Role of the $\alpha_{1D}$-Adrenergic Receptor in the Development of Salt-Induced Hypertension

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Abstract—In an attempt to elucidate whether there is a specific $\alpha_{1}$-adrenergic receptor ($\alpha_{1}$-AR) subtype involved in the genesis or maintenance of hypertension, the $\alpha_{1D}$-AR subtype was evaluated in a model of salt-induced hypertension. The $\alpha_{1D}$-AR–deficient ($\alpha_{1D}^{-/-}$) and control ($\alpha_{1D}^{-/+}$) mice (n=8 to 14 in each group) were submitted to subtotal nephrectomy and given 1% saline as drinking water for 35 days. Blood pressure (BP) was monitored by tail-cuff readings and confirmed at the end point by direct intraarterial BP recording. The $\alpha_{1D}^{-/-}$ mice had a significantly ($P=0.0004$) attenuated increase in BP response in this protocol (baseline 94.6±2.8 versus end point 107.4±4.5 mm Hg) compared with that of their wild-type counterparts ($\alpha_{1D}^{+/+}$), from a baseline 97.4±2.9 to an end point 139.4±4.5 mm Hg. Seven of 15 $\alpha_{1D}^{-/-}$ mice died with edema, probably owing to renal failure, whereas 14 of 15 $\alpha_{1D}^{-/+}$ mice were maintained for 35 days. Body weight, renal remnant weight, and residual renal function were similar in the 2 groups, whereas the values of plasma catecholamines (epinephrine, norepinephrine, and dopamine) were higher in $\alpha_{1D}^{+/+}$ than in the $\alpha_{1D}^{-/-}$ mice. These data suggest that $\alpha_{1D}$-AR plays an important role in developing a high BP in response to dietary salt-loading, and that agents having selective $\alpha_{1D}$-AR antagonism could have significant therapeutic potential in the treatment of hypertension. (Hypertension. 2002;40:101-106.)

Key Words: receptors, adrenergic ■ mice ■ hypertension, sodium-dependent ■ nephrectomy
on the $\alpha_2$-AR as an important sympathetic component in this interaction, whereas much less attention has been paid to the role of the $\alpha_1$-AR. To assess the functional roles of $\alpha_{1D}$-AR subtypes in salt-induced hypertension, genetically altered mice lacking the $\alpha_{1D}$-AR were examined by a dietary salt-loading study that was initiated after subtotal nephrectomy.

**Methods**

**Animals**

Two groups of male mice, age 7 to 9 weeks and weighing 23.2 to 28.2 g, were used in the present study. Homozygous ($\alpha_{1D}^{+/+}$) knockout mice for the $\alpha_{1D}$-AR subtype with the genetic background of 129Sv and C57Black6/J strains were used, along with their wild-type controls ($\alpha_{1D}^{-/-}$), which were littermates of the $\alpha_{1D}^{+/+}$ mice. All mice were housed in the animal quarters with a 12-hour light/dark cycle and were provided food and distilled water ad libitum. After subtotal nephrectomy, drinking water was replaced with 1% saline. All experiments were conducted in accordance with guidelines for the care and use of animals approved by the National Center for Child Health and Development Research Institute.

**Animal Genotyping**

Inactivation of the $\alpha_{1D}$-AR gene involved insertion of the PGK-Neo cassette. Genotypes were determined from DNA isolated from the tail using the following primers: $\alpha_{1D}^{+/+}$-1521R (CAGCTGCACCTCAG-TAGGATGCTA), $\alpha_{1D}^{-/-}$-1239F (CCGTGTGTTGAGGACCCGG-CAGCGG), and Neo-2401-F (CTCTACATTTGAGGACAAAG-GATTT) were used to detect the intact (282 bp) or interrupted (637 bp) $\alpha_{1D}$-AR gene. The $\alpha_{1D}^{+/+}$-1521R primer was located within the first exon of the $\alpha_{1D}$-AR gene, and the $\alpha_{1D}^{-/-}$-1239F primer was within the region of the first exon replaced with the Neo in the mutant allele. The Neo-2401-F primer was within the Neo gene. Each sample contained the upstream and downstream primers (10 pmol of each), 0.25 mmol/L of each dNTP, 50 mmol/L KCl, 10 mmol/L Tris-HCl pH 8.6, 1.5 mmol/L MgCl$_2$, and 2.5 U of Taq DNA polymerase (Takara Shuzo Co). Thermal cycling was performed for 1 minute at 94°C, 1 minute at 56°C, and 2 minutes at 72°C for 30 cycles. Bands were separated on 2% agarose gels.

