Increased $\alpha$-Adrenergic Receptor Affinity in Resistance Vessels from Hypertensive Rats

Niels C. B. Nyborg and John A. Bevan

SUMMARY $\alpha$-Adrenergic receptor-related properties, specifically, norepinephrine affinity, occupancy and reserve during contraction, were determined in segments of rat resistance arteries. These were obtained from the superior mesenteric bed of spontaneously hypertensive rats and Wistar-Kyoto strain controls. Receptor affinity for norepinephrine in the spontaneously hypertensive rats was significantly greater than that for the Wistar-Kyoto controls. There were no differences in the estimates of receptor occupancy and reserve. This finding taken together with other studies is consistent with the conclusion that increased $\alpha$-adrenergic receptor-mediated sensitivity of vascular smooth muscle of the spontaneously hypertensive rat reflects differences in the agonist site on the $\alpha$-adrenergic receptor. (Hypertension 11: 635–638, 1988)

Key Words • receptor • affinity • norepinephrine • spontaneously hypertensive rats • vascular smooth muscle

Increased sensitivity of vascular smooth muscle to norepinephrine (NE) has been proposed as a factor responsible at least in part for the increased blood pressure of the spontaneously hypertensive rat (SHR). This increased sensitivity may result from differences in a number of factors, including cell membrane properties, level of membrane potential, receptor density, type of receptor, and excitation-contraction coupling. Although vascular smooth muscle cells from SHR are less polarized in their resting state, and this may contribute to the increased sensitivity of receptor-mediated contraction, attempts to characterize features of the postjunctional $\alpha$-adrenergic receptor itself have failed to establish notable differences using conventional in vitro analysis by receptor antagonists or radioligand binding.

The sensitivity of the contraction of a blood vessel to NE has been shown to be influenced by $\alpha$-adrenergic receptor number and affinity. These studies suggest that these receptor variables are cell-regulated attributes. Thus, they may not necessarily be identical in the same artery from different strains, such as the SHR and the Wistar-Kyoto rat (WKY), particularly when these arteries exhibit different sensitivities to NE.

The present experiments were undertaken to determine if there is a receptor-related basis for the increased NE sensitivity of vascular smooth muscle of mesenteric resistance arteries from SHR compared with normotensive WKY. The results suggest that $\alpha$-adrenergic receptor affinity, but not receptor reserve, may be responsible for the increased sensitivity of the vascular smooth muscle in the SHR to NE.

Materials and Methods

Three-month-old male SHR and WKY (Charles River, St.-Constant, Quebec, Canada) were anesthetized with ketamine (Ketalar, Parke-Davis), and their mean blood pressures were determined by direct cannulation of the aorta (SHR, 136 ± 14 mm Hg; WKY, 82 ± 10 mm Hg; $n = 3$ measurements/group; $p < 0.05$). The proximal part of the jejunum was removed and placed in oxygenated (5% CO$_2$, 95% O$_2$) physiological saline solution (PSS) with the following composition (mM): NaCl, 119; NaHCO$_3$, 25; KCl, 4.7; CaCl$_2$, 1.6; MgSO$_4$, 1.2; glucose, 11; EDTA, 0.023; ascorbic acid, 0.113; pH 7.4. The K-containing PSS (K-PSS) had the same composition, except that KCl was exchanged on an equimolar basis for NaCl. A small vessel segment was dissected from the second or third branch of the superior mesenteric artery and mounted on two 32-$\mu$m tungsten wires on a small vessel myograph. The wires were connected to a
force transducer (Model G10B, Statham, Oxnard, CA, USA) and micrometer, respectively. The vessels were equilibrated at 37°C for 30 minutes. On the basis of the passive wall force–internal circumference relationship of the vessels, the internal circumference was set to 90% of that achieved when relaxed and under a transmural pressure of 100 mm Hg. At this circumference, maximal active force is developed.

The vessels were activated three times with K-PSS containing NE (10^-6 M). The last of these three contractions was taken as the maximal mechanical tissue response. Two cumulative NE concentration-response curves were made in the presence of propranolol (3 × 10^-6 M; Sigma Chemical, St. Louis, MO, USA) and cocaine (3 × 10^-6 M) to block β-adrenergic receptors and neuronal NE uptake, respectively. Previous experiments have shown that uptake of extra-neuronal uptake) blockade does not influence the NE sensitivity of this tissue. Before the second NE concentration-response curve determination, the vessels were incubated with benextramine (3 × 10^-9 M; Sigma) for 15 minutes, followed by a 30-minute period in which the PSS bathing the tissue was frequently changed. Benextramine has a similar mode of action to phenoxybenzamine; it is an irreversible α-adrenergic receptor antagonist 15-16 and may have an advantage over phenoxybenzamine in measurement of the agonist dissociation constant (Kd) in that it has a lower lipid and higher water solubility and is more selective for the α-adrenergic receptor. Exposure to benextramine blocks irreversibly a fraction of the α-adrenergic receptors on the vascular smooth muscle cells. A depression in maximal NE response of 20 to 30% was sought.

