Endothelium-Dependent Mechanical Properties of the Carotid Artery in WKY and SHR
Role of Angiotensin Converting Enzyme Inhibition

Bernard I. Levy, Joelle Benessiano, Pierre Poitevin, and Michel E. Safar

An experimental model of in situ isolated carotid arteries has been used to evaluate the static mechanical properties of the arterial wall in 12-week-old Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). The effects of endothelium removal and of local incubation with the converting enzyme inhibitor lisinopril (ICI Pharma 209000) on the carotid compliance (CC) were compared with the effects of total abolition of the vascular smooth muscle tone by potassium cyanide. CC measured for pressures ranging from 50 to 175 mm Hg had maximal values (0.22±0.07 μl/mm Hg and 0.13±0.03 μl/mm Hg, respectively, for WKY and SHR, p<0.001) for pressure values close to the operating pressures in both groups. Maximal values of CC were increased by 35% and 45% in WKY and SHR, respectively, after potassium cyanide poisoning (p<0.01). The endothelium removal induced a significant increase in CC compared with their control values (+37%, p<0.01, and +25%, p<0.01, respectively, in WKY and SHR). CC measured after endothelium removal did not significantly differ from its values measured after potassium cyanide poisoning in normotensive animals. In contrast, in hypertensive animals, CC was significantly lower after endothelium removal than after potassium cyanide poisoning (p<0.01). In the presence of intact endothelium, local incubation with converting enzyme inhibitor increased CC by 23% (p<0.05) in WKY rats and by 14% (p<0.01) in SHR. In contrast, after endothelium removal, converting enzyme inhibitors did not significantly increase further CC in either strain. The present results suggest that the mechanical properties of the wall of the carotid artery are endothelium dependent. The relative constricting role of the endothelium seems to be more predominant in normotensive than in genetically hypertensive rats. The vascular converting enzyme seems to be involved in the endothelium dependent wall properties because endothelium removal and local application of converting enzyme inhibitors had similar effects on CC. (Circulation Research 1990;66:321–328)

In 1980, Furchgott and Zawadski demonstrated that the vasodilation produced by acetylcholine required the presence of the endothelium. Since then, many reports have been published describing the endothelium dependence of a large number of vasodilators and the antagonistic effect of the endothelium on the action of several vasoconstrictors. Endothelial cells also play a vasoconstrictive role; several investigators have reported that endothelium-dependent vascular contractions were induced by several stimuli. In 1988, endothelin, an endothelium-derived polypeptide with potent vasoconstrictive effects, was demonstrated and isolated by Yanagisawa et al.

In parallel, attention has been directed to the renin angiotensin (RA) system localized in the vascular tissues rather than to the circulating RA system in an attempt to explain certain types of hypertension. Endothelial cells are also involved in RA system because angiotensin converting enzyme (ACE) is largely localized on the luminal surface of the arterial endothelium. Thus, the arterial wall and especially the endothelium play a major role in the local control of vasomotor tone by complex and intricate mechanisms.

For many years, changes in vasomotor tone have been shown to modify the mechanical properties of the arterial wall. However, the specific contribution of the endothelium to changes in arterial mechanical properties has never been investigated.
We have recently developed an experimental model that allows us to measure accurately the static mechanical properties of the in situ isolated carotid artery in rats in control conditions and after abolition of smooth muscle tone.\textsuperscript{17,18} The goal of this work was to study 1) the role of the endothelium in the determination of the static mechanical properties of the carotid arterial wall, and 2) the specific influence of the vascular RA system on the compliance of the carotid artery. We have thus measured the acute effects of endothelium removal and of a locally applied converting enzyme inhibitor on the passive mechanical properties of the in situ isolated carotid artery in normotensive and hypertensive rats.

