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Evaluation of the ability of irbesartan to cross the blood–brain barrier following acute intragastric treatment

Carlo Polidori [a,*], Roberto Ciccocioppo [a], Dino Nisato [b], Catherine Cazaubon [b], Maurizio Massi [a]

[a] *Department of Pharmacological Sciences and Experimental Medicine, University of Camerino, Via Scalzino 3, 62032 Camerino (MC), Italy*
[b] *Cardiovascular Research Department, Sanofi Recherche, Montpellier Cedex 04, France*

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Abstract

The present study evaluated in functional tests the ability of the angiotensin AT_1 receptor antagonist irbesartan, 2-*n*-butyl-3-[(2′-(1*H*-tetrazol-5-yl)-biphenyl-4-yl)methyl]-1,3-diaza-spiro[4,4]non-1-en-4-one, in comparison to losartan, 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′(1*H*-tetrazol-5-yl) bi-phenyl-4-yl)methyl]imidazole, to cross the blood–brain barrier following acute intragastric administration. Two tests were used: the dipsogenic response to intracerebroventricular injection of angiotensin II, and Na^+ intake in response to adrenalectomy. In normotensive rats, irbesartan reduced the dipsogenic response to angiotensin II, 10 pmol per rat, at the dose of 90 mg/kg, but not at lower doses. Losartan significantly reduced angiotensin II-induced drinking at 30 mg/kg, but not at a lower dose. In spontaneously hypertensive rats, irbesartan reduced the response to angiotensin II at 50 mg/kg, but not at lower doses, while losartan significantly inhibited angiotensin II-induced drinking even at 10 mg/kg. In adrenalectomized rats, the intake of 2% NaCl was inhibited by the intragastric administration of losartan 30 or 50 mg/kg, while irbesartan did not reduce it in doses up to 50 mg/kg. The results of the present study consistently indicate that after acute intragastric administration, the ability of irbesartan to cross the blood–brain barrier is lower than that of losartan.

Keywords: Angiotensin AT_1 receptor; Irbesartan; Losartan; Water intake; Salt intake

1. Introduction

Angiotensin II is known to be involved in the physiopathological control of blood pressure not only by means of peripheral effects, such as vasoconstriction, stimulation of aldosterone secretion, direct actions on the renal tubule, but also by means of central effects, such as an increase in 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; Phillips, 1987; Unger et al., 1988). In the central nervous system, angiotensin II may also be involved in the control of the release of adrenocorticotropic and reproductive hormones, in cognitive processes, anxiety and analgesia (Barnes et al., 1990; Phillips, 1987 and Wright and Harding, 1994, for review). Therefore, it is of interest to know whether, and under which conditions, peripherally administered drugs that influence the renin–angiotensin system are able to cross the blood–brain barrier to affect central angiotensinergic mechanisms.

The aim of the present study was to evaluate in functional tests whether the angiotensin AT_1 receptor antagonist irbesartan (Cazaubon et al., 1993, 1994; Christophe et al., 1995; Lacour et al., 1994) is able to influence the central renin–angiotensin system after acute intragastric administration in comparison to losartan (Timmermans et al., 1993). The following functional tests in rats were chosen: the dipsogenic response induced by intracerebroventricular (i.c.v.) injection of angiotensin II and the Na^+ intake response induced by adrenalectomy.

The drinking response to i.c.v. angiotensin II is an appropriate functional test, because it is mediated by angiotensin AT_1 receptors (Fregly and Rowland, 1991; Beresford and Fitzsimons, 1992; Sakai et al., 1994; Polidori et al., 1995), apparently in brain sites inside the blood–brain barrier. The subfornical organ, a circumven-

* Corresponding author. Tel.: +39-737-403307; fax: +39-737-630618; e-mail: polidori@cambio.unicam.it

