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Brain Angiotensin II: New Developments, Unanswered Questions and Therapeutic Opportunities

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SUMMARY

1. There are two Angiotensin II systems in the brain. The discovery of brain Angiotensin II receptors located in neurons inside the blood brain barrier confirmed the existence of an endogenous brain Angiotensin II system, responding to Angiotensin II generated in and/or transported into the brain. In addition, Angiotensin II receptors in circumventricular organs and in cerebrovascular endothelial cells respond to circulating Angiotensin II of peripheral origin. Thus, the brain responds to both circulating and tissue Angiotensin II, and the two systems are integrated.

2. The neuroanatomical location of Angiotensin II receptors and the regulation of the receptor number are most important to determine the level of activation of the brain Angiotensin II systems.

3. Classical, well-defined actions of Angiotensin II in the brain include the regulation of hormone formation and release, the control of the central and peripheral sympathoadrenal systems, and the regulation of water and sodium intake. As a consequence of changes in the hormone, sympathetic and electrolyte systems, feed back mechanisms in turn modulate the activity of the brain Angiotensin II systems. It is reasonable to hypothesize that brain Angiotensin II is involved in the regulation of multiple additional functions in the brain, including brain development, neuronal migration, process of sensory information, cognition, regulation of emotional responses, and cerebral blood flow.

4. Many of the classical and of the hypothetical functions of brain Angiotensin II are mediated by stimulation of Angiotensin II AT_1 receptors.

5. Brain AT_2 receptors are highly expressed during development. In the adult, AT_2 receptors are restricted to areas predominantly involved in the process of sensory information. However, the role of AT_2 receptors remains to be clarified.

6. Subcutaneous or oral administration of a selective and potent non-peptidic AT_1 receptor antagonist with very low affinity for AT_2 receptors and good bioavailability blocked AT_1 receptors not only outside but also inside the blood brain barrier. The blockade of the complete brain Angiotensin II AT_1 system allowed us to further clarify some of the central actions of the peptide and suggested some new potential therapeutic avenues for this class of compounds.

7. Pretreatment with peripherally administered AT_1 antagonists completely prevented the hormonal and sympathoadrenal response to isolation stress. A similar pretreatment prevented the development of stress-induced gastric ulcers. These findings strongly suggest that blockade of brain AT_1 receptors could be considered as a novel therapeutic approach in the treatment of stress-related disorders.

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8. Peripheral administration of AT_1 receptor antagonists strongly affected brain circulation and normalized some of the profound alterations in cerebrovascular structure and function characteristic of chronic genetic hypertension. AT_1 receptor antagonists were capable of reversing the pathological cerebrovascular remodeling in hypertension and the shift to the right in the cerebral autoregulation, normalizing cerebrovascular compliance. In addition, AT_1 receptor antagonists normalized the expression of cerebrovascular nitric oxide synthase isoenzymes and reversed the inflammatory reaction characteristic of cerebral vessels in hypertension. As a consequence of the normalization of cerebrovascular compliance and the prevention of inflammation, there was, in genetically hypertensive rats a decreased vulnerability to brain ischemia. After pretreatment with AT_1 antagonists, there was a protection of cerebrovascular flow during experimental stroke, decreased neuronal death, and a substantial reduction in the size of infarct after occlusion of the middle cerebral artery. At least part of the protective effect of AT_1 receptor antagonists was related to the inhibition of the Angiotensin II system, and not to the normalization of blood pressure. These results indicate that treatment with AT_1 receptor antagonists appears to be a major therapeutic avenue for the prevention of ischemia and inflammatory diseases of the brain.

9. Thus, orally administered AT_1 receptor antagonists may be considered as novel therapeutic compounds for the treatment of diseases of the central nervous system when stress, inflammation and ischemia play major roles.

10. Many questions remain. How is brain Angiotensin II formed, metabolized, and distributed? What is the role of brain AT_2 receptors? What are the molecular mechanisms involved in the cerebrovascular remodeling and inflammation which are promoted by AT_1 receptor stimulation? How does Angiotensin II regulate the stress response at higher brain centers? Does the degree of activity of the brain Angiotensin II system predict vulnerability to stress and brain ischemia? We look forward to further studies in this exiting and expanding field.

KEY WORDS: renin angiotensin system; angiotensin II receptors; AT_1 receptors; AT_2 receptors; stress; ischemia; gastric ulcers; sympathetic system; hormones; brain development; sensory systems; cerebrovascular circulation.

BRAIN ANGIOTENSIN II SYSTEMS

Angiotensin II (Ang II) was initially described as a peripheral hormone (Braun-Menéndez *et al.*, 1940; Page and Helmer, 1940), mediating the effects of the classical Renin-Angiotensin System (RAS) (Page, 1987). Circulating Ang II induced vasoconstriction, aldosterone release, sodium and water retention, increased fluid intake and had a key role in the regulation of blood pressure and fluid homeostasis (Fig. 1) (Page, 1987).

Injection of Ang II into the general circulation elicited effects in the central nervous system, indicating the presence of receptors for this peptide in the brain (Buckley, 1988). Circulating Ang II does not penetrate into the brain, and receptors responding to blood borne Ang II were initially located, after peripheral injection of radiolabeled peptide, in the circumventricular organs outside the blood brain barrier (Van Houten *et al.*, 1980). Central receptor stimulation induced fluid and salt intake and increased blood pressure (Mendelsohn *et al.*, 1984; Saavedra, 1992; Phillips and Sumners, 1998), effects integrated with water and electrolyte retention in the kidney.

Ang II is formed in many tissues including most peripheral organs and the brain, and local Ang II systems are regulated independently from the classical RAS (Bumpus *et al.*, 1988; Millan and Aguilera, 1988; Ganten *et al.*, 1989; Ganong, 1993; Jonsson *et al.*, 1994; Pieruzzi *et al.*, 1995; Leung and Carlsson, 2001; Hirasawa *et al.*, 2002; Jones and Woods, 2003). Both circulating and locally formed Ang II could

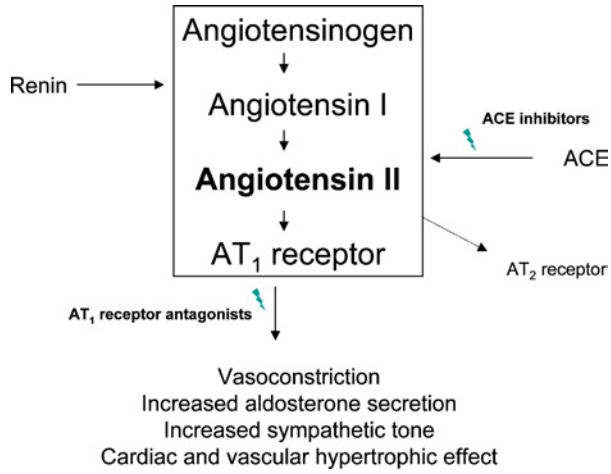

Fig. 1. Angiotensin II formation through the Renin-Angiotensin system, classical mayor actions, physiologically active receptors and principal sites for inhibition of Angiotensin II effects. The precursor Angiotensinogen is transformed by kidney renin into the inactive precursor Ang I, which in turn is transformed into the active principle Ang II by Angiotensin Converting Enzyme (ACE). Most of Ang II effects, including vasoconstriction, increased aldosterone secretion and sympathetic tone and cardiac and vascular hypertrophy, are mediated through AT_1 receptor stimulation. The functions of the AT_2 receptor are not yet clearly defined. Decreased activity of Ang II can be achieved through blockade of its synthesis by ACE inhibition, or AT_1 receptor blockade with specific nonpeptidic antagonists.

influence many, if not all tissues, and that the effects of the peptide may be more complex and widespread than originally envisioned.

All components of the classical RAS, such as angiotensinogen, renin, angiotensin-converting enzyme (ACE), and Ang II (Fig. 1) are present in the brain (Ganten *et al.*, 1984; Saavedra, 1992; Phillips and Sumners, 1998). However, localization studies have revealed a puzzling picture, because there is not a single brain cell where all RAS components are normally expressed. The postulate of brain Ang II formation through a classical RAS system requires multiple-cell interaction at long distances, enough to make the system unrealistically complex and inefficient. Conversely, the selective receptor localization clearly demonstrates that stimulation of receptors by Ang II is likely to result in specific, selective effects. Alternative hypothesis are necessary to explain how brain Ang II is produced and how the peptide reaches its receptors at physiologically active concentrations. One of such hypothesis is that the brain possesses alternative enzymatic mechanisms for the formation of Ang II, distinct from those discovered early for the classical RAS. However the subject has not been clarified and the way for Ang II to reach its physiologically active receptors remains a mystery. For this reason it is wiser to refer to a brain "Angiotensin II system" than to a brain "RAS system."

With the development of autoradiographic methods (Mendelsohn *et al.*, 1984; Gehlert *et al.*, 1986; Healy *et al.*, 1986; Saavedra *et al.*, 1986; Tsutsumi and Saavedra, 1991a), it was possible to precisely determine the location of all Ang II receptors in the brain. The comparison of the distribution of brain Ang II receptors and brain Ang II immunoreactivity revealed that most of the brain Ang II, and most of the brain Ang II receptors, were localized in neurons (Lind *et al.*, 1985; Tsutsumi and Saavedra, 1991a). It became clear that Ang II, present in the brain, is able to reach receptors inside the blood brain barrier, not accessible to the circulating peptide. Of particular interest was the discovery of a forebrain band of Ang II receptors, linking circumventricular organs such as the subfornical organ, the organum vasculosum of the lamina terminalis and the median eminence, with structures within the blood brain barrier such as the hypothalamic paraventricular nucleus and the lamina terminalis (Figs. 2 and 3) (Shigematsu *et al.*, 1986). Such a pathway is one anatomical substrate for a physiological connection between the circulating or "peripheral" and the brain or "central" Ang II systems (Saavedra 1992).

