Pulsation-induced dilation of subendocardial and subepicardial arterioles: effect on vasodilator sensitivity

OANA SOROP, JOS A. E. SPAAN, AND ED VANBAVEL
Department of Medical Physics, Academic Medical Center,
University of Amsterdam, 1100 DE Amsterdam, The Netherlands

Received 11 April 2001; accepted in final form 6 September 2001

Sorop, Oana, Jos A. E. Spaan, and Ed VanBavel.
Pulsation-induced dilation of subendocardial and subepicardial arterioles: effect on vasodilator sensitivity. Am J Physiol Heart Circ Physiol 282: H311–H319, 2002.—Coronary vessels are squeezed by the surrounding myocardium during systole, impeding blood flow specifically in the subendocardium. To study the myocardial compression effect, we applied pulsatile transvascular pressure to isolated, cannulated subendocardial (Endo) and subepicardial (Epi) resistance arterioles. Pressure pulsation at 0.5 to 2.5 Hz between 20 and 100 mmHg induced dilation of preconstricted vessels that was somewhat larger in Epi arterioles. In four Epi and five Endo arterioles loaded with fura 2, pulsation led to a small increase in intracellular calcium. Pulsion induced a significant decrease in IC50 for bradykinin (BK) (5.9 ± 0.6 vs. 27.3 ± 3.2 nM in Epi vessels and 7.6 ± 0.3 vs. 302 ± 9 nM in Endo vessels), compared with steady pressure. The adenosine (Ado) sensitivity was not significantly affected (2.21 ± 0.08 vs. 3.76 ± 0.4 μM) in Epi arteries but was enhanced during pulsations in Endo vessels (3.1 ± 0.3 vs. 10.1 ± 0.6 μM). When pulsation-induced dilation was compensated by a higher concentration of the preconstrictor (U-46619), a significantly larger dilation to BK or Ado was found during pulsations. In conclusion, pulsation-induced dilation occurs at physiologically relevant frequencies and amplitudes in Endo vessels. The process does not involve intracellular calcium reduction and increases vasodilator sensitivity.

Address for reprint requests and other correspondence: E. VanBavel, Dept. of Medical Physics, Univ. of Amsterdam, Academic Medical Center, PO Box 22700, 1100 DE Amsterdam, The Netherlands (E-mail: e.vanbavel@amc.uva.nl).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

It is well known that cardiac contraction impedes coronary blood flow. In systole, intramyocardial vessels are squeezed due to either the stiffening of the activated cardiac muscle (11–13) or the development of an interstitial pressure surrounding the vessels (19). The compressive effect is dominant in subendocardial (Endo) vessels, as evidenced by measurements of microsphere distribution in beating versus arrested hearts and scarce direct observations of Endo arterioles in the beating heart (3, 5, 21). Systolic Endo flow impediment is thought to contribute to the vulnerability of this layer. Compression as mimicked by a pulsatile transmural pressure was recently shown to cause direct dilation of isolated resistance vessels. Using isolated porcine subepicardial (Epi) arterioles, we found a substantial dilation on applying pulsatile pressure at 1 Hz while keeping mean pressure constant at 60 mmHg. The effect was endothelium-independent (6). Recchia et al. (17) observed a similar dilation in freshly isolated porcine carotid artery segments also shown to be endothelium independent.

Because myocardial interstitial pressure near the endocardium reflects left ventricular pressure (8, 10, 21), extravascular pressure excursions in the order of 100 mmHg are to be expected for left ventricular Endo arterioles. At the onset of systole, such pressure is immediately transmitted to the intravascular compartment, preventing acute compression. However, in the course of systole, the vessels empty through retrograde flow back to the main coronaries, and transvascular pressure (inside-outside) gradually falls. In the presence of stenosis, however, systolic backflow is hampered, and consequently the amplitude of transvascular pressure oscillations is reduced. On the basis of the above studies on Epi and noncoronary vessels, one could argue that pulsation-induced dilation is present also in Endo vessels and provides a continuous state of partial dilation, supporting perfusion in this area. This mechanism would then be impaired in the presence of a stenosis, aggravating the consequences for the subendocardium. Alternatively, one could argue that these vessels, being the only ones in the body experiencing such large pulsation, might have developed compensating mechanisms desensitizing them to the pulsation. However, a direct study of the behavior of isolated Endo arterioles under pulsation has not yet been performed and it is, therefore, unknown whether these vessels are sensitive to pulsatile transvascular pressure.

