Endothelin and mechanical properties of the carotid artery in Wistar–Kyoto and spontaneously hypertensive rats

Giuseppe Marano a,*, Mauro Grigioni b, Sergio Palazzesi a, Alberto U. Ferrari c

a Laboratorio di Farmacologia, Istituto Superiore di Sanità, Viale Regina Elena 299, 00116 Rome, Italy
b Laboratorio di Ingegneria Biomedica, Istituto Superiore di Sanità, Rome Italy
c Cattedra di Cardioangiologia Medica, Centro Fisiologia Clinica e Ipertensione, University of Milan, CNR and IRCSS Ospedale Maggiore, Milan, Italy

Received 2 April 1998; accepted 13 July 1998

Abstract

The aim of this study is to evaluate the role of endothelin in the control of the static mechanical properties of in vitro carotid arteries from 14-week-old Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). An in vitro preparation in which the artery was allowed to longitudinally elongate similarly to the in situ carotid artery was employed. The diameter of in vitro carotid arteries subjected to static pressures (from 25 to 200 mmHg in 25 mmHg steps) was determined by videomicroscopy and computer-assisted image analysis, the cross-sectional compliance and distensibility–pressure curves being then derived. The role of endothelin was assessed by incubating carotid arteries with the selective ETA and ETB endothelin receptor antagonists BQ123 and BQ788, respectively. These effects were compared with those observed under control conditions, as well as with those following complete abolition of vascular smooth muscle tone by potassium cyanide (KCN). Carotid diameter was significantly larger, and compliance and distensibility significantly smaller, in SHR compared to WKY rats. Local incubation with BQ123 was associated with significant dilations as well as significant increases in cross-sectional compliance and distensibility in both strains. This was even more pronounced with KCN, while BQ788 had no effect. The results of the present study suggest that: (i) endothelin exerts a tonic stiffening effect on the in vitro common carotid artery; (ii) this effect is mediated via the ETA endothelin receptor, and (iii) the stiffening effect of endothelin is exerted to a similar extent in the carotid arteries of normotensive WKY and SHR rats.

Keywords: Endothelin; Arterial distensibility; Wistar–Kyoto rat; Spontaneously hypertensive rat; Vascular biology

1. Introduction

The mechanical properties of large elastic arteries have been extensively studied in hypertensive compared to normotensive animals and human beings. However, in spite of the use of sophisticated in vivo and in vitro techniques, controversial findings have been reported concerning crucial aspects of hypertension-related changes in arterial mechanical behaviour, such as their time of onset during the course of the disease, their distribution in the various portions of the arterial tree, and perhaps even more importantly, their underlying mechanisms [1–6]. The reasons for these discrepancies may be multifold and may relate to differences in the hypertensive model and/or stage of hypertension examined, as well as to differences in the experimental techniques used to assess arterial wall mechanics.

Recent attention has been given to the possibility that changes in vasomotor tone, due to an imbalance in the release of vasoconstrictor and vasodilator substances from the endothelium, significantly contribute to the mechanical behaviour not only of small-sized resistance arteries, but also of larger arterial conduits [1,2,4–7]. Endothelin which is a potent vasoconstrictor peptide released by the endothelium [8,9], is known to contribute to arteriolar tone in

*Corresponding author. Tel.: +39-6-4990-2395; Fax: +39-6-4938-7104; E-mail: gmarano@net.iss.it

Time for primary review 34 days.
normotensive subjects [10,11] as well as to the increased arteriolar tone that characterises resistance vessels in some types of human and experimental hypertension [12–15]. We thus hypothesise that endothelin may influence the mechanical properties of large elastic arterial segments, and may do so to varying degrees under normotensive vs. hypertensive conditions. However, to the best of our knowledge, neither issue has so far been directly investigated.

The purpose of the present study was therefore to examine the role of endothelin in the control of common carotid artery mechanical properties in normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive (SHR) rats. This was pursued by analysing the complete pressure–diameter relationship in vitro, with the notable technical feature of using a preparation that allows the artery, when put under pressure, to undergo a longitudinal elongation similar to that of the in situ carotid artery. Furthermore, as functional and molecular studies have demonstrated that two distinct endothelin receptor subtypes (ETA and ETB) are distributed in the arterial wall [16], an additional goal of the experiments was to evaluate which subtype is involved in the control of common carotid mechanical behaviour.

