Heart Rate-Dependent Stiffening of Large Arteries in Intact and Sympathectomized Rats

Luca Mircoli, Arduino A. Mangoni, Cristina Giannattasio, Giuseppe Mancia, Alberto U. Ferrari

Abstract—In the anesthetized rat, acute increases in heart rate are accompanied by a reduction in arterial distensibility, which is a significant phenomenon in elastic-type vessels such as the common carotid but much less evident in muscle-type vessels such as the femoral artery. Because the sympathetic nervous system importantly reduces arterial distensibility, the present study aimed to determine whether sympathetic influences (1) are involved in the heart rate–dependent changes in arterial distensibility and (2) exert differential effects on elastic-type versus muscle-type arteries. To address this issue, 9 sympathectomized (6-hydroxydopamine) and 10 vehicle-treated, 12-week-old, pentobarbitone-anesthetized Wistar-Kyoto rats were subjected to atrial pacing via a transjugular catheter at 5 different randomly sequenced rates (280, 310, 340, 370, and 400 bpm). After each step, spontaneous sinus rhythm was allowed to return to normal. Common carotid and femoral artery diameters were measured by an echo Doppler device (NIUS 01), and blood pressure was measured via catheter inserted into the contralateral vessel. Arterial distensibility was calculated over the systolic-diastolic pressure range according to the Langewouters formula. In the common carotid artery, progressive increases in heart rate determined progressive and marked reductions of distensibility (range, 15% to 43%) in sympathetomized and intact rats. In the femoral artery, the stiffening effect of tachycardia was present in sympathectomized rats (range, 21% to 42%), at variance with the inconsistent changes observed in intact rats. In conclusion, our experiments support the notions (1) that in predominantly elastic-type arteries, the stiffening effect of tachycardia is exerted independently of sympathetic modulation of the vessel wall properties and (2) that in predominantly muscle-type arteries, removal of sympathetic influences unmasks the stiffening effect of tachycardia.

Key Words: sympathetic nervous system ◼ heart rate ◼ pacing, atrial ◼ ultrasonography ◼ arterial pressure ◼ rats

We have recently shown that in rats, acute increases in heart rate by pacing are accompanied by marked reductions in the distensibility of the common carotid artery, whereas the distensibility of the femoral artery is much less consistently affected.1 The mechanisms by which pacing-induced tachycardia reduces distensibility in some but not all arteries have never been investigated and clarified. A plausible hypothesis, however, is that the heart rate–dependent stiffening of the arterial wall to a large extent is due to its viscous and thus inertial response to a distending force.2–4 Another plausible hypothesis is that this response is less apparent in vessels such as the femoral artery in which distensibility is tonically restrained by a high smooth muscle tone determined both by the abundance of smooth muscle tissue and by a more dense nerve supply of such tissue5 than in vessels such as the carotid artery, in which the influence of the much scantier smooth muscle tissue is less important.6–9

In the present study, we examined the effects of pacing-induced tachycardia on the common carotid and femoral arteries before and after sympathectomy (an intervention that markedly reduces vascular smooth muscle tone10–12).

Methods

Rat Preparation and Measurements

We studied 9 Wistar-Kyoto rats aged 12 weeks, which were supplied by Charles River (Calco, Italy); all procedures followed were in accordance with institutional guidelines of the Centro di Fisiologia Clinica e Ipertensione. Each rat was sympathetomized by pretreatment with 6-hydroxydopamine (100 mg · kg⁻¹ · IP 2 times during 5 to 6 days), a method that allows almost complete destruction of peripheral sympathetic nerve terminals without affecting the behavior of the animal. In the animals studied, this was confirmed by the maintenance of the original body weight (290±8 g, mean±SEM) and the marked reduction of the pressor (−88%) and tachycardic (−92%) response to tyramine injection (100 mg/kg IV)13,14 performed before collection of the arterial distensibility data (see below). Data from these animals were compared with those obtained in rats pretreated with vehicle alone during 5 to 6 days. In each rat, anesthesia was induced by sodium pentobarbitone (40 mg/kg IP); polyethylene catheters were inserted into a common carotid and a femoral artery for continuous blood pressure measurement by a Statham P23 D pressure transducer. The blood pressure signal was

