Baroreflex sensitivity during the development of spontaneous hypertension in rats

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Summary
1. Baroreflex sensitivity was studied in relation to the development of spontaneous hypertension in rats (SH rats), with normotensive Wistar-Kyoto (WKY) rats as controls. Conscious, freely moving animals were studied at different times, ranging from 4 to 20 weeks after birth.

2. The youngest SH rats (4-6 weeks; n = 10) already had significantly (P < 0.01) higher mean arterial blood pressure (112 ± 2 mmHg) than WKY rats of corresponding age (95 ± 4 mmHg; n = 10). Baroreflex sensitivity did not differ at this age (0.37 ± 0.04 vs 0.38 ± 0.05 ms/mmHg).

3. Mean arterial pressure increased rapidly in SH rats during further development, reaching a value of 166 ± 3 mmHg in 12-20 week old animals (n = 25). In equally old WKY rats blood pressure was significantly (P < 0.001) lower (110 ± 6 mmHg, n = 25). Baroreflex sensitivity did not change during development of SH rats (0.40 ± 0.03 ms/mmHg in 12-20 weeks old SH rats), whereas it increased two- to three-fold in WKY rats (0.93 ± 0.08 ms/mmHg, P < 0.001).

4. It is concluded that an increase in baroreflex sensitivity is part of the development of a normotensive cardiovascular system, whereas in SH rats responsiveness of the baroreceptor reflex remains depressed during the development and stabilization of hypertension.

Key words: baroreflex sensitivity, baroreflex control, spontaneous hypertension.

Abbreviations: BRS, baroreflex sensitivity; MAP, mean arterial pressure; SH, spontaneously hypertensive; WKY, Wistar-Kyoto.

Introduction
The baroreflex is an important factor in the control of the cardiovascular system. It has been shown that the range of control exerted by baroreceptors adapts to maintained high pressure both in hypertensive man [1-3] and in several forms of experimental hypertension in animals [4-9]. The adaptation of baroreceptors to a maintained high pressure has been associated with a decrease of the overall baroreflex sensitivity (BRS). Cumulative evidence over the last decade supports the presence of reduced BRS in borderline [10, 11] and essential human hypertension [2, 3, 12]. In animal studies reduced BRS was shown in renal hypertensive [9] as well as spontaneously hypertensive (SH) rats [8, 13-15].

Although it seems well established that the sensitivity of the baroreflex is reduced in hypertension, relatively little is known about the dynamics of this process in relation to the development of hypertension. Angell-James and co-workers [16, 17] studied the development of BRS in renal hypertensive and medial sclerotic rabbits. The sensitivity was reduced progressively by a factor 2-3 in both groups over a period of 16 weeks parallel to the development of hypertension. In contrast, Jones & Floras [9] recently showed that in renal hypertensive rats BRS is reduced by a factor 2 in an early phase of the development of hypertension. With a further increase in pressure, sensitivity was reduced progressively to four to five times below control values.
The present study was designed to follow BRS during the development of a spontaneous, genetically determined form of experimental hypertension. The Japanese strain of spontaneously hypertensive (SH) rats was studied, with progenitor Wistar–Kyoto (WKY) rats as controls.

Methods

Male spontaneously hypertensive rats and progenitor normotensive Wistar–Kyoto controls (WKY rats) of ages ranging from 4 to 20 weeks were obtained from the Centraal Proefdierenbedrijf T.N.O., Zeist, The Netherlands. They were housed individually and had free access to standard laboratory food (Hope Farms, RMHTM) and water.