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tricular organ outside the barrier, which is essential for the drinking response elicited by blood-borne angiotensin II, has little if any role in the dipsogenic response to i.c.v. angiotensin II (Lind and Johnson, 1982; Perfumi et al., 1986; Johnson and Edwards, 1990). Regional obstruction or lesion of periventricular tissues of the antero-ventral third ventricular region (including the organum vasculosum of the lamina terminalis, the median preoptic nucleus and the preoptic periventricular nuclei) abolishes the drinking response elicited by i.c.v. angiotensin II (Buggy et al., 1975). However, lesion of the organum vasculosum of the lamina terminalis, a circumventricular organ outside the barrier, has no effect on the drinking response to i.c.v. angiotensin II. Nuclei inside the barrier, such as the median preoptic nucleus or the preoptic periventricular nuclei, are mostly involved in the response (Gardner and Stricker, 1985; Johnson and Edwards, 1990). Angiotensin II induces intense expression of c-Fos protein in the anterior region of the third ventricle, including the organum vasculosum of the lamina terminalis, the subfornical organ and the median preoptic nucleus (Herbert et al., 1992; Lebrun et al., 1995). However, the i.c.v. injection of angiotensin II induces c-Fos expression mainly in the median preoptic nucleus (McKinley et al., 1995), and only in periventricular parts of the two circumventricular organs. These organs show tight junctions over the ependymal surface that preclude rapid access of molecules from the cerebrospinal fluid (McKinley et al., 1990).

Salt intake evoked by adrenalectomy in rats may be another functional test to evaluate the ability of angiotensin AT_1 receptor antagonists to influence the central nervous system. Even though the neuroanatomical substrates for this effect are not well defined yet, the work of Sakai and Epstein (1990) has shown that salt appetite in the adrenalectomized rat is inhibited by i.c.v., but not by intravenous infusion of peptide angiotensin II antagonists. These findings suggest that the receptors that mediate the effect may be inside the blood–brain barrier. Also, the sodium intake induced by angiotensin II is mediated by angiotensin AT_1 receptors (Galaverna et al., 1996; Beresford and Fitzsimons, 1992; Rowland et al., 1992).

The experiments concerning the dipsogenic response to i.c.v. angiotensin II were carried out also in spontaneously hypertensive rats (SHR) because these animals may show altered regulation of the brain renin–angiotensin system (Wright and Harding, 1994), and because the permeability of the blood–brain barrier may be altered by acute or chronic hypertension (Mayhan, 1990; Tang et al., 1992).

2. Materials and methods

2.1. Animals

The experiments concerning the dipsogenic response to i.c.v. angiotensin II were carried out in: (a) male Wistar rats (Charles River, Italy), weighing 325 to 350 g at the moment of intracranial surgery, and (b) male SHR rats (Charles River), 11 to 12 weeks old at the moment of intracranial surgery.

The experiments with adrenalectomized rats were carried out with male Wistar rats (Charles River), weighing 325 to 350 g at the moment when adrenalectomy was performed.

Rats were housed in individual stainless steel cages in a room with 12:12-h light:dark cycle (lights off at 1800 h), controlled temperature (20 to 21°C) and humidity (45 to 55%). They were offered free access to food pellets (4RF18, Mucedola, Settimo Milanese, Italy) and tap water.

2.2. Drugs

Losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′(1*H*-tetrazol-5-yl) biphenyl-4-yl) methyl] imidazole potassium salt) was a gift of DuPont Merck, Research and Development, Wilmington, DE, USA Irbesartan, 2-*n*-butyl-3-[(2′-(1*H*-tetrazol-5-yl)-biphenyl-4-yl)methyl]-1,3-diazaspiro [4,4]non-1-en-4-one, was a gift of Sanofi Recherche, Montpellier, France. Ile^5-angiotensin II was purchased from Novabiochem-Inalco, Milan, Italy; carbachol was purchased from Sigma Italia, Milan, Italy.

The solutions of irbesartan and losartan for both intragastric and i.c.v. administration were made up in water, to which was added 2 M NaOH to bring the pH of the solution to 8.

2.3. Intracranial surgery

Rats were anesthetized by intraperitoneal injection of 100 to 150 μl per 100 g body weight 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 per rat, intramuscularly) before surgery. Using a David Kopf stereotaxic instrument, a guide cannula, aimed at the left lateral ventricle, was implanted and cemented with acrylic cement to the skull. The coordinates for the guide cannula were: AP = 1 mm behind the bregma, $L = 2$ mm from the sagittal suture, $V = 2$ mm from the surface of the skull. Rats were allowed to recover from surgery for a week. For i.c.v. injections a stainless steel injector temporarily inserted into the guide cannula and protruding 2.5 mm beyond the cannula tip was used.

2.4. Validation of cannula placement

Before experiments, i.c.v. cannula placement was validated by evaluating the drinking response to i.c.v. angiotensin II, 10 pmol per rat. Only rats that drank at least 6 ml 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.

