β-Adrenergic Relaxation in Mesenteric Resistance Arteries of Spontaneously Hypertensive and Wistar-Kyoto Rats: The Role of Precontraction and Intracellular Ca$^{2+}$

W. Matthijs Blankesteijn, *Nicolaas J. H. Raat, †Peter H. G. M. Willems, and Theo Thien

Department of Medicine, Division of General Internal Medicine, University Hospital Nijmegen, and Departments of *Physiology and †Biochemistry, University of Nijmegen, The Netherlands

Summary: An attenuated β-adrenergic vasodilation of small arteries may help explain the increased peripheral resistance in hypertension. To investigate this, we compared the isoprenaline-induced relaxation of mesenteric resistance arteries of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) using a small vessel myograph. The arteries had similar diameters, but the contractile force induced by cumulative addition of K$^+$ (10–130 μM) was 1.3-fold higher for the SHR. The β-adrenoceptor-mediated relaxation of arteries precontracted with 40 μM K$^+$, was significantly less in SHR (41 ± 3%, n = 11) than in WKY (56 ± 3%, n = 15, p = 0.003), and the pD$_2$ value for isoprenaline was significantly lower in SHR (7.13 ± 0.09 vs. 7.41 ± 0.07, p = 0.02). In contrast, when precontracted with phenylephrine (PE, α₁-adrenoceptor agonist, 3–10 μM), isoprenaline relaxation was almost complete in both SHR and WKY, and the pD$_2$ value for isoprenaline did not differ between strains. Forskolin induced complete relaxation of both precontractions. Because the β-adrenergic relaxation of the mesenteric resistance arteries was attenuated only after K$^+$-precontraction, we conclude that alterations in this precontracting mechanism in SHR rather than a defect in the β-adrenoceptor system may provide an explanation for the decreased relaxation in these vessels. Intracellular Ca$^{2+}$ measurements and a review of the literature support this conclusion. Key Words: β-Adrenergic relaxation—Mesenteric resistance arteries—Isoprenaline—Forskolin—Spontaneously hypertensive rats—Intracellular Ca$^{2+}$ concentration—Precontraction.

Increased peripheral vascular resistance (PVR) is common in hypertension (1). Because stimulation of β-adrenoceptors on the smooth muscle cells (SMC) of the vascular wall results in vasodilation, a blunted β-adrenergic response may help explain the increased peripheral resistance of the blood vessels in hypertension (2,3). Alterations in the β-adrenoceptor system in hypertension have been investigated quite extensively. For most of these studies, animal models of hypertension, such as the spontaneously hypertensive rat (SHR), have been used. In studies of conduit arteries of SHR, such as aorta and femoral artery, Cohen and Berkowitz (4) and Asano and co-workers (5–10) showed that the β-adrenergic responsiveness was decreased. The main part of the vascular resistance, however, is generated in the smaller arteries or resistance vessels (11). Therefore, these vessels appear to be more appropriate for investigation of alterations in β-adrenergic responsiveness in hypertension. In the present study, we examined the β-adrenoceptor-mediated relaxation in mesenteric resistance arteries from SHR and Wistar-Kyoto rats (WKY) with the β-adrenoceptor agonist isoprenaline.

The contraction and relaxation of vascular SMC (VSMC) is the result of a change in the intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) or a modulation of the sensitivity of the contractile apparatus to Ca$^{2+}$ (12). The contraction of mesenteric resistance arteries with K$^+$ has been shown to be mainly the result of

Received March 15, 1995; revision accepted August 28, 1995.

