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Effects of Losartan and Irbesartan administration on brain angiotensinogen mRNA levels

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Abstract

Losartan, 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′(1*H*-tetrazol-5-yl)-biphenil-4-yl)methyl]imidazole, and 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, are two angiotensin AT_1 receptor antagonists largely used in human health care as antihypertensive agents. Their ability to cross the blood–brain barrier and to influence the central renin–angiotensin system are widely investigated, but how this brain system responds to the subchronic and chronic block of the angiotensin AT_1 receptor is still unknown. Normotensive rats were intragastrically implanted for 7- and 30-day administration, with a dose of 3 and 30 mg/kg body weight. Treatments were shown to influence, in a dose-, time- and brain-area-dependent manner, angiotensinogen mRNA levels in scanned areas. This study showed a general up-regulation of angiotensinogen mRNA expression after 7 days and a widespread down-regulation or basal level of expression after a 30-day administration of two angiotensin AT_1 receptor antagonists.

Keywords: Angiotensinogen mRNA; Brain renin–angiotensin system; Angiotensin AT_1 receptor antagonist; Losartan; Irbesartan

1. Introduction

Angiotensinogen is the precursor of the active forms of various angiotensins. Angiotensin II is the most important peptide as final effector in the endocrine, paracrine, autocrine and intracrine renin–angiotensin system (Re, 1989, Baker et al., 1992).

The several angiotensins are produced by angiotensinogen via different enzymatic pathways. Angiotensin I, II, III, IV, and angiotensin (1–7) (Wright and Harding, 1997; Ferrario and Iyer, 1998; Kucharewicz et al., 2002) act through several angiotensin receptors such as AT_1 (AT_{1a} and AT_{1b} in the rat), AT_2, AT_4 (Clauser et al., 1996; Wright and Harding, 1997; Unger, 1999; Carey et al., 2001; De Gasparo et al., 2000; Sadoshima, 2000; Kaschina and Unger, 2003), the dual angiotensin II/vasopressin receptor (Ruiz-Opazo et al., 1995; Gonzalez et al., 1997; Hurbin et al., 2000) and the angiotensin (1–7) non-AT_1/AT_2 receptor, recently identified as the G protein-coupled receptor Mas that mediates the antidiuretic action of this peptide (Santos et al., 2003).

The renin–angiotensin system has been extensively studied in the brain, and has become a major point of interest for its pivotal functional role in various physiological effects. More specifically, AngII, AngIII and the angiotensinogen are involved in the hypertensive disease (Cox and Bishop, 1991; Steckelings et al., 1992; Bottari et al., 1993; Zini et al., 1996; Brooks, 1997; Song et al., 1997; Wright and Harding, 1997; Reaux et al., 2001; Sangaletti et al., 2004). Moreover, it is known that central, but not peripheral, angiotensin AT_1 receptor blockade by non-peptide angiotensin AT_1 receptor antagonist Irbesartan has antihypertensive effects (Leenen and Yuan, 2001). Non-peptide angiotensin AT_1 receptor antagonists such as Losartan and Irbesartan, have been employed as primary treatment in hypertension and used in several studies concerning their permeability towards the central nervous system. Losartan and its active metabolite EXP3174 (Wong et al., 1990) are known to cross the blood–brain barrier (Fregly and Rowland, 1991; Song et al., 1991; Li et al., 1993; Zhuo et al., 1994; Polidori et al., 1996, 1998; Culman et al. 1999; Wang et al., 2003). On the other hand, Irbesartan is known to cross the

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blood–brain barrier and to influence the brain renin–angiotensin system (Lacour et al., 1995; Culman et al., 1999; Leenen and Yuan, 2001). Yet, controversial findings arise from studies on the brain renin–angiotensin system after either treatment with Losartan or Irbesartan (Polidori et al., 1998; Culman et al., 1999). In the past, there has also been a controversy regarding the permeability of Losartan to the blood–brain barrier and its influence on blood pressure, vasopressin release, and water intake (Wong et al., 1990; Bui et al., 1992). The discrepancies of these studies are probably linked to the complexity of the renin–angiotensin system, particularly the renin–angiotensin system of the brain.

Using transgenic rats, Schinke et al. (1999) showed a direct correlation between brain angiotensinogen mRNA and blood pressure. Their hypertensive rats express high levels of angiotensinogen mRNA in the brain, so that when this mRNA is being turned down, blood pressure also decreases.

So, based upon these findings, the aim of this study was to investigate the effects of Losartan and Irbesartan after subchronic and chronic peripheral administration on the angiotensinogen mRNA expression in different brain regions of normotensive rats. So far, two studies investigated the mRNA expression of angiotensin AT_1 receptor in the CNS after treatment with angiotensin AT_1 receptor antagonists (Nishimura et al., 2000), whereas no investigation of angiotensinogen mRNA expression in CNS has yet being reported.

