Reducing Pulse Pressure in Hypertension May Normalize Small Artery Structure

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To investigate the relation between the small artery structure and different blood pressure parameters, spontaneously hypertensive rats were treated from 4 to 24 weeks of age (20 weeks in total) with five different antihypertensive therapies: two angiotensin converting enzyme inhibitors (perindopril and captopril), a calcium antagonist (isradipine), a β-blocker (metoprolol), and a vasodilator (hydralazine). At 24 weeks of age, 24-hour blood pressure was measured, and two mesenteric resistance vessels were taken from each animal. Blood pressure was 227/135 mm Hg (systolic/diastolic) and 161/106 mm Hg in untreated hypertensive and normotensive control rats, respectively. Heart rates were 376 min⁻¹ and 295 min⁻¹ for the two strains. All treatments reduced all blood pressure parameters except for metoprolol, which did not reduce pulse pressure. In the small arteries, the media cross-sectional area was unaffected by the treatments. When a simple correlation analysis was made, pulse pressure was found to correlate more closely (r=0.64, p<0.001) to the resistance vessel media/lumen ratio than any of the other pressure parameters studied: systolic (r=0.51, p=0.011), mean (r=0.41, p=0.05), or diastolic (r=0.28, p=0.19). When an analysis of covariance was performed that included pulse pressure, mean blood pressure, and heart rate, which also correlated significantly to the media/lumen ratio, 81% of the variation in the media/lumen ratio could be accounted for by the variation in the three covariates (p<10⁻⁵), pulse pressure being the major factor. In conclusion, it is indicated that a reduction in pulse pressure and heart rate during antihypertensive treatment may be important in preventing the development of abnormal small artery structure in hypertension. (Hypertension 1991;18:722–727)

Recently, there has been an increasing interest in the effect of cyclic pressure loading of the heart and blood vessels in hypertension. It has been shown that elevated pulse pressure (PP) correlates well with an increased weight of the left ventricle, and the role of PP in essential hypertension has been discussed. Antihypertensive drugs that have a beneficial effect on PP have proved particularly effective in reducing left ventricular hypertrophy, and elevated PP has proved to be a separate risk factor in hypertension for early death from cardiovascular disease. So far, the effect of PP on the small arteries has not been described.

In a recent study, we showed that antihypertensive treatment of the spontaneously hypertensive rat (SHR) reduced both mean blood pressure (MBP) and small artery media/lumen ratio, although the correlation between the parameters was not particularly impressive. The present article analyzes a subgroup of that study in which 24-hour blood pressure measurements were taken. We analyzed the relation between parameters describing small artery structure, primarily media/lumen ratio, but also media thickness, lumen diameter, and media area in relation to parameters describing the 24-hour blood pressure: MBP, PP, systolic (SBP) and diastolic (DBP) blood pressure, and 24-hour heart rate (HR).

**Methods**

**Animals**

Male SHRs and normotensive Wistar-Kyoto (WKY) rats aged 4 weeks were obtained from the Moellegaard Breeding Centre, Lille Skensved, Denmark. They were given free access to food pellets and drinking water, and they were kept on sawdust bedding in 40×30×20-cm cages. There were two rats in each cage, and lights were on from 6:00 AM to 6:00 PM.

**Antihypertensive Treatment**

We used five different antihypertensive drugs: two angiotensin I converting enzyme inhibitors, perindopril (Servier, Neullly, France) and captopril...
(Squibb, Princeton, N.J.); a calcium antagonist, isradipine (Sandoz, Basle, Switzerland); a vasodilator, hydralazine (CIBA-GEIGY, Basle, Switzerland); and a β-blocker, metoprolol (Hoechst, Moehlndal, Sweden). The doses were 1.5, 60, 40, 25, and 130 mg/kg per day, respectively. Isradipine was dissolved in 96% ethanol and was emulsified in polysorbate 20 (obtained from Sygehusapoteket, Aarhus County Hospital). Both ethanol and polysorbate 20 had a concentration of 1% in the final solution. All other drugs could be dissolved in water directly. (For further details, see Reference 5.)

Protocol

The SHR were assigned to six groups (five treated and one control) with four rats in each group. The antihypertensive drugs were given as monotherapy in the drinking water from 4 to 24 weeks of age (in total 20 weeks of treatment), after which 24-hour blood pressure was measured. Finally, a small biopsy containing two mesenteric small arteries for morphometric measurements was taken from each rat (see below). The WKY rats and the control SHRs followed the same protocol, only no drugs were added to the drinking water.