Address correspondence and reprint requests to Professor Dr. Th. Thien at Department of Medicine, Division of General Internal Medicine, University Hospital Nijmegen St. Radboud, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

The present address of Drs. Blankesteijn and Raat is Department of Pharmacology, University of Limburg, Maastricht, The Netherlands.
an increase in [Ca\(^{2+}\)]\(i\), whereas phenylephrine (PE, \(\alpha_1\)-adrenoceptor agonist) mainly induces a sensitization to Ca\(^{2+}\) (15). To determine the influence of different mechanisms of contraction on the \(\beta\)-adrenoceptor-mediated relaxation, we contracted the arteries with either K\(^+\) or PE and then exposed them to isoprenaline. The effect of K\(^+\), PE, isoprenaline and forskolin on the intracellular Ca\(^{2+}\) concentration of VSMC muscle cells was investigated by digital imaging microscopy of Fura-2-loaded mesenteric resistance arteries from WKY.

**MATERIALS AND METHODS**

**Materials**
Isoprenaline was purchased from Lanksberg BV (Uden, The Netherlands), PE from Genfarm BV (Maarssen, The Netherlands), and forskolin from Molecular Probes (Eugene, OR, U.S.A.). All other reagents were of analytical quality.

**Preparation of the mesenteric arteries**
Male SHR and WKY were obtained from Harlan Olac, Bicester, U.K. At the age of 12–18 weeks, the rats were killed by cervical dislocation. The intestine was removed and stored for 24 h at 4°C in phosphate-buffered saline solution (PSS: NaCl 120 mM, KCl 4.7 mM, CaCl\(_2\) 2.5 mM, NaHCO\(_3\) 25 mM, KH\(_2\)PO\(_4\) 1.2 mM, MgCl\(_2\) 1.17 mM, EDTA 2.7 mM, and glucose 5.5 mM). Storage of the intestine for 24 h did not affect any of the properties determined in this study. Parts of the small intestine, 7–15 cm from the stomach, were fixed on a wax plate and stored at room temperature for 2 h in the dark. The incubation solution was then changed, followed by a second incubation for 2 h, in which the artery was transferred to a thermostated (30°C) perfusion chamber (Leiden chamber). The incubation volume was ~300 μl. The tissue was superfused at a flow rate of 1 ml/min with PSS and thoroughly gassed with 95% O\(_2\)/5% CO\(_2\). The test substances were dissolved in the medium and delivered to the tissue by means of superfusion. To remove nonhydrolyzed Fura-2-AM and pluronic F127, the arteries with either K\(^+\) or PE and then exposed to isoprenaline. The effect of K\(^+\), PE, isoprenaline and forskolin on the intracellular Ca\(^{2+}\) concentration of VSMC muscle cells was investigated by digital imaging microscopy of Fura-2-loaded mesenteric resistance arteries from WKY.

**Contraction and relaxation of resistance arteries**
After normalization, the resistance arteries were contracted for 1 min by exchanging the PSS for K-PSS, in which all NaCl was replaced by KCl (total K\(^+\) concentration 125 mM), followed by rinsing twice with PSS and repeated after 15 min. A cumulative concentration-response curve was recorded by increasing the K\(^+\)-concentration stepwise from 10 to 125 mM K\(^+\). After two

ADRENERGIC RELAXATION IN SHR AND WKY

FIG. 1. Concentration-response curve for the contraction of mesenteric resistance arteries from spontaneously hypertensive rats (SHR, solid line, n = 11) and Wistar-Kyoto rats (WKY, dashed line, n = 15) to cumulative addition of K+. Curves were constructed by fitting the averaged data from the individual experiments. Error bars indicate SE.

U.S.A.). Sensitivities to drugs, expressed as pD2 values, and maximal effects were calculated on the basis of data from individual arteries. The responses to relaxing agents were expressed as the absolute decrease in tension (mN) and as a percentage of the precontraction. The data are mean ± SE. The results were analyzed statistically with a two-sample Wilcoxon rank sum test for comparison of the SHR and WKY arteries, and a paired Wilcoxon signed-rank test for comparison of the different precontractions. A p-value < 0.05 (two-sided) was considered statistically significant, insignificant differences are indicated by NS in the text.