Under ether anaesthesia a cannula was advanced into the left femoral artery for blood pressure measurement. A silastic 0.020 inch internal diameter (i.d.) cannula with a 3 cm piece of silastic 0.030 inch i.d. tubing at the distal end (total volume 0.02 ml) was brought into the right jugular vein to allow intravenous injection. The cannulae were filled with a heparin solution in saline [250 units/ml of sodium chloride solution (154 mmol/l)]. Rats were allowed minimally 1 day to recover before the start of experiments. They were allowed to move freely in their cages during the experiments. Experiments were performed during day time. Rats usually are at rest in their cages during that time. The arterial blood pressure signal was measured with a Statham P23 Db or CTC-CPO1 strain gauge and was recorded continuously with a Grass 7P Polygraph. The natural frequency response of the catheter manometer system is larger than 60 Hz. Mean arterial pressure (MAP) was calculated as the diastolic pressure + [systolic – diastolic pressure]. Heart period was determined on a beat-to-beat basis as the reciprocal value of heart rate, which was measured from a Narco Biotachometer (type 7302, position beat-to-beat) triggered with the blood pressure signal.

According to the original method of Smyth et al. [18] BRS is measured by plotting the pulse interval of each beat against the systolic pressure of the preceding beat during an increase in blood pressure induced by the pressor agents angiotensin or phenylephrine. In later studies the same authors indicated that use of the α-adrenoceptor-stimulating agent phenylephrine is preferable and that mean arterial pressure could be used equally well as systolic blood pressure [3]. In a subsequent study Pickering & Davies [19] investigated the shift in pulse interval that gave the highest correlations between pressure and heart period. They found that a shift 0 (i.e. correlating pressures with the periods of the beat in which they occur) gave the best correlations in some cases, whereas sometimes a shift +1 (i.e. using periods of succeeding beats) gave the best results. This phase shift is related to the latency of the baroreflex, which is close to 1 s in man [19, 20]. This allows the use of a phase shift of 0 or +1.

In the rat the high heart frequency of 6–9 beats/s in combination with approximately 1 s latency in the reflex-mediated change in heart rate [21] does not allow a simple phase shift of 0 or +1.

Fig. 1 gives an example of plots of MAP against heart period at different phase shifts during a phenylephrine injection in a normotensive WKY rat. The corresponding slopes of the linear regression lines for the rising phase of blood pressure as a measure of BRS are also indicated with their correlation coefficients. This analysis indicates the arbitrary value for BRS obtained when applying the original method of Smyth et al. [18] in the rat. We therefore used an adaptation of this method by injecting six to nine doses of phenylephrine ranging from 0.1 to 30 μg/kg (0.005–1.5 nmol/kg) in 0.05 ml of saline. After each dose the cannula was flushed with 0.05 ml of saline. Cardiac slowing in terms of the maximal heart period during the first 3 s after a phenylephrine injection was plotted against maximal MAP in that period (Fig. 2). The relationship heart period/MAP (ms/mmHg) was expressed as a regression coefficient. Only observations with a correlation coefficient greater than 0.70 and a P value less than 0.05 were used; 4% of the correlations had to be rejected on the basis of this criterion. Results are expressed as means ± 1 SEM. Linear regression for determination of BRS was performed with the least-squares method by using a Texas Instruments TI 59 calculator. Significances of differences between mean values was tested by using Student’s unpaired t-test.

Results

Rats were divided in four groups on the basis of their age: 4–6 weeks, 6–9 weeks, 9–12 weeks and 12–20 weeks. There were no significant differences in body weights for the SH and WKY rats in equal age groups (Table 1). MAP in the youngest SH rats (112 ± 2 mmHg, n = 10) was already significantly (P < 0.01) higher than in 4–6 weeks old WKY rats (95 ± 4 mmHg; n =
Baroreflex sensitivity in SH rats

Fig. 1. Plots of the mean arterial pressure (MAP) against heart period at different phase shifts during a phenylephrine-induced increase in blood pressure in a WKY rat (MAP = 112 mmHg) according to the method of Smyth et al. [18]. For both the increase and return of blood pressure during each consecutive heart beat MAP is plotted against heart period during the pulse interval after that heart beat (shift +1) or the nth interval after that heart beat (shift +n). Baroreflex sensitivity (BRS) is expressed as the slope of the linear regression for each rising phase of MAP. \( r \) is the corresponding correlation coefficient.