2. Methods

2.1. Vessel preparation

Rats were housed and taken care of in compliance with the guidelines of the Council of European Communities (86/609/EEC). Fourteen-week-old SHR (n=30) and age-matched WKY (n=30) were anaesthetised with intraperitoneal urethane (1.5 g/kg). After induction of anaesthesia, the animals were mechanically ventilated through a tracheal cannula connected to a rodent ventilator (mod. 680, Harvard Apparatus). After midsternal thoracotomy and cervicotomy, both left and right common carotid arteries were dissected and exposed. Arterial blood pressure was recorded via a carotid catheter connected to a pressure transducer (P23ID, Statham, Gould). Each artery was cannulated at both ends, and flushed with Krebs solution (mmol/l: NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.17, NaHCO3 25, KH2PO4 1.2 and glucose 5.6) containing albumin (4%) to maintain both a pressure of about 100 mmHg and a physiological osmotic pressure gradient across the arterial wall. A portion of carotid artery, about 20 mm in length, was excised in order to obtain a length / width ratio greater than 10 to minimise the end effects, as in standard in vitro reference for compliance measurements. The artery was then immersed in an oxygenated thermostatic bath containing Krebs solution at 37°C and gassed with 95% O2–5% CO2. One cannula was connected to the pressurisation system, while the other was connected to a sliding set up with an appropriate weight (see Fig. 1) in order to reproduce the in situ distance of a couple of markers positioned before the excising, and to allow the artery to be longitudinally adjusted during pressure increases similarly to the in situ tethered artery. At the end of each experiment the anatomic and functional integrity of the carotid endothelium was tested by flushing Evan’s blue (0.03%) albumin solution into the vessel and by ensuring that acetylcholine-induced dilation was preserved.

2.2. Measurement of arterial diameter

The carotid artery was exposed under a binocular inverted microscope (×100) (Axiovert, Zeiss). The microscope was connected to both a video camera and a tape recorder, allowing the entire experiment to be recorded for image analysis.

The vessel was subjected to stepwise increases in pressure of 25 mmHg each, from 25 to 200 mmHg according to Lichtenstein et al. [3]. The recorded images were analysed using specific software allowing the digitalisation and measurements of diameters (Optilab/Pro). The system reached an accuracy of up to 10 mm, stated by micrometer sample before each measurement series. Diameters were measured at each pressure level, after 4 min, to let the vessel reach a steady condition [3]. Diameters were calculated as the mean of ten successive measurements along the recorded piece of artery, standardising table steps and magnification levels.

Cross-sectional compliance (Cs) was calculated as the change in cross-sectional area per unit pressure change (Cs=ΔS/ΔP) where ΔP represents an interval of 25 mmHg. Arterial cross-sectional distensibility (Ds) was calculated as the compliance value normalised for the cross-sectional area at 25 mmHg with the vessel extended to in situ length (Ss). Thus, cross-sectional distensibility is defined as Ds=(1/Ss)×(ΔS/ΔP).

2.3. Experimental protocol

In a preliminary set of experiments three different concentrations (10, 1 and 0.1 μmol/l) of BQ123 or BQ788 were tested. Based on the results of this concentration–response study and on data reported in literature, we performed measurement of the carotid artery diameter from WKY and SHR rats under (i) control conditions, (ii) after 30 min of intraluminal incubation with BQ123 (1 μmol/l) [17,18] (Alexis), an ETA receptor antagonist, or with BQ788 (1 μmol/l) [17] (Alexis), an ETB receptor antagonist, and (iii) after 30 min of incubation with a saline solution of KCN (100 mg/l) which suppresses smooth muscle tone completely.

2.4. Statistical analysis

Results are expressed as mean±SE. We used two-way
Fig. 1. Schematic diagram of experimental set up. The diameter of the vessel was monitored by a video camera mounted on the microscope. The vessel was irrigated with thermostated (37°C), oxygenated Krebs solution: (1) to stop flow when changing during solution; (2) to control oxygenated Krebs solution flow; (3) to prevent distortion of carotid artery during expansion.

3. Results

Table 1 shows the values of mean arterial pressure, heart rate, and body weight in WKY and SHR on the day of the experiment. Mean arterial pressure was significantly higher in SHR than in WKY (p<0.01).

The cross-sectional compliance and distensibility values obtained in SHR and WKY rats under control conditions are shown in Table 2. Cross-sectional compliance values were significantly reduced in SHR compared to WKY rats for pressures levels between 50 and 150 mmHg (Table 2), indicating a stiffer carotid wall in SHR. Distensibility values were significantly smaller in SHR than in WKY rats over the entire range of pressures (Table 2). In both groups, the maximal Cc and Dc values were observed at pressures between 75 and 100 mmHg.

As shown in Fig. 2, KCN poisoning induced a significant upward shift of the diameter–pressure relationship in both strains (Fig. 2A), indicating the existence of a basal vasomotor tone opposing the pressure-induced distension of the carotid artery. Accordingly, KCN poisoning was followed in both SHR and WKY rats by significant increases in Cc (Fig. 2B) and Dc (Fig. 6A). The magnitude of these effects was related to transmural pressure, but unrelated to differences between animal strains. Indeed, after KCN poisoning, it was observed that increases in Cc or Dc were not significantly different between WKY and SHR.