Ang II receptors are also localized in endothelial cells lining the brain capillaries and microvessels (Fig. 4) (Ando *et al.*, 2004). Thus, Ang II receptors in the circumventricular organs and cerebrovascular endothelial cells respond to circulating Ang II; receptors located inside the blood barrier are activated by Ang II formed in the brain and/or transported to the brain from the circulation.

BRAIN ANGIOTENSIN II RECEPTOR TYPES

There are two Ang II receptor types, namely the AT_1 and AT_2 receptors. With similar binding affinity for Ang II, the different types are identified on the basis of their selective affinity for different nonpeptidic ligands (Timmermans *et al.*, 1993; De Gasparo *et al.*, 2000). Both receptor types belong to the superfamily of seven membrane-spanning G-protein coupled receptors (Sasaki *et al.*, 1991; Kambayashi *et al.*, 1993), but they only share a 32–34% identity at the amino acid level (Clauser *et al.*, 1996).

The well-known biological actions of Ang II, such as contraction of smooth muscle leading to vasoconstriction and increases in blood pressure, increase in water and sodium intake, renal sodium retention and secretion of vasopressin and aldosterone are mediated by the AT_1 type (Fig. 1) (Timmermans *et al.*, 1993). In the brain, both the hormonal control and the regulation of the sympathetic system are also under AT_1 receptor control (Saavedra, 1992). In rodents, but not in other mammals or in humans, there are two Ang II AT_1 receptor subtypes, the AT_{1A} and AT_{1B} receptors, and these subtypes are selectively localized and regulated (Inagami *et al.*, 1994; Kakar *et al.*, 1992). In the brain, most if not all the AT_1 receptors belong to the AT_{1A} receptor subtype. The AT_{1A} and AT_{1B} receptors express similar affinities for the natural agonist Ang II and for the selective AT_1 receptor antagonists. For this reason, differentiation between AT_{1A} and AT_{1B} receptors could not be achieved by binding techniques but only with the use of *in situ* hybridization, taking advantage of the significant differences in the untranslated regions (UTRs)

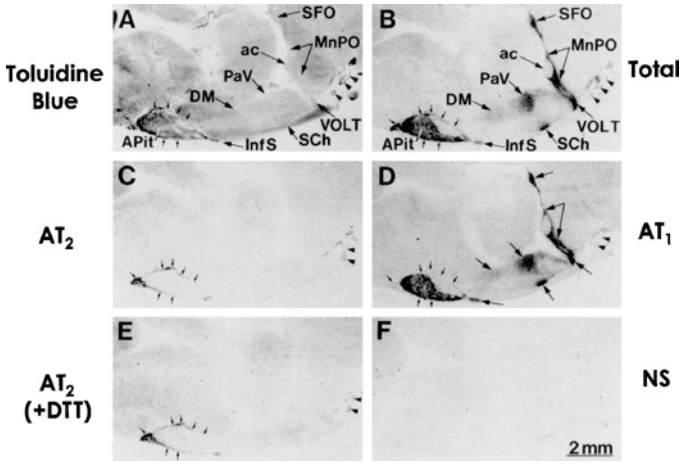

Fig. 2. Expression of Angiotensin II AT_1 receptors in areas related to the control of the reaction to stress: circumventricular organs, the midline receptor pathway, paraventricular hypothalamic nucleus and anterior pituitary. Figures represent parasagittal sections of the rat brain at the level of the hypothalamic area in the basal rat forebrain. A—staining with toluidine blue. B—autoradiography of Ang II receptor binding in a consecutive section, incubated in the presence of [^{125}I] Sarcosine^1-Ang II to reveal the total number of Ang II receptors. C—consecutive section incubated as in B in the presence of the selective AT_1 blocker losartan which displaces binding to AT_1 receptors, to reveal the localization of AT_2 receptors. D—consecutive section incubated as in B in the presence of the selective AT_2 ligand CGP 42112, which displaces binding to AT_2 receptors, to reveal the localization of AT_1 receptors. E—consecutive section incubated as in B in the presence of dithiothreitol, to reveal the localization of AT_2 receptors. The addition of dithiothreitol eliminates binding to AT_1 receptors through dissociation of their disulfide bridges. F—consecutive section incubated as in B, in the presence of excess unlabeled Angiotensin II. Binding to both AT_1 and AT_2 receptors has been displaced. Small arrows point to the dura mater surrounding the pituitary gland. Arrowheads point to the anterior cerebral artery. SFO—subfornical organ; MnPO—median preoptic nucleus; ac—anterior commissure; PaV—paraventricular nucleus; DM—dorsomedial hypothalamic nucleus; APit—anterior pituitary; VOLT—vascular organ of the lamina terminalis; SCh—suprachiasmatic nucleus' InfS—infundibulum stem (Tsutsumi and Saavedra, 1991).

of the AT_{1A} and AT_{1B} genes. By constructing riboprobes hybridizing to selective UTR domains, it is possible to reveal AT_{1A} or AT_{1B} selectively (Jöhren *et al.*, 1995).

Both AT_1 and AT_2 receptor types were found, with a distribution similar, although not identical, in all mammalian species studied (Tsutsumi and Saavedra, 1991a; Tsutsumi *et al.*, 1991a,b; Jöhren *et al.*, 1995, 1996; Jöhren and Saavedra, 1996a; Häuser *et al.*, 1998), including humans (Barnes *et al.*, 1993). While AT_1 receptors predominate in adult animals, AT_2 receptors are highly expressed in the developing brain (Tsutsumi and Saavedra, 1991a; Tsutsumi *et al.*, 1991a,b; Jöhren and Saavedra, 1996a). AT_1 receptors were identified in areas related to neuroendocrine control and autonomic regulation of cardiovascular function and the limbic

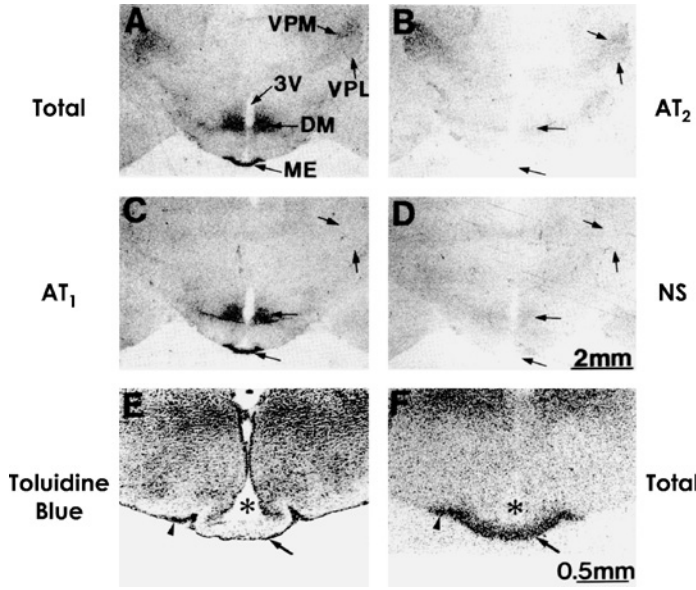

Fig. 3. Expression of Angiotensin II AT_1 receptors in the median eminence. Figures represent coronal sections of the rat brain at the level of the hypothalamic area in the basal rat forebrain and including the median eminence. A—autoradiography of Ang II receptor binding, incubated in the presence of [^{125}I] Sarcosine^1-Ang II to reveal the total number of Ang II receptors. B—consecutive section incubated as in A in the presence of the selective AT_1 blocker losartan which displaces binding to AT_1 receptors, to reveal the localization of AT_2 receptors. C—consecutive section incubated as in A in the presence of the selective AT_2 ligand CGP 42112, which displaces binding to AT_2 receptors, to reveal the localization of AT_1 receptors. D—consecutive section incubated as in A in the presence of excess unlabelled Ang II. Binding to both AT_1 and AT_2 receptors has been displaced. E—enlargement from A, stained with toluidine blue. F—enlargement from A, revealing the total number of Ang II receptors in the median eminence. Arrows point to the external layer of the median eminence, the dorsomedial nucleus, the ventral posterolateral thalamic nucleus, the ventral posteromedial thalamic nucleus and the third ventricle. Arrowheads point to the lateral part of the median eminence. DM—dorsomedial hypothalamic nucleus; VPM—ventral posteromedial thalamic nucleus; VPL—ventral posterolateral thalamic nucleus; ME—median eminence. Asterisks indicate the third ventricle (Tsutsumi and Saavedra, 1991).

system (Figs. 2 and 3) (Tsutsumi and Saavedra, 1991a,b) and in these areas, rodents express predominantly the AT_{1A} subtype (Jöhren *et al.*, 1995).

