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Influence of graded changes in vasomotor tone on the carotid arterial mechanics in live spontaneously hypertensive rats

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1 The contribution of vasomotor tone to the increased stiffness of carotid arteries in living spontaneously hypertensive rats (SHR) is largely unknown. Whether a reduced vascular tone is associated with an increase or a decrease in arterial stiffness *in vivo* remains to be determined. The goal of the present investigation was to show that a decrease in vascular tone is associated with a decrease in arterial stiffness, independent of the structural composition of the arterial wall.

2 New high resolution echo-tracking techniques were used to evaluate pulsatile changes of carotid blood pressure and diameter following transient and graded changes of vasomotor tone produced by the dihydropyridine derivative, isradipine. Treatment for 8 weeks was given to groups of SHR rats either with a low (0.6 kg day^{-1}) or a high (2.6 mg kg^{-1} day^{-1}) dose. Another SHR group received an acute dose of 2.6 mg kg^{-1} day^{-1}. Results were compared to those of placebo-treated Wystar-Kyoto (WKY) and SHR rats. Whatever the dosage, acute or chronic calcium blockade caused a decrease in blood pressure which was maximal 1 h after administration and disappeared after the 16th h. Carotid arterial thickness and the composition of the arterial wall was determined from histomorphometry.

3 In placebo-treated SHR, the inverse relationship relating blood pressure to carotid arterial distensibility was significantly shifted toward higher values of blood pressure compared to the curve of normotensive placebo-treated WKY rats. The curve of SHR receiving chronically a non antihypertensive (0.6 mg kg^{-1} day^{-1}) isradipine dose prolonged that of placebo-treated SHR toward lower values of blood pressure, so that carotid distensibility was significantly higher than in WKY for the same diameter and blood pressure level (145 mmHg). With administration of a chronic antihypertensive dose (2.6 mg kg^{-1} day^{-1}) causing a significant decrease in arterial thickness, the curve of SHR was transiently shifted towards the WKY curve, resulting in a normal arterial function. Acute antihypertensive calcium blockade with a single isradipine dose (2.6 mg kg^{-1} day^{-1}) caused a similar shift in the pressure-distensibility curve toward the WKY curve although the histomorphometric composition of the arterial wall differed significantly from that of chronically treated animals.

4 The study provides evidence that, in living SHR submitted to calcium blockade, (i) a low dose of isradipine causing no substantial antihypertensive effect is associated with a significant elevation of carotid arterial distensibility for the same pressure and diameter as normotensive controls, and (ii) an acute or chronic dose causing a substantial antihypertensive effect is associated with a transient shift of the SHR distensibility-pressure curve toward a physiological arterial function, increasing carotid distensibility for the same pressure and diameter as WKY controls. Since such findings were observed independently of the histomorphometric composition of the arterial wall, they imply that the transient decrease in arterial stiffness produced by calcium blockade should involve specific changes in the connections between arterial smooth muscle and extracellular matrix.

Keywords: Rat carotid arterial mechanics; vasomotor tone; structure of the arterial wall; echo-tracking techniques

Introduction

In vitro studies have shown that hypertensive carotid arteries in spontaneously hypertensive rats (SHR) are more rigid than those from normotensive controls, due to functional and structural changes involving increased arterial smooth muscle mass and collagen content (Cox, 1979; Nichols & O'Rourke, 1990; Levy *et al.*, 1994). Carotid artery studies in living rats have also shown that, for the same range of static transmural pressure, carotid compliance was lower in hypertensive than in normotensive animals, even after total relaxation of arterial smooth muscle (Levy *et al.*, 1994). However, in both of these *in vitro* and *in vivo* preparations, flow was interrupted and carotid arterial mechanics were investigated under increasing levels of static and not pulsatile pressure. Flow, principally when pulsatile, contributes greatly to the modulation of arterial smooth muscle tone, principally through changes in endothelial function and release of vasoactive substances (Milnor, 1982; Pohl *et al.*, 1986; Nichols & O'Rourke, 1990). Thus, the question is raised as to whether the presence of pulsatile pressure and flow, with their specific consequences on arterial smooth muscle tone, could modulate the viscoelastic properties of the carotid arterial wall differently from what has been described experimentally (Nichols & O'Rourke, 1990; Levy *et al.*, 1994). The recent development of high resolution echo-tracking devices allows adequate measurements to be obtained of pulsatile changes in carotid arterial diameter in small living rodents (Tardy *et al.*, 1991; Hayoz *et al.*, 1992).

Early works of Dobrin (1983) and of Gow (1980) on arterial tone, calibre and distensibility suggest that an increase in arterial smooth muscle decreases arterial stiffness whereas a decrease in smooth muscle tone is associated has an opposite effect. This major point is, however, beset by conceptual and methodological snags, including the value taken to be initial length (or diameter), whether comparisons should be made at the same diameter or at the same distending pressure, and the effects of change of wall thickness at different diameters. Moreover, the problem was studied exclusively *in vitro*, and there is no study in conscious hypertensive rats taking into

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account these parameters together, in conditions of operational pulsatile pressure and flow. The most recent works on this subject (Safar *et al.*, 1990) indicate that a reduction in arterial tone in man might have the opposite effect to that suggested by Gow (1980) and actually decreases arterial stiffness.

Studies of changes in arterial vasomotor tone *in vivo* require the use of powerful compounds such as nitrates, converting enzyme inhibitors or calcium entry-blockers. For the present investigation, calcium-blockade has two definite advantages. Firstly, the haemodynamic changes produced by both antihypertensive and non-antihypertensive dosages may be obtained easily (Kazda *et al.*, 1982), making it possible to evaluate the changes in carotid arterial mechanics following graded changes of vasomotor tone. Secondly, the drug effects are quite transient, allowing determination for the same change in vasomotor tone of the resultant change in carotid mechanics at different degrees of wall thickness, as commonly estimated from histomorphometry.

The purpose of the present study was, in living normotensive Wistar-Kyoto rats (WKY) and SHR (i) to determine the changes in arterial stiffness of the carotid artery produced by graded changes in vasomotor tone due to calcium-blockade, and (ii) to evaluate to what extent some changes in the structure of the arterial wall may modify the observed alterations in carotid arterial mechanics. It will be shown that a decrease in arterial smooth muscle tone is associated with a significant reduction in arterial stiffness and that the acute and transient haemodynamic responses produced by calcium blockade are poorly influenced by the histomorphometric composition of the arterial wall.

