Role of Endothelium in Dilator Responses of Spontaneously Hypertensive Rat Arteries

Makoto Konishi, M.D. and Che Su, Ph.D.

SUMMARY The possible role of endothelium in the vascular responses to vasodilator drugs was studied in relation to experimental hypertension. Short ring segments of the thoracic aorta and femoral artery were removed from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. They were rubbed on the intimal surface to destruct endothelial cells or unrubbed, and bathed in Krebs-bicarbonate solution for isometric recording of contractile and relaxant responses. The relaxant response to acetylcholine was abolished by rubbing all arteries tested. Rubbing also significantly attenuated the relaxation induced by adenosine in the SHR aorta and SHR and WKY femoral artery, and attenuated the relaxation by isoproterenol in the SHR femoral artery. In contrast, contractile response to norepinephrine was augmented by intimal surface rubbing in all arteries except the aorta of WKY. It is suggested that an endothelial compensatory mechanism develops to offset the diminished relaxant and/or increased contractile responsiveness of smooth muscle cells in the hypertensive rat arteries. (Hypertension 5: 881–886, 1983)

Key Words • endothelium • vasodilation • hypertension • spontaneously hypertensive rats

Blood vessels of spontaneously hypertensive rats (SHR) have been reported to differ from those of normotensive Wistar-Kyoto (WKY) rats in response to drugs. The thoracic aorta of SHR gave a smaller relaxant response to acetylcholine (ACh), adenosine (ADO), and isoproterenol (ISO) than did the aorta of WKY. Other investigators found that the ADO- and ISO-induced relaxant responses of the thoracic aorta, perfused mesenteric artery, and perfused hindlimb of SHR were greater than those of WKY. Finally, the contractile response to norepinephrine (NE) of the SHR aorta was either equal to or smaller than that of the WKY aorta, but the response of the SHR hindquarters was greater than the WKY counterparts. The discrepancies remain unexplained but it now appears that the role of the endothelium needs to be considered.

In recent years, Furchgott and his associates have demonstrated that the endothelial cells participate in the vasodilator effects of a variety of drugs. They have postulated that ACh, for example, releases from endothelial cells a substance that relaxes vascular smooth muscle. However, ADO and ISO and some other agents do not require endothelial participation for relaxation of the rabbit thoracic aorta, according to some investigators.

The present work was undertaken to reassess the possible contribution of endothelium to the responses of rat arteries to ACh, ADO, and ISO and, second, to the differences between arteries of SHR and WKY. In addition to the commonly used thoracic aorta and relaxant responses, the femoral artery and NE-induced contraction were studied for comparison purposes.

Methods

Male 15- to 16-week-old age-matched SHR and WKY were used. Mean body weights of these rats were 293 ± 5.5 g (n = 17) and 317 ± 4.3 g (n = 20) respectively. Mean systolic blood pressure of SHR and WKY were 186.1 ± 2.3 mm Hg (n = 17) and 135.4 ± 2.0 mm Hg (n = 20) (p < 0.001) by the tail-cuff method in the unanesthetized state. The rat was anesthetized by intraperitoneal pentobarbital (50 mg/kg) and exsanguinated. The thorax was opened, and the descending thoracic aorta and femoral artery were immediately excised. After removal of loose connective tissue, a 3 mm-long cylindrical segment was cut from each artery. An adjacent segment was taken after destruction of endothelium; the intimal surface of the aorta was gently rubbed by a wooden rod 1 mm in diameter and that of the femoral artery by a 24-gauge
steel wire. Two stainless steel wires (0.1 mm outside diameter) were inserted through the lumen of the vessel ring; one was anchored to a stationary support and the other connected to a Statham force displacement transducer coupled to a Grass polygraph. The preparation was bathed in Krebs-bicarbonate solution aerated with a mixture of 95% O₂ and 5% CO₂ and maintained at 37°C. The composition of the Krebs-bicarbonate solution was (in mM): NaCl, 120; KCl, 5.2; CaCl₂·2H₂O, 2.4; MgSO₄·7H₂O, 1.2; NaHCO₃, 25; Na₂EDTA, 0.03; dextrose, 11 (pH, 7.4). A resting stretch of 2 g, optimum for both the aorta and femoral artery of WKY or SHR, was applied to the ring, which was allowed to equilibrate for 90 minutes during which time the bath medium was replaced every 20 minutes. The contractile response to 30 mM KCl was first obtained.

