Structural and Functional Changes in Cerebral Arteries from Spontaneously Hypertensive Rats
RAYMOND J. WINQUIST, PH.D., AND DAVID F. BOHR, M.D.

SUMMARY Segments of basilar arteries from both spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats were studied in vitro utilizing a microvessel apparatus. At similar levels of passive force, basilar arteries from SHR developed less force in response to depolarizing solution (130 mM K+) compared to basilar arteries from WKY. Arterial segments from the hypertensive animals required less stretch to achieve each level of passive force. Basilar arteries from SHR but not WKY typically displayed both phasic and tonic spontaneous activity which was inhibited in a reversible manner by washing the tissues in physiological salt solution without added Ca++ (EGTA, 1 mM). There was a significant shift to the left in the EC50 of serotonin and a greater maximal response to this agonist in basilar arteries from SHR compared to those from WKY (p < 0.01). The EC50 to Ca++ (added to a depolarizing solution) was shifted to the right in the arteries from SHR compared to the normotensive controls (p < 0.05). There was no difference between the arteries from the two groups of animals in the relaxation response produced by isoproterenol. However, contracted basilar arteries from SHR were less sensitive to the relaxant effects of elevated Ca++ than contracted basilar arteries from WKY (p < 0.05). These results demonstrate the existence of both structural and functional difference between cerebral vessels of SHR and WKY. Our findings also demonstrate the complex nature of the changes in calcium dynamics in blood vessels from hypertensive animals. (Hypertension 5: 292-297, 1983)

Key Words • contractility • spontaneous activity • serotonin • calcium • depolarization • isoproterenol

Cerebral vascular disease is often associated with hypertension,1,2 yet little is known about cerebral vascular responsiveness in this association. Cerebral vessels from the rat, an animal with which many well-accepted models of hypertension have been developed, have heretofore been too small for in vitro methodologies. Measurements of cerebral blood flow in these models are often difficult to interpret1 and may primarily reflect the presence of structural adaptations of the vascular wall to the elevated pressure.4,5 Although many studies have demonstrated functional changes in isolated blood vessels from hypertensive animals,6 these changes differ both qualitatively and quantitatively among the various vascular beds.5-8

The recent development of small vessel instrumentation has permitted an accurate monitoring of the responsiveness of small muscular arteries.9 We have found that this apparatus is suitable for studying isolated basilar arteries of the rat.10 As this vessel is believed to have an important effect on cerebral blood flow,11-13 we compared the responsiveness of basilar arteries from spontaneously hypertensive rats with those of their normotensive controls.

Methods
Animals were adult (3 to 5 months old) spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats (Taconic Farms) selected for similar body weights. The SHR throughout this age range exhibit similar vascular wall/lumen ratios.9 Systolic blood pressure was measured in the unanesthetized rat by the indirect tail-cuff technique with a piezoelectric transducer. The rats were then anesthetized with ether and exsanguinated. The brain was carefully removed and immediately placed in cold physiological salt solution (PSS) of the following composition (mM): NaCl (130), KCl (4.7), CaCl2 (1.6), KH2PO4 (1.18), MgSO4,}

From the Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan. Supported by USPHS Grants HL 06080 and HL 18575. Address for reprints: Raymond J. Winquist, Ph.D., Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486. Received June 30, 1982; revision accepted October 27, 1982.

292
(1.17), NaHCO₃ (14.9), CaNa₂EDTA (0.03), glucose (5.5), pH 7.4, gassed with 95% O₂ and 5% CO₂. With the aid of a dissecting microscope, the entire basilar artery was removed from the confluence of the vertebral arteries to its bifurcation into the posterior cerebral arteries. This artery was then transferred to a microvessel apparatus, similar in design to that developed by Mulvany and Halpern.¹⁴ Our apparatus was comprised of two separate chambers, each containing approximately 15 cc PSS maintained at 37°C. This permitted vessels from one SHR and one WKY to be studied simultaneously.

A basilar artery segment was threaded onto a fine tungsten wire (Westinghouse, 32 μm diameter), one end of which was secured on one of two tissue support blocks. After a second wire had been passed through the vessel segment both wires were fastened to the support blocks and the tissue was trimmed, leaving a basilar artery ring approximately 1 mm long (distal to the vertebral confluence) to be aligned in the chamber. One of the support blocks was mounted on a micrometer displacement device, the other to a Kistler-Morse DSC-6 force-displacement transducer which was electrically coupled to a Grass Model 7 polygraph to record circumferential force development. These vessel rings were allowed to equilibrate for 1 hour during which warm, oxygenated PSS was continuously pumped through the microvessel chamber.