RESULTS

Concentration-response curves for K+

A cumulative increase in K+ concentration induced a concentration-dependent contraction of the mesenteric resistance arteries, as shown in Fig. 1. The maximal contractile force was 7.1 ± 0.5 mN (n = 11) for SHR arteries and 5.5 ± 0.7 mN (n = 15) for WKY arteries, a difference which was statistically significant (p = 0.03). The pD2 values for K+ were 1.33 ± 0.04 and 1.35 ± 0.03 (NS) for SHR and WKY, respectively.

Relaxation of the arteries with isoprenaline

Isoprenaline-mediated relaxations were studied after precontraction of the arteries with 40 mM K+, a precontraction that was stable for at least 40 min. As shown in Fig. 2A, the precontraction was slightly but insignificantly greater in arteries from SHR. The absolute isoprenaline-mediated relaxation (in mN) was not significantly different between the two groups. However, when expressed as a percentage of the precontraction with 40 mM K+ (Fig. 2B and Table 1), a significant attenuation of the relaxing effect of isoprenaline was observed in SHR as compared with WKY. The pD2 value for isoprenaline was significantly lower in the SHR arteries, precontracted with 40 mM K+ (Table 1).

To study the influence of the nature of precontraction on isoprenaline-mediated relaxations, we also used the α1-selective adrenergic agonist phenylephrine (17) as a precontracting agent. The contraction induced by PE (3–10 μM in PSS) was significantly stronger than the response to K+ in WKY arteries, but significantly weaker in SHR arteries (Table 2). PE induced an oscillating contraction in all arteries tested, a phenomenon which was also observed with oxymethazoline, norepinephrine, and serotonin (data not shown). In some of the preparations (3 of 11 SHR, 3 of 15 WKY) the PE response was transient and did not reach sufficient stability to allow study of the isoprenaline-mediated relaxation. In contrast to K+ precontraction, the β-adrenoceptor-mediated relaxation was almost complete in PE-precontracted arteries (Fig. 3A). The relative relaxation shown in Fig. 3B was similar in SHR and WKY, although the concentration-response curve for WKY was shifted to the right. This resulted in a tendency toward a higher pD2 value for isoprenaline in SHR (Table 1), which just failed to reach statistical significance.

Relaxation of the mesenteric resistance arteries with forskolin

Cumulative addition of forskolin induced an almost complete relaxation of mesenteric resistance arteries precontracted with 40 mM K+. As shown in Table 1, no marked differences in forskolin-mediated relaxation were evident between SHR and WKY arteries. Forskolin also completely relaxed


## TABLE 1. Comparison of the relaxations induced by isoprenaline and forskolin in SHR and WKY mesenteric resistance arteries

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>WKY</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isoprenaline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precontraction (mN)</td>
<td>5.7 ± 0.5</td>
<td>5.2 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum relaxation (mN)</td>
<td>2.3 ± 0.2</td>
<td>2.9 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum percentage of relaxation</td>
<td>41 ± 3</td>
<td>36 ± 3</td>
<td>0.003</td>
</tr>
<tr>
<td>pD₂ (M)</td>
<td>7.13 ± 0.09</td>
<td>7.41 ± 0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Precontraction (mN)</strong></td>
<td>4.7 ± 0.6</td>
<td>6.5 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum relaxation (mN)</td>
<td>4.4 ± 0.7</td>
<td>6.2 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum percentage of relaxation</td>
<td>93 ± 2</td>
<td>91 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>pD₂ (M)</td>
<td>7.05 ± 0.11</td>
<td>6.76 ± 0.11</td>
<td>0.07</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Forskolin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precontraction (mN)</td>
<td>4.8 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum relaxation (mN)</td>
<td>4.0 ± 0.4</td>
<td>3.7 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum percentage of relaxation</td>
<td>81 ± 3</td>
<td>84 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>pD₂ (M)</td>
<td>7.08 ± 0.12</td>
<td>7.01 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

**SHR**, spontaneously hypertensive rats; **WKY**, Wistar-Kyoto rats; **PE**, phenylephrine.

Data are mean ± SE calculated on the basis of the results of curve fits from individual arteries, with statistical analysis by Wilcoxon rank-sum test for unpaired samples.

