Divergent functions of angiotensin II receptor isoforms in the brain

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The renin-angiotensin system (RAS) plays a critical role in cardiovascular and fluid homeostasis. The major biologically active peptide of the RAS is angiotensin II, which acts through G protein–coupled receptors of two pharmacological classes, AT1 and AT2. AT1 receptors, expressed in brain and peripheral tissues, mediate most classically recognized actions of the RAS, including blood pressure homeostasis and regulation of drinking and water balance. In rodents, two highly homologous AT1 receptor isoforms, termed AT1A and AT1B receptors, are expressed at different levels in major forebrain cardiovascular and fluid regulatory centers, with AT1A expression generally exceeding AT1B expression, but the relative contributions of these receptor subtypes to central angiotensin II responses are not known. We used gene targeting in combination with a unique system for maintaining catheters in the cerebral ventricles of conscious mice to test whether there are differential roles for AT1A and AT1B receptors in responses elicited by angiotensin II in the brain.

Here we show that the blood pressure increase elicited by centrally administered angiotensin II can be selectively ascribed to the AT1A receptor. However, the drinking response requires the presence of AT1B receptors. To our knowledge, this is the first demonstration of a primary and nonredundant physiological function for AT1B receptors.


Introduction

The renin-angiotensin system (RAS) plays a critical role in cardiovascular and fluid homeostasis. In addition to its importance in regulating normal physiology, enhanced activity or dysregulation of the RAS can lead to maladaptive responses and significant cardiovascular disease. The RAS is an ancient system that is present in lower organisms including amphibians, and many of its features have been conserved across evolution. The major biologically active peptide of the RAS is angiotensin II, and its major actions are mediated by G protein–coupled angiotensin receptors.

These receptors can be divided into two pharmacological classes, AT1 and AT2 (1). Most of the classically recognized actions of the RAS in fluid and blood pressure homeostasis are mediated by AT1 receptors. AT1 receptors are expressed in brain and peripheral tissues. The central nervous system (CNS) receptors play a key role in blood pressure regulation and in regulation of drinking and water balance (2).

The discovery of two AT1 receptor isoforms in rats and mice, termed AT1A and AT1B receptors, uncovered an additional level of complexity of the system (3–5). These receptors are highly homologous with indistinguishable binding profiles, but are products of distinct genes (Agtr1a and Agtr1b) that are differentially expressed and regulated (6–8). Although a single report has suggested the existence of AT1B receptors in humans (9), most investigators believe that humans have only a single AT1 receptor gene. Despite this apparent genetic difference, the physiological actions of AT1 receptors in humans and rodents are virtually identical. Because pharmacological AT1 receptor antagonists cause equivalent inhibition of both AT1 isoforms, the relative physiological roles of AT1A and AT1B and their relationship to human AT1 receptor functions have been difficult to identify. Recent gene-targeting experiments have clarified the relative role of the AT1A and AT1B receptors in the periphery, demonstrating a predominant role for AT1A receptors in regulation of vascular tone (10, 11). In the brain as in the periphery, expression of AT1A exceeds that of AT1B, with the exception of the pituitary gland, where AT1B expression predominates (12–14). AT1A expression is especially prominent in major forebrain cardiovascular and fluid regulatory centers (12). AT1B expression is highest in the anterior pituitary (12–14). However, the relative contributions of these AT1 receptor subtypes to central angiotensin II responses are not known. We used gene targeting in combination with a unique system for maintaining catheters in the cerebral ventricles of conscious mice to test whether there are differential roles for AT1A and AT1B receptors in responses elicited by angiotensin II in the CNS.
Methods

Animals. Mice with targeted deletions of the AT1A (Agtr1a−/−) or AT1B (Agtr1b−/−) receptors were generated using homologous recombination in embryonic stem cells as described previously (10, 15, 16). Knockout mice were maintained on the original 129Sv/C57Bl hybrid background, and wild-type controls were littersmates of each of the respective knockout models. All mice were fed standard mouse chow (LM-485; Teklad Premier Laboratory Diets) and water ad libitum. Care for the mice used in the experiments exceeded the standards set forth by the National Institutes of Health in their guidelines for the care and use of experimental animals. All procedures were approved by the University Animal Care and Use Committee at the University of Iowa.

Surgical procedures. Mice were implanted with intracerebroventricular (ICV) cannulae and carotid arterial catheters for brain microinjection of drugs and for direct measurement of pulsatile and mean arterial pressure (MAP), respectively, as described by us in detail previously (17). All experiments were performed in conscious, freely moving mice. Animals were allowed 4 days’ recovery before in vivo testing for proper ICV cannula placement by intraventricular injection of the muscarinic agonist carbachol (50 ng). Carbachol elicits a characteristic pressor and bradycardic response in rodents when injected intraventricularly (17). Mice that did not respond to carbachol were not used in the studies (n = 2).

Experimental protocols. Two days after in vivo testing of cannula placement with carbachol, the blood pressure effects of ICV angiotensin II (50, 100, and 200 ng) were examined in the home cage following the protocol described previously (10). Mice were allowed 60 minutes’ adaptation after connection to recording equipment and insertion of the ICV injector. The volume of each injection was 300 nL. Thirty minutes was allowed before administration of the next dose. The number of drinking episodes was also recorded during the 30 minutes after each dose of angiotensin II. A single drinking episode was defined as the mouse approaching and then drinking from the water tube. Removal of the tongue from the sipper tube defined the end of an episode. This approach was taken for the 30 minutes after each dose of angiotensin II. A single drinking episode was defined as the mouse approaching and then drinking from the water tube. Removal of the tongue from the sipper tube defined the end of an episode. This approach was taken because of the difficulty in accurately measuring small quantities of water acutely consumed by mice. The miniature glass burette drinking tube was placed on the home cage before surgery and remained throughout the experiment.

