Arterial Baroreceptor Reflex Function in Borderline Hypertensive Rats

Gerald F. DiBona and Susan Y. Jones

With increased dietary NaCl intake (8% NaCl), the borderline hypertensive rat develops hypertension, thus expressing the phenotype of the spontaneously hypertensive parent. Since arterial baroreceptor reflex function is impaired in the spontaneously hypertensive parent, it was the objective of this study to examine arterial baroreceptor reflex function in the borderline hypertensive rat made hypertensive by increased dietary NaCl intake. Borderline hypertensive rats were fed either 1% or 8% NaCl from age 4 to 16 weeks. Borderline hypertensive rats fed 8% NaCl (n=10) were hypertensive compared with borderline hypertensive rats fed 1% NaCl (n=11) (141±3 versus 120±4 mm Hg, p<0.01). They were chronically instrumented for the recording of arterial pressure, heart rate, and renal sympathetic nerve activity. The percent change from control in heart rate and renal sympathetic nerve activity resulting from increases (phenylephrine) and decreases (nitroglycerine) in arterial pressure were measured in conscious freely moving animals. With respect to arterial baroreceptor reflex control of heart rate, 8% NaCl borderline hypertensive rats had a similar range (75±4%) and maximal gain (−2.72±0.24%/mm Hg) as 1% NaCl borderline hypertensive rats (70±4%; −2.78±0.50%/mm Hg). With respect to arterial baroreceptor reflex control of renal sympathetic nerve activity, 8% NaCl borderline hypertensive rats had values for range (205±22%) and maximal gain (−3.92±0.93%/mm Hg) that were not significantly different from those for 1% NaCl borderline hypertensive rats (167±33%, −2.76±0.62%/mm Hg). Thus, in borderline hypertensive rats made hypertensive by increased dietary NaCl intake, the arterial baroreceptor control of both heart rate and renal sympathetic nerve activity is parallel shifted along the arterial pressure axis in proportion to the increased level of arterial pressure without changes in range or maximal gain. (Hypertension 1992;19:56–61)

The borderline hypertensive rat (BHR) is a genetic model of environmentally induced hypertension. The BHR is the first generation offspring of a mating between a spontaneously hypertensive rat (SHR) and a normotensive Wistar-Kyoto (WKY) rat and possesses genetic information from both a normotensive WKY and a hypertensive SHR parent. As described by Lawler and colleagues,1–5 the BHR become permanently hypertensive when subjected to a time-limited period of exposure to environmental stress or to increased dietary sodium intake. Renal denervation, performed early but not late, can prevent the development of environmental stress-induced hypertension in BHR.6

Increased dietary sodium intake causes the BHR to exhibit multiple characteristics of the phenotype of the hypertensive SHR parent. In addition to the development of sustained hypertension, BHR subjected to increased dietary sodium intake also manifest exaggerated natriuretic and renal sympathoinhibitory responses to intravenous isotonic saline loading,7 enhanced efferent renal sympathetic nerve activity (ERSNA) and antinatriuretic responses to environmental stress,7 and increased responsiveness of central nervous system α2-adrenergic receptors regulating ERSNA,8 each of which is similar to the response of the hypertensive SHR parent.8–12

Abnormalities of arterial baroreceptor reflex function have been described in SHR compared with WKY rats.13–20 Although there seems to be general agreement that arterial baroreceptor reflex control of heart rate (HR) is impaired in SHR, there is a diversity of opinion regarding the arterial baroreceptor reflex control of peripheral sympathetic nerve...
activity in SHR. From these studies in SHR and WKY rats, the parents of the BHR, it might be predicted that hypertensive BHR would have impaired arterial baroreceptor reflex control of HR but the status of arterial baroreceptor reflex control of (renal) sympathetic nerve activity would be difficult to predict. In this regard, a study by Lawler et al\(^{20}\) suggested that the arterial baroreceptor reflex control of HR in BHR was impaired by 11 but not 5 weeks of chronic stress.