While the collected evidence indicates that the classical actions of Ang II, both in the periphery and in the brain, are mediated through AT_1 receptor activity, stimulation of AT_2 receptors may offset or oppose, by cross-talk mechanisms, the AT_1 mediated actions of Ang II on cell growth, blood pressure regulation, vascular reactivity and fluid intake, suppressing tissue and cellular growth, inducing neuronal differentiation and supporting apoptosis (Gallinat *et al.*, 2000). In addition, the expression of the brain AT_2 receptors suggests a role in brain organogenesis and in the

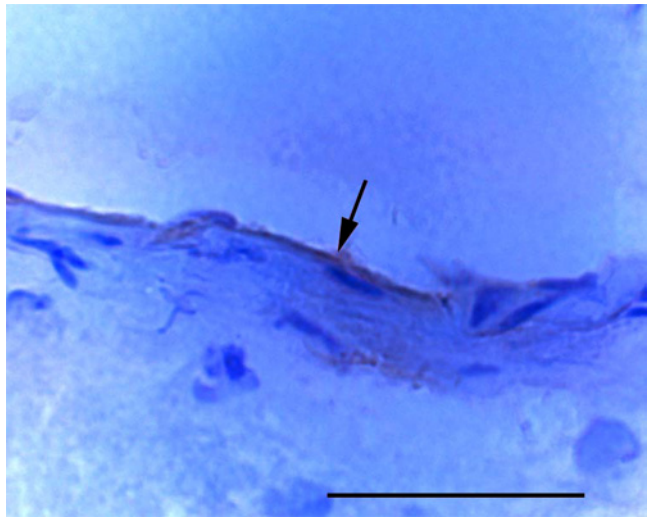

Fig. 4. Localization of AT_1 receptors in cerebrovascular endothelial cells. AT_1 receptors are localized to the endothelium of a cerebrovascular arteriole, as revealed by immunocytochemistry using an antimouse monoclonal antibody (courtesy: Dr. Hans Imboden, Bern, Switzerland). Arrow points to AT_1 receptors located at the surface on endothelial cells.

function of the sensory and motor systems (Tsutsumi and Saavedra, 1991a; Jöhren *et al.*, 1995, 1996; Jöhren and Saavedra, 1996a). However, the physiological functions of the AT_2 type are still uncertain (De Gasparo and Siragy, 1999; Gallinat *et al.*, 2000).

Thus, the role of brain Ang II appears to be multiple and complex. In addition to a regulatory role in the control of the autonomic and hormone systems, receptor localization suggests participation in brain development, sensory processes, cognition and in the regulation of cerebrovascular flow (Saavedra, 1992).

NOVEL CENTRAL FUNCTIONS OF ANGIOTENSIN II

(1) *The control of the reaction to stress. Blockade of Angiotensin II AT_1 receptors protects against stress and reduces anxiety.* The AT_1 receptors are remarkably concentrated in all key hypothalamic areas belonging to the hypothalamic-pituitary-adrenal axis, such as the parvocellular portion of the hypothalamic paraventricular nucleus (Fig. 5), the site of corticotrophin-releasing hormone (CRH) formation (Shigematsu *et al.*, 1986; Tsutsumi and Saavedra, 1991a), and the median eminence (Fig. 3), from where CRH is released to the portal circulation to stimulate pituitary ACTH secretion (Tsutsumi and Saavedra, 1991a). There are many AT_1 receptors in the subfornical organ (Tsutsumi and Saavedra, 1991a; Shigematsu *et al.*, 1986) sending projections to the paraventricular nucleus (Bain and Ferguson, 1995).

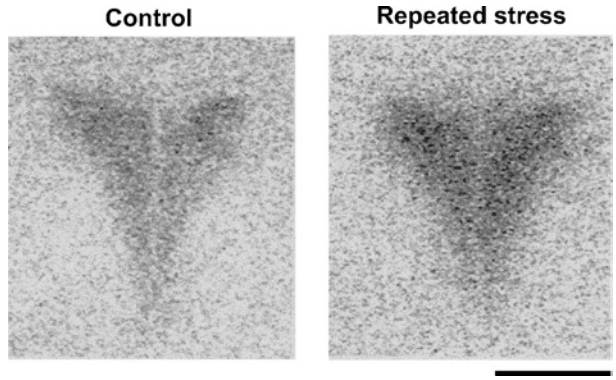

Fig. 5. Increased Angiotensin II receptor expression in the paraventricular nucleus after repeated restraint stress. The figure represents a typical autoradiography image of Ang II receptor binding as determined after incubation in the presence of [^{125}I]-Sarcosine^{1}-Ang II, in control animals and in animals submitted to seven repeated sessions of two hours restraint. Binding is concentrated in the parvocellular region of the paraventricular nucleus. Note the intense signal generated after repeated restraint stress. Bar is 0.5 mm (modified from Castrén and Saavedra, 1988).

AT_1 receptors are also highly expressed in the pituitary and adrenal glands (Tsutsumi and Saavedra, 1991b; Israel *et al.*, 1995). Thus, AT_1 receptors are concentrated in all key areas of the HPA axis. All AT_1 receptors located in the HPA axis belong to the AT_{1A} subtype (Kakar *et al.*, 1992; Jöhren *et al.*, 1995; Jöhren and Saavedra, 1996b; Leong *et al.*, 2002). The exception is the adrenal cortex, expressing in rodents both AT_{1A} and AT_{1B} receptor subtypes (Kakar *et al.*, 1992), and the pituitary gland, expressing AT_{1B} receptors in nonstressed adult rodents (Leong *et al.*, 2002).

Stress, through peripheral sympathetic nerve stimulation, increases renin activity and therefore the production of circulating Ang II (Fig. 6) (Xang *et al.*, 1993; Yang *et al.*, 1996). Both the peripheral and the central Ang II systems are stimulated, with increases in circulating Ang II levels and AT_1 receptor expression. The expression of AT_1 and AT_2 receptors in the adrenal zona glomerulosa and medulla and in the anterior pituitary is increased during isolation or restraint stress, although these changes are dependent on the type and duration of the stressor (Armando *et al.*, 2001; Leong *et al.*, 2002). Circulating or locally formed Ang II, by stimulating AT_1 receptors, contributes to the secretion of ACTH from the pituitary gland, aldosterone from the adrenal zona glomerulosa, and catecholamines from the adrenal medulla (Ganong and Murakami, 1987; Jezova *et al.*, 2003). Adrenomedullary AT_2 receptors, the major receptor type in this tissue, may interact with AT_1 receptors regulating basal catecholamine synthesis and stress-induced release (Jezova *et al.*, 2003).

Higher circulating Ang II during stress stimulate brain AT_1 receptors located in the circumventricular organs (Tsutsumi and Saavedra, 1991b) to increase thirst, fluid retention, blood pressure, cardiac rhythm and hormone release (Saavedra,

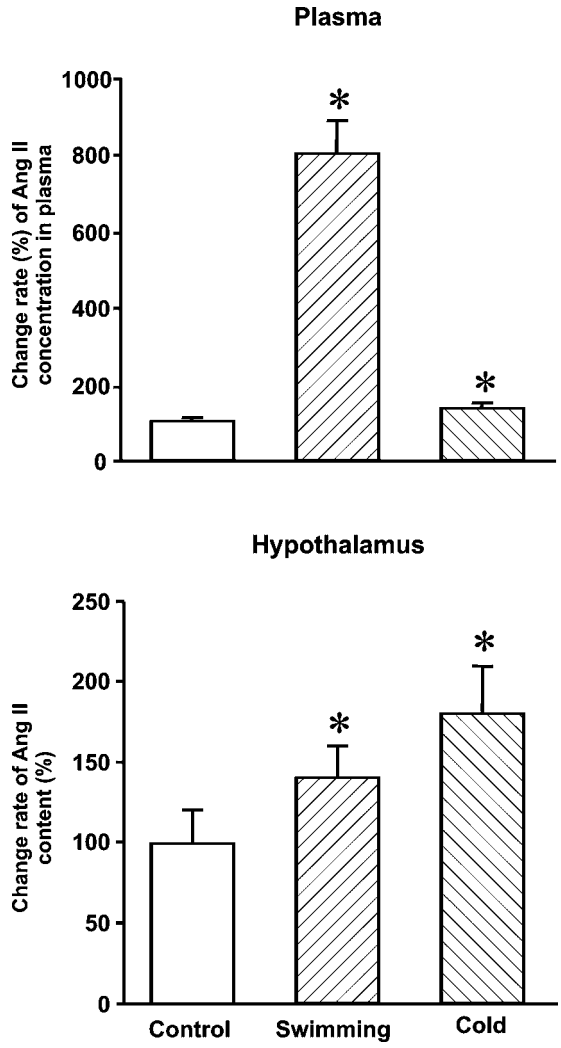

Fig. 6. Increased plasma and hypothalamic Angiotensin II concentrations during stress. Both the plasma and hypothalamic Ang II concentrations are substantially increased during different types of stress. The relative increase is dependent on the type of stress. $^{*}P < 0.05$ from control values (modified from Xang *et al.*, 1993).

1992). In addition, stress increases the concentration of brain Ang II (Fig. 6) (Xang *et al.*, 1993; Yang *et al.*, 1996; Peng and Phillips, 2001). Acute stress increases Ang II content in many brain regions including the hypothalamus (Xang *et al.*, 1993) and AT_1 receptor expression in the parvocellular portion of the paraventricular nucleus, the subfornical organ, the median eminence and the anterior pituitary (Fig. 5) (Castrén and Saavedra, 1988; Aguilera *et al.*, 1995a; Jezova *et al.*, 1998; Leong *et al.*, 2002). The resulting stimulation of the brain and pituitary Ang II systems, together

with the increased AT_1 receptor expression, activates the HPA axis, enhances CRH formation and release (Sumitomo *et al.*, 1991; Aguilera *et al.*, 1995b), increases pituitary ACTH release, and increases adrenal corticosterone formation and release (Ganong and Murakami, 1987). Increased corticosterone secretion during stress increases AT_1 receptor expression in the paraventricular nucleus, through stimulation of glucocorticoid response elements (GRE) in the receptor promoter region (Guo *et al.*, 1995). Glucocorticoids are not only able to stimulate AT_1 receptor expression during stress, but they are also necessary to maintain basal AT_1 receptor expression (Castrén and Saavedra, 1989; Aguilera *et al.*, 1995b). Stress-induced upregulation of AT_1 receptors in the paraventricular nucleus (Castrén and Saavedra, 1988) (Fig. 5) is one of the important factors modulating the increased CRH production which is followed by HPA axis stimulation.

