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.

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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.1,2 Dysregulation of one or more of these physiological pathways is associated with the development and maintenance of hypertension.2-4 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.2 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).5,5 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.6 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.7 For example, α/γ heterodimers of adducin have been demonstrated to regulate protein kinases involved in vesicular trafficking, calcium mobilization, and transmitter release.7 In addition, its interaction with Na+/K+ ATPase regulates the pump activity and thus regulates neuronal activity.8,9 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.6 This hypothesis is consistent with association studies indicating a linkage in α, β, and γ adducin polymorphisms with hypertension.10-12 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.13,14 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.15 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).16

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 solution), and acepromazine (0.5 mL of 10 mg/mL solution). This rodent cocktail was injected intramuscularly at a dose of 0.5 to 0.7 mL/kg. One DOCA pellet was inserted subcutaneously between the shoulder blades, during which time the left kidney was also removed as described.17,18 After recovery, rats were maintained on a 1% saline drinking water in place of tap water ad libitum. A control group of SD rats (n=6) underwent a sham surgical protocol, and others.21,22 Both 120-kDa and 105-kDa bands were used to represent γ-adducin levels for normalization with tubulin. In preliminary experiments, we had determined that there was a linear relationship between total proteins loaded on the gel (5 to 50 μg protein) with the amount of γ-adducin and tubulin immunoreactivity. Thus, all our subsequent experiments were performed with 20 μg lysate proteins.

**Experimental Groups and Statistical Analysis**

Six animals per group and 5 culture dishes per data point were used. Neurons from each culture dish originated by pooling cells from 2 to 4 animals. Images from autoradiographs were captured with GS700 densitometer (Bio-Rad Laboratories). The immunoreactivity bands were quantitated using Quantity One Analysis software (Bio-Rad Laboratories) and corrected for equal sample loading by normalizing with standard protein. Data are presented as mean±SE. Comparison between control and experimental groups were made using the Student t test with statistical software.

**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.15 Establishment of this hypertensive state in the current study was associated with a 70% decrease in hypothalamic γ-adducin levels. Neurons from each culture dish originated by pooling cells from 2 to 4 animals. Images from autoradiographs were captured with GS700 densitometer (Bio-Rad Laboratories). The immunoreactivity bands were quantitated using Quantity One Analysis software (Bio-Rad Laboratories) and corrected for equal sample loading by normalizing with standard protein. Data are presented as mean±SE. Comparison between control and experimental groups were made using the Student t test with statistical software.
(Figure 1B and C). Administration of losartan attenuated the development of high BP. This attenuation prevented a decrease in hypothalamic \( \gamma \)-adducin levels (Figure 1C). In contrast to the hypothalamus, levels of \( \gamma \)-adducin in the brainstem of Ang II-induced hypertensive rats did not decrease and thus were not different from control rats (Figure 1D and E).

Next, we used a DOCA-salt rat model of hypertension. This treatment resulted in an increase in BP of 43 mm Hg (116±4 mm Hg in control rats versus 159±8 mm Hg in DOCA-salt rats; \( n=6 \)) (Figure 2A). The DOCA-salt-induced hypertension was associated with a 70% decrease in \( \gamma \)-adducin in the hypothalamus (Figure 2B and C). In contrast, brainstem \( \gamma \)-adducin levels did not change between the two groups (Figure 2D).

**Effect of Ang II on \( \gamma \)-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. 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. Neuronal cultures were treated in a long-term basis with 100 nmol/L Ang II for 4 hours. This resulted in a 70% decrease in the levels of \( \gamma \)-adducin (Figure 3). Co-incubation with 1 \( \mu \)mol/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 \( \gamma \)-adducin. Similarly, 1 \( \mu \)mol/L PD123319, an AT2 receptor antagonist, showed little effect on Ang II-induced decrease in \( \gamma \)-adducin. These data indicated that Ang II-induced decrease in \( \gamma \)-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 \( \gamma \)-adducin in the brain is linked to hypertension. 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. Consistent with our hypothesis, we found that induction of a hypertensive state was associated with a decrease in the levels of hypothalamic \( \gamma \)-adducin. However, in contrast to genetic models, \( \gamma \)-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 \( \gamma \)-adducin in the hypothalamic nuclei is an initial event directing the dysregulation of the CNS mechanisms leading to hyperten-
sion. However, a prolonged hypertensive state, seen only in the genetic models, may induce secondary alterations associated with a decrease in the brainstem γ-adducin. Alternatively, the difference could simply be genetic versus non-genetic. Irrespective of the mechanisms, it is evident that the regulation of γ-adducin in the hypothalamus seems 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 regulation of cardiovascular functions is imprinted in the multifunctional hypothalamus. Irrespective of the mechanisms, it is evident that the possibility that γ-adducin expression by gene transfer/ manipulation techniques would prevent neurogenic hypertension on a long-term basis.

Perspectives
Adducin is a cytosolic protein with multifunctional properties. They include vesicular transport, Ca\(^{2+}\) 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.

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References
6. Yang H, Francis SC, Sellers K, DeBarros M, Sun C, Summers C, Ferrario CM, Katovich MJ, Muro AF, Raizada MK. Hypertension-linked decrease in γ-adducin expression by gene transfer/manipulation techniques from prehypertensive SHR brain exhibit a decrease in γ-adducin expression, an increase in AT\(_1\) 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 imparted in the multifunctional properties of this protein. 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. It is known that heterodimerization of adducins is needed for their activity. 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 Ca\(^{2+}\) 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; 6,7 (3) MARCKS phosphorylation has been shown to stimulate vesicular transport in neurons; 28 and (4) γ-adducin directly influences neuronal activity.

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.


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