**Subtotal Nephrectomy and BP Monitoring**

Mice (n=15 in each group) were subjected to subtotal nephrectomy and handled as described. Briefly, both poles of the left kidney were excised under anesthesia with intraperitoneal sodium pentobarbital (50 mg/kg), leaving a small amount of residual renal tissue around the hilum and preserving the ureter and hilar vessels. The excised renal tissues were weighed, and the ratios of these organs to the body weight were calculated individually. After a 7-day recovery period, the right kidney was removed, leaving 25% of the total renal mass. Twenty-four hours after the second operation, the animals were placed and maintained on 1% saline as drinking water for 35 days.

Tail-cuff systolic BP (SBP) and heart rate (HR) measurements were obtained by use of a computerized tail-cuff system (BA-98A system, Softron Co) as described elsewhere. Mice were monitored for 35 days or until they became hypertensive; ie, their tail-cuff SBP reached 150 mm Hg, or an increase of >40 mm Hg above baseline was recorded and sustained for 3 consecutive days. BP measurements of the last 3 days were averaged, and the resulting mean value was considered the end point tail-cuff BP for the animal. For animals that failed to develop hypertension as defined above during the 35-day period, the end point tail-cuff BP was calculated by averaging the measurements recorded for the last 3 days. The end point tail-cuff BP was confirmed by direct measurement via arterial catheterization at the end of the study, as described previously.

**Statistical Analysis**

All values are expressed as mean±SEM. P<0.05 according to a Student’s t test was considered statistically significant. Cumulative survival curves were constructed by the Kaplan-Meier method, and difference between the curves was tested for significance with the use of the log-rank statistic.

**Results**

**Measurement of Plasma Catecholamines and Creatinine**

Blood was drawn slowly from the arterial line and used for measuring plasma catecholamine levels (epinephrine, norepinephrine, and dopamine) and creatinine levels. Catecholamine levels were determined by high-pressure liquid chromatography using commercially available reagents (Tosho Co) as described elsewhere. Plasma creatinine was determined by use of a commercially available colorimetric kit from Sigma Diagnostics.

**Blood**

Four of 15 $\alpha_{1D}^{+/+}$ mice died within 4 days, and 3 $\alpha_{1D}^{+/+}$ mice died at the seventh, 12th, or 35th day. One $\alpha_{1D}^{-/-}$ mouse died on the 26th day.

**Survival**

The Kaplan-Meier analysis detected a favorable effect of $\alpha_{1D}^{-/-}$ gene ablation on cumulative survival. The difference in survival rates calculated by log-rank test was significantly better in the mutant mice group (53% for $\alpha_{1D}^{+/+}$ and 93% for $\alpha_{1D}^{-/-}$; P=0.0121).

No significant differences were observed between genetically altered mice and their controls in regard to body weight at baseline and end point, ratios of excised or remnant kidney weight to body weight at the end point, and ratios of heart.
weight to body weight at the end point. There were also no differences in plasma creatinine levels (Table), indicating that the residual renal function after subtotal nephrectomy was similar in the 2 groups and within a normal range, compared with another report. Plasma creatinine levels and ratios of heart weight to body weight in both groups were significantly (P<0.05) increased compared with those of controls that did not undergo salt-loading (creatinine: 7.25±0.05 μmol/L in α₁D−/−, n=8, 7.07±0.05 μmol/L in α₁D+/−, n=12; heart-weight/body-weight ratio: 4.62±0.31 mg/g, in α₁D+/−, n=8, 4.82±0.23 mg/g, in α₁D−/−, n=12, respectively), indicating that this kind of salt loading could affect the renal or cardiac functions.

