Abnormal Baroreflex Control of Heart Rate in Prehypertensive and Hypertensive Dahl Genetically Salt-Sensitive Rats

FRANK J. GORDON, PH.D., HIDEYO MATSUGUCHI, M.D., AND ALLYN L. MARK, M.D.

With the technical assistance of DONALD A. MORGAN

SUMMARY This study tested the hypothesis that in the Dahl model of genetic hypertension abnormal baroreflex function may precede and contribute to the development of hypertension. Sensitivity of baroreflex control of heart rate was assessed in conscious sensitive (S) and resistant (R) Dahl rats fed a high or low salt diet. Sensitivity of baroreflex control of heart rate was lower in S rats fed low salt although arterial pressure was not different from that of R rats. Hypertensive S rats showed resetting of baroreflexes to the higher pressure level without any further change in sensitivity. Pressor responses to phenylephrine were augmented in both prehypertensive and hypertensive S rats compared to R rats. Ganglionic blockade abolished this difference. In hypertensive S rats, ganglionic blockade decreased arterial pressure to levels not different than similarly treated R rats, indicating that neurogenic mechanisms contribute importantly to the early stages of hypertension in the Dahl model. These studies have identified impaired baroreflex function in prehypertensive and hypertensive Dahl S rats. This defect augments responses to pressor stimuli and may contribute to the development of salt-induced hypertension in the Dahl strain. (Hypertension 3 (supp I): 1-135—1-141, 1981)

KEY WORDS • Dahl rats • baroreflex • heart rate • genetic hypertension • pressor response • ganglionic blockade

ABNORMALITIES in the baroreflex control of heart rate have been identified in both experimental and human hypertension.1-4 These abnormalities are generally thought to occur as a result of elevated arterial pressure and consequent decreases in arterial distensibility.5-6 However, it is possible that a genetic defect in baroreflex function could precede and contribute to hypertension.

The purpose of the present study was to examine baroreflex control of heart rate in the Dahl strain of genetically salt-sensitive rats in both the prehypertensive and hypertensive stages. There are two specific reasons for studying baroreflex mechanisms in Dahl rats. First, neurogenic mechanisms contribute importantly to the development of vasoconstriction and hypertension in Dahl rats.7-8 Abnormalities in baroreflex control might play a role in these neurogenic alterations. Second, while Dahl S rats are genetically predisposed to develop hypertension when fed a high sodium diet, they remain normotensive when maintained on a low sodium diet. Thus, unlike other genetic models of hypertension, such as the spontaneously hypertensive rat, genetic factors that might contribute to hypertension in the Dahl strain can be studied in a well-defined prehypertensive stage. This affords an opportunity to examine baroreflex mechanisms in hypertension-prone animals prior to the development of hypertension.

Methods

Animals

The animals used in this study were female Dahl salt-sensitive (S) and salt-resistant (R) rats obtained from Brookhaven National Laboratories, Upton, New York. The rats were fed either a low or high NaCl diet (ICN Pharmaceutical) from a few days post-weaning until the time of experimentation. The low and high salt diets contained 0.4% and 8.0% NaCl per unit of dry weight respectively. Potassium chloride content was 1.3% for both diets.
Baroreflex Control of Heart Rate

Dahl rats were anesthetized with Equithesin (2.1 ml/kg, i.p.; Jensen-Salsbery) for insertion of indwelling arterial and venous catheters for measurements of arterial pressure and injection of drugs respectively. The arterial catheter (PE 50) was threaded via a femoral artery into the lower abdominal aorta so that its tip resided 1 to 2 cm below the bifurcation of the renal arteries. A femoral vein was also catheterized (PE 10). The catheters were then tunneled subcutaneously to exit from the skin between the scapulae, filled with 200 U/ml heparin-saline, and occluded with a short piece of 23-gauge wire. The catheters were flushed 24 hours later to maintain patency.