**Materials and Methods**

**Carotid Artery Compliance**

Twenty-four 12-week-old spontaneously hypertensive rats (SHR) were compared with matched normotensive control rats (Wistar Kyoto, WKY). Animals were anesthetized with intraperitoneal inactine (100 mg/kg) and kept at a constant body temperature by a thermostated operating table. After anesthesia, the trachea was cannulated and connected to a rodent respirator (model 680, Harvard Apparatus, South Natick, Massachusetts). A midsternal thoracotomy was performed, and the root of the left carotid artery was exposed.

Arterial blood pressure was recorded via a right carotid catheter connected to a P23ID pressure transducer (Statham, Gould, Cleveland Ohio). The upper end of the left carotid artery was catheterized with an 80-cm long nylon tube (0.6 mm i.d.) filled with Tyrode’s solution mixed with albumin (4%) and Evans blue (0.03%). The presence of protein in flushing and incubating solutions preserved the endothelium\textsuperscript{19} and maintained a physiological osmotic pressure gradient across the vessel wall. The tube was connected to a manometer pressurized at adjustable pressure levels. A three-way tap was connected between the manometer and the nylon tube, permitting a part of the tube to be filled in order to observe the position of the meniscus. The root of the left carotid artery was dissected, and a removable clamp was positioned at the junction of the aortic arch and the carotid artery. This preparation allowed us to exclude in situ 18–20 mm of nonexposed carotid (Figure 1).

To start the measurements, the segment of isolated artery was submitted to the atmospheric pressure for 5 minutes and the position of the meniscus was noted. The artery was then submitted to a pressure step of 25 mm Hg. The movement of the meniscus, representing changes in the contained volume within the artery, was followed and noted every 10 seconds for 5 minutes. During the first 30–45 seconds, the inflow was rapid and then became linear with time. The initial transient increase in volume with pressure was assumed to result from viscoelastic behavior of the tissue and relaxation of vascular smooth muscle.

The later linear inflow within the carotid artery after this initial increase in arterial volume could be attributed to the fluid filtration through the vascular wall.\textsuperscript{20} An estimate of the initial increase in volume free of viscoelastic effects was obtained by extrapolating the linear portion of the inflow curve to the time when the pressure step was applied (Figure 2). These measurements were repeated for pressures ranging from 25 to 175 mm Hg in steps of 25 mm Hg. The static compliance of the isolated segment of artery (carotid compliance [CC] measured in microliters per millimeter of mercury) was calculated for each level of pressure as the quotient of the extrapolated volume increase and the pressure step imposed (25 mm Hg). In preliminary experiments, we have verified that the CC values were not different when measured for increasing pressure steps (from 25 to 175 mm Hg) or for decreasing pressures (from 175 to 25 mm Hg). In the same way, we performed two series of CC measurements separated by a 1-hour interval, which showed that CC values measured for the same transmural pressures were not affected by a 1-hour delay. The pressure was maintained at each level during 5 minutes.

**Experimental Design**

The WKY and SHR were randomized into two groups. In the first (E+), the left carotid artery was flushed with CEI, a lysine analogue of the enalapril acid solution (lisinopril, ICI Pharma 209000, 2.3 \( \mu \)g/ml). The lisinopril solution was maintained in the carotid artery for 20 minutes, after which the pressure-volume relation of the left carotid was recorded again. Previous experiments have shown that longer incubation with CEI does not affect CC.

In the second group (E−), the left carotid endothelium was removed by introducing a blunt catheter
guide into the whole vessel to the root of the carotid. The carotid artery was flushed to wash from it the removed endothelial cells. CC was measured 20 minutes after removal of endothelium and again 20 minutes after lisinopril incubation for the same range of transmural pressures (25 to 175 mm Hg).

In all groups, the clamp on the carotid excluding the root of the left carotid artery was then removed and the artery was washed and filled with a saline solution of potassium cyanide (KCN 100 mg/liter). The KCN solution was maintained in the carotid artery for 30 minutes, a period sufficient to poison the vascular smooth muscle.16 After isolation of the same segment of carotid as used previously by re-clamping the root, the measurement of the pressure-volume relation was performed in the KCN-treated vessel.

