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Peripherally administered Angiotensin II AT₁ receptor antagonists are anti-stress compounds *in vivo*

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Abstract

Angiotensin II AT₁ receptor blockers (ARBs) are commonly used in the clinical treatment of hypertension. Subcutaneous or oral administration of the ARB candesartan inhibits brain as well as peripheral AT₁ receptors, indicating transport across the blood brain barrier. Pretreatment with candesartan profoundly modifies the response to stress. The ARB prevents the peripheral and central sympathetic activation characteristic of isolation stress and abolishes the activation of the hypothalamic-pituitary-adrenal axis during isolation. In addition, candesartan prevents the isolation-induced decrease in cortical corticotropin-releasing factor 1 and benzodiazepine receptors induced by isolation. When administered before cold-restraint stress, candesartan totally prevents the production of gastric ulcerations. This preventive effect of candesartan is the consequence of profound anti-inflammatory effects, reduction of sympathetic stimulation, and preservation of blood flow to the gastric mucosa. The ARB does not reduce the hypothalamic-pituitary-adrenal axis stimulation during cold-restraint. Preservation of the effects of endogenous glucocorticoids is essential for protection of the gastric mucosa during cold-restraint. Administration of the ARB to non-stressed rats decreases anxiety in the elevated plus-maze. Our results demonstrate that Angiotensin II, through AT₁ receptor stimulation, is a major stress hormone, and that ARBs, in addition to their anti-hypertensive effects, may be considered for the treatment of stress-related disorders.

Keywords

Renin Angiotensin System; inflammation; brain; gastric ulcers; Corticotropin releasing hormone; GABA_A; sympathetic system

INTRODUCTION

A large body of evidence indicates that brain and peripheral Angiotensin II (Ang II), the acting principle of the Renin-Angiotensin System (RAS) is a major stress hormone. 1· 2 However, Ang II is not usually considered as an active participant in stress, in part because its role in the regulation of blood pressure and kidney function 3 overshadows other potential effects of Ang II in the brain and the periphery. The first firm indication of a participation of brain Ang II in stress was the discovery of increased Ang II receptors in the paraventricular nucleus of the hypothalamus (PVN) during immobilization stress. 4 This was followed by reports of increased plasma and brain Ang II during stress, 5· 6 and localization studies revealing the presence of Ang II AT₁ receptors in all brain areas involved in the central control of the hormonal and sympathetic response to stress. 7· 8 The

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hypothesis of a central role of Ang II in the reaction to stress was firmly established with the demonstration that inhibition of peripheral and brain Ang II AT₁ receptors by peripheral administration of Ang II AT₁ receptor blockers (ARBs) prevented the hormonal and sympathoadrenal response 9 and the response of higher cortical centers to stress, reducing anxiety in rodents. 2 This review and commentary will summarize the evidence for anti-stress and anti-anxiety properties of ARBs, substantiating the proposal to consider administration of ARBs as a novel therapy for stress-induced disorders.

MATERIALS AND METHODS

To quantify Ang II receptor types (AT₁ and AT₂ receptors) we used quantitative autoradiography incubating brain sections in the presence of [¹²⁵I]Sar¹-Ang II and selective AT₁ and AT₂ receptor ligands (losartan and PD123319, respectively). 7 We studied the response to stress after sustained blockade of peripheral and brain AT₁ receptors with the selective, potent, insurmountable AT₁ antagonist candesartan. 10· 11 We selected the stress of isolation, a model resulting, in rodents, from the restriction from freely regulating exposure to novel surroundings and access to familiar territory, because of its clinical relevance, to study the effect of pretreatment with an ARP on the hormonal and sympathetic response to stress. 9 To determine whether or not AT₁ receptor blockade could be of therapeutic benefit, we studied the effects of candesartan on the development of a stress-induced disorder, the formation of gastric ulcers induced by a marked stress, that of cold-restraint in the rat. 12 To test the effect of ARBs on higher centers regulating the stress response, we studied corticotropin-releasing factor receptor 1 (CRF₁ receptors) and benzodiazepine receptors by quantitative autoradiography and incubation of brain sections with [¹²⁵I]sauvagine or [³H]flunitrazepam, respectively. 2 To determine the effect of ARBs on anxiety, we used the elevated plus-maze, and quantified the number of entries to and the time spent in the open arms.

