Baroreceptor Function and Changes in Strain Sensitivity in Normotensive and Spontaneously Hypertensive Rats

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SUMMARY Baroreceptor resetting during hypertension has been attributed to a reduction in the distensibility of the vessel wall in which the receptors are located. According to this hypothesis, a simple increase in pressure is all that is required to overcome the increase in vessel wall stiffness. However, previous work from our laboratory suggested a more complicated situation. Measurements of both vessel wall mechanical properties and baroreceptor discharge characteristics in spontaneously hypertensive rats (SHR) showed that distortion thresholds for the receptors also undergo changes. Here we have examined the time course of resetting, measuring both aortic distensibility and baroreceptor properties in SHR and normotensive Wistar-Kyoto rats (WKY) from 5 to 30 weeks in age. An in vitro aortic arch-aortic nerve preparation was used. We found that for a given pressure the aortic radii of WKY were increasing much more rapidly than the aortic radii for SHR and, beyond 5 weeks of age, were much more distensible. The lower distensibility in SHR was accompanied by increased wall thickness. The discharge characteristics of single baroreceptors were expressed in terms of both pressure and distortion or circumferential wall strain. The change in distensibility of WKY aortas from 5 to 30 weeks was suitably matched by an increase in the strain threshold for discharge of WKY baroreceptors resulting in a constant pressure threshold for discharge. The lower distensibility of SHR aortas was accompanied by lower threshold strains in SHR baroreceptors, but the changes were not suitably matched, and progressive resetting of SHR baroreceptors to higher threshold pressures occurred. The two sets of receptors appear to be different as early as 5 weeks of age when blood pressures are similar and, furthermore, these differences are accentuated by age and hypertension.


ARTERIAL baroreceptor function is abnormal in all forms of established hypertension; greater distending pressures are required to activate the receptors, and the suprathreshold sensitivity to pressure changes is reduced (McCubbin et al., 1956). Since distensibility of the vessel wall in which the receptors are located is reduced at this time, the resetting of baroreceptor function to higher operating pressures has been attributed to the confinement or “splinting” of receptors within a stiffer vessel (Angell-James, 1973; Nosaka and Wang, 1972; Sapru and Wang, 1976; Kedzi, 1976; Jones, 1977).

Investigations of the time course of baroreflex resetting during experimental renal hypertension indicate that the threshold pressure and/or sensitivity for reflex activation can shift within hours to days of the onset of hypertension (Krieger, 1970; Jones and Floras, in press). These changes are faster than might be expected for changes in vessel wall composition (Jones, 1977) and suggest that some other mechanism might be responsible for the early phase of baroreflex resetting.

Previous work in our laboratory suggested that resetting in the initial stages of hypertension in spontaneously hypertensive rats (SHR) may actually be due to intrinsic differences in the SHR baroreceptors themselves (Andresen et al., 1978). Here, we examine the changes between 5 and 30 weeks of age of aortic mechanical properties and baroreceptor function in normotensive rats [Wistar-Kyoto strain (WKY)] and in SHR. We found that differences in transduction between SHR and WKY baroreceptors appear as early as 5 weeks and that these differences are accentuated by development and hypertension leading to the familiar resetting of SHR baroreceptors with increased baroreceptor pressure threshold and reduced suprathreshold sensitivity. WKY baroreceptors maintain a constant threshold pressure for activation during a period of great growth of the aorta, by matching the increasing vessel caliber and distensibility with progressive increases in the vessel wall distortion required for activation. SHR baroreceptors, by contrast, are more sensitive to distortion, and are activated by smaller strains than those of WKY but are not as well matched to the changing vessel wall properties. Thus mechanotransduction in SHR baroreceptors...
is abnormal at the earliest of ages and remains abnormal throughout development so that simple splinting of the receptors is not solely responsible for SHR resetting. In the accompanying paper, we hypothesize that abnormal throughout development so that simple splinting of the receptors is not solely responsible for SHR resetting. In the accompanying paper, we hypothesize that the abnormal development of the baroreceptors is responsible for the abnormal blood pressure regulation in SHR. Antihypertensive drug therapy held at normotensive levels by antihypertensive drugs to maintain blood pressure (BP) during development was analyzed to determine the role of the baroreceptors in blood pressure regulation.