The aim of the present study was to compare the effect of pulse pressure on tone of Endo versus Epi resistance vessels in vitro. We tested to what extent amplitude, frequency, and waveform of the applied pressure pulses, resembling different conditions to which the coronary vasculature may be subjected in the beating heart, affect pulsation-induced dilation. To follow one of the possible mechanisms involved, we also measured the intracellular calcium changes while sub-
jecting the vessel to pressure pulses. Finally, we tested whether pulsation affects the sensitivity to vasodilators.

MATERIALS AND METHODS

Experimental preparation. Thirty female Yorkshire pigs, 12–18 wk old, weighing 17–26 kg, were anesthetized by 4% halothane, followed by ketamine (20 mg/kg), midazolam (1 mg/kg im), and atropine (0.05 mg/kg). After the animal was intubated and artificially ventilated (O_2/N_2O, 1:2), the ear vein was cannulated and midazolam (0.2 mg/kg) was administered intravenously. A midsternal thoracotomy was performed. The pericardium was opened, and the heart was exposed. After heparinization (0.1 ml/kg iv), the heart was fibrillated, excised, and immediately placed in cold (4°C) MOPS-buffered Ringer solution composed of the following (in mmol/l): 145.0 NaCl, 4.7 KCl, 1.17 MgSO_4·7H_2O, 2.0 CaCl_2·H_2O, 1.2 Na_2HPO_4·H_2O, 5.0 glucose, and 2.0 pyruvate; the solution was equilibrated with air, pH 7.35 ± 0.02. Coronary microcirculation was visualized by injecting an India ink-gelatin-physiological saline solution into the left anterior descending and circumflex arteries. It was previously shown (14) that ink-gelatin perfusion does not alter normal endothelial function. Dissection was performed in MOPS-buffered Ringer solution containing 1% albumin at 4°C, using a dissection microscope with epi-illumination. Arterioles from the epicardial and endocardial layer were dissected and cleared as much as possible from the surrounding tissue and then placed in the cannulation chamber in MOPS buffer. The vessel was cannulated at both ends with glass micropipettes, using two nylon filaments, tested for any leaks, and set to its in situ length.

Each cannula was supplied by a reservoir with MOPS buffer containing 1% bovine serum albumin. Both reservoirs were simultaneously pressurized by a single Venturi valve (Fairchild T5200–50), driven by a command voltage generated by a computer program or a pulse generator. Internal diameter of the vessels was continuously measured using a video technique. During the whole experiment, the vessel was superfused with MOPS buffer at a rate of 3 ml/min using a peristaltic pump. The temperature was controlled and maintained constant at 36.8 ± 37°C. All vascular agonists used during the experiments were added to the superfusion medium.

Internal diameter of the vessels was between 100 and 200 μm when fully dilated and pressurized at 60 mmHg mean pressure. Unlike vessels below 100 μm, such vessels do not always develop a substantial level of basal tone. To have a consistent level of tone in all protocols, we preconstricted all vessels with 1 μM of U-46619, a thromboxane analog.

Protocol 1: Effect of pulse pressure on Endo versus Epi arterioles. The purpose of this set of experiments was to test the hypothesis that pulse pressure, resembling systolic compression, causes dilation of vessels isolated from the myocardium and to test whether there exist any differences in the sensitivity of Epi versus Endo vessels to systolic compression. We simulated such compression by applying transvascular pressure variations with similar characteristics to the cannulated vessels. Because applying an extravascular pressure to the vessels is not very practical considering that superfusion has to be maintained and drugs have to be added, this transvascular pressure waveform was obtained by reducing intravascular pressure from the diastolic value rather than increasing extravascular pressure. It should be stressed here that the mechanical loading of the vessel depends primarily on the transvascular pressure gradient and hardly on the absolute pressure level. The Fairchild electrically driven Venturi valve used in the current study allowed for rhythmic pressure variations driven by a computer-generated command voltage. The use of a square command voltage in combination with the low-pass transfer function of this valve from the command voltage to pressure resulted in pressure waveforms qualitatively resembling in vivo transvascular pressure variations. Luminal pressure variations were applied with frequencies between 0.5 and 2.5 Hz. The command for the valve was varied between 100 and 20 mmHg, resulting in amplitudes of pressure variations that fall with frequency and waveforms that become more sinusoidal at higher heart rates, as would occur in vivo. The wave characteristics as measured by in-line pressure transducers are summarized in Fig. 1, which shows the amplitude of recorded pressure variations for the five frequencies, expressed as actual peak-peak and root mean square value. The latter represents the square root of the power contained in the pressure variations. Figure 1 also presents the root mean square amplitude of the base harmonic for each of the five applied frequencies, as obtained from Fourier analysis of the recorded pressures. Actual waveforms at 1 and 2 Hz are also depicted in the example tracings of Fig. 3.