2. Materials and methods

2.1. Animals

Normotensive male Wistar Kyoto rats (n=at least 6 per group) (Charles River, Italy), weighing 325 to 350 g at the moment of the catheter intragastric implant were used. Animals were individually housed in cages in a room with controlled temperature (20–22 °C), humidity (45% to 55%) and 12:12 h light/dark cycle (light off at 18:00 h). They were offered free access to food pellets (diet 4RF18, Mucedola, Settimo Milanese, Italy) and tap water. All adopted procedures were in adherence to the European Community Council Directive for Care and Use of Laboratory Animals.

2.2. Drugs administration

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-diaza-spiro[4,4]non-1-en-4-one, was a gift of Sanofi Recherche, Montpellier, France. The solutions of Irbesartan and Losartan for intragastric administration were made up in water, to which a 2 M NaOH was added to bring the pH of the solution to 8.

Drugs were administered intragastrically once daily for 7 or 30 days at 3 and 30 mg/kg body weight per day. Five groups of animals were used for each set of experiment, the subchronic (7 days) and the chronic one (30 days). Groups of the two experiments were Control (physiological saline), Losartan low and high dose (3 and 30 mg/kg), and Irbesartan low and high dose (3 and 30 mg/kg). Doses from the literature are chosen as functional in lowering pressure in acute oral treatment after central i.c.v. stimulation with AngII (Culman et al., 1999).

2.3. Intragastric surgery

Rats were anesthetized by intraperitoneal injection of 100 to 150 μl per 100 g body weight of a solution containing tiletamine chlorohydrate and zolazepam chlorohydrate.

Catheters were implanted into the stomach wall and shifted up subcutaneously to the scapula (Lukas and Moreton, 1979). The drug administration began 1 week after the implant.

2.4. Tissue preparation and In situ hybridization histochemistry

On the morning of days 8 and 31 (24 h after the last drug administration), rats were killed by decapitation and their brains immediately removed, frozen in CO_2 ice pellets and stored at −80 °C. Subsequently, 14-μm-thick coronal section were cut at −20 °C in a cryostat, collected on gelatin-coated diethylpyrocarbonate-treated slides and stored at −80 °C until hybridization. Prior to hybridization, sections were fixed for 10 min at 4 °C in 4% paraformaldehyde/0.1 M sodium phosphate buffer for 1–2 min (pH 7.2) and then washed in ice-cold 0.5× SSC buffer for 1–2 min (1× SSC=0.15 M sodium chloride/0.015 M sodium citrate buffer, pH 7.0). Sections were air-dried at room temperature and hybridized at 37 °C in a humidified environment with a solution consisting of 0.2% (w/v) bovine serum albumin, 0.1% (w/v) polyvinylpyrrolidone, 4× SSC buffer, 50% formamide, 100 mg/ml of salmon sperm DNA and 6×10^6 cpm/ml of [^{33}P]-labeled synthetic oligonucleotide probe using terminal transferase. The synthetic oligodeoxyribonucleotide employed to detect angiotensin II mRNA was 5′dTGCCTCACTCAGCATCTTGTACATGCGGAA3′, which corresponds to the nucleotide sequence coding for amino acids 106–115 of the rat angiotensinogen (Yongue et al. 1991).

The hybridization mix was added at 100 μl per slide. Glass cover slips were applied and sections were incubated overnight (16 h) at 37 °C in humidified covered plastic trays. Following an overnight hybridization, cover slips were removed and slides were first rinsed in 1× SSC buffer at room temperature for 60 min and then for 60 min at 42 °C. They were then dipped in Kodak NTB-2 emulsion and permitted to air dry before they were stored in light-tight slides boxes containing Drierite (Acros Organics, USA). The slides were exposed for 3 weeks at 4 °C. Slides were finally developed to evaluate the silver grain numbers by using standard procedure: slides were developed in Kodak Dektol, rinsed in water, fixed in Kodak fixer and washed. Sections were counterstained with 0.1% cresyl violet and cover-slipped with Clarion mounting medium (Biomeda, USA).

In situ hybridization results as number of grains per cell were examined with a Nikon Optiphot-2 microscope. Sections

were examined at a magnification of 40× under bright- and dark-field microscopy. Images of reduced silver grains over cells were acquired at 40× magnification with a CCD video camera (DAGE/MTI Model 72). Images were digitized and stored on a microcomputer (Imaging Research, St. Catherines, Canada) (Fig. 1A–B) with a 486 microprocessor following a protocol for in situ hybridization analysis (Lucas et al., 1994).

