REVIEW —Current Perspective—

Role of the Angiotensin II Type-2 Receptor in the Mouse Central Nervous System

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ABSTRACT—There are two known major angiotensin II receptor subtypes, type 1 (AT₁) and type 2 (AT₂), both of which are present in the brain. AT₁ and AT₂ receptors occur in characteristic distributions that are highly correlated with the distribution of angiotensin II-like immunoreactivity in nerve terminals. Acting through the AT₁ receptor in the central nervous system, angiotensin II has effects on fluid and electrolyte homeostasis, neuroendocrine systems, autonomic pathways regulating cardiovascular function and behavior. While the role of the AT₂ receptor in the brain is less well understood, recent knockout studies point to their involvement in behavioral and cardiovascular functions. We discuss here evidence regarding the function of the AT₂ receptor in the brain, determined using mice lacking the AT₂ receptor.

Keywords: Angiotensin, AT₁ receptor, AT₂ receptor, Neuromodulation, Anxiogenic behavior

Introduction

The brain has at least two major angiotensin II receptor subtypes, type 1 (AT₁) and type 2 (AT₂) (1). Cloning studies revealed AT₁ and AT₂ receptors to be different molecules (2), both of which are of similar size (359 amino acids for the AT₁ versus 363 amino acids for the AT₂). Hydropathy analyses of the sequences revealed that both have a seven transmembrane-spanning domain structure, which is consistent with G-protein-coupled receptors (1, 2). However, AT₁ and AT₂ receptors are only 32–34% identical, based upon the amino acid sequence (1, 2). In light of these differences, it is to be expected that AT₁ and AT₂ receptors have different functions.

The majority of studies on AT₁ receptor signaling have been performed in cultured vascular smooth muscle cells or transfected COS or CHO cells (1–3). The AT₁ receptors can engage many second messenger systems via heterotrimeric G-proteins, predominantly G_q, with activation of phospholipase C_β, leading to increased inositol triphosphate and diacylglycerol generation and subsequent increases in intracellular Ca²⁺ concentrations and protein kinase C activity (2, 3). The AT₁ receptors may also mediate a decrease in adenylate cyclase via G_i, stimulate tyrosine phosphorylation of Jak2 (Janus kinase

2), activating the Jak-STAT (signal transducers and activators of transcription) pathway, and activate the mitogen-activated protein kinase pathway, leading to increased *c-fos* transcription (2, 3). On the other hand, signaling pathways used by the AT₂ receptor vary between cell types studied (2, 4). Recent evidence suggests the AT₂ receptor associates with G_{α12} and G_{α13}, stimulates potassium channel activity and inhibits mitogen activated protein kinase activity via G_{αi} and protein phosphatase 2A (5). AT₂-receptor stimulation potentiates voltage-dependent potassium currents in neurons isolated from the hypothalamus and medulla of newborn rats and increases calcium channel activity in NG108-15 cells (2, 4).

The distribution of AT₁ and AT₂ receptors in the rat brain has been defined in binding studies using radioligands (1, 6). Two-week-old rats express the AT₁ receptor in selective limbic system areas, structures involved in cardiovascular and fluid regulation, parts of the hippocampal formation, and in the choroid plexus (6). In these young rats, the AT₂ receptor is concentrated in areas involved in learning and control of motor activity, sensory areas, and selective limbic system structures (6). The cingulate cortex, the molecular layer of the cerebellar cortex, and the superior colliculus has both AT₁ and

AT₂ receptors (6). The number of AT₁ receptors in most areas of 8-week-old rats is similar to or even higher than that found in young rats (6). In contrast, AT₂ receptors were always much fewer in the number of adult rats, and in some areas, there is even a lower amount in adults (6). In the mouse (7), the distribution of AT₁ receptors is similar to that observed in the rat. AT₁ receptors were found in areas involved in the regulation of fluid homeostasis and hormone release such as the subfornical organ and the paraventricular nucleus (7). AT₁ was also found in the caudate nucleus, an area where it was absent in rats (7). AT₂ receptors were detected in sensory and motor related areas such as the superior colliculus and the inferior olive where the AT₂ receptor was absent in AT₂-deficient mice (7). The locus coeruleus of the rat has the AT₂ receptor (6); angiotensin II binding sites were detected in both wild-type and AT₂-null mice; and angiotensin II binding sites in the mouse locus coeruleus were sensitive to the AT₁-specific antagonist losartan (7).

