 h after i.p. losartan injection. (Fig. 3).

Table 1
ID_{50} on drinking induced by i.c.v. angiotensin II, 10 pmol/rat, of angiotensin AT_1 receptor antagonists given by i.p. or i.c.v. injection

Antagonist	I.p. ID_{50} (μmol/kg)	I.c.v. ID_{50} (pmol/rat)	Ratio (i.p./i.c.v. ID_{50})
EXP3174	6.7	3.9	1.7×10^6
Losartan	49.9	357	0.13×10^6
GR117289	12.0	25.5	0.47×10^6
LR-B/081	> 54	25.9	$> 2.0 \times 10^6$

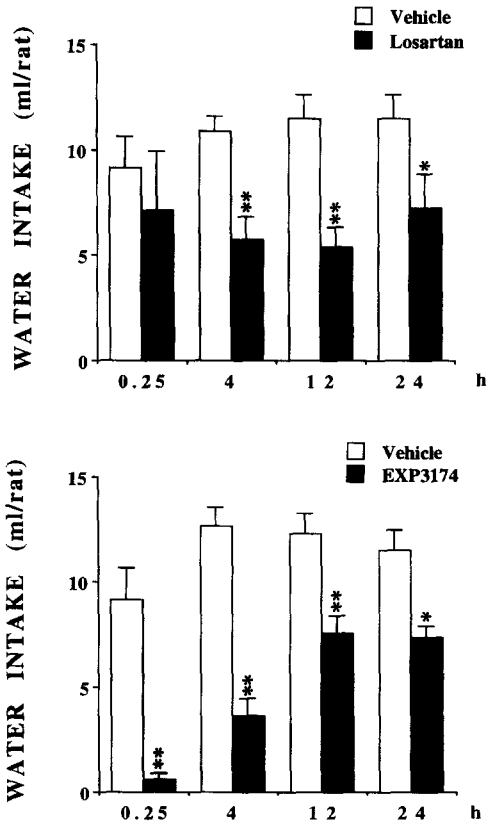

Fig. 3. Water intake for 15 min after i.c.v. injection of angiotensin II, 10 pmol/rat, in animals i.p. pretreated with vehicle (controls) or with 20 μmol/kg of losartan (upper panel) or EXP3174 (lower panel) at different times before angiotensin injection. Values are means ± S.E.M. of 6–14 values. Difference from controls: * $P < 0.05$; ** $P < 0.01$; where not indicated difference from controls was not statistically significant.

EXP3174 significantly reduced the angiotensin II-induced water intake at each time of observation. The analysis of variance revealed a statistically significant treatment effect [$F(1,61) = 100.21$; $P < 0.0001$] and significant effect of the time at which the antagonist was administered [$F(3,61) = 8.88$; $P < 0.001$] (Fig. 3).

The analysis of variance of GR117289 data revealed a statistically significant treatment effect [$F(1,61) = 76.12$; $P < 0.0001$], a non-significant effect of the time at which the antagonist was administered [$F(3,61) = 0.51$; $P > 0.05$], but a significant treatment-time interaction $F(3,61) = 3.07$; $P < 0.05$]. A significant reduction in water intake was observed at 4, 12 and 24 h after i.p. GR117289 injection (Fig. 4).

The analysis of variance of LR-B/081 data revealed a statistically significant treatment effect [$F(1,81) = 17.26$; $P < 0.001$], effect of the time at which the antagonist was administered [$F(3,81) = 3.40$; $P < 0.05$] and treatment-time interaction [$F(3,81) = 3.43$; $P < 0.005$]. A significant