Two days after surgery, baroreflex control of heart rate was studied in conscious freely-moving rats. The arterial catheter was connected to a transducer (Statham P231a) for arterial pressure recording. Heart rate (beats/min) was measured by a carotid tonometer (Beckman 9857B) triggered from the arterial pulse pressure signal, and converted to pulse interval (msec) by the equation 60,000/heart rate.

The venous catheter was filled with saline (0.9%), or a solution of phenylephrine (Neo-Synephrine, Winthrop) or nitroglycerin (Nitrostat, Parke-Davis) with the concentration adjusted for each rat according to body weight. Graded doses of phenylephrine (0.25 to 4.0 μg/kg) and nitroglycerin (5 to 40 μg/kg) were administered by bolus injections of 2.5 to 40 μl to raise or lower arterial pressure. The order in which the two drugs were given was randomized between rats. A 40 μl injection of saline was employed as the control condition. Saline injection had no appreciable effect on blood pressure (BP) or heart rate.

The rats were placed in a test chamber (20 × 20 × 30 cm), and extraneous room noise was masked by an 80 db white noise source. The rats were allowed at least 30 minutes to stabilize; resting measurements for BP and heart rate were recorded, and the drug injection protocols were begun. Peak pressor and depressor responses and the associated reflex changes in heart rate were recorded for each drug dose. Repeated drug injections were not made until heart rate was within 15 beats/min of the initial resting value, and arterial pressure had returned to baseline. The slope (gain) of the baroreflex control of heart rate (Δ pulse interval (PI)/Δ mean arterial pressure (MAP)) was determined for each rat by fitting a linear regression line through the points Δ PI vs Δ MAP over a wide range of pharmacologically-induced arterial pressure alterations (−30 to +60 mm Hg). Baroreflex slopes were determined separately for increases and decreases in arterial pressure produced by phenylephrine and nitroglycerin respectively. The linear correlation coefficients (mean ± SEM) between Δ pulse interval and Δ mean arterial pressure for phenylephrine injections were 0.98 ± 0.01 and 0.96 ± 0.01 for R and S rats respectively. The linear correlation coefficients for nitroglycerin injections were 0.91 ± 0.04 and 0.94 ± 0.02 for R and S rats.

Experimental Groups

Three groups of Dahl S and R rats were studied. The first group of rats was fed low salt diet for 4 weeks, and during the fifth week baroreflex control of heart rate was determined in conscious animals, as described. The second group was fed the low salt diet, and the third high salt diet, for 4 weeks, and baroreflex control of heart rate examined. After baroreflex function had been determined for these latter two groups, 20 mg/kg chlorisondamine (Ecolid, CIBA) was administered intravenously to the unanesthetized rats over a period of 5 minutes to produce autonomic ganglion blockade. All doses of phenylephrine as well as the largest dose of nitroglycerin were administered again following ganglionic blockade. Ganglionic blockade was employed in these studies for three reasons: 1) to demonstrate that the heart rate response to phenylephrine and nitroglycerin were neurogenically mediated; 2) to assess pressor responses to phenylephrine in the presence and absence of autonomic buffering influences; 3) to determine the extent of the neurogenic contribution to arterial pressure in hypertensive S rats.

Statistical Analysis

Statistical analyses were performed by analysis of variance for factorial experiments or Student's t test for orthogonal pair-wise mean difference tests. Significance statements for dose-response curves relate to differences between groups over the range of doses tested. Numerical values cited in the text refer to the mean ± SEM.