Invasive 24-Hour Blood Pressure Measurements

In brief, a polythene tube was inserted, via the left femoral artery, into the abdominal aorta just below the kidney arteries, taken subcutaneously to the nape, extruded, and, via a swivel device that allowed rotation around the catheter axis, was connected to a pressure transducer. Each time a new measurement was initiated, the recording system was adjusted to be critically damped and proved fast enough for measuring SBP and DBP accurately for 24 hours.6 MBP was continuously registered, and PP and HR were measured every 4–5 hours. The rats could move freely, had free access to food and water, and were neither handled nor under the influence of any drugs except for the antihypertensive drugs. The tubes were constantly perfused with saline at a rate of 0.6 ml/hr, which raised the transducer pressure by 1 mm Hg. This was not corrected for. Twenty-four-hour SBP and DBP were calculated from the measurements of MBP and PP (DBP = MBP – 0.46 PP; SBP = DBP + PP).6 By doing this, it was possible to avoid the variations in blood pressure with respiration and thus to have an exact measure not just of PP but also of SBP and DBP on a 24-hour basis.

Vascular Structure

As described previously,5 a vessel segment biopsy was taken from the mesentery when the 24-hour measurements of MBP were terminated. By choosing the most distal vessels in each side of the biopsy, it was possible to obtain vessels from a specific anatomic location that was at a constant distance from the intestinal wall. The vessels were the second or third branch off the superior mesenteric artery. The vessels were held fully relaxed (i.e., unable to dilate further even in a Ca2+-free environment) in a physiological saline solution (PSS) consisting of (mmol/l): NaCl 139, KCl 4.7, NaHCO3 25, CaCl2 2.5, KH2PO4 1.18, MgSO4 1.17, ethylenediaminetetraacetic acid 0.026, and glucose 5.5. The PSS was bubbled with 95% O2 and 5% CO2 to give a pH of 7.4 and was kept at 37°C. Two small arteries were taken, one from each side of the biopsy as distal as possible. The operator was unaware of the type of rat being investigated. Each vessel was mounted as a ring preparation on an isometric myograph,7 and structural parameters were determined.7,8 Media area was determined, and the vessel was set to 90% of the internal circumference expected from a transmural pressure of 100 mm Hg (L0). Lumen diameter was taken as L0/3.14, and the corresponding media thickness was calculated on the basis that the media area remained constant. From these data, media/lumen ratio was calculated. The vessels had lumen diameters ranging from 0.180 to 0.330 mm (mean 0.225 mm).

Statistics

Regression analysis between pressure and structural parameters was performed. One 24-hour average value for the pressure parameter and the average media/lumen ratio, media thickness, lumen diameter, or media area of the two small arteries from the same rat were taken as a single value for that rat. From the number of points and the coefficient of correlation (r), a probability value was calculated. All SHR treated and untreated were included in the regression; WKY rats were excluded. Furthermore, using the same data, an analysis of covariance9 was performed with media/lumen ratio as base parameter and PP, HR, and MBP as covariates representing the level of blood pressure and the cyclic pressure loading (PP, amplitude and HR, frequency). From this analysis, it was possible to determine to which degree the variation in the covariates could account for the simultaneous variation in the base parameter. The effect of the drugs on the small artery structure and function has been described in detail in an earlier article including larger groups of rats.5

Results

At 24 weeks of age, when treatments had lasted 20 weeks, DBP for WKY rats ranged from 98 to 114 mm Hg compared with 122–145 mm Hg for untreated SHRs. For the treated SHRs, DBP ranged from 82 to 122 mm Hg. No single 24-hour measurement of SBP, DBP, MBP, or PP in an SHR control overlapped the WKY rat range and vice versa. In all five treated groups, all four pressure parameters were significantly reduced except for PP in the metoprolol group, which was unchanged. Three treatments reduced the average DBP to the same value as that of the WKY rats (namely, hydralazine, captopril, and metoprolol), although it was notable that metoprolol had little effect on PP (Table 1). The largest effects on PP were seen in the perindopril- and captopril-
TABLE 1. Twenty-Four-Hour Blood Pressure Parameters and 24-Hour Heart Rate