2.5. Brain areas

Almost all analyzed brain areas express physiological angiotensinogen mRNA: horizontal limb diagonal band of Broca, medial septal nucleus, bed nucleus of stria terminalis, medial preoptic nucleus, subfornical organ, anteroventral thalamic nucleus, anterior paraventricular thalamic nucleus, paraventricular hypothalamic nucleus, supraoptic hypothalamic nucleus, arcuate hypothalamic nucleus, suprachiasmatic nucleus, and ventromedial hypothalamic nucleus.

2.6. Statistical analysis

The numbers of grains per cell per region per rat were averaged to yield a mean value. Statistical analysis of mean number of grains per cell was done via a simultaneous test of analysis of variance (ANOVA) with treatment as between-subject and brain regions for angiotensinogen mRNA as within-subject factors. Post-hoc follow-up tests of main effects (Student–Newman–Keuls) and interaction effects were done as required with a two-tailed cut-off at $P<0.05$ and $P<0.01$ vs. control for significance.

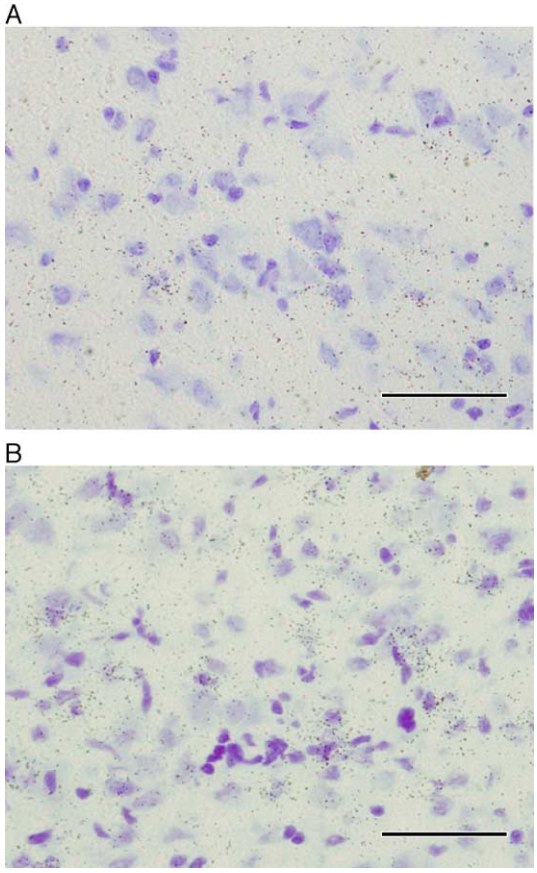

Fig. 1. In situ hybridization. Effects of 7-day drug treatment in the paraventricular hypothalamic nucleus. (A) Angiotensinogen mRNA expression in animals treated with vehicle. (B) Angiotensinogen mRNA expression in animals treated with Losartan high dose. Bar: 50 μm.