Results

The MAP and heart rate for Dahl R and S rats fed low salt diet (Group 1) were not significantly different (R = 95 ± 4 vs S = 100 ± 4 mm Hg; table 1). Figure 1 plots the relationship between the changes in arterial pressure produced by phenylephrine and nitroglycerin and the associated reflex mediated alterations in pulse interval for R and S rats in Group 1. Although reflex tachycardia produced by nitroglycerin (shown as a decrease in pulse interval) was the same for both R and S rats, Dahl S rats demonstrated a blunted bradycardic response for equivalent increases in arterial pressure. For statistical analysis, the slope of the relationship between Δ pulse interval (msec)/Δ mean arterial pressure (mm Hg) was derived for individual R and S rats in each group, and the group means of the individual slopes were compared. The slope for pulse interval prolongation when arterial pressure was raised with phenylephrine was significantly (p < 0.01) lower in S than in R rats (R = 1.63 ± 0.12 vs S = 1.07 ± 0.08 msec/mm Hg). The slope of pulse interval shortening during decreases in arterial pressure produced by nitroglycerin was not different for the two groups (R = 1.31 ± 0.17 vs S = 1.45 ± 0.27 msec/mm Hg). Conscious S rats also demonstrated significantly (p < 0.001) greater pressor...
responses to phenylephrine (table 1). The depressor responses to intravenous injections of nitroglycerin were not different for R and S rats. In the second group of R and S rats fed the low salt diet for 4 weeks, baroreflex slopes of conscious animals during phenylephrine were 1.53 ± 0.14 msec/mm Hg for R rats and 0.96 ± 0.07 msec/mm Hg for S rats (p < 0.01). In this group of rats, pressor responses to phenylephrine were compared before and after ganglionic blockade produced by intravenous infusion of 20 mg/kg chlorisondamine. Ganglionic blockade virtually eliminated reflex bradycardic responses produced by increases in arterial pressure. Before chlorisondamine administration, heart rate decreased more than 100 beats/min for both R and S rats injected with the highest dose of phenylephrine (4.0 µg/kg). After ganglionic blockade, pressor responses to phenylephrine were again observed (fig. 2), but heart rate decreased by only −6 ± 1 and −4 ± 5 beats/min in R and S rats respectively for the 4 µg/kg dose of phenylephrine. As seen in figure 2 left, phenylephrine produced significantly greater pressor responses in prehypertensive Dahl S rats compared to R rats before ganglionic blockade (p < 0.001), replicating the results of the previous experiment (table 1). After ganglionic blockade, the pressor action of phenylephrine was not different between the two groups (fig. 2 right). Thus, removing the reflex buffering influence of the autonomic nervous system by peripheral ganglionic blockade eliminated the difference in pressor responses to phenylephrine between Dahl S and R rats.

When Dahl rats were fed the high salt diet for 4 weeks, S rats became significantly hypertensive (p < 0.001) without a significant change in heart rate (fig. 3). Like S rats on the low salt diet, hypertensive S rats fed the high salt diet had a significant (p < 0.001) deficit in phenylephrine associated reflex bradycardia (R = 1.84 ± 0.12 vs S = 1.11 ± 0.06 msec/mm Hg; fig. 3). The baroreflex slope derived from the relationship between hypotension produced by nitroglycerin and the reflex mediated decrease in pulse interval was not different for R and S rats fed the high salt diet. The slope (msec/mm Hg) for R rats was

Figure 1. Scatter plot of the relationship between peak changes in mean arterial pressure produced by intravenous bolus injections of phenylephrine, nitroglycerin, and saline plotted against reflex mediated changes in pulse interval for conscious Dahl R and S rats fed low salt diet. Linear regression equations were fit through these points for Dahl R rats (solid lines) and Dahl S rats (broken lines). Regression lines were calculated separately for phenylephrine (upper right), and nitroglycerin (lower left) provoked changes in arterial pressure. Dahl S rats demonstrated less lengthening of pulse interval as shown by the distribution of the open circles and the decreased slope of the broken regression line. Nitroglycerin produced similar hypotension and decreases in pulse interval for both groups. The number of animals in each group is shown in parentheses.
**Figure 2.** Pressor responses (mean ± SEM) of Dahl R and prehypertensive Dahl S rats to graded doses of phenylephrine before and after ganglionic blockade produced by intravenous infusion of 20 mg/kg chlorisondamine. Left graph: Before ganglionic blockade, S rats had significantly greater pressor responses than R rats (p < 0.001). Right graph: After ganglionic blockade, the pressor responses of R and S rats were not different. The number of animals in each group is shown in parentheses.

