Decrease in Hypothalamic Gamma Adducin in Rat Models of Hypertension

Hong Yang, Phyllis Y. Reaves, Michael J. Katovich, Mohan K. Raizada

Abstract—We have previously shown that a decrease in hypothalamic gamma adducin (γ-adducin) is associated with hypertension in the spontaneously hypertensive rat (SHR). In view of many inherent issues with SHR, our objective in the present study was to provide proof of this concept with the use of 2 nongenetic rat models of hypertension. Subcutaneous angiotensin II (Ang II) infusion for 2 weeks (55 ng/kg per day) resulted in an increase in blood pressure (BP) of 18 mm Hg. This was associated with a 70% decrease in hypothalamic γ-adducin. Concomitant administration of losartan attenuated the development of hypertension and a decrease in γ-adducin. Deoxycorticosterone acetate salt-induced hypertension also caused a 70% decrease in hypothalamic γ-adducin. Finally, neuronal cultures from neonatal rat brains were incubated with 100 nmol/L Ang II for 4 hours to mimic the in vivo Ang II infusion rat model. This chronic incubation with Ang II resulted in a 60% decrease in the neuronal γ-adducin. Taken together, these observations strengthen our hypothesis that a decrease in hypothalamic γ-adducin is linked to hypertension.

(Hypertension. 2004;43[part 2]:324-328.)

Key Words: angiotensin ■ hypertension ■ brain ■ hypothalamus

The central nervous system (CNS) plays a critical role in the control of many cardiovascular functions by regulating such physiological mechanisms as sympathetic nerve activity, vasopressin release, and baroreceptor reflexes. Dysregulation of one or more of these physiological pathways is associated with the development and maintenance of hypertension. For example, an increased sympathetic nerve activity that is linked to animal models and human hypertension has been identified as one of the key physiological mechanisms that is altered in neurogenic hypertension. Despite abundant evidence supporting dysregulated CNS function, the therapeutic potential of these findings in the control and treatment of neurogenic hypertension has not been fully explored. In part, this may be because of the fact that little is known about the cellular and molecular basis of this alteration in hypertension.

Our research group has been involved in filling this important gap in knowledge by identifying and characterizing genes and signaling pathways that are uniquely altered in the brain of the spontaneously hypertensive rat (SHR). These studies have demonstrated that the expression of gamma-adducin (γ-adducin), a cytosolic protein, is reduced in the hypothalamic-brainstem areas of the SHR. γ-Adducin belongs to a family of cytosolic proteins whose dimerization with an α isoform plays a critical role in the control of cytoskeletal-mediated cellular processes. For example, α/γ heterodimers of adducin have been demonstrated to regulate protein kinases involved in vesicular trafficking, calcium mobilization, and transmitter release. In addition, its interaction with Na+/K+ ATPase regulates the pump activity and thus regulates neuronal activity. These observations, together with our data on γ-adducin expression, lead us to propose that a decrease in the expression of this protein is one of the key early molecular events that could be linked to the development and maintenance of neurogenic hypertension.

This hypothesis is consistent with association studies indicating a linkage in α, β, and γ adducin polymorphisms with hypertension. Thus, our objective in the present study was to validate our hypothesis that a decrease in brain γ-adducin is linked to hypertension by using two nongenetic rat models of hypertension that are known to have a neurogenic component. This is critical in recognizing the therapeutic potential of this linkage in view of many inherent issues related to the genetic background of the SHR and its normotensive control. Our studies demonstrate that hypertension-induced by either Ang II infusion or deoxycorticosterone acetate (DOCA)/salt treatment in normotensive rats is linked to a decrease in the levels of hypothalamic γ-adducin.

Methods

Animals

Twelve-week-old male Sprague-Dawley (SD) rats were used for all in vivo studies. They were obtained from Charles River Laboratories...
Subcutaneous Ang II infusion was performed for 2 weeks to induce hypertension in male SD rats. Twelve SD rats were fitted with ALZET 2004 minipumps (Durect Corp, Cupertino, Calif) as described previously. Minipumps were filled with either physiological saline (n=6) or Ang II (n=6) in such a way that pumps delivered 0.25 μL/h for 2 weeks for a final dose of Ang II of 55 ng/kg per day. In a subgroup of rats, losartan was administered in the drinking water during Ang II infusion. This dose of losartan was effective in normalizing Ang II-induced blood pressure (BP).