Methods

The study was performed in 72 male SHR and 23 male normotensive rats (WKY) (Iffa-Credo, Fréjus, France). The experiments involved both blood pressure measurements in conscious animals and arterial function studies under pentobarbitone anaesthesia (60 mg kg^{-1}, i.p.). The dihydropyridine derivative, isradipine (Isr), was given at two different doses. From pharmacological data in SHR (Hamilton, 1987; Hof & Ruegg, 1988), both doses are known to alter vasomotor tone, the higher dose (2.6 mg kg^{-1} day^{-1}) causing a substantial antihypertensive effect and the lower dose (0.6 mg kg^{-1} day^{-1}) causing minor changes in blood pressure. Isr was given orally each day using 5 ml kg^{-1} of water with 2% gelatin added. Both acute and chronic studies were performed, with only a 2.6 mg kg^{-1} dose being used in the acute studies.

In a pilot experiment in conscious animals (see below), the Isr lower-dose (0.6 mg kg^{-1} day^{-1}) and higher dose (2.6 mg kg^{-1} day^{-1}) were given acutely as a single dose to two different groups of SHR ($n=8$ for each group) and respectively compared to a SHR placebo group ($n=5$). With the lower dose, mean blood pressure was 163 ± 3 mmHg 1 h after administration and 188 ± 7 mmHg for the corresponding control group ($P<0.01$). With the higher dose, the values of mean blood pressure were respectively 189 ± 3 and 157 ± 4 mmHg ($P<0.01$). In three SHR, we checked, according to previous pharmacodynamic data (Hof & Ruegg, 1988; Hamilton, 1987) that the duration of action of the higher dose was below or equal to 16 h, as confirmed further in the results of the study.

Experimental protocols

For chronic treatment, 6 week-old rats were divided into 4 groups: Group I: placebo-treated SHR rats (2% gelatin in 5 ml kg^{-1} of water); Group II: SHR administered the lower Isr dose (0.6 mg kg^{-1} day^{-1}); Group III: SHR administered the higher Isr dose (2.6 mg kg^{-1} day^{-1}); Group IV: placebo-treated WKY rats (2% gelatin in 5 ml kg^{-1} of water). All animals underwent 2 administrations per day (10 h 00 min and 17 h 00 min) and had free access to food and water in an environment at constant temperature (25°C). Measurements were done after 8 weeks treatment, when the rats were 14 weeks old. Thus, three sets of experiments, using different groups of animals, were done: the first involved the blood pressure and arterial measurements 1 h after the last administration (done at 10 h 00 min); the second involved the same measurements 16 h after the last administration (done at 17 h 00 min); and the third was performed for the histomorphometric studies in SHR and WKY. The number of animals are indicated in Tables 1, 2 and 3.

For assessing the effect of acute Isr administration, 14-week old SHR were divided into two groups: one control group ($n=7$) received acute placebo; the SHR-treated group ($n=9$) received a single dose of 2.6 mg kg^{-1} Isr. Blood pressure and arterial mechanics were measured 1 h after administration. For comparison with normotensive controls, the untreated WKY were those studied from the first experimental protocol.

Blood pressure measurements in conscious animals

Two days before the age of 14 weeks, rats were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.). A catheter (PE-50 fused to PE-10: Clay Adams, Parisppany, NJ, U.S.A.) was placed in the lower abdominal aorta (via the femoral artery) for direct measurement of arterial pressure. Arterial pressure was recorded with a Statham pressure transducer (P23 Db) and a Gould pressure processor. The catheter was filled with heparinized saline (50 units ml^{-1}) and was tunneled subcutaneously under the skin of the back and exited between the scapulae. The animals were then allowed to recover from anaesthesia for 48 h.

Arterial pressure measurements were performed in conscious, freely moving, 14-week-old rats in their home cage. The blood pressure signal was recorded continuously on a four-channel digital audio tape recorder, Biologic (DTR-1201), over a 1 h period after an equilibration period of at least 30 min. During the recording, or while replaying the tape, 30 min of the signal was sampled at 1 kHz and stored on a PC-486 microcomputer. An algorithm was developed to identify the cardiac cycles and to calculate, for each cycle, the values of the diastolic, systolic, mean and pulsed arterial pressures (mmHg) and the heart rate (b min^{-1}). Artifacts were either removed manually or eliminated automatically by a dedicated filter.

Determination of the carotid artery pressure-diameter relationship

Under pentobarbitone anaesthesia administered 24 to 48 h after the above measurements, the common carotid artery diameter-pressure relationship was established from the simultaneous recording of arterial diameter (left side) and blood pressure (right side). The technique of arterial diameter measurement, with an ultrasonic echo-tracking device (NIUS-O1, Asulab SA, Neuchâtel, Switzerland), has been described previously in man and rats (Tardy *et al.*, 1991; Hayoz *et al.*, 1992).

Briefly, this device measures internal arterial diameter and its systolic-diastolic variations with a precision close to 50 and 1 μm, respectively. This degree of resolution is made possible by oversampling (5,000 arterial diameter measurements s^{-1}) and averaging 16 consecutive values. A 10 mHz focalized transducer is stereotactically positioned over the left common carotid artery, 1 cm below the carotid bifurcation, using gel as transmitting medium. The Doppler technique is used to place the probe perpendicular to the arterial axis, in its largest cross-sectional dimension. After the transducer is switched to radiofrequency-mode, the basckscattered echoes from both anterior and posterior walls are visualized on an oscilloscope screen. The radiofrequency signals of both walls exhibiting a high signal-to-noise ratio are then easily tagged by an electronic tracker so that their movement can be continuously tracked. Recording of the tracker's displacements allows a digitized signal of diameter variation to be derived. The blood

pressure is measured as described above at the right common carotid artery, simultaneously to the determination of the arterial diameter, using a non-occlusive catheter introduced via the external carotid artery.

From the two simultaneous and continuous signals of pulsatile changes in arterial diameter and blood pressure, the computerized acquisition system fits the diameter-pressure curve within the diastolic-systolic range of blood pressure, and then calculates the distensibility-pressure curve. The relationship between the pressure, P, and the lumen diameter, D, was fitted with the model of Langewouters *et al.* (1986), using an arctangent function and three optimal fit parameters, α, β and γ:

$$D(P) = \alpha \frac{3.14}{2} + \tan g^{-1} \frac{P-\beta}{\gamma} \qquad [1]$$

where P is pressure and D is the internal diameter, assuming a cylindrical vessel. From a mathematical viewpoint, the three parameters, α, β and γ fully characterize each individual diameter-pressure curve. Any statistical difference between α, β or γ coefficients between two sets of curves means that significant differences may be assessed between the two functions. In this analysis, the changes in coefficients have only mathematical and statistical relevance and cannot be specifically interpreted from a pathophysiological viewpoint. Nevertheless, as shown in Figure 1, an increase in α shifts the curve towards higher diameter values whereas an increase in β shifts the curve towards lower diameter. A change in γ slightly modifies the curvature of the pressure-diameter curve.

