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Targeting brain angiotensin and corticotrophin-releasing hormone systems interaction for the treatment of mood and alcohol use disorders

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Abstract

The brain renin–angiotensin system (RAS) participates importantly in the regulation of endocrine, autonomic, and behavioral response to stress. Recent data indicate that central action of AT₁ receptor antagonists can reduce anxiety symptoms in experimental animals. Furthermore, central inhibition of RAS activity decreases ethanol intake in an animal model of alcoholism. Pathological anxiety responses and the development of substance dependence are both critically mediated through corticotrophin-releasing hormone (CRH) systems, and the RAS is positioned to interact both with hypothalamic as well as extrahypothalamic CRH systems. The thesis of this paper is that the RAS is part of the neurochemical dysregulation underlying negative affective states, anxiety disorders, and ethanol dependence and that medications targeting the RAS should be considered to augment the treatment of these disorders.

Keywords

Angiotensin receptor; ACE; Alcoholism; Anxiety; Gene expression

Introduction

In addition to its role as a peripheral hormone regulating cardiovascular functions, angiotensin II (Ang II) is a pleiotropic neuropeptide that, through stimulation of its cognate AT₁ receptors, is the main effector of the brain renin–angiotensin system (RAS) and that participates importantly in the regulation of endocrine, autonomic, and behavioral response to stress [1]. There is substantial evidence that Ang II participates in both the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic system response to stress. *First*, AT₁ receptors are selectively expressed in all components of the HPA axis [2]. In the hypothalamic paraventricular nucleus (PVN), AT₁ receptors are localized in corticotrophin-releasing hormone (CRH) producing parvocellular neurons and their expression is glucocorticoid dependent. AT₁ receptors are also highly expressed in areas of the brain regulating central sympathetic activity [2] and in peripheral sympathetic nerve terminals [3]. AT₁ receptor stimulation enhances catecholamine formation and release from the adrenal medulla during stress. *Second*, the AT₁ receptor expression in PVN is increased in several stress models, contributing to enhanced CRH and vasopressin production and release and stimulating the central and peripheral sympathetic systems [2].

In addition, cortical and limbic system mechanisms control the behavioral stress and anxiety responses [4]. *Extrahypothalamic CRH systems* (cortical and subcortical) are major

mediators of these responses [5]. Important structures include the amygdala and the brain stem. In contrast to HPA regulation, extrahypothalamic CRH signaling through the CRH₁ receptor is quiescent under a wide range of conditions but becomes activated in the presence of uncontrollable stress.

Recent evidence indicates that also the influence of brain RAS on the response to stress is not limited to its role on the central regulation of the HPA axis and the sympathetic response. Receptors for Ang II are highly and selectively expressed in all central structures involved in the extrahypothalamic control of the stress response, including the amygdala, the locus coeruleus, the hippocampus, and the medullary centers involved in the regulation of the central sympathetic activity [2]. Similarly, the HPA axis and the extrahypothalamic CRH systems are distinct systems which are differentially regulated. For example, corticosterone provides negative feedback on CRH expression in the hypothalamic paraventricular nucleus as part of the HPA axis regulation but acts as a positive signal for CRH expression in the central amygdala [4].

Ang II–CRH systems interaction may take place on the level of the peptides, between the receptors, or through separate signaling mechanisms. An analysis of angiotensinogen (*Agt*) mRNA expression in the mouse brain provides interesting insight into potential sites of interactions. The highest forebrain *Agt* mRNA levels are found in the medial amygdala (Fig. 1), in close proximity to the locus of highest extrahypothalamic *Crh* mRNA expression. Interestingly, CRH₁ receptors appear upregulated in the medial amygdala during states of increased behavioral sensitivity to stress [6]. However, most of the cellular and molecular interactions between these systems are still unknown.

We found that pretreatment with a centrally acting AT₁ receptor antagonist (candesartan) prevented the isolation-induced alterations in cerebrocortical CRH₁ and benzodiazepine receptors, part of the main inhibitory gamma-aminobutyric acid (GABA)_A complex [7]. A similar treatment decreased anxiety in rats and prevented the stress-induced enhanced tyrosine hydroxylase transcription in the locus coeruleus, a measurement of central sympathetic stimulation [7,8]. These results implicate interactions between the central RAS, extrahypothalamic CRH systems, and GABA_A in the regulation of anxiety and the behavioral responses to stress.

