Effects of \(\alpha_1\)-Blockade on Arterial Compliance in Normotensive and Hypertensive Rats

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The effects of blockade of \(\alpha_1\)-adrenergic receptors on the mechanical properties of the arterial wall were studied in 10 spontaneously hypertensive rats (SHR) as compared with 10 matched normotensive Wistar-Kyoto (WKY) rats. Ascending aortic pressure and flow were recorded in open-chest anesthetized rats, and the systemic arterial compliance was calculated. Intravenous injection (1 mg/kg) of Urapidil, a selective \(\alpha_1\)-adrenergic antagonist, induced a significant decrease in arterial pressure (\(-26\%\), \(p<0.01\) and \(-37\%\), \(p<0.001\) in WKY rats and SHR, respectively) without significant changes in cardiac output. In control conditions, systemic arterial compliance was lower in SHR (3.29 ± 1.52 \(\mu\)l/mm Hg) than in WKY rats (4.35 ± 1.35 \(\mu\)l/mm Hg, \(p<0.01\)). Urapidil injection induced significant increases in systemic arterial compliance values in both strains (\(p<0.001\)). In another set of experiments (15 WKY rats and 15 SHR), the carotid compliance (\(\mu\)l/mm Hg) was determined from the arterial volume-pressure relation under control conditions, after local incubation with Urapidil, and after total abolition of the vascular smooth muscle by KCN. In WKY rats, the carotid compliance increased markedly after incubation with Urapidil at doses corresponding to 1 mg/kg (+31\%, \(p<0.01\)). A further increase in the carotid compliance was observed after KCN poisoning (+11\%, \(p<0.05\)). In SHR, incubation with Urapidil at doses corresponding to 2 mg/kg were necessary to induce a significant increase in compliance (+38\%). At this dosage, there was no further increase in compliance after KCN poisoning. The present study suggests that \(\alpha\)-blockade influences the arterial compliance in normotensive and hypertensive rats independently of its effects on the arterial blood pressure. (Hypertension 1991;17:534–540)

The role of \(\alpha\)- and \(\beta\)-adrenergic receptors on the control of vascular tone has been extensively studied in hypertensive animals. The respective contributions of \(\beta\)-receptors and of \(\alpha_1\)- and \(\alpha_2\)-receptors have been widely investigated by using administration of selective antagonist compounds. However, most of the investigations have only studied the vascular tone of peripheral small arteries. Several lines of evidence from experimental studies suggest that changes in arterial smooth muscle activity, such as those produced by the autonomic nervous system, affect the dimensions as well as the stiffness of the arterial wall. Gerova and Gerov have shown that electrical stimulation of the lumbar sympathetic nerves induces a significant reduction of the diameter of the femoral artery without changes in arterial pressure; similarly, norepinephrine produces a contraction of the aortic smooth muscle with a reduction in diameter. In the same way, phenox ybenzamin abolishes the constrictive response to hemorrhage and causes an increase in aortic diameter. More recent studies suggest that changes in the activity of the sympathetic nervous system could modify the vascular wall smooth muscle tone differently in large and small arteries. First, although the aorta contains highly sensitive vascular smooth muscle cells and an excess of receptors, the sensitivity decreases with the reduction in distal arteries, and in some areas yet more peripheral, the receptor number decreases. Second, the pharmacological investigation of isolated human vessels indicates that the resistance and conduit portions of the vasculature react differently to a number of vasoactive agents, including agonists and antagonists of \(\alpha\)-receptors. Finally, since the postsynaptic \(\alpha_2\)-adrenergic receptor subtype appears to be located close to the intima of blood vessels, it may trigger the release of vasoactive substance from the endothelium.
to investigate the active changes of the large arteries produced by blockade of adrenergic receptors in hypertensive animals. Urapidil (Byk Gurbet Pharmazeutika, Konstanz, FRG), a phenylpiperazine-substituted derivative of uracil, has a selective α2-adrenergic receptor antagonist effect in isolated vessels. Given intravenously, it decreases blood pressure and peripheral resistance. In addition to the α,-adrenergic receptor blocking potency, a central mechanism could participate to some extent in an antihypertensive effect. This central mechanism, distinct from that of clonidine, could involve a 5-hydroxytryptamine agonistic potency. In the present investigation, the antihypertensive effect of intravenous Urapidil was studied in normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). To specifically investigate the direct effects of Urapidil on the arterial wall properties, the static mechanical properties of the carotid arterial wall were also measured after local incubation with Urapidil.