Since the arterial and the blood pressure signals were not determined on the same side, we checked that there was no time delay between the diameter and the pressure signals, due to the electronic processing. For that purpose, we measured *in vitro* and at the same place (Boutouyrie *et al.*, 1994), the displacement of a purely elastic membrane (with the echotracking system) and the distending pressure (with Millar probe). Under these conditions, and with a sampling of 2500 Hz, no time delay was measured, indicating that it was lower than 0.4 ms (the sampling rate of the device). Then, using the same procedure, we calculated the time delay between the pressure signal recorded with the catheter (PE10+PE50), and the pressure signal recorded with Millar probe. The time delay was 9.2 ms. In anaesthetized rats, we observed a small hysteresis of the pressure-diameter curve principally due to a lag of pressure behind diameter during systole. Since the fitting of the pressure-diameter curve based on the arc tangent function is principally influenced by the points of the diastolic portion of the curve, the residual hysteresis was practically eliminated by the fitting (Hayoz *et al.*, 1992; Tardy *et al.*, 1991). Finally, we verified in groups of anaesthetized rats that the pressure-diameter curve did not differ whether the diameter signal was recorded on the right side and the pressure signal recorded on the left side of the common carotid artery, or vice versa.

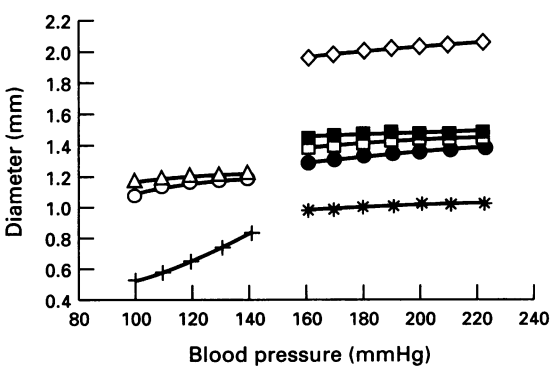

Figure 1 Arctangent model: diameter-pressure curve within the operational range of blood pressure obtained by changing the α and γ parameters in a spontaneously hypertensive rat (SHR) (□) and the β parameter in a normotensive rat (WKY) (○). As examples, for WKY, β has been divided by 2 (Δ) and multiplied by 2 (+). For SHR: α has been divided by 2 (✱) and multiplied by 2 (◇); γ has been divided by 2 (■) and multiplied by 2 (●).

The reproducibility of the method was studied in 9 Sprague-Dawley rats using the coefficient of variation (standard deviation expressed as a percentage of the mean of 10 successive measurements). The reproducibility of carotid diameter measurements and their systolic-diastolic variation was assessed over 5 measurements, each performed by two observers over a 30 min period. Under these conditions the mean intra-observer coefficients of variation were $3 \pm 1\%$ and $6 \pm 2\%$.

Evaluation of carotid arterial distensibility

The distention of an artery (change in volume) during a cardiac cycle depends on the elastic characteristics of the vessel wall (and the surrounding tissue) and the local pulse pressure (Milnor, 1982; Nichols & O'Rourke, 1990). Local arterial cross-sectional distensibility, assuming a constant length of a cylindrical vessel, is defined by the relative change in luminal cross-sectional area ($\triangle$LCSA/LCSAd) for a given change in intravascular pressure ($\triangle$P), $\triangle$ representing the systolic-diastolic variation and LCSAd the diastolic cross-sectional area of the vessel. In relation to the nonlinearity of the cross-section-pressure curve, arterial distensibility decreases curvilinearly as blood pressure increases. Thus, the distensibility-pressure curve over the systolic-diastolic range was established by deriving the equation of the pressure-LCSA curve, allowing the evaluation of distensibility at any given value of arbitrary pressure. On the other hand, operational distensibility was defined as the distensibility corresponding to the operational steady-state mean arterial pressure of each particular group of animals. As for distensibility, it was possible to define from the diameter-pressure curve an operational value of diameter and to calculate the diameter at a given value of pressure. Compliance-pressure curves gave similar results to those of distensibility-pressure curve (data not shown).

Histomorphometric study of the common carotid artery

Morphological analysis was performed in placebo WKY and in SHR after chronic treatment with placebo or Isr 2.6 mg kg^{-1} day^{-1} according to standard techniques (Tod *et al.*, 1983; Thorball & Tranum-Jansen, 1983). After the rats had been killed, the carotid artery was removed and fixed at operating pressure (the mean arterial pressure of each rat) in a 4% formaldehyde saline solution and then embedded in paraffin. Three successive sagittal sections of 5 μm thickness were specifically stained to obtain monochromatic views of the various structures studied in the arterial media. Sirius red was used for collagen staining, orcein for elastin, and haematoxylin after periodic acid oxidation for nucleus staining. Slides underwent automated image processing (NS 1500, Nachet-Vision Paris) based upon morphological principles (Todd *et al.*, 1983; Thorball & Tranum-Jensen, 1983; Matthews *et al.*, 1990). Different algorithms were developed to analyze each of the three structures shown by the staining in each of the three successive sections. The first algorithm analyzed the mean media thickness by measuring the distance between the internal and external elastic laminae (70 measurements per section). From media thickness and lumen cross-sectional area, medial cross-sectional area was calculated. Due to the incompressibility of the artery this parameter is not influenced by the level of blood pressure. The medial elastin network was analyzed in terms of relative area (density), and content (μm^2 mm^{-1} carotid section) and mean thickness (μm) of elastin lamella and lamina. The measurements and calculations were performed in 10 fields in each section. The second algorithm analyzed the collagen matrix by measurement of relative area

density and content in 20 continuous fields in each stained section. The third algorithm counted the number of nuclei per mm of carotid length and measured the mean area of each nucleus. For each algorithm, repetitive measurements were performed, pooled and averaged for the three algorithms in the corresponding stained section of the aortic wall media of each animal.

Statistical analysis (Morrison, 1976)

All values were averaged and expressed as mean ± s.e.mean. Variance analysis was performed to compare the different groups during either placebo or acute or chronic Isr administration. Differences between entire diameter- and distensibility-pressure curves were evaluated by comparing statistically the three independent parameters (α, β and γ) characterizing each of the different curves, by one-way ANOVA. Thereafter, to compare distensibility at the same blood pressure level in two (or several) significantly different groups, respective values of distensibility for the given value of blood pressure were compared by Student's *t* test. The latter procedure was initiated by calculating the cross-sectional area under the distensibility-pressure curves corresponding to the overlap between the studied curves, i.e. for their common pulse pressure range. The mean ± s.e.mean of the cross-sectional area was then compared by Student's *t* test, as though they were raw data (18). Differences were considered significant for values of $P < 0.05$.

