Structural and Functional Alterations of Mesenteric Vascular Beds in Spontaneously Hypertensive Rats

Tsutomu Inoue, Takako Masuda, and Koichiro Kishi, Ph.D.

SUMMARY

The morphology and reactivity of mesenteric arteries from spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar Kyoto rats (WKY) were investigated. Isolated, perfused mesenteric vascular beds were prepared from 6-, 11- and 18-week-old SHR and WKY. At these ages, the walls and media of large mesenteric arteries were significantly thicker in SHR than in WKY. The number of smooth muscle cell layers in the media was significantly larger in SHR than in WKY. This difference between SHR and WKY increased as rats grew older, in parallel with differences in the blood pressure.

Flow rate-perfusion pressure curves indicated that the vascular basal resistance to flow increased more profoundly in SHR preparations than in WKY preparations as rats grew older. This may be related to the structural alterations of the resistance vessel wall in SHR. The pressor responses to KCl were greater in SHR preparations than in WKY preparations as rats grew older. This may be caused partly by the increase of the number of smooth muscle cell layers in the media of SHR resistance vessels.

The pressor response to norepinephrine (NE) was significantly higher in SHR preparations than in WKY preparations at all ages investigated. In marked contrast to the vascular basal resistance and the pressor response to KCl, the pressor response to NE was extremely exaggerated in SHR at the age of 6 weeks. This extremely high NE response in younger SHR may not be caused by the structural alteration in resistance vessels. It may be caused by a functional change, which is regulated by the signal transduction process in smooth muscle cells of resistance vessels. These results suggest that the development of hypertension in SHR may be caused by genetic structural and functional abnormalities of resistance vessels. Both abnormalities may be caused by the hyperreactivity to NE through an altered signal transduction process in smooth muscle cells of resistance vessels in SHR.

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The elevated blood pressure of spontaneously hypertensive rats (SHR), a model for human essential hypertension, is associated with an increased peripheral resistance. The increased peripheral resistance can be induced by the morphological and/or functional alterations in resistance vessels. Mulvany et al. and Lee observed media thickening in mesenteric resistance vessels in SHR. Lee et al. suggested that trophic effects of an overactive sympathetic nervous system may cause a hyperplastic change in the smooth muscle cells in the reactive and resistance vessels in SHR. However, there is still controversy as to whether structural alterations of blood vessels, such as thickening of the vessel wall, are observed in very young SHR. The pressor response of the mesenteric vascular bed to norepinephrine (NE), the primary transmitter, was shown to be higher in SHR than in Wistar Kyoto rats (WKY). Mulvany and Nyborg and Cauvin et al. suggested that Ca$^{2+}$ influx pathways activated by NE in mesenteric resistance vessels may be altered in SHR. However, there is no study which completely shows the time course of changes in the vascular structure, vascular basal resistance and responses to NE and KCl in resistance vessels from SHR. This study observed the course of changes in the structure, vascular basal resistance and responses to NE and KCl of mesenteric arteries in early, developing and established stages of hypertension development in SHR. These observations are important to elucidate possible mechanisms of the development and maintenance of hypertension in this animal model.

**Materials and Methods**

**Rats:**

Male SHR (6-, 11- and 18-week-old) and age-matched WKY were used. All rats were fed normal chow (MR-3-A; Nihon Nosan, Yokohama, Japan) and tap water ad libitum. In advance of experiments, the systolic blood pressure was measured by the tail cuff method (model KN-210-1; Natsume Seisakusho, Tokyo, Japan).

**Preparation of mesenteric vascular bed:**

Rats were fasted for 24 hr before the experiments. Rats were anesthetized with pentobarbital sodium (Nembutal; Abbott Laboratories, North
Fig. 1. A diagram depicting the mesenteric arteries.

Chicago, USA, 50 mg/kg body weight, i.p.). The mesenteric loop preparation was prepared by the modified method of Castellucci et al.\textsuperscript{8,13} An isolated mesenteric vascular bed, which included three main branches of large mesenteric arteries with their dominating intestine, was used (Fig. 1).