**Tail-Cuff Measurements**

Figure 2 shows the course of tail-cuff HR and SBP changes during the 1% saline drinking period after subtotal nephrectomy in the 2 groups. Figure 2A presents HR changes and shows that in both groups, HR did not differ and significantly increased compared with the baseline (baseline versus end point: 557±23 bpm and 629±26 bpm in α₁D+/−, n=8, P=0.041 and 502±23 bpm and 597±16 bpm, in α₁D−/−, n=14, P=0.0004). On the other hand, Figure 2B presents SBP changes in the α₁D+/− (n=8) and the α₁D−/− (n=14) mice and shows that SBP in the α₁D+/− mice increased significantly by the end of the 35-day period, whereas in the α₁D−/− mice did not change significantly. Hypertension, as defined in the Methods section, developed within 3 weeks on average in the α₁D+/− mice, not including the 7 mice that died. Only 2 out of 14 α₁D−/− mice that survived developed hypertension (their SBP reached 150 mm Hg), whereas 6 out of 8 surviving α₁D+/− mice developed hypertension (2 SBPs were increased >40 mm Hg from the baseline, and 4 reached 150 mm Hg).

Figure 3 summarizes BP and HR measurements of 2 groups of mice at the baseline and end point. Figure 3A shows that there was no difference in baseline SBP (ie, before surgery) between the α₁D−/− (n=14) and control α₁D+/− (n=8) mice (97.4±2.8 mm Hg and 94.6±2.9 mm Hg in α₁D+/− and α₁D−/− mice, respectively, P=0.46). However, an attenuated hypertensive response to subtotal nephrectomy and salt loading was observed in the α₁D−/− mice, resulting in a significantly lower end point SBP compared with that of their wild-type controls (139.4±4.5 mm Hg and 107±4.5 mm Hg in α₁D+/− and α₁D−/− mice, respectively, P=0.0004) (Figure 3B). Figure 3D and 3E present mean tail-cuff HR measurements for the 2 groups. Tail-cuff HR levels at baseline and end point tended to be somewhat higher in the α₁D−/− mice, but these differences were not significant. A significant increase in tail-cuff HR relative to baseline was observed in both groups after subtotal nephrectomy and 1% saline loading (according to a paired t test).

**Intraarterial BP and HR Measurements**

The end point direct mean arterial pressure (MAP) and HR measurements for each group are shown in Figure 3C and 3F. Consistent with the end point tail-cuff BP measurements, direct MAP was significantly lower in the α₁D−/− (n=14) group compared with the α₁D+/− mice (n=8) (124.1±7.6 mm Hg in the α₁D−/− versus 161.9±6.8 mm Hg in the α₁D+/− mice; P=0.016). Comparison of end point tail-cuff SBP measurements with direct MAP values showed that direct MAP values were higher than tail-cuff SBP measurements. Direct HR tended to be higher in the α₁D+/− mice, but this difference was not significant (565±46 bpm in the α₁D−/− versus 634±18 bpm in the α₁D+/− mice; P=0.14).
Figure 3. BP and HR at the baseline and end point for each group of mice. Tail-cuff BP at baseline (A) and end point (B), and tail-cuff HR at baseline (D) and end point (E) for each group of mice. Direct intraarterial MAP (C) and HR (F) at the end point are indicated. Open bars denote wild-type (α_{1D}−/−) mice; closed bars denote the α_{1D}-AR-deficient (α_{1D}−/−) mice. *P<0.05 α_{1D}−/− vs α_{1D}+/− mice.