Methods

Sixty normotensive rats (WKY) served as controls for sixty SHR (Okamoto-Aoki strain), all of which were obtained from Taconic Farms, Inc. The rats were male and 4 weeks of age upon receipt. Both groups were housed in the same room and fed the same rat chow diet.

Beginning at 5 weeks of age, tail blood pressures were measured in warmed unanesthetized animals by the indirect, tail cuff method. Measurements were made on a rotating basis so that the blood pressure of each rat was measured once each 2 weeks. Animals were weighed weekly. For experiments, rats were chosen at random from each group.

Five age groups were used for study: 5 weeks, 8 weeks, 14 weeks, 20 weeks, and 30 weeks. Experiments were conducted starting 5 days before these age levels were reached and completed within 5 days following these ages. These 10-day age windows were considered the shortest periods in which the required experiments could be completed.

For both baroreceptor and vessel wall measurements, we have used an in vitro aortic arch-aortic nerve preparation which has been described previously (Brown et al., 1976; 1978). Under pentobarbital anesthesia (30-50 mg/kg), the aortic arch and nerve are exposed. Metal cannulas are placed in the innominate artery and the descending aorta, and ligatures are placed on the ascending aorta, left common carotid and left subclavian arteries. The aortic arch and nerve are then removed and transferred to a warmed perfusion bath where the vessel is fixed to approximate its in situ length and shape.

Measurement of Vessel Wall Mechanics

Four rats from each group were used for the portion of this study on vessel wall mechanics. Aortic segments identical to those excised for the receptor studies were mounted in a perfusion chamber, fixed to approximate the in situ condition, and bathed in Krebs-Henseleit solution. This solution was also perfused through the vessel lumen until measurements were made.

Vessel diameter was measured with an ocular micrometer as previously described (Andresen et al., 1978). Briefly, an observer read diameters from the ocular micrometer at the command of a second person who manipulated the transmural pressure. Measurements were made generally between 10 and 30 seconds after a step change in pressure, and random staircase changes were repeated until two or more readings at a given pressure ranging from 0 to 200 mm Hg agreed. Measurements made in this way are in agreement with those made with a piezoelectric sonomicrometer as we have already reported (Andresen et al., 1978). We have found the results to be reproducible using either method. Following the completion of these measurements of diameter, the aortic segment was trimmed to include only that part of the segment inside the limits of the ligatures. This trimmed aortic segment was then patted dry and weighed.

Calculations of Vessel Wall Parameters

A number of parameters could be calculated from the static measurements of the external aortic radius, \( R_E \), and aortic segment weight. Assuming a constant vessel wall volume, the internal radius, \( R_i \), was calculated from the following expression:

\[
R_i = R_E^2 - \frac{w}{\pi d \rho \ell}^{1/2}
\]

where \( w \) is the segment weight, \( \ell \) is the segment length, and \( d \) is the tissue density of 1.06 (MacDonald, 1974). The wall thicknesses (h) derived in this manner, \( R_E - R_i \), agree well with direct histological measurements made on aortic segments fixed under known pressures (Andresen et al., 1978).
Circumferential wall stress, \( \sigma \), was calculated as:

\[
\sigma = P \frac{R}{h} \tag{2}
\]

where \( P \) is the distending pressure, \( R \) is the midwall radius, and \( h \) is the calculated wall thickness. Circumferential wall strain, \( \epsilon \), was calculated as the ratio of the change in vessel radius to the initial, zero pressure radius. The incremental elastic modulus, \( E_{inc} \), was calculated according to Bergel's formula (Bergel, 1961) as:

\[
E_{inc} = \frac{(\Delta P/\Delta r)}{2(1-\nu)^2} \frac{R_R^2/(R_R^2-R_i^2)} {R E R^2_A - R^2} \tag{3}
\]

where \( \nu \), the Poisson ratio, was assumed to be 0.5 and \( \Delta P \) was always 20 mm Hg. Vessel wall parameters at 100 mm Hg and at the prevailing systolic blood pressure are plotted for comparisons.