The AT₁ and AT₂ receptors were initially identified on the basis of differential pharmacological properties (1, 6, 7). Briefly, AT₁ receptors have a high affinity for losartan, whereas AT₂ receptors have a high affinity for PD123,177, PD123,319 and CGP42112A (6, 7). Numerous studies were done on the effect of both AT₁ and AT₂ receptor antagonists on various responses to centrally administered angiotensin II (1, 8). With regard to water drinking, salt appetite, vasopressin secretion and pressor responses to intracerebroventricular (i.c.v.) administration of angiotensin II, central administration of the AT₁ but not AT₂ antagonists, AT₁ receptor antibody or antisense oligonucleotide blocks these responses (8). AT₁ receptors may mediate anxiety (9), while either AT₁ receptors or AT₂ receptors (10) were reported to enhance cognition without influencing anxiety in laboratory animals. In contrast, Shepherd et al. (11) reported that both AT₁ (losartan) and AT₂ (PD123,177) receptor antagonists were without significant effect in animal models of anxiety and memory. Thus, studies on the central function of AT₂ receptors yielded diverse and often conflicting results.

AT₂ receptor-deficient mouse strains (12)

The disrupted mouse AT₂ gene contains the entire coding region in the third exon in embryonic stem (ES) E14-1 cells and generated chimaeric mice. Because the AT₂ gene is on the X chromosome and ES cells are XY, all F₁ male mice were wild-type and all F₁ females were heterozygous for the mutation. The heterozygous females, which appeared to be healthy, were mated with F₁ males, giving rise to an F₂ generation that included hemizygous males and heterozygous females. Homozygous females were obtained in the F₃ generation by mating these F₂ mice.

The hemizygous males and homozygous females appeared to be healthy and were fertile. The total RNA from hemizygous males was analyzed by northern blotting to confirm the absence of the wild-type AT₂ transcript in fetuses and in adult brains, both of which normally express AT₂ at high levels. Levels of AT₁ mRNA were not affected in kidneys or brain (12).

Mutant mice lacking the gene encoding the AT₂ receptor exhibited an increase in blood pressure, increased sensitivity to the pressor action of angiotensin II, and attenuated exploratory behavior (12, 13).

Behavioral properties in AT₂-deficient mice

Anxiety-like behavior: One of the currently accepted anxiety tests is based on exploratory behavior, either implicitly or explicitly, used as an index of anxiety (14, 15). AT₂-deficient mice exhibited a selective reduction of exploratory but not locomotor activities (12, 16). It can be difficult to assess an animal's exploratory behavior, independently of other behavior such as locomotion (17).

The defecation rate in rodents is considered to be an index of their "emotionality" (18), and reduction in defecation rate following diazepam administration, a drug widely prescribed for treatment of anxiety and related disorders, was seen to be consistent with its anxiolytic action (19). The number of defecations in AT₂-deficient mice increased significantly compared with wild-type mice (16). Therefore, AT₂-deficient mice showed an exaggerated response to a novel environment, findings consistent with an increased anxiogenic behavioral state.

The stress-induced hyperthermia paradigm in mice allows for screening of putative anxiolytic drugs (20). AT₂-deficient mice and wild-type mice both showed typical circadian rhythms with respect to their body temperature and physical activity (21). During the latter half of the dark period, AT₂-deficient mice exhibited a lower body temperature than did wild-type mice (21). In comparison with wild-type mice, however, AT₂-deficient mice had a significantly greater hyperthermia during cage-switch stress (21).

