Calcium channel blockade prevents pressure-dependent inward remodeling in isolated subendocardial resistance vessels

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Sorop, Oana, Erik N. T. P. Bakker, Adrian Pistea, Jos A. E. Spaan, and Ed VanBavel. Calcium channel blockade prevents pressure-dependent inward remodeling in isolated subendocardial resistance vessels. Am J Physiol Heart Circ Physiol 291: H1236–H1245, 2006. First published March 24, 2006; doi:10.1152/ajpheart.00838.2005.—The capacity for myocardial perfusion depends on the structure of the coronary microvascular bed. Coronary microvessels may adapt their structure to various stimuli. We tested whether the local pressure profile affects tone and remodeling of porcine coronary microvessels. Subendocardial vessels (~160 μm, n = 53) were cannulated and kept in organoid culture for 3 days under different transvascular pressure profiles: Osc 80: mean 80 mmHg, 60 mmHg peak-peak sine wave pulsation amplitude at 1.5 Hz; St 80: steady 80 mmHg; Osc 40: mean 40 mmHg, 30 mmHg amplitude; St 40: steady 40 mmHg. Under the Osc 80 profile, modest tone developed, reducing the diameter to 81 ± 14% (mean ± SE, n = 6) of the maximal, passive diameter. No inward remodeling was found here, as determined from the passive pressure-diameter relation after 3 days of culture. Under all other profiles, much more tone developed (e.g., Osc 40: to 26 ± 3%, n = 7). In addition, these vessels showed eutrophic (i.e., without a change in wall cross-sectional area) inward remodeling (e.g., Osc 40: passive diameter reduction by 24 ± 3%). The calcium blocker amlodipine induced maintained dilation in St 40 vessels and reversed the 22 ± 3% (n = 6) inward remodeling to 15 ± 3% (n = 8) outward remodeling toward day 3. Vessels required a functional endothelium to maintain structural integrity in culture. Our data indicate that reduction of either mean pressure or pulse pressure leads to microvascular constriction followed by inward remodeling. These effects could be reversed by amlodipine. Although microvascular pressure profiles distal to stenoses are poorly defined, these data suggest that vasodilator therapy could improve subendocardial microvascular function and structure in coronary artery disease.

coronary circulation; microcirculation; vascular remodeling; amlodipine

LOCAL MYOCARDIAL PERFUSION depends on both the structure of the coronary microvascular bed and the tone of the small arteries and arterioles. Coronary microvascular tone is believed to depend on, among others, local pressure (9). Thus, in acute experiments on isolated coronary arterioles, myogenic dilation is observed when pressure is reduced. In addition, pulsating pressure induces acute vasodilation (16). This is of relevance for the vessels in the subendocardium that in systole are compressed by the surrounding myocardium; this compression is thought to increase the vulnerability of this layer for ischemia. However, in the above isolated vessel experiments, the pressure profiles are typically maintained only for a few minutes to hours. Very little is known about the long-term effects of pressure on coronary microvascular tone or about possible remodeling of these vessels. Such long-term changes may occur in the coronary circulation as a result of hemodynamically significant stenoses. A microvascular component to myocardial underperfusion in the presence of coronary stenosis has been suggested. Thus, using histology, Hong and colleagues (8) observed luminal narrowing of microvessels distal to a stenosis. Others have observed a compensatory decrease in microvascular resistance in the presence of mild stenoses, evident after balloon dilation (17). However, it is not clear to what extent the local pressure profile is involved in such changes.