In summary, WKY and SHR were thus separated into four groups of 12 animals: WKY E+, WKY E−, SHR E+, and SHR E−. In all groups, CC was measured in control conditions, after lisinopril incubation, and after potassium cyanide poisoning. Furthermore, in the E− groups, CC was measured before and after endothelial removal.

At the end of each experiment within the E+ groups, endothelial cell integrity was verified by excising and longitudinally opening the carotid artery to expose the endoluminal area. After washing, the absence of fixation of Evans blue indicated that the endothelial surface remained unaltered.21 Experiments were rejected if the endothelial surface was colored at the end of the experiment.

To verify that the muscular reactivity of the in situ isolated carotid artery was unaffected by the experimental design, we performed two additional series of experiments. In the first one (10 normotensive rats), we have checked the effects of acetylcholine (10−6 M) with intact endothelium and after endothelial removal. In the presence of endothelium, acetylcholine induced a significant relaxation of the carotid wall. After endothelial removal, acetylcholine did not modify the carotid compliance. In another series of experiments (five WKY), we have verified that after endothelial removal, flushing with norepinephrine (10−7 M) induced a marked shift of the pressure-volume relation toward the pressure axis, indicating that the smooth muscle reactivity in the carotid wall was not altered by the endothelium stripping.

**Statistical Analysis**

Results are expressed as mean±SD. The experimental design allowed us to use a two-way analysis of variance with repeated measurements to provide evidence of differences related to experimental models and/or treatment and interaction. Differences between groups were evaluated using the Newman-Keuls test.22

**Results**

The operating systemic blood pressure values were recorded 10 minutes after induction of anesthesia. The systolic, diastolic, and mean arterial pressure values were 131±16, 92±14, and 105±14 mm Hg for WKY and 180±17, 143±15, and 154±15 mm Hg for SHR, respectively.

**Carotid Arterial Compliance (Table 1)**

With intact endothelium. Figure 3 shows the carotid pressure-volume relations (Figure 3A) and corresponding compliance values (Figure 3B) obtained in the WKY and SHR groups. CC ranged from 0.07±0.02 to 0.22±0.07 μl/mm Hg in the WKY group and from 0.05±0.01 to 0.13±0.03 μl/mm Hg in the SHR group. Minimal CC values were obtained for the lower and the higher pressure levels when the pressure-volume relation was flat. The maximal value of CC corresponded to the 100 mm Hg step level for WKY and the 220 mm Hg level for SHR group. Mean values of arterial wall compliance were larger at specific pressure levels in the carotids from WKY and smaller in the carotids from the SHR. For pressure values ranging from 75 to 150 mm Hg, the CC values in SHR and WKY were significantly different (p<0.001); that is, the carotid arterial wall was significantly stiffer in the SHR group.

Abolition of vascular smooth muscle tone by 30 minutes incubation with KCN solution resulted in significant increases in the values of carotid compliance at specific values of transmural pressure relative...
TABLE 1  Summary of Mean Values and Standard Deviations of
Carotid Compliance (µl/mm Hg) Measured for Increasing Press-
ure Steps

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Normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were separated into four groups of 12: WKY E+, WKY E−, SHR E+, and SHR E− (E+, intact endothelium; E−, endothelium removed). In all groups, carotid compliance was measured in control conditions, after lisinopril incubation (+CEI), and after potassium cyanide poisoning (+KCN). In the E− groups, carotid compliance was measured before and after endothelium removal.

to control conditions (p<0.01 in both strains). The magnitude of this effect varied substantially with transmural pressure and animal group. No significant variations in CC were observed for the lower and higher pressure values. In contrast, for transmural pressure values from 75 to 150 mm Hg, KCN induced a significant increase in CC (Figure 4). Maximal values of CC were multiplied by 1.35 and 1.45, respectively, in WKY and SHR after abolition of the smooth muscle tone by KCN. There was a significant difference between these two increases in CC mean values (p<0.01). Therefore, the smooth muscle appears to be more effective in controlling the static elastic properties of the carotid artery in SHR than in WKY.