RESULTS

Peripheral administration of an ARB blocks brain AT₁ receptors

Administration of the ARB candesartan, subcutaneously, blocked AT₁ receptors in peripheral organs and in the brain, including the subfornical organ and the PVN (Fig. 1A,B). 11

Peripheral administration of an ARB prevents the hormonal and sympathetic stimulation during isolation stress

Administration of candesartan for 2 weeks before isolation, prevented the increased AT₁ receptor expression in the PVN, the increased pituitary ACTH, decreased pituitary vasopressin, and increased adrenal corticosterone, aldosterone, catecholamines and the adrenal TH transcription (Fig. 1C) produced by isolation. 9

Peripheral administration of an ARB prevents ulceration of the gastric mucosa during cold-restraint stress

We found that pretreatment with candesartan dramatically decreased the number of ulcerations produced by cold-restraint stress (Fig. 1D). However, AT₁ blockade did not prevent the increase in adrenal corticosterone produced by cold-restraint stress. 12

Peripheral administration of an ARB prevents changes in CRF₁ and benzodiazepine receptor expression in the rat cortex

Candesartan pretreatment prevented the isolation stress-induced decrease cortical CRF₁ (Fig. 2A) and benzodiazepine (Fig. 2B) receptor expression. 2

Peripheral administration of an ARB has anti-anxiety effects

Candesartan administration increases the time spent in and the number of entries to the open arm of an elevated plus-maze (Fig. 2C,D). 2

DISCUSSION

On the basis of neuroanatomical, physiological and pharmacological evidence, we proposed circulating and brain Ang II as an important regulatory hormone in stress. 1· 2· 13 During stress, enhanced sympathetic activation leads to increased renin production and this generates increased blood levels of Ang II. 5· 6 Increased circulating Ang II augments the stimulation of its physiologically active AT₁ receptors, 14 contributing to increase anterior pituitary ACTH, adrenal glucocorticoid, aldosterone and catecholamine formation and release during stress. 13· 15· 16· 17 This is associated with stress-induced stimulation of the brain, pituitary and adrenal local RAS. 9· 13· 18· 19· 20

There is an intimate connection between peripheral (circulating) Ang II and brain Ang II. In the brain, AT₁ receptors are concentrated in all forebrain areas belonging or related to the hypothalamic-pituitary-adrenal (HPA) axis, including the parvocellular PVN, the site of CRF formation, 8· 21· 22 the median eminence, from where CRF is released to the portal circulation to stimulate pituitary ACTH secretion, and the subfornical organ (SFO). 7· 21 AT₁ receptors are also expressed in the anterior pituitary, adrenal cortex and adrenal medulla 8· 23 and in noradrenaline terminals. 24 By stimulation of AT₁ receptors outside the blood-brain barrier such as the SFO, circulating Ang II activates stress pathways in the brain intimately connected to the PVN, a crucial site in the HPA axis. 13· 25 In the PVN, AT₁ Ang II receptors are localized to CRF-producing parvocellular neurons, 26 and Ang II regulates CRF production directly and indirectly by actions on GABA and glutamate interneurons. 27 The SFO-PVN connection is a central pathway of interaction between circulating Ang II with effects in SFO neurons and brain Ang II activating PVN neurons and spinal cord neurons contributing to regulate specific motor responses (Pavel et al., submitted).

The stress-mediated effects of circulating Ang II are associated with increased activity of the brain RAS. 13 Stress increases not only the local formation of Ang II in the hypothalamus and other parts of the brain, 5· 6· 28 but also the expression of Ang II receptors in the PVN and SFO. 4· 9

While the HPA axis and the sympathetic stimulation are final common pathways during the reaction to stress, higher supra-hypothalamic centers located in multiple limbic structures and in the cortex integrate the response to stress, determining its timing and extent, and regulating adaptation to the stress stimuli. The principal regulatory systems include the central sympathetic system, supra-hypothalamic CRF systems with a major role of CRF₁ receptors, 29 and the GABA_A system. AT₁ receptors are localized to limbic and central sympathetic pathways, 7 and ARBs exert profound effects on hypothalamic CRF and the central sympathetic activation during stress. Because of this, we hypothesized that ARBs could also contribute to regulate supra-hypothalamic structures during stress.

To test the role of brain AT₁ receptors, we used candesartan, an ARB which, when administered peripherally, crossed the blood-brain barrier and inhibited central as well as peripheral AT₁ receptors. 11 The results obtained after administration of candesartan support our hypothesis of a major role for Ang II and its AT₁ receptors in stress. Sustained blockade of peripheral and brain AT₁ receptors with candesartan prevented the hormonal and sympathetic response to isolation stress. The isolation-induced increase in ACTH, glucocorticoid, vasopressin and catecholamine formation and release were significantly

reduced or abolished in ARB-treated rats, 9 in parallel with the inhibition of stress-induced alterations in CRF concentration and transcription in the PVN. 30 These observations confirmed that Ang II AT₁ receptor stimulation was necessary for the HPA axis response, the enhanced AVP release from the posterior pituitary, 9 and for the peripheral sympathetic stimulation during stress. 13 The effects of ARBs were not limited to restraining the hypothalamic and sympathoadrenal responses to stress. Candesartan also prevented the stress-induced increase in tyrosine hydroxylase (TH) transcription in the locus coeruleus, and the cortical decrease in CRF₁ and benzodiazepine receptor binding during isolation. 2 These results indicate that AT₁ receptor stimulation is necessary for the full response of supra-hypothalamic structures during stress. AT₁ receptor activation is required for the stress-induced increase in central sympathetic activity, for the response of cortical CRF pathways, and for the inhibition of the GABA_A system during isolation stress.