The distance between the outer edges of the tungsten wires (referred to as interwire distance) was measured with the aid of a monocular microscope. Following the equilibration period, each ring was stretched in increments in O-Ca ++ PSS (EDTA, 1 mM) to prevent any effects of intrinsic tone on the measurement of resting force. Once the new relationship between length and passive force had been established, the O-Ca ++ PSS was replaced by a depolarizing solution (130 mM KCl substituted for 130 mM NaCl, in otherwise standard, Ca-containing PSS). This procedure was continued until a maximal amount of force in response to the depolarizing solution was obtained; tissues were then kept at that level of stretch for subsequent experiments.

Contactile responses to agonists were expressed as a percentage of the maximal active force generated in response to the depolarizing solution. Perfusion of PSS through the chambers was terminated prior to the commencement of drug additions; aeration was provided through small PE tubing immersed in the chambers. Drugs were dissolved in deionized-distilled water and added cumulatively in volumes not exceeding 30 μL. Concentrations are expressed as moles/liter final concentration in the tissue chamber.

To examine differences in isoproterenol sensitivity (i.e., changes in EC₅₀), the maximum relaxation to this agent was taken as 100% relaxation for each individual tissue. Experiments with increased bath concentrations of CaCl₂ utilized PSS containing 6.0 instead of 14.9 mM NaHCO₃ to prevent precipitation of Ca (HCO₃)₂.

The sources of the drugs were Elkins-Sinn (isoproterenol hydrochloride), Ciba (Regitine, phentolamine mesylate) and Sigma (serotonin).

Statistical evaluation of the data was by the Students t test for unpaired samples using geometric means of the EC₅₀ when applicable. The 0.05 level of probability was regarded as significant.

Results

At the time of experimentation the systolic blood pressures of SHR were significantly higher than those of WKY (207 ± 5 vs 131 ± 3 mmHg, n = 12 in each group; p < 0.001). There was no significant difference in rat body weight (SHR = 317 ± 26 g; WKY = 301 ± 20 g). The measured interwire distance of flattened, unstretched (without applied passive force in O-Ca ++ PSS) vessel rings was smaller in SHR compared to WKY basilar arteries (SHR: 221 ± 8 μm, n = 7; WKY: 255 ± 10 μm, n = 8, p < 0.05, fig. 1). Data demonstrating the relationship between passive and
active forces are shown in figure 1, along with the associated changes in interwire distances required to achieve the values of passive force. Cerebral arteries from the hypertensive animals required less stretch to achieve a given passive force than did arteries from control animals. At each level of passive force, basilar arteries from WKY developed significantly greater levels of active force in response to the depolarizing solution than did those from the SHR (fig. 1). This difference between the two groups was not influenced by the inclusion of phenotamine \(10^{-6}\) M in the depolarizing solution to eliminate the component of the response to the depolarizing solution caused by norepinephrine released from intrinsic nerves. The average values of active force generated in two phentolamine-treated preparations were 522, 785, and 840 mg (SHR) and 885, 1060, and 1050 mg (WKY) for 50, 150, and 250 mg passive force, respectively.

Isolated basilar arteries from SHR often developed phasic activity superimposed on a tonic contraction (eight of 14 arteries, fig. 2). These intrinsic changes in force were observed only infrequently in isolated arteries from WKY (two of 14). Both phasic and tonic contractions were inhibited when the vessels were washed in O-Ca++ PSS (EGTA, 1 mM) but appeared when regular PSS was returned to the chamber.

Cerebral arteries from the two groups of animals were also exposed to various constrictor and dilator agents. The contractile dose-response curves to serotonin are shown in figure 3. Arteries from SHR were more sensitive to serotonin (SHR \(EC_{50} = 3.3 \times 10^{-8}\) M; WKY \(EC_{50} = 14 \times 10^{-8}\) M, \(p < 0.01\)) and contracted to a greater percentage of the depolarizing contraction in response to \(10^{-8}\) M serotonin (SHR maximum: 108% ± 3%; WKY maximum: 86% ± 5%, \(p < 0.001\)) compared to arteries from WKY. However, these differences in contractility were not found in the dose-response curves to Ca++ in depolarizing solution (fig. 4). Basilar arteries from WKY were more sensitive to the constrictor effects of added Ca++ than were those from SHR (WKY \(IC_{50} = 6.6 \times 10^{-5}\) M; for SHR = 12 \(\times 10^{-5}\) M, \(p < 0.05\)). The maximum response to the readdition of Ca++ in depolarizing solution was significantly greater in the arteries from WKY than in those from SHR (WKY maximum = 1380 ± 150 mg; SHR = maximum 940 ± 70 mg, \(p < 0.05\)).