Preliminary experiments showed that 30 mM KCl induced 65% to 75% of the maximum KCl contractions, and endothelium removal reduced the maximum response without shifting the concentration-effect curve. After repeated rinsing, the arterial segment was maintained in a contracted state with a near ED₃₀ (median effective) concentration of NE (10⁻⁸ to 10⁻⁷ M) to study the relaxant responses to ACh, ADO, and ISO, in that order. Each agent was applied in cumulative concentrations and followed by a 40-minute resting interval with two rinsings. Papaverine 10⁻⁴ M, which was previously determined to cause the maximum relaxation, was subsequently added. The relaxant responses to ACh, ADO, and ISO were expressed as % of the response to papaverine. Finally, NE (10⁻⁹ to 10⁻³ M) was cumulatively applied to obtain contractile responses.

Results are presented in mean values ± SEM, and statistical analyses were made by Student's t test. Drugs used were L-norepinephrine bitartrate, L-isoproterenol bitartrate, adenosine, papaverine, acetylcholine chloride (Sigma Chemical Company, St. Louis, Missouri), and potassium chloride.

Results

WKY Arteries

ACh (10⁻⁸ to 10⁻⁵ M) relaxed the thoracic aorta and femoral artery from either WKY or SHR in a concentration-dependent manner. Following rubbing of the arterial internal surface, the effect of ACh was practically abolished, as illustrated in figure 1. The relaxant effect was maximum at about 10⁻⁶ M ACh, although a slight contractile effect may have counteracted the relaxant at this concentration and at 10⁻³ M. No appreciable contraction, however, resulted in the rubbed arteries (fig. 2). In all arteries tested, the ACh-induced relaxation was completely prevented by 10⁻⁷ M atropine, which did not affect the relaxation responses to adenosine or isoproterenol.

Adenosine (10⁻³ to 3 × 10⁻⁴ M) was relaxant in both arteries with or without rubbing. While rubbing had no influence at all in the aorta, it significantly

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**Figure 1.** Relaxant responses to acetylcholine (ACh) and papaverine (PA) of unrebed (left) and rubbed (right) femoral artery and thoracic aorta from SHR. The preparations were precontracted with norepinephrine (NE). Molar concentrations of drugs are indicated.
attenuated the relaxation by adenosine at $10^{-3}$ and $10^{-4}$ M in the femoral artery (fig. 3). Isoproterenol ($10^{-8}$ to $10^{-3}$ M) elicited the largest relaxation of the three agents tested at relatively low concentrations. Rubbing the intimal surface of the aorta or femoral artery did not significantly alter the concentration-effect relationship of this agent (fig. 4).

Norepinephrine ($10^{-8}$ to $10^{-5}$ M) contracted both arteries (fig. 5). Rubbing the intimal surface slightly but not significantly increased the aortic contractile responses, but it considerably increased the femoral arterial responses to NE ($10^{-7}$ and $10^{-6}$ M).

**SHR Arteries**

In response to ACh ($10^{-6}$ and $10^{-3}$ M), the unrubbed aorta from SHR relaxed significantly less, and the SHR femoral artery relaxed more, compared to their WKY counterparts. The apparent ED$_{50}$ did not differ between SHR and WKY for either artery (fig. 2). A greater contractile component was possibly associated with the SHR aorta than the WKY aorta, as figure 2 indicates.

