Role of the $\alpha_{2B}$-Adrenergic Receptor in the Development of Salt-Induced Hypertension

Konstantinos P. Makaritsis, Diane E. Handy, Conrado Johns, Brian Kobilka, Irene Gavras, Haralambos Gavras

Abstract—Salt sensitivity is a common trait in patients with essential hypertension and seems to have both an inherited and an acquired component (eg, is influenced by aging and renal insufficiency). Experimental evidence suggests that salt loading induces hypertension via a neurogenic mechanism mediated by the $\alpha_2$-adrenergic receptors ($\alpha_2$-AR). To explore the $\alpha_2$-AR subtype involved in this mechanism, we studied 2 groups of mice genetically engineered to be deficient in one of the 3 $\alpha_2$-AR subtype genes (either $\alpha_{2B}$-AR $+/-$ or $\alpha_{2C}$-AR $-/-$ knockout mice) compared with their wild-type counterparts. The mice (n=10 to 14 in each group) were submitted to subtotal nephrectomy and dietary salt-loading study. Blood pressure (BP) was monitored by tail-cuff readings and confirmed at the end point by direct intra-arterial BP recording. The $\alpha_{2B}$-AR–deficient mice had an attenuated BP response in this protocol (baseline 101.8±2.7 versus end point 109.9±2.8 mm Hg), whereas the BP of their wild-type counterparts went from a baseline 101.9±2.3 to an end point 141.4±7.1 mm Hg. The other 2 groups had BP increases of 44.6±5.17 and 46.7±7.01 mm Hg, with no difference between the mice deficient in the $\alpha_{2C}$-AR gene subtype versus their wild-type counterparts. Body weight, renal remnant weight, and residual renal function were no different among groups. These data suggest that a full complement of $\alpha_{2B}$-AR genes is necessary to raise BP in response to dietary salt loading, whereas complete absence of the $\alpha_{2C}$-AR subtype does not preclude salt-induced BP elevation. It is unclear whether the mechanism(s) involved in this process are of central origin (inability to increase sympathetic outflow), vascular origin (inability to vasoconstrict), or renal origin (inability to retain excess salt and fluid). (Hypertension. 1999;33:14-17.)

Key Words: receptors, adrenergic, alpha $\blacksquare$ mice $\blacksquare$ hypertension, sodium-dependent

A common characteristic among essential hypertensive patients is excessive sensitivity to salt, with a prevalence estimated to be >50%.1 Although factors such as aging and diminished renal function enhance salt sensitivity, there is also evidence that this is a heritable trait that is genetically determined.2 In support of this notion is the familial aggregation and the higher prevalence of salt sensitivity in certain ethnic groups, eg, African Americans.

The mechanisms by which salt loading raises blood pressure (BP) are still incompletely understood. Increasing evidence in recent literature suggests that the prevailing mechanism is a neurogenic one involving an early interaction between vasopressinergic and adrenergic neurons in the central nervous system (CNS), leading to a later persistent hyperadrenergic state.3 A large body of experimental data4–7 suggests that the sympathetic component that plays a pivotal role in this interaction is the $\alpha_2$-adrenergic receptor ($\alpha_2$-AR). Notably, in vitro8 and in vivo9 studies in the past have also indicated that the sodium ion can affect the $\alpha_2$-AR function by altering the sensitivity and responsiveness of these receptors to agonist neurotransmitters.

Because radioligands cannot discriminate between $\alpha_2$-AR subtypes, the subtype involved in these salt-mediated effects could not be further dissected by pharmacological techniques. Recently, however, genetically engineered mice in which either the $\alpha_{2B}$-AR or the $\alpha_{2C}$-AR gene has been selectively deleted became available.10,11 The following experiments were designed to explore the role of each one of these $\alpha_2$-AR subtypes in salt sensitivity by use of genetically altered mice lacking one or both copies of each of the $\alpha_2$-AR subtypes in a subtotal nephrectomy and dietary salt-loading study.

Methods

Animals

Four groups of male mice aged 7 to 9 weeks and weighing 20.2 to 27.2 g were used in the present study. One group of homozygous (−/−) knockout mice for the $\alpha_{2C}$-AR subtype and 1 group of heterozygous (+/−) $\alpha_{2B}$-AR subtype gene knockout mice were used, along with their wild-type controls (+/+). Homozygous $\alpha_{2B}$−/− gene knockout mice were not available in sufficient numbers, as they do not breed well. Heterozygous $\alpha_{2B}$−/− gene knockout mice were deemed acceptable as they have been shown to have a lower level of expression of the $\alpha_{2B}$-protein.11 All mice were housed in the...
animal quarters with a 12-hour light/dark cycle and were provided food (Purina, Certified Rodent Chow 5002) 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 Boston University Medical Center.