Table 1 Chronic effects of isradipine on blood pressure and heart rate in conscious rats

Parameters	*SHR controls*	*SHR-Isr* (0.6 mg kg^{-1} 24 h^{-1})	*SHR-Isr* (2.6 mg kg^{-1} 24 h^{-1})	*WKY controls*
1 h after treatment	(*n* = 7)	(*n* = 7)	(*n* = 7)	(*n* = 7)
SAP (mmHg)	191 ± 2	179 ± 5	171 ± 5**	143 ± 2***†††*fff*
DAP (mmHg)	150 ± 2	141 ± 5	131 ± 4**	112 ± 2***†††*ff*
MAP (mmHg)	171 ± 1	161 ± 5	152 ± 4*	127 ± 2***†††*fff*
PP (mmHg)	41 ± 2	39 ± 3	41 ± 4	32 ± 1*
HR (b min^{-1})	371 ± 6	363 ± 10	371 ± 12	397 ± 13
16 h after treatment	(*n* = 6)	(*n* = 5)	(*n* = 6)	(*n* = 6)
SAP (mmHg)	193 ± 5	185 ± 5	184 ± 5	135 ± 2***†††*fff*
DAP (mmHg)	149 ± 3	140 ± 2	143 ± 3	105 ± 3***†††*fff*
MAP (mmHg)	172 ± 4	163 ± 3	164 ± 4	120 ± 3***†††*fff*
PP (mmHg)	45 ± 3	46 ± 2	41 ± 2	30 ± 2**††*f*
HR (b min^{-1})	354 ± 9	363 ± 17	347 ± 8	423 ± 10***†††*fff*

Values are given ± s.e.mean. SAP = systolic arterial pressure; DAP = diastolic arterial pressure; MAP = mean arterial pressure; PP = pulse pressure; HR = heart rate.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: vs SHR controls.
††$P < 0.01$, †††$P < 0.001$: vs Isr (0.6 mg kg^{-1} 24 h^{-1}).
f$P < 0.05$, *ff*$P < 0.01$, *fff*$P < 0.001$: vs Isr (2.6 mg kg^{-1} 24 h^{-1}).

Table 2 Effect of chronic administration of isradipine (0.6 and 2.6 mg kg^{-1} day^{-1} orally) during 8 weeks on operational carotid arterial parameters, 1 and 16 h following gavage

Parameters	*SHR controls*	*Isr* (0.6 mg kg^{-1} 24 h^{-1})	*Isr* (2.6 mg kg^{-1} 24 h^{-1})	*WKY controls*
1 h	(*n* = 8)	(*n* = 9)	(*n* = 7)	(*n* = 7)
MAP (mmHg)	194 ± 6	163 ± 8	132 ± 12***	135 ± 5***
HR (beats min^{-1})	383 ± 8	381 ± 10	354 ± 16	357 ± 13
Dm (μm)	1046 ± 28	977 ± 25	886 ± 34*	929 ± 38
PP (mmHg)	47 ± 6	41 ± 5	43 ± 2	28 ± 1
Ds-Dd (μm)	59 ± 3	87 ± 8	126 ± 13***/+	70 ± 4*ff*
Ds-Dd (%)	5.6 ± 0.3	9.2 ± 0.9	14.7 ± 1.9***/+	7.6 ± 0.4*ff*
Dist (mmHg^{-1} × 10^{-3})	2.56 ± 0.29	4.61 ± 0.73	8.1 ± 1.4***/+	5.8 ± 0.4
α (AU)	0.36 ± 0.01	0.35 ± 0.01	0.29 ± 0.02	0.31 ± 0.02
β (AU)	122 ± 6	116 ± 5	97 ± 6*	98 ± 2*
γ (AU)	54 ± 10	53 ± 7	42 ± 6	45 ± 7
16 h	(*n* = 5)	(*n* = 5)	(*n* = 6)	(*n* = 5)
MAP (mmHg)	197 ± 7	187 ± 7	187 ± 14	125 ± 7***†††*ff*
HR (beats min^{-1})	376 ± 6	358 ± 13	367 ± 14	373 ± 13
Dm (μm)	1086 ± 26	1019 ± 44	1012 ± 53	838 ± 50**
PP (mmHg)	47 ± 5	56 ± 3	44 ± 5	27 ± 1*†††*f*
Ds-Dd (μm)	52 ± 3	67 ± 7	64 ± 12	74 ± 5
Ds-Dd (%)	4.8 ± 0.4	6.6 ± 0.8	6.7 ± 1.8	9.0 ± 1.0
Dist (mmHg^{-1} × 10^{-3})	2.30 ± 0.36	2.68 ± 0.40	3.41 ± 1.15	6.88 ± 0.6**††*ff*
α (AU)	0.37 ± 0.01	0.34 ± 0.02	0.34 ± 0.02	0.25 ± 0.03**f*
β (AU)	128 ± 5	121 ± 6	126 ± 7	95 ± 5*†*ff*
γ (AU)	41 ± 8	45 ± 7	47 ± 9	36 ± 6

Values are given ± s.e.mean. Dm = diameter; Ds = systolic diameter; Dist = distensibility; Dd = diastolic diameter; AU = arbitrary unit.
*$P < 0.05$, **$P < 0.01$. ***$P < 0.001$: vs SHR controls.
†$P < 0.05$, ††$P < 0.01$, †††$P < 0.01$: vs Isr 0.6 mg kg^{-1} 24 h^{-1}.
f$P < 0.05$, *ff*$P < 0.01$: vs Isr 2.6 mg kg^{-1} 24 h^{-1}.
Abbreviations: see Table 1.

Results

Chronic isradipine administration

Table 1 shows the blood pressure and heart rate changes obtained in the conscious state in all groups of chronically treated animals. Mean blood pressure was decreased in both SHR groups receiving Isr, but only significantly with the high Isr dose (2.6 mg kg^{-1} day^{-1}) and only at 1 h after administration ($P<0.01$). By comparison with untreated WKY rats, mean blood pressure did not reach the normotensive level (152±4 versus 127±2 mmHg; $P<0.001$). There was no significant change in heart rate with chronic Isr therapy.

Table 2 shows the mean values of carotid arterial parameters at the operational mean arterial pressure of the corresponding anaesthetized animals successively studied 1 and 16 h after isradipine administration. In Table 2, the drug-induced changes are compared, on the left with SHR controls, and on the right, with the normotensive values of WKY rats.

With the low Isr doses, blood pressure obtained 1 h after administration was slightly lowered but the decrease was not significant in comparison with SHR controls. Sixteen hours after administration, blood pressure was similar to controls. Both 1 and 16 h after Isr administration, operational carotid arterial parameters did not change significantly.

