Hypertension in β-Adducin–Deficient Mice

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Abstract—Polymorphic variants of the cytoskeletal protein adducin have been associated with hypertension in humans and rats. However, the direct role of this protein in modulating arterial blood pressure has never been demonstrated. To assess the effect of β-adducin on blood pressure, a β-adducin–deficient mouse strain (−/−) was studied and compared with wild-type controls (+/+). Aortic blood pressure was measured in nonanesthetized, freely moving animals with the use of telemetry implants. It is important to note that these mice have at least 98% of C57Bl/6 genetic background, with the only difference from wild-type animals being the β-adducin mutation. We found statistically significant higher levels of systolic blood pressure (mm Hg) (mean±SE values: −/−: 126.94±1.14, n=5; +/+: 108.06±2.34, n=6; P≤0.0001), diastolic blood pressure (−/−: 83.54±1.07; +/+: 74.87±2.23; P≤0.005), and pulse blood pressure (−/−: 43.32±1.10; +/+: 33.19±1.96; P=0.001) in β-adducin–deficient mice. Western blot analysis showed that as a result of the induced genetic modification, β-adducin was not present in heart protein extracts from −/− mice. Consequently, this deficiency produced a sharp decrease of α-adducin and a lesser reduction in γ-adducin levels. However, we found neither cardiac remodeling nor modification of the heart function in these animals. This is the first report showing direct evidence that hypertension is triggered by a mutation in the adducin gene family. (Hypertension. 2000;36:449-453.)

Key Words: hypertension, genetic ■ adducin ■ mice, knockout ■ echocardiography ■ cytoskeleton ■ telemetry ■ electrocardiography

The adducin protein family is composed of 3 members encoded by closely related genes: α-, β-, and γ-adducin.1,2 The α and β subunits, but not the γ subunit, are found in membrane cytoskeletons of human erythrocytes at the actin-spectrin junctions as a mixture of heterodimers and heterotrimerers. Combinations of αβ and αγ oligomers are found in the actin cytoskeleton at cell-cell contact sites in other cells.2,3

Adducin, an unexpected cytoskeletal player as blood pressure (BP) modulator, has been the subject of several association studies that attempted to link adducin variants to hypertension. Since the publication of the first positive association of adducin polymorphism with human hypertension,4 contradictory results have appeared in the literature. In fact, the human α-adducin gene polymorphism (G460W) has been found to be significantly associated with hypertension and salt sensitivity in certain patient groups,4–8 although this observation was not confirmed by other groups.9–14

The attention to adducin in hypertension was originally drawn by a rat model (Milan hypertensive strain [MHS]), after our characterization of the α- and β-adducin–specific variants associated with high BP levels.15 These mutated adducins lead to a higher level of filamentous actin, enhanced actin bundling in cell-free systems, and increased Na-K pump activity when transfected into kidney epithelial cells.16 However, in both humans and rats, there has been no proof for direct involvement of any of the adducins in the modulation of BP. Furthermore, adducin polymorphism cosegregation analysis and kidney cross-transplantation experiments in the MHS rat model showed that, in a manner similar to that observed in humans,4,5,7 adducin accounted for only a portion of the hypertension.15 Therefore, the simultaneous action of other genes and other organs may be involved in the primary cause of hypertension in both human and rats.15,17

The knockout technology allows elimination of all differences in genetic background except for the desired mutation. Taking advantage of this possibility, we have created a β-adducin–deficient mouse strain by targeted disruption of the β-adducin gene.18 The shape and osmotic fragility of red blood cells (RBCs) of homozygous mutant animals were altered, and consequently the mice suffered from a mild anemia with compensated hemolysis, similar to human spheroctytic hereditary elliptocytosis.18

Our knockout model also gave us the opportunity to study the direct association between β-adducin and hypertension in a well-defined genetic background. Therefore, we studied the effect of β-adducin deficiency on arterial BP, and we found that β-adducin–mutant animals developed hypertension. We
also studied cardiac remodeling and heart function through postmortem analysis and in vivo dynamic assessment of left ventricular dimensions and electrocardiography to discover any possible pathology associated with hypertension or a possible effect of the $\beta$-adducin deficiency on excitatory cell membrane ion channels.