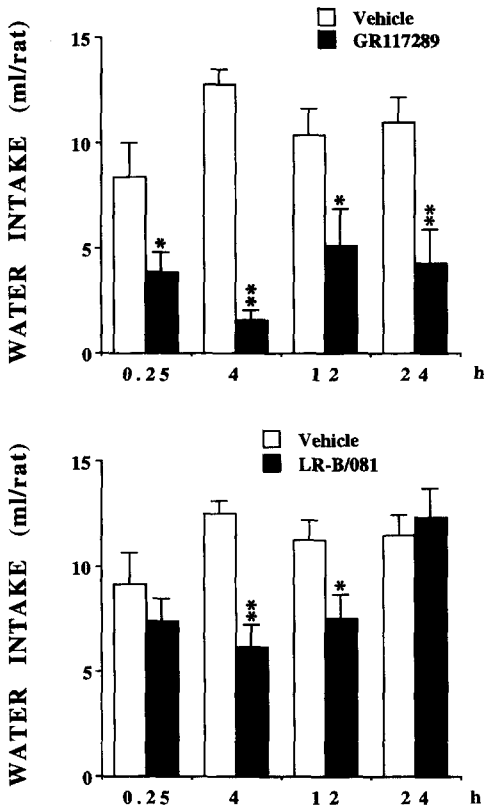

Fig. 4. Water intake over 15 min after i.c.v. injection of angiotensin II, 10 pmol/rat, in animals i.p. pretreated with vehicle (controls) or with 20 μmol/kg of GR117289 (upper panel) or LR-B/081 (lower panel) at different times before angiotensin injection. Values are means ± S.E.M. of 6–16 values. Difference from controls: * $P < 0.05$; ** $P < 0.01$; where not indicated difference from controls was not statistically significant.

reduction in water intake was observed only at 4 and 12 h after i.p. LR-B/081 injection (Fig. 4).

3.4. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on carbachol-induced drinking

LR-B/081, losartan, EXP3174 or GR117289, at the i.p. dose of 20 μmol/kg did not significantly modify carbachol-induced water intake. For instance, 4 h after i.p. injection the controls took 11.2 ± 1.0 ml of water/rat in 15 min after i.c.v. carbachol injection, while the treated rats took 10.05 ± 0.71 (LR-B/081), 13.97 ± 1.49 (losartan), 9.13 ± 1.73 (EXP3174) and 9.96 ± 1.94 ml/rat (GR117289).

3.5. Effect of i.p. injections of angiotensin AT_1 receptor antagonists on the pressor response to i.v. angiotensin II

Following i.p. injection of 20 μmol/kg of each angiotensin AT_1 receptor antagonist tested, the hypertensive effect of i.v. angiotensin II was almost completely abolished (more than 90% inhibition) in the first 4 h of observation (Fig. 5). Afterwards a slow recovery in the hypertensive response to angiotensin II was observed, but

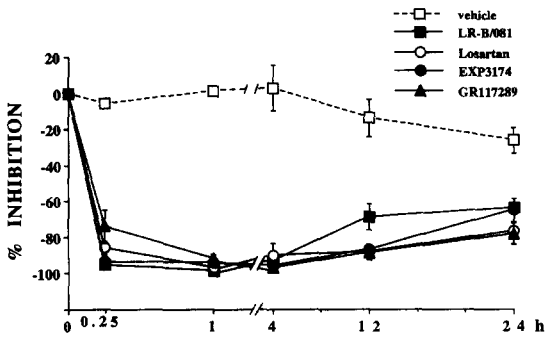

Fig. 5. Percent inhibition of the pressor effect of i.v. angiotensin II, 0.15 nmol/kg, in rats i.p. pretreated with vehicle (controls) or with 20 μmol/kg of the 4 antagonists tested. Values are means ± S.E.M. of 3 values. Difference from controls for each antagonist was statistically highly significant ($P < 0.01$) from 0.25 to 24 h.

the inhibition of its effect remained above 70% for each antagonist. No statistically significant difference was observed between the effects of the 4 angiotensin AT_1 receptor antagonists at any time of observation.