We have developed techniques for the organ culture of cannulated, pressurized arterioles. In a study on rat cremaster arterioles, we observed rapid changes in vascular structure, and a causal link was found between maintained tone and inward remodeling (4). Although this link occurred essentially independent of the mode of contractile activation, pressure-induced activation formed an exception: in cremaster arterioles we observed more inward remodeling at 50 compared with 100 mmHg, despite the higher tone during culture at the higher pressure (3). In the same study, we demonstrated that pulsatile pressure keeps cultured cremaster vessels in a more dilated state and partially suppresses inward remodeling. However, effects of changes in pressure and pressure pulsations, as could possibly occur in microvessels distal to coronary stenoses, have not been tested yet on coronary arterioles in organoid culture. Here we test whether long-lasting changes in the pressure profile affect tone and remodeling of these coronary vessels and whether long-lasting pharmacological vasodilation leads to a structural enlargement under these conditions.

METHODS

Experimental Preparation

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and with the guidelines on animal experiments of our institution. The experimental preparation of the blood vessels is described in detail elsewhere (16). Briefly, 26 female Yorkshire pigs weighing 18–23 kg, 12–18 wk old, were anesthetized, intubated, and artificially ventilated, and hearts were excised. From each heart, two subendocardial arterioles were dissected free of surrounding tissue and cannulated at both ends with glass micropipettes under sterile conditions.

Cannulated subendocardial arterioles were cultured for 3 days (72 h) using the technique for organ culture of cannulated cremaster muscle arterioles developed by Bakker and coworkers (4). Briefly, after cannulation, the arterioles were perfused with Leibovitz culture medium supplemented with 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 0.2 mg/ml ciprofloxacin, and 10% heat-inactivated FCS. Each
cannula was independently pressurized by a Venturi valve (FAIRC-CHILD T5200–50), driven by a command voltage. We simulated the extravascular compression exerted by the contracting myocardium on subendocardial arterioles by applying transvascular pressure variations to the cannulated vessels. This transvascular pressure waveform was obtained by reducing intravascular pressure rather than increasing extravascular pressure. A small pressure gradient was established between cannulas to ensure the refreshment of the intraluminal fluid (shear stress <1 dyn/cm²). The vessels were superfused with Leibovitz medium with the same composition but without the FCS. Each cultured vessel was placed on an inverted microscope built in our laboratory, and internal and external diameters of the vessels were regularly measured during culture using charge-coupled device (CCD) cameras attached to the microscopes and automated video analysis. The temperature was maintained constant at 37°C. All drugs were added to the superfusion medium.

Protocols

**Experimental group 1: Effect of pressure profiles.** Subendocardial vessels were isolated, cannulated, and kept in organun culture under four different pressure profiles: Osc 80 group: vessels were subjected to a mean pressure of 80 mmHg and pressure pulsations of 60 mmHg peak-peak amplitude with sinusoidal waveform and 1.5 Hz frequency. This pressure profile combines values of steady and pulsating pressure thought to approximate the profile in subendocardial arterioles; St 80 group: vessels were kept at 80 mmHg steady mean pressure without transvascular pressure pulsations; Osc 40 group: vessels were subjected to a low mean pressure of 40 mmHg, and pulsation of 75% peak-peak amplitude, as in the Osc 80 group; St 40 group: arterioles were kept at 40 mmHg mean pressure and no pressure pulsations. These data were then further analyzed by ANOVA over six groups (fresh, four pressure profiles, and the Amlo group). These values were then averaged over the experiments. Paired and unpaired t-tests, one-way ANOVA, or repeated-measures ANOVA were used for statistical analysis (SPSS version 11.5.0; SPSS, Chicago, IL). EM pictures were analyzed using ImageJ software (National Institutes of Health). For each vessel, EC coverage was quantified from the combined ~20 images that covered the circumference of a single section. Approximately 10 evenly spaced images were then used for further quantitation. In these images, we determined thickness of EC, internal elastic lamina, and media, as well as the number of SMC layers and matrix content of the media, and averaged the results over these images to yield a single value of these quantities per vessel. These data were then further analyzed by ANOVA over six groups (fresh, four pressure profiles, and the Amlo group).

Significance was accepted for *P* < 0.05. All results are indicated as means ± SE.