**Methods**

**$\beta$-Adducin–Deficient Mice**

The $\beta$-adducin gene was disrupted (knocked out) as described previously. This strain was crossed with C57Bl/6 mice for 5 generations to obtain a homogeneous background (theoretically 98%). Mice used in this study (wild-type and $\beta$-adducin–mutant mice) were 8 to 10 months old.

**BP Determinations**

Arterial BP was measured in $5^{-/-}$ and $6^{+/+}$ nonanesthetized, freely moving animals with the use of telemetry implants (PA-C20 BP device, Data Sciences International) for a period of 6 hours. BP determinations started 30 minutes after discontinuation of halothane anesthesia. The first hour was considered from 30 minutes after discontinuation of anesthesia until 59 minutes later, the second hour was from 60 minutes after discontinuation of anesthesia until 119 minutes later, and so on every 60 minutes. During this time, abdominal aortic BP waveforms were continuously sampled, and the values are means of 5-second periods every 3 minutes (a total of twenty 5-second periods per hour, except that in the first hour only ten 5-second periods were considered). The device calculated a mean of all individual pulses ($\approx$40 pulses) during that time. Therefore, every hourly mean includes $\approx$800 pulses.

**Electrocardiography**

Two platinum needle electrodes were implanted in the subcutaneous tissue overlying the right scapula and the apex of the heart in $5^{-/-}$ and $7^{+/+}$ halothane-anesthetized mice. Halothane was then discontinued; the ECG was recorded for 30 minutes with the use of ICM-01 amplifiers on a Gilson polygraph and digitized with a Labmaster A-D board at 10 KHz. Analysis of ECG parameters was performed with Axotape software (Axon, Inc.).

**Heart Morphology and Mass**

Measurements of heart mass and volume of ventricular walls were made by 2 methods: planimetry of histological section images and weight of dissected ventricles. In the first (6 $^{-/-}$ and 5 $^{+/+}$ mice), hearts were removed, flash-frozen in methylbutane at dry ice temperature, embedded in OCT compound (Miles Laboratories), serially cut in 20-µm slices perpendicular to their long axis on a Microselect cryostat (Carl Zeiss), and stained with hematoxylin–eosin, and images of slices were digitized. Thickness and total area of the ventricular walls were measured with IPPLUS software (Media Cybernetics, Inc). Volume of ventricular walls was calculated from slice areas and interslice distances.

In a separate series of animals ($7^{-/-}$ and 8 $^{+/+}$ mice), hearts were dissected to eliminate the great vessels and atria. The remaining left and right ventricles were opened to discard any blood contents and weighed.

**Echocardiography**

Mice ($6^{+/+}$ and 5 $^{-/-}$) were sedated with Avertin (2,2,2-tribromethanol, 2.5% solution, 0.016 mL/g body mass, Aldrich Chemical Co). Two-dimensionally guided M-mode and Doppler images were obtained with a 7.5-MHz probe and 10-mm standoff on an ATL Apogee CX200. Animals were positioned in the supine or left decubitus position. The images were digitized for analysis (SigmaScan software, Systat, Inc) of left ventricular dimensions during systole and diastole. Ejection times were determined from flow velocity profiles of the aorta.

**Western Blot Analysis**

Heart homogenates were electrophoresed on a 10% Laemmli gel, blotted onto nitrocellulose membranes, probed with rabbit anti-α-, β-, and γ-adducin polyclonal antibodies (1:500, 1:1000, and 1:1000 dilution, respectively), and analyzed by densitometry, as previously described. The experiments were repeated, and identical results were obtained with 2 independent protein preparations.

**Creatinine and Kidney Weight Analysis**

Fresh blood from wild-type and $\beta$-adducin–mutant mice (10 mice per group) was collected in Eppendorf tubes. Creatinine levels were analyzed according to standard methods. For kidney weight analysis, mice were killed (9 mice per group) and weighed; then the kidneys were removed, and left and right kidneys were weighed separately. Data of kidney weight correspond to the sum of both kidneys and are expressed as percentage of total animal weight.