**Morphology:**
Mesenteric vascular bed preparations were perfused with 0.9% NaCl solution for 10 min and with 10% formalin for 20 min at a flow rate of 1.0 ml/min. The fixed tissues were embedded in paraffin. Two-μm-thick cross-sections of the large mesenteric artery (Fig. 1) were cut with a microtome. The thin cross-sections were stained with a modified Azan stain. Three to four cross-sections from each rat were studied morphometrically at the light microscopic level. In most cases, the arterial preparations presented a relatively circular profile in cross section, and the internal elastic lamina was not folded. Therefore, it is likely that the arteries were not contracted.\textsuperscript{14}

**Vascular reactivity:**
Isolated mesenteric vascular bed preparations were perfused with physiological saline solution (PSS, mM: NaCl 118.4, KCl 4.7, MgSO\textsubscript{4} 1.2, CaCl\textsubscript{2} 1.4, NaHCO\textsubscript{3} 25.0, KH\textsubscript{2}PO\textsubscript{4} 1.2, glucose 11.7, pH 7.4). The perfusion fluid was bubbled with a 95% O\textsubscript{2}, 5% CO\textsubscript{2} mixture at 37°C, and maintained at the flow rate of 1.0 ml/min by a peristaltic pump (model Minipulse 2; Gilson Medical Electronics, Villiers-le-Bel, France), unless otherwise stated. The perfusion pressure was monitored with a pressure transducer (model CP-01; Century Technology, Inglewood, USA) connected to a pressure amplifier (model PA-011; Star Medical, Tokyo, Japan) and a chart recorder (model R-52; Rikadenki Kogyo Co. Ltd., Tokyo, Japan). A 30-min equilibration period was allowed before starting an experiment. KCl (0.1–10.0 mg/preparation) and norepinephrine (NOR-ADRENALIN; Sankyo Co. Ltd., Tokyo, Japan, 0.03–3.0 μg/preparation) were injected intra-
luminally into the perfusion fluid in a volume of 0.1 ml. NE and KCl injections induced a transient increase of perfusion pressure for 5–10 min; the peak height was recorded as NE- and KCl-induced responses.

In our preparations, sympathetic nerve terminals were left intact. It was suggested that when NE is introduced intraluminally there is less chance for neuronal uptake to impede access to the vascular smooth muscle \(\alpha\)-receptors and thus sensitivity differences are seen without uptake blockade. In this study, sensitivity and reactivity differences of the NE-induced response were seen without uptake blockade. Furthermore, KCl-induced responses were not affected by the addition of guanethidine (1 \(\mu\)M) into the perfusion fluid, which interferes with the release of NE from peripheral sympathetic nerve terminals.

**Statistics:**

All data are shown as means±SD. The statistical significance was determined by the Student’s t-test. Furthermore, in the analysis for dose-response curves, a two-way analysis of variance was followed by the Bonferroni method for significance for inter-strain comparison.

**RESULTS**

**Blood pressure:**

The systolic blood pressure was significantly higher in SHR than in age-matched WKY at all ages investigated (Table I). The difference in blood pressure between SHR and WKY increased with age.

**Morphology:**

The results of morphometric studies of the large mesenteric arteries

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age (weeks)</th>
<th>No. of rats</th>
<th>No. of SM* cell layers in media</th>
<th>Media thickness ((\mu)m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>6</td>
<td>6</td>
<td>3.2±0.4</td>
<td>10.6±0.6</td>
</tr>
<tr>
<td>SHR</td>
<td>6</td>
<td>6</td>
<td>4.4±0.4**</td>
<td>14.4±1.2**</td>
</tr>
<tr>
<td>WKY</td>
<td>11</td>
<td>6</td>
<td>3.5±0.5</td>
<td>10.7±0.6</td>
</tr>
<tr>
<td>SHR</td>
<td>11</td>
<td>6</td>
<td>5.0±0.3**</td>
<td>15.0±2.1**</td>
</tr>
<tr>
<td>WKY</td>
<td>18</td>
<td>6</td>
<td>3.6±0.3</td>
<td>12.8±2.1</td>
</tr>
<tr>
<td>SHR</td>
<td>18</td>
<td>6</td>
<td>5.2±0.7**</td>
<td>19.0±1.7**</td>
</tr>
</tbody>
</table>

* SM=smooth muscle.