Methods

Biological Model

The study was performed in two groups of 12-week-old rats supplied by CERJ (Le Genest, St. Isle, France): 25 WKY rats weighing 320±25 g and 25 SHR weighing 290±20 g.

Hemodynamic Study

The effects of Urapidil on the hemodynamic parameters were investigated in 10 WKY rats and 10 SHR. The basic surgical preparation and hemodynamic measurements have been described elsewhere and are presented here in brief. After induction of anesthesia with pentobarbital (50 mg/kg i.p.), the trachea was cannulated and then was connected to a rodent respirometer (model 680, Harvard Apparatus, South Natick, Mass.). A Teflon catheter (0.9 mm i.d.) connected to a Statham P23 ID (Gould Statham, Oxnard, Calif.) pressure transducer was introduced into the right carotid artery and positioned into the ascending aorta. The catheter manometer system was checked with both time and frequency domain measurements, and it showed a flat response beyond 40 Hz. A midsternal thoracotomy was performed and the ascending aorta was isolated; an adapted ultrasonic Doppler flow probe was then placed around the isolated aorta for measurement of mean (cardiac output minus coronary blood flow) and phasic instantaneous aortic blood flow. Ascending aortic blood flow and pressure were simultaneously recorded and analyzed by a microcomputer system coupled with an analog/digital converter (Apple IIe and A2D). At least 10 minutes after surgical preparation, when hemodynamics were stable, pressure and flow curves were recorded and digitized (500 Hz). Hemodynamic parameters were calculated for successive cardiac cycles for 30 seconds and then averaged to provide hemodynamic values. Aortic pressure and flow were recorded under control conditions; Urapidil was then injected intravenously and subsequent recordings were made 5, 10, 15, 20, 30, and 45 minutes later. In a preliminary study, we observed that intravenous injection of Urapidil induced a significant drop in blood pressure in SHR within 10 minutes. The average decrease in mean arterial pressure was 30% with 0.5 mg/kg and 35% with 1 and 2 mg/kg. For that reason, systemic hemodynamics were studied only with the 1 mg/kg dosage.

Basic study parameters were the systolic, diastolic, and mean arterial blood pressures, the cardiac output, and the heart rate. Total peripheral resistance was determined as the quotient of mean arterial blood pressure and cardiac output.

The systemic arterial compliance was computed from a simple elastic model that discharges during diastole into a single resistance representing the total peripheral resistance. Equations of the model are obtained from its electric analog, the RC model, which associates in series a capacitance element (systemic arterial compliance) and a resistive element (total peripheral resistance). Such a model has two fundamental characteristics: 1) it discharges mono-exponentially as a function of time, and 2) the time constant of the system, that is, the reciprocal of the exponential slope discharge, represents the product of the capacitance and the resistance. Thus, systemic arterial compliance is calculated as aortic compliance according to the formula

\[ SAC = \frac{\text{tho}}{\text{TPR}} \]

where SAC is systemic arterial compliance, tho is the time constant, and TPR is total peripheral resistance.

We have previously shown that, in hypertensive rats, systemic arterial compliance was strongly correlated with the characteristic impedance of the ascending aorta and thus with its elastic properties.