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visualized on a Grass 7D recorder and continuously acquired by a personal computer. Heart rate was derived as the reciprocal of the interval between 2 consecutive systolic peaks. A unipolar catheter was inserted into the right internal jugular vein and positioned into the right atrium. The external wire of the catheter was connected to a Grass S88 stimulator, and stimuli were applied at a tension of 3 V, a duration of 2 milliseconds, and a frequency of 4.66 to 6.67 Hz, to allow the heart to be paced at 280 to 400 bpm. Correct delivery of the pacing stimulus to the right atrium was verified by the ECG signal displayed on an oscilloscope by the unchanged shape and duration of the P-Q interval and QRS complex compared with the sinus rhythm (Figure 1). Pacing rates >400 bpm were not considered, because at these high frequencies, most animals displayed reduced pulse pressure, thereby altering the force responsible for changes in arterial diameter and distensibility.

Arterial distensibility was measured according to the method described and validated in previous reports. Briefly, an ultrasonic 10-MHz transducer (NIUS 01 System, Asulab) connected to a pulsed Doppler device (Capital Medical Service) was positioned over the common carotid and femoral arteries contralateral to the cannulated vessel to track the movements of the proximal and distal arterial wall over the entire systolic-diastolic cycle. In the common carotid artery, a high-quality signal was obtained transcutaneously, whereas in the femoral artery, it was obtained after a superficial skin incision, without severing muscle fasciae. In both vessels, sound transmission was optimized by interposition of ultrasound gel. Care was taken to avoid vessel deformation by the probe and to position it perpendicularly to the longitudinal axis and on the largest cross-sectional dimension of the artery at a site corresponding to the tip of the contralateral catheter. For the carotid or the femoral artery, the vessel wall signals were acquired in continuum (at 300 Hz) and stored in the computer used for acquisition of the blood pressure signal. The space resolution of the ultrasonic system used in these experiments is 2.5 μm. In thin-walled vessels such as those of the rat, no attempt is made to identify the echoes reflected from the intimal and adventitial surface; either wall (proximal and distal) is taken as a single point, and wall thickness is not considered. The computer was programmed to calculate the diameter-pressure curve of the vessel both for the increasing blood pressure values from diastole to systole and for the decreasing blood pressure values from systole to diastole, according to its fitting with the arctangent model of Langewouters. This model is based on the following formula:

\[ S = \alpha \left( \pi/2 + \tan^{-1}\left(\frac{P-\beta}{\gamma}\right) \right) \]

where \( S \) is the cross-sectional area of the vessel, \( P \) is the intravascular pressure, and \( \alpha \), \( \beta \), and \( \gamma \) are 3 optimal parameters describing the spatial position of the diameter-pressure curve. From this formula, cross-sectional compliance \( C = \Delta S/\Delta P \) was calculated as follows:

\[ C = \frac{\alpha}{\gamma}(1 + \left(\frac{P-\beta}{\gamma}\right)^2) \]

and expressed as the compliance-pressure curve. Cross-sectional distensibility was calculated by normalization of compliance with respect to arterial diameter values. The area under the curve relating arterial distensibility to blood pressure was normalized with respect to pulse pressure and referred to as the “distensibility index.”

### Protocol and Data Analysis

The carotid and femoral arteries were studied in a random order during a single experimental session. After the measuring devices were positioned, a 10- to 20-minute period of stabilization was allowed. With the rat in sinus rhythm, blood pressure and arterial diameter data were acquired during 5 periods of 4 seconds each; each data acquisition was separated from the next by an interval of 30 seconds. The atria were then paced in 5 randomly sequenced steps at the rates of 280, 310, 340, 370, and 400 bpm. During each step, blood pressure and arterial diameter data were acquired over 10 periods of 4 seconds each. After each pacing step, spontaneous sinus rhythm was allowed to return and a series of 5 data acquisitions of 4 seconds each was again obtained.