Ang II is also involved in the stress-induced enhanced vasopressin release from the posterior pituitary (Armando *et al.*, 2001), and in the central and peripheral sympathetic stimulation characteristic of acute stress (Saavedra, 1992). Vasopressin release is under control of brain Ang II, through AT_1 receptor stimulation at the paraventricular nucleus and the median eminence (Saavedra, 1992).

Brain Ang II enhances central sympathetic activity, leading to increased adrenomedullary and peripheral sympathetic catecholamine release (Saavedra, 1992). In rats, Ang II receptors in the locus coeruleus are of the AT_2 type (Tsutsumi and Saavedra, 1991a), as they are most of the adrenomedullary Ang II receptors (Israel *et al.*, 1995). This implies a participation of AT_2 receptors in the regulation of central and peripheral sympathetic stimulation during stress (Saavedra, 1999; Jezova *et al.*, 2003). On the other hand, the paraventricular nucleus projects to the locus coeruleus, and AT_1 receptor stimulation in the adrenal medulla is sufficient to produce adrenomedullary catecholamine release (Wong *et al.*, 1990).

Thus, the selective localization of Ang II receptors, the increase in circulating and brain Ang II and the enhanced expression of AT_1 receptors during stress strongly suggested the participation of Ang II in the stimulation of all components of the HPA axis, and the central and peripheral sympathetic systems during stress.

We studied the response to stress after sustained blockade of peripheral and brain AT_1 receptors. A selective, potent, insurmountable AT_1 antagonist such as candesartan (Morsing, 1999; Sever, 1999; Timmermans, 1999), when administered peripherally for 2 weeks significantly decreased AT_1 receptor binding, not only in circumventricular organs, but also in the hypothalamic paraventricular nucleus and in the nucleus of the solitary tract, indicating that this compound readily penetrated the blood brain barrier (Nishimura *et al.*, 2000a).

We selected the stress of isolation, a clinically relevant model resulting, in rodents, from the restriction from freely regulating exposure to novel surroundings and access to familiar territory. We studied the effect of AT_1 receptor blockade with candesartan administered for 2 weeks before isolation. Twenty-four hours of isolation enhanced AT_1 receptor expression in the paraventricular nucleus (Armando *et al.*, 2001) to an extent similar to the increase in AT_1 receptors that occurs during repeated immobilization stress (Castrén and Saavedra, 1988). Isolation also increased pituitary ACTH, decreased pituitary vasopressin, and increased adrenal corticosterone,

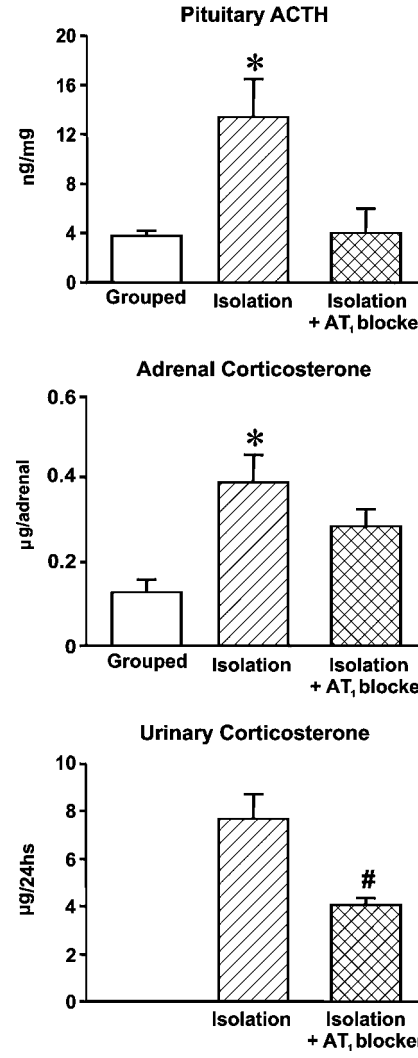

Fig. 7. Effect of pretreatment with an AT_1 receptor antagonist on the stress-induced pituitary ACTH and adrenal corticosterone content and urinary corticosterone excretion. Isolation stress increases pituitary ACTH and corticosterone content. Pretreatment with candesartan prevents the increase in pituitary ACTH, reduces the increase in adrenal corticosterone, and decreases the urinary excretion of corticosterone. $^{*}P < 0.05$, vs. grouped control and isolated animals pretreated with candesartan. $^{\#}P < 0.05$ vs. isolated animals treated with vehicle (Armando *et al.*, 2001).

aldosterone, catecholamines and the adrenal transcription of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, hallmarks of the stress reaction (Armando *et al.*, 2001). Pretreatment with candesartan blocked AT_1 receptor binding after isolation not only in peripheral tissues but also in the brain, in a manner similar to that previously observed in unstressed animals, prevented the increase in pituitary ACTH and adrenal corticosterone (Fig. 7) and the decrease in pituitary vasopressin during isolation, decreased the adrenomedullary catecholamine response, including the isolation-induced increase in tyrosine hydroxylase transcription, and decreased the urinary excretion of catecholamines, corticosterone (Fig. 7) and vasopressin (Armando *et al.*, 2001).

Our results demonstrated that simultaneous antagonism of peripheral and brain AT_1 receptors could represent an advantage in the control of the stress reaction. If blockade of pathologically enhanced responses to stress has beneficial effects, centrally acting insurmountable AT_1 antagonists such as candesartan, could have a place in the therapy of stress-related disorders.

To establish whether or not AT_1 receptor blockade could be of therapeutic benefit, we initiated a study of the effects of candesartan on the development of a stress-induced disorder, the formation of gastric ulcers induced by cold-restraint in the rat (Bregonzio *et al.*, 2003). Maintenance of gastric blood flow is important to protect the mucosa from endogenous and exogenous damaging factors, and Ang II, through AT_1 receptor stimulation, increases vascular tone in resistance arteries (Griendling *et al.*, 1996) including those of the gastric vasculature (Heinemann *et al.*, 1999) leading to decreased blood flow and ischemia. We speculated that AT_1 receptor inhibition with candesartan could protect gastric blood flow during stress and reduce gastric ulcer formation.

We found that candesartan dramatically decreased the number of ulcerations produced by cold-restraint stress (Fig. 8) (Bregonzio *et al.*, 2003). Several interrelated

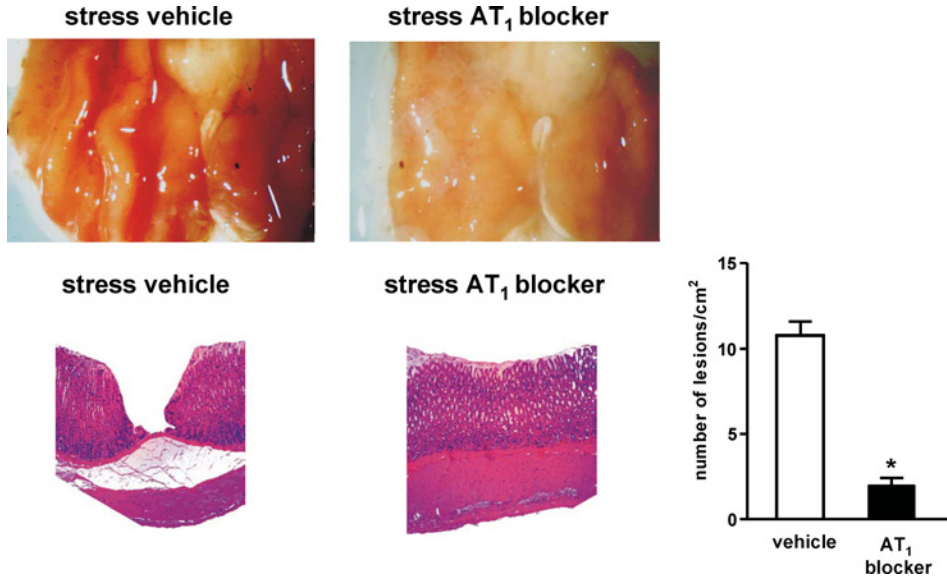

Fig. 8. Prevention of gastric mucosal lesions induced by cold restraint stress by pretreatment with AT_1 receptor antagonists. Top: Gastric mucosa corresponding to the glandular portion of the stomach from rats submitted to cold-restraint. Left: treated with vehicle; right: treated with the AT1 receptor blocker candesartan, for 2 weeks before cold-restraint. Bottom: Microphotographs of hematoxylin-eosin-stained sections of the glandular portion of the stomach in animals submitted to cold-restraint stress treated with vehicle (left) or with candesartan (right), for 2 weeks before the stress. The lesion in the animal submitted to stress and treated with vehicle (left) involves the entire depth of the gastric mucosa. Pretreatment with candesartan for 2 weeks (right) prevented the development of stress-induced gastric ulcers. Bar is 100 μm. Bar graph: number of lesions counted in the glandular portion of the stomach; open bars, animals treated with vehicle; closed bars: animals pretreated with candesartan (modified from Bregonzio *et al.*, 2003).

mechanisms are probably involved in the protective effect of the AT_1 antagonist, including the reduction of the stress-induced adrenomedullary catecholamine formation and release, increase in gastric blood flow and anti-inflammatory effects (Bregonzio *et al.*, 2003). The protection of gastric blood flow after administration of AT_1 receptor antagonists is probably mediated by inhibition of receptors localized to the endothelium of arteries located in the gastric mucosa (Bregonzio *et al.*, 2003), and is similar to the protective effect on cerebral blood flow during brain ischemia (Nishimura *et al.*, 2000b; Ito *et al.*, 2002).