Beta-adrenoceptor-mediated inhibition of contraction was examined in cerebral vessels contracted by 40 mM KCl (fig. 5). There was no significant difference in the \(IC_{50}\) for isoproterenol of cerebral arteries from SHR and WKY (SHR \(IC_{50} = 2.6 \times 10^{-7}\) M; WKY \(IC_{50} = 1.6 \times 10^{-7}\) M). The maximal amount of relaxation in response to isoproterenol was greater in basilar arteries from WKY than in those from SHR (WKY maximum = 51% ± 9%; SHR maximum = 30% ± 4%, \(p < 0.05\)). Cerebral arteries contracted with 40 mM KCl relaxed in a concentration-dependent fashion when calcium was added to the bath (fig. 6). The concentration of calcium required to inhibit the KC1 contraction by 60% (\(IC_{50}\)) was significantly less in arteries from WKY than in those from SHR (Ca++ \(IC_{50}\) for WKY = 8.3 \(\times 10^{-3}\) M; for SHR = 12 \(\times 10^{-3}\) M, \(p < 0.05\)). The maximum relaxation produced by 20 mM calcium was greater in arteries from WKY; however, this difference was not significant (WKY maximum relaxation = 91 ± 4%; SHR = 76% ± 6%, \(p < 0.1\)).
FIGURE 3. The contractile dose-response curve to serotonin for basilar arteries from WKY and SHR. The contractile responses were expressed as percent of the maximal response to depolarizing solution in each tissue. There was a significant shift in the serotonin EC₅₀ and maximal response in basilar arteries from SHR compared to arteries from WKY (p < 0.01). Values are means ± SEM, with the number of animals given in parentheses.

FIGURE 4. The contractile dose-response curve to the addition of Ca²⁺ to a Ca²⁺-free (1 mM EGTA) depolarizing medium (130 mM K⁺) of basilar arteries from WKY and SHR. There was a significant shift to the right in the EC₅₀ to added Ca²⁺ in arteries from SHR compared to those from WKY. The ordinate is as explained under figure 3. Values are means ± SEM, with the number of animals given in parentheses.

FIGURE 5. The relaxant dose-response curve to isoproterenol in contracted basilar arteries (40 mM K⁺) from WKY and SHR. The maximal relaxation to isoproterenol in each tissue was used as 100% relaxation in each tissue. There was no difference in the EC₅₀ to isoproterenol between the two groups of arteries. Values are means ± SEM, with the number of animals given in parentheses.

FIGURE 6. The relaxant dose-response curve to elevations in the bath concentration of Ca²⁺ in contracted basilar arteries (40 mM K⁺) from WKY and SHR. Responses are expressed as percent changes in the level of force with 40 mM K⁺ at 1.6 mM Ca²⁺. There was a significant shift to the right in the EC₅₀ to elevated Ca²⁺ in basilar arteries from SHR. Values are means ± SEM, with the number of animals given in parentheses.
Discussion

Both morphometric and hemodynamic studies have suggested that cerebral vessels undergo structural changes in hypertensive man and animals. We have extended these observations by examining isolated cerebral artery segments under conditions where both structural and functional abnormalities can be elucidated. Functional changes in vascular smooth muscle from hypertensive animals are not always pressure dependent and may be of importance in the pathogenesis of cerebrovascular disease.

Cerebral arteries from SHR were less compliant when compared to the same arteries from WKY (fig. 1). The arteries from SHR had smaller flattened, unstretched diameters and required less stretch to achieve a particular level of passive force. These findings are consistent with the greater cerebrovascular resistance during maximum vasodilatation in SHR compared to WKY. Morphometric analysis suggests that these results may be explained by an increased wall-to-lumen ratio in vessels from hypertensive animals. The diminished maximum relaxation to isoproterenol in basilar arteries from SHR compared to WKY is in agreement with these studies. Folkow et al. have long championed an increased medial thickness as a factor in the elevated peripheral resistance in hypertension.