At the hypothalamic level, candesartan prevents the CRH–adrenocorticotrophic hormone–glucocorticoid response to isolation stress demonstrating AT₁ receptors contribution in controlling the endocrine response to stress in this paradigm [9,10]. Also, pretreatment with candesartan effectively prevented the gastric erosions produced by acute cold-restraint stress in the rat [11]. The therapeutic effect of candesartan was the result of protection of gastric blood flow, prevention of local inflammatory responses, and decreased sympathetic activation. In addition, maintenance of the HPA axis response allows the protective effect of glucocorticoids on the gastric mucosa because in our model the increase in corticosterone inhibits the stress-induced gastric erosions [11]. Thus, AT₁ receptor blockade does not appear to inhibit the HPA axis response when it is important as protective, anti-inflammatory response. [11]. These experiments demonstrate that AT₁ receptor antagonism could be effective in stress-induced disorders.

A role for the central RAS emerges also in the development of alcoholism, a disorder that evolves over 5–10 years and that is characterized by uncontrolled heavy drinking and a chronic relapsing course. The involvement of the RAS in ethanol consumption behavior has been long debated, but elucidation was mired in the complexity of interacting peripheral and central mechanisms and available pharmacological tools [12–14]. Recent studies in animals with genetic modification in various components of the RAS demonstrate that Ang II via

AT₁ action is a positive modulator of spontaneous ethanol consumption in rodents [15,16]. Furthermore, increased expression of *Agt* was found in microarray studies of brains from different rodent lines selectively bred for high ethanol preference (HAP mice, P and AA rats) compared to their respective controls [17-19]. Bioinformatic analysis of known gene-gene interactions among differential expressed genes in these animals identified *Agt* as a key node in the interaction network. Functional validation for the increased *Agt* expression is provided by the demonstration that lisinopril or ceranapril, two inhibitors of angiotensin-converting enzyme (ACE), reduce voluntary ethanol consumption in P rats [18,20].

However, voluntary consumption in laboratory rodents rarely leads to blood alcohol concentrations required for induction of dependence, and thus the predictive validity of consumption at this level for human alcoholism is unclear [21]. To model the neurochemical changes of alcoholism and to induce persistent behavioral changes in laboratory animals, we used repeated cycles of ethanol intoxication and withdrawal over prolonged periods of time, thereby paralleling the clinical course of alcoholism and achieving pharmacological validity for target identification and medication development [22]. The hallmarks of the resulting behavioral syndrome, collectively labeled as the *postdependent state*, are excessive ethanol consumption and increased behavioral sensitivity to stress that persist long beyond acute withdrawal (Fig. 2 [6,23]. As predicted from the **allostatic concept of addiction** (Box 1) we found upregulated CRH activity in the central amygdala of postdependent animals during acute withdrawal and many weeks thereafter, contributing to increased ethanol intake, anxiety, and ethanol-seeking behavior, all of which can be alleviated by blockade of CRH₁ receptors [6,23-26]. In fact, the excessive ethanol consumption appears to be a means to downregulate amygdala CRH₁ receptors [27].

Interestingly, postdependent animals show significantly increased *Agt* mRNA and a trend level downregulation of *Agtr1a* mRNA expression in the medial prefrontal cortex but not in nucleus accumbens and amygdala [28]. The regional specificity is interesting and in line with the microarray studies in high-preferring rats [18,19]. Inhibition of AngII production by 5 mg/kg spirapril, an ACE inhibitor that efficiently crosses the blood brain barrier and that according to experiments in *Agt* knockout mice reduces ethanol consumption via central Ang II action [16], was more potent in reducing ethanol intake in postdependent rats compared to controls [28].

We then asked whether low central RAS activity might provide a protective mechanism for the development of ethanol dependence. TGR(ASrAOGEN)680 rats, which exhibit more than 90% reduced *Agt* levels in the brain [29] and show reduced voluntary ethanol consumption [16], were exposed to the cyclic vapor intoxication procedure to induce a postdependent state. We found that blockade of central RAS activity is not sufficient to halt the progression into ethanol dependence [28]. Together, these data suggest that the central RAS is sensitized in its modulatory control of ethanol consumption in the dependent state, but neither pharmacological nor genetic blockade of this system appears to be sufficient to prevent the progression into dependence.

In summary, inhibition of the CRH₁ receptor subtype has been proposed as an attractive target for medication development in anxiety, depression, and addiction [23,30,31] and promising compounds are in development [6,25,32]. On the other hand, a wide variety of safe and effective RAS-targeting medications exist, but their potential as adjuvant medications for these disorders is largely unexplored. Our results show that AT₁ receptor blockade selectively modulates the sympathetic and HPA axis responses to stress, maintaining the HPA axis response when necessary for the welfare of the individual, and reduces anxiety probably as a consequence of prevention of extrahypothalamic stimulation of CRH₁ receptors while protecting the inhibitory activity of the GABA_A complex.