Measurements of the Compliance of the Carotid Artery

In two other groups of animals (15 WKY rats and 15 SHR), we measured the static mechanical properties of the carotid artery. The upper end of the left carotid artery was catheterized with a nylon tube 80 cm long (0.6 mm i.d.) filled with a Tyrode's solution with albumin (4%) and Evans blue (0.03%). The presence of protein in flushing and incubating solutions served to preserve the endothelium 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 so that the position of the meniscus could be observed. The root of the carotid artery was then 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, 18–22 mm of nonexposed carotid (Figure 1).
isolated carotid
meniscus
air
liquid

FIGURE 1. Schematic representation of the experimental system for measurement of the volume-pressure relation in the in situ isolated carotid artery.

At baseline the segment of isolated artery was at normal atmospheric pressure for 5 minutes, and the position of the meniscus was noted. The artery was then submitted to an incremental pressure increase of 50 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 displacement of the meniscus 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 constant inflow within the carotid artery after this initial increase in arterial volume could be attributed to fluid filtration through the vascular wall. The later constant inflow within the carotid artery after this initial increase in arterial volume could be attributed to fluid filtration through the vascular wall. 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 incremental pressure increase was applied. These measurements were repeated for pressures ranging from 50 to 200 mm Hg in increments of 50 mm Hg. The static compliance of the isolated segment of artery (carotid compliance: μL/mm Hg) was calculated for each level of pressure as the quotient of the extrapolated volume increase and the pressure increment imposed (50 mm Hg) (Figure 2).

The clamp on the carotid excluding the root of the left carotid artery was then removed, and the artery was washed and filled with the same solution (Tyrode's, albumin, and Evans blue) containing Urapidil. In preliminary experiments, plasma volume was measured in WKY rats and SHR using bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) labeled in vitro with iodine-125 (Amersham, 91944 Les Ulis, France). The peak plasma Urapidil concentrations after intravenous injection of 0.5, 1, or 2 mg/kg were calculated as the ratio of the quantity of injected Urapidil to the plasma volume. The mean values of the peak plasma concentration and thus the in situ concentrations of Urapidil were 0.043, 0.086, and 0.129 mg/ml corresponding to intravenous injections of 0.5, 1, or 2 mg/kg. After isolating the same segment of carotid as used previously by clamping the root again, the measurement of the pressure-volume relation was performed during local incubation with Urapidil using these three concentrations (5 WKY rats and 5 SHR for each dosage).

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

In preliminary experiments, we verified that the carotid compliance values were not different when consecutively measured for increasing pressure increments (from 50 to 200 mm Hg) or for decreasing pressures (from 200 to 50 mm Hg). In the same way, we performed two series of carotid compliance measurements separated by a 90-minute interval; carotid compliance values measured for the same transmural pressures were not affected by a 1½-hour time delay.

Statistical Analysis

Results are expressed as mean±1 SD. The experimental design allowed us to use a two-way analysis of variance (ANOVA) with repeated measures to provide
FIGURE 3. Line graphs showing hemodynamic changes (mean±1 SD) after administration of Urapidil in normotensive Wistar-Kyoto rats (○) and spontaneously hypertensive rats (●). Panel A: Mean arterial pressure (MBP). Panel B: Cardiac output (CO). Panel C: Total peripheral resistances (TPR). Panel D: Heart rate (HR). See text for statistical evaluation.

Results

Systemic Hemodynamics

The antihypertensive effects of Urapidil occurred immediately in both strains; 5 minutes after injection, the mean arterial pressure fell from the initial value of 95±15 mm Hg down to 70±12 mm Hg in WKY rats (p<0.01) and from 139±13 mm Hg down to 88±17 mm Hg in the SHR (p<0.001). Subsequently, from the fifth minute until the end of the experiment, mean blood pressure remained stable at the reduced pressure level in both groups (Figure 3A).

Cardiac output did not change significantly in either group (Figure 3B). Total peripheral resistances were significantly higher in SHR than in WKY rats (p<0.001) and fell after Urapidil injection only in SHR (p<0.05) (Figure 3C). Heart rate was significantly higher in SHR than in WKY rats and fell after Urapidil injection from the 10th to the 45th minute of the investigation both in WKY rats (p<0.01) and in SHR (p<0.001) (Figure 3D).