The results of three series of measurements of vessel wall properties over a 5-year period in equivalent groups of animals are similar. The accuracy of the length, weight, and wall thickness measurements is within plus or minus 7%, 3%, and 7%, respectively, of the mean values (Table 1, Andresen et al., 1978).

Statistical Comparisons

The data for both the receptor and vessel wall measurements were submitted to an analysis of variance. A priori contrasts were judged significant if \( P \leq 0.05 \).

Results

Blood Pressures

Blood pressure (BP) measurements began at 5 weeks of age, and the group mean of SHR tail systolic BP was not different from that of WKY, although the range of BP was greater in SHR (WKY, 95.7 ± 3.9; SHR, 96.5 ± 6.7; mean ± SE). By 6 weeks, however, the SHR had significantly greater BP than WKY, but BP still was lower than the stable adult WKY levels (SHR, 127.9 ± 3.0 mm Hg; WKY, 109.4 ± 2.4 mm Hg; \( P < 0.001 \)). In general, BP rose during the initial weeks of the study to reach a plateau of stable BP which then was maintained for the remainder of the study (Fig. 1). By about 10 weeks of age, WKY BP leveled out at about 125 mm Hg. SHR BP, however, continued to rise until reaching roughly 200–220 mm Hg at 16–18 weeks of age, where it remained through 30 weeks of age.

Vessel Wall Distensibility during Development

Vessel wall development in SHR appears retarded as reported previously (Andresen et al., 1978). External and internal aortic radii at 100 mm Hg in SHR 5 and 8 weeks of age (\( R_E \) and \( R_i \), Fig. 2) were not significantly different than in WKY, but beyond this age the differences became significant (\( P < 0.01 \)). The greatest increases in radius occurred between 8 and 14 weeks of age, and it was during this period that the differences between WKY and SHR were greatest. Beyond 14 weeks, the rate of radius increase slowed in WKY and resulted in a reduction in the difference in radius between WKY and SHR groups.

The vessel wall thickness, \( h \), of the aortic segment increased much more rapidly in the hypertensive rats (Fig. 2). After an initial thinning from 5 to 8 weeks, \( h \) in WKY increases gradually throughout
the 8- to 30-week age period. In contrast, h for SHR was already significantly greater (P < 0.006) at 8 weeks of age and increased rapidly up to 20 weeks of age where h was nearly 50% greater than for WKY. The time course of the increases in vessel wall thickness of SHR (Fig. 2) closely paralleled the development of hypertensive blood pressure (Fig. 1) including the plateau occurring beyond 20 weeks of age. The increases in vessel wall radius and thickness with age resulted in relatively constant wall stress values at 100 mm Hg, $\sigma_{100}$ (Fig. 3A). Throughout this time, however, SHR $\sigma_{100}$ was lower in SHR than in WKY (P < .03).

A vessel wall descriptor that is important to our analysis of baroreceptor function is circumferential wall strain, $\varepsilon$. As WKY mature, $\varepsilon_{100}$ increases until age 20 weeks (Fig. 3B). Between 20 and 30 weeks of age, $\varepsilon_{100}$ declined in WKY, although not significantly. During the entire age range from 5 to 30 weeks, $\varepsilon_{100}$ for SHR was lower than for WKY (P < 0.03) and did not change appreciably with age.

In summary, we found that up to early adulthood, the baroreceptor-containing segment of the rat aortic arch in WKY increases in radius, vessel wall thickness, and wall strain. This period of growth in WKY is characterized by an accelerated phase from 8 to 14 weeks, followed by a more gradual increase from 20 to 30 weeks. In contrast to WKY, aortic growth in SHR appears stunted. The rapid phase is shortened, and radius increases more slowly. Both wall strain and wall stress are smaller in SHR and fairly stable from 5 to 30 weeks of age. Wall thickness, however, increases much more rapidly in SHR.