**Figure 3.** Mean arterial pressure, heart rate, and baroreflex slope (mean ± SEM) for Dahl R and S rats fed high salt diet for 4 weeks. Dahl S rats became significantly hypertensive (p < 0.001) on the high salt diet and demonstrated a significant (p < 0.001) decrease in the slope (sensitivity) for reflex bradycardia produced by increasing arterial pressure with phenylephrine. Basal heart rate for R and S rats was not different. The number of animals in each group is listed in the bars.

**Figure 4.** Baroreflex slope for Dahl R and S rats fed either a low salt diet (left graph) or high salt diet (right graph) for 4 weeks. Black circles represent for Dahl R rats the peak arterial pressure and pulse interval (mean ± SEM) resulting from intravenous bolus injections of saline and five doses of phenylephrine. Black solid lines represent the least squares linear regression equations fit through these points. The open circles and broken lines depict these data for Dahl S rats. Dahl S rats fed the low salt diet (left) had a decreased slope (sensitivity) for baroreflex control of pulse interval. Dahl S rats fed the high salt diet (right) showed a parallel shift to the right of the function relating pulse interval to arterial pressure without any further change in slope when compared to S rats fed low salt diet. Baroreflex function was not different for Dahl R rats fed either low or high salt diet. The number of animals in each group is shown in parentheses.
BAROREFLEXES IN DAHL HYPERTENSION/Gordon et al.

Discussion

The principal new finding of this study is that the sensitivity of the baroreflex control of heart rate is impaired in Dahl S rats prior to the development of hypertension. Resetting and impairment of baroreflexes in hypertension have generally been attributed to increases in arterial pressure and attendant decreases in arterial distensibility. However, these functions may not account for the impairment of baroreflexes in the Dahl S rats fed low salt for the following reasons. First, arterial pressure was not significantly different between R and S rats fed low salt diet. Second, previous studies have shown that vascular resistance during maximal vasodilation in perfused hindquarter and renal beds is not different between R and S rats fed low salt diets, indicating a lack of significant structural changes in resistance vessels in S rats compared to R rats.

1.16 ± 0.18 and for S rats, 1.33 ± 0.14. Depressor responses produced by nitroglycerin were also not different between the two groups.

Hypertensive S rats demonstrated increased pressor responses to phenylephrine (p < 0.001), and, as with the normotensive animals, this difference was eliminated by ganglionic blockade.

To compare baroreflex control of heart rate in normotensive and hypertensive Dahl rats, the data from the first group fed the low salt diet as well as the rats fed the high salt diet were plotted in figure 4. The six points for each group of rats represent the mean (± SEM) of the peak level of arterial pressure produced by five doses of phenylephrine plotted against the mean (± SEM) pulse interval associated with these changes in BP. The lowest point on each curve represents the values associated with the saline control injection.

Dahl S rats fed the low salt diet had arterial pressures that were not significantly different than those of R rats (table 1). However, the slope (sensitivity) for baroreflex control of heart rate was decreased. Baroreflex slopes of R and S rats were not changed when these rats were fed the high salt diet, but the function relating pulse interval to arterial pressure in the hypertensive S rats was shifted to the right, indicating that the baroreceptors in the hypertensive animals had been reset to a higher pressure level without any further change in sensitivity.

As seen in figure 5, ganglionic blockade produced a profound fall in arterial pressure in both R and S rats fed high salt. Before ganglionic blockade, S rats were significantly hypertensive (p < 0.001). After ganglionic blockade, arterial pressure was not significantly different for R and S rats. Furthermore, removal of neural vasoconstrictor tone by autonomic blockade produced not only a greater absolute fall in arterial pressure in hypertensive S rats (S = -63 ± 4 mm Hg vs R = 45 ± 1 mm Hg; p < 0.001) but also a significantly greater percent decrease in BP (R = -46 ± 2% vs S = -52 ± 2%; p < 0.05).