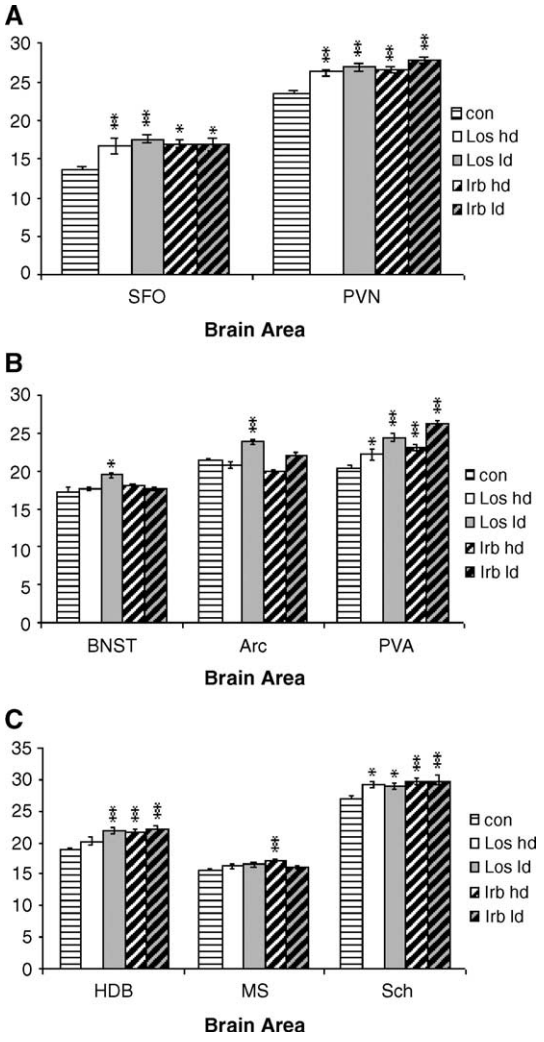

Fig. 2. (A–C) Effects of subchronic (7 days) Losartan (Los) and Irbesartan (Irb) administration on angiotensinogen mRNA in discrete brain nuclei. Los high dose (hd)=30 mg/kg per day, Los low dose (ld)=3 mg/kg per day, Irb hd=30 mg/kg per day, Irb ld=3 mg/kg per day. Data are shown as mean±S.E.M., number of grains/cells, groups of six–eight animals. *$P<0.05$, **$P<0.01$, where P values are vs. control. SFO=subfornical organ; PVN=paraventricular hypothalamic nucleus; BNST=bed nucleus of stria terminalis; Arc=arcuate hypothalamic nucleus; PVA=anterior paraventricular thalamic nucleus; HDB=horizontal limb diagonal band of Broca; MS=medial septal nucleus; Sch=suprachiasmatic nucleus.

3. Results

3.1. Effects of subchronic (7 days) intragastric administration of Losartan and Irbesartan on brain angiotensinogen mRNA expression

As shown in Fig. 2A–C, subchronic intragastric drug administrations of two angiotensin AT_1 antagonists (3 or 30 mg/kg

body weight/day) influence angiotensinogen brain expression in some of the mapped areas, up-regulating the mRNA level, while showing no effects in others.

Fig. 2A shows a statistically significant increase of angiotensinogen mRNA expression on subfornical organ cells of high- and low-dose Losartan-treated rats ($F=4.234$; $P<0.01$) by 23.5% and 30%, respectively. Similarly, Irbesartan treatment ($P<0.05$) shows a 25% with high and 24.8% increase with low dose of angiotensinogen mRNA.

In the paraventricular hypothalamic nucleus, there is a statistically significant increase ($F=10.827$; $P<0.01$) of angiotensinogen mRNA expression of about 12.9% and 15.8%, respectively, for Losartan high and low dose, of about 14.2% and 19.5% following Irbesartan high and low treatment (Fig. 2A).

Fig. 2B shows an increase in the angiotensinogen mRNA level in the paraventricular thalamic nucleus ($F=15.334$; $P<0.01$) after treatment with Irbesartan low and high dose and with Losartan low and high dose of about 29%, 13.4%, 20% and 9% ($P<0.05$), respectively.

Losartan low dose treatment highlights an increase ($F=5.553$; $P<0.01$) of about 12.6% of angiotensinogen mRNA expression (Fig. 2B) in the bed nucleus of stria terminalis.

Losartan low dose yields an increase ($F=14.126$) of about 11.2% ($P<0.01$) (Fig. 2B) of angiotensinogen mRNA in arcuate hypothalamic nucleus.

Fig. 2C shows an increase in the angiotensinogen mRNA in treated rats ($F=8.485$) in the horizontal limb diagonal band of Broca with Losartan high dose ($P<0.05$) and low dose ($P<0.01$) and for Irbesartan high and low dose treatments ($P<0.01$) of about 7.5%, 16.4%, 15.5% and 17.1%, respectively.

At the suprachiasmatic nucleus level, data show an increase in angiotensinogen mRNA in treated rats ($F=4.137$; $P<0.01$) of about 10.4% and 10.1% for Irbesartan low and high doses, respectively, while ($P<0.05$) of about 7.1% and 8% for Losartan low and high doses, respectively (Fig. 2C).

Furthermore, Fig. 2C shows a 9.8% increase in angiotensinogen mRNA in medial septal nucleus, only following Irbesartan high dose ($F=3.398$; $P<0.01$) administration.

With regard to the medial preoptic nucleus, anteroventral thalamic nucleus, supraoptic hypothalamic nucleus and ventromedial hypothalamic nucleus, experimental data resulted in no statistically significant change in treated versus control rats.

3.2. Effects of chronic (30 days) intragastric administration of Losartan and Irbesartan on brain angiotensinogen mRNA expression

As shown in Fig. 3A–C, chronic intragastric drug administrations of the two angiotensin AT_1 antagonists (3 or 30 mg/kg body weight/day) influence angiotensinogen brain expression in some mapped areas, down-regulating the mRNA level, whereas no effects have been detected in the remainders.

Fig. 3A shows a statistically significant decrease of angiotensinogen mRNA in the horizontal limb diagonal band of Broca cells ($F=10.323$; $P<0.01$) by 25.4% with Losartan high dose.