**RESULTS**

Seven vessels were subjected to each of the four pressure profiles. The *d* max was 181 ± 16 (St 40), 172 ± 19 (Osc 40), 156 ± 9 (St 80), and 200 ± 11 (Osc 80) μm; these vessel calibers were not significantly different (*P* = not significant [NS], 1-way ANOVA).

Figure 1A shows the course of basal tone development during culture under the four pressure profiles. A significantly progressive constriction developed over the days in St 40 (*n* = 7, *P* < 0.01, repeated-measures ANOVA); Osc 40 (*n* = 7, *P* < 0.01), and St 80 (*n* = 6, *P* < 0.01). In contrast, less (not significantly progressive) constriction was present in the Osc 80 group (*n* = 6, *P* = NS). Thus diameter at *day 3* was 81 ± 14% of the initial passive diameter in the Osc 80 vessels vs. 49 ± 10% (St 80), 26 ± 3% (Osc 40), and 32 ± 9% (St 40).
Differences in active diameter were significant at days 2 and 3 (P < 0.05 and P < 0.005, respectively, 1-way ANOVA on each separate day). Some vessels had rhythmic (few cycles/min) vasomotion (1 in St 80, 4 in Osc 80, 2 in St 40, and 4 in Osc 40). Such vasomotion continued for 1–3 days. Slower transients were incidentally observed. We found no correlations between such dynamic behavior and remodeling.

At day 3 toward the end of the experiment, endothelial function was tested from the dilation to 10^{-6} M bradykinin. To compare the vessels, all groups were pressurized to a steady 80 mmHg during this intervention. Figure 1B plots the diameter, normalized to the final passive diameter at this operating pressure. As can be seen, vessels from all groups dilated to nearly maximal values. The dilation was significant for St 40 (n = 6, P < 0.005, paired t-tests), Osc 40 (n = 6, P < 0.001), and St 80 (n = 7, P < 0.02) but not for Osc 80 because of the already large initial diameter (n = 7, P = NS). Diameters in the presence of bradykinin were not significantly different between the four groups (1-way ANOVA).

Remodeling was assessed from the shift in passive pressure-diameter relations between start and end of culture. These data are depicted in Fig. 2. A substantial and significant inward remodeling was found in St 40 (n = 7, P < 0.001, repeated-measures ANOVA; Fig. 2A), Osc 40 (n = 7, P < 0.001; Fig. 2B), and St 80 (n = 6, P < 0.05; Fig. 2C) groups but not in the Osc 80 vessels (n = 6, P = NS; Fig. 2D). As an example, passive diameter on day 3 at an operating pressure of 80 mmHg was reduced to 81 ± 1% (St 40), 76 ± 3% (Osc 40), and 89 ± 5% (St 80). In contrast, in the Osc 80 group, diameter was 102 ± 4% of the day 0 value. When comparing the passive diameters at day 3 between groups, we found that Osc 80 vessels remained significantly larger than St 40 and Osc 40 vessels (P < 0.001, repeated-measures ANOVA on all groups at day 3). For vessels maintained at 40 mmHg, we tested whether subsequent culture for another 3 days at full vasodilation, induced by 10^{-4} M papaverine, would reverse the inward remodeling. As can be seen in Fig. 2, A and B (day 6), this was only partly the case. Thus, between days 3 and 6, passive diameter at 80 mmHg operating pressure increased from 81 ± 1 to 89 ± 1% (St 40, n = 6) and from 76 ± 3 to 82 ± 1% (Osc 40, n = 6) of the original day 0 diameter. The remaining inward remodeling at day 6 was still highly significant (P < 0.001 in both groups, repeated-measures ANOVA). Thus, once developed, inward remodeling was not readily reversible.

Figure 3 shows distensibility as calculated from the passive pressure-diameter relations (see METHODS). The inward remodeling in St 40, Osc 40, and St 80 was associated with a decrease in distensibility that was significant for all pressures in the tested range between 14 and 120 mmHg. In contrast, distensibility remained constant in Osc 80 (P = NS for all tested pressures).