Catecholamines Levels

Plasma catecholamine levels for both groups are shown in the Table. Plasma catecholamines for both groups increased after salt loading compared with those measured in α_{1D}−/− or α_{1D}−/− mice without salt loading (basal levels of plasma total catecholamines; 31.05±2.82 nmol/L in α_{1D}−/−, 27.43±4.57 nmol/L, in α_{1D}−/−). Plasma epinephrine levels tended to be higher in α_{1D}−/+ (n=8) than in the α_{1D}−/− mice (n=14), but this difference was not significant. Norepinephrine, dopamine, and total catecholamine levels were significantly higher in α_{1D}−/+ (n=8) than in the α_{1D}−/− mice (n=14).

Discussion

The functional role of the α_{1D}-AR subtype in the development of high BP was investigated using a model of salt-induced hypertension. One percent saline was given as drinking water to mice genetically lacking α_{1D}-AR after subtotal nephrectomy. The α_{1D}−/− mice had a significantly attenuated increase in MAP compared with that of α_{1D}−/− mice. The present study provides clear evidence that α_{1D}-AR plays an important role in developing high BP in response to dietary salt loading. In addition to our observations, several reports support the idea that α_{1D}-AR could be involved in the pathogenesis and/or maintenance of hypertension.19,27–30

We observed that 7 α_{1D}−/+ mice died with general edema during 1% saline drinking after subtotal nephrectomy, whereas only 1 α_{1D}−/− mouse died in the same period. This might suggest that α_{1D}−/− mice are less susceptible to acute renal failure caused by subtotal nephrectomy followed by 1% saline drinking. As the amount of excised kidney in the dead α_{1D}−/− mice was comparable to that in surviving α_{1D}−/− mice (the ratio of excised kidney weight to body weight was 3.31±0.02 mg/g in surviving mice, n=8, and 3.33±0.04 mg/g in dead mice, n=7), our view is that the less amount of remaining kidney was not the direct cause of death. Although the exact reason for the different death rates is uncertain, the present study clearly shows that the lack of α_{1D}-AR leads to a better prognosis.

Interestingly, relative heart weights significantly increased even in the α_{1D}−/− mice without hypertension. It is generally accepted that cardiac hypertrophy develops in response to increased cardiac workload as a compensatory mechanism. Various factors, including mechanical and biochemical ones, are involved in the development of cardiac hypertrophy.21 As the α_{1D}−/− mice did not develop hypertension by salt loading, an enhanced adrenergic effect on the heart, rather than the mechanical stress owing to hypertension, might be directly related to the increased heart weight in the α_{1D}−/− mice. Although studies have suggested that α_{1D}-ARs are pivotal in promoting cardiac hypertrophy, most studies favor the α_{1A}-AR as the dominant subtype,22 with little cardiac role of α_{1D}-AR. This is in good agreement with our observation that the heart was enlarged in the α_{1D}−/− mice under high circulating catecholamines. Besides the enhanced adrenergic system, biochemical alterations such as saltwater imbalance, other exogenous factors could be involved. Further study is required to clarify the functional role of α_{1D}-AR in this type of acute renal failure.

The present experiment also clearly indicates that α_{1D}-AR is a prerequisite for development of salt-induced hypertension. Possible explanations for the observation that mice lacking the α_{1D}-AR gene are less susceptible to developing salt-induced hypertension could include (1) decreased vascular reactivity to enhanced adrenergic stimuli that accompanies salt loading, (2) altered sympathetic activity in the CNS, and (3) altered functional activity of renal α_{1D}-AR in reabsorption of sodium.