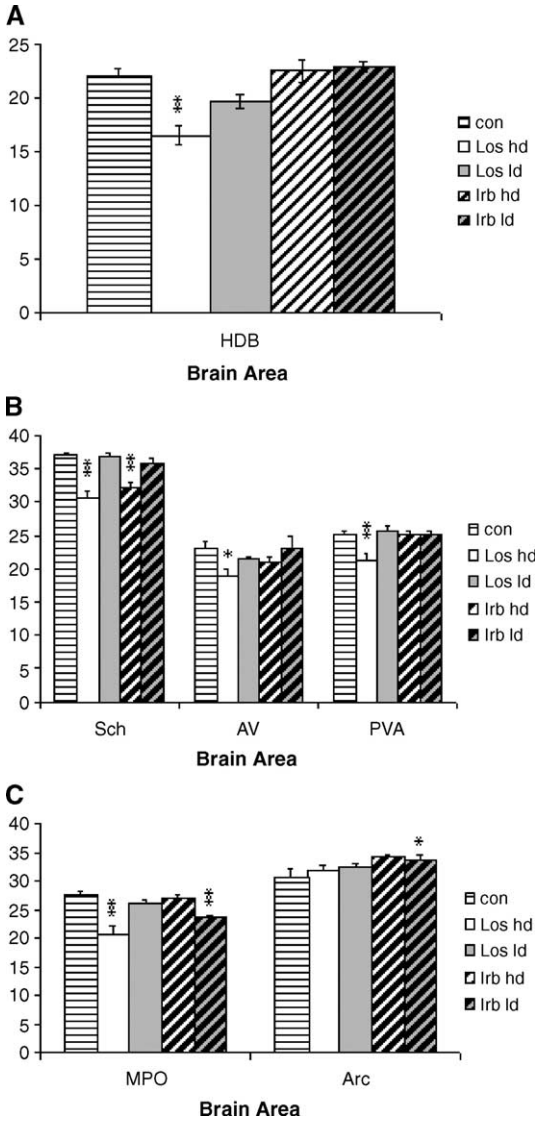

Fig. 3. (A–C) Effects of chronic (30 days) Losartan (Los) and Irbesartan (Irb) administration on angiotensinogen mRNA in discrete brain nuclei. Los high dose (hd)=30 mg/kg per day, Los low dose (ld)=3 mg/kg per day, Irb hd=30 mg/kg per day, Irb ld=3 mg/kg per day. Data are shown as mean±S.E.M., number of grains/cells, groups of six–eight animals. *$P<0.05$, **$P<0.01$, where P values are vs. control. HDB=horizontal limb diagonal band of Broca; Sch=suprachiasmatic nucleus; AV=anteroventral thalamic nucleus; PVA=anterior paraventricular thalamic nucleus; MPO=medial preoptic nucleus; Arc=arcuate hypothalamic nucleus.

These findings also showed a 17.4% and 12.9% statistically significant decrease in the suprachiasmatic nucleus ($F=13.209$; $P<0.01$) following Losartan and Irbesartan high dose treatments, respectively (Fig. 3B).

Fig. 3B shows also a 17.9% decrease of angiotensinogen mRNA level in the anteroventral thalamic nucleus ($F=2.888$) after Losartan high dose ($P<0.05$).

The paraventricular thalamic nucleus also shows a decrease ($F=5.782$) following Losartan high dose ($P<0.01$) by 15.7% (Fig. 3B).

The medial preoptic nucleus shows a decrease of angiotensinogen mRNA after Losartan high dose and Irbesartan low dose treatments ($F=10.094$) of about 25.1% ($P<0.01$) and 14.6% ($P<0.05$), respectively (Fig. 3C).

On the other hand, as in Fig. 3C, these studies resulted in an up-regulation of angiotensinogen mRNA levels after 30-day chronic treatment. In fact, the arcuate hypothalamic nucleus shows an increase ($F=2.921$; $P<0.05$) by 11.7% after administration of Irbesartan high dose.

Angiotensinogen mRNA levels did not show any statistically significant effects in treated versus control rats in the medial septal nucleus, the bed nucleus of stria terminalis, the subfornical organ and the paraventricular hypothalamic nucleus.

4. Discussion

This study has shown a brain angiotensinogen mRNA regulation induced by subchronic and chronic systemic administration of the angiotensin AT_1 receptor antagonists Losartan and Irbesartan. A widespread up-regulation of angiotensinogen mRNA expression has been found in discrete areas after a subchronic 7 days treatment, as shown in Table 1. On the contrary, following 30-day chronic treatment, angiotensinogen mRNA levels were reduced to control value or even below the basal level of the up-regulated region during the subchronic treatment (Table 1).