Separate experiments were performed to assess the role of non-AT1 receptors in the dipsogenic action of angiotensin II. Studies were carried out as already described here, with mice being allowed 60 minutes’ adaptation before ICV injection of the largest dose of angiotensin II (200 ng, 300 nL). The number of drinking episodes were recorded for 30 minutes after injection. The ICV injector was then removed and replaced with one for microinjection of the selective AT1 receptor antagonist losartan (10 μg, 500 nL). Ten minutes later, the ICV angiotensin II injection (200 ng, 300 nL) was repeated and drinking behavior was recorded for 30 minutes. The dose and time course for losartan blockade of AT1 receptors has been established in previous studies (17).

Results

Six days after cannulation of the lateral brain ventricles and the carotid artery in mice with a gene targeted deletion of the AT1A (Agtr1a−/−) or the AT1B (Agtr1b−/−) receptor or in wild-type littersmates, we measured intra-arterial pressures in conscious freely moving mice. MAPs were similar in wild-type (114 ± mmHg) and Agtr1b−/− mice (111 ± mmHg; P > 0.05). However, basal blood pressure in the Agtr1a−/− mice (99 ± mmHg) was significantly lower than that of wild-type controls (P < 0.01), confirming a role for the AT1A receptor in normal maintenance of blood pressure (10).

The vasopressor effect of central angiotensin II has been suggested to be a critical component in both chronic blood pressure regulation and the pathogenesis of hypertension (2). As shown in Figure 1, ICV injection of angiotensin II caused an acute, dose-dependent increase in blood pressure in wild-type mice. This response is identical to that seen in other species (2). The vascular effects of ICV injection of angiotensin II in Agtr1b−/− mice was virtually identical to those seen in controls (Figure 1), suggesting that the AT1B receptor does not have an essential contribution to this response. By contrast, ICV injection of angiotensin II induced only a very minor increase in blood pressure in Agtr1a−/− mice (Figure 1). Thus, the CNS actions of angiotensin II to increase blood pressure are primarily mediated by AT1A receptors.

Along with its effects to raise blood pressure, angiotensin II that is generated in the CNS has potent dipsogenic actions. These effects are believed to stim-
ulate drinking behavior during states of volume depletion (2). As shown in Figure 2, ICV administration of angiotensin II to wild-type mice elicited a robust drinking response that is similar to that reported in many other species (2). We next examined the effects of AT1 receptor gene mutations on the dipsogenic effects of angiotensin II. Compared to wild-type mice, there was a small but statistically significant ($P < 0.05$) reduction in the drinking response to the highest doses of angiotensin II in the AT1A receptor-deficient mice. By contrast, in the AT1B-deficient mice, drinking evoked by central angiotensin II was markedly diminished compared with either wild-type ($P < 0.001$) or $\text{Agtr1a}^{-/-}$ animals ($P < 0.01$). These data indicate that AT1B receptors are the primary mediators of the central dipsogenic actions of angiotensin II, whereas AT1A receptors make only a modest contribution to this response. This is the first demonstration of a primary and nonredundant physiological function of AT1B receptors.

Most previous studies have suggested that AT1 receptors are responsible for the entire dipsogenic action of angiotensin II. However, a few studies have suggested roles for AT2 and other non-AT1 receptors in these responses (e.g., ref. 18). To test for a contribution of non-AT1 receptors to this response in wild-type mice, and to evaluate whether these responses might be more pronounced in AT1A-mutant animals, we examined the effects of losartan, a specific AT1 receptor antagonist with equivalent potency for AT1A and AT1B receptors. As shown in Figure 3, the AT1 receptor antagonist completely blocked angiotensin II-induced drinking in wild-type and AT1A receptor-deficient mice. Likewise, the small residual response in the $\text{Agtr1b}^{-/-}$ mice was also abrogated by losartan. These data indicate that in mice, non-AT1 receptors play no significant role in the stimulation of drinking by angiotensin II.

Discussion

Our data show that AT1A and AT1B receptors have distinct functions in mediating the central actions of angiotensin II. The blood pressure effects are produced exclusively by activation of AT1A receptors, whereas AT1B receptors are responsible for a major portion of the drinking response. The mechanism of this functional divergence between these two highly homologous receptors in the brain remains to be determined. Because the ligand specificities and signal-effector coupling are virtually identical for the two receptors, differential localization and/or unique neural circuitry for AT1A and AT1B in the brain may explain these differential functions. Although the coding regions for these receptors are nearly identical, their upstream sequences, including regulatory sequences, have little identity. Moreover, differential regulation of expression of the two receptors in peripheral tissues has been reported previously (4–8).

The physiological functions of the RAS are very similar in rodents and humans. Although there is a report suggesting that AT1B receptors might also exist in humans (9), to our knowledge this has not been independently confirmed. Our findings raise the question of whether the separate functions mediated by brain AT1A and AT1B receptors in lower mammals have been merged and are provided by a single receptor in humans. If so, it is interesting that the global actions of AT1 receptors in the CNS are very similar in humans and rodents. Alternatively, there may be a yet unidentified angiotensin receptor in human brain that is important in blood pressure and/or body fluid regulation. Nonetheless, this divergence of function between AT1A and AT1B receptors in brain provides a scenario to identify separately the more distal signals involved in vasomotor and drinking responses stimulated by angiotensin II.
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