The α_{1D}−/− mice had a significantly attenuated increase in MAP with salt loading compared with that of α_{1D}−/− mice. Catecholamines induce vasoconstriction mainly via stimulation of vascular α_{1}-AR family, α_{1D}-AR in particular.33 Our recent characterization of α_{1D}−/− mice showed that the vascular contractile response and the pressor response to α_{1}-AR stimulation are mediated primarily by α_{1D}-AR, among the 3 α_{1}-AR subtypes.14 Thus, α_{1}-AR antagonists such as prazosin are considered to exert their hypotensive effect mainly by inhibiting vascular α_{1D}-AR. Hence, mice lacking the α_{1D}-AR are less susceptible to developing salt-induced hypertension, and this could partly be owing to their reduced vascular reactivity to α_{1}-AR stimulation. In addition to α_{1D}-AR, it was previously reported that mice lacking α_{1D}-AR, which is abundant in the vasculature rather than in CNS and is responsible for a peripheral vasoconstrictive action,34 are less susceptible to develop hypertension in the same salt-loading hypertension model.22 Hence, these studies may indicate that
an enhanced sympathetic activity is of importance in developing and maintaining this salt-loading hypertension. Moreover, the studies show that $\alpha_{1D}$-AR and $\alpha_{2A}$-AR, those mediating adrenergic vasoconstriction, can be potentially important therapeutic targets in this type of hypertension. Further studies with selective pharmacological tools will clarify the relative contribution of each receptor in this hypertension model.

The lesser increase in BP observed in $\alpha_{1D}$-/- mice can also be explained by a suppressed sympathetic activity. $\alpha_1$-AR has been implicated to regulate sympathetic outflow in the CNS, and the $\alpha_1$-AR blocker prazosin has been implicated to act in the CNS to suppress sympathetic outflow. In fact, we observed that the increase in circulating catecholamines with salt loading was less in $\alpha_{1D}$-/- mice compared with $\alpha_{1D}$$^+/+$ mice, although the basal levels of circulating catecholamines were not much different between $\alpha_{1D}$$^+/+$ and $\alpha_{1D}$-/- mice. As most of patients with hypertension have a higher noradrenaline level, this is reminiscent of the previous report that prazosin appears to depress baroreflex function, in hypertensive patients in particular. Hence, $\alpha_1$-AR, which is abundant in the CNS, might play an important role in the regulation of sympathetic outflow, and mice lacking the $\alpha_1$-AR might be less susceptible to develop salt-induced hypertension, primarily because they cannot respond to salt loading with the normal increase in sympathetic outflow. Hence, ablating $\alpha_1$-AR may lead to antihypertensive effects salt-induced hypertension, partly by reducing the amount of endogenous agonist as well as by modulating vascular reactivity to them. The role of $\alpha_2$-AR in developing a hyperadrenergic state in salt-sensitive hypertension is clearly of great interest and needs to be further investigated.

Besides the 2 above-mentioned possibilities, altered $\alpha_1$-AR function in renal reabsorption of sodium should be taken into account. Renal $\alpha_1$-ARs have been implicated in BP control and hypertension in rats. They apparently mediate Na$^+$/H$^+$ exchanger, although the responsible subtypes are considered to be $\alpha_1A$-AR and $\alpha_1F$-AR, but not $\alpha_1D$-AR. As in the case of rats, murine kidney expresses $\alpha_1A$-AR and $\alpha_1D$-AR and very low levels of $\alpha_2$-AR. However, $\alpha_2$-AR could be induced and would have an important regulatory activity in subtotal nephrectomy/salt-loading. Therefore, the potential functional significance of $\alpha_1$-AR in the pathophysiological setting of salt-induced hypertension is still uncertain in the present study.

The results presented here support the idea that $\alpha_1$-AR can play a role in the development of essential hypertension or hypertension with salt-sensitivity. Furthermore, it was reported that functional expression of $\alpha_1$-AR in the resistance vessels increases with age. These observations make it worth exploring whether genetic differences in $\alpha_1$-AR structure, density, or function or alterations due to aging and other factors, are associated with differences in salt sensitivity in humans. Also, the present study strongly suggests that $\alpha_1$-AR antagonism could have therapeutic potential in the treatment of hypertension.

**Perspective**

Using $\alpha_{1D}$-AR knockout mice, the present study shows that $\alpha_{1D}$-AR plays an important role in developing the dietary salt-loading hypertension. Selective $\alpha_{2D}$-AR antagonism could have significant therapeutic potential in the treatment of hypertension.

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