In the chronic experiments Losartan high dose has a predominant effect; in fact, in the horizontal diagonal band of Broca, paraventricular thalamic nucleus and anteroventral thalamic nucleus, angiotensinogen expression was down-regulated only by Losartan high dose (Table 1). Suprachiasmatic nucleus and medial preoptic nucleus are both down-regulated by Losartan high dose, being the former also down-regulated by the Irbesartan high dose. Moreover, the latter is down-regulated by Irbesartan low dose (Table 1).

By comparing the results between subchronic and chronic angiotensin AT_1 antagonists administration, one may postulate an up and down mechanism of regulation of the angiotensinogen gene expression, which can substantially contribute to decrease blood pressure in 4–6 weeks of therapeutic treatment (Wang et al., 1991; Reeves et al., 1998), as Schinke and coworkers' findings have shown a direct correlation between brain angiotensinogen mRNA and blood pressure.

The down-regulation of angiotensinogen mRNA expression after chronic drug administration of functional and physically related areas such as the subfornical organ and the paraventricular hypothalamic nucleus (Table 1) may be in accordance with the works of Tanaka and Nomura (1993) and Lippoldt et al. (1993, 1995), which showed a negative feedback regulation by the angiotensin II via projections from the lateral hypothalamic region to the subfornical organ, thus lowering the angiotensinergic system sensitivity in presence of angiotensin II arousal. In fact, in the first 7 days, a wide general up-regulation of angiotensinogen after administration of the two angiotensin AT_1 receptor antagonists has occurred in the brain. These two areas are involved in the control of blood pressure, drinking, body fluid homeostasis and cardiovascular control (Wright and Harding, 1992; Saavedra, 1992; Phillips and Summer, 1998; Badoer, 2001). Also, if they are differently involved in vasopressin release, in excitatory inputs to the sympathetic pathway and in different feedback with the baroreflex system, circumventricular organs and hypothalamic nuclei, they show a similar angiotensinogen regulation after angiotensin AT_1 receptors block.

The suprachiasmatic nucleus and the horizontal diagonal band of Broca show a 7-day up-regulation and a 30-day down-regulation, or back shift to basal-level angiotensinogen mRNA expression (Table 1). In the horizontal diagonal band of Broca (7 days experiment), Losartan high dose seems to have the lowest influence in the up-regulation, whereas at 30 days it is more effective in the down-regulation. Hence, it is possible that Losartan high dose causes an early up-regulation, thus showing a milder up-regulation at 7 days, since, at this time, it is already in a down-regulation trend thoroughly manifested at 30 days. Suprachiasmatic nucleus following 7 days of treatment has an equivalent rate of up-regulation for each treatment. After 30 days, high-dosage drugs down-regulate the mRNA

Table 1
Effects of irbesartan and losartan on angiotensinogen mRNA in discrete brain nuclei

Brain area	Losartan treatments			
	30 mg/kg per day		3 mg/kg per day	
	7 days (%)	30 days (%)	7 days (%)	30 days (%)
SFO	+23[a]	–	+30[a]	–
PVN	+13[a]	–	+16[a]	–
BNST	–	–	+13[b]	–
Arc	–	–	+11.2[a]	–
PVA	+9[b]	−15.7[a]	+19[a]	–
AV	–	−18[a]	–	–
Sch	+8[b]	−17.4[a]	+7[b]	–
HDB	+7[b]	−25.4[b]	+16[b]	–
MS	–	–	–	–
MPO	–	−25[a]	–	–

Brain area	Irbesartan treatments			
	30 mg/kg per day		3 mg/kg per day	
	7 days (%)	30 days (%)	7 days (%)	30 days (%)
SFO	+25[b]	–	+25[b]	–
PVN	+14[a]	–	+19[a]	–
BNST	–	–	–	–
Arc	–	+12[b]	–	–
PVA	+13[a]	–	+29[a]	–
AV	–	–	–	–
Sch	+10[a]	−12.9[a]	+10[a]	–
HDB	+15[a]	–	+17[a]	–
MS	+9.8[a]	–	–	–
MPO	–	–	–	−14.6[a]

Data are shown as mean, number of grains/cells, groups of six–eight animals. SFO=subfornical organ; PVN=paraventricular hypothalamic nucleus; BNST=bed nucleus of stria terminalis; Arc=arcuate hypothalamic nucleus; PVA=anterior paraventricular thalamic nucleus; AV=anteroventral thalamic nucleus; Sch=suprachiasmatic nucleus; HDB=horizontal limb diagonal band of Broca; MS=medial septal nucleus; MPO=medial preoptic nucleus.
[a] $P<0.01$ vs. control.
[b] $P<0.05$ vs. control.