We found that stress markedly increased the expression of the proinflammatory cytokine tumor necrosis factor α(TNF-α), the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) and the number of infiltrating neutrophils in the gastric mucosa (Bregonzio *et al.*, 2003), which play crucial roles in the progression of gastric injury (Hamaguchi *et al.*, 2001). Pretreatment with the AT_1 receptor antagonist prevented these changes (Fig. 9). This indicated that the anti-inflammatory effects of AT_1 blockade could be relevant for the protection of stress-induced lesions (Bregonzio *et al.*, 2003). Inhibition of AT_1 receptors, by combined local and systemic mechanisms, protects gastric blood flow, inhibits the pro-inflammatory cascade preventing the gastric ischemia and inflammation characteristic of a major stress response and protecting the gastric mucosa from stress-induced ulcerations.

AT_1 blockade did not prevent the increase in adrenal corticosterone produced by cold-restraint as it did in response to isolation (Yang *et al.*, 1996; Bregonzio *et al.*, 2003). This demonstrated that Ang II regulates the stress reaction differently depending on the kind and intensity of the stress. Preservation of the glucocorticoid response during stress may contribute to the therapeutic effect of candesartan, because corticoids have been proposed to protect against gastric ulceration (Filaretova *et al.*, 1998).

Thus, our experiments demonstrate a clear protective, antistress effect of candesartan in an acute stress-induced disorder.

The regulation of the stress response by AT_1 receptors is not limited to their influence on the HPA axis and the sympathoadrenal system, and includes regulatory effects at higher central levels. CRH acts as a modulator, predominantly through CRH_1 receptor stimulation, in centers higher than the hypothalamus to influence and integrate stress-induced behaviors (Brunson *et al.*, 2002). We found that isolation stress in rats decreases CRH_1 receptor expression in the frontal, parietal and cingulate cortex, an effect prevented by AT_1 receptor inhibition prior to stress (Fig. 10). The stress-related decrease in receptor binding can be interpreted as increased receptor occupancy due to enhanced CRH release during stress (Keck and Holsboer, 2001; Brunson *et al.*, 2002), which is prevented by prior AT_1 receptor blockade.

Isolation stress decreased flunitrazepam binding in the frontal and parietal cortex, a characteristic response to stress (Medina *et al.*, 1983; Bremmer *et al.*, 2000). The stress-induced decrease in flunitrazepam binding was also prevented by prior AT_1 receptor blockade (Fig. 11). The benzodiazepine binding site is part of the $GABA_A$ receptor complex, regulating, in higher centers, the response to anxiety (Serra *et al.*, 1999; Smith, 2001). For this reason we postulated that AT_1 Ang II receptors may be involved in higher regulatory mechanisms controlling the behavioral and cognitive responses to stress and anxiety. We tested this hypothesis in a plus-maze, with

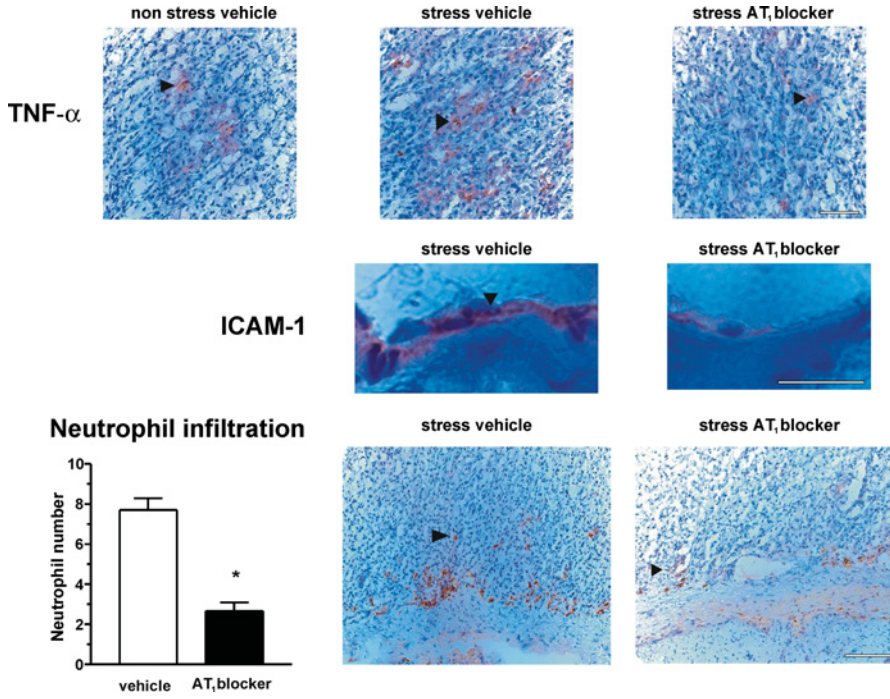

Fig. 9. Decreased TNF-α and ICAM-1 expression and neutrophil infiltration in the gastric mucosa after cold-restraint by pretreatment with AT_1 receptor antagonists. Top—immunocytochemistry of TNF-α. Arrowheads point to TNF-α immunoreactivity. Note the increased immunoreactivity in animals submitted to cold restraint, and the decreased expression of TNF-α in stressed animals pretreated with the AT_1 receptor antagonist. Middle: Immunocytochemistry of intercellular adhesion molecule-1 (ICAM-1). Black arrowheads point to ICAM-1 localized to the endothelium of a small artery located in the gastric mucosa. Note at the marked reduction in ICAM-1 immunoreactivity in stressed rats pretreated with candesartan. Bar is 20 μm. Bottom: Neutrophil infiltration. Left—gastric mucosa of stressed rats pretreated with vehicle. Right—-gastric mucosa of stressed rats pretreated with the AT_1 receptor antagonist. Note the marked reduction in the neutrophil number in stressed rats pretreated with candesartan. Arrowheads point to infiltrating neutrophils. Bars are 20 μm. Bar figures represent the number of infiltrating neutrophils in gastric mucosa from SHR pretreated with vehicle or the AT_1 receptor antagonist for 2 weeks before cold-restraint stress. $^*P < 0.05$. Note that the number of infiltrating neutrophils was significantly reduced by pretreatment with the AT_1 receptor antagonist (modified from Bregonzio *et al.*, 2003).

rats pretreated with candesartan or vehicle. Candesartan treatment significantly enhanced the time spent in the open arm, indicating a clear anxiolytic effect of the AT_1 receptor antagonist (Fig. 12).

Hyperactivity of the HPA axis and of CRH neurons regulating higher brain centers are confirmed findings in anxiety and in stress-related affective disorders (Keck and Holsboer, 2001). Our observations indicated that antagonism of brain Ang II AT_1 receptors (Figs. 13 and 14) could open a new lead in the treatment of anxiety and other stress-related psychiatric conditions.

(2) *The control of the cerebral circulation and participation in mechanisms of brain ischemia and neuronal injury. Blockade of Angiotensin II AT_1 receptors*

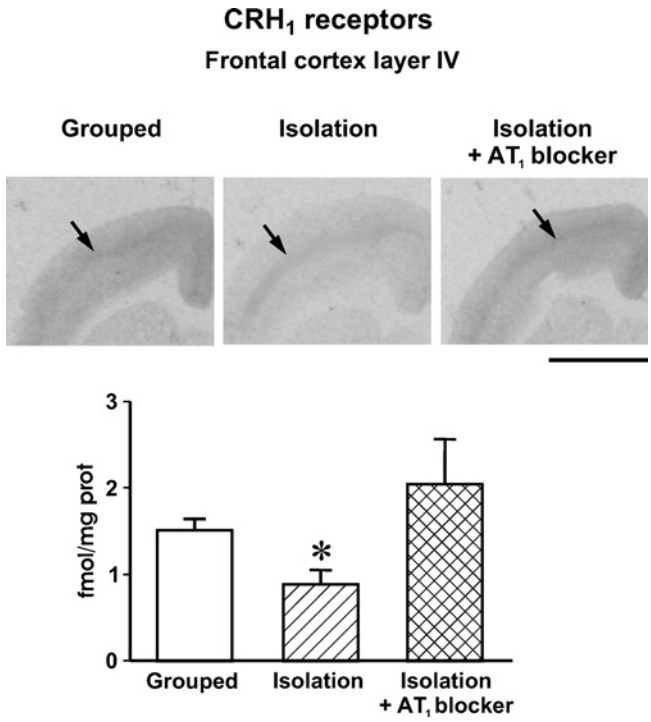

Fig. 10. Effect of AT_1 receptor blockade on expression of CRH_1 receptor binding in the cortex of rats submitted to isolation stress. Figures represent autoradiographic images of cortical sections incubated in the presence of 0.2 nM of [^{125}I]-sauvagine to reveal CRH receptors. Arrows point to cortex, layer IV. Bar is 3 mm. Columns are quantitative autoradiographic measurements of CRH_1 receptor binding, specifically displaced by addition of 13 nM antalarmine. Results are means ± SEM, for groups of six animals measured individually. $^*P < 0.05$ vs. control grouped and isolated, candesartan-treated animals, one-way ANOVA followed by Neuman Keul's test.

improves cerebrovascular compliance in hypertension, reversing pathological cerebrovascular remodeling, normalizing the expression of nitric oxide synthase isoenzymes and reducing cerebrovascular inflammation. Ang II modulates blood flow to peripheral tissues and increases systemic blood pressure by AT_1 receptor stimulation in peripheral arteries followed by vasoconstriction (Timmermans *et al.*, 1995). In the brain, circulating or locally formed Ang II, through AT_1 and perhaps AT_2 (Tsutsumi and Saavedra, 1991c) receptor stimulation in cerebral vessels and sympathetic nerves, exerts a profound influence in the control of cerebrovascular flow (Edvinsson, 1975; Edvinsson *et al.*, 1979; Brecher *et al.*, 1981; Speth and Harik, 1985; Tsutsumi and Saavedra, 1991c; Saavedra and Nishimura, 1999). In the spontaneously hypertensive rats (SHR) both the brain Ang II and sympathetic systems are stimulated (Saavedra, 1992). In SHR, the resulting increased vasoconstrictor tone and arterial thickness with smooth muscle proliferation leads to decreased vascular compliance

and decreased capacity of cerebral vessels to dilate during hypoperfusion, increasing their vulnerability to ischemia (Hajdu *et al.*, 1991; Fujii *et al.*, 1992; Näveri *et al.*, 1994; Blezer *et al.*, 1998; Nishimura *et al.*, 1998).