Animal Genotyping

Inactivation of each of the α2-AR genes involved insertion of the pGK.Neo.Bpa cassette. For each α2 gene, specific primers that flank the site of the pGK.Neo cassette insertion and a compatible primer specific for the pGK promoter were synthesized. Genotypes were determined from DNA isolated from tail or spleen by use of these primers in 1 polymerase chain reaction (PCR) to detect the intact gene (α2-primer pair) and the interrupted gene (α2-primer/pGK-primer pair).

To screen the α2B-AR lines, MB.GF2 (ATCCTCACCGTG-GCTCATTG), MB.GB2 (TGGAGGCTGGGGGTCCATTAG), and PGK0.1 (CAGAAAGGCAAGGACAA-AGC) primers were used to detect the intact (365 bp) or interrupted (750 bp) α2B-AR gene. To screen the α2B-AR lines, MC.GF1 (CACCTGTGGTGCATTAGTCTGGAC), MC.GB1 (TGCCCGACCCATTCTCTG), and PGK0.3 (CATTGTTGACGGTCCTGACGAG) were used to detect the intact (377 bp) or interrupted (540 bp) α2C-AR gene. Presence of the PGK.neo.Bpa insert was confirmed by use of the neo.F1(TGGAAGAGCTTCGCTAGACG) and neo.B3 (CAC-CATGATTCGCAAGACGAG) primers to produce a 548-bp band by PCR. Each 25-μL PCR contained 0.2 μmol/L each primer, 0.2 mmol/L each dNTP, 2 mmol/L Mg2+, 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, and 0.025 U AmpliTaq Gold (Perkin Elmer) and was incubated as follows: 95°C for 12 minutes followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 75°C for 1 minute 30 seconds followed by 75°C for 5 minutes. Bands were separated on 3% to 4% NuSieve agarose (FMC) gels.

Subtotal Nephrectomy and BP Monitoring

Mice were submitted to subtotal nephrectomy and handled as described elsewhere. In short, under anesthesia with intraperitoneal sodium pentobarbital, both poles of the left kidney were excised, leaving a small amount of residual renal tissue around the hilum and preserving the ureter and hilar vessels. After a 7- to 10-day recovery period, the right kidney was removed, leaving 20% to 25% of the total renal mass. Twenty-four hours after the second operation, the animals were placed and maintained on 1% NaCl as drinking water for a maximal period of 35 days.

Tail-cuff systolic BP (SBP) and heart rate (HR) measurements were obtained by use of a computerized tail-cuff system (BP 2000 Visitech Systems) described elsewhere.

Mice were followed up for a maximal period of 35 days or until they became hypertensive, ie, their tail-cuff SBP reached 150 mm Hg or an increase by ≥40 mm Hg from baseline was recorded and sustained for 3 consecutive days. BP measurements of the last 3 days were averaged and the mean was considered the end point tail-cuff BP for the animal. In animals that failed to develop hypertension as defined above during the 35-day period, the end point tail-cuff BP was calculated by averaging measurements of 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 elsewhere.

Statistical Analysis

All data are presented as mean±SEM. Student’s t tests for paired and unpaired data were used as appropriate. The Mann-Whitney rank sum test was used for nonparametric data. Differences at P<0.05 were considered significant.
Salt Sensitivity and $\alpha_{2b}$-Adrenergic Receptor

Changes in Body Weight, Ratio of Remnant Kidney Weight to Body Weight, Days on 1% NaCl, and Plasma Creatinine Levels in Subtotally Nephrectomized Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\alpha_{2b}^{+/+}$</th>
<th>$\alpha_{2b}^{-/-}$</th>
<th>$\alpha_{2c}^{+/+}$</th>
<th>$\alpha_{2c}^{-/-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BW, g</td>
<td>20.2±0.9</td>
<td>22.3±0.9</td>
<td>27.1±1.1</td>
<td>24.5±0.7</td>
</tr>
<tr>
<td>(n=12)</td>
<td>(n=10)</td>
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<tr>
<td>End point BW, g</td>
<td>22.6±1.1</td>
<td>22.8±1.4</td>
<td>25.8±2.2</td>
<td>25.0±0.6</td>
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<tr>
<td>RKW/BW, mg/g</td>
<td>6.5±0.3</td>
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<td>Days on 1% NaCl</td>
<td>29.7±2.4</td>
<td>31.5±2.5</td>
<td>12.8±1.9</td>
<td>16.3±2.8</td>
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<td>Creatinine, μmol/L</td>
<td>85±5.3</td>
<td>77±11.5</td>
<td>98±20.3</td>
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BW indicates body weight; RKW, remnant kidney weight.

and in ≈4 weeks in the $\alpha_{2b}^{+/+}$. All $\alpha_{2b}^{-/-}$ mice were maintained for 35 days, except 2 mice that died a few days earlier without appreciable change in BP.