Furthermore, central RAS inhibition decreases ethanol consumption in dependent animals. However, it has to be pointed out that these drugs have so far not been evaluated in a series of validated animal models of these conditions. These include models of anxiety under low- and high-stress conditions (e.g., exploration of open field or elevated plus maze, social defeat, and conflict tests). The lack of predictive validity for alcoholism is particularly true for most investigations of RAS compounds on ethanol consumption. There is a lack of experiments that address the effect of RAS compounds on the motivational properties of ethanol in dependent and nondependent animals and, most importantly, in highly validated models of drug craving and relapse behavior (Box 2).

Given the high comorbidity of hypertension with stress, anxiety, and alcohol use disorders, systematic exploration of new generation, central acting AT₁ receptor blockers, and ACE inhibitors for their potential to augment existing pharmacotherapy for these conditions appears highly relevant.

Box 1. Allostasis—a key mechanism in chronic stress and development of addiction

The classical hallmark of the stress response is the stimulation of the HPA axis and the central and peripheral sympathetic systems. Acutely, this response allows to quickly mobilize resources and is regulated within homeostatic boundaries [4]. However, chronic stress increases energetic needs which may not simply be satisfied by homeostatic (dys)regulation but rather require dynamic adjustment of the regulatory set point of these systems, a process known as *allostasis* (stability through change) [33]. Thus, an allostatic mechanism does not simply defend homeostasis but continuously tries to match resources to needs by adjustment toward new set points, thereby not only regulating the initially challenged neurochemical system but recruiting further systems into the process. The concept of allostatic regulation has proven useful in understanding the worsening of chronic disorders such as hypertension because it provides a mechanism on how repeated or prolonged deviation from homeostasis leads over time to wear and tear on the body (“allostatic load”) and ultimately to pathology. Allostatic remodeling of brain structures involved in emotionality and motivation is hypothesized to underlie altered physiological, behavioral, and drug responses [4].

Many brain structures work in an integrated manner to regulate the behavioral and hormonal response to stress. The response of the cerebral cortex and subcortical regulatory centers determines the behavioral and emotional responses and influences the hypothalamus and brain stem to regulate the physiological (hormonal and sympathetic) responses. Dysregulation of HPA axis and of sympathetic activity have long been recognized as key substrates of an allostatic mechanism involved in stress-related mood and anxiety disorders. A similar view to the allostatic concept of maladaptive stress responses can be applied to the neuroadaptive processes that drive ethanol dependence. Here, homeostatic regulation of the emotional state by the mesocorticolimbic dopamine system and the extended amygdala is challenged by repeated drug use causing overactivation of brain reward systems (e.g., dopamine, opioid peptides, GABA, and glutamate) and recruitment of antireward and brain stress systems (e.g., CRH, noradrenaline, and dynorphine) that results in an allostatic shift toward a negative hedonic affective set point that ultimately leads to compulsive drug seeking and drug taking that define addiction as a chronic, relapsing disorder [34]. Thus, as dependence develops through a history of repeated cycles of intoxication and withdrawal, the motivational and neural substrates shift from initially positive to negative reinforced drug taking.

In particular for alcoholism, it has become evident that neuroadaptations in the amygdala CRH system are driving the dependent state and are a major target for its treatment [23]. There is a role for brain RAS in the regulation of the extrahypothalamic (cortical, subcortical) and hypothalamic (PVN) responses to stress [2]. The activation of the RAS is integrated with stimulatory CRH, arginine vasopressin, and catecholamine and with inhibitory GABA_A and natriuretic (atrial natriuretic factor) systems, influencing both the hypothalamic and extrahypothalamic regulatory centers. Excessive stress or individual vulnerabilities may ultimately result in allostatic load. Blockade of CRH and Ang II receptors may improve adaptation mechanisms, restore the balance, and promote the return to homeostasis.

Box 2. Animal models of alcohol consumption, craving, and relapse

- **Voluntary consumption:** Under certain conditions, experimental animals will voluntarily consume ethanol solutions to pharmacologically relevant levels. Animals can freely choose between ethanol and water under continuous or limited access conditions.
- **Operant self-administration:** Animals are trained to emit an operant response (usually to press a lever) for a distinct amount of the drug. By varying the conditions for this task the experimenter can assess how much work an animal is willing to perform for obtaining the drug, which is interpreted as a measure of motivation for the drug. Importantly, this procedure can easily be combined with conditioning paradigms.