In SHR, selective inhibition of Ang II AT_1 receptors by acute intravenous (Vraamak *et al.*, 1995) or prolonged subcutaneous (Nishimura *et al.*, 2000b) administration of candesartan shifts their cerebrovascular autoregulatory response to the left, in the direction of lower blood pressures. We found that chronic AT_1 blockade significantly reduced the volume of ischemia and tissue swelling resulting from middle cerebral artery (MCA) occlusion with reperfusion, predominantly in cortical areas (Nishimura *et al.*, 2000a). This effect correlated with inhibition of brain and cerebral artery AT_1 receptors (Nishimura *et al.*, 2000b). We proposed that the resulting increased capacity of the cerebral arteries to dilate, by increasing collateral flow, reduced the loss of CBF in the periphery of the zone of ischemia and this

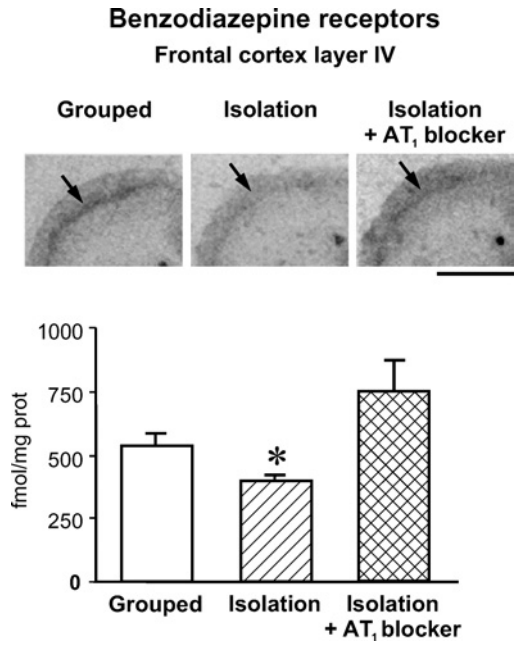

Fig. 11. Effect of AT_1 receptor blockade on expression of flunitrazepam binding in the cortex of rats submitted to isolation stress. Figures represent autoradiographic images of cortical sections incubated in the presence of 1 nM ^{3}H-flunitrazepam. Arrows point to cortex, layer IV. Bar is 3 mm. Columns are quantitative autoradiographic measurements of flunitrazepam binding, selectively displaced by 1 μM clonazepam. Results are expressed as means $\pm$ SEM, for groups of six animals measured individually. $^*P < 0.05$, vs. control grouped and isolated, candesartan-treated animals, one-way ANOVA followed by Neuman Keul's test.

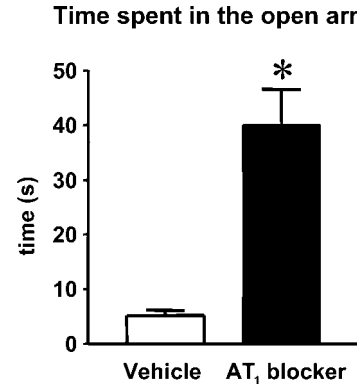

Fig. 12. Anxiolytic effect of candesartan. The figure shows the increase in the time spent in the open arm of a plus-maze by rats pretreated for 14 days with 1.0 mg/kg/day of candesartan, administered orally, a sign of decreased anxiety. $^{*}P < 0.05$, vs. vehicle-treated rats.

contributed to the neuroprotective effect of the AT_1 antagonists (Nishimura *et al.*, 2000a).

To clarify the mechanism of the protective effect of AT_1 receptor blockade during brain ischemia, we pretreated SHR with candesartan followed by permanent distal MCA occlusion (Ito *et al.*, 2002), a model that results in a profound decrease in blood flow predominantly localized to the ipsilateral cortical areas leading to relatively reproducible cortical infarct volumes. MCA occlusion produced, after

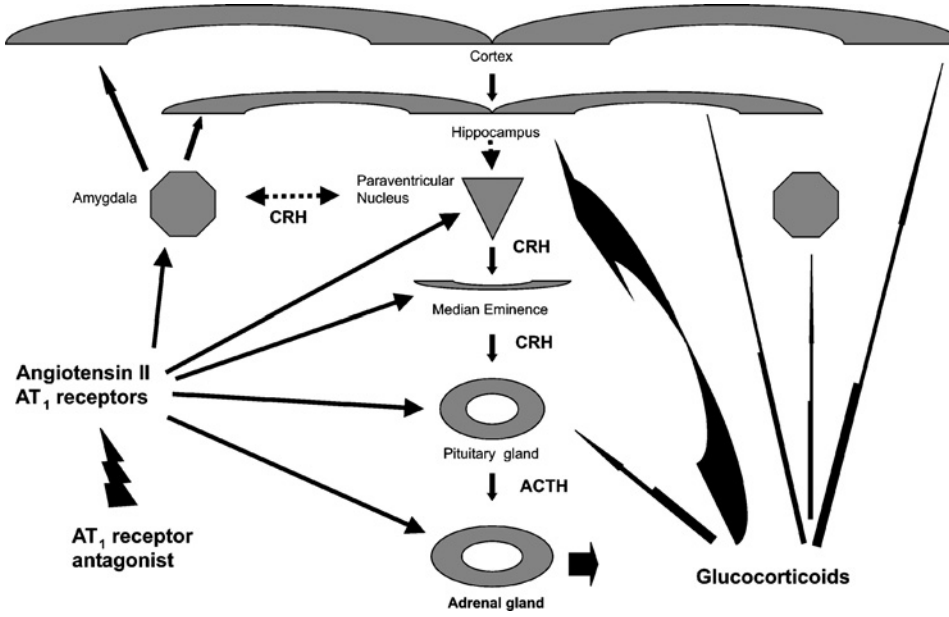

Fig. 13. Sites of effect of AT_1 receptor antagonists on the regulation of peripheral and brain components of the stress reaction. Left arrows—AT_1 receptor antagonists block AT_1 receptor stimulation in the adrenal and pituitary glands, the median eminence, paraventricular nucleus, amygdala, hippocampus and cerebral cortex. Right arrows—sites of glucocorticoid feedback regulation during stress in the pituitary gland, the median eminence, paraventricular nucleus, hippocampus, and cortex.

Effect of AT_1 receptor blockade on the brain CRH systems during stress

	Mild stress (isolation)	Severe stress (cold-restraint)
HPA axis Response	Prevented	Conserved *
Cortical CRH_1 receptor Response	Prevented **	Prevented **

* Protects from gastric mucosal damage ** Anxiolytic effects

Fig. 14. Effect of AT_1 receptor blockade on the brain CRH systems during stress. Blockade of AT_1 receptors prevents the hypothalamic–pituitary–adrenal axis response only when the stress is mild, such as during 24 h of isolation. During severe stress, such as cold-restraint, the hypothalamic–pituitary–adrenal axis response is not prevented, and this is one of the mechanisms protecting from devastating gastric mucosal damage. On the other hand, the response of higher cortical structures, revealed by changes in cortical expression of CRH_1 receptors, is blocked by pretreatment with AT_1 receptor antagonists during mild or severe stress, correlating with antianxiety effects. The effect of the AT_1 receptor antagonist on cortical CRH_1 (and benzodiazepine, see text) receptors is the basis for its anxiolytic effect.

24 h, a cortical ischemic lesion and tissue swelling in the ipsilateral hemisphere, with a substantial decrease in cerebral blood flow (CBF) in the ipsilateral hemisphere (Ito *et al.*, 2001, 2002). We found that candesartan significantly decreased the size of the infarct and tissue swelling and reduced the decrease in CBF after MCA occlusion in the cortex. (Fig. 15) (Ito *et al.*, 2002). Preservation of CBF above a crucial threshold of 0.50 mL/g/min (Fig. 16) as a result of AT_1 receptor blockade was essential for tissue survival, and the area where CBF was below 0.50 mL/g/min correlated well with the total area of ischemia, (Ito *et al.*, 2002).