Since the normal blood pressures in the two rat types are so different at each age, comparisons were also made at the measured tail systolic BP. Figure 4 shows that SHR have normal (WKY-like) $R_E$ and $R_i$ at systolic BP at any age. Differences between SHR and WKY for h and $\varepsilon_{SBP}$ are reduced when compared to the systolic BP rather than 100 mm Hg (Fig. 5) but the differences remain significant after 5 weeks of age (P < 0.05). The relationship for $\sigma_{SBP}$ is reversed, the wall stress for SHR aortas being significantly increased at systolic BP. Despite this, $\varepsilon_{SBP}$ is reduced in SHR aortas. Thus, although the aortas of SHR and WKY operate at similar radii, their distensibility characteristics are quite different at the prevailing BP. Figure 6 shows that the incremental elastic modulus in SHR was significantly higher than in WKY at all ages beyond 5 weeks, indicating diminished wall distensibility.

**Baroreceptor Functional Properties during Development**

**Receptor Pressure Characteristics**

The steady state discharge characteristics of 87 WKY and 102 SHR single unit baroreceptors were recorded. At 5 and 8 weeks of age, the mean of the pressures at which these regularly discharging receptors began to fire ($P_{th}$) was not different between SHR and WKY baroreceptors (Fig. 7A). $P_{th}$ remained fairly constant in WKY from 5 to 30 weeks of age. In SHR, however, $P_{th}$ reset with age, becoming significantly greater by 14 weeks, and did not appear to have reached a plateau even at 30 weeks (Fig. 7A) although BP had stabilized at about 200

**Figure 3** Relationships of the circumferential vessel wall stress, $\sigma$ (A), and wall strain, $\varepsilon$ (B), at 100 mm Hg, with age. Points are means of four animals. Bars are ± SE; * designates significant differences in the means of SHR and WKY (P ≤ 0.05).

**Figure 4** Relationship of aortic radius at the prevailing systolic blood pressure with age. Points are means of four rats. Bars are ± SE; * designates significant differences in the means of SHR and WKY (P ≤ 0.05). A: External radius, $R_E$, in mm; B: internal radius, $R_i$, in mm (see text).
mm Hg by 16-18 weeks of age (Fig. 1). WKY baroreceptor sensitivity near threshold pressure ($S_{th}$) increased steadily with age (Fig. 7B). $S_{th}$ in SHR was slightly lower at 5 and 8 weeks of age but failed to increase during subsequent weeks, so that beyond 8 weeks of age $S_{th}$ was significantly lower for SHR than for WKY ($P < 0.05$). Thus, during maturation, SHR baroreceptors were reset to operate at higher pressures with lower sensitivities to suprathreshold pressures.

**Receptor Strain Characteristics**

$P_{th}$ and $S_{th}$ were converted from pressure terms to their equivalents in terms of vessel wall distortion, or circumferential wall strain, to illustrate more clearly the contribution of vessel wall distensibility to baroreceptor discharge characteristics. Figure 8A shows the mean value of circumferential wall strain at threshold pressure, $e_{th}$, at various ages, and Figure 8B shows the mean value of suprathreshold strain sensitivity, $S_{E}$, at the same ages. In normotensive WKY, baroreceptors require progressively greater vessel wall distortions to reach threshold, $e_{th}$, as development proceeds from 5 to 20 weeks of age (Fig. 8A). From 20 to 30 weeks of age, $e_{th}$ shows a small fall which was not statistically significant. SHR baroreceptors, however, require less vessel wall strain for activation even at the youngest age ($P < 0.01$). $e_{th}$ rose during the subsequent 25 weeks but remained much lower than WKY ($P < 0.01$). The strain sensitivity $S_{E}$ of WKY and SHR baroreceptors changed little after 8 weeks, rising slightly between ages 20 and 30 weeks (Fig. 8B). WKY $S_{E}$ was significantly lower than SHR $S_{E}$ at all ages after 8 weeks ($P < 0.002$). Thus, aortic baroreceptors of SHR have lower distortion thresholds at all ages and greater distortion sensitivity than WKY baroreceptors.
FIGURE 8 Relationships of the baroreceptor strain characteristics with age. Points are means of the same numbers of regularly discharging, single fiber baroreceptors from SHR and WKY described in the previous figure legend. Bars are ± SE; * designates significant differences in the means of SHR and WKY (P < 0.05).

A: Strain threshold, e th (see text for definition). B: Suprathreshold strain sensitivity, S th expressed as the increase in discharge per 1% increase in wall strain above threshold (see text) for the same receptors shown in A.