Rubbing rendered the SHR aorta much less responsive to adenosine $10^{-4}$ and $3 \times 10^{-4}$ M, and less responsive than the rubbed or unrubbed WKY aorta at all relaxant concentrations. The unrubbed SHR aorta was also less responsive than the WKY aorta to adenosine $10^{-3}$ M (fig. 3 left). Similar relationships held for the femoral artery, except that the unrubbed SHR artery gave smaller responses to all concentrations of adenosine than did the WKY artery (fig. 3 right).

The relaxation elicited by ISO in the SHR aorta was slightly though not significantly diminished by rubbing, except at the highest ISO concentration tested ($10^{-3}$ M) (fig. 4 left). In the femoral artery, also, rubbing significantly attenuated the effect of ISO ($10^{-8}$, $10^{-7}$, and $10^{-5}$ M) in the SHR but not WKY preparations (fig. 4 right). The unrubbed SHR aorta and femoral artery were equally responsive as the WKY preparations. The rubbed SHR femoral artery was less responsive than the rubbed WKY artery to ISO ($10^{-8}$ to $10^{-5}$ M). The ISO-induced relaxation was completely blocked in all four arteries tested by pretreatment with $10^{-6}$ M propranolol, which did not alter responses to ACh or ADO.

The NE-induced contraction (in mg developed force) of the unrubbed thoracic aorta and femoral artery from SHR was not significantly different from those from WKY. Rubbing significantly potentiated the contractions induced by NE at concentrations $10^{-8}$ to $10^{-5}$ M in the aorta of SHR and $10^{-8}$ to $10^{-7}$ M in the femoral artery of SHR (fig. 5).

Unlike the NE-induced contraction, the KCl-induced contraction tended to decrease with rubbing. Consequently, the ratio between the two was greater rubbed than unrubbed for either the aorta or femoral artery of WKY or SHR (table 1).
FIGURE 3. Dose-response curves for adenosine in thoracic aorta (left) and femoral artery (right). WKY arteries are denoted by ○ (unrubbed) and ● (rubbed), and SHR arteries by △ (unrubbed) and ▲ (rubbed). *p < 0.05, **p < 0.01, WKY or SHR rubbed artery vs unrubbed; ††p < 0.01, rubbed or unrubbed SHR artery vs WKY artery. Vertical bars represent SEM.

FIGURE 4. Dose-response curves for isoproterenol in thoracic aorta (left) and femoral artery (right). WKY arteries are denoted by ○ (unrubbed) and ● (rubbed), and SHR arteries by △ (unrubbed) and ▲ (rubbed). *p < 0.05, **p < 0.01, WKY or SHR rubbed artery vs unrubbed; ††p < 0.01, rubbed or unrubbed SHR artery vs WKY artery. Vertical bars represent SEM.
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Discussion

ACh, ADO, and ISO all relaxed the untreated isolated rat aorta and femoral artery. The results support the reports of smaller effects of ACh and ADO in the SHR aorta than in WKY aorta.2 2 A similar relationship was found to hold for ADO, but the reverse was true for ACh, in the femoral artery. Contrary to some reports,1-6 ISO was equipotent in the unrubbed aorta or femoral artery of SHR and WKY. Finally, unlike the smaller NE contractile response of SHR aorta than of WKY aorta observed by other investigators,4 8 no significant difference was seen in the present study.

It is noteworthy that an appreciable relaxant effect of ACh was observable only in the unrubbed arteries presumably with largely intact endothelial cells. These cells are highly fragile. It is possible that, in the previously reported studies, endothelial losses occurred unwittingly at times to affect the results and this, among other factors, contributed to the discrepancies. For example, with injured endothelial cells, the relaxant response to ACh may be too small or virtually absent to permit accurate comparisons between arteries of SHR and WKY. Conversely, the effects of ADO and ISO, which are otherwise comparable at least in the aortas of WKY and SHR, may be disproportionate-ly mitigated by endothelial injury in the SHR arteries. It seems that vasoactive drug effects on blood vessels in relation to hypertension must take the endothelial intactness into account.