The elevated plus-maze task and the light/dark exploration task were used as validated animal models of anxiety that are predictive of drug responses in humans (15). These tests, which are based on the natural aversion of rodents to open or light spaces, are sensitive to the effects of both anxiolytic and anxiogenic agents in rodents (15). As in the novel environment, clear group differences were observed between AT₂-deficient mice and wild-type mice, using these test paradigms. The time spent in the light area and that in open arms area was significantly reduced in AT₂-deficient mice compared with findings in wild-type mice in the light/dark exploration task and the elevated plus-maze task, respectively (16). Reduction in time spent

in the light area in the light/dark exploration task was reversed by anxiolytic diazepam (22). In addition, the hexobarbital-induced sleeping time and duration of immobility in the behavioral despair test did not differ significantly between AT₂-deficient and wild-type mice (16), suggesting that AT₂-deficient mice did not exhibit non-specific central nervous system depression and depressant-like behavior. These findings strongly suggest that AT₂-deficient mice specifically showed an anxiety-like state.

A considerable body of evidence supports the view that anxiety disorders may be, at least in part, related to excessive norepinephrine (NE) activity (23) and functional interaction between angiotensin II and the neuronal noradrenergic system may occur (2, 24). Moreover, the amygdala appears to play a crucial role in conditioned fear and probably anxiety (24). Both angiotensin II (AT₁ and/or AT₂) and NE receptors are located in the amygdala (1, 6, 7). Among modulators of NE neurotransmission, the anxiety-like behavior seen in AT₂-deficient mice was suppressed only by the α_1 adrenoceptor antagonist prazosin (16). AT₂-deficient mice showed downregulation of the α_1 adrenoceptor in the amygdala (16), perhaps induced by an excessive NE release as a result of the absence of AT₂ receptors, an event that would result in activation

of the cerebral α_1 adrenoceptor. Thus, the anxiety-like behavior observed in AT₂-deficient mice may be mediated by interaction between the α_1 adrenoceptor and angiotensin II (AT₁ and/or AT₂) receptors in the amygdala (Fig. 1). Stress-induced anxiety-like behavior may reflect excessive NE activity via central the α_1 adrenoceptor (14).

There is much evidence that direct activation of the locus coeruleus, which may reflect dysfunction of inhibitory α_2 adrenoceptor, can produce signs of arousal in laboratory animals and which strongly resembles those seen in human anxiety states (23). However, the absence of AT₂ receptors in the locus coeruleus was noted in histochemical studies of both AT₂-deficient and wild-type mice (7). Neither the α_2 adrenoceptor antagonist yohimbine nor the α_2 adrenoceptor agonist clonidine attenuated the anxiety-like behavior in AT₂-deficient mice (16).

Corticotropin-releasing factor (CRF), a 41 amino acid residue peptide that regulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary (25), has been shown to mediate stress-induced changes in the autonomic nervous system, neuroendocrine functions and behavior (22, 25, 26). The i.c.v. administration of CRF to laboratory animals produces anxiety-like behav-

Fig. 1. Schema of the anxiety-like behavior in AT₂-deficient mice. The anxiety-like behavior observed in AT₂-deficient mice may be mediated by interaction between the α_1 adrenoceptor and angiotensin II (AT₁ and/or AT₂) receptors in the amygdala. The AT₁-receptor antagonist losartan (9) and the angiotensin I converting enzyme (ACE) inhibitor captopril (27) produced anxiolytic activity, findings which suggest that the anxiolytic action may reflect the inhibition of AT₁ receptors. Thus, one possible explanation for the anxiety-like behavior in AT₂-deficient mice may be activation of AT₁ receptors as AT₂ receptors are absent in the amygdala. AII: angiotensin II, AT₁: AT₁ receptor, AT₂: AT₂ receptor, NE: norepinephrine.

ior, such as altered locomotor activity (25) and an increased anxiety-like behavior (22). The CRF antagonist α -helical CRF₉₋₄₁ had no effect on the reduced time spent in the light area in AT₂-deficient mice (16). The number of [¹²⁵I]-CRF binding sites in the amygdala was not significantly attenuated in AT₂-deficient mice, compared with wild-type mice (16). In addition, plasma ACTH and corticosterone concentrations did not differ significantly between AT₂-deficient and wild-type mice, thus indicating no endocrine abnormalities involving the hypothalamic-pituitary-adrenal axis (16). The anxiety-like behavior seen in AT₂-deficient mice may be related to abnormalities in the central noradrenergic neuronal system but not to the CRF neuronal system.