Discussion

We have studied a subset of the entire population of aortic baroreceptors, those with regularly discharging fibers, in both normotensive (WKY) and genetically hypertensive rats (SHR) from ages 5 to 30 weeks. The results indicate that alterations in mechanotransduction with age are critically involved in the function of WKY and SHR baroreceptors. The changes enable WKY baroreceptors to maintain a constant pressure threshold in spite of large increases in radius and distensibility. Resetting in SHR appears to result from a mismatch between distensibility and mechanotransduction. The likelihood that selective destruction of low threshold baroreceptors has occurred is remote. Baroreceptors with myelinated axons have lower thresholds than baroreceptors with unmyelinated axons, and histological studies show that the ratio of myelinated to unmyelinated axons in the aortic nerves of WKY and SHR is unchanged.

Some Comments on Regularly Discharging Baroreceptors

Aortic baroreceptors can be separated into two major functional classes by the regularity of their discharge patterns (Brown et al., 1976; Thoren et al., 1977). The standard deviation of the interspike interval of regular baroreceptors is generally less than 10%, whereas for irregular baroreceptors it can exceed 50% (Thoren et al., 1977). We have sampled only regular baroreceptors in this study as reflected in the relatively narrow distributions of threshold pressures for both WKY and SHR (Fig. 9). The mean P th values reported presently are lower for both WKY and SHR than in earlier reports (Sapru and Wang, 1976; Andresen et al., 1978). This could reflect differences both in technique and/or in sampling. The continuous measurement of P th with slow ramps accounts for only a small downward shift in the P th estimate and should be the same for both WKY and SHR. The most likely explanation is that irregularly discharging baroreceptors which have higher thresholds (Thoren et al., 1977) were deliberately excluded from the present investigation. By selecting for regularly discharging fibers, we have followed the development of a well-defined group and have limited the influence of mixing two groups of baroreceptors with distinctly different threshold discharge characteristics.

Pressure-Response Characteristics of Regularly Discharging Baroreceptors

The threshold pressure for activation (P th) of WKY baroreceptors was remarkably stable from 5 to 30 weeks of age. Systolic blood pressure (BP) was still increasing to its mature plateau level during the two earliest samplings (5 and 8 weeks of age), and a small though statistically insignificant increase in P th occurred as well. The receptor gain or suprathreshold pressure sensitivity (S th) in WKY showed a small gradual increase from 5 to 30 weeks.
of age. In the hypertensive rats, marked resetting of $P_{th}$ was evident by age 14 weeks and was not complete even at age 30 weeks, 10 weeks after BP had stabilized at about 200 mm Hg. SHR baroreceptor gain, $S_{th}$, failed to increase with age. These data on threshold agree in general with previous studies on SHR resetting (Sapru and Wang, 1976; Sapru and Krieger, 1979), although the thresholds were somewhat lower probably for reasons discussed above.

Wall Strain and the Baroreceptor Response during Maturation and Aging

The mechanical properties of the aortic arch segment innervated by the left aortic depressor nerve develop very differently in WKY and SHR animals. The properties are somewhat similar in the youngest animals (here 5 weeks of age) but follow very different courses with further development. In the WKY group, at 100 mm Hg vessel caliber, $R_{k}$ and $R_{c}$, and circumferential wall strain, $\epsilon$, increase substantially with age, the most rapid changes occurring between 5 and 14 weeks. The changes in SHR at 100 mm Hg are reduced; their aortas have significantly smaller $R_{k}$, $R_{c}$, and $\epsilon$ by 8 weeks, do not show the accelerated growth phase at 14 weeks, and grow much more slowly at later stages. Vessel wall thickness, $h$, however, increases much more rapidly, leading to lower wall strain and stress at 100 mm Hg. At systolic blood pressure, both groups operated at similar radii, but SHR still had consistently lower strains and lower distensibilities than WKY, although wall stress was greater, significantly so between 15 to 30 weeks. An earlier report (Andresen et al., 1978) found $\epsilon_{100}$ only slightly lower in SHR than in WKY at 10 weeks and the differences were not significant. In the present studies, WKY and SHR alike had consistently lower body weights and smaller vessel radii than in the earlier study. We feel that this, combined with the greater intragroup variability of the earlier study (compare Figure 5A, Andresen et al., 1978, and Figure 3B, present study), account for the present finding.