The inhibition of TH transcription, the rate-limiting enzyme in the synthesis of catecholamines, in the locus coeruleus, the origin of the major catecholamine innervation to the forebrain, indicates that AT₁ receptor blockade prevents the contribution of the central sympathetic system to the increased adrenomedullary and peripheral sympathetic catecholamine release during stress. 13 The ARB effects on TH transcription may be indirect, through AT₁ receptor inhibition in the PVN, an area projecting to the locus coeruleus. 31

In addition to its role in the regulation of the HPA axis, CRF, predominantly through CRF₁ receptor stimulation, modulates the activity of limbic and cortical structures to influence and integrate stress-induced behaviors. 29 Stress increases cortical and limbic CRF release, leading to excess CRF₁ receptor activation. 29, 32 Stress characteristically decreases cortical benzodiazepine binding. 33, 34 The benzodiazepine binding site is part of the GABA_A receptor complex, and reduced benzodiazepine binding is evidence of decreased activity of this major central inhibitory system. 35, 36 The responses of the central sympathetic system and the limbic and cortical CRF and GABA_A systems during stress are integrated. It has been proposed that enhanced central sympathetic and CRF activity, combined with decreased GABA_A function are causally related to the development of anxiety as a consequence of stress. Pretreatment with the ARB prevented the central sympathetic, CRF and GABA_A responses to stress. For this reason we postulated that AT₁ receptor stimulation was necessary for the full development of the response of higher regulatory mechanisms controlling the behavioral and cognitive responses to stress and anxiety. When tested in an elevated plus-maze, rats pretreated with candesartan exhibited decreased anxiety, as revealed by the significant enhancement of the time spent in and of the number of entries to the open arm. 2

We studied the effects of ARBs on the development of a stress-induced disorder, the formation of gastric ulcers induced by cold-restraint stress in the rat, to determine if ARB treatment of stress-induced disorders could be of therapeutic benefit. 12 Candesartan dramatically decreased the number of ulcerations produced by cold-restraint stress. This therapeutic effect is the consequence of several interrelated mechanisms, the prevention of the stress-induced reduction on gastric blood flow, a marked anti-inflammatory effect in the gastric mucosa, and a partial reduction of the sympathoadrenal stimulation produced by cold-restraint. 12 In addition, AT₁ blockade did not prevent the increase in adrenal corticosterone produced by cold-restraint stress. 12 Preservation of the glucocorticoid response during a major stress contributes to the therapeutic effect of ARBs, because glucocorticoids protect against gastric ulceration. 37

CONCLUSIONS

1. Ang II is an important stress hormone. Stress stimulates the circulating and brain RAS by enhancing Ang II formation and increasing Ang II AT₁ receptor expression.
2. Peripheral administration of ARBs crossing the blood brain barrier blocks brain Ang II AT₁ receptors and allows to study the contribution of the central RAS to stress.
3. Hyperactivity of the HPA axis and of CRF neurons regulating higher brain centers, and the central sympathetic system, and inhibition of GABA_A transmission are confirmed findings in anxiety and in stress-related affective disorders. Administration of ARBs prevents the central and peripheral sympathetic stimulation and the response of the HPA axis to isolation stress. In addition, ARBs prevent the cortical alterations on CRF₁ and benzodiazepine receptors during isolation, and exert powerful anti-anxiety effects.
4. Treatment with ARBs prevent the ulceration of the gastric mucosa characteristic of cold-restraint stress by a number of mechanisms, including anti-inflammatory effects, preservation of blood flow to the gastric mucosa, partial inhibition of central and peripheral sympathetic stimulation and preservation of the HPA axis response and protective effects of glucocorticoids.
5. ARBs with peripheral and central actions may be considered of potential therapeutic use in stress-related and anxiety disorders.

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Figure 1.

Peripheral administration of an ARBs (**B**) blocks AT₁ receptors in the SFO and the PVN (**A,B**), prevents the isolation stress-induced increase of adrenal TH mRNA (**C**), and dramatically decrease the number of ulcerations produced by cold-restraint stress (**D**). SFO – the subfornical organ, PVN – the paraventricular nucleus, TH – tyrosine hydroxylase.

* $p < 0.05$ vs. to all others groups. Modified from Nishimura et al. 2000 (A,B); Armando et al. 2001 (C); Bregonzio et al. 2003 (D). Scale is 1mm.

Figure 2.

Pretreatment with peripherally administered ARB candesartan prevents the decrease of cortical CRF₁ (A) and benzodiazepine (B) receptor expression during isolation stress. Behavior of the grouped undisturbed rats pretreated with vehicle or ARB in the elevated plus-maze (C,D). Increased number of open arm entries and increased time spent in the open arm are signs of decreased anxiety. *p<0.05 vs. all other groups. (from Saavedra et al. 2006).