The Kd and receptor occupancy were determined using the approach proposed by Furchgott. The reciprocals of equieffective concentrations of agonist before (A) and after benextramine (A') in the range of 20 to 80% of maximal response were determined. The slope and y-intercept of the regression line of 1/A against 1/A' were used to calculate Kd (Kd = slope – 1/intercept). Receptor occupancy, the fraction of receptors responsible for eliciting maximum response in vessels after partial irreversible blockade, was taken as 1/slope. The contractions in response to NE that were used in the calculation of Kd were invariably blocked completely by prazosin (10^-7 M). Relative receptor occupation, receptors occupied by agonist/total available receptors (R/R0), was calculated for the EC50 by substituting the calculated Kd values in the equation derived by Furchgott and Bursztyn, where R/R0 = EC50/Kd + EC50, when these values are expressed in molar concentrations. An alternative measurement of receptor reserve, –antilog (pD2 - pKd), was also used. PD2 and pKd are the negative logs of the agonist ED50 and dissociation constants, respectively, when expressed in molar concentrations.

Receptor dissociation constants and vessel sensitivity to NE are given in terms of pKd and pD2 values, respectively, where pKd = – log Kd and pD2 = – log EC50. Results are presented as means ± SE, and differences between the strains have been analyzed using Student’s t test, with the level of significance set below 0.05.

Results

Arterial segments taken from WKY, although they were from anatomically similar positions, were significantly larger than those from SHR. Equilibrium dose-concentration effect curves for NE were typical for this artery and have been published previously (see, for example, References 2 and 3). Based on the pD2 value — the negative log of the ED50 — SHR vessels were about three times more sensitive to NE than were those from WKY (p < 0.01; Table 1). The mean maximal responses to NE were 87 ± 5% (n = 5) and 70 ± 9% (n = 5) of maximal tissue responses to K-PSS in the SHR and WKY, respectively. Treatment with benextramine, an irreversible antagonist of the α-adrenergic receptor, had the classic effect of an irreversible antagonist; it reduced the slope and the maximum value of the NE dose-response curves. The maximal contraction to NE decreased to 81 ± 4% (n = 5) and 72 ± 4% (n = 5) of control in SHR and WKY mesenteric resistance vessels, respectively. Neither of these latter pairs of values were significantly different from each other. The regression analysis involved in the determination of the NE dissociation constant, Kd, and receptor occupancy showed a high correlation coefficient (r^2 > 0.99).

The pKd — the negative log of the dissociation constant — for the SHR was statistically greater than that for the WKY, representing the increased affinity of the small mesenteric artery α-adrenergic receptor in the hypertensive compared with the normotensive rat. Receptor occupation for maximal response after treatment with benextramine was equal in the two groups of vessels. The relative receptor occupation for half-maximal NE response was 39 ± 6% in SHR and 39 ± 8% in WKY. This finding suggests that the α-adrenergic receptor reserve, if present, is of the same size in the two groups of vessels. However, receptor reserve assessment based on the index – antilog (pD2 - pKd) indicates that there are no free or unoccupied α-adrenergic receptors in these tissues when they

### Table 1. Characteristics of Rat Mesenteric Arteries

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective lumen diameter (μm)</td>
<td>258 ± 15</td>
<td>209 ± 9*</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pD2</td>
<td>5.57 ± 0.07</td>
<td>6.10 ± 0.10*</td>
</tr>
<tr>
<td>pKd</td>
<td>5.37 ± 0.16</td>
<td>5.89 ± 0.07*</td>
</tr>
<tr>
<td>q (%)</td>
<td>64 ± 8</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>r^2</td>
<td>0.99 ± 0.00</td>
<td>0.99 ± 0.00</td>
</tr>
</tbody>
</table>

Values are means ± SE of five vessels in each group. Vessel norepinephrine (NE) sensitivity and affinity are given in terms of pD2 and pKd values, respectively, where pD2 = – log EC50 and pKd = – log Kd, when they are measured in molar concentrations. q = receptor occupancy; r^2 = correlation coefficient for linear regression line of plots of reciprocal values of equieffective concentrations of NE before and after treatment with benextramine.

*p < 0.05, †p < 0.01, compared with WKY values (by Student's t test).
are maximally contracted to NE, since pD$_{2}$ and pK$_{a}$ for the two strains were not significantly different.

Linear regression analysis showed a significant ($p < 0.05$) correlation between pD$_{2}$ and pK$_{a}$ values when the data were pooled. The slope of the data regression line (0.766) was not significantly different from unity (Figure 1). The slope of unity represents the theoretical expected relationship when sensitivity is entirely dictated by affinity.

**Discussion**

The present study confirms previous findings that arteries from SHR are more sensitive to NE than are arteries from WKY when all known factors that influence the action of NE on the $\alpha_1$-adrenergic receptor are blocked or eliminated. We have found that this increased sensitivity is associated with an increased NE dissociation constant ($K_{a}$) for the $\alpha_1$-adrenergic receptor, but not with differences in receptor reserve, or relative receptor occupation. This latter parameter is a well-known source of variation in tissue sensitivity. $^{19,20}$ Restriction of receptor number is known to occur in small blood vessels, and evidence has been accumulated to show some small artery responses may be receptor-number limited. $^{1}$

A relationship between affinity of the $\alpha_1$-adrenergic receptor in vascular smooth muscle for NE and the sensitivity of the arterial contractile response has been demonstrated in a series of 12 rabbit arteries. $^{5,21}$ As the correlation was found to be highly significant and the regression line had a slope indistinguishable from unity, the variation in affinity in this series was sufficient to account for the differences in sensitivity. It occurred in arteries after endothelium removal. The results of the present study of the basis of sensitivity differences between the small mesenteric arteries from the two species of rats are consistent with the prior publication.