Protection of CBF by candesartan treatment was associated with decreased media thickness and external diameter/media thickness ratio (Fig. 15). Thus, preservation of CBF above a crucial threshold during ischemia is necessary for end organ protection, and is a crucial factor in the protection from ischemia following AT_1 blockade. Improved CBF is most probably related to dilation of collateral vessels, prominent in cortical areas. We postulated that the protection of CBF during ischemia and the normalization of cerebrovascular autoregulation that follows pretreatment with AT_1 antagonists might have its basis on the inhibition of the growth promoting effects of Ang II in cerebral arteries (Griffin *et al.*, 1991) resulting in increased arterial compliance and increased capacity of brain arteries to dilate during ischemia. This was supported by our findings of increased MCA external diameter and reduced media thickness after candesartan treatment (Fig. 15). Because adrenergic receptor blockade (Nishimura *et al.*, 2000a) or calcium channel inhibition

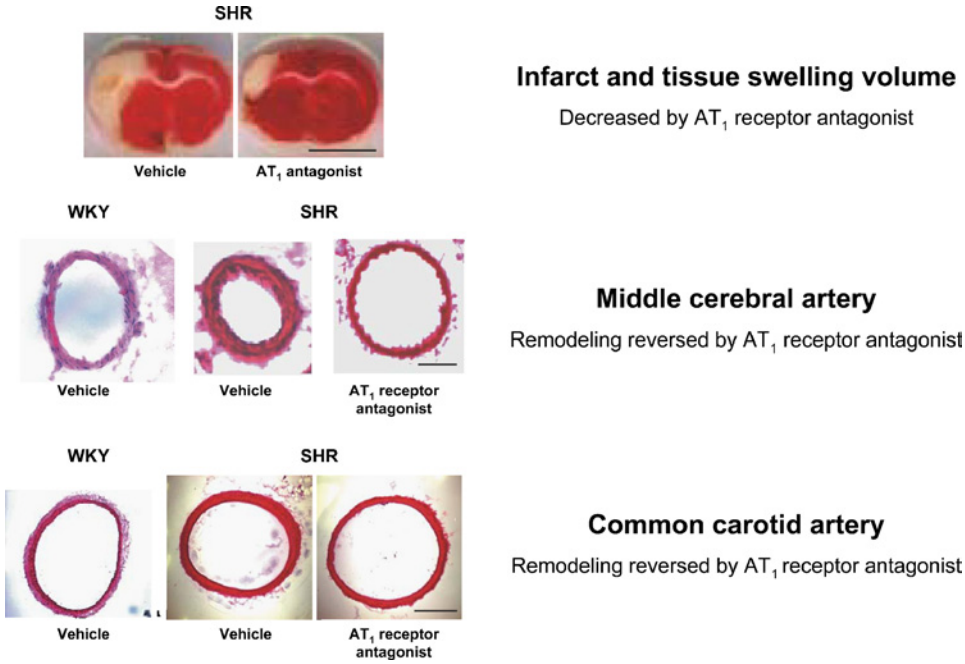

Fig. 15. Prevention of brain ischemia by Angiotensin II AT_1 receptor antagonism parallels inhibition of arterial remodeling. Top: Figures reveal the infarction volume and tissue swelling produced in an SHR treated with vehicle (left) or the AT_1 receptor antagonist candesartan (right) for 2 weeks, and submitted to middle cerebral artery occlusion. Ischemic tissue appears in white. Note the significant decrease in infarct volume and tissue swelling in the brain of the rat pretreated with the AT_1 antagonist. Middle panel—Middle cerebral artery. Lower panel: common carotid artery. Photographs of are of representative histological cross sections of rat middle cerebral artery stained with hematoxylin and eosin. Note the increased media thickness and increased media thickness/luminal diameter ratio in SHR treated with vehicle (middle figures) when compared with normotensive WKY rats (left figures). These changes are evidence of pathological remodeling during hypertension. Note the normalization of both the media thickness and the media thickness/luminal ratio in SHR treated with the AT_1 receptor blocker (right figures). Bars represent 1 cm (upper panel) 50 μm (middle panel), and 200 μm (lower panel) (modified from Ito *et al.,* 2002; Yamakawa *et al.*, 2003)

(Ito *et al.*, 2002) did not protect from ischemia, it appears that inhibition of the Ang II system is essential to ensure tissue survival after MCA occlusion.

Cerebrovascular compliance may respond to changes in nitric oxide (NO) production at specific cellular sites. NO participates in the peripheral vascular alterations in hypertension and the Ang II and NO systems are intimately associated (Briones *et al.*, 2002) Ang II increases reactive oxygen species, enhancing scavenging of NO (Intengan and Schiffrin, 2001). To clarify these mechanisms, we studied eNOS and iNOS protein and mRNA in common carotid artery, principal arteries forming the Willis polygon and cerebral microvessels of SHR and their normotensive WKY controls treated with the AT_1 antagonist candesartan. In all brain vessels studied, SHR expressed lower eNOS mRNA and protein and higher iNOS mRNA and protein than WKY (Fig. 17) (Yamakawa *et al.*, 2003). AT_1 receptor blockade increased eNOS mRNA and protein, and decreased iNOS mRNA and protein, in all brain

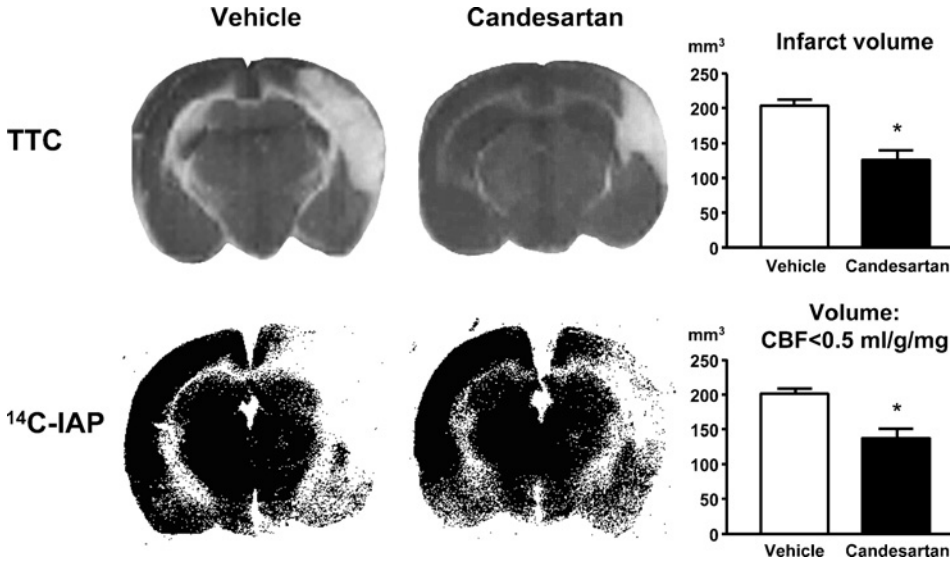

Fig. 16. Relationship between cerebral blood flow decrease and the area of ischemia in SHR after left middle cerebral artery occlusion and pretreatment with an AT_1 receptor antagonist. Figures represent typical images from rats treated with vehicle (left) or with the AT1 receptor antagonist candesartan for 2 weeks, and submitted to middle cerebral artery occlusion. Upper figures—images of sections revealing the area of infarct, which appears white in the figures. Lower figures—images of sections obtained after determination of blood flow by the ^{14}C-iodoantipyrine method, and analyzed with the use of the image processing system and computerized image analysis to reveal the area corresponding to a cerebral blood flow below the 0.50 mL/g/min thresholds. Scale bar is 1 cm. Bars represent the quantification of the area volumes of infarct, (upper bars) and the quantification of area volumes of cerebral blood flow below the 0.50 mL/g/min threshold (lower bars). $^*P < 0.05$, compared with vehicle-treated group. Note that the decreased area of ischemia after candesartan treatment correlates well with the decreased area where blood flow is lower than the 0.5 mL/g/mg threshold (Ito *et al.*, 2002).

vessels from SHR. The net result of the treatment was the elimination of the difference in eNOS and iNOS protein expression between SHR and WKY rats (Fig. 17) (Yamakawa *et al.*, 2003). From our results it can be concluded that normalization of NO production at key endothelial and adventitia sites is important for the regulation of arterial compliance and is under control of AT_1 receptor activation.

Chronic inflammation of blood vessels may also be important for the vulnerability to stroke in patients suffering from hypertension and arteriosclerosis (Ross, 1993). An initial step in the process of arteriosclerosis is endothelial dysfunction with endothelial macrophage adhesion, followed by their infiltration into the blood vessel wall, and reduction of nitric oxide (NO) production leading to vasoconstriction. (Ross, 1993; Harrison, 1997). To investigate whether or not Ang II AT_1 receptor antagonists exert anti-inflammatory effects in brain vessels, we studied ICAM-1 expression, perivascular macrophage infiltration and endothelial macrophage adherence in brain microvessels and carotid artery of SHR (Ando *et al.*, 2004).

We found increased expression of endothelial AT_1 receptors in brain microvessels and middle cerebral artery of SHR when compared to normotensive controls, a signal of increased AT_1 receptor stimulation (Fig. 18) (Ando *et al.*, 2004).