The applied pressures, as measured by the in-line transducers, may be damped by the cannula resistances in combination with the compliance of the cannulated vessel. Previous calculations (4) revealed a cut-off frequency of around 30 Hz for such damping, indicating that this is not a concern. In a separate experiment, we tested whether indeed the intravascular pressure truly resembles the generated pressure. Intraluminal pressure of the cannulated arteriole was recorded with a servonull micropipette system, using a glass micropipette with a tip diameter of 2 μm punctured through the vessel wall. The servonull signal obtained was identical to that of the in-line transducers for all applied frequencies.

Tone in all vessels was induced by 1 μM of U-46619 and maintained for 15 min at constant pressure before pulsation. Pulsatile pressures were then applied for 15 s (see also Fig. 3). Baseline pressure before and immediately after pulsation was set to 100 mmHg to mimic long diastole. In addition, the same pulsations were applied from a baseline pressure of 60 mmHg to study the degree of pulsation-induced dilation when average pressure remains the same. Finally, a baseline
pressure of 20 mmHg was applied to have a symmetric set of pressure protocols. For each pulsation period, we analyzed the difference between the inner diameter just before applying the pulse and immediately (first 2 s) after the pulse was stopped, both values thus being obtained at the baseline pressure. The 15 pressure protocols (5 frequencies × 3 baseline pressures) were applied in random sequence and separated by 3 min at constant pressure, allowing the vessel diameter to return to the baseline value. Endo and Epi vessels were studied in random sequence.

In a separate set of experiments, vessels were subjected to pulsatile pressure of 1.5 Hz and varying amplitude, using either the above waveform or true sinusoidal pressure variations. Baseline pressure and average pressure during pulsation were kept at 60 mmHg.

**Protocol 2: changes of intracellular Ca$^{2+}$ in response to pressure pulses.** After cannulation, vessels were loaded with the calcium indicator fura 2 according to procedures previously described in detail (20). In short, 50 µg of fura 2-acetoxyxymethylester (AM) was dissolved in 50 µl of DMSO containing 2% pluronic and suspended in 5 ml physiological saline solution. This loading solution was superfused for 1 h at 30°C, followed by a 30-min washout period at 37°C. With the use of a photomultiplier tube, an integral measurement of intracellular calcium was made over the full vessel wall. The calcium signal was measured simultaneously with diameter just before, during, and after finishing a 30-s period of pulsation between 20 and 100 mmHg with a frequency of 1 Hz. These experiments were performed for baseline pressures of 20, 60, and 100 mmHg in vessels that had developed basal tone or were preconstricted by 1 µM of U-46619. At the end of the experiment, 2 µM of ionomycin was added and fura 2 emission was determined in the presence and absence of extracellular calcium and after quenching with manganese. The intracellular calcium is expressed here as R = R$_{min}$, where R is the ratio of 515 nm emission on excitation with 340 and 380 nm, using the emission levels after quenching for background subtraction, and R$_{min}$ is the value in the presence of ionomycin and absence of extracellular calcium. In addition, the dynamic range of fura measurements was determined as R$_{max}$ - R$_{min}$ with R$_{max}$ being the ratio in ionomycin and high calcium.