Biochemical and electrophysiological evidence suggests that AT₁ and AT₂ receptors have antagonistic effects (2). Activation of AT₁ and AT₂ receptors elicited stimulatory and inhibitory effects, respectively, on mitogen-activated protein kinase in neurons (2–5). Within the brain, blockade of periventricular AT₂ receptors potentiated AT₁-receptor-mediated stimulation of drinking and vasopressin secretion (1, 8). In catecholaminergic neurons, angiotensin II elicited an AT₁-receptor-mediated decrease in the delayed rectifier K⁺ current (I_K) followed by an AT₂-receptor-mediated increase in I_K (2). These studies collectively suggest that functional interactions between the two receptor subtypes have a key role in angiotensin II-induced neuromodulatory actions in neurons. The AT₁-receptor antagonist losartan (9) and an angiotensin I converting enzyme (ACE) inhibitor captopril (27), used to abolish endogenous angiotensin II, produced anxiolytic activity, a finding which suggests that the anxiolytic action may reflect inhibition of AT₁ receptors. Thus, one possible explanation for the anxiety-like behavior in AT₂-deficient mice may be activation of AT₁ receptors as AT₂ receptors are absent in the brain (Fig. 1). In angiotensin II-induced blood pressure responses in AT₂-deficient mice, AT₂ antagonized the AT₁-mediated pressor action of angiotensin II (12, 13).

Learning and memory: Angiotensin may also be involved in learning and memory (1). AT₂ receptors are expressed in areas related to learning and control of motor activity (1, 6). The ACE inhibitor captopril (28), the AT₁-receptor antagonist losartan and the AT₂-receptor antagonist PD123,177 (11) have a cognitive enhancing action in rodents. However, the step through latency in AT₂-deficient mice did not differ from that of wild-type mice in the passive avoidance task (S. Okuyama et al., unpublished observation). Thus, the AT₂ receptors may not be directly involved in mediating cognitive function.

Acute cortical injury: The transient upregulation of AT₂ receptors mRNA occurs in the cortex and hippocampus after ischemic injury and these changes may be re-

lated to molecular events that lead to delayed neuronal cell death (29). Angiotensin II inhibits *N*-methyl-D-aspartate (NMDA)-mediated nitric oxide (NO) and cGMP production through a mechanism involving an AT₂-receptor subtype (30). No significant differences were found between AT₂-deficient and wild-type cerebra in the extent of edema after injury related to cold (S. Okuyama et al., unpublished observation). Cold-induced brain injury has been well established as a model of vasogenic brain edema (31). As the accumulation of glutamate was observed in interstitial fluids after vasogenic edema, it has been suggested that glutamate neurotoxicity may be involved in the tissue damage caused by trauma (31). Thus, AT₂ receptors may not be related to the glutamate neurotoxicity seen after traumatic cerebral injury.

Conclusion

It is generally accepted that there are two major subtypes of angiotensin II receptors. AT₁ receptors in the hypothalamus and brain stem mediate the regulatory effects of angiotensin II on many biological events including altered baroreflex function, blood pressure, drinking and vasopressin release (1–4). These effects of angiotensin II involve activation of central catecholaminergic pathways (1–4). In contrast, the physiological functions associated with AT₂ receptors have not been fully delineated (1–4). Mice generated with a targeted disruption of the AT₂ receptors show attenuation of exploratory behavior, in addition to enhanced pressor responses to angiotensin II (12, 13). The absence of AT₂ receptors induces anxiety-like behavior that may result, in part, in neuromodulation of central α_1 adrenoceptors via AT₁ receptors (16).

For patients on ACE inhibitors for control of blood pressure, anxiolytic effects become apparent when the blood pressure is lowered (32). (mRen-2)27 transgenic rats, a model of hypertension dependent on increased expression of the renin angiotensin system, showed a greater anxiogenic profile than did control rats (33). Administration of captopril to (mRen-2)27 transgenic rats reversed the anxiety-like behavior (33). Blood pressure in AT₂-deficient mice is higher than that of wild-type mice (12, 13). The anxiety-like behavior seen in AT₂-deficient mice was suppressed by captopril (16). Thus, AT₂-deficient mice can serve as a model reflecting the human clinical situation and may be appropriate for studies on effects of anti-hypertensive drugs in patients with elevated blood pressure.

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