The difference increased during the further development of the animals, reaching values of 166 ± 3 mmHg for the 12–20 weeks old SH rats \((n = 25)\) and 110 ± 6 mmHg in the WKY rats \((n = 25)\) (Table 1). Heart rate was significantly \((P < 0.001)\) higher in the youngest SH rats \((453 ± 13\) beats/min) than in WKY rats \((381 ± 9\) beats/min). The rate decreased during the further development of SH rats; in the 9–12 and 12–20 weeks old animals it was no longer significantly different from WKY rats’ heart rates.

Baroreflex sensitivity is plotted against age for the different groups in Fig. 3. Data indicate that young SH and WKY rats had equally low BRS values, ranging from 0.18 to 0.60 ms/mmHg. Mean BRS values are equal for the youngest SH \((0.37 ± 0.04\) ms/mmHg) and WKY rats \((0.38 ± 0.05\) ms/mmHg). BRS increased with age in WKY rats, stabilizing at a mean value of 0.93 ± 0.08 ms/mmHg in the 12–20 weeks old animals. This value is significantly \((P < 0.001)\) higher than that of 12–20 weeks old SH rats \((0.40 ± 0.03\) ms/mmHg). In SH rats a low BRS is retained throughout the entire age range studied, with a relative maximum of 0.45 ± 0.03 ms/mmHg in the 6–9 weeks old rats.
TABLE 1. Body weight, mean arterial pressure (MAP) and heart rate of spontaneously hypertensive (SH) rats and Wistar-Kyoto normotensive (WKY) rats at different ages

Group results are means ± SEM. Significances of differences between corresponding SH and WKY rats: *P < 0.05; **P < 0.01; ***P < 0.001.

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Age (weeks)</th>
<th>n</th>
<th>Body wt. (g)</th>
<th>MAP (mmHg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
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<td>SH</td>
<td>4-6</td>
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<td>106 ± 5</td>
<td>112 ± 2**</td>
<td>453 ± 13***</td>
</tr>
<tr>
<td>WKY</td>
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<td>107 ± 8</td>
<td>95 ± 4</td>
<td>381 ± 9</td>
</tr>
<tr>
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<td>136 ± 5***</td>
<td>395 ± 8**</td>
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<tr>
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<td>109 ± 3</td>
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<tr>
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<td>161 ± 4***</td>
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<tr>
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<td>112 ± 3</td>
<td>378 ± 12</td>
</tr>
<tr>
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<td>322 ± 7</td>
<td>166 ± 3***</td>
<td>370 ± 7</td>
</tr>
<tr>
<td>WKY</td>
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<td>25</td>
<td>309 ± 8</td>
<td>110 ± 6</td>
<td>369 ± 11</td>
</tr>
</tbody>
</table>

Fig. 2. Plots of the change in mean arterial pressure (MAP) against heart period during the injection of different doses of phenylephrine in an adult, conscious SH rat (MAP = 155 mmHg) and WKY rat (MAP = 110 mmHg). Baroreflex sensitivity (BRS) was determined as the slope of the linear regression between MAP and heart period.

Discussion

We have used an adaptation of the method described originally by Smyth et al. [18] to measure baroreflex sensitivity during the development of hypertension in SH rats. The original BRS method was developed for use in humans and used one bolus of phenylephrine. Systolic or mean blood pressures of successive arterial pulses is plotted in that method against each pulse interval that begins with the next beat. This can be done in man, since the latency of the cardiac reflex response in many species, including man, is 0.5-2 s [19, 21, 22]. Since normal heart rate in man approximates to 1 beat/s, correlations with a 0 or +1 phase shift give an estimate of BRS in man [19]. In the rat, however, normal heart rate is 6-9 beats/s, with a latency of the cardiac baroreceptor-mediated reflex of 0.5-1 s [21]. This means that phase shifts of +4 to +10 may have to be used to obtain optimal correlations. Jones & Floras [9] recently described a method for measuring BRS in rats in which 'the observer was given the facility to include delays of several cardiac cycles when computing the regression of pulse interval against preceding mean arterial pressure'. With this method they found a BRS of 0.95 ms/mmHg in normotensive rats. This value corresponds well to the value of 0.93 ms/mmHg we found in normotensive adult WKY rats. Coleman [21] measured BRS recently in conscious normotensive rats by a method comparable with that we have used. Although he expressed his results in a different manner,
have shown that the adaptation of baroreceptors lower in the tension than in with phenylephrine is independent of the dose used, confirming the linearity we find in our correlations of maximal heart period against maximal MAP.