Arteries precontracted with PE (data not shown).

### [Ca²⁺]i measurements

Measurements of the intracellular Ca²⁺ concentration were performed under conditions similar to those in the myograph. Loading of the arteries with Fura-2 resulted in fluorescent staining of the SMC spanning the arterial lumen. A tracing of a [Ca²⁺]i measurement is shown in Fig. 4. Superfusion with 40 mM K⁺ induced an increase in the 340/380 ratio from 0.87 ± 0.10 to 0.98 ± 0.12 (n = 4); this increase was rapidly reversible by superfusion with PSS. Superfusion with PE (10 µM) resulted in an average increase in the basal 340/380 ratio of 0.05 in three experiments, whereas in a fourth experiment no increase in ratio was detected. In three experiments, isoprenaline (10 µM) was added to the superfusate while the 340/380 ratio was increased with 40 mM K⁺, but this did not induce a decrease in the ratio. Similar results were obtained with forskolin (10 µM) and dibutyryl-cyclicAMP (100 µM).

### DISCUSSION

In the present study, the vasorelaxant properties of the β-adrenoceptor system of mesenteric resistance arteries of SHR and WKY rats were compared. To our knowledge, this is the first time that this receptor system has been studied in resistance vessels, which contribute strongly to vascular resistance and are therefore of primary interest in hypertension research.

The maximal contraction of the mesenteric resistance arteries, induced by K⁺, was significantly higher in SHR as compared with WKY. This finding is in agreement with results of other studies (8–10) in which strips of conducting arteries, mainly femoral arteries, from SHR and WKY were used.

## TABLE 2. Effect of different precontractions on the relaxing properties of isoprenaline

<table>
<thead>
<tr>
<th></th>
<th>K⁺</th>
<th></th>
<th>PE</th>
<th></th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR (n = 8)</td>
<td>Precontraction (mN)</td>
<td>5.8 ± 0.5</td>
<td>4.7 ± 0.6</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum relaxation (mN)</td>
<td>2.4 ± 0.2</td>
<td>4.4 ± 0.7</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum percentage of relaxation</td>
<td>41 ± 3</td>
<td>93 ± 2</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pD₂ (M)</td>
<td>7.14 ± 0.07</td>
<td>7.05 ± 0.11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY (n = 12)</td>
<td>Precontraction (mN)</td>
<td>5.6 ± 0.8</td>
<td>6.5 ± 1.0</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relaxation (mN)</td>
<td>3.1 ± 0.5</td>
<td>6.2 ± 1.1</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relaxation (%)</td>
<td>56 ± 3</td>
<td>91 ± 3</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pD₂ (M)</td>
<td>7.37 ± 0.05</td>
<td>6.76 ± 0.11</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

Statistical analysis by Wilcoxon signed-rank test for paired observations.
Superfusion with K+ induced an increase in the intracellular Ca\(^{2+}\) concentration, as shown. Unless otherwise indicated with light from 340 and 380 nm (340/380 ratio), a measure for increase in 340/380 ratio as compared with K+.

(FORSK, 10 \(i\) not be reversed with isoprenaline (ISO, 10 \(i\) phenylephrine by cumulative addition of isoprenaline. Spontaneously hypertensive rats (SHR, solid line, \(n = 8\)); Wistar-Kyoto rats (WKY, dashed line, \(n = 12\)). Details as in legend to Fig. 2.

These results indicate that both in conducting arteries and in resistance arteries of SHR, an abnormality exists, causing an increased K\(^+\)-induced contractile force. Besides an increased ratio of wall to lumen in SHR, Kojima and associates (18) described a malfunction of the sarcoplasmic reticulum (SR) in femoral arteries from SHR as compared with WKY that leads to an increased intracellular Ca\(^{2+}\) concentration on contractile stimulus.