This finding does not imply that this difference represents the basis of the hypertension found in the SHR. It does suggest that the vascular smooth muscle sensitivity difference can be accounted for by a difference of affinity. Increased vascular smooth muscle adrenergic receptor affinity seems to be just one of a number of manifestations of sympathetic hyperactivity, including an increased discharge rate$^{22}$ and innervation density found in this animal. $^{2}$ The cellular factors that regulate $\alpha_1$-adrenergic receptor affinity and number are not known.

A previous attempt$^{4}$ to characterize the postjunctional $\alpha_1$-adrenergic receptor in SHR vasculature failed to show any alteration in the antagonist dissociation constant for the $\alpha_1$-adrenergic receptor (pD$_{2}$) for phenotolamine, nor have differences been found using $\alpha_1$-adrenergic receptor radioligand binding techniques. $^{5,6}$ Such findings are not necessarily at odds with these present observations. In studies on rabbit arteries, where agonist affinity was found to vary over more than two orders of magnitude, the affinity of the antagonist prazosin was the same in all vessels studied. $^{21}$ The receptor affinity variation is related to the agonist, not necessarily to the antagonist, action. This statement seems to imply a lack of identity of the recognition sites for the two drugs; the recognition site for the agonist exhibiting a variation from tissue to tissue and that for the antagonist not showing variation. The findings of Clineschmidt et al. $^{4}$ are consistent with these observations. However, little additional effort has been made to characterize the $\alpha_1$-adrenergic receptor in vascular smooth muscle of resistance vessels from the SHR, $^{23}$ and rigorous studies to show the functional identity or lack of identity of the receptor from the two strains, requiring receptor purification, have not been performed.

The receptor-occlusion method used in these experiments provides information about the agonist-recognition site of the $\alpha_1$-adrenergic receptor population. It may be assumed that the $K_{a}$ value for the same full agonist, in this case NE, is a constant when it interacts with the same type of receptor and that this should be independent of tissue localization or species. This assumption, however, does not hold true in rabbit arteries, $^{5}$ and in the arteries from the two rat strains.

The small but significant difference in NE sensitivity found in these rats (about threefold) is in accordance with previous findings of greater NE sensitivity in vascular smooth muscle from SHR. $^{1,2}$ This difference is revealed when the influence of uptake, (neuronal uptake) during ED$_{50}$ determination is removed. NE sensitivity of vascular smooth muscle in mesenteric resistance vessels, like that of other blood vessels, $^{24}$ has been shown to vary with their circumferential distention. $^{25}$ However, such a possible influence was avoided in the present study by setting the vessels at their optimal lumen diameter for active force development. $^{11}$ The maximum responses of the two groups of arteries to NE were not different and were less than the tissue maximum. This latter phenomenon, based on studies of rabbit arteries, may indicate that contraction

---

**Figure 1. Relationship between norepinephrine dissociation constant ($pK_{a}$) and sensitivity ($pD_{2}$) of mesenteric resistance vessels from SHR (•) and WKY (○).** The equation of the regression line was $y = 0.766x + 1.163$ ($p < 0.05$). The line designated (data regression) is that for the collective data. The interrupted line (theoretical regression) is the line equating the $pK_{a}$ with $pD_{2}$. Note that it falls within the prediction band for the data.
Cell membrane differences, such as microviscosity and liquidity, particularly in the microenvironment of the receptor, may influence receptor properties, including agonist affinity. This possibility extends to other cell membrane characteristics, including ionic transport, enzymatic function, and physical deformability. A number of biomembrane differences between the SHR and WKY have been documented, including not only vascular smooth muscle cells, but other cell types whose membranes are more easily harvested and analyzed, such as erythrocytes, where differences in cholesterol content, Na⁺,K⁺-adenosine triphosphatase activity, and erythrocyte spin label motion have been described.

References

11. Mulvany MJ, Hansen PK, Aalkjaer C. Direct evidence that the greater contractility of resistance vessels in spontaneously hyper- tensive rats is associated with a narrowed lumen, a thick- ened media and an increased number of smooth muscle cell layers. Circ Res 1978;43:856–864
18. Furchgott RF, Bursztyn P. Comparison of dissociation cons- stants and of relative efficacies of selected agonists acting on parasympathetic receptors. Ann NY Acad Sci 1967;144:882–889
Increased alpha-adrenergic receptor affinity in resistance vessels from hypertensive rats.
N C Nyborg and J A Bevan

Hypertension. 1988;11:635-638
doi: 10.1161/01.HYP.11.6.635

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/11/6_Pt_2/635

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/