**Protocol 3: the effect of pulsatile pressure on response to adenosine and bradykinin.** Vessels were pressurized alternately with 60-mmHg constant pressure and with sinusoidal pressure variations at 1.5 Hz between 20 and 100 mmHg, using a sinusoidal Fairchild command voltage. To have a stable level of constriction, 1 µM of U-46619 was kept for at least 15 min in the vessel bath. Cumulative concentration-response curves for adenosine (Ado) and bradykinin (BK) were recorded in the presence and absence of pulsation. All concentrations were maintained in the superfusion for 3 min. The order of pulse or no pulse was randomized.

In further experiments, we tested whether differences in vasodilator sensitivity could be due to the lower level of preconstriction in the presence versus absence of pulsation. During pulsation, the concentration of U-46619 was increased to 4 µM to obtain a level of preconstriction similar to that of 1 µM of the thromboxane analog in the absence of pulsation. A 15-min stabilization period was then allowed for each condition, and single concentrations of Ado or BK close to their IC$_{50}$ values were applied.

**Drugs.** Ado, BK, and U-46619 were purchased from Sigma (St. Louis, MO). Fura 2-AM was obtained from Molecular Probes (Eugene, OR).

**Data analysis.** All arteriolar diameters and all changes in diameter were normalized to the passive diameter at 60 mmHg as obtained in the absence of preconstrictors and the presence of 10$^{-7}$ M BK. Results are reported as means ± SD, unless otherwise indicated. We used SPSS software to test in a general linear model whether dilation depended significantly on pressure, frequency, and their product (interaction term). This regression analysis was done separately for Endo and Epi vessels. A simple binomial test was used for assessing whether an overall difference existed between Endo and Epi vessels over the 15 interventions (three baseline pressures and five frequencies), followed by unpaired t-tests for each of these interventions. IC$_{50}$ values were determined using sigmoid curve fitting with variable Hill slope on individual vessels, followed by averaging per group. Where appropriate, paired or unpaired two-sided t-tests were applied. Results were considered statistically significant at P < 0.05.

**RESULTS**

**Effect of pulse pressure on Endo versus Epi arterioles.** Six Endo and six Epi arterioles of similar size [166 ± 15 and 147 ± 36 µm passive inner diameter, respectively; P = not significant (NS)] were cannulated and subjected to the pulse pressure protocols, as described in MATERIALS AND METHODS. Basal tone developed in some of these vessels but was rather variable. Therefore, 1 µM of U-46619 was used to obtain consistent and stable levels of preconstriction. Figure 2 summarizes these levels of preconstriction at the three pressure levels before pulsation. As can be seen, Epi vessels compensated to some extent to the higher pressure, i.e., had weak myogenic adaptation of the induced tone, whereas Endo vessels had larger diameters at higher pressures. The level of constriction was significantly deeper in Epi versus Endo vessels at 60 and 100 mmHg. Figure 3 denotes typical responses of these preconstricted vessels to pulsatile pressure. As can be seen, the vessels showed dilation in response to pulsation. The pulsation-induced dilation started almost instantaneously, reaching a plateau value after ~10 s. Such rapid dilation was found in Epi as well as Endo vessels, and this was the case for all frequencies and baseline pressures. After arrest of the pulsation, vessels regained their initial levels of tone in the course of a few minutes. The peak-peak amplitude of diameter excursions during pulsation ranged from 6% of the dilated diameter at 2.5 Hz to 13% at 0.5 Hz. At 60 and
In both Epi and Endo vessels, dilation became significantly less at higher frequencies (regression analysis: $P = 0.007$ and $P = 0.02$, respectively) and higher baseline pressures ($P < 0.001$ and $P < 0.001$), whereas interaction between the effects of pressure and frequency was not significant in either group (for regression analysis, see MATERIALS AND METHODS). When comparing Endo and Epi vessels, we noticed a larger dilation in the Epi vessels for 13 of the 15 interventions, indicating that these vessels are more sensitive to pulsation ($P = 0.004$, binomial test). The difference is largest at baseline pressures of 60 and 100 mmHg and significant for 5 of 10 interventions at these pressures (unpaired $t$-tests). Because Epi vessels had a

100 mmHg, Endo vessels had a somewhat higher amplitude that we attributed to the less deep preconstriction in this group (data not shown in detail). Figure 4 summarizes the degree of pulsation-induced dilation.