Incubation with BQ123 induced in both strains significant changes consisting of a moderate increase in diameter (Fig. 3A), and a more clearcut increase in Cc and Dc (Fig. 3B and Fig. 6B); the effect of the ETA antagonist was

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Mean arterial pressure (mmHg)</th>
<th>Heart rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=30)</td>
<td>320±10</td>
<td>91±3</td>
</tr>
<tr>
<td>SHR (n=30)</td>
<td>310±10</td>
<td>153±4*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. (* p<0.01, SHR vs. WKY).
Table 2
Cross-sectional compliance and distensibility values for each pressure step in Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) under basal conditions

<table>
<thead>
<tr>
<th>Pressure step (mmHg)</th>
<th>Cross-sectional compliance (mm²/mmHg) $\times 10^{-3}$</th>
<th>Distensibility (mmHg$^{-1}$) $\times 10^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>25–50</td>
<td>2.2±0.1</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>50–75</td>
<td>6.7±0.2$^*$</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>75–100</td>
<td>8.0±0.2$^*$</td>
<td>5.5±0.3</td>
</tr>
<tr>
<td>100–125</td>
<td>5.7±0.1$^*$</td>
<td>4.7±0.3</td>
</tr>
<tr>
<td>125–150</td>
<td>4.7±0.2$^*$</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>150–175</td>
<td>3.6±0.3</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>175–200</td>
<td>2.2±0.2</td>
<td>2.3±0.2</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE. $^*$ $P<0.05$, WKY vs. SHR.

dose-dependent, with the maximum effect being reached at the 1 μmol/l concentration (Fig. 4). At variance with BQ123, the ETB receptor antagonist BQ788 did not affect $C_s$ and $D_s$ produced by BQ123 was significantly smaller than that produced by KCN, the difference attaining statistical significance for all three parameters in both normotensive and hypertensive rats. Again, it was observed that the

![Fig. 2. Effects of smooth muscle poisoning by KCN on pressure–diameter relations (A) and cross-sectional compliance values (B) in normotensive and hypertensive rats. WKY, normotensive rats in control conditions; SHR, hypertensive rats in control conditions; WKY+KCN, normotensive rats 30 min after incubation with KCN; SHR+KCN, hypertensive rats 30 min after incubation with KCN. (*) $P<0.05$, WKY+KCN or SHR+KCN vs. control.]

![Fig. 3. Effects of incubation with BQ123 on pressure–diameter relations (A) and cross-sectional compliance values (B) in normotensive and hypertensive rats. WKY, normotensive rats in control conditions; SHR, hypertensive rats in control conditions; WKY+BQ123, normotensive rats 30 min after incubation with an ETA receptor antagonist; SHR+BQ123, hypertensive rats 30 min after incubation with an ETA receptor antagonist. (*) $P<0.05$, WKY+BQ123 or SHR+BQ123 vs. control.]

...after KCN. The magnitude of the changes in diameter, $C_s$, and $D_s$ produced by BQ123 was significantly smaller than that produced by KCN, the difference attaining statistical significance for all three parameters in both normotensive and hypertensive rats. Again, it was observed that the
changes in $C_s$ and $D_s$ induced by the ETA receptor antagonist were similar, irrespective of the rat strain as well as of the pressure range (Fig. 3B, Fig. 6B).

4. Discussion

Our study was conducted in isolated carotid arteries positioned in a device that made them able, when pressurised, to elongate similarly to the in situ carotid arteries. Under these biophysical conditions, the most important finding was that endothelin exerts on the common carotid artery a tonic stiffening influence that contributes importantly to determining the mechanical properties of this vessel. Obviously, whether step increases in pressure and stretch are related to a step release of endothelin is unknown. However, taking into account that pressure and stretch do release endothelin from endothelial cells [19,20], and that the diameter–pressure curves obtained with vs. without BQ123 were progressively divergent, it is reasonable to hypothesize that increases in pressure can be associated to a significant release of endothelin.

The present results were obtained in 14-week-old normotensive WKY rats and, with a quite similar pattern, in age-matched SHR rats, i.e., in the early established hypertensive stage. Thus the data confirm the basic hypothesis of the study, i.e., that endothelin affects not only small resistance arteries but also much larger, conduit-functioning arterial vessels, and further suggest that the stiffening effect of this peptide is exerted irrespective of the presence or absence of high blood pressure, at least as far as the SHR model is concerned. Finally, reevaluation of the pressure–diameter relationship in the presence of specific ETA and ETB receptor antagonists showed that the effects of endothelin on the common carotid artery are largely mediated via ETA receptors. The ET receptor antagonist data also indicate that endothelin is unlikely to contribute to the increased arterial stiffness displayed by the hypertensive animals (see below). To the best of our knowledge, these findings provide the first characterization of the role of endothelin in the mechanical behaviour of large arteries.