FIGURE 3. Top: Mean values of the carotid volume-pressure relation measured, with intact endothelium, in normotensive (WKY) and spontaneously hypertensive rats (SHR). Bottom: Compliance of the isolated carotid artery (CC) vs. pressure calculated as the slope of the volume-pressure curves (ΔV/ΔP) in WKY and SHR (mean±SD).

FIGURE 4. Effects of smooth muscle poisoning by potassium cyanide (KCN) on the carotid compliance (CC) in normotensive and hypertensive rats with intact endothelium (mean±SD). WKYE+, normotensive rats with intact endothelium in control conditions; WKYE+KCN, normotensive rats with intact endothelium 30 minutes after incubation with potassium cyanide; SHRE+, hypertensive rats with intact endothelium in control conditions; SHRE+KCN, hypertensive rats with intact endothelium 30 minutes after incubation with potassium cyanide.
FIGURE 5. Effects of endothelium removal on the carotid compliance (CC) in normotensive and hypertensive rats (mean±SD). WKYE+, normotensive rats with intact endothelium in control conditions; WKYE−, normotensive rats with damaged endothelium; SHRE+, hypertensive rats with intact endothelium in control conditions; SHRE−, hypertensive rats with damaged endothelium.

Effect of Endothelium Removal

In both normotensive and hypertensive strains, the removal of the endothelium induced a significant increase in CC compared with their control values (WKYE E+ vs. WKYE E−, p<0.01; SHR E+ vs. SHR E−, p<0.01; Figure 5). In the lower and higher range of transmural pressure (50 and 175 mm Hg), there was no significant effect of endothelium removal on CC either in normotensive or in the hypertensive groups. In contrast, for a transmural pressure of 100 mm Hg (maximal value for CC) the mean value of CC calculated was increased by +37% in the WKY group. In the SHR group the mean value for the maximal CC value (150 mm Hg) was increased by +25% after endothelium removal. In the WKY group, there was no significant difference between CC values measured after endothelium removal and after KCN poisoning. In contrast, the CC values measured in the hypertensive group after endothelium removal were significantly lower than those measured after smooth muscle poisoning by KCN (Figure 6). It thus appears that the smooth muscle relaxation induced by endothelium removal was similar to that obtained after total abolition of vascular smooth muscle tone in WKY. In contrast, in SHR, a significant muscle wall tone persisted after endothelium removal compared with the completely relaxed arterial wall.

Effects of Local Incubation of ACE

CEI has been tested both in control conditions and after removal of the endothelium. In control conditions (E+), local incubation of lisinopril produced a significant increase in CC in both WKY and SHR (p<0.05 and p<0.01, respectively; Figure 7). Once again, the maximal relaxing effect was obtained for transmural pressure values varying from 75 to 150 mm Hg (at 100 mm Hg, CC was increased by +23% in WKY and +14% in SHR); for lower and higher pressure values, CEI did not produce significant changes in CC in either strains. In contrast, after stripping of endothelium, the converting enzyme inhibitor did not induce significant changes in CC.

FIGURE 6. Effects of smooth muscle poisoning by potassium cyanide (KCN) on the carotid compliance (CC) in normotensive and hypertensive rats with damaged endothelium (mean±SD). WKYE−, normotensive rats with damaged endothelium; WKYE−KCN, normotensive rats with damaged endothelium 30 minutes after incubation with potassium cyanide; SHRE−, hypertensive rats with damaged endothelium; SHRE−KCN, hypertensive rats with damaged endothelium 30 minutes after incubation with potassium cyanide.