Figure 4 depicts the change in CSAw after 3 days of culture. These data were calculated from the inner and outer diameter during culture. A slight, nonsignificant tendency for wall hypertrophy (defined here as an increase in CSAw, irrespective of the cellular or matrix base) was observed in the groups that showed inward remodeling (P = NS, t-tests vs. constant CSAw, n = 7). The change in CSAw was not different between groups (P = NS, 1-way ANOVA).

Experimental Group 2: Effect of Amlo on Tone and Remodeling

Eight vessels were cultured at 40 mmHg steady pressure in the continuous presence of 10^{-7} M Amlo in the superfusion medium. Six additional vessels were simultaneously cultured in the absence of the calcium blocker (St 40A). The d_{max} was 175 ± 13 μm (Amlo) vs. 159 ± 8 μm (St 40A). These calibers were not significantly different (P = NS, unpaired t-test). Figure 5 depicts the development of tone during culture. As was the case for experimental group 1, the St 40A vessels (n = 7, tone was not monitored in one set) developed a progressive tone. In contrast, Amlo vessels (n = 5) remained maximally

Fig. 1. A: basal tone development during 72 h in culture in vessels subjected to various pressure profiles (n = 6–7 in each group). Active diameter is normalized to the passive value at day 0; initial values less than unity are due to the result of onset of tone and elastic recoil of the 40 mmHg vessels. Osc 80 vessels, subjected to a mean pressure of 80 mmHg and pressure pulsations of 60 mmHg peak-peak amplitude with sinusoidal waveform and 1.5 Hz frequency; St 80, vessels kept at 80 mmHg steady mean pressure without transvascular pressure pulsations; Osc 40, vessels subjected to a low mean pressure of 40 mmHg, and pulsation of 75% peak-peak amplitude; St 40, arterioles kept at 40 mmHg mean pressure and no pressure pulsations. Osc 80 vessels had significantly larger diameters compared with the other groups at day 3.

B: bradykinin-induced dilation at day 3 of culture in vessels subjected to different pressure profiles. The diameter is normalized to the maximal response to papaverine recorded on the same day (n = 6–7 in each group). NS, not significant. *P < 0.05 and **P < 0.005.

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dilated. The difference in diameter during culture was significant at days 2 and 3 ($P < 0.01$, unpaired $t$-tests). In incidental cases, we established that Amlo vessels were still reactive to endothelin-1 after washout of the calcium blocker (data not shown).

Figure 6A depicts the remodeling of vessels cultured in the presence vs. absence of Amlo. Amlo prevented the inward remodeling. Rather, in Amlo-treated vessels, the maintained dilation was paralleled by a substantial outward remodeling. Thus, at day 3, the passive diameter at an operating pressure of...
80 mmHg was 115 ± 3% (Amlo, n = 8) vs. 78 ± 3% (St 40A, n = 6) of the original diameter. Both the outward remodeling in the Amlo group and the inward remodeling in the St 40A group were highly significant (repeated-measures ANOVA, $P < 0.001$ for both groups). Figure 6A indicates the associated changes in CSAw. As was the case for experimental group 1, a slight tendency for wall hypertrophy was observed that was not significant ($P = \text{NS}, t$-test against constant CSAw, n = 7 and 5).

**EM in Experimental Groups 1 and 2**

Figure 7 shows representative EM microphotographs of a freshly isolated vessel (top) and vessels cultured at 40 mmHg steady pressure without (middle) and with (bottom) Amlo. As can be seen, general morphology of the vessels was maintained during culture, with an intact endothelial coverage, and three to four layers of spindle-shaped smooth muscle cells (SMC). There were no signs of neointima formation or intimal thickening. Apoptosis was not observed. There were no signs of increased interstitial space. Table 1 summarizes histological data as quantitated from these images. There were no significant differences in any of these characteristics between the various conditions (ANOVA). Also when the inwardly remodeling (St 40, Osc 40, and St 80) and nonremodeling groups (Osc 80 and fresh) were pooled, these histological parameters remained similar ($P = \text{NS}, \text{ANOVA}$s). Thus, on the basis of these quantitative data, the vessels maintain a normal ultrastructure in culture and during remodeling.