Table I. Contractile Responses to Norepinephrine (NE, 10^-5 M) and KCl (30 mM)

<table>
<thead>
<tr>
<th></th>
<th>Thoracic aorta</th>
<th>Femoral artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unrubbed</td>
<td>Rubbed</td>
</tr>
<tr>
<td>NE</td>
<td>2.0±0.1</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>KCl</td>
<td>1.7±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Ratio NE/KCl</td>
<td>1.3±0.1</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

NE = norepinephrine; KCl = potassium chloride; WKY = Wistar-Kyoto rat; SHR = spontaneously hypertensive rat. Values are means ± SEM developed force (g).

*p < 0.05, compared with the unrubbed artery.

†p < 0.01, compared with the unrubbed artery.
The obligatory endothelial role in the ACh-induced relaxation has been demonstrated by many investigators in all blood vessels examined. Abolition of this relaxation by rubbing the arterial internal surface in the present study suggests effective endothelial cell destruction by rubbing. Our scanning electron microscopy also indicated destruction or removal of nearly all endothelial cells in rubbed arteries (unpublished data). Furchgott and his associates\textsuperscript{12-14} and Chand and Altura\textsuperscript{17} reported that the relaxant responses of the rabbit aorta and canine pulmonary artery to ISO were unaffected by intimal surface rubbing. Similarly, ADO and AMP, unlike ADP and ATP, were said to act directly on vascular smooth muscle cells in these vessels.\textsuperscript{12, 14, 18} In the present work, however, internal surface rubbing significantly reduced the ISO and ADO effects on the SHR aorta and femoral artery. Although these findings may in part reflect a genetic oddity associated with SHR, the femoral artery of WKY was also significantly affected by rubbing in its response to ADO. The disparity between the present and published results requires further clarification.

Intimal rubbing potentiated contractile responses to some concentrations of NE in the aorta of SHR and femoral artery of SHR and WKY. This is contrary to the contraction by 30 mM KCl, which was uniformly suppressed by rubbing. Similarly, DeMey and Vanhoutte\textsuperscript{18} observed a significant diminution of KCl-induced, but not NE-induced, contraction of the canine femoral artery. Since KCl is considered to act directly on smooth muscle cells, the reduced contraction possibly indicates injury to some muscle cells associated with rubbing. This injury presumably equally affected the action of NE on smooth muscle cells, and without such injury, the potentiation of NE by rubbing would even be greater. Our recent studies indicate that another alpha-adrenoceptor agonist, phenylephrine, is also augmented by destruction of endothelium (Koshi and Su, unpublished data).

The rubbed aorta and femoral artery of SHR are relaxed less by ADO and ISO than the WKY arteries. This probably represents diminished responsiveness of vascular smooth muscle in the absence of endothelium to the two dissimilar agents and possibly also other vasodilators. ADO is thought to be a circulatory regulator in several vascular beds including skeletal muscle,\textsuperscript{19} and the vasodilator effect of ISO presumably can be mimicked by circulating epinephrine to some extent. The attenuated smooth muscle responsiveness to physiological vasodilators may thus contribute to the elevated blood pressure in SHR. The endothelium apparently plays a greater role in the drug-induced relaxation of arteries from SHR than those from WKY. Furthermore, endothelial destruction had a greater potentiating effect on the NE-induced contraction in the aorta of SHR (fig. 5). In other words, it seems that endothelial cells tend to promote vascular muscle relaxation (with ADO and ISO) and counter its contraction (with NE), generally to a greater extent in SHR than WKY. This endothelial function may possibly be a compensatory mechanism developed to offset the diminished relaxant and/or enhanced contractile responsiveness of smooth muscle cells in the hypertensive rat arteries.

References

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