* Significantly different from age-matched WKY (p<0.05).
ALTERATIONS OF MESENTERIC VASCULAR BEDS IN SHR

Table I. Systolic Blood Pressure

<table>
<thead>
<tr>
<th>Strain</th>
<th>Systolic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-week-old</td>
</tr>
<tr>
<td>WKY</td>
<td>133.3±8.0  (n=23)</td>
</tr>
<tr>
<td>SHR</td>
<td>152.6±10.7** (n=24)</td>
</tr>
</tbody>
</table>

** Significantly different from age-matched WKY (p<0.01).

Fig. 2. Light micrographs of cross-sections of large mesenteric arteries from 6-week-old SHR and WKY (magnification bars = 50 μm).

ments of Large Mesenteric Arteries

<table>
<thead>
<tr>
<th>Wall thickness (μm)</th>
<th>Lumen diameter (μm)</th>
<th>Media/Lumen ratio</th>
<th>Wall/Lumen ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.4±2.6</td>
<td>188.9±9.6</td>
<td>0.05±0.01</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>23.3±1.5**</td>
<td>188.8±18.8</td>
<td>0.08±0.01**</td>
<td>0.13±0.01**</td>
</tr>
<tr>
<td>19.9±0.6</td>
<td>189.6±6.9</td>
<td>0.06±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>26.7±5.2**</td>
<td>190.9±8.1</td>
<td>0.08±0.01**</td>
<td>0.14±0.01**</td>
</tr>
<tr>
<td>21.4±3.3</td>
<td>189.8±16.5</td>
<td>0.07±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>28.1±3.2**</td>
<td>200.7±17.9</td>
<td>0.10±0.01**</td>
<td>0.14±0.03*</td>
</tr>
</tbody>
</table>

** Significantly different from age-matched WKY (p<0.01).
Fig. 3. Flow rate-perfusion pressure curves in the isolated perfused mesenteric vascular beds from 6- (6w), 11- (11w) and 18-week-old (18w) SHR and WKY. There was a significant difference between WKY and SHR curves at the age of 18 weeks (two-way analysis of variance followed by the Bonferroni method; p<0.01). *, ** Significantly different from age-matched WKY (Student’s t-test; p<0.05, 0.01, respectively).

are summarized in Table II. The media of the vascular wall was thicker in SHR than in WKY at all ages investigated. The difference was apparent even in 6-week-old rats (Fig. 2). The number of smooth muscle cell layers in the media was larger in SHR than in WKY (Table II). This difference between SHR and WKY increased with age, in parallel with blood pressure. The vascular wall was thicker in SHR than in WKY. However, there
Fig. 4. Pressor responses to KCl in the isolated perfused mesenteric vascular beds from 6- (6w), 11- (11w) and 18-week-old (18w) SHR and WKY. There were significant differences between WKY and SHR curves at the ages of 11 and 18 weeks (two-way analysis of variance followed by the Bonferroni method; p<0.05 and p<0.05, respectively). *, ** Significantly different from age-matched WKY (Student's t-test; p<0.05, 0.01, respectively).

Fig. 5. Pressor responses to norepinephrine in the isolated perfused mesenteric vascular beds from 6- (6w), 11- (11w) and 18-week-old (18w) SHR and WKY. There were significant differences between WKY and SHR curves at the ages of 6, 11 and 18 weeks (two-way analysis of variance followed by the Bonferroni method; p<0.01, p<0.01 and p<0.05, respectively). *, ** Significantly different from age-matched WKY (Student's t-test; p<0.05, 0.01, respectively).
was no significant difference in lumen diameter between SHR and WKY. The ratios of media thickness and wall thickness to lumen diameter were larger in SHR than in WKY.

**Vascular reactivity:**

Flow rate-perfusion pressure curves are presented in Fig. 3. There was no significant difference in the vascular basal resistance to flow between 6-week-old SHR and age-matched WKY. The vascular basal resistance became higher in SHR than in WKY as rats grew older. In 18-week-old SHR, the vascular basal resistance was significantly higher than in age-matched WKY.

The pressor responses to KCl are shown in Fig. 4. There was no significant difference in the pressor response to KCl between 6-week-old SHR and age-matched WKY. The pressor response to KCl increased more noticeably in SHR preparations than in WKY preparations as rats grew older. However, there was no significant difference in the sensitivity to KCl between SHR and WKY.