Hypertension was also induced in the SD rats by administration of DOCA (Sigma, St Louis, Mo) compounded into 25 mg pellets. Animals (n=6) were anesthetized with a rodent cocktail containing ketamine (1.5 mL of a 100 mg/mL solution), xylazine (1.5 mL of 20 mg/mL aprotinin), and 0.7 mL/kg. One DOCA pellet was inserted subcutaneously between the shoulder blades, during which time the left kidney was also removed and the right kidney was isolated. Animals underwent a sham surgical protocol, and a group of SD rats (n=6) underwent a sham surgical protocol, and a group of SD rats (n=6) were anesthetized with a rodent cocktail containing ketamine (1.5 mL of a 100 mg/mL solution), xylazine (1.5 mL of 20 mg/mL aprotinin), and 0.7 mL/kg. One DOCA pellet was inserted subcutaneously between the shoulder blades, during which time the left kidney was also removed and the right kidney was isolated. Animals underwent a sham surgical protocol, and a group of SD rats (n=6) were anesthetized with a rodent cocktail containing ketamine (1.5 mL of a 100 mg/mL solution), xylazine (1.5 mL of 20 mg/mL aprotinin), and 0.7 mL/kg. One DOCA pellet was inserted subcutaneously between the shoulder blades, during which time the left kidney was also removed and the right kidney was isolated. 

Neuronal Cultures

Neuronal cultures were prepared from the Wistar Kyoto strain of normotensive rat brains exactly as described previously. Dissociated brain cells (3 × 10⁶ cell/35-mm-diameter dish) were plated onto poly l-lysine precoated culture dishes in Dulbecco modified eagles medium containing 10% horse serum and enriched for neuronal cells as described previously. Ten-day-old culture, which contained ~90% neurons and ~10% glial cells, was used for all the experiments.

Western Blotting for γ-Adducin

Hypothalamic and brainstem tissue blocks or neuronal cultures were homogenized in the lysis buffer (50 mmol/L Tris, pH7.4, 150mmol/L NaCl, 10% glycerol, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton x-100, 2 mmol/L EDTA, 1 mmol/L phyllethylsulfonyl fluoride, 10 μg/mL aprotinin, and 2 μg/mL leupeptin). The lysates were centrifuged at 5000g and 20 μg of proteins from the supernatants were separated by SDS-PAGE. Proteins were transferred to a nitrocellulose membrane and used for Western blotting with the use of γ-adducin–specific antibody as follows; membranes were incubated with a primary antibody against γ-adducin at 4°C overnight, followed by a secondary antibody for 2 hours. Membranes were then developed with an ECL kit.

Results

γ-Adducin in the Brains of Hypertensive Rats

Two nongenetic rat models of hypertension have been used to compare the levels of γ-adducin and to provide proof of the concept that a decrease in this cytosolic protein is linked to hypertension. Ang II infusion for 2 weeks in SD rats resulted in the development of hypertension. Mean BP was 128±2 mm Hg (n=6) in Ang II-infused rats, whereas it was 110±4 mm Hg (n=6) in saline-infused rats (Figure 1A). We have previously reported a 60% increase in the heart weight-to-body weight ratio after a similar 2-week Ang II infusion. Establishment of this hypertensive state in the current study was associated with a 70% decrease in hypothalamic γ-adducin levels.
Effect of Ang II on γ-Adducin in Neuronal Cultures

Neuronal cells in primary culture have been used to demonstrate those cellular and molecular changes in the brain that are linked to a hypertensive state from those that are induced by high BP.2,19 Because these cultures are prepared from 1-day-old prehypertensive rats, changes induced as a result of high BP are easily eliminated in the culture.2,19 Neuronal cultures were treated on a long-term basis with 100 nmol/L Ang II for 4 hours. This resulted in a 70% decrease in the levels of γ-adducin (Figure 3). Co-incubation with 1 μmol/L losartan completely attenuated the Ang II-induced decrease in this protein. Losartan alone did not have significant effect on the basal levels of neuronal γ-adducin. Similarly, 1 μmol/L PD123319, an AT2 receptor antagonist, showed little effect on Ang II-induced decrease in γ-adducin. These data indicated that Ang II-induced decrease in γ-adducin is mediated by activation of AT1 receptor subtype.

Discussion

The most significant conclusion of the present study is that it provides a conceptual support for our previous hypothesis that a decreased expression of γ-adducin in the brain is linked to hypertension.6 We believe that it was critical to validate this concept with the use of nongenetic rat models of hypertension. This gene appears to provide a novel target for the central control of hypertension.

Ang II infusion and DOCA-salt rats provide ideal models for validation, because the CNS also plays a critical role in the development and establishment of hypertension in these models, as has been shown for the SHR and renin transgenic (Ren-2) rats.23–26 Consistent with our hypothesis, we found that induction of a hypertensive state was associated with a decrease in the levels of hypothalamic γ-adducin. However, in contrast to genetic models, γ-adducin levels in the brainstem did not change in both nongenetic rat models. The physiological relevance of this difference remains speculative at the present time. It may be that the decrease in γ-adducin in the hypothalamic nuclei is an initial event directing the dysregulation of the CNS mechanisms leading to hyperten-

Figure 2. Effect of DOCA-salt-induced hypertension on hypothalamic γ-adducin. A, Effect of BP. Six control (C) and 6 DOCA-salt (DOCA) rats were prepared essentially as described in Methods. Indirect BP were measured 4 weeks after treatment. Data are mean±SE; n = 6; *P<0.05. B and C, Western blotting of hypothalamic γ-adducin. Hypothalamic areas from control (C) and DOCA-salt (DOCA) rats were dissected and subjected to Western blotting as described in Methods. B, Representative autoradiogram. C, Quantitation of γ-adducin bands. *Significantly different from control (P<0.01; n = 3). D, Levels of γ-adducin in the brainstem of DOCA-salt rats. Brainstems from each group of rats were dissected and proteins subjected to quantitation of γ-adducin after Western blotting. Data are derived from 3 animals.