With the high Isr dose, substantial modifications were observed. In comparison with SHR controls, SHR receiving high-dose Isr had: decreased mean arterial pressure ($P<0.001$) but unmodified pulse pressure; significantly decreased operational mean diameter (886±34 versus 1046±28 μm; $P<0.05$); increased percentage stroke change in diameter (14.7±1.9 versus 5.6±0.3; $P<0.001$); increased operational distensibility (8.1±1.4 versus 2.56±0.29 $mmHg^{-1} \times 10^{-3}$; $P<0.001$). In comparison with WKY controls, SHR receiving high-dose Isr did not differ regarding operational mean diameter and distensibility; mean arterial pressure was also similar. All these results were observed 1 h after administration and had completely disappeared 16 h after administration.

In addition to the results with Isr low and high dose, Table 2 shows a statistical comparison between WKY and SHR controls both 1 and 16 h after placebo administration: SHR rats had significantly higher mean arterial pressure and mean diameter and lower operational distensibility ($P<0.01$), particularly for the rats measured at 16 h.

Figures 2 and 3 describe the diameter-pressure and distensibility-pressure relationships in WKY and SHR animals 1 and 16 h after administration. For WKY and SHR controls investigated one and 16 h after placebo, the SHR pressure-diameter curve seemed to prolong the WKY curve toward higher values of blood pressure, but the statistical evaluation clearly indicated that the two curves were substantially different, with significant differences in the α and β coefficients; for instance, 16 h after administration, these coefficients were respectively 0.25±0.03 and 95±5 in WKY controls and 0.37±0.01 and 128±5 in SHR controls ($P<0.05$) (See Table 2). Subsequently, in these untreated animals, a significant shift

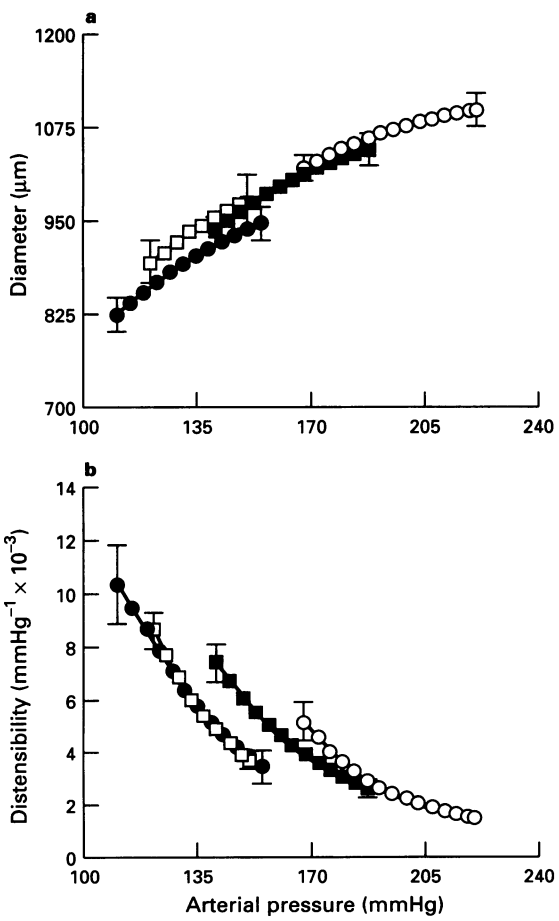

Figure 2 Diameter-pressure curves (a) and distensibility-pressure curves (b) established, 1 h after the treatment, in SHR treated with isradipine for 8 weeks: 0.6 mg kg^{-1} day^{-1} ($n=9$) (■) and 2.6 mg kg^{-1} day^{-1} ($n=7$) (●) in comparison with control SHR ($n=8$) (○) and WKY ($n=7$) (□). Values are means±s.e.mean.

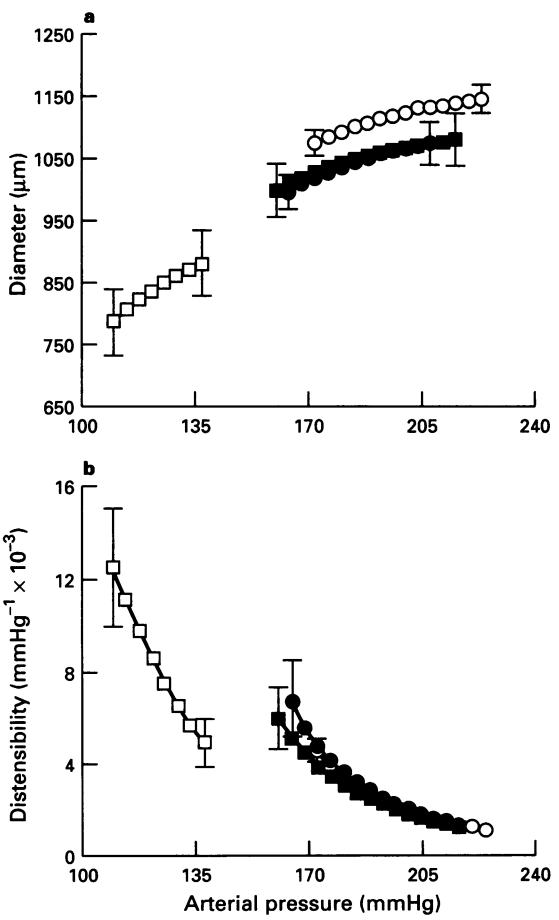

Figure 3 Diameter-pressure curves (a) and distensibility-pressure curves (b) established 16 h after the treatment, in SHR treated with isradipine for 8 weeks: 0.6 mg kg^{-1} $24\,h^{-1}$ ($n=5$) and 2.6 mg kg^{-1} day^{-1} ($n=6$) in comparison with control SHR ($n=5$) and WKY ($n=5$). Values are means±s.e.mean. Same symbols as Figure 2.

of the SHR distensibility-pressure curve toward higher values of blood pressure was observed by comparison with the WKY curve.

As shown in Figure 2, the low dose of Isr (0.6 mg kg^{-1} day^{-1}) given 1 h after administration did not significantly change the pressure-diameter and pressure-distensibility curves by comparison with the curves of untreated SHR. However, the pressure-diameter and distensibility curves prolonged those of the untreated SHR toward lower values of blood pressure. Figure 2 indicates that, for the same given level of blood pressure (145 mmHg), the low dose of Isr (0.6 mg kg^{-1} day^{-1}) induced a significantly ($P<0.05$) higher distensibility than in WKY controls. At this pressure, arterial diameter was the same.