**Experimental Group 3: Removal of Endothelial Cells**

We attempted to remove EC from eight vessels of four pigs. Initial constriction to $10^{-8}$ M endothelin was not affected: vessels constricted to a normalized diameter of 0.24 ± 0.08 (n = 8 vessels, without EC) vs. 0.25 ± 0.05 (n = 7 vessels with EC, $P = \text{NS}, t$-test). After endothelial rubbing, six of eight vessels still dilated to some extent to $10^{-6}$ M bradykinin, but the mean dilation was significantly impaired. Thus relative dilation by bradykinin during endothelin preconstriction (0 for no reaction and 1 for full dilation) averaged

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Fig. 4. Wall CSA did not change during culture under the various pressure profiles (n = 7 in each group). Shown is the ratio of wall area at days 3 and 0. This was not significantly different from unity for any of the groups (dashed line).

Fig. 5. Five arterioles cultured in the presence of amloipine remained fully dilated during culture. Seven control vessels developed progressive basal tone. w/wo, With/without. *$P < 0.05$.

Fig. 6. A: amlodipine (AMLO) treatment induced outward remodeling during 3 days in culture (n = 8), whereas control arterioles showed inward remodeling (n = 6). B: remodeling was not associated with a change in wall CSA (n = 5 without amloipine and n = 7 with amloipine). **$P < 0.001$.**
during endothelin at day 3 was 0.65 ± 0.13 (n = 8 without EC, 3 of these vessels were fully unresponsive) vs. 0.13 ± 0.02 (n = 7 with EC, P < 0.001). The three unreactive vessels also did not respond to a 10-fold higher (10⁻⁶ M) concentration of endothelin. Denuded vessels were unresponsive to 10⁻⁶ M bradykinin at day 3: relative dilation was −0.06 ± 0.02 (n = 5 without EC, excluding the 3 vessels that could not be tested because of lack of preconstriction) vs. 0.37 ± 0.13 (n = 7 with EC, P < 0.05). Altogether, vessels had unaltered endothelin responsiveness immediately after denudation but became unresponsive during culture. Any remaining reactivity to bradykinin also disappeared during culture.

Figure 9, A and B, shows EM pictures of two vessels denuded at day 0 and harvested at day 3. As expected, EC have disappeared. Moreover, the whole vessel wall became highly disorganized with substantial swelling of the interstitial space and presence of many small cells or cell fragments. This was not the case in vessels cultured with EC (Fig. 9C, also see Fig. 7). These functional and EM data suggest that, at least in the present setting, an intact endothelium is required for culture of porcine coronary vessels. These data preclude the drawing of conclusions on possible endothelium-dependent effects of Amlo.

**DISCUSSION**

This study demonstrates that the caliber of coronary arterioles is influenced by the transvascular pressure profile. Thus reduction of mean pressure or absence of pressure pulsations induced inward remodeling and a reduction of distensibility in the course of 3 days. Inward remodeling was associated with tone, whereas the calcium blocker amlodipine maintained vasodilation and also reverted the inward remodeling to outward remodeling. Vessels cultured without EC lost responsiveness after 3 days, whereas the wall of these vessels became disorganized.

**Limitations of the Current Study**

There are multiple ways to characterize remodeling. We chose to quantitate the change in passive inner diameter, which is the ultimate parameter of interest when considering consequences for myocardial perfusion. Because vessels are distensible, a full passive pressure-diameter relation was made for all vessels at the start and end of culture, and these relations were compared for each individual vessel. This in vitro approach, the ability to monitor individual vessels over time, and the paired comparison of pressure-diameter relations allowed for a very sensitive assessment of remodeling, detecting changes in vascular caliber as small as 1%.