The current study was performed to simultaneously examine the arterial baroreceptor reflex control of HR and ERSNA in conscious BHR fed either 1% NaCl or 8% NaCl for 12 weeks from age 4 to 16 weeks.

**Methods**

**Animals**

This study used male BHR which were the first generation offspring of SHR females and WKY males purchased from Taconic Farms, Germantown, N.Y. The rats were weaned at 4 weeks of age. Standard laboratory rat chow and tap water were available to all rats until the dietary regimens were instituted as described below.

**Anesthesia**

The rats were anesthetized with methohexital (Brevital, 20 mg/kg i.p. supplemented with 10 mg/kg i.v. as needed; Eli Lilly and Company, Indianapolis, Ind.).

**Procedures**

**Chronic catheterization.** The rats were instrumented with catheters (Tygon, Fisher Scientific Int., Chicago, Ill.) in the left carotid artery and jugular vein. The catheters were tunneled to the back of the neck, flushed and filled with heparinized saline (100 units/ml; Elkins-Sinn, Cherry Hill, N.J.), and plugged with stainless steel pins.

**Renal sympathetic nerve activity recording electrode.** The left kidney was exposed through a left flank incision via a retroperitoneal approach. With the use of a dissecting microscope (×25), a renal nerve branch from the aorticorenal ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a bipolar platinum wire (Cooner Wire Company, Chatsworth, Calif.) electrode. Renal sympathetic nerve activity was amplified (10,000–50,000 times) and filtered (low, 30; high, 3,000 Hz) with a bandpass amplifier (model P511, Grass Instrument Co., Quincy, Mass.). The amplified and filtered signal was channeled to an oscilloscope (model 5113, Tektronix, Inc., Beaverton, Ore.) and a polygraph (model 7DA, Grass) for visual evaluation, to an audio amplifier/loudspeaker (model AM 8 audio monitor, Grass) for auditory evaluation, and to a rectifying voltage integrator (model 7P10, Grass) and a frequency discharge counter (Scope Raster/Stepper model 140A, W-P Instruments, Inc., New Haven, Conn.). The integrated voltage, frequency discharge, and renal neurogram signals were displayed on the Grass polygraph. The quality of the renal sympathetic nerve activity signal was assessed by examining its pulse synchronous rhythmicity and by examining the magnitude of decrease in recorded renal sympathetic nerve activity during sinoaortic baroreceptor loading with an intravenous injection of norepinephrine (3 μg). The renal sympathetic nerve activity remaining after maximum inhibition following norepinephrine administration was similar to the background noise observed approximately 30 minutes postmortem; this value was subtracted from all experimental values of renal sympathetic nerve activity.

When an optimal renal sympathetic nerve activity signal was observed, the recording electrode was fixed to the renal nerve branch with a silicone cement (Wacker Sil-Gel 604, Wacker-Chemie, Munich, FRG). The electrode cable was then secured in position by suturing it to the abdominal trunk muscles. Finally, the electrode cable was exteriorized, and the flank incision was closed in layers.

**Experimental Protocol**

At 4 weeks of age the rats were randomly assigned to one of two dietary groups, 1% or 8% NaCl, with tap water drinking solution ad libitum. At 16 weeks of age, all rats underwent chronic catheterization and implantation of a renal sympathetic nerve activity recording electrode.

After allowing 24 hours for recovery from surgery, rats were returned to their home cages (31 cm L×36 cm W×15 cm H) which permitted free movement. An intravenous infusion (58 μl/min) of isotonic saline was then started and was allowed to continue throughout the duration of the experimental protocol. Four to 6 hours after habituation and the start of isotonic saline infusion, the arterial catheter was flushed and attached to a pressure transducer (model P23Db, Statham, Oxnard, Calif.). After stabilization of mean arterial pressure (MAP) and HR, the quality of the renal sympathetic nerve activity recording was again tested with an intravenous injection of norepinephrine (3 μg) as previously described to ensure the absence of noise due to mechanical movement, respiration, or HR. If the quality of the renal sympathetic nerve activity recording was the same as that observed when the electrode was implanted, then the experiment commenced.