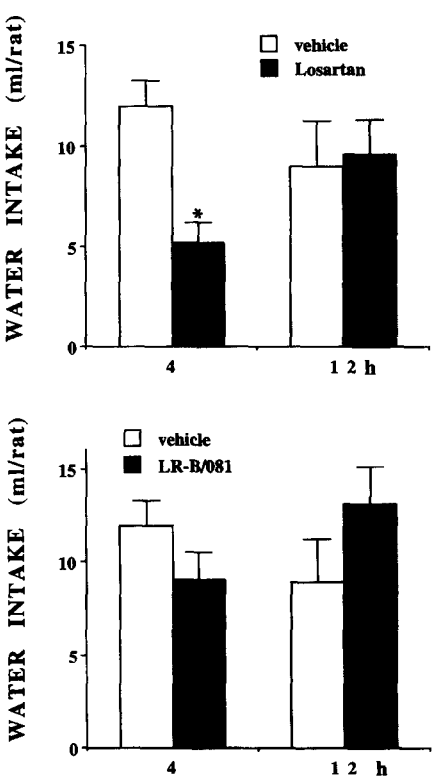

Fig. 6. Water intake over 15 min after i.c.v. injection of angiotensin II, 10 pmol/rat, in animals p.o. pretreated with vehicle (controls) or with 20 μmol/kg of losartan (upper panel) or LR-B/081 (lower panel) at 4 or 12 h before angiotensin injection. Values are means ± S.E.M. of 9 (4 h) and 5 (12 h) values. Difference from controls: * $P < 0.05$; ** $P < 0.01$; where not indicated difference from controls was not statistically significant.

3.6. Effect of p.o. administration of losartan or LR-B/081 on angiotensin II-induced drinking

As shown in Fig. 6, the p.o. administration of LR-B/081, 20 μmol/kg, 4 h before i.c.v. angiotensin II injection slightly, but not significantly, reduced angiotensin II-induced drinking. The analysis of variance revealed the absence of treatment effect [$F(1,8) = 4.87$; $P > 0.05$] and of treatment-time (15 and 30 min time of observation) interaction. On the other hand, at the same dose, losartan given 4 h before i.c.v. angiotensin II reduced the water intake. The analysis of variance revealed a significant treatment effect [$F(1,8) = 30.80$; $P > 0.001$].

When 20 μmol/kg of either LR-B/081 or losartan was given 12 h before i.c.v. angiotensin II, neither drug significantly modified the drinking response to angiotensin II.

4. Discussion

The results of the present study showed that, following i.p. injection, each of the 4 angiotensin AT_1 receptor antagonists tested was able to inhibit the dipsogenic effect of i.c.v. angiotensin II, providing functional evidence that they can cross the blood-brain barrier. However, it should be emphasized that the i.p. doses required to inhibit drinking induced by central angiotensin II were in the order of milligrams, while the i.c.v. doses were in the order of nanograms, suggesting that the ability to cross the blood-brain barrier is not very pronounced even for the antagonists most potent after i.p. injection.

Differences were observed in the potency and time course of the inhibitory effect of the 4 antagonists. The ID_{50} determined by i.p. injection of the antagonists 15 min before i.c.v. angiotensin II, gave the following order of potency: EXP3174 > GR117289 > losartan > LR-B/081 (see Table 1).

Clearly, the ID_{50} in our functional study was influenced by two main factors: (1) the ability of the drug to cross the blood-brain barrier; and (2) the ability of the drug to block central receptors. Since angiotensin AT_1 receptors that mediate angiotensin II-induced drinking are easily accessible from the ventricle, the effect of the antagonists given by i.c.v. injection can be considered a measure of their ability to block central AT_1 receptors. Thus, the ratio of i.p./i.c.v. ID_{50} of each antagonist can be taken as a relative measure of the ability to cross the blood-brain barrier.

GR117289 showed an i.p./i.c.v. ratio, lower than that of EXP3174 and of LR-B/081, suggesting that GR117289 can cross the barrier more readily than the other 2 antagonists. These results concerning GR117289 are consistent with those of previous studies (Marshall et al., 1992; Dennes et al., 1993).

The i.p./i.c.v. ratio for EXP3174 was only about 3 times higher than that of GR117289, indicating that EXP3174 also readily crosses the blood-brain barrier.

LR-B/081 showed an i.p./i.c.v. ratio higher than that of the other antagonists. As stated in the Results, it was impossible to exactly determine its value, suggesting that the drug does not easily cross the barrier. This finding is rather surprising in relation to the high lipophilicity of LR-B/081 ($K_p = 127.2$; Dr. G. Heinrich, Berlin Chemie, Berlin, Germany, personal communication). The K_p determined by the same author under the same experimental conditions was 0.32 for losartan (potassium salt) and 11.7 for losartan (acid). However, it is interesting to note a recent report that the transplacental transfer of losartan and of angiotensin converting enzyme inhibitors does not parallel their lipid solubility (Stevenson et al., 1995).