In the initial state, systemic arterial compliance was considerably lower in SHR (3.29±1.52 μl/mm Hg) (p<0.01). Urapidil caused a significant increase in the systemic arterial compliance in both groups (p<0.001) (Figure 4). Fifteen minutes after Urapidil injection, systemic arterial compliance reached 6.49±2.47 μl/mm Hg in SHR and 6.41±3.71 μl/mm Hg in WKY rats. ANOVA showed that there were significant differences in the systemic arterial compliance values between WKY rats and SHR during the whole experiment (before and 45 minutes after Urapidil injection).

FIGURE 4. Line graph showing changes in systemic arterial compliance (SAC) after administration of Urapidil in normotensive Wistar-Kyoto (WKY) rats (○) and spontaneously hypertensive rats (SHR) (●). See text for statistical evaluation.
Carotid Compliance

Figure 5 shows a typical example of the changes in carotid compliance values for the different pressure steps in untreated animals. Both in WKY rats and SHR, a parabolic curve was observed in baseline conditions with maximal values for the pressure step between 100 and 150 mm Hg. At the whole range of pressure, the carotid compliance was lower in SHR than in WKY rats. After incubation with Urapidil, carotid compliance increased with maximal changes observed at the 100–150 mm Hg pressure step value. For that reason, only these results are presented in detail.

In WKY rats, carotid compliance at the 100 mm Hg pressure level increased markedly (+31%) after Urapidil administration at doses corresponding to 1 mg/kg i.v. (p<0.01) (Figure 6). A further significant increase in carotid compliance (+11%) was observed after KCN poisoning (p<0.05).

In SHR, carotid compliance did not significantly change after incubation with Urapidil corresponding to 1 mg/kg i.v. (Figure 7A) but increased markedly (+38%) with the dosage equivalent to 2 mg/kg (Figure 7B, p<0.01). At this dosage, there was no further increase in the values of the carotid compliance after KCN poisoning. Variance analysis showed that carotid compliance was constantly lower in SHR than in WKY rats whatever the experimental conditions (baseline conditions, Urapidil incubation, and KCN poisoning).

Discussion

The antihypertensive effect of Urapidil no longer needs to be demonstrated.13 The initial effect, occurring shortly after injection, seems to be a vasodilation with more pronounced effects in SHR than in WKY rat strains. The induced fall in blood pressure was not associated with a change in cardiac output or heart rate in either strain, suggesting an inhibition of the baroreceptor reflex response to arteriolar vasodilation.21 In addition to the pronounced decrease in systemic vascular resistances, a significant increase in arterial compliance was observed, which is the main focus of this study. The systemic arterial compliance, as measured in these experiments, was considerably smaller in SHR than in WKY rats under control conditions. This increased stiffness of the arterial wall in SHR has been previously documented14,16,22 and may be attributed to different associated factors such as 1) a difference in intrinsic mechanical wall properties due to structural differences, especially in arterial smooth muscle mass and in the elastin-to-collagen ratio, 2) a higher vasomotor tone of smooth muscle cells in the arterial wall of SHR, and 3) the mechanical response of the vessels to a higher operating pressure in SHR. After Urapidil administration, arterial blood pressure decreased and arterial compliance increased, suggesting the latter factor could be determinant. In a previous study, using another α-blocking drug (Nicergoline, Rhone Poulenc-Rorer, Autony, France), we showed that α-blockade could indeed modify arterial smooth muscle tone and therefore arterial compliance independently of the mechanical response of the vessel wall to the distending arterial pressure level.16 In that regard, the present findings on the compliance of the isolated carotid artery are important to consider.