After 15 minutes of control recording of MAP, HR, and ERSNA, a graded intravenous infusion of nitroglycerin was administered to lower MAP to approximately 50 mm Hg over a period of 3–5 minutes. The nitroglycerin infusion was stopped and MAP, HR, and ERSNA were allowed to return to control levels for a period of 30 minutes. Then, a graded intravenous infusion of phenylephrine was administered to raise MAP to approximately 200 mm Hg over a period of 3–5 minutes. The phenylephrine infusion was stopped and MAP, HR, and ERSNA were allowed to return to control levels for a period of 30 minutes. This protocol was then repeated in each animal.
HR was determined with a linear cardiotachometer (model 7P4, Grass) driven by the arterial pressure waveform. Data acquisition (MAP, HR, integrated ERSNA) was performed with an analog-to-digital converter (model DT2801, Data Translation Inc., Marlborough, Mass.) using LABTECH NOTEBOOK 4.2 software (Laboratory Technologies Corp., Wilmington, Mass.) and an IBM PC-XT computer. HR and ERSNA were expressed as percent change from control and plotted against MAP. The resulting sigmoidal curves were analyzed (SIGMAPLOT 4.0, Jandel Scientific, Corte Madera, Calif.) with a four parameter logistic regression equation

$$y=p_4+p_1/(1+\exp[p_2(x-p_3)])$$

where $y$ is percent change in HR or ERSNA and $x$ is MAP. The four parameters represent: $p_1$, range of change in $y$; $p_2$, coefficient for calculation of gain; $p_3$, midrange of curve; $p_4$, minimum value of $y$. The instantaneous gain at any value of MAP is calculated from the first derivative of the equation:

$$\text{set point gain (at control MAP)}=[p_2(x)-p_4]^2/p_1$$

and

$$\text{maximal gain (where MAP=}}p_3)=-(p_1)(p_2)/4$$

The values for the two runs in each animal were averaged to provide a single data point for that animal. For the single comparison between the two groups, 1% NaCl BHR and 8% NaCl BHR, the $t$ test was used, and $p<0.05$ was considered statistically significant.

Results

Twelve weeks of increased dietary NaCl intake increased MAP from 120±4 mm Hg in 1% NaCl BHR to 141±3 mm Hg in 8% NaCl BHR ($p<0.01$). The difference in HR (429±15 beats per minute in 1% NaCl BHR versus 381±12 beats per minute in 8% NaCl BHR) was not statistically significant. Body weights at the time of instrumentation were not significantly different, 410±8 g for 1% NaCl BHR and 428±7 g for 8% NaCl BHR.

Figure 1 represents a single run in a 1% NaCl BHR with a control MAP of 124 mm Hg and control HR of 395 beats per minute. Figure 1A shows the percent change in HR and Figure 1B shows the percent change in ERSNA as MAP was varied between approximately 50 and 200 mm Hg. The responses were generally sigmoidal with relative plateaus at the lowest and highest levels of MAP. The four parameters generated by the logistic regression equation are shown in each panel, and the group averages are presented in Table 1. These values were used to generate the group curves shown in Figures 2–5.

As illustrated in Figure 2, the arterial baroreceptor reflex curve for control of HR was shifted parallel to the right along the MAP axis in 8% NaCl BHR compared with 1% NaCl BHR. As seen in Table 1, the range and minimum values were not different between the two groups. MAP at midrange, $p_3$, was slightly but not significantly higher than the control level of MAP (set point) for both groups. MAP at midrange, $p_3$, was significantly higher in 8% NaCl BHR than 1% NaCl BHR. The intergroup difference for the MAP at midrange (19 mm Hg) was similar to the intergroup difference for MAP at set point (21 mm Hg). The values for instantaneous and maximal gain (Figure 3, Table 1) were not significantly different between the two groups.
TABLE 1. Summary of Data on Arterial Baroreceptor Reflex Control of Efferent Renal Sympathetic Nerve Activity and Heart Rate in Borderline Hypertensive Rats Fed Either 1% or 8% NaCl