Discussion

Subtotal nephrectomy is a long-accepted experimental procedure used over the years to accentuate salt-induced hypertension equivalent to that accompanying human chronic renal failure. Previous studies from our laboratory and others have established that this hypertension is associated with indices of sympathetic overactivity and that seems to be mediated by altered $\alpha_{1}$-AR function in CNS structures. The present experiments suggest that the $\alpha_{2b}$-AR subtype plays a crucial role in this situation, because mice lacking a full complement of the $\alpha_{2b}$-AR gene were unable to raise their BP in response to chronic salt loading aided by subtotal nephrectomy. Although missing only 1 copy of the $\alpha_{2b}$-AR gene, these animals have been shown in the past to be deficient in $\alpha_{2b}$-AR protein levels. The data do not permit conclusions as to the mechanism(s) by which NaCl may affect $\alpha_{2b}$-AR, ie, whether it causes the gene itself or some regulatory protein to respond by altering the numbers of generated $\alpha_{2b}$-AR or whether it alters the functional status of the $\alpha_{2b}$-AR in terms of its affinity to neurotransmitters. They do indicate, however, that adequate expression of normally functioning $\alpha_{2b}$-AR is a prerequisite for development of salt-induced hypertension. In contrast, subtotally nephrectomized mice with complete lack of the $\alpha_{2c}$-AR gene developed salt-induced hypertension to the same extent as their wild-type counterparts.

In a number of recent publications, it was suggested that the $\alpha_{2a}$-AR subtype, which is abundantly distributed throughout the CNS and highly concentrated in the brain stem, is directly involved in regulating sympathetic outflow. On the contrary, the $\alpha_{2b}$-AR is restricted only in a limited area of the CNS, namely the thalamus and the nucleus tractus solitarii area of the brain stem, but is abundant in the vascular smooth muscle cells of the arterial wall and mostly responsible for a peripheral vasoconstrictive action, whereas the $\alpha_{2c}$-AR have an “elusive, mysterious character” with no clearly defined function so far. A separate study of $\alpha_{2a}$-AR

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** HR at baseline and end point for each group of mice. Bar symbols as in Figure 1.

(104.3±2.71 versus 135.0±7.03 mm Hg, respectively; *P*<0.002). Comparison of end point tail-cuff SBP measurements with direct MAPs by regression analysis showed a close correlation between the 2 readings for all mice studied (*r*=0.746, *P*<0.001, *n*=36).

Other Parameters

Table 1 shows no differences between genetically altered mice and their wild-type counterparts.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Direct intra-arterial BP at end point for each group of mice. Bar symbols as in Figures 1 and 2. *P*<0.01 between genetically altered mice and their wild-type counterparts.

Body Weight, Days on 1% NaCl, and Plasma Creatinine Levels

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knockout mice versus their wild counterparts will be reported elsewhere (B.K. Kobilka, unpublished data).

The fact that mice lacking 1 copy of the α2α-AR gene are unable to develop salt-induced hypertension could have several potential interpretations: An obvious one is that lack of functional peripheral α2α-AR on the vascular wall diminishes the capacity of resistance arteries to constrict in response to adrenergic stimuli. This is unlikely, however, because catecholamines induce vasoconstriction mainly via stimulation of the α1α-AR, which constitute the majority of the vascular wall α2-AR.19 For this reason, selective α1α-AR antagonists like prazosin, terazosin, etc. have a major and consistent hypotensive effect, whereas α2α-AR antagonists (e.g., yohimbine) cause minimal vasodilation overshadowed by a centrally mediated hypertensive action.

A second possibility is that lack of adequately functional renal α2α-AR precluded reabsorption of sodium. The α2α-AR are the numerically predominant AR type in the kidney,20 and in rats they belong mostly to the α2α-AR subtype.21 Several investigators have proposed that increased sodium reabsorption leading to salt-induced hypertension is a function of renal α2α-AR,22–24 Therefore, it is possible that the α2α-AR–deficient animals were unable to retain sodium and, hence, never did attain a salt-loaded state. This possibility cannot be excluded without metabolic studies to calculate salt intake and output of each subgroup.

A third potential explanation is that lack of functional central α2α-AR may be responsible for inability to respond to salt loading with the expected increase in sympathetic outflow. Even though this report is rather a widely accepted effect,16 it is possible that the strategically located α2α-AR in the thalamus and brain stem17 may play a modulating role on the α2α-AR responses. In the absence of some physiological indicator of sympathetic activity, such as circulating catecholamine levels or nerve conduction studies, this possibility cannot be confirmed or refuted. Nevertheless, the lack of α2α-AR–mediated vasoconstriction would suggest absence of excessive catecholaminergic stimulation of CNS origin.

It is tempting to speculate on the potential significance of this finding in terms of genetic predisposition to salt sensitivity and essential hypertension. Although results from genetic epidemiological studies have so far been inconsistent, there have been suggestions that hypertensive African Americans may have a higher frequency of α2-AR gene polymorphisms.25,26 Further, aging was associated with diminished affinity status of α2-AR in elderly black normotensive subjects compared with their white counterparts or to age- and race-matched hypertensive subjects.27 These intriguing bits of information make it worth exploring whether genetic differences in α2α-AR subtype numbers, structure, or function or alterations due to aging and other factors are associated with differences in salt sensitivity in humans.

Acknowledgments

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References


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