Finally, losartan gave the lowest i.p./i.c.v. ratio. The drug showed a far lower i.c.v. potency in comparison to the other antagonists, while its i.p. potency was only slightly inferior. This finding, however, may be to a large extent related to conversion to its active metabolite EXP3174, which is a very potent inhibitor of i.c.v. angiotensin II-induced drinking following peripheral administration. In this regard, it should be noticed that the i.p. injection of losartan took place 15 min before i.c.v. angiotensin II administration, while the i.c.v. antagonist injection took place just 5 min before i.c.v. angiotensin. The longer interval after i.p. injection and the higher metabolic activity in the periphery might have allowed a greater conversion to EXP3174. The time course of the effect of losartan is consistent with this hypothesis. The drug was not active at 15 min after i.p. injection, suggesting that adequate time for conversion is required.

Our findings indicating that i.p. losartan can influence central angiotensin mechanisms are consistent with the results of ex vivo binding studies (Song et al., 1991; Marshall et al., 1992; Zhuo et al., 1994) and of functional studies (Fregley and Rowland, 1991; Dennes et al., 1993; Li et al., 1993; Morien et al., 1994; Palmer et al., 1994; Wayner et al., 1993).

As far as the time course of the effect is concerned, our results indicate that the inhibitory effect of EXP3174 was prompt and long-lasting, since it was evident at 15 min after i.p. administration and remained statistically significant even at 24 h after i.p. injection. The effect of losartan was slower in onset than that of EXP3174, but then became similar to that of EXP3174, both in intensity and in duration, lasting up to 24 h.

The effect of GR117289 was prompt, being evident at 15 min after administration, reached a maximum at the 4 h and remained very pronounced even at 24 h.

The effect of LR-B/081 was slow in onset, was statistically significant at 4 and 12 h and was no longer detected at 24 h. The absence of effect of LR-B/081 on i.c.v. angiotensin II-induced drinking at 24 h cannot be ascribed to a different half-life of the drug in comparison to the

other antagonists, since 24 h after i.p. injection of 20 μmol/kg of LR-B/081 was still able to inhibit the pressor effect of i.v. angiotensin II to almost the same extent as the other antagonists. Therefore, it seems reasonable to think that the time course of the LR-B/081 effect also reflects a lower ability of this drug to cross the blood-brain barrier.

Following p.o. administration of 20 μmol/kg, losartan reduced drinking when given 4, but not 12 h before i.c.v. angiotensin II; LR-B/081 did not inhibit drinking either at 4 or 12 h. These results indicate that also following p.o. administration losartan can influence central angiotensin mechanisms, although for a shorter period of time than after i.p. injection. Indeed, Wong et al. (1990a) have shown that a single p.o. administration of losartan, 10 mg/kg, does not influence the hypertensive response in the 3 h following i.c.v. angiotensin II, suggesting that, on p.o. administration, the drug does not affect central angiotensin receptors. Moreover, Bui et al. (1992) have shown that in rats given 3 mg/kg of losartan in the drinking water for 3 days the dipsogenic and pressor response to i.c.v. angiotensin II was not modified. However, the experimental conditions were different in these 2 studies and in the present one: different dose and modality of administration in the study of Bui et al. (1992); different functional test and different times of observation in the study of Wong et al. (1990a). In relation to the time of observation, the present study emphasized the opportunity of evaluating the central effects of peripheral administration of these angiotensin AT_1 receptor antagonists for several hours after their administration.

As far as LR-B/081 is concerned, the results of p.o. administration, in keeping with those obtained following i.p. injection, indicate that LR-B/081 has a lower ability than losartan to exert central effects.

The results of the present study can be of interest for research in relation to the use of angiotensin AT_1 receptor antagonists as pharmacological tools to investigate the physiopathological functions of the central renin-angiotensin system. The present findings should stimulate further investigation of the beneficial and/or negative implications of central actions of angiotensin AT_1 receptor antagonists in the course of antihypertensive therapy.

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