Fig. 3. Examples of pulsation-induced dilation for trains of pulsations with 1-Hz frequency, 100 mmHg baseline (A) and 60 mmHg baseline pressure (B) and 2-Hz frequency, 60 mmHg baseline pressure (C).

Fig. 4. The dependence of pulsation-induced dilation in 6 Endo and 6 Epi vessels on frequency and baseline pressure. The dilation in response to pressure pulses decreased with increasing baseline pressure for both vessel types. Increasing frequency also resulted in less dilation in both types of vessels. *Significant differences Endo vs. Epi vessels, $t$-test.
deeper tone at these pressures before pulsation, we considered the possibility that the Endo-Epi difference is caused by the initial tone rather than by sensitivity to pulsation. However, when inspecting individual responses within the Epi as well as within the Endo groups, no significant correlation nor tendency for correlation was found between initial level of tone and degree of dilation, and this was the case at both 60 and 100 mmHg (data not shown).

The above data show that pulsation-induced dilation occurred in both vessel types, although Endo vessels had an intrinsically lower sensitivity to pulsation. To test the effect of amplitude and waveform of the pulse applied to the vessel, four Epi arterioles kept at 60 mmHg were subjected to pressure pulses of 1.5 Hz and increasing amplitudes, using either harmonic-containing or sinusoidal pressure variations. Figure 5 illustrates that the responses to these two waveforms are similar \( (P = \text{NS} \text{ at any amplitude, paired } t\text{-test}) \). In both cases, oscillations with peak-peak amplitudes up to 25–30 mmHg remained without effect, whereas 35–40 mmHg was sufficient to induce a dilation.

Intracellular calcium during pulsation. Dilation in response to pulsating pressure may result from intracellular signaling events or from a direct mechanical effect on the contractile elements. To dissociate between these two possibilities, the effect of pulsation on intracellular calcium was measured. A 30-s period of pulsation \( (1 \text{ Hz, } 20–100 \text{ mmHg}) \) resulted in substantial dilation in four Epi and five Endo fura-loaded vessels with basal tone (Fig. 6), and this was the case for baseline pressures of 20, 60, and 100 mmHg. This dilation was not associated with a reduction in intracellular calcium. Rather, calcium expressed as \( \frac{R - R_{\text{min}}}{R_{\text{min}}} \) rose somewhat in both Epi and Endo vessels in response to pulsation. Although this rise was significant for three of six cases, the only substantial rise was found for Endo vessels at a baseline pressure of 20 mmHg. Baseline calcium before pulsation increased with pressure in Endo but not Epi vessels. Similar results were found for vessels preconstricted with U-46619 (data not shown). The sensitivity of the fura emission ratio for changes in intracellular calcium was evident from the dynamic range \( (R_{\text{max}} - R_{\text{min}}) \) as recorded at the end of the experiments, which averaged \( 0.59 \pm 0.14 \) and \( 0.73 \pm 0.09 \), for Epi and Endo, respectively \( (P = \text{NS}) \), and from responses to vasoconstrictors (not shown).
Vasodilator sensitivity during pulsation. A further series of experiments was performed to determine whether pulsatile pressure influences sensitivity to the vasodilators BK and Ado. Nine preconstricted Epi (passive diameter = 129 ± 27 μm) and five Endo (passive diameter = 169 ± 61 μm) arterioles were subjected to sinusoidal pressure waves with amplitude of 80 mmHg, frequency of 1.5 Hz, and baseline pressure of 60 mmHg. The concentration-response curves for both agonists were recorded in the presence of pulsations and at 60 mmHg steady pressure. Figure 7 summarizes the results. Preconstriction by 1 μM of U-46619 was comparable in both vessel types and for both vessels was significantly less deep during pulsation (Endo: 79.5 ± 2.9% in presence of pulsations vs. 66.5 ± 2.3% at steady pressure, Epi: 77.8 ± 3.6 vs. 55.4 ± 1.2%). Both vessels became significantly more sensitive to BK during pulsation: IC50 was 7.60 ± 0.27 nM vs. 302 ± 9 nM in Endo vessels in the presence and absence of pulsation, respectively (P < 0.05). Note that the sensitivity to BK is remarkably low under static pressure in Endo vessels, whereas pulsation caused a ×40 shift in sensitivity. During these experiments, the initial U-46619-induced constriction was less deep in the presence of pressure pulses. Because the level of preconstriction rather than the pulsation might have affected the vasodilator sensitivity, we performed a separate group of experiments where we tuned the constriction to similar values, using 4 μM and 1 μM of U-46619 in the presence and the absence, respectively, of pulsatile pressure, and then we tested the effect of a single concentration of BK, close to the above IC50 values. Figure 8 presents the results: whereas preconstriction levels were identical (P = NS, paired t-test), in the presence of BK diameter was larger during pulsation than at steady pressure (P < 0.05) in Endo as well as Epi arterioles.