<table>
<thead>
<tr>
<th>Strain or treatment</th>
<th>MBP (mm Hg)</th>
<th>HR (min⁻¹)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>PP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>177±6</td>
<td>376±18</td>
<td>227±6</td>
<td>135±5</td>
<td>92±2</td>
</tr>
<tr>
<td>WKY</td>
<td>132±4</td>
<td>295±15</td>
<td>161±3</td>
<td>106±4</td>
<td>55±1</td>
</tr>
<tr>
<td>PER</td>
<td>121±7*</td>
<td>358±10</td>
<td>153±8*</td>
<td>94±6*</td>
<td>60±3*</td>
</tr>
<tr>
<td>CAP</td>
<td>137±2*</td>
<td>376±16</td>
<td>172±2*</td>
<td>107±2*</td>
<td>65±1*</td>
</tr>
<tr>
<td>HYD</td>
<td>140±5*</td>
<td>332±6</td>
<td>178±3*</td>
<td>107±5*</td>
<td>71±2*</td>
</tr>
<tr>
<td>ISR</td>
<td>149±3*</td>
<td>333±13</td>
<td>190±3*</td>
<td>115±4*</td>
<td>75±1*</td>
</tr>
<tr>
<td>MET</td>
<td>146±8*</td>
<td>392±26</td>
<td>193±7*</td>
<td>105±8*</td>
<td>88±2</td>
</tr>
</tbody>
</table>

Values show group mean±SEM for four rats per treated group. MBP, SBP, DBP, and PP, mean, systolic, diastolic, and pulse pressures, respectively; HR, heart rate; SHR, spontaneously hypertensive rat control group; WKY, Wistar-Kyoto rat control group; PER, CAP, HYD, ISR, and MET, perindopril-, captopril-, hydralazine-, isradipine-, and metoprolol-treated SHRs, respectively.

*Significantly reduced by the treatment (p<0.05).

treated groups. HR was not influenced significantly by the treatments. These differences were tested by one-way analysis of variance followed by Student's t test between treated and untreated SHRs. Figure 1 shows diagrams of small artery media/lumen ratio and the blood pressure parameters. PP was the best correlate, followed by (in order) SBP, MBP, and HR. DBP was not significantly correlated to media/lumen ratio. By analysis of covariance, using PP, HR, and MBP as covariates, the variation in media/lumen ratio was divided into two groups: residual (19.3%) and accounted for (80.7%; F=7.816; f1=8; f2=15; p<10⁻⁵). PP came out as the major factor (F=15.5; f1=1; f2=15; p=0.001), HR as a minor factor (F=4.3; f1=1; f2=15; p=0.056), whereas MBP could not account for any of the variation in media/lumen ratio (F=0.27; f1=1; f2=15; p=0.612). This somewhat surprising finding is discussed below. In the present subgroup as well as in the large study, the media areas did not differ either between SHR and WKY rats or between untreated and untreated and

FIGURE 1. Scatterplots show resistance vessel media/lumen ratio (m: l) plotted against 24-hour blood pressure parameters (panels A–D) and 24-hour heart rate (panel E). ○, Hydranaline-treated rats; □, metoprolol-treated rats; ▲, isradipine-treated rats; ▲, perindopril-treated rats; ▲, captopril-treated rats; □, group mean±SEM for untreated control rats. r, Coefficient of correlation; P, corresponding probability value; DBP, diastolic blood pressure; MBP, mean blood pressure; SBP, systolic blood pressure; PP, pulse pressure; HR, heart rate.
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the treatments did not change media thickness significantly, and only perindopril increased the lumen diameter. Details on the effects of the treatments on vascular structure are given in Reference 5. In Table 3, a correlation analysis of the pressure parameters and media thickness, lumen diameter, or media area is shown. For lumen diameter, the correlation of media/lumen ratio to PP was not closer as it was to MBP, whereas PP was the only significant correlate to media thickness. The media area was not correlated to any of the pressure parameters.

Discussion

The present study is the first long-term antihypertensive treatment study presenting 24-hour measurements of MBP, PP, and HR together with morphometric measurements of the small artery structure. Although the number of animals was relatively small and five different antihypertensive drugs were used, it seems clear that reduction in the cyclic pressure loading (especially the amplitude but also the frequency) on the small arteries may be considered an important factor for the level of media/lumen ratio during antihypertensive treatment. The correlation (p < 0.001) between media/lumen ratio and PP was close even though only two resistance vessels were examined per animal.