In contrast with the low dose, the high dose of Isr (2.6 mg kg^{-1} day^{-1}) shifted the pressure-diameter and pressure-distensibility curves toward that of the WKY curves (Figure 2). WKY and SHR curves became almost identical. Thus, at the given pressure of 145 mmHg, carotid distensibility was higher with the low dose of Isr than with the high dose for the same carotid diameter ($P<0.05$).

Figure 3 shows that the diameter and distensibility-pressure relationships observed 16 h after administration with either dose of Isr were identical to those of SHR controls.

Table 3 summarizes the histomorphometric changes in SHR carotid artery following 8 weeks' treatment with Isr 2.6 mg kg^{-1} 24 h^{-1}. The medial cross-sectional area was decreased (0.12 ± 0.01 versus 0.14 ± 0.01 mm^2; $P<0.05$), as were the elastin (10434 ± 323 versus 12419 ± 551 μm^2 mm^{-1}; $P<0.01$) and collagen contents (6086 ± 196 versus 7824 ± 298 $\mu m^2 mm^{-1}$; $P<0.001$). By comparison with WKY controls, collagen content remained significantly higher ($P<0.01$).

Single acute isradipine administration

In conscious animals, Isr (2.6 mg kg^{-1}) significantly decreased mean blood pressure (146 ± 8 versus 173 ± 3 mmHg; $P<0.01$), and increased heart rate ($P<0.05$) (Table 4) by comparison with untreated SHR controls. Blood pressure did not reach the normotensive WKY levels.

Table 5 shows that, in anaesthetized animals, 1 h after administration, Isr 2.6 mg kg^{-1} decreased mean arterial pressure ($P<0.001$) without any change in pulse pressure (comparison with untreated SHR controls). The stroke change in diameter and operational distensibility increased significantly ($P<0.001$), and this increase was comparable to that of chronic treatment at this dose. By comparison with the WKY controls, the Isr group showed no significant difference in mean arterial pressure and operational diameter but had a slightly higher operational distensibility ($P<0.05$).

Figure 4 shows the diameter- and distensibility-pressure curves in SHR receiving acute Isr, in SHR and in WKY control animals. Curves of Isr-treated animals were significantly different from those of SHR controls, based on the change in the β coefficient (127 ± 8 versus 83 ± 6; $P<0.001$) (Table 5), resulting in a shift of the distensibility curve toward lower values of blood pressure after acute Isr administration. The shifted distensibility-pressure curve of Isr-treated animals was not significantly different from that of WKY controls in terms of α, β and γ coefficients (Table 5).

Discussion

In the present study, the mechanical properties of the carotid artery were studied in living SHR following long term treatment by the calcium-entry blocker Isr. Given twice a day, the drug produced transient haemodynamic changes which differed with lower and higher doses. With the lower dose, mean blood pressure decreased slightly and operational distensibility did not change significantly 1 h after Isr administration. However, the comparison with WKY indicated that carotid distensibility was increased for the same pressure (145 mmHg) and diameter as control normotensive animals. With the higher dose, mean blood pressure decreased and operational distensibility increased, both significantly. The pressure-distensibility curve was shifted transiently towards the normotensive WKY curve, indicating the same carotid distensibility at any given value of pressure and diameter. In untreated SHR, the same shift was obtained with an acute and identical single dose of Isr, but with a significantly different histomorphometric composition of the arterial wall.

This study used recent technological progress to obtain reliable *in vivo* results. Earlier experiments were done in large animals such as dogs and required vascular surgery to position an ultrasonic dimensiometer on the vascular tissue (Milnor, 1982; Nichols & O'Rourke, 1990; Cabrera-Fischer *et al.*, 1991). With the present echotracking techniques, it is possible to obtain similar determinations non-invasively in small rodents. The resolution of the method reaches 2.5 μm. Reproducibility is very high but requires non-moving animals and therefore anaesthesia. This consideration is important since pentobarbitone may theoretically alter arterial stiffness through its

Table 3 Histomorphometric changes in SHR carotid artery following isradipine (Isr, 2.6 mg kg^{-1} day^{-1}) given orally for 8 weeks

Parameters	*SHR controls* ($n=9$)	*Isr* (2.6 mg kg^{-1} day^{-1}) ($n=8$)	*WKY controls* ($n=10$)
Dimension			
LCSA (mm^2)	0.35 ± 0.03	0.34 ± 0.03	0.37 ± 0.02
MCSA (mm^2)	0.14 ± 0.01	0.12 ± 0.01*	0.12 ± 0.01*
Media thickness (mm)	54.6 ± 2.3	46.9 ± 1.3*	41.7 ± 1.4***
LCSA/MCSA	2.5 ± 0.2	2.9 ± 0.2	3.1 ± 2*
Elastin			
Density (%)	22.8 ± 0.7	22.3 ± 0.5	21.8 ± 1.1
Content (μm^2 mm^{-1}, CCA section)	12419 ± 551	10434 ± 323*	9025 ± 449***
Collagen			
Density (%)	14.4 ± 0.3	13.9 ± 0.3*	11.6 ± 0.3***f
Content (μm^2 mm^{-1}, CCA section)	7824 ± 298	6086 ± 196***	4858 ± 249***ff
Nuclei			
Number (mm^{-1} CCA section)	244 ± 18	207 ± 9	156 ± 6***ff
Mean cross-sectional area (μm^2)	7.0 ± 0.5	7.1 ± 0.3	8.0 ± 0.4

Values are given ± 1 s.e.mean.
*$P<0.05$, ***$P<0.01$: vs SHR controls.
$fP<0.05$, $ffP<0.01$: vs Isr (2.6 mg kg^{-1} day^{-1}).
LCSA: luminal cross-sectional area, MCSA: medial cross-sectional area, CCA: common carotid artery.

Table 4 Effects of acute isradipine administration on blood pressure and heart rate one hour after treatment in conscious rats (WKY controls of Table 1 are indicated for comparison)

Parameters	*SHR controls* ($n=8$)	*SHR-Isr* (2.6 mg kg^{-1} day^{-1}) ($n=9$)	*WKY controls* ($n=7$)
SAP (mmHg)	195±3	168±7**	143±2***†
DAP (mmHg)	149±3	124±8*	112±2***
MAP (mmHg)	173±3	146±8**	127±2***
PP (mmHg)	46±3	44±2	32±2***††
HR (beats min^{-1})	354±7	399±13*	397±13*

Values are given ± 1 s.e.mean.
*$P<0.05$, **$P<0.01$, ***$P<0.001$: vs SHR controls.
†$P<0.05$, ††$P<0.01$: vs Isr (2.6 mg kg^{-1} day^{-1}).
Abbreviations: see Table 1.