Figures 9 and 10 depict the results for Ado. Epi but not Endo vessels fully dilated to the highest Ado concentrations. Pulsation caused sensitization to Ado that was significant for Endo vessels (IC50: 3.11 ± 0.35 μM vs. 10.05 ± 0.58 μM in Endo, P < 0.05, in presence and absence, respectively, of pulsations and 2.21 ± 0.08 μM vs. 3.76 ± 0.40 μM in Epi, P = NS). As for BK, a single concentration of Ado caused larger dilation during pulsation in vessels with tuned preconstriction, and this was the case in both Endo and Epi vessels.

DISCUSSION

Results of the current study show that isolated Endo and Epi resistance arteries dilate in response to pulsatile pressure. In both vessel types, intracellular calcium is essentially unaffected, whereas vasodilator sensitivity is increased during pulsation.

It is well established that cardiac contraction limits predominantly Endo flow, whereas Epi flow is less affected (5). The flow limitation is thought to result from compression of the vessels through either development of high levels of intramyocardial pressure during systole (19) or systolic myocardial elastance (11). There are clear differences between both concepts, but these concern the nature of the extravascular force
rather than the vascular consequences. The sequence of events during compression is best explained on the basis of intramyocardial pressure. At the onset of systole, extravascular pressure is immediately transmitted to the intravascular compartment, because fluid is incompressible. Transvascular pressure (inside-outside) consequently is initially unchanged. Subsequently, the raised intravascular pressure results in a displacement of the fluid to regions of the coronary circulation where the compression is absent, i.e., back to the major coronaries. During the displacement, the transvascular pressure and the diameter are simultaneously reduced. Thus the effect of extravascular compression on transvascular pressure is low-pass filtered due to coronary resistance and compliance. We mimicked such compression by applying pulsatile transvascular pressure of similar waveform to the cannulated segments. The amplitude of diameter oscillations during pulsation was found to be comparable to those seen in a limited amount of in vivo observations in Endo vessels. Thus the average diameter amplitude during our experiments was 11% of the active diameter in Endo vessels at 1.5 Hz and 60 mmHg baseline pressure, whereas Merkus (16) observed a diameter amplitude of 13% of the active diameter amplitude in Endo arterioles in anesthetized open-chest dogs. In the beating porcine heart, Yada and colleagues (10, 22) observed a 20% decrease in diameter of Endo vessels in systole, whereas Epi vessels were compressed by only 2%. This comparison indicates that the pressure oscillations we applied (Figs. 3 and 4) are indeed realistic for the Endo vessels. They are too large for the Epi vessels. However, we wanted to be able to compare the vessels under identical conditions. Lower amplitudes of pressure variation, as would occur in the subepicardium, did not cause dilation (Fig. 5). Thus at an amplitude of 20 mmHg, a diameter pulsation was observed of 2.2%, comparable to the 2% observed by Kajiya et al. (10), but dilation after onset of this pressure variation was absent. Our data thus indicate that pulsation-induced dilation occurs in the beating heart in the Endo regions only.