These observations suggest that the impairment of baroreflexes in Dahl S rats is not related to the level of arterial pressure or structural changes in small blood vessels, but they do not exclude the possibility that large arteries may be less distensible in S rats. Since rapid reflex bradycardia in the rat is mediated almost exclusively by vagal activation, 11 we examined in other experiments sinus node responses to direct cholinergic stimulation. Methacholine-produced cardiac slowing was not significantly different between strains, and, if anything, was somewhat greater in S than R rats. This observation suggests that a decreased cholinergic sensitivity of the heart in S rats cannot explain the attenuated bradycardia observed during reflex stimulation. The most likely explanation for the defect in baroreflex sensitivity observed in S rats is that this abnormality is of neural rather than nonneural origin.

Before discussing the significance of the abnormality in baroreflex mediated bradycardia associated with increases in arterial pressure, we should note that differences in baroreflex mediated tachycardia between R and S rats were not found when arterial pressure was lowered by nitroglycerin injections. Why baroreflex abnormalities appeared only when arterial pressure was raised and not lowered is not immediately apparent. It is possible that differences in the relative magnitude of the maximum BP changes induced by phenylephrine and nitroglycerin (+60 vs -30 mm Hg) contributed to this difference, or that vessel wall geometry and thus the stretch exerted on baroreceptor nerve endings differs when arterial pressure changes in opposite directions from baseline levels. Another contributing factor might be the activation of the efferent autonomic mechanisms con-
trolling heart rate (sympathetic vs parasympathetic) are different for R and S rats, and that abnormal baroreflexes are revealed only when arterial pressure is raised and not lowered.

Systemic pressor responses to phenylephrine were greater in Dahl S than R rats when these animals were fed either low or high salt diet. This difference was eliminated when the reflex buffering influence of the autonomic nervous system was removed by ganglionic blockade. These results indicate that the defect in baroreflex buffering in S rats was responsible for their enhanced pressor responsiveness to phenylephrine.

This observation may also resolve the paradoxical findings obtained when vascular responses have been assessed by different methods. Early investigations by Dahl et al. showed that intact ether-anesthetized S rats were more sensitive to the pressor action of intravenously injected norepinephrine and angiotensin than were R rats. Later studies indicated that vascular reactivity was similar for R and S rats when various vasoconstrictor agents were tested in isolated perfused vascular beds or in vitro arterial smooth muscle preparations. The major methodological difference between these types of studies is that, in the intact animal, baroreflex modulation of systemic pressor responses remains operative, whereas in isolated preparations the nervous system cannot buffer direct vasoconstrictor effects. Thus, the divergent outcomes described above are analogous to the results obtained in our present experiments when pressor responses to phenylephrine were examined both before and after autonomic ganglion blockade. These observations, taken as a whole, suggest that: 1) the sensitivity of vascular muscle is not increased in Dahl S rats; and 2) the augmented responses to systemic injection of pressor agents in S rats results from impairment in baroreflex buffering capacity.

Impairments in baroreflex control have been shown to accompany both human and experimental hypertension. These abnormalities have generally been thought to be secondary to hypertension rather than contributing to its genesis. The results of the present study indicate that Dahl S rats, which are genetically predisposed to develop hypertension, have impaired baroreflex control in the prehypertensive stage, suggesting that this defect may contribute to their propensity to develop hypertension. The functional consequence of impaired baroreflex buffering was revealed in the experiments which examined pressor responses to phenylephrine. Pressor responses were greater in prehypertensive S rats, and it was shown that this was the result of their failure to adequately buffer the direct vasoconstrictor action of the drug. We have observed increased pressor responses in S rats for exogenously administered norepinephrine and vasopressin as well as phenylephrine. These results suggest that for any pressor stimulus S rats will respond with a greater increase in arterial pressure. Such enhanced responsiveness might accelerate secondary pathological changes in blood vessels and/or renal function in the face of excess sodium, contributing to the development of sustained hypertension.