In tissues from SHR and WKY precontracted with 40 mM K+, cumulative addition of isoprenaline induced a partial relaxation of the mesenteric resistance arteries. In a previous study, we showed that this relaxation was not the result of a release of endothelium-derived relaxing factor (EDRF) and therefore is probably the result of a direct stimulation of \(\beta\)-adrenoceptors on VSMC (19). When expressed as a fraction of the precontraction, the isoprenaline-induced relaxation was significantly less in SHR arteries. Moreover, the \(pD_2\) value for isoprenaline was significantly lower in SHR as compared with WKY. These results are similar to those of Cohen and Berkowitz (4) and Asano and co-workers (5–10) in studies of conducting arteries of SHR, in which the \(\beta\)-adrenoceptor mediated relaxation was expressed as a fraction of the papaverine-induced relaxation.

To investigate the effect of the type of precontraction on the \(\beta\)-adrenergic relaxation, the \(\alpha_1\)-adrenoceptor agonist PE was also used as a precontracting agent. The compound induces contraction through inositol phospholipid metabolism (20), without the \(\alpha_2\)-adrenoceptor–mediated inhibition of cyclicAMP production (21). Because the latter would interfere with the isoprenaline-mediated stimulation of cyclicAMP production, it would hamper the interpretation of the experiments. When the mesenteric resistance arteries from either SHR or WKY were precontracted with PE, isoprenaline could almost completely relax them, and no difference in relaxation was observed between the two groups. Moreover, the \(pD_2\) value for isoprenaline was no longer decreased in SHR. These results indicate that the \(\beta\)-adrenoceptor-mediated relaxation is dependent on the type of precontraction used. These results are in agreement with the data of Abe and Karaki (22), who showed that dibutylcyclic AMP could relax rat aorta strips more effectively after \(\alpha\)-adrenoceptor–mediated precontraction than after depolarization with K+

The stronger isoprenaline-mediated relaxation of PE-precontracted arteries as compared with K\(^+\)-precontracted arteries may be explained by the mechanisms by which these compounds induce contraction. Jensen and colleagues (13) showed that K+ induces contraction of mesenteric resistance arteries mainly by increasing the intracellular Ca\(^{2+}\) concentration, whereas norepinephrine induces contraction mainly by increasing the sensitivity of the contractile apparatus for Ca\(^{2+}\). Relaxation by cyclicAMP-generating agents can result from a desensitization of the contractile apparatus for Ca\(^{2+}\) (12); in the present study, this finding was supported by the measurements of the intracellular Ca\(^{2+}\) concentration in WKY. Therefore, the attenuated isoprenaline-mediated relaxation observed in SHR arteries precontracted with K+ is more likely to result from an increased contraction of these arteries to K+ than from a defect in the \(\beta\)-adrenergic system. At 40 mM K+, the SHR arteries tend to contract more strongly than those of WKY, which can be explained by a malfunction of the SR (18). Due to this malfunction, K+ induces a stronger increase in the intracellular Ca\(^{2+}\) concentration in SHR arteries.
which can less effectively be counteracted by isoprenaline, leading to an attenuated isoprenaline-induced relaxation of SHR arteries with the compound. Although one of the limitations of our study is that the [Ca$^{2+}$] measurements were made only in WKY arteries, we suggest that the attenuated $\beta$-adrenergic relaxation in SHR arteries precontracted with K$^+$ is better explained by an alteration in intracellular Ca$^{2+}$ handling in SHR arteries than by a defect in the $\beta$-adrenergic system itself. This finding is in agreement with recent reports concerning $\beta$-adrenoceptor density and function in human primary hypertension (23,24), in which no alterations in this receptor system were noted.

The $\beta$-adrenergic relaxation of mesenteric resistance arteries from SHR was attenuated after precontraction with K$^+$. This finding could not be reproduced with a PE precontraction and can probably be explained by the reported malfunction of the SR in SHR arteries. The $\beta$-adrenergic relaxation itself is likely to be unaltered in mesenteric resistance arteries of SHR.

Acknowledgment: We thank Dr. J. F. Rodrigues de Miranda and E. Nagtegaal for helpful discussions.

REFERENCES