Pulsation-induced dilation was found for all tested frequencies, ranging between 0.5 and 2.5 Hz and covering the resting heart rate of the pigs (1.5–2 Hz). We have not attempted to increase the frequency above 2.5 Hz and, therefore, cannot make any conclusions on the effect of pulsation in heavily exercising animals where heart rate is elevated. Pulsation-induced dilation became somewhat less over the range between 0.5 and 2.5 Hz. We attribute this to the reduced actual pressure amplitude at higher frequency (Fig. 1), which was applied to mimic the low-pass filtering effect of proximal resistance and intramyocardial compliance on transmural pressure excursions as seen in the beating heart. We believe this mimicking was not unrealistic, even though the branching coronary circulation is clearly not a linear first-order system. Thus the estimated cut-off frequency for the pressure driver, 2 Hz, is between apparent cut-off frequencies estimated from arterial inflow and myocardial blood volume variations during heart contraction (9). Hence, these data suggest...

Fig. 9. The concentration-response curves for Ado in the absence and presence of pressure pulses in 5 Endo (A) and 9 Epi (B) arterioles. Dilation in response to Ado was normalized to the passive diameter.

Fig. 10. The dilation obtained with $10^{-5}$ M Ado in 5 Endo (A) and 9 Epi (B) arteries, when starting at the same level of preconstriction with and without pressure pulses. The vessel was constricted with 1 $\mu$M of U-46619 at a constant pressure of 60 mmHg and with 4 $\mu$M of U-46619 during pressure pulses. *$P < 0.05$, t-test.
that the contribution of pulsation-induced dilation to tone of Endo vessels may indeed become less at higher heart rates.

We (6) previously considered the possibility that pulsation-induced dilation could actively contribute to autoregulation of blood flow. Thus we suggested that an increased heart rate or increased inotropic state could lead to extra vasodilation through this mechanism. Our current data leave little room for control of tone at changing heart rates, because an increased frequency did not result in more dilation. Considering the inotropic state, we did observe an increased response at higher amplitudes over the range considered to be relevant for Endo vessels (Fig. 5). Thus pulsation-induced dilation might indeed actively contribute to Endo autoregulation under conditions of varying inotropic state. In addition to being a possible active control mechanism, pulsation-induced dilation appears to be an influence always present in the subendocardium but not in the subepicardium. This influence forms an important contributor to tone especially because the vasodilator sensitivity is increased. Moreover, in the presence of a severe coronary artery stenosis, Endo pulsatility is greatly reduced due to the increased resistance for the systolic back flow. A reduction of pulsation to <40 mmHg would result in absence of this response and this might contribute to the vulnerability of the Endo layer.

The mechanism of pulsation-induced dilation remains unclear. The effect could either result from intracellular signaling in the smooth muscle cells or reflect a direct mechanical influence on the contractile elements. It was not the purpose of the current study to fully unravel the mechanisms involved, but we did want to discriminate between these two possibilities. Intracellular calcium was found not to be reduced by pulsation, strongly arguing for a direct effect on the contractile elements. For several reasons, we do not believe that such a direct effect would represent irreversible damage by rupturing during pulsation, as was suggested by Busse and Fleming (1). First, the response to pulsation was reversible and vessels regained their original level of constriction after ending the pulsation. This was the case for the preconstricted vessels as well as vessels that had substantial basal tone (data not shown). Second, pulsation-induced dilation occurred also when pressure was switched from 100 mmHg steady to a pulsation between 20 and 100 mmHg. It is hard to envision that such a reduction in mean pressure would damage the vessel. Third, as explained above, pressure excursions of these amplitudes are believed to occur under normal physiological conditions in the beating heart.

We found a threshold amplitude for pulsation-induced dilation of around 40 mmHg peak-peak, irrespective of the waveform of the pulsation. Using carotid artery segments, Recchia et al. (17) found a similar threshold. Also in vivo data provide support for enhanced coronary flow at pulsations of aortic pressure above around 40 mmHg (18). The coincidence of these thresholds suggests that a similar mechanism of dilation is present in these experiments. Recchia et al. (17) argued that a plastic rearrangement of the vascular wall occurred with sustained pulsatile pressure. A non-linear viscoelasticity, with more viscosity occurring during pressure decay, would indeed give the observed dilation. However, it is unclear how viscoelastic effects can lead to the increased vasodilator sensitivity that we observed during pulsation and to regional differences in pulsation-induced dilation. A possibility worth studying would be the contribution of the cytoskeleton to both mechanics and signaling during pulsation.