**Statistical Methods**

Data analysis was performed with the use of the software package Stata, version 6.0 (StataCorp, 1999). The data were summarized with the mean as a measure of central tendency and the standard error as a measure of dispersion. The difference in BP between the wild-type and mutant mice was assessed by a repeated-measures ANOVA according to a split-plot factorial design. A $P$ value of 0.05 was chosen as the limit of statistical significance. Student’s $t$ test was used for the rest of the analyzed parameters.

**Results**

We studied the effect of $\beta$-adducin deficiency on arterial BP by comparing wild-type and $\beta$-adducin knockout mice. The mutant strain was backcrossed with C57Bl/6 mice for 5
Figure 2. Western blot analysis of α-, β-, and γ-adducin in heart protein extracts. A, Heart protein extracts (20 μg) from adult wild-type (+/+; lanes 1 and 4) and mutant (−/−; lanes 2 and 5) mice were analyzed with a rabbit anti-mouse β-adducin polyclonal antibody (lanes 1 and 4) and the recombinant deleted β-adducin18 (lanes 3 and 6) are marked by black arrows. B and C, The same protein extract was analyzed with anti–α-adducin antibody18 (lanes 1 and 4) and mutant (−/−; lanes 3 and 6) are marked by black arrows. B and C, The same protein extract was analyzed with anti–α-adducin antibody18 (lanes 1 and 4) and mutant (−/−; lanes 3 and 6) are marked by black arrows. B and C, The same protein extract was analyzed with anti–α-adducin antibody18 (lanes 1 and 4) and mutant (−/−; lanes 3 and 6) are marked by black arrows. B and C, The same protein extract was analyzed with anti–α-adducin antibody18 (lanes 1 and 4) and mutant (−/−; lanes 3 and 6) are marked by black arrows. B and C, The same protein extract was analyzed with anti–α-adducin antibody18 (lanes 1 and 4) and mutant (−/−; lanes 3 and 6) are marked by black arrows.

Figure 3. Measurements of left ventricular wall thickness and muscle volume. A, Measurements of left ventricular wall thickness (mean and SE), B, Measurements of left ventricular muscle volume (mean and SE), C, M-mode and Doppler echocardiography, D, Electrocardiogram.

ANOVA (Figure 1). However, heart rate was not significantly higher in mutant mice (Figure 1, inset). Repeated-measures ANOVA of the BP data showed that there was a nonsignificant effect for time and for the interaction mutation-by-time in BP determinations (data not shown), except for a significant effect for time in the case of pulse BP. Further data analysis showed that the differences between the time point at hour 1 and the time points from hours 3 to 6 account for the overall significant effect of time for pulse BP. In fact, if the data between hours 2 to 6 are considered, there is no effect of time for pulse BP (Figure 1), suggesting that it might be generated by a residual effect of anesthesia in the first hour after its discontinuation.

We analyzed the expression levels of the different adducin subunits (α, β, and γ) in heart protein extracts by Western blot. No β-adducin (normal or deleted form) was detected in total heart homogenates of β-adducin−/− mice (Figure 2A). By using an anti–α-adducin antibody, we detected a sharp decrease (70%) in the amount of this subunit in −/− mice (Figure 2B). In addition, when we used an anti–γ-adducin antibody, we observed a downregulation (50%) of γ-adducin levels in these mice (Figure 2C). Although adducin mRNAs had been previously found in hearts of some organisms,21,22 the presence of α-, β-, and γ-adducin protein in this tissue has never been reported.