The pressor responses to NE (Fig. 5) were significantly higher in SHR preparations than in WKY preparations at all ages investigated. In marked contrast to the vascular basal resistance and the pressor response to KCl, the pressor response to NE was significantly elevated in SHR at the age of 6 weeks. The sensitivity to NE was significantly higher in SHR than in WKY only at the age of 6 weeks (pD\textsubscript{2} (=-\log D\textsubscript{50}(g)); 6.52±0.16 in WKY (n=5), 6.71±0.09 in SHR (n=5), p<0.05).

**DISCUSSION**

In this study, we showed clearly that there are structural and functional alterations in mesenteric vascular beds from SHR in the early stages of hypertension development. Isolated, perfused mesenteric vascular bed preparations from 6-week-old SHR were much more reactive to NE than those from WKY. The structural alteration of mesenteric arteries increased in older SHR, in parallel with the development of hypertension. Since the mesenteric vascular beds and arteries investigated in this study are responsible for the peripheral resistance, our results suggest that both functional and structural alterations in resistance vessels may be important in the development and maintenance of hypertension. This may be associated with the increased sympathetic activity reported in SHR.

At all ages investigated, the walls and media of large mesenteric arteries were thicker in SHR than in WKY. These results support the findings of
Lee[4], [5] and Warshaw et al.[8] but do not support the work of Limas et al.[7]. Furthermore, we found that the media was thicker in SHR than in WKY at the age of 3 weeks (media thickness; 6.4±0.3 μm in WKY (n=5), 10.1±0.8 μm in SHR (n=5), p<0.01). Since the number of smooth muscle cell layers in the media was larger in SHR than in WKY and this difference increased with age, this study supports a progressive hyperplasia of smooth muscle cells in the media of large mesenteric arteries in SHR.[14] Flow rate-perfusion pressure curves indicated that the vascular basal resistance to flow became higher in SHR preparations than in WKY preparations as rats grew older (Fig. 3). This difference may be caused by a structural alteration of the resistance vessel wall in SHR (Table II).

There was no significant difference in pressor responses to KCl between 6-week-old SHR and age-matched WKY (Fig. 4). This result is consistent with those of Mulvany and Nyborg[11] and Whall et al.[19] and suggests that the potassium (potential)-dependent regulation of contraction in mesenteric resistance vessels from SHR is not altered.[11], [12] The pressor response to KCl became higher in SHR preparations than in WKY preparations as rats grew older (Fig. 4). This may partly reflect an increase in the number of smooth muscle cell layers in media of resistance vessels in SHR (Table II), because thicker media may produce higher total tension and higher perfusion pressure as indicated by Laplace's law.[20]

In marked contrast to the vascular basal resistance and the pressor response to KCl, the pressor response to NE was greatly elevated in SHR preparations at the age of 6 weeks (Fig. 5). This extremely high NE response in younger SHR may not be caused by a structural change in resistance vessels, but may indicate a functional change. Such a physiologic change in smooth muscle cells of resistance vessels may be mediated through α1-adrenoceptors,[21] and may be important in the development of hypertension in SHR. Nyborg and Bevan showed an increased α-adrenoceptor affinity for NE in resistance vessels from SHR.[22] Mulvany and Nyborg[11] and Cauvin et al.[12] suggested that Ca²⁺ influx pathways activated by NE in mesenteric resistance vessels may be altered in SHR. Uehara et al.[23] reported that the activity of phospholipase C is enhanced in the aorta of young and adult SHR. Turla and Webb[24] suggested that increased vascular responsiveness in stroke prone SHR (SHRSP) may result from enhanced activity of the protein kinase C branch of the Ca²⁺ messenger system. Therefore, these alterations of the signal transduction process may cause functional changes in the resistance vessels. However, further research is needed to elucidate the role of these factors in detail. It is also possible that both structural and functional alterations are caused by the hyperreactivity to NE through
an altered signal transduction process in smooth muscle cells of resistance vessels, because the NE signal transduction process may induce contraction and cell proliferation in these cells.\(^2\)

In conclusion, this study has demonstrated both functional and structural alterations in mesenteric resistance vessels from SHR in the early stage of hypertension development. Both alterations may be caused by hyperreactivity to NE through an altered signal transduction process in smooth muscle cells of resistance vessels, and may be important in the development and maintenance of hypertension. Further research is needed to elucidate the cause of the hyperreactivity to NE of resistance vessels in SHR. In future research, the extremely high pressor response to NE of mesenteric vascular beds in very young SHR will be a noteworthy phenomenon.

**REFERENCES**