FIGURE 7. Effects of local incubation with lisinopril (IEC) on the carotid compliance (CC) in normotensive and hypertensive rats with intact endothelium (mean±SD). WKYE+, normotensive rats with intact endothelium in control conditions; WKYE+IEC, normotensive rats with intact endothelium 20 minutes after incubation with converting enzyme inhibitor; SHRE+, hypertensive rats with intact endothelium in control conditions; SHRE+IEC, hypertensive rats with intact endothelium 20 minutes after incubation with converting enzyme inhibitor.
Discussion

The calculation of hemodynamic parameters generally used to appreciate the mechanical properties of the arterial walls (characteristic impedance, pulse wave velocity, time constant of the diastolic pressure decay) requires simplification of assumptions. In contrast, the measurement of CC provides direct information about the mechanical behavior of the carotid wall in different experimental conditions. In the present study, CC was measured in situ in nonisolated, nonexposed vessels maintained at physiological longitudinal stress. These experimental conditions allowed us to measure more physiological values than could be obtained from strips or from in vitro arteries.

Arterial pressure-diameter relation has been reported to be curvilinear by several investigators. This curve is concave to the volume axis in the low pressure range, has an inflection point at intermediate pressure values, and then becomes concave toward the pressure axis in the high pressure range. CC is calculated as the derivative (ΔV/ΔP) of this volume-pressure curve. Its maximal value is thus obtained at the inflection point. In our experiments, it is striking that the maximal value for CC was obtained in both groups for pressure values close to the mean arterial operating pressure. This can be compared with a study of the rat carotid artery where Cox reports that the minimal value of characteristic impedance was observed for the operating mean arterial pressure in both WKY and SHR groups. The characteristic impedance (Zc) is related to the arterial compliance by

$$Z_c = \frac{1}{A} \sqrt{\frac{rVAP}{\Delta V}}$$

where A is the cross-sectional area of the ascending aorta, r is the density of blood, and ΔV/ΔP is the distensibility of the ascending aorta. From Equation 1, minimal Zc corresponds to maximal ΔV/ΔP, that is, maximal CC. Stone and Dujardin obtained similar results in the dog aorta; the pressure at which Zc was minimized (or compliance maximized) was located close to the mean arterial pressure. This finding supports the hypothesis that the normal operating value of transmural pressure in living animals determines the variation of arterial stiffness with transmural pressure by influencing arterial mechanical and geometric properties.

Inactivation of carotid smooth muscle tone by KCN poisoning induced a larger increase in CC in SHR than in the WKY group. This is in agreement with results reported by Drobrin and Rovick. Furthermore, Cox has shown that the activation of smooth muscle in carotids from the SHR produced larger effects compared with carotids from WKY. The significant differences in CC measured after abolition of vascular smooth muscle tone may be related to differences in protein content of the extracellular matrix of the arterial wall, to an increase in smooth muscle mass, or to a combination of both factors.

Under control conditions and after abolition of the vascular smooth muscle tone, there was undoubtedly a higher stiffness in SHR carotid arteries than in WKY carotid arteries. There is general agreement that an increase in arterial wall stiffness occurs in association with the development of sustained hypertension. We have previously shown, in renovascular and in spontaneously hypertensive rats, that the increase in stiffness was related to altered artery wall thickness and smooth muscle mass, to altered passive stiffness of the vessel walls, and to altered muscle tone.

Removal of the endothelium markedly increased CC in both normotensive and hypertensive strains (p<0.01). A direct mechanical role of the endothelial monocellular layer cannot explain the increase in CC induced by the endothelium removal, but subtle damage of the internal elastic lamella cannot be excluded. In contrast, there is evidence that the endothelium releases various vasoactive agents that induce changes in local vasomotor tone and thus the mechanical properties of the arterial wall. Our results may be compared with those obtained by Harder, who reported that an endothelium vaso-
constrictive factor was observed in cat cerebral arteries only with intact endothelium. Similarly, Katusic et al.\textsuperscript{11} reported that isolated canine basilar arteries developed an active tension when a stretch was applied to arterial rings with endothelium but not in those in which the endothelium had been removed.