The mechanism for the increased MBP in rat genetic and human essential hypertension is unknown but may be related to or mediated through structural changes in the resistance vessels. It has been shown that raised blood pressure level is associated with increases in the thickness of the smooth muscle layer and with decreases in lumen diameter of resistance vessels, resulting in an increase in media/lumen ratio and thus increased minimal vascular resistance. However, treatment studies do not entirely support the hypothesis that MBP and media/lumen ratio are closely related. When the blood pressure is decreased by antihypertensive treatment, although corresponding reductions in media/lumen ratio do sometimes occur, these are not always as pronounced as could be expected from the values measured in untreated hypertensive and normotensive subjects and the degree of blood pressure reduction. All these studies have, however, only examined the relation between vascular structure and a single pressure parameter representing the blood pressure level. The present data may help to explain why, although MBP is reduced, vascular structure is not always reduced by long-term antihypertensive treatment; the cyclic pressure loading represented by PP and HR may still have been elevated. It has been shown in humans that the pulsatile pressure persists as far as the capillary level; around 40% of both intravascular MBP and PP are transmitted down to the capillary level and depends primarily on factors such as peak ejection velocity and compliance of the large arteries. However, the resistance vessels can also affect PP through their effect on MBP because an increased MBP implies decreased compliance of the central arteries, which results in an increased PP.

<table>
<thead>
<tr>
<th>Strain or treatment</th>
<th>Media (µm)</th>
<th>Lumen (µm)</th>
<th>m:l (%)</th>
<th>Media area (µm²×10⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>15.3±1.2</td>
<td>212±9</td>
<td>7.2±0.5</td>
<td>11.1±1.1</td>
</tr>
<tr>
<td>WKY</td>
<td>12.1±1.4</td>
<td>233±14</td>
<td>5.2±0.4*</td>
<td>9.5±1.6</td>
</tr>
<tr>
<td>PER</td>
<td>13.1±1.0</td>
<td>297±14*</td>
<td>4.4±0.2*</td>
<td>12.8±1.8</td>
</tr>
<tr>
<td>CAP</td>
<td>15.0±0.8</td>
<td>236±25</td>
<td>6.4±0.4</td>
<td>11.9±1.8</td>
</tr>
<tr>
<td>HYD</td>
<td>15.4±1.0</td>
<td>240±17</td>
<td>6.4±0.4</td>
<td>12.5±1.7</td>
</tr>
<tr>
<td>ISR</td>
<td>14.0±0.5</td>
<td>150±17</td>
<td>5.6±0.2*</td>
<td>11.8±1.0</td>
</tr>
<tr>
<td>MET</td>
<td>16.2±0.7</td>
<td>203±11</td>
<td>8.0±0.2</td>
<td>11.2±0.9</td>
</tr>
</tbody>
</table>

Values show group mean±SEM for four rats per treated group. m:l, Media/lumen ratio; SHR, spontaneously hypertensive rat control group; WKY, Wistar-Kyoto rat normotensive control group; PER, CAP, HYD, ISR, and MET, perindopril-, captopril-, hydralazine-, isradipine-, and metoprolol-treated SHRs, respectively.

*Significantly changed by the treatment (p < 0.05).

The present data do not directly help in understanding in what way the cyclic pressure is connected to media/lumen ratio. PP is generated in the central parts of the circulation, since routine measurements of PP in small arteries are not realistic in the clinic.

The present data do not directly help in understanding in what way the cyclic pressure is connected to media/lumen ratio. PP is generated in the central circulation and depends primarily on factors such as peak ejection velocity and compliance of the large arteries. However, the resistance vessels can also affect PP through their effect on MBP because an increased MBP implies decreased compliance of the central arteries, which results in an increased PP
assuming that all else is equal. That PP should be decreased through an effect of the treatments on MBP in the present study appears unlikely, for one would then expect a closer correlation between MBP and media/lumen ratio rather than between PP and media/lumen ratio. Other factors are important for the level of PP including the duration of diastole and the rate of the pressure decline in diastole.

In the present study, there was no change in HR due to the treatments. Also, the decreased media/lumen ratio found in some of the treated groups would tend to increase the speed of the pressure drop in diastole because of a reduction in peripheral resistance and thus would tend to increase the PP. It therefore seems likely that PP determines the level of media/lumen ratio rather than the reverse, although no final conclusion can be drawn from the abovementioned arguments.