2.5. Experiment 1: effect of acute intragastric administration of irbesartan or losartan on water intake induced by i.c.v. injection of angiotensin II

Angiotensin II, 10 pmol per rat, was injected i.c.v. in 1 μl per rat of isotonic saline. The angiotensin AT_1 receptor antagonists were given intragastrically by gavage, in a volume of 2.5 ml/kg, 1, 4, 12 or 24 h before angiotensin II. Water intake was measured 15 and 30 min following angiotensin II injection (most drinking occurred in the first 15 min). In two experimental sessions in counterbalanced order, each rat received, by intragastric administration at a single time before i.c.v. angiotensin (1, 4, 12 or 24 h), vehicle and one dose of one antagonist.

2.6. Experiment 2: effect of acute intragastric administration of irbesartan or losartan on water intake induced by i.c.v. injection of carbachol

The effect of irbesartan and losartan was also tested on drinking induced by i.c.v. carbachol (which does not involve angiotensinergic mechanisms; Fitzsimons, 1979). Carbachol, 300 ng per rat, was i.c.v. injected in 1 μl of isotonic saline. Water intake was measured 15 and 30 min later. Each rat received vehicle, irbersatan or losartan at intervals of 7 days.

2.7. Experiment 3: effect of i.c.v. administration of irbesartan on water intake induced by i.c.v. injection of angiotensin II

Irbesartan or its vehicle was i.c.v. administered in a volume of 1 μl per rat, 5 min before i.c.v. injection of angiotensin II, 10 pmol per rat. Water intake was measured 15 and 30 min following angiotensin II injection. The experiment was carried out according to a Latin Square design, in which each animal received, in different experimental sessions, i.c.v. administration of vehicle and the 3 doses of irbesartan.

2.8. Experiment 4: effect of acute intragastric administration of irbesartan or losartan on adrenalectomy-induced salt intake

Under ketamine/acepromazine anesthesia, bilateral dorsal incisions were made at the level of the last rib and the adrenal glands were removed, taking care to ensure that the adrenal capsulas remained intact. For 7 days after surgery, animals had free access to 2% NaCl solution, in addition to food pellets and tap water. From the 8th day, 2% NaCl was offered only 2 h/day. Experiments began after 7 days of limited access to salt.

Irbesartan and losartan were given by gavage, in 2.5 ml/kg of vehicle, 4 h before access to salt. The 4-h interval was chosen on the basis of the results of Experiment 1. Salt intake was measured 15, 30, 60 and 120 min after access to salt. To reduce the influence of variability among rats in salt intake, the experiments were carried out according to a within subject design. Each rat received, in different experimental sessions, intragastric administration of vehicle and the different doses of one antagonist, according to a Latin Square design.

2.9. Statistical analysis

The intake (milliliter per rat) of each rat was analyzed statistically. In Experiments 1 and 2, data for each antagonist were analyzed by split-plot analysis of variance, with between groups comparisons for time of administration (1, 4, 12 or 24 h) before the test, and within groups comparisons for pharmacological treatment (vehicle or dose of antagonist) and for period of observation (15 or 30 min). In Experiments 3 and 4, data for each antagonist were analyzed by means of a two-way multifactor analysis of variance (repeated measures). Planned pairwise comparisons were carried out by means of the Student's *t*-test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Experiment 1: effect of acute intragastric administration of irbesartan or losartan on water intake induced by i.c.v. injection of angiotensin II

Control normotensive rats responded to the i.c.v. injection of angiotensin II, 10 pmol per rat, with a mean water intake ranging between 9.6 to 13.0 ml per rat in 15 min and 10.4 to 13.5 ml per rat in 30 min.

A dose of 90 mg/kg of irbesartan was needed to significantly reduce water intake in response to angiotensin II ($F(1,7) = 22.37$; $P < 0.01$). The effect was statistically significant both 15 and 30 min after angiotensin II injection. Irbesartan, 10, 30 or 50 mg/kg did not significantly modify angiotensin-induced drinking ($F(1,24) = 0.60$; $P > 0.05$; $F(1,26) = 2.80$; $P > 0.05$; $F(1,19) = 0.01$; $P > 0.05$, respectively) (Fig. 1).