Figure 3. Effect of Ang II on γ-adducin levels in WKY-rat brain neuronal cultures. Neuronal cells from WKY rats were established in primary culture as described in Methods. Cultures were treated with PBS (C) 100 nmol/L Ang II (Ang II), or 100 nmol/L Ang II containing 1 μmol/L losartan (Los), 1 μmol/L losartan alone, or 100 nmol/L Ang II with 1 μmol/L PD123319 (Ang II + PD) for 4 hours at 37°C. Proteins were isolated and subjected to Western blotting for γ-adducin as described in Methods. A, Representative autoradiogram. B, Quantitation of γ-adducin band.
to be key in the expression of hypertension. Further studies will be needed to identify the specific hypothalamic nuclei responsible for this decrease for selective gene targeting.

It has been well established that the brain renin–angiotensin system plays a critical role in the neural control of BP and that its dysregulation is one of the mechanisms that contribute to hypertension.2,7 These observations coupled with our data beg the following question: Is γ-adducin a relay gene that translates dysregulated renin–angiotensin system into a hypertensive state? Our data suggest this possibility: (1) the brain renin–angiotensin system is shown to contribute to hypertension in all four rat models;2,23–27 (2) neuronal cultures from prehypertensive SHR brain exhibit a decrease in γ-adducin expression, an increase in AT1 receptors, and an increase in the neuromodulatory actions of Ang II;6 (3) long-term exposure of neuronal cultures to Ang II causes a decrease in γ-adducin; and (4) attenuation of hypertension by losartan prevents a decrease in γ-adducin in the hypothalamus.

Finally, we believe that the ability of γ-adducin to regulate cardiovascular functions is imprinted in the multifunctional properties of this protein.7 Thus, it is tempting to suggest that a following sequence of events leads to a hypertensive state. A decrease in γ-adducin would reduce its overall cellular activity by decreasing its interactions with both α and β subunits of adducins.7 It is known that heterodimerization of adducins is needed for their activity.7 A reduction in its activity would be translated into alterations in the activation of key protein kinases such as MARCKS and a subsequent modulation of the neuronal cytoskeleton. These would, in turn, modulate vesicular transport and increase the release of neurotransmitters/neuromodulators, thus stimulating neuronal activity. Increased neuronal activity would be a key cellular change that would relay adducin to signal downstream in the form of an increased sympathetic nerve activity and other dysregulated cardiovascular functions in hypertension. Some evidence to support this cascade of events are (1) levels of adducins regulate cellular and cytoskeletal activities including Ca2+ influx;7 (2) a decrease in adducin is demonstrated to stimulate Na+/K+ pump activity, which would be key in the stimulation of neuronal activity;5,7 (3) MARCKS phosphorylation has been shown to stimulate vesicular transport in neurons;28 and (4) γ-adducin directly influences neuronal activity.7

In conclusion, our studies support that a decrease in the expression of hypothalamic γ-adducin is linked to neurogenic hypertension. However, functional studies are needed to establish a cause-and-effect relationship between γ-adducin decreases and development of hypertension. This leaves open the possibility that γ-adducin may be a marker rather than a cause of neurogenic hypertension.

Perspectives
Adducin is a cytosolic protein with multifunctional properties. They include vesicular transport, Ca2+ mobilization, cytoskeletal reorganization, Na+/K+ ATPase regulation, and neuronal activity. Thus, adducin is ideally poised to be a key player in the central control of cardiovascular functions. This suggestion is supported by our earlier data that a decrease in the brain γ-adducin subtype is associated with hypertension in genetic rat models. The significance of our present findings is that it provides conceptual support for our hypothesis. Hypothalamic decrease of this gene could be responsible for hypertension in genetic and nongenetic models. This puts us in a unique position to determine if normalization of γ-adducin expression by gene transfer/manipulation techniques would prevent neurogenic hypertension on a long-term basis.

Acknowledgments
We thank Nichole Herring for her assistance in the preparation of this manuscript and Fan Lin for the preparation of neuronal cultures. This work was supported by National Institutes of Health grants HL33610 and HL56921.

References
17. Baylis C, Qu C, Engels K. Comparison of L-type and mixed L- and T-type calcium channel blockers on kidney injury caused by...