Our results show that BRS is significantly lower in the SH rats with established hypertension than in WKY rats. Similar conclusions were reached by others on the basis of different methods [8, 13–15]. Moreover, many authors have shown that the adaptation of baroreceptors to a maintained high blood pressure is associated with reduced BRS in other forms of clinical and experimental hypertension (see the Introduction). As a cause of reduced BRS several authors have suggested that during hypertension the distensibility of the vessels in which baroreceptors are located decreases. Others [15] favour an abnormality in the central nervous system processing of baroreceptor reflex activity. On the other hand, it has been suggested that a change in baroreceptor elements themselves plays a role in the reduced sensitivity of the baroreflex. Such mechanisms could involve a change in membrane permeability to Na+ or K+, or a change in the membrane Na+ electrogenic pump [23, 24]. Recent evidence favouring such a non-structural cause for reduced BRS in hypertension came from Jones & Floras [9], who studied the time course of BRS changes in renovascular hypertension in rats. They found a significant diminution of BRS within 3 days after clamping the renal artery, at a time when structural changes in the heart or vasculature were not yet present.

The primary purpose of our study was to follow the dynamics of the change in BRS during the development of spontaneous hypertension. The most surprising result was that during the development of hypertension BRS did not decrease in SH rats, but rather increased in the WKY rats. During the phase of maturation of the animals (cf. the increase in body weight) BRS increased by a factor 2–3 in WKY rats, whereas it remained relatively constant in SH rats. In fact, sensitivity of SH rats increases slightly up to weeks 6–9 and then diminishes again. These results do not point to a BRS decrease in SH rats as a consequence of structural vascular changes after hypertension. Rather, they could indicate a difference in the development of baroreceptor properties during maturation of the two strains of rats. Interestingly, Andresen et al. [25] by measuring single aortic baroreceptor fibre discharges to increasing pressures, as an index of receptor gain, found a gradual increase in sensitivity in WKY rats from 5 to 30 weeks of age, whereas SH rat baroreceptor sensitivity failed to increase. These authors speculated that the large changes in vessel (aorta) morphology during maturation are matched in WKY rats by changes in the mecano-transduction of the baroreceptors, thus preventing changes in the pressure threshold [25, 26]. In the SH rats, on the other hand, the different pattern of development of the aorta (smaller internal and external radii, smaller circumferential wall strain) is not matched by an adequate adaptation of mecano-transduction in SH rat baroreceptors, according to Brown's group [24–26]. The same group speculated earlier that a change in membrane permeability to Na+ or K+, or changes in the membrane Na+ electrogenic pump, might underlie this abnormality in SH rat baroreceptors [23, 24].

The implications of these findings for our insight into the pathogenesis of spontaneous hypertension remain to be further investigated. Our SH rats were slightly hypertensive already before major changes in baroreceptor reflex properties seemed to take place. On the other hand, the period of drastic rise in pressure coincides with the time when baroreceptor properties apparently adapt to keep pressure normal in WKY rats. A similar increase in the sensitivity of baroreflexes was described by Vatner & Manders [27] during early postnatal development of normotensive dogs. It thus seems that increase in BRS is part of the development of a normotensive cardiovascular system, whereas in SH rats the responsiveness of the baroreceptor reflex remains depressed.

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References