In normotensive rats, losartan, 30 mg/kg, significantly reduced water intake ($F(1,20) = 207.87$; $P < 0.001$) (Fig. 1). The effect was statistically significant when losartan was administered 1, 4 or 12 h, but not 24 h, before angiotensin II. Losartan, 10 mg/kg, did not significantly reduce angiotensin II-induced drinking ($F(1,21) = 1.13$; $P > 0.05$).

SHR responded to i.c.v. angiotensin II, 10 pmol per rat, with a mean water intake ranging between 5.8 to 8.4 ml per rat in 15 min and 6.1 to 9.2 ml per rat in 30 min.

In SHR, (Fig. 2) irbesartan, 50 mg/kg, significantly reduced water intake induced by angiotensin II ($F(1,14) =$

17.96; $P < 0.005$). The effect was significant when irbesartan was administered 4 h, but not 1 h, before angiotensin II. Irbesartan, 10 or 30 mg/kg, did not modify angiotensin-induced drinking ($F(1,25) = 0.001$; $P > 0.05$ or $F(1,30) = 3.53$; $P > 0.05$; respectively). The analysis revealed neither a significant interaction between treatment and interval (1, 4, 12 or 24 h) between administration of the 2 drugs, nor a significant interaction between treatment and time of observation.

In SHR, losartan, 10 mg/kg, significantly reduced water intake induced by angiotensin II ($F(1,24) = 6.35$; $P < 0.05$), when given 4 h before angiotensin II (Fig. 2). Losartan 30 mg/kg reduced water intake ($F(1,23) = 38.52$; $P < 0.001$), when injected 1, 4 or 12, but not 24, h before angiotensin II. No significant treatment effect was observed in response to losartan, 3 mg/kg, given 4 h before angiotensin II ($F(1,6) = 0.13$; $P > 0.05$).

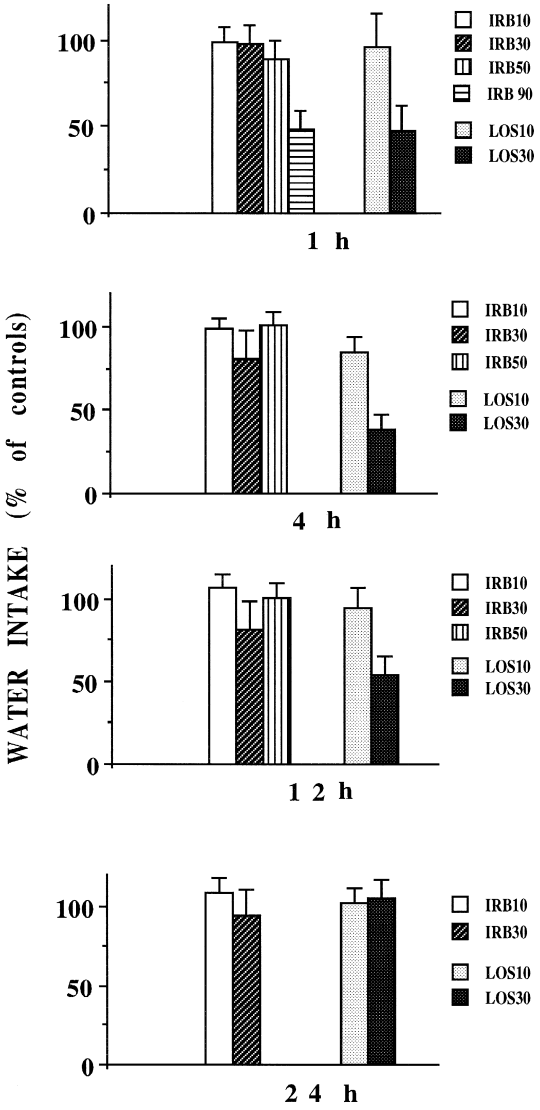

Fig. 1. Cumulative water intake (percentage of controls) 15 min after i.c.v. injection of angiotensin II, 10 pmol per rat, in normotensive rats intragastrically pretreated (1, 4, 12 or 24 h before) with either irbesartan or losartan. Values are means ± S.E.M. for 6 to 9 subjects. Statistical difference from controls is reported in Section 3.