A further significant result of our study is that the carotid arteries of SHR rats have larger diameters but
SHR rats does not depend on abnormalities in the release of endothelin, because the magnitude of the changes in compliance and distensibility after BQ123 was similar in both strains. In addition, the results of the experiments performed in vessels poisoned by KCN, i.e., in the absence of any degree of vasomotor tone, underline the important role of the structural characteristics of the arterial wall in determining the differences in carotid mechanical properties between the two strains.

A comment is also warranted to discuss some possible limitations of our study. First, the experiments were performed in lack of flow, which is likely to alter the balance of the vasoconstrictor/vasodilator influences of the endothelium, especially as far as nitric oxide production is concerned. However, the alterations detected in the hypertensive compared to the normotensive animals are unlikely to have been influenced by the lack of nitric oxide, because (i) the alterations were shown to depend on structural rather than functional abnormalities and (ii) at the 12–14 week stage the degree of nitric oxide-dependent vasodilation was shown to be similar in SHR compared to WKY rats [22], so that the effects, if any, of this factor should have been similar in the vessels from the two strains. In addition, at the end of each experiment, we assessed the anatomic and functional integrity of the endothelium, by flushing Evan’s blue into the vessel and by making sure that acetylcholine-induced dilation was preserved.

A second problem relates to the fact that endothelins are a family of 21 amino acid peptides with similar biological activities, and we did not attempt to determine exactly which isopeptide is involved in the control of the mechanical properties of the carotid artery. It is nonetheless reasonable to hypothesise that endothelin-1 played a dominant role because it is the most abundant isopeptide reduced cross-sectional compliance and distensibility compared to age-matched normotensive WKY rats, the alterations being evident throughout the 25–200 mmHg pressure range. This observation may be viewed as confirming data previously obtained on elastic arteries either in vitro by means of videomicroscopy [1,3] or in vivo by ultrasonic echotracking techniques [4,21]. It should however be noted that this was not universally agreed upon, and that according to some reports [5,6] carotid artery compliance of hypertensive animals is not significantly different from that of normotensive animals. These controversial results could originate from the fact that in vivo compliance is a dynamic cross-sectional compliance, while the compliance determined in situ or in vitro from step increases in distending pressure is a static (cross-sectional or volumetric) compliance. In the authors’ opinion, the peculiar technical conditions under which our data have been collected add strength to the notion that spontaneous hypertension is associated with altered common carotid artery mechanical properties. Our experiments also indicate that the increased carotid artery stiffness observed in

Fig. 6. Effects of incubation with KCN (A) or BQ123 (B) on distensibility—pressure relations in normotensive and hypertensive rats. Arterial cross-sectional distensibility was calculated as the compliance value normalised for the cross-sectional area at 25 mmHg with the vessel extended to in situ length. (* P<0.05, after KCN or BQ123 vs. control).
this discussion to dissect out all possible factors accounting for these controversial results, it is quite clear that in this very complex but only recently explored area great caution is needed in the interpretation of the data and any unsubstantiated extrapolation may be unsafe.

A final consideration arising from our findings relates to their possible clinical implications. It has been previously suggested [10,11,27] that endothelin contributes to the regulation of vasomotor tone in healthy subjects as well as in patients with cardiovascular disease. In addition, the known trophic effect of endothelin is also a potential adverse feature of this compound: our current finding that the action of endothelin extends to the functional behaviour of large arteries further enhances the potential (patho)physiological relevance of this peptide in terms of circulatory homeostasis and of progression of cardiovascular structural and functional alterations. For example, it may first all contribute to the genesis/worsening of atherosclerosis of the vessels themselves. In addition, since the mechanical properties of large, conduit-functioning arteries can significantly affect reflection waves and left ventricular afterload, the action of endothelin on these vessels may contribute to the development of ventricular hypertrophy in hypertension, to an adverse hemodynamic outcome in acute myocardial infarction, to the deterioration of ventricular function in heart failure, etc. If confirmed, these so far speculative considerations may soon become of practical importance due to the recent introduction of nonpeptidic endothelin receptor antagonists as cardiovascular therapeutic agents.

In conclusion, the results of the present study indicate that (i) endothelin exerts a tonic stiffening effect on the in vitro common carotid artery; (ii) this effect is mediated via the ETA endothelin receptor, and (iii) the stiffening effect of endothelin is exerted to a similar extent in the carotid arteries of normotensive WKY and of SHR rats.

References