<table>
<thead>
<tr>
<th>Variable</th>
<th>1% NaCl</th>
<th>8% NaCl</th>
<th>1% NaCl</th>
<th>8% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>p1 (%)</td>
<td>167±33</td>
<td>205±22</td>
<td>70±5</td>
<td>75±4</td>
</tr>
<tr>
<td>p3 (mm Hg)</td>
<td>120±5</td>
<td>138±3*</td>
<td>129±6</td>
<td>148±1*</td>
</tr>
<tr>
<td>p4 (%)</td>
<td>-89±10</td>
<td>-95±9</td>
<td>-56±5</td>
<td>-55±4</td>
</tr>
<tr>
<td>Max gain</td>
<td>-2.76±0.62</td>
<td>-3.92±0.93</td>
<td>-2.78±0.50</td>
<td>-2.72±0.24</td>
</tr>
</tbody>
</table>

ERSNA, efferent renal sympathetic nerve activity; HR, heart rate; n, number of rats; p1, range; p3, midrange; p4, minimum value; Max gain, maximal gain.

*p<0.05 compared with respective 1% NaCl value.

As illustrated in Figure 4, the arterial baroreceptor reflex curve for control of ERSNA was shifted to the right along the MAP axis in 8% NaCl BHR compared with 1% NaCl BHR. As shown in Table 1, the range and minimum values, although higher in 8% NaCl BHR than in 1% NaCl BHR, were not statistically significantly different. MAP at midrange, p3, was not significantly different from the control level of MAP (set point) for both groups. MAP at midrange, p3, was significantly higher in 8% NaCl BHR than 1% NaCl BHR. The intergroup difference for the MAP at midrange (18 mm Hg) was similar to the intergroup difference for MAP at set point (21 mm Hg). The values for instantaneous and maximal gain (Figure 5, Table 1), although slightly greater in 8% NaCl BHR, were not significantly different from those in 1% NaCl BHR.

Discussion

The present studies demonstrated that the increase in arterial pressure produced in BHR by 12 weeks of increased dietary NaCl intake (8% NaCl from age 4 to 16 weeks) results in a parallel resetting of the arterial baroreceptor reflex control of HR and ERSNA proportional to the higher level of arterial pressure (upward shift of the set point) when compared with age-matched normotensive BHR consuming 1% NaCl. The extent of resetting, indicated by the similarity between the increase in the value for MAP at midrange (p3) of 18-19 mm Hg and the increase in control MAP (set point) of 21 mm Hg, was essentially complete. Furthermore, the values for range, minimal value, and instantaneous and maximal gain were not different between BHR fed 1% NaCl and 8% NaCl for either HR or ERSNA.

These studies may be contrasted with those reported by Lawler et al, who examined the changes in HR in response to increases in MAP in conscious chronically stressed BHR. In the BHR subjected to stress, the curves relating pulse interval to MAP were shifted to the right (shift in set point) along the MAP axis compared with the control BHR. The slope of the line relating interbeat interval to MAP (msec/mm Hg) was 0.336 in control BHR, 0.278 in BHR stressed for 5 weeks, and 0.263 in BHR stressed for 11 weeks. Visual inspection of the figures suggests
that basal MAP in the control BHR was approximately 130 mm Hg and increased to approximately 135 and 142 mm Hg in BHR stressed for 5 and 11 weeks, respectively. The authors concluded that chronic stress in a genetically predisposed animal produces a gradual decrease in the sensitivity (reduction of the gain) of arterial baroreceptor reflex control of HR. There are several differences between the two studies that may account for the apparent discrepancy. Lawler et al. used chronic tail shock stress to increase MAP in BHR; these authors examined the cardiac portion of the arterial baroreceptor reflex in response to increases in MAP using bolus injections of angiotensin II. With respect to the current studies, increased dietary NaCl intake was used to increase MAP in BHR, and the cardiac portion of the arterial baroreceptor reflex was examined during both ramp increases (phenylephrine) and decreases (nitroglycerine) in MAP. In addition, since the baroreceptor reflex control of HR is not a uniformly valid index of baroreceptor reflex control of peripheral sympathetic nerve activity,23 the (renal) sympathetic neural portion of the arterial baroreflex was also examined during similar changes in MAP.