Blood pressure, arterial diameter, and arterial distensibility were computed for each 4-second data acquisition period. Data were averaged for each sinus rhythm and each atrial pacing period. Results from individual rats were averaged to obtain values of mean±SEM separately for the sympathectomized and the intact groups.

Comparisons were made between values obtained during each pacing step and the corresponding sinus rhythm period. The statistical significance of the differences in mean values was assessed by the Student 2-tailed \( t \) test for paired observations. A value of \( P<0.05 \) was considered statistically significant.

### Results

Compared with vehicle-treated rats, sympathectomized rats showed a trend to lower values of systolic blood pressure (105.1±3.5 versus 116.5±3.7 mm Hg), diastolic blood pressure (77.0±2.2 versus 80.5±3.5 mm Hg), mean arterial pressure (86.2±2.4 versus 94.3±4.3 mm Hg), and heart rate (228.2±20.0 versus 262.1±7.0 bpm). However, in no case did the differences attain statistical significance. In both groups, heart rate was virtually constant during the different periods of sinus rhythm. This was the case also for systolic, diastolic, and mean arterial pressures, which also were not appreciably modified during any of the atrial pacing steps (data not shown).

### Common Carotid Artery Mechanical Properties

In vehicle-treated rats, the diameter of the common carotid artery was not substantially affected by pacing (Figure 2, top left), which, in contrast, significantly reduced the arterial distensibility index at each pacing step (Figure 2, bottom left). The reduction in arterial distensibility tended to be greater as the pacing rate increased, as was evident throughout the systolic-diastolic pressure range (Figure 3, left). In sympathectomized rats, carotid artery diameter was similar (Figure 2, top right) whereas the arterial distensibility index was significantly greater (Figure 2, bottom right) compared with vehicle-treated rats. As in vehicle-treated rats, pacing-induced tachycardia was associated with no significant change in carotid artery diameter and with a significant reduction in the arterial distensibility index. The reduction was evident throughout the systolic-diastolic pressure range at all pacing steps (Figure 2, right).
Femoral Artery Mechanical Properties

In vehicle-treated rats, femoral artery diameter and distensibility were not sizably affected by pacing (Figure 4, left). In sympathectomized rats with spontaneous sinus rhythm, diameter was moderately and distensibility index was markedly and significantly greater than in vehicle-treated rats (Figure 4, right). Pacing-induced tachycardia did not affect diameter but, at variance with intact rats, it markedly reduced the distensibility index, the reduction being evident throughout the systolic-diastolic pressure range at all pacing steps (Figures 4 and 5).

Discussion

In our vehicle-treated rats, pacing-induced tachycardia reduced common carotid artery distensibility, although it had no effect on a stiffer, muscle-type vessel such as the femoral artery. A different pattern was observed in sympathectomized animals, however, inasmuch as after sympathectomy, the carotid artery showed a significant increase in distensibility during sinus rhythm and maintained sensitivity to the stiffening effect of tachycardia. In contrast, in the femoral artery sympathectomy also brought about an increase in distensibility during sinus rhythm that was, however, accompanied by the appearance of a rate-dependent stiffening effect that had not been observed in the sympathetically intact rats.

These data are consistent with the hypothesis that the rate-dependent decrease in arterial distensibility is primarily due to the viscous nature of the vessel wall and thus to its inertial response to changes in intravascular pressure; this is in agreement with the data of Boutouyrie et al.\textsuperscript{19} that arterial wall viscosity is more strongly influenced by mechanical load than by smooth muscle tone. Our data are also consistent with the hypothesis that the rate-dependent arterial stiffening is less evident in muscle-type than in elastic-type arteries, because in the former, distensibility is already kept to remarkably low levels by the high smooth muscle tone that characterizes these vessels.\textsuperscript{20–22}