Once a given level of passive force was obtained, basilar arteries from SHR contracted less to depolarizing solution than basilar arteries from WKY (fig. 1). The "passive force" vs "active force" relationship for basilar arteries from both SHR and WKY was not affected by the inclusion of phenolamine (10^-6 M) during the experiment nor by differences in the level of intrinsic tone (as all vessels were incubated in O-Ca++ PSS during the application of passive force). A lesser force-generating ability of vascular smooth muscle from hypertensive animals has long been recognized and may be due to a decreased smooth muscle cell volume or a decreased smooth muscle cell myosin content. Conversely, Mulvany et al. and Whall et al. have reported that small mesenteric arteries from SHR generate more force than these arteries from normotensive animals. However, recent preliminary findings show that cerebral but not the mesenteric vessels from SHR had significantly lower myosin levels compared to the corresponding vessels from WKY. Therefore, structural changes in the cerebrovascular smooth muscle from SHR may account for both the decreased vascular compliance, which may be common to most vascular beds, and the quantitative difference in force-generating ability.

Functional differences in cerebrovascular responsiveness were also observed between the preparations from SHR and WKY. Basilar arteries from SHR were more sensitive to the contractile effects of serotonin but actually less sensitive to added calcium (in depolarizing medium) when compared to arteries from WKY (figs. 3 and 4). A selective change in agonist sensitivity in vascular smooth muscle from hypertensive animals has been noted previously and cannot be explained solely on grounds of structural alterations such as vascular hypertrophy. Interestingly, the vasoconstrictor response to serotonin is usually enhanced to a greater degree than is that to other agonists in such preparations. Mulvany and Nyborg reported an increased "calcium sensitivity" for small mesenteric arteries from SHR. These opposite results may be caused by vessel individuality or by the fact that we used a depolarizing solution instead of norepinephrine (as did Mulvany and Nyborg) as the stimulant for calcium influx. The rat basilar artery relaxes in response to norepinephrine. It is possible that voltage-controlled (depolarized muscle) and receptor-controlled (norepinephrine-activated) calcium channels may be differentially affected in hypertension. Fundamental differences between these types of calcium channels have been demonstrated for vascular smooth muscle.

It does appear, however, that there exists functional changes in calcium sensitivity in cerebral vessels from SHR. Figure 2 displays the spontaneous vasomotor activity that was frequently recorded from these vessels. Such activity was typically absent in basilar rings from WKY. An increased myogenic activity in vessels from hypertensive animals has been attributed to an increased plasma membrane permeability to calcium. The spontaneous activity is inhibited in Ca++-free PSS as we observed in our study (fig. 2).

Increases in extracellular calcium caused relaxation of basilar arteries that had been made to contract by depolarization (fig. 6). The calcium-induced relaxation was attenuated in basilar arteries from hypertensive animals which is consistent with studies using larger blood vessels. This difference was not due to a decreased sensitivity to dilator agents since the isoproterenol EC50 for relaxation was similar in basilar arteries from the two groups of animals (fig. 5). If the spontaneous activity in basilar arteries from SHR reflects an increased membrane permeability to calcium, this phenomenon is not apparent in completely depolarized preparations (fig. 4). The observation is compatible with the hypothesis that there are fewer calcium binding sites responsible for membrane stabilization in the SHR. These functional differences exemplify the complexities in ascertaining changes in vascular calcium sensitivity in hypertension.

Our results demonstrate that cerebral vessels from hypertensive rats exhibit both structural and functional abnormalities during the established phase of genetic hypertension. The structural modification in cerebrovascular compliance may serve a protective role against stroke by reducing wall stress. Conversely, in disease states that affect large cerebral arteries, the regulation of cerebral blood flow may be compromised. The increased sensitivity to serotonin may be a functional change predisposing cerebral vessels to spasm in hypertensive animals. An altered membrane function, which could be common to cells from diverse morphological origin in hypertension, may underlie many of the similar findings in responsiveness amongst different vessels in hypertension.
References


25. Mulvany MJ, Hansen PK, Aalkjaer C: Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media and an increased number of smooth muscle cell layers. Circ Res 43: 854, 1978


Structural and functional changes in cerebral arteries from spontaneously hypertensive rats.
R J Winquist and D F Bohr

Hypertension. 1983;5:292-297
doi: 10.1161/01.HYP.5.3.292
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 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/5/3/292

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/