BHR made hypertensive by increased dietary NaCl intake differ from their hypertensive SHR parent in respect to the arterial baroreceptor reflex control of HR. Arterial baroreceptor reflex control of HR is impaired in SHR.13,14 This impairment in arterial baroreceptor reflex control of HR is dependent on the development of hypertension since prevention of the hypertension with captopril is associated with an increase in arterial baroreceptor sensitivity in comparison with age-matched untreated SHR.24 Increased dietary NaCl intake in the BHR results in the expression by the BHR of several manifestations of the phenotype of the hypertensive SHR parent. These include the development of hypertension,5,7,8 an exaggerated natriuretic response to volume expansion,7 augmented responses to acute environmental stress,7 and enhanced responsiveness of central nervous system €-adrenergic receptors governing ERSNA.6 However, the current studies demonstrate that the development of an impaired arterial baroreceptor reflex control of HR does not accompany the induction of hypertension by increased dietary NaCl intake in BHR. Thus, the dietary NaCl intake induced expression in the BHR of manifestations of the phenotype of the hypertensive SHR parent does not appear to be nonspecific.

There is a divergence of opinion concerning the arterial baroreceptor reflex control of peripheral sympathetic nerve activity in SHR. For example, some find the arterial baroreceptor reflex control of renal sympathetic nerve activity to be normal in SHR,13,17 whereas others find it to be abnormal.15,16 With regard to other peripheral sympathetic nerves, arterial baroreceptor reflex control of lumbar sympathetic nerve activity has been found to be abnormal in SHR,19 whereas arterial baroreceptor reflex control of splanchnic nerve activity is reported to be normal or even hyperresponsive to changes in MAP in stroke-prone SHR.18 It is possible that this diversity of opinion can be accounted for by differences in experimental variables (e.g., anesthetized versus conscious preparations, different nerves with varying contributions of preganglionic and postganglionic fiber elements, multifiber versus single fiber recordings, and bolus versus ramp administration of agents that alter MAP). In the current studies, the arterial baroreceptor reflex control of ERSNA was slightly augmented in 8% NaCl BHR compared with 1% NaCl BHR. Although the differences did not achieve statistical significance, the values for range and max-

**Figure 4.** Line graph shows percent change from control in efferent renal sympathetic nerve activity (% Delta ERSNA) versus mean arterial pressure (MAP) in borderline hypertensive rats (BHR) fed 1% NaCl for 12 weeks (n=11) and in BHR fed 8% NaCl for 12 weeks (n=10).

**Figure 5.** Line graph shows gain for baroreceptor reflex control of efferent renal sympathetic nerve activity (% Delta ERSNA) versus mean arterial pressure (MAP) in borderline hypertensive rats (BHR) fed 1% NaCl for 12 weeks (n=11) and in BHR fed 8% NaCl for 12 weeks (n=10).
imum gain (Table 1) were greater in 8% NaCl BHR than in 1% NaCl BHR. These results do not support the view that the arterial baroreceptor reflex control of ERSNA is impaired in BHR with hypertension induced by increased dietary NaCl intake for 12 weeks.

In summary, hypertension induced in BHR by 12 weeks of increased dietary NaCl intake (8% NaCl) is associated with a parallel upward resetting along the MAP axis of the arterial baroreceptor reflex control of both HR and ERSNA without any change in range or gain. These results suggest that the induction of hypertension by increased dietary NaCl intake in BHR is not dependent on a primary abnormality in arterial baroreceptor function. We cannot state if a prolonged period of hypertension would result in the appearance of impaired arterial baroreceptor reflex control of HR and ERSNA in BHR. However, if this were the case, this would indicate that the impairment of arterial baroreceptor function was a secondary consequence of the hypertension.

References