Epi and Endo vessels differed in several respects. A first difference was that Epi vessels had more induced tone at 60 and 100 mmHg compared with Endo vessels, whereas vasoconstriction was equal at 20 mmHg. Thus the active pressure-diameter relation was essentially flat in Epi vessels while Endo vessels were still distended when increasing pressure. Such a difference in pressure sensitivity was previously also observed by Kuo et al. (15) who found myogenic vasoconstriction to be greater in Epi vessels. Interestingly, though, intracellular calcium rose with baseline pressure in the Endo but not the Epi vessels. Thus it appears that Epi-Endo differences exist with respect to myogenic calcium handling. It was, however, not the purpose to study the myogenic behavior of these vessels, and these observations, therefore, need to be addressed in future work. Second, pulsation-induced dilation was larger in the Epi vessels, especially at 60 and 100 mmHg baseline pressure. One could argue that this difference results from the above mentioned diverging preconstriction at these pressures. However, within the groups, no correlation was present between preconstriction and the degree of dilation. It thus seems that intrinsic quantitative differences exist between these vessels also with respect to pulsation-induced dilation, even though the responses are qualitatively similar. Third, under static pressure, Endo vessels were remarkably insensitive to BK (Fig. 7). Pulsation caused a 40-fold leftward shift in IC50, and under these conditions, Endo and Epi vessels had comparable sensitivities to BK as well as Ado. The huge shift in BK sensitivity of Endo vessels with pulsation underlines the importance of interaction between mechanical effects and vasodilator sensitivity in determining Endo perfusion. The mechanisms responsible for this interaction await further study.

Evidence exists for an endothelium-dependent component to pulsation-induced dilation in isolated rabbit carotid artery (1). Pulsation has also been shown to cause release of an endothelial cell hyperpolarizing factor from coronary vessels. The factor is believed to be a cytochrome P-450 metabolite (1, 2). Previously, we showed that physical removal of the endothelial cells does not prevent pulsation-induced dilation in the current model (6). Similarly, Recchia et al. (17) showed that pulsation-induced dilation of carotid arteries is endothelium-independent. These data, therefore, indicate that if an endothelium-dependent contribution to pulsation-induced dilation indeed exists, it is masked by a direct effect on the smooth muscle cells in at least
some models. Because in our experiments smooth muscle intracellular calcium did not decrease during pulsation, no obvious role for endothelium-derived hyperpolarizing factor could be identified. We also considered the possibility that the small increase in fura ratio during pulsation might stem from possible endothelial fura loading. Yet the observed rise in calcium was slower than the pulsation-induced dilation and thus seems not to be an initial event in the dilation.

We cannot rule out the fact that the increased BK sensitivity during pulsation is related to endothelial mechanisms, especially because such a strong shift of sensitivity to this endothelium-dependent dilator was found during pulsation. Ado sensitivity was also increased with pulsation. Reported values for Ado sensitivity remain remarkably heterogeneous, but it has been established that vasodilation at lower concentrations ($\leq 10^{-8}$ M) is exclusively mediated by endothelial NO production, whereas higher concentrations ($> 10^{-7}$ M) of Ado act also directly on the smooth muscle cells (7). Because in our experiments, the IC$_{50}$ value for Ado was in the range of $10^{-8}$ M, we believe that this reflects a direct effect on the smooth muscle cells. This does, however, not exclude the possibility that the increased sensitivity during pulsation has an endothelial origin also for Ado, either through endothelial Ado receptors or through interaction between endothelial factors and Ado acting on the smooth muscle cells. Experiments on deendothelialized and fura-loaded vessels will be required to further disclose the role of this layer in vasodilator sensitivity and associated calcium handling.

In conclusion, Endo and Epi vessels dilated to pulsation. Even though the response was somewhat less in the Epi vessels, the large difference in pulsatile transmural pressure regime indicates that in the beating heart Endo vessels are preferentially dilated by this effect. Moreover, during pulsation the vessels became more sensitive to vasodilators. It should be stressed that endothelium-dependent and -independent vasodilator influences form part of normal tone control in these vessels. Therefore, the sensitization to vasodilators during pulsation, which we expect to occur continuously in the subendocardium, may provide a base for future vasodilator therapy aimed at improving specifically subendocardial flow.

REFERENCES