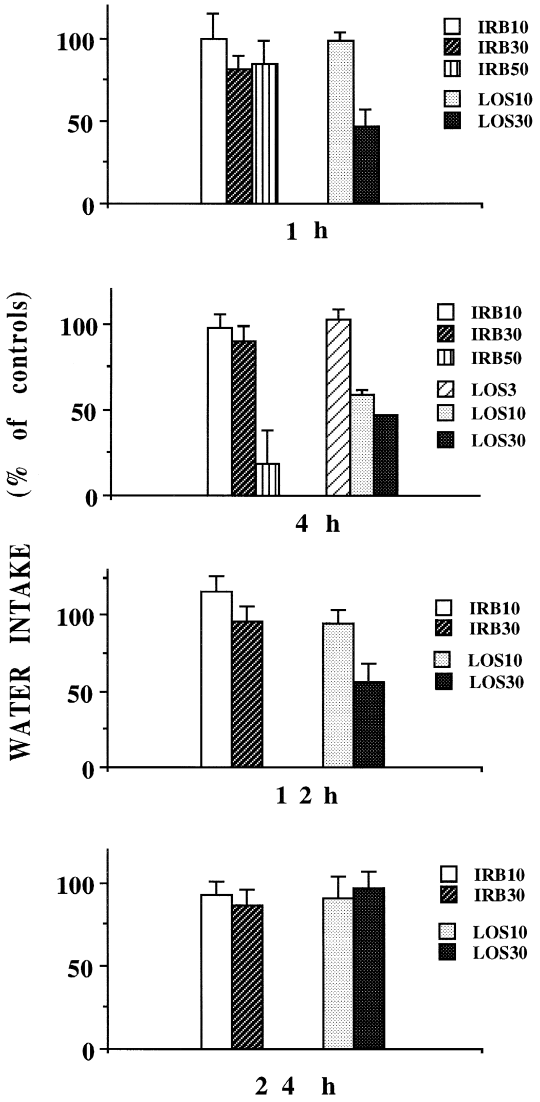

Fig. 2. Cumulative water intake (percentage of controls) 15 min after i.c.v. injection of angiotensin II, 10 pmol per rat, in SHR intragastrically pretreated (1, 4, 12 or 24 h) before with either irbesartan or losartan. Values are means ± S.E.M. for 6 to 10 subjects. Statistical difference from controls is reported in Section 3.

3.2. Experiment 2: effect of acute intragastric administration of irbesartan or losartan on water intake induced by i.c.v. injection of carbachol

In normotensive rats neither irbesartan, 90 mg/kg, nor losartan, 30 mg/kg, significantly modified drinking elicited by i.c.v. carbachol, 300 ng per rat, (vehicle = 7.8 ± 0.8 ml per rat, irbesartan 90 mg/kg = 8.8 ± 0.9 ml per rat; vehicle = 7.8 ± 0.8 ml per rat, losartan 30 mg/kg = 9.2 ± 0.7 ml per rat).

Also, in SHR neither irbesartan, 50 mg/kg, nor losartan, 30 mg/kg, significantly modified the drinking response to i.c.v. carbachol, 300 ng per rat, given 4 h after irbesartan or losartan (vehicle 6.0 ± 0.5 ml per rat, irbesartan 50 mg/kg $= 7.8 \pm 1$ ml per rat; vehicle 6.0 ± 0.5 ml per rat, losartan 30 mg/kg $= 7.4 \pm 0.8$ ml per rat).

These findings provide evidence that the effect of losartan and irbesartan is selective for angiotensin II-induced drinking.

3.3. Experiment 3: effect of i.c.v. administration of irbesartan on water intake induced by i.c.v. injection of angiotensin II

In the range of i.c.v. doses of 1 to 100 ng per rat, irbesartan significantly inhibited angiotensin II-induced water intake ($F(3,18) = 19.70$; $P < 0.001$). Even 1 ng per rat produced a highly significant and pronounced (more than 40%) inhibition, while 100 ng per rat almost completely suppressed water intake (Fig. 3).

3.4. Experiment 4: effect of acute intragastric administration of irbesartan or losartan on adrenalectomy-induced salt intake

Control adrenalectomized rats had a mean salt intake of 13.3 to 15.6 ml per rat at 15 min and 22.2 to 25.7 ml per rat at 120 min.