expression of angiotensinogen, whereas it diminishes to basal level following low dosage (Table. 1). In general, where Losartan high dose shows the major effects, we can suppose, like Polidori et al. (1996), that, due to its higher lipophilic character, the metabolite of Losartan, the EXP3174, an angiotensin AT_1 receptor antagonist, is more able to cross the blood–brain barrier, thus evoking an early down-regulation of angiotensinogen mRNA expression after 7 days, when compared to other treatments. In the suprachiasmatic nucleus, the two drugs have similar influence on angiotensinogen mRNA expression. It is therefore likely that this mRNA regulation is also brain-region-dependent. The horizontal diagonal band of Broca and the suprachiasmatic nucleus are related to the limbic portion that can explain their similar expression except for the drug dependence which is present in the horizontal diagonal band of Broca but not in the suprachiasmatic nucleus. The suprachiasmatic nucleus represents the central pacemaker of the mammalian circadian clock, controlling the rest–activity cycle, the blood-pressure rhythm, as well as the temporal organization of many physiological and endocrine functions in mammals (Moore, 1995; Witte et al., 1998), while horizontal diagonal band of Broca has a role in dipsogenic responses to hypovolemic stimuli, probably in basal autonomic function and in basal vasopressin release (Cunningham et al., 1994; Sullivan et al., 2003).

Interestingly, after 7 days of treatment, the paraventricular thalamic nucleus as the suprachiasmatic nucleus and Horizontal diagonal band of Broca shows an up-regulation at all doses, even if the paraventricular thalamic nucleus, at high dosage seem to evoke a milder increase in angiotensinogen when compared to low doses. In the Horizontal diagonal band of Broca, only Losartan high dose evokes the same response at 7 days and, in the suprachiasmatic nucleus, no differences in up-regulation are detectable. Conversely, after chronic treatment, only Losartan high dose is effective in reducing gene expression below the basal level as in the horizontal diagonal Band of Broca, while no differences in drug influence is present in the suprachiasmatic nucleus where also Irbesartan high dose shows the same effect (Table 1). The thalamic portion takes part in arousal and awareness processes. The paraventricular thalamic nucleus is involved in viscero-limbic functions and has also deep interactions with the suprachiasmatic nucleus and has been therefore implicated in the regulation of the biological rhythms (Van der Werf et al., 2002; Herman et al., 2002). Due to this, the paraventricular thalamic nucleus, the horizontal diagonal band of Broca and the suprachiasmatic nucleus have a very similar regulation after both subchronic and chronic administration also if, for unknown reasons, the former two regions discriminate between the two drugs in the chronic treatment while the latter does not (Table 1).

The anteroventral thalamic nucleus does not show any significant variations after 7 days of treatment, whereas Losartan high dose evokes an angiotensinogen gene expression reduction after 30 days of administration. This nucleus is a component of the anterior thalamic nuclei and of the limbic system, and has a direct connection with the retina, participating in learning and memory functions (Lindroos et al., 1992). Moreover, the anteroventral thalamic nucleus is related to the locus coeruleum, which is considered an alarm system that receives the sensory information necessary to maintain body integrity and relation functions (Aston-Jones, 1985). As other regions related to the limbic system, the anteroventral thalamic nucleus has a Losartan high dose down-regulation at 30 days but no up-regulation have been detected at 7 days. In this case, a regulation of angiotensinogen mRNA expression, which is also a brain-region-dependent after angiotensin AT1 receptor block, is present.

The bed nucleus of the stria terminalis and the arcuate hypothalamic nucleus show an up-regulation following subchronic treatment only in the case of Losartan low dose, while after chronic treatment there is a back shift to the basal level of expression in the former nucleus and a permanent up-regulation by Irbesartan high dose (the unique up-regulation data after 30 days of administration) in the latter one. The bed nucleus of the stria terminalis is involved in salt intake in response to sodium depletion, but not in water intake (Zardetto-Smith et al., 1994; Johnson et al., 1999). This area receives neural inputs from the subfornical organ and from the organum vasculosum of the lamina terminalis (Sunn et al., 2003) and has been linked to electrolyte control and cardiovascular regulation (Dunn and Williams, 1995). In addition, it is known that the bed nucleus of stria terminalis is polysynaptically linked to sympathetic pathways (Westerhaus and Loewy, 1999).

In the arcuate hypothalamic nucleus, the up and down-regulation still remain controversial (Table 1). This area is involved in the release of prolactin by an indirect mechanism upon stimulation of dopamine by the renin–angiotensin system (Johren et al., 1997). This nucleus is also part of the hypothalamic–pituitary–adrenal axis (HPA), which is activated in response to stress, as well as in the regulation of angiotensin receptors by restraint stress (Castren and Saavedra, 1988; Aguilera et al., 1995; Leong et al., 2002;), thus implying an important role of the renin–angiotensin system in the regulation of the activity of the HPA axis. Angiotensin has been shown to regulate corticotropin-releasing factor, adrenocorticotropic hormone (ACTH), and corticosterone synthesis and secretion (Rivier and Vale, 1983; Sumitomo et al., 1991; Jezova et al., 1998).