Using the present experimental model of “in situ” isolated carotid artery, we have previously shown that the carotid compliance may be evaluated at different pressure levels.23,24 In several experiments, we have shown that the carotid compliance-pressure curve had a parabolic shape with maximum values nearly at the operating arterial pressure of the animal. At each pressure level, carotid compliance was significantly lower in SHR than in WKY rats (p<0.001), indicating that the principal factors affecting the arterial compliance were either structural factors (increase in smooth muscle mass and collagen content) or a change in arterial smooth muscle tone, or both. Interestingly, after KCN administration, the functional factor is completely abolished, enabling an adequate quantitative evaluation of carotid compli-
ance reserve in WKY rats and SHR. It is within this framework that the local effect of Urapidil may be evaluated on arterial smooth muscle tone and consequently on arterial compliance. Therefore, the changes in carotid compliance induced by local incubation with Urapidil reflect the local effects of selective α tone adrenergic receptor blockade.

The main finding of the present study was that α-blockade induced by Urapidil, at the dosage corresponding to intravenous injection of 0.5 and 1 mg/kg, significantly increased carotid compliance in normotensive rats. This effect was obviously independent of the arterial distending pressure and therefore was related to modifications of the vascular smooth muscle tone. Since similar results were observed with 0.5 and 1 mg/kg equivalent dosages, it seems that a maximal blockade of the α tone was attained in the WKY rat strain (Figure 4). The increase in carotid compliance induced by Urapidil was lower than that measured after total abolition of smooth muscle tone by KCN poisoning, indicating that α tone was only a part of compliance reserve in WKY rats and that other neurohumoral factors might be involved in the mechanisms influencing the carotid compliance in the WKY rat strain. We have shown in a previous study, that the compliance of the carotid artery is endothelium-dependent and that the angiotensin converting enzyme might be involved in the basal tone of the arterial wall in normotensive animals.24

In contrast with normotensive rats, α-blockade caused by Urapidil slightly influenced the carotid compliance in SHR. Indeed, Urapidil given at 1 mg/kg equivalent dosage did not change the carotid compliance (Figure 7A). This finding agrees with those reported in hypertensive human brachial arteries: intravenous Urapidil did not change arterial compliance despite a significant blood pressure reduction.25 Furthermore, there is a discrepancy between the large increase in systemic arterial compliance in SHR compared with WKY rats and the small increase in the in situ carotid compliance in SHR compared with WKY rats. This result suggests that the increase in systemic arterial compliance is related to the arterial pressure decrease induced by the α-blocker (pure physical mechanism) and to the decrease of the arterial smooth muscle tone. This apparent discrepancy emphasizes that it is essential to compare the mechanical properties of a vessel for the same level of transmural pressure.

In SHR, only the higher dosage (2 mg/kg) markedly increased the carotid compliance values, which reached levels similar to those attained after KCN poisoning. Such results suggest that the α tone was of more significant importance in the carotid arterial wall of the SHR than in that of normotensive animals. However, it cannot be ruled out that other mechanisms might be involved in the reduced compliance in SHR. We have shown in a previous study using the same experimental model that the release of endothelial relaxing substances had less influence on the carotid compliance in SHR than in WKY rats.24 Furthermore, the hypertrophy of the smooth muscle cells observed in the arterial wall in SHR could also account for the reduced compliance in hypertensive rats.

In conclusion, the present study has shown that α-blockade due to Urapidil influences arterial compliance in normotensive and hypertensive rats independently of its effect on the arterial blood pressure. Higher dosages of Urapidil were necessary, in situ, in hypertensive than in normotensive rats to similarly increase the arterial compliance, suggesting a significant contribution of α tone, associated with other factors, in the reduced compliance observed in hypertensive rats.

References

FIGURE 7. Bar graphs showing carotid compliance (CC) at the 100–150 mm Hg pressure level in spontaneously hypertensive rats under control conditions, after incubation with Urapidil with dosage corresponding to 1 mg/kg (panel A) and 2 mg/kg (panel B), and after potassium cyanide (KCN) poisoning. **p<0.01 vs. control conditions.

**KEY WORDS** • arteries • compliance • sympatholytics • rat studies
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