The analysis of variance revealed no significant effect on salt intake of irbesartan, 10 mg/kg ($F(1,8) = 2.46$; $P > 0.05$), but a significant treatment-time interaction ($F(3,24) = 4.35$; $P < 0.05$). In irbesartan treated rats salt intake was slightly, but significantly higher than that of controls at 60 min after access to salt (Fig. 4). No significant effect of irbesartan, 30 mg/kg, was observed ($F(1,8) = 0.00$; $P > 0.05$) and there was no treatment-time interaction. Irbesartan, 50 mg/kg, significantly increased salt intake ($F(1,9) = 5.45$; $P < 0.05$) at 120 min after access to salt.

A pronounced inhibition of salt intake was observed following losartan, 30 mg/kg ($F(1,7) = 7.65$; $P < 0.05$) or 50 mg/kg ($F(1,6) = 18.38$; $P < 0.01$). Salt intake was significantly lower than that of controls at 30 and 60 min after access to salt. In rats treated with losartan 50 mg/kg, salt intake was significantly lower than that of controls at 15, 30, 60 and 120 min after access to salt. In response to 10 mg/kg losartan, neither a significant treatment effect ($F(1,8) = 0.01$; $P > 0.05$) nor a significant treatment-time interaction was observed.

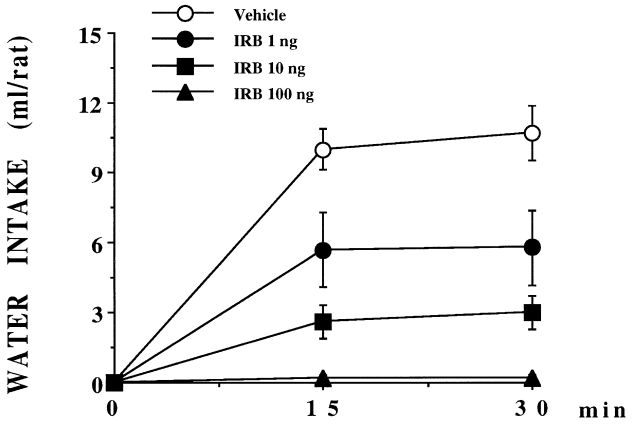

Fig. 3. Cumulative water intake (milliliter per rat) 15 min after i.c.v. injection of angiotensin II, 10 pmol per rat, in normotensive rats i.c.v. pretreated 5 min before with vehicle or irbesartan at 1 to 100 ng per rat. Values are means ± S.E.M. for 7 subjects. Statistical difference from controls is reported in Section 3.

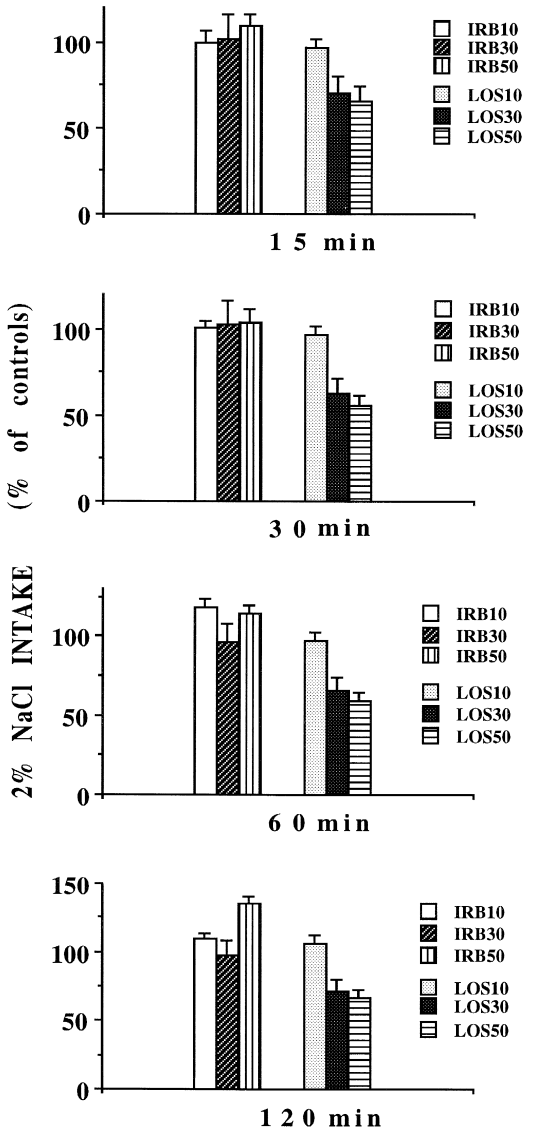

Fig. 4. Cumulative 2% NaCl intake (percentage of controls) in adrenalectomized rats intragastrically pretreated 4 h before access to salt with either irbesartan or losartan. Values are means ± S.E.M. for 9 subjects. Statistical difference from controls is reported in Section 3.