Irbesartan high dose increases gene expression after 7 days of treatment in the medial septal nucleus, but no changes are detectable after 30 days. The medial septal nucleus has connection with the subfornical organ and the paraventricular hypothalamic nucleus, and is involved in water and sodium homeostasis and arterial pressure (Camargo et al., 2002; Saad and Camargo, 2003) but it shows a different pattern of angiotensinogen regulation after angiotensin AT_1 receptors block.

No variations in gene expression have been detected in the medial preoptic nucleus after 7 days, whereas chronic treatment decreases angiotensinogen mRNA levels following Losartan high dose and Irbesartan low dose administration. The medial preoptic nucleus has multiple efferent and afferent connections with the subfornical organ and it is involved in the dipsogenic and pressor responses. The subfornical organ stimuli cause release of noradrenaline in the medial preoptic nucleus. Moreover, it receives γ-aminobutyric acid (GABA) neurons from

organum vasculosum of the lamina terminalis and its GABAergic projections to supraoptic nucleus, inhibiting the excitability of both vasopressin- and oxytocin-secreting cells (Lind et al., 1984; Tanaka, 1989; Tanaka et al., 2003; Ushigome et al., 2004). Hence, in the medial preoptic nucleus, the angiotensinogen gene expression regulation after angiotensin AT_1 receptor block is brain-region-dependent (Table 1).

Amongst the mapped brain areas, some may differ from our speculative model, i.e. up- and down-regulation, in that they may be involved in different neuroanatomical and biochemical circuitries.

Based upon these findings, it is difficult to find a strict correlation between the function of the mapped areas and the different effects arousing from the angiotensin AT_1 receptors block. We may only speculate that some areas involved in the same neuroanatomical and biochemical pathways show similar trend of regulation after angiotensin AT_1 receptors antagonism.

Moreover, the chemical–physical characteristic of the antagonists are probably responsible for the different influence in the gene expression. Several reports have taken into account the ability of these two antagonists, to cross the blood–brain barrier and to influence the renin–angiotensin system's physiological response. Lacour et al. (1995), Polidori et al. (1998), Culman et al. (1999) and other authors tried to evaluate the differences between Losartan and Irbesartan in eliciting their effects throughout the brain, either outside and/or inside the blood–brain barrier. Yet, data are still equivocal: doses, functional tests and methods employed by researches have been different, thus making comparisons difficult. Besides, the effects of this two angiotensin AT_1 receptor antagonists on the central and peripheral renin–angiotensin system is difficult to be interpreted due to the several numbers of angiotensin peptides, their receptors and the interactions with other neurochemical, and endocrine pathways.

Therefore, the study of the angiotensinogen gene expression appears to be a straightforward method to evaluate the effects of the angiotensin AT_1 receptor blockade. So far, only Nishimura et al. (1998, 2000) and Sangaletti et al. (2004) have studied brain gene expression following angiotensin AT_1 receptor antagonist administration, using Candesartan (CV-11974) and Losartan, respectively. In Nishimura experiments, no variation in angiotensin AT_1 receptor gene expression has been detected after 14 days of administration in mapped areas at doses employed.

Thus, in light of our findings, it is possible that angiotensin AT_1 receptor gene expression, after blockade, may not correlate with angiotensinogen gene expression, or that the experimental conditions used and the areas analyzed by Nishimura do not show a similar pattern of regulation.

Sangaletti et al. (2004), either in absence or in presence of the coarctation hypertension model, show 1) a positive correlation between pressure and angiotensinogen mRNA in bulbar areas, and 2) a diminution or no variation in bulbar areas of angiotensin AT_{1a} receptor and angiotensinogen mRNA expression after 9 days of Losartan treatment. So it is possible that this is in accordance with our studies, although these authors may have used different conditions.

In conclusion, our results show a peculiar mechanism of up- and down-regulation of angiotensinogen gene expression in the central renin–angiotensin system following angiotensin AT_1 receptor Losartan and Irbesartan antagonists peripheral administration, which seems to be drug-, dose-, time- and area-dependent. Interestingly, the central down-regulation of the angiotensinogen gene expression after chronic administration is probably an important factor in lowering blood pressure, during 4 to 6 weeks of therapeutic treatment with angiotensin AT_1 receptor antagonists.

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