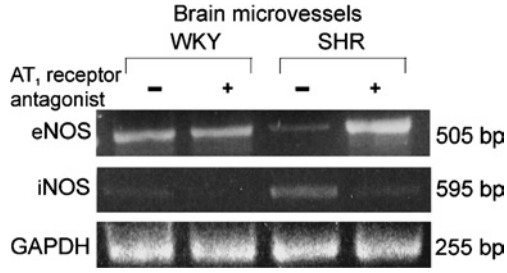

Fig. 17. Normalization of eNOS and iNOS mRNA in brain microvessels of SHR after treatment with an Angiotensin II AT_1 antagonist. There were parallel changes in NOS isoenzyme mRNA expression between the strains and after treatment, with opposite differences in the eNOS and iNOS mRNA expression between untreated SHR and WKY. eNOS mRNA was decreased and iNOS mRNA was increased in brain microvessels of untreated SHR. In brain microvessels, AT_1 receptor antagonism markedly stimulated eNOS mRNA expression in SHR to levels higher than those present in WKY. Treatment with the AT_1 receptor antagonist, which did not modify iNOS mRNA in WKY rats, normalized its expression in brain microvessels of SHR. Thus, treatment with the AT_1 receptor antagonist normalized both the expression of eNOS and iNOS mRNA in cerebral microvessels from SHR (Yamakawa *et al.,* 2003).

Endothelial ICAM-1 expression in microvessels and carotid artery was remarkably increased in SHR compared with WKY rats and decreased after AT_1 receptor blockade to a level similar to that of WKY rats (Ando *et al.*, 2004). Macrophage infiltration was observed surrounding cerebral microvessels only in SHR (Ando *et al.*, 2004). In addition, there were some ED1-positive macrophages attached to the carotid artery endothelium of SHR (Ando *et al.*, 2004). The number of perivascular infiltrating macrophages was reduced in SHR after treatment with the AT_1 receptor blocker (Fig. 18) (Ando *et al.*, 2004).

Our results highlight the role of the Ang II system in the cerebrovascular response to ischemia and demonstrate the protective effect of AT_1 receptor blockade (Nishimura *et al.*, 2000b; Ito *et al.*, 2002; Yamakawa *et al.*, 2003; Ando *et al.*, 2004). AT_1 antagonists, by normalizing the capacity of cerebral arteries from hypertensive animals to dilate, improve collateral flow and reduce the loss of CBF in the periphery of the zone of ischemia (Ito *et al.*, 2002). We demonstrate that normalization of brain arterial compliance requires prolonged AT_1 receptor blockade (Ito *et al.*, 2002) paralleling the reversal of the decreased lumen diameter and increased medial thickness characteristic of hypertension-induced cerebrovascular pathological remodeling (Baumbach and Heistad, 1992; Mulvany *et al.*, 1996; Intengan and Schiffrin, 2001) improved cerebrovascular compliance in response to cerebral ischemia is a function of the normalization of cerebrovascular morphometry that follows long term inhibition of AT_1 receptors.

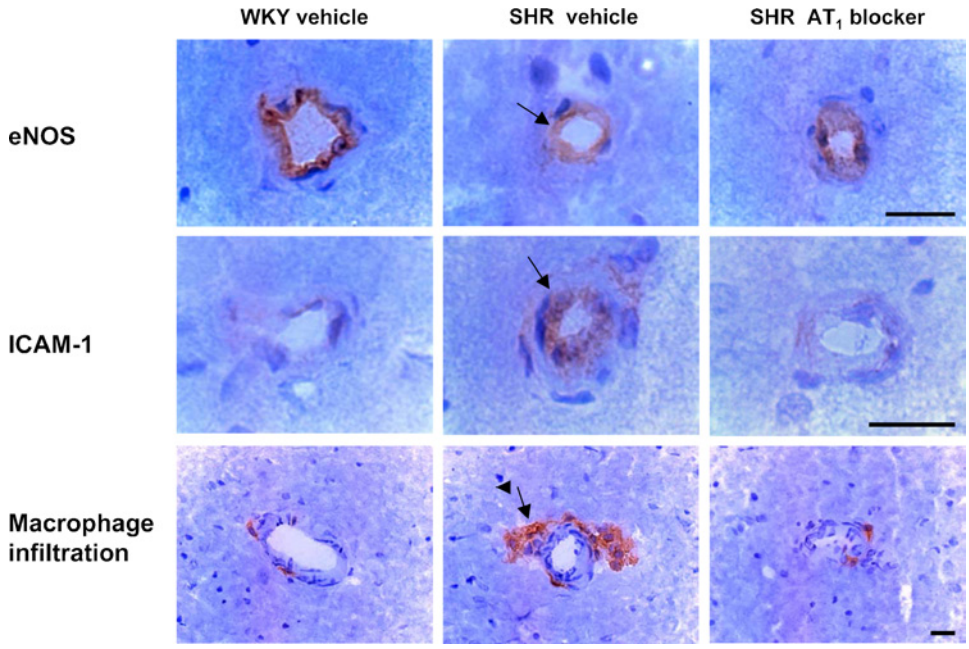

Fig. 18. Localization of eNOS, and ICAM-1, and macrophage infiltration in brain microvessels from WKY and SHR. Top Figures. Endothelial eNOS immunoreactivity in brain microvessels from WKY rats (left) and SHR (middle) treated with vehicle for 2 weeks, and SHR treated with the AT_1 receptor antagonist (right). Arrow points to decreased eNOS immunoreactivity in microvessels from SHR. Bar is 20 μm. Middle Figures. Endothelial ICAM-1 immunoreactivity in brain microvessels from WKY rats (left) and SHR (middle) treated with vehicle for 2 weeks, and SHR treated with the AT_1 receptor antagonist (right). Arrow points to increased ICAM-1 immunoreactivity in microvessels from SHR. Bar is 20 μm. Bottom Figures. Arrow points to rounded macrophages detached from the microvessel wall in an SHR treated with vehicle. Note the absence of detached macrophages in microvessels from WKY rats or SHR treated with the AT_1 receptor antagonist. Bar is 20 μm (Ando *et al.*, 2004)

Our studies may help to clarify the controversial role of NOS isoenzymes in cerebrovascular control during hypertension and brain ischemia. AT_1 receptor antagonism normalizes the hypertension-induced alterations in cerebrovascular expression of NOS isoenzymes. These results agree with the hypothesis of a balanced interaction between the Ang II and NO systems (Cahill *et al.*, 1995; Millatt *et al.*, 1999). Decreased cerebrovascular eNOS expression during genetic hypertension might be responsible for the decreased capacity to vasodilate in response to ischemia (Fujii *et al.*, 1992) and might influence the development of pathological remodeling by non-hemodynamic actions (Chou *et al.*, 1998; Rudic *et al.*, 1998; Rudic and Sessa, 1999). Increased iNOS expression, on the other hand, may result in up regulation of NO production, leading to induced generation of reactive oxygen species and inflammation (Cromheeke *et al.*, 1999; Yogo *et al.*, 2000). By restoring the balance in the expression of NOS isoenzymes, AT_1 receptor antagonism may contribute to the normalization of NO function. Restoration of eNOS expression may stimulate NO production by the endothelium, improving vasodilatation, reversing the pathological arterial remodeling, an important mechanism for neuroprotection during

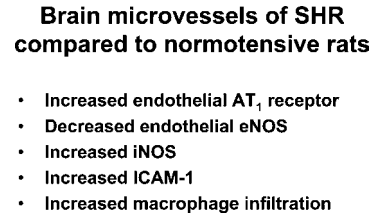

Fig. 19. Angiotensin II AT_1 receptor-dependent inflammation and hypertrophy in cerebral vessels in hypertension. Microvessels from untreated SHR show increased endothelial AT_1 receptor expression, decreased endothelial eNOS, increased iNOS and ICAM-1 expression, and macrophage infiltration. Blockade of AT_1 receptors restores eNOS, iNOS and ICAM-1 expression and prevents macrophage infiltration. This indicates profound anti-inflammatory effects of AT_1 receptor antagonists in the cerebral vasculature.

ischemia (Yogo *et al.*, 2000; Amin-Hanjani *et al.*, 2001; Leker *et al.*, 2001). Inhibition of iNOS may reduce Angiotensin II-induced generation of reactive oxygen species, decreasing NO scavenging, cellular damage and inflammation (Griendling *et al.*, 1994; Rajagopalan *et al.*, 1996). The reversal of the eNOS-iNOS ratio (Figs. 17 and 18), a restoration of the balance lost during hypertension, occurs throughout the entire cerebrovascular system and is associated with the normalization of brain arterial morphology and with protection against ischemia.

Alterations in eNOS expression in SHR correlate with increased endothelial Ang II AT_1 receptor and ICAM-1 expression, higher numbers of endothelium-adhering macrophages in cerebral microvessels and carotid artery, and increased number of perivascular infiltrating macrophages in microvessels of SHR, evidence of cerebrovascular inflammation (Fig. 19) (Ando *et al.*, 2004). The reversal of the cerebrovascular inflammation by AT_1 receptor blockade was a major finding in our study, suggesting AT_1 receptor overstimulation as a molecular mechanism leading to brain inflammation. The suppression of inflammation in brain vessels suggests important therapeutic advantages of AT_1 receptor antagonists not only in the prevention of brain ischemia but also in the treatment of inflammatory diseases of the brain.

CONCLUSIONS

Our studies suggest the possibility of novel therapeutic effects of Ang II AT_1 receptor blockade. First, AT_1 receptor blockade antagonizes the effects of AT_1 receptor stimulation in peripheral organs integrating, together with hypothalamic structures, the HPA axis, and in higher brain centers involved in the processing of sensory information and the behavioral response to stress. The use of AT_1 antagonists with central as well as peripheral effects may decrease the vulnerability and sensitivity to stress and anxiety disorders. Second, long term AT_1 receptor inhibition improves cerebrovascular compliance, reverses pathological remodeling, normalizes NO production, and reverses cerebrovascular inflammation, characteristics of vulnerability to ischemia and stroke in hypertension. For this reason the AT_1 receptor antagonists can be considered as potentially effective therapeutic agents in ischemic and inflammatory diseases of the brain.

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