Table 5 Effect of acute isradipine administration (2.6 mg kg^{-1}, orally) on operational carotid arterial parameters in SHR one hour after gavage (WKY controls of Table 2 are indicated for comparison)

Parameters	*SHR control* ($n=7$)	*Isr* (2.6 mg kg^{-1}) ($n=9$)	*WKY controls* ($n=7$)
MAP (mmHg)	183±11	108±7***	135±5**
HR (beats min^{-1})	377±11	389±16	357±13
Dm (μm)	1142±15	1000±34**	929±38***
PP (mmHg)	51±5	50±6	28±1*f
Ds-Dd (μm)	62±11	164±14***	70±4fff
Ds-Dd (%)	5.5±0.9	16.6±1.7***	7.6±0.4fff
Dist (mmHg^{-1} × 10^{-3})	2.6±0.7	8.0±0.6***	5.8±0.4**f
α (AU)	0.42±0.03	0.38±0.03	0.31±0.02*
β (AU)	127±8	83±6***	98±2**
γ (AU)	40±12	38±4	45±7

Values are given ± 1 s.e.mean.
*$P<0.05$, **$P<0.01$, ***$P<0.001$:vs SHR controls.
$fP<0.05$, $fffP<0.001$:vs Isr (2.6 mg kg^{-1} 24 h^{-1}).
Abbreviations: see Tables 1 and 2.

influence on the sympathetic nervous system and smooth muscle tone (Altura & Altura, 1975). Bearing in mind this limitation, the rat carotid artery was studied in the presence of maintained pulsatile pressure and blood flow and without any dissection and/or alteration of the innervation of the arterial wall. Using this procedure, the curves obtained in untreated animals were quite similar to those previously described by Hayoz *et al.* (1992), using the same methodology. The untreated SHR pressure-diameter curve seemed to prolong that of the WKY curve. However, statistical comparison of the α, β and γ coefficients characterizing each set of normotensive and hypertensive curves clearly indicated that the arc tangent functions corresponding to normotensive and hypertensive animals were significantly different, resulting in a shift of the diameter-pressure curve in SHR toward higher values of blood pressure and diameter. Subsequently, a significant shift of the distensibility-pressure curve was also observed, permitting adequate evaluation of the changes produced by calcium blockade due to Isr.

In this investigation, low-dose Isr did not significantly modify systemic blood pressure and operational distensibility. This finding may be partly due to a loss of statistical power, as a consequence of a relatively small number of rats. Nevertheless, the distensibility-pressure curve in these animals slightly prolonged the curve of the untreated hypertensive animals toward lower blood pressure values. Based on this observation, the statistical analysis of Figure 2 indicates that distensibility at a given pressure was significantly altered. Firstly, it was significantly higher in hypertensive than in normotensive WKY animals for the same values (145 mmHg) of blood pressure. Secondly, it was significantly higher than in SHR treated with a high dose of Isr for the same pressure (145 mmHg). In both situations, at this particular pressure, values of arterial diameter were similar. Thus, in living SHR, the decrease in vasomotor tone produced by calcium blockade was associated with a drug-induced increase in carotid distensibility for the same pressure and diameter as normotensive WKY controls. This change was even more pronounced with the low dose of Isr, making carotid distensibility even higher in hypertensive than in normotensive animals for the same pressure. Such findings clearly show that the presence of cyclic flow and *in vivo* vasomotor tone greatly modify the mechanical properties of the hypertensive arteries of living animals. These striking modifications had probably been underestimated by the flow interruption characteristic of prior *in vitro* and *in vivo* preparations (Cox, 1979; Nichols & O'Rourke 1990; Milnor, 1982; Dobrin, 1983; Levy *et al.*, 1993; 1994).

Following a high dose of Isr, the changes in carotid operational arterial mechanics observed 1 h after administration were similar in the context of either a single acute or repeated chronic administration: mean blood pressure and arterial diameter decreased significantly; pulse pressure did not change, while pulsatile changes in arterial diameter increased significantly, resulting in an increase in operational carotid distensibility. Similar findings have been previously reported with use of ultrasonic techniques in dogs after acute nifedipine and diltiazem administration (Masafumi *et al.*, 1989; London *et al.*, 1992). However, in the present paper, the pressure-distensibility curves provided more insight concerning the drug-induced mechanical changes and their time-course. Firstly, the curve was shifted toward the normotensive WKY curve, indicating that changes in vasomotor tone in hypertensive rats

may completely normalize the SHR pressure-distensibility curve to achieve physiological operating ranges. Secondly, the arterial changes were transient, since they disappeared 16 h after administration of Isr.

In the present study, the mechanisms of the changes in the pressure-diameter and distensibility curves with a high dose of Isr are difficult to identify. In relation with the non-linearity of the pressure-diameter curves, the blood pressure reduction itself produced by the high Isr dose may have favoured an increase in carotid arterial distensibility. On the other hand, because blood pressure predominantly bears on elastin and arterial smooth muscle at the high Isr dose, the observed increase in distensibility might have been obtained not only through blood pressure reduction (i.e. through purely mechanical factors), but also through drug-induced arterial smooth muscle relaxation (i.e. through associated changes in carotid arterial vasomotor tone). Indeed, studies done *in vivo* with carotid preparations involving interruption of flow and evaluating static (and not pulsatile) pressure-diameter relationships have extensively shown that acute and long-term calcium-blockade relaxes arterial smooth muscle and increases carotid compliance at any given value of transmural pressure (Masafumi *et al.*, 1989; London *et al.*, 1992; Levy *et al.*, 1993; 1994). The last possibility to consider is that the factors influencing the present pulsatile pressure-diameter relationship are partly different from those modulating the static pressure-diameter relationship. In contrast with the static relationship, the pulsatile relationship is known to be poorly influenced by smooth muscle tone at any given value of wall stress (Bauer *et al.*, 1982). In addition, because arteries *in vivo* are submitted to everlasting cyclic loading and unloading, making the arteries stiffer in cyclic than in static conditions, pulsatile compliance is highly frequency-dependent and influenced both by the viscous properties of the arterial wall and by vasomotor tone (Bergel, 1961; Milnor, 1982; Nichols & O'Rourke, 1990). Clearly some changes in the connections between smooth muscle cells and collagen fibres might play a major role in explaining such changes in the viscosity of the arterial wall.