4. Discussion

The results of the present study indicate that irbesartan does not readily influence central angiotensinergic mechanisms after acute intragastric administration. In normotensive rats, 90 mg/kg was needed to inhibit drinking in response to angiotensin II; however, this is a very high dose since irbesartan almost completely abolishes the pressor response to intravenous angiotensin II at acute oral doses of 10 to 30 mg/kg (Cazaubon et al., 1993). In SHR, irbesartan reduced drinking in response to angiotensin II at 50 mg/kg. In adrenalectomized rats, irbesartan did not reduce salt intake in doses up to 50 mg/kg.

However, the i.c.v. injection of irbesartan potently inhibited angiotensin II-induced drinking. The potency of i.c.v. irbesartan was similar to that measured after i.c.v. injection of the angiotensin AT_1 receptor antagonist EXP 3174 (2-*n*-butyl-4-chloro-1-[(2′ (1*H*-tetrazol-5-yl) biphenyl-4-yl) methyl] imidazole-5-carboxylic acid) (ID_{50} = 3.9 pmol per rat, about 2 ng per rat), while it was far superior to that of losartan (ID_{50} = 357 pmol per rat, about 700 ng per rat) (Polidori et al., 1995). These findings suggest that the low potency of irbesartan to inhibit the central action of angiotensin II might be related to the fact that irbesartan does not readily cross the blood–brain barrier after acute intragastric administration.

Lacour et al. (1995) reported that a 4-day administration of irbesartan, 30 mg/kg per day, by the intragastric route significantly inhibited drinking in response to i.c.v. angiotensin II, 100 ng per rat. This finding suggests that the duration of treatment may influence the ability of irbesartan to exert central effects.

Interestingly, in the present study a significant inhibition of angiotensin II-induced drinking was observed in SHR at 50 mg/kg, while 90 mg/kg was required in normotensive rats. These findings are in keeping with the hypothesis that the permeability of the blood–brain barrier is different in normotensive and hypertensive rats (Tang et al., 1992).

A low accessibility of irbesartan to the central nervous system after acute intragastric administration might explain why irbesartan increased salt intake in adrenalectomized rats. Angiotensin AT_1 receptor antagonists are known to increase plasma levels of components of the renin–angiotensin system by blocking the peripheral feedback mechanism. Angiotensin I may cross the barrier and generate angiotensin II in the brain, thus increasing salt intake, if central angiotensin AT_1 receptors are not blocked (Fregly and Rowland, 1986). Salt intake has also been reported after peripheral injection of low doses of captopril (Elfont et al., 1984), doses which do not block the central conversion of angiotensin I to angiotensin II (Evered et al., 1980).

The present results indicate that following acute intragastric administration losartan influences central angiotensinergic mechanisms more potently than irbesartan, both in normotensive, SHR and adrenalectomized rats. Accordingly, several studies have shown that peripheral losartan administration influences central angiotensinergic mechanisms (Song et al., 1991; Polidori et al., 1996; Rowland et al., 1996). Irbesartan is highly lipophylic and has an about 10-fold higher affinity for angiotensin AT_1 receptors than losartan. Therefore, the ability of peripherally administered losartan to influence central angiotensin mechanisms more than irbesartan might be likely accounted for by its conversion to EXP3174, which is about 20 times more potent than losartan to inhibit central angiotensin AT_1 receptors and which apparently crosses the blood–brain barrier rather well (Polidori et al., 1996).

In conclusion, the results of the present study, as well as those of the study of Polidori et al. (1996), show marked differences in the ability of angiotensin AT_1 receptor antagonists to influence central angiotensinergic mechanisms following acute intragastric administration. These findings can be of interest for experimental research in which angiotensin AT_1 receptor antagonists are used as pharmacological tools to investigate the physiopathological functions of the central renin–angiotensin system. Moreover, the present findings warrant further investigation of the beneficial and/or negative implications of the central actions of angiotensin AT_1 receptor antagonists in the course of the antihypertensive therapy.

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