Graft studies, in which veins have been implanted into the arterial system, appear to provide some of the most convincing evidence that the blood pressure level can change vascular structure. However, the possibility cannot be excluded that it may be the increase in pulsatile pressure loading rather than the increased blood pressure level that gives rise to the change in vascular structure. After all, in those experiments, the vessel grafts were subjected to the largest possible relative change in pulsatile pressure: from almost zero in the veins to arterial PP. Mechanisms for the morphological changes in grafted veins have recently been investigated in dogs. Femoral veins were implanted end-to-end or end-to-side into the arterial system. Small cuffs were used for restricting graft dilatation locally or for creating a proximal stenosis to reduce the pressure load. In 3-month-old grafts, histological studies showed a marked increase in media area that appeared to be delayed by using cuffs to prevent distension of the grafts. It was concluded that distension stimulates the medial hyperplasia. Conversely, altering the flow rate through the grafts did not lead to differences in medial hyperplasia. Finally, the medial hyperplasia was not delayed by lowering MBP (by 21%) and PP (by 38%) simultaneously. This is in agreement with our findings of no change in media area despite a substantial pressure reduction. Further comparison with our data is not possible since data on lumen diameters were not shown.

At the cellular level, it is known that cells in vitro respond to cyclic stretching with an increased mitotic activity, perhaps explaining the mechanism by which the cyclic pressure loading could influence the structure of the resistance vessel wall. Furthermore, during antihypertensive treatment of human essential hypertension it has been observed that it may be dangerous to reduce DBP too much since this increases the mortality from ischemic heart disease. It was suggested that this could be ascribed to the perfusion pressure in diastole becoming too low and leading to infarction of the heart muscles; an alternative explanation may be that the PP was increased too much by the treatment, maintaining an important stimulus for preservation or aggravation of the abnormal vascular structure and thus for this reason inducing ischemia. I believe this possibility deserves further investigation.

Recent observations in a special kind of vasculature, aortic aneurysms, indicate that their growth rate is strongly related to PP. Aneurysm expansion rate was positively correlated to SBP and negatively correlated to DBP.

In conclusion, the reduction in small artery media/lumen ratio due to antihypertensive treatment was accomplished by combined effects on media and lumen with no significant change in media area (muscle mass). It is indicated that changes in intraluminal mechanical factors may account for most of the variation in the resistance vessel media/lumen ratio, even in a mixed treatment study using five different antihypertensive drugs, when parameters representing both the blood pressure level and the cyclic pressure loading are included. This furthermore speaks against the importance of drug-specific effects on resistance vessel structure independent of intravascular pressure. Based on this, one might point to the potential importance of not just aiming antihypertensive treatment efforts at DBP, but also of trying to reduce PP and HR to reduce the cyclic pressure load on the resistance vessels if normalization of their structure (media/lumen ratio) is desired. Already, it has been shown that reducing PP in hypertension is beneficial concerning cardiac hypertrophy and the incidence of cardiovascular death in hypertension. Clearly, much further work is necessary to clarify the issue.

Acknowledgments
CIBA-GEIGY Corporation, AB Haessle, Sandoz Pharmaceuticals Corporation, Laboratoires Servier, and E.R. Squibb & Sons, Inc., are thanked for their generous gifts of the antihypertensive agents. Eli Lilly and Company is thanked for providing the anesthetic Brietal. I am indebted to Dr. Michael John Mulvany, Institute of Pharmacology, University of Aarhus, for patiently discussing data and manuscripts with me, for providing laboratory facilities, for technical help with the morphological measurements on the small arteries, and for his help in many other ways with this study. Dr. Lennard "Tang" Jespersen, Department of Clinical Physiology, Aarhus City Hospital, is thanked for fruitful discussions in the early stages of the study. Finally, Klinik Foto, Holstebro Centralsygehus, is thanked for their assistance with the graphs.

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**KEY WORDS** • chronic hypertension • pulse pressure • heart rate • vascular resistance • blood pressure monitoring • antihypertensive therapy • spontaneously hypertensive rats • Wistar-Kyoto rats
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Hypertension. 1991;18:722-727
doi: 10.1161/01.HYP.18.6.722

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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