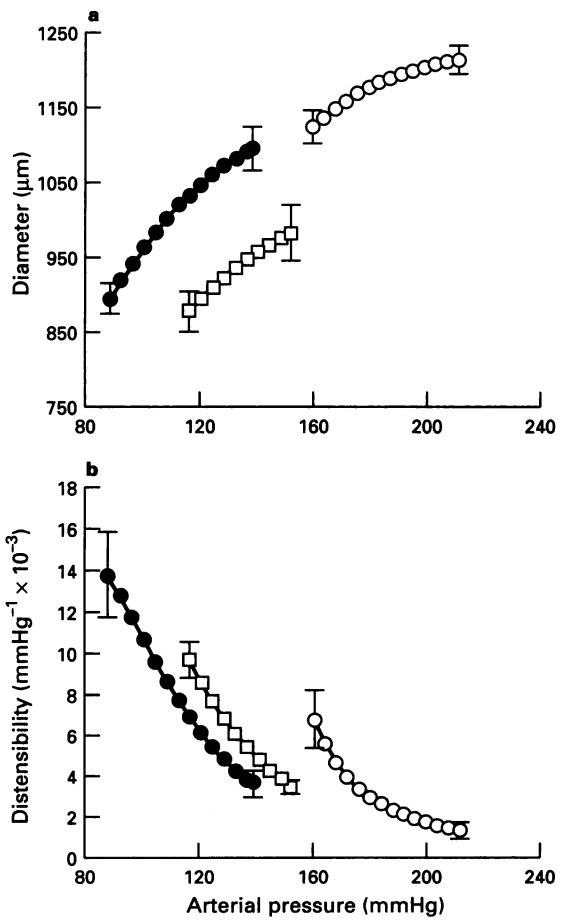

Figure 4 Diameter-pressure curves (a) and distensibility-pressure curves (b) established 1 h after the treatment, in SHR treated with a single acute administration of isradipine (2.6 mg kg^{-1} day^{-1}; $n=9$) in comparison with control SHR ($n=7$) and WKY ($n=7$). Values are means ± s.e.mean. Same symbols as in Figure 2.

Whereas the changes in carotid arterial smooth muscle tone were intermittent during chronic Isr administration, we showed that the drug-induced structural modifications of the vasculature were obviously sustained. Histomorphometric studies in SHR have shown that, in comparison to WKY rats, untreated hypertension is associated with increased carotid arterial thickness due to medial smooth muscle hypertrophy and enhanced extracellular matrix involving principally collagen (Cox, 1979; Milnor, 1982; Dobrin, 1983; Nichols & O'Rourke, 1990; Levy *et al.*, 1994). Our present histomorphometric findings in placebo-treated WKY and SHR were obtained from the classical perfusion-fixation technique and were very similar to those previously reported in the literature (Owens, 1985; Kratky *et al.*, 1991; Benetos *et al.*, 1993; Levy *et al.*, 1994). Following chronic calcium blockade, decreased medial thickness was observed in association with a substantial reduction in elastin and collagen content, which however did not completely return toward normal values. Similar structural modifications have been widely observed following antihypertensive therapy and generally attributed to the blood pressure reduction (Levy *et al.*, 1994; Owens, 1995). In this study, there were several observations suggesting that the structural changes cannot be explained on the basis of the blood pressure reduction alone. Firstly, the blood pressure changes observed following acute and chronic Isr were relatively modest (Table 1) and not maintained throughout the day (Table 2). Here, the intra-arterial blood pressure measurements that we obtained in conscious and moving animals, add considerably to the discussion. Indeed, whether continuous versus intermittent changes in blood pressure may have the same consequences on structural arterial damage remains to be explored. Secondly, the structural changes following Isr were different from those obtained with other antihypertensive agents, such as converting enzyme inhibitors, which did not modify substantially the elastin content of the vascular wall (Safar *et al.*, 1990; London *et al.*, 1992). Finally, in hypertensive animals, calcium blockade by Isr is not constantly associated with the presently observed structural changes (Table 3), depending on the territories involved and on the age of animals at the beginning of treatment (Levy *et al.*, 1994). Taken together, such observations suggest that factors unrelated to the blood pressure level (as those related to growth factors) might indeed contribute to the observed histomorphometric alterations.

The principal finding of the study was that the changes in pressure-diameter and distensibility curves following acute and chronic calcium blockade were quite similar whatever the structural characteristics of the arterial wall. Both acute and chronic Isr curves tended to be shifted towards the physiological WKY curves, independently of the characteristics of the medial cross-sectional area, smooth muscle mass and elastin and collagen content. Since the decrease in vasomotor tone produced by high dose Isr was associated with an increased carotid distensibility (both operational and at a given pressure) and restored transiently a normal arterial function in the presence of different compositions of the vascular wall, the mechanism by which smooth muscle relaxation improves distensibility cannot be explained solely on the basis of histomorphometric changes.

A mechanism has been suggested (Nichols & O'Rourke, 1990) whereby, at normal distending pressure, smooth muscle in the arterial wall is in series with some of the stiffer components of collagen but in parallel with the elastic lamellae. Contraction of smooth muscle tenses the collagen components,

whereas dilation transfers stresses to the elastic lamellae. Such an explanation is consistent with the classical arrangement of elastin, smooth muscle and collagen within the arterial wall. The collagen's lattice within the wall would permit the wall to behave in this way by closing (and elongating) when muscle relaxes and opening (and shortening) when muscle contracts. Nevertheless, the mechanism involved here remains difficult to explain. The fact that it occurs within hours and is reversible appears to implicate arterial smooth muscle. For instance, it may be due to release of endothelial-derived relaxing factor and so may have a similar explanation to the effect of nitrates through activation of guanylate cyclase (Nichols & O'Rourke, 1990). Alternatively, as discussed above from the study of the pulsatile pressure-volume relationship, the viscous properties of the arterial wall may be involved. Since calcium blockade restores a normal arterial function with different compositions of the arterial wall, the amount of smooth muscle and collagen are not, by themselves, directly implicated. We speculate that their connections were modified, particularly through adhesive molecules. Studies of atherosclerotic plaques have shown that adhesive molecules may be expressed within arterial smooth muscle cells, particularly under the stimulus of specific growth factors (Janat *et al.*, 1992; Couffinhal *et al.*, 1994) as those implicated with calcium-blockade.

In conclusion, the present study represents the first evaluation of the mechanical properties of hypertensive carotid arteries in living rats under conditions of maintained pulsatile blood pressure and flow, before and following graded changes in vasomotor tone produced by calcium blockade. The data presented here clearly indicate that, in living hypertensive rats, a decrease in arterial smooth muscle tone is associated with a decrease in arterial stiffness for the same pressure and diameter as normotensive controls. Following either acute or chronic calcium blockade with anti-hypertensive doses, the SHR pressure-diameter and distensibility curves may be shifted transiently toward the physiological range of the WKY curve. This shift may be obtained whatever the histomorphometry of the arterial wall. This observation implies intrinsic adjustments between two major components of the arterial wall: smooth muscle cells and collagen fibres.

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