In the present study, the endothelium removal induced an increase in the carotid compliance comparable with that obtained after muscle cell poisoning in the WKY group. In contrast, in SHR, the increase in CC induced by the removal of endothelium was significantly lower than that obtained after ablation of the vascular smooth muscle tone. Thus, the increase in CC induced by the endothelium removal appeared to be maximal in normotensive rats but incomplete in hypertensive animals. In WKY, the similar CC values measured after endothelium removal and after smooth muscle cell poisoning suggest that endothelium-dependent vasomotor tone was predominant in this strain. In the SHR group, the significantly lower CC value measured after endothelium removal than that obtained after KCN poisoning may be related to a non–endothelium-mediated arterial smooth muscle tone. Several hypotheses may be proposed in this respect. Luscher et al.\textsuperscript{30} have suggested an impaired vascular responsiveness to endothelium-derived relaxing factor in the carotid artery of SHR compared with WKY. The persistence of sympathetic tone, higher in SHR than in WKY and preserved in these nondi- sected and nonexposed endothelial arteries, may be hypothesized.\textsuperscript{30} This suggests that the relative importance of the sympathetic tone and RA system activity in WKY and SHR strains are different.

Several investigators have shown that endothelium may release prostaglandins when submitted to acetylcholine.\textsuperscript{31–33} A possible action of prostaglandins on the increase in CC observed after endothelium removal in our experiments may be ruled out because 1) there was no acetylcholine in the solution used to perfuse the isolated carotid arteries, and 2) a possible suppression of a release of prostaglandins by endothelial cells would induce a decrease and not an increase in CC as observed during our experiments.

The RA system plays an important role in maintaining cardiovascular homeostasis particularly via the contractile state of the blood vessel.\textsuperscript{34} Substantial evidence has been reported that components of the RA system are present in local tissues such as blood vessel walls, the heart, the kidney, the brain, etc.\textsuperscript{13} Thus, the RA system may act locally within the vascular wall to modify the arterial smooth muscle tone. Modifications of smooth muscle tone may induce changes in the mechanical properties of the walls of the large arteries. We have reported such modifications after chronic therapy in animal experiments\textsuperscript{37} and in clinical studies.\textsuperscript{35} Converting enzyme inhibitors may act directly on the smooth muscle cells or via the endothelium. ACE is largely localized on the luminal surface of the arterial endothelium.\textsuperscript{14,15} Furthermore, the long-term effects of ACE inhibitors in blood pressure lowering were reported to be unrelated to the pretreatment plasma renin level.\textsuperscript{36} The more likely explanation is that ACE inhibitors not only block serum ACE but also markedly inhibit tissue ACE. Several other experimental and clinical studies have shown that the fall in blood pressure induced by inhibitors of ACE is more closely related to inhibition of tissue or vascular ACE activity than to inhibition of the serum enzyme.\textsuperscript{37–39} The relative contribution of tissue RA system may depend on multiple experimental conditions and the hypertensive model studied; furthermore, during in vivo experiments with ACE inhibitors, systemic treatment invariably antagonizes the plasma RA system as well as tissue RA systems. Thus, attempts to experimentally address this question in vivo have encountered major difficulties, and it is generally difficult to isolate the specific role of the vascular RA system. The present experiment suggests that in the isolated carotid artery, the ACE inhibitor acts via the endothelial cells. Other pharmacological actions of CEI on endothelium-derived constricting factors and/or endothelin cannot be ruled out.

In conclusion, this study has shown that 1) the static mechanical properties of the in situ isolated carotid artery in WKY and SHR are endothelium dependent, 2) the relative constriciting role of the endothelium compared with that due to other factors seemed to be higher in normotensive than in spontaneously hypertensive rats, and 3) mechanical removal of the endothelium and local incubation with converting enzyme inhibitor similarly relaxed the carotid arterial wall in normotensive and hypertensive rats. The vascular converting enzyme seems thus to be involved in the endothelium-dependent tone. Our results suggest that converting enzyme inhibitors could directly inhibit the vascular wall RA system.

Acknowledgments

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