Qualitative histological analysis showed no obvious morphological differences between hearts from wild-type and mutant mice (not shown). The quantitative histological study of frozen hearts did not show any difference in dimensions of ventricular walls (Figure 3A and 3B). This result was confirmed by the measurement of left and right combined ventricular masses in a separate series of animals used for ECG recording (0.401±0.019% of body mass for −/− and +/+ mice, respectively). In addition, the in vivo M-mode echocardiography study did not reveal differences among the 2 strains in septum and posterior left ventricular wall thickness for mutant and wild-type control (+/+; +/−) mice, are shown. Heart rates (mean±SE) were as follows: +/−: 517±5.7 bpm; −/−: 522±7.7 bpm. Units of measure are as follows: ventricular septum thickness (VST), posterior wall thickness (PWT), end-diastolic diameter (EDD), and end-systolic diameter (ESD), mm; left ventricular fractional shortening (LVFS), %; ejection time (ET), ms; velocity of circumferential shortening (VCF), 1/s. D, Electrocardiography. ECG waveform intervals (mean±SE), measured from β-adducin deficient (−/−) and wild-type control (+/+; +/−) mice, are shown. Heart rates (mean±SE) were as follows: +/−: 517±5.7 bpm; −/−: 522±7.7 bpm. Body temperatures were as follows: +/−: 37.2±0.11°C; −/−: 36.7±0.35°C. None of the differences among group means were statistically significant.
ventricular wall thickness, end-diastolic and end-systolic diameters, and left ventricular fractional shortening (Figure 3C). The Doppler echocardiography study also showed similar values of ejection time in both strains (Figure 3C). ECG demonstrated ECG waveforms characterized by a QRS complex with a small or absent Q wave, a prominent R wave, and an S wave followed immediately by a T wave with no discernible ST interval in both mice strains. Values of QRS complex duration and of PR and QT intervals did not differ among the 2 strains (Figure 3D). These ECG data indicate that the absence of β-adducin does not produce significant changes in the muscle cell membrane ion channels of the heart to induce changes in the ECG parameters. It is noteworthy that despite the marked changes in adducin expression observed, no alterations of heart morphological, mechanical, or electric properties have been detected in this model, suggesting a lack of involvement of these proteins in heart function.

Discussion

Studies in humans and rats have associated adducin with hypertension, but the direct role of this protein in BP has never been demonstrated. To investigate the role of β-adducin in BP regulation, we generated mice homozygous (−/−) for a targeted disruption of the β-adducin gene and transferred this gene mutation to a homogeneous genetic background (theoretically, 98% identical to that of the control animals). This is the first report showing strong evidence that hypertension is triggered by a mutation in the β-adducin gene.

One of the clear consequences of the absence of β-adducin in some tissues (References 18 and 22 and data not shown) is the concomitant modification of α- and γ-adducin levels, supporting the hypothesis that the abnormal adducin complex assembly might be an important determinant of BP.23 Hence, β-adducin deficiency could be directly responsible for BP changes, or it may be acting through the consequent modification of α- and γ-adducin levels or other as yet undetected effects. β-Adducin–deficient mice have a lower hematocrit than wild-type animals (43.5 versus 46.1; \(P = 0.005\)), and this cannot explain the change in BP.

Although in hearts of −/− mice, β-adducin deficiency produced altered α- and γ-adducin levels, we have found neither cardiac remodeling nor modification of the heart function in these animals. This phenomenon is probably related to the moderate magnitude of the change in arterial BP present and/or the relatively young age of the animals under study. However, we cannot exclude the involvement of adducin on heart function under different types of load and stress.

The involvement of the kidney in the sequence of events connecting adducin polymorphism to the development and maintenance of hypertension was shown in the MHS strain of rats17,24 and was suggested in humans.6,8 However, preliminary analysis of kidneys of our β-adducin–deficient mice showed no difference by gross histological analysis with +/+ mice, and the kidney weight of −/− mice was similar to that of +/+ animals (0.56 ± 0.09% and 0.58 ± 0.06% of animal weight for +/+ and −/− animals, respectively [mean ± SD]; n = 9 per group; \(P = 0.226\), Student’s \(t\) test). Moreover, serum creatinine levels showed no difference between −/− and +/+ animals (0.416 ± 0.044 mg/dL and 0.419 ± 0.025 mg/dL for +/+ and −/− animals, respectively [mean ± SD]; n = 10 per group; \(P = 0.853\), Student’s \(t\) test).

The interest of our work for clinical research lies primarily in the definitive proof of the direct involvement of adducin in BP modulation. Contrary to the partial contribution of adducin genes to hypertension in both different human populations and MHS rats, the mutation of the β-adducin gene in the knockout mouse is the only one responsible for BP changes in this strain. Considering this and the very high degree of sequence homology between mouse and human adducins (Reference 18 and A.F. Muro, unpublished data, 1999), it is possible that the molecular mechanisms of hypertension in these mice are relevant to humans. Furthermore, since in our mouse model RBC abnormalities were present, causing mild hemolytic anemia, it will be of great interest to search for adducin polymorphisms in selected groups of patients with elevated BP accompanied by erythrocyte changes similar to those found in our mouse model.

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References


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