Both methods are valuable tools to study the mechanisms regulating moderate ethanol consumption but have limited predictive validity for drug discovery in alcoholism.

It is problematic that the positively reinforcing effects of voluntary ethanol consumption are difficult to disentangle from a variety of confounds driving intake, such as taste, smell, calorie, etc. Voluntary consumption is also affected by genetic factors (innate preference) and can be increased during dependence. Studies in *postdependent* animals have shown that such excessive ethanol intake seems fundamentally different from basal level consumption.

The clinical defining features of alcoholism are uncontrolled heavy drinking and its chronic relapsing course. As the motivational drive to consume ethanol shifts from initial positive, pleasurable drug effects to relief of negative affective states, so does the mechanism of drug craving, that is, from *reward craving* to *relief craving*. This clinical perspective is reflected in the two animal models below.

- **Reinstatement of alcohol-seeking behavior:** Animals are first trained to respond for ethanol in an operant self-administration paradigm, usually in the presence of a discrete cue (e.g., a light or tone). This is followed by an extinction period, when no reinforcer is delivered during the self-administration sessions. Once lever pressing is extinguished, ethanol seeking, as seen as increased lever pressing in the absence of ethanol can be reinstated after presentation of the conditioned cue or a small dose of ethanol or (this can be interpreted as a *model of reward craving*). Alternatively, stress (e.g., foot shock) can reinstate responding (*relief craving model*).
- **Alcohol deprivation effect:** Transiently increased alcohol intake is seen in animals after a period of prolonged deprivation, probably as a result of dysphoria or a negative affective state (*relief craving model*).

Different neural circuits and pharmacological targets seem to be involved in reward and relief craving. Interestingly, AT₁ receptor blockade could be helpful both in models of

reward and relief craving because AT₁ receptors, besides the RAS–CRH system interaction proposed here, may also affect dopamine release [16]. Compiled from [21,23,35].

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Fig. 1.

Expression atlas for *Agt* in the brain of C57Bl/6J mice. The main view shows voxels (volume elements) with high signal intensity and frequency of *Agt* expression. The locus of highest *Agt* expression in the forebrain is the MeA (*encircled*), a brain region that shows upregulation of CRH₁ receptors in *postdependent animals* (see text and Fig. 2). Several key structures of addiction are shown for orientation. The *insert* in the upper left corner shows the diffuse pattern of *Agt* expression throughout the brain. Expression intensities are color-coded from *blue* to *red* representing low to high values, respectively. Data are from Allen Brain Atlas (www.brain-map.org) and are analyzed using BrainExplorer v1.4.1 software. The atlas is shown in the horizontal plan, the compass is at bregma (*red line*: rostral–caudal direction, *green*: dorsal–ventral, *yellow*: medial–lateral). NAc nucleus accumbens, BNST bed nucleus of the stria terminalis, CeA central amygdalar nucleus, MeA medial amygdalar nucleus, VTA ventral tegmental area

Fig. 2.

Targeting CRH and RAS in the dependence-induced drinking model. Forced intermittent ethanol exposure can be used to induce excessive dependence-related ethanol consumption, which appears to have improved predictive validity for medication development than assessment of basal ethanol consumption [21]. In our model, dependence is induced by daily cycles of ethanol vapor intoxication and withdrawal. After a 3-week resting period to alleviate acute withdrawal symptoms and to allow the behavioral and neuroplasticity to stabilize, animals show a persistent phenotype of excessive voluntary ethanol consumption and increased behavioral sensitivity to stress (*postdependent state*). **a** The circadian blood alcohol level (BAL) profile during a single intoxication cycle shows intoxication above 150 g/dl for several hours which over a period of 7 weeks is sufficient to induce dependence. **b** Postdependent animals (14 weeks after the last ethanol intoxication) are still more sensitive to a mild shock in the punished drinking test compared to controls, as seen in the reduction of approaches to the drinking spout (licks per minute). The behavior is completely normalized by pretreatment with the novel CRH₁ antagonist MTIP (10 mg/kg, i.p.). **c** The excessive ethanol consumption in postdependent animals (10 weeks after the last ethanol intoxication) is significantly reduced by oral administration of the angiotensin converting enzyme inhibitor spirapril (5 mg/kg). Data are taken from [6,28]