Although abnormalities in baroreflex control may contribute to or exacerbate hypertension in Dahl S rats, this defect cannot be a sufficient condition or sole cause for hypertension since S rats maintained on a low salt diet remain normotensive. However, impaired baroreflex control in S rats would tend to interact with other hypertensinogenic stimuli. This possibility is supported by the results of other investigators who have shown augmentation of several forms of experimental hypertension in Dahl S rats.

When arterial baroreflexes have been assessed in hypertension, the curve relating reflex activity to arterial pressure is reset to a higher pressure. Dahl S rats fed the high salt diet for 4 weeks developed hypertension. The baroreflex curve for these rats was shifted to the right, indicating that the baroreceptors had been reset to the higher pressure level. No significant difference in the baroreflex slope was found for S rats fed a high salt diet compared to normotensive S rats fed a low salt diet, indicating that baroreflex sensitivity was not changed in the early stage of salt-induced hypertension.

In Dahl S and R rats fed a low salt diet with comparable levels of arterial pressure, the sensitivity for baroreflex control of heart rate was diminished. This result indicates that abnormalities in baroreflex control of heart rate in S rats occurs independent of hypertension. This finding also suggests that this defect may be of genetic origin rather than being secondary to elevated arterial pressure. Brown and colleagues have indicated baroreceptor abnormalities may also occur in the absence of structural vascular alterations in another form of genetic hypertension, the spontaneously hypertensive rat. These observations raise the possibility that alterations in baroreflex function might contribute to the development of genetic hypertension in the Dahl strain.

Initial studies of the Dahl strain implicated humoral and renal factors in the pathogenesis of salt-induced hypertension. More recent investigations have shown that neurogenic mechanisms play an important role in Dahl hypertension since destruction of the peripheral sympathetic nervous system prevents the development of hypertension, and neurogenic vasoconstrictor tone makes a large contribution to vascular resistance in the early stages of hypertension in this model. In addition, the central nervous system may contribute to hypertension in the Dahl strain since centrally-mediated pressor responses are greater in S than R rats.

The present study strengthens the concept that neurogenic factors contribute importantly to the early stages of Dahl hypertension. We assessed the neurogenic contribution to elevated arterial pressure in the Dahl model of hypertension by interrupting sympathetic neural transmission with ganglionic blockade. When neural vasoconstrictor tone was eliminated by ganglionic blockade in conscious Dahl R and S rats fed a high salt diet, MAP fell to levels that were not different for the two groups. This result indicates that the elevated BP of Dahl S rats fed a high salt diet was maintained in large measure by neuro-
genic factors. A recent report by Touw et al. proposes that it may be important to consider the percentage as well as absolute change in BP produced by ganglionic blockade to determine if a greater proportion of the total level of arterial pressure is maintained by neural factors in hypertensive animals. In this regard, ganglionic blockade in hypertensive Dahl S rats produced both greater absolute and percent decreases in BP when compared to R rats fed the same high salt diet.

In summary, the results of our experiments show that the sensitivity for baroreflex control of heart rate is impaired in prehypertensive Dahl S rats, suggesting that this defect may be of genetic origin rather than a result of elevated arterial pressure. Furthermore, baroreflex abnormalities appear to account for the increased pressor responsiveness of Dahl S rats and may contribute toward their propensity to develop hypertension. Finally, removal of neurogenic vasoconstrictor tone in hypertensive Dahl S rats reduced arterial pressure to levels not different than similarly treated R rats. These results support the view that neurogenic factors contribute importantly to early hypertension in the Dahl strain.

Acknowledgments

We thank Dr. Junichi Iwai and David McChesney for kindly providing the animals used in this study, Jan Ellsworth for secretarial assistance, and Linda Godfrey for preparing the illustrations.

References

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F J Gordon, H Matsuguchi and A L Mark

_Hypertension_. 1981;3:I135
doi: 10.1161/01.HYP.3.3_Pt_2.I135

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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