It is possible to quantify the contribution of these mechanical properties to receptor activity by expressing the discharge characteristics of the baroreceptors in terms of total vessel wall distortion. The remarkable stability of $P_{th}$ in WKY appears to result from a progressive increase in the degree of wall distortion required for receptor activation, $\epsilon_{th}$. This increase in $\epsilon_{th}$, with age effectively compensates for increases in radius and distensibility. From 20 to 30 weeks of age, there is an actual decline in $\epsilon$ at both 100 mm Hg and systolic BP, indicating perhaps the beginning of the gradual decline in distensibility observed in aging rats (Berry et al., 1975), and this decline is accompanied by a drop in receptor $\epsilon_{th}$. Thus, WKY receptor output is kept constant for normotensive pressures by a reduction in the sensitivity of receptor distortion or transduction in the face of rapid growth of the aorta. This phenomenon of strain-resetting is probably very important, for adaptation of this sort would have to occur either in the central nervous system or in the peripheral receptors, to maintain blood pressure at normal levels during the aortic growth associated with maturation. Our studies show that resetting occurs in the receptors. The studies also indicate that strain-resetting may occur during aging as well as during maturation and, appropriately, it is in the opposite direction, i.e., increased receptor strain sensitivity associated with reduced wall distensibility. An assumption in these interpretations is that the overall wall properties we measured are equivalent to those in the vicinity of the receptors; this appears to be the case in structural studies of rat aortic baroreceptors (Krauhs, 1979). The question of whether the different changes shown by SHR receptors are genetically produced or whether BP is the main factor in the dynamics of strain-receptor matching will be examined in the accompanying paper (Andresen et al., 1980).

The Splinting Hypothesis

One possible explanation for baroreceptor resetting which has frequently been suggested is that, during hypertension, the vessel wall in which the receptors are located becomes less distensible so that the strain on the receptor at any given pressure is reduced (Sapru and Wang, 1976; Angell-James, 1971; Kezdi, 1965; Jones, 1977). According to this hypothesis, the result is that higher pressures are required to reach distortion levels which activate the receptors. Implicit in this argument is that the receptors aren’t or needn’t be functionally altered for resetting to occur. Clearly, the $\epsilon_{th}$ data for SHR demonstrate that these receptors are not transducing normally and, in fact, have lower distortion thresholds and greater strain sensitivity than WKY baroreceptors. SHR receptors require significantly less wall distortion to reach threshold at all ages from 5 to 30 weeks. Hence the splinting hypothesis is not valid at the macroscopic level when strain threshold for discharge is correlated with strain of the total vessel wall. Splinting on a microscopic scale of the elements that couple the receptor to the vessel wall remains a possibility. If one uses a model similar to the one proposed by Franz (1969), this would require that the reduction in distensibility of the coupling elements be greater than the overall reduction in distensibility of the vessel wall. Moreover, creep would have to be unaffected, since we have already shown that the dynamic properties of baroreceptors from mature SHR and WKY are similar (Brown et al., 1978). Another possibility is an intrinsic change in the baroreceptors themselves. The mechanisms could involve a change in membrane permeability to Na or K ions, or a change in the membrane Na$^+$ electrogenic pump; they have been discussed in more detail in recent articles (Andresen et al., 1978; Brown, 1980).
The differences in strain threshold between SHR and WKY baroreceptors are smallest at 5 and 8 weeks, increase during the growth between 8 and 20 weeks, and are reduced at 30 weeks. In SHR, these changes are not sufficient to compensate completely for the alterations in vessel wall associated with developmental and hypertensive processes. From these considerations we are led to the conclusion that the mismatch between receptor properties and wall properties increases with age and results in pressure resetting. Possible underlying mechanisms for the mismatch could be genetic, pressure-induced, or a combination of the two, namely, genetic susceptibility to changes in membrane function triggered by changes in blood pressure. In the following paper, we examine the contribution of blood pressure to the resetting process (Andresen and Brown, 1980).

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