Several other aspects of our study are worth comment. First, the femoral artery distensibility in sympathectomized rats was greater than that in intact rats during spontaneous sinus rhythm but not during pacing-induced tachycardia, whereas the carotid artery distensibility in sympathectomized rats was greater than that in intact rats irrespective of heart rate (ie, during both sinus rhythm and pacing-induced tachycardia). In other words, the stiffening influence of tachycardia was able to completely offset the antistiffening effect of sympathectomy in the femoral but not in the carotid artery.
Second, as mentioned above, after sympathectomy, arterial distensibility was increased both in the femoral and in the carotid artery, which indicates that sympathetic tone has a stiffening effect not only in arteries where muscle tone is prominent but also in arteries that have a predominantly elastic structure.\textsuperscript{10–12,23} This effect presumably is due to the fact that elastic-type arteries have a significant amount of smooth muscle tissue supplied by sympathetic nerve fibers\textsuperscript{24} (although to a lesser extent compared with muscle-type arteries,\textsuperscript{5,25}); this supply is responsible for a contraction that elevates the elastic modulus of the vessel.

Third, in the carotid artery, the stiffening effect of tachycardia was similar in intact and sympathectomized rats, regardless of whether distensibility was smaller or larger. This indicates that sympathetic activity has a prominent stiffening effect on this vessel but also indicates that this effect is independent of the one due to tachycardia, with which it does not interact. On the other hand, the behavior of the femoral artery was in several ways at variance with that of the carotid artery; ie, in the former vessel, distensibility was also enhanced after sympathectomy, but this was only seen during sinus rhythm. Furthermore, the stiffening effect of tachycardia was evident in sympathectomized but not in intact rats. Thus, tachycardia and sympathetic nerve activity are important modulators of wall mechanics in muscle-type arteries; however, in these vessels, either factor alone is capable of a near-maximal effect, which prevents the influence of the other from manifesting.

Our study has some potential limitations. First, intact and sympathectomized animals have markedly different circulating levels of several substances, such as angiotensin, vasopressin, catecholamines,\textsuperscript{26} and possibly endothelin and natriuretic peptides, that have vasoactive properties and thus may affect arterial distensibility. However, it is unlikely that these differences were primarily involved in the acute effect of tachycardia on arterial distensibility due to the rapid (seconds) onset of the pacing-related alterations.

Second, our data were collected while the animals were anesthetized, a condition known to alter autonomic tone.\textsuperscript{27,28} Thus, our findings will have to be confirmed in conscious animals, in which measurement of arterial distensibility is technically demanding and less precise because it involves the disruption of periarterial tissue.

Third, our experiments cannot answer the question of which components of vascular smooth muscle tone are responsible for the differences in the behavior of the carotid versus the femoral artery; ie, whether the neurally dependent or intrinsic tone of the vessel is responsible. However, it is reasonable to propose that both components are involved: a role for the former factor is suggested by the observation that
sympathectomy had more prominent antistiffening effects on the femoral than on the carotid artery; on the other hand, a role for the latter is indicated by the fact that even after sympathectomy, the femoral artery remained much stiffer than the carotid artery.

Fourth, caution needs to be exerted as to the clinical implications of our findings, because pacing-induced tachycardia may be different from everyday tachycardias, which are mediated by neural mechanisms that may concurrently alter vascular smooth muscle tone. In a recent study in a large number of human subjects, overall arterial compliance was shown to be inversely related to heart rate.29 This may be due to the fact that overall compliance depends to a major degree on elastic-type vessels.

In conclusion, our experiments in anesthetized normotensive rats provided evidence to support the notions (1) that in predominantly elastic-type arteries, the stiffening effect of tachycardia is exerted independently of sympathetic modulation of the vessel wall properties and (2) that in predominantly muscle-type arteries, removal of sympathetic influences unmasks the stiffening effect of tachycardia; this effect is not directly demonstrable in the intact animal, presumably because of an already prominent stiffening effect of sympathetically mediated smooth muscle tone in this vessel type.

References


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