Pharmacological tests at the start of culture were limited because it is not clear whether these would influence the subsequent behavior and remodeling of the vessels. Thus concentration-response curves were avoided but rather we used single doses of endothelin and bradykinin to test for SMC and endothelial reactivity. We therefore cannot draw conclusions on changes in sensitivity. It is quite likely that sensitivity to many agonists is up- or downregulated during culture, and substantial work needs to be done to be able to correlate culture conditions with changes in expression or activity of receptors and other elements of the cell signaling cascades.
We have not addressed myogenic responsiveness to step changes in pressure. Kuo et al. (9) observed very weak myogenic responsiveness to acute pressure steps in these subendocardial vessels. Therefore, addressing whether myogenic responses to step changes in pressure are maintained in culture could better be tested on other vessel types. In concordance with the weak myogenic response in acute experiments, the vessels in culture had smaller steady-state diameters at the lower pressures. In this respect, the coronary vessels behave differently from rat cremaster arterioles, which do maintain more constriction at higher pressure when kept in culture (3). We also did not test how acute changes in pulsation affect vasoconstriction. Such experiments were done before (6, 16) and revealed a small (~6–7%) dilation above 40 mmHg peak-peak pressure pulsations. Interestingly, maintaining pulsation of similar amplitude in our experiments (Osc 80 vs. St 80) led to a much larger (~65%) active diameter by day 3.

Table 1. Histological characteristics of porcine subendocardial vessels as determined from electron microscopy

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>EC Coverage, %</th>
<th>EC Thickness, µm</th>
<th>IEL Thickness, µm</th>
<th>Media Thickness, µm</th>
<th>IM Thickness, µm</th>
<th>Media Matrix, %</th>
<th>Media SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>St 40</td>
<td>4</td>
<td>100±0</td>
<td>1.97±0.43</td>
<td>1.03±0.22</td>
<td>7.5±1.1</td>
<td>10.5±1.8</td>
<td>12.3±2.1</td>
<td>3.21±0.17</td>
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<tr>
<td>Osc 40</td>
<td>2</td>
<td>100±0</td>
<td>1.87±0.24</td>
<td>0.62±0.02</td>
<td>4.2±0.3</td>
<td>6.7±0.5</td>
<td>9.2±3.8</td>
<td>2.35±0.26</td>
</tr>
<tr>
<td>St 80</td>
<td>3</td>
<td>100±0</td>
<td>1.84±0.04</td>
<td>0.58±0.01</td>
<td>4.4±0.4</td>
<td>6.8±0.5</td>
<td>21.4±11.3</td>
<td>2.46±0.13</td>
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<tr>
<td>Osc 80</td>
<td>3</td>
<td>93±7</td>
<td>1.49±0.39</td>
<td>0.65±0.17</td>
<td>3.6±1.1</td>
<td>5.7±1.5</td>
<td>11.2±3.0</td>
<td>2.52±0.22</td>
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<tr>
<td>Amlo</td>
<td>2</td>
<td>100±0</td>
<td>2.35±0.36</td>
<td>0.92±0.03</td>
<td>5.8±2.2</td>
<td>9.1±2.5</td>
<td>14.7±9.7</td>
<td>2.59±0.45</td>
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<td>Fresh</td>
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<td>89±9</td>
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<td>0.82±0.07</td>
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<td>10.2±0.6</td>
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<td>3.49±0.41</td>
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<tr>
<td>Inward</td>
<td>9</td>
<td>100±0</td>
<td>1.90±0.18</td>
<td>0.79±0.12</td>
<td>5.7±0.7</td>
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<td>14.7±3.8</td>
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<td>Nonremodeled</td>
<td>7</td>
<td>91±6</td>
<td>1.70±0.17</td>
<td>0.75±0.08</td>
<td>5.8±0.9</td>
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<td>10.7±1.5</td>
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Values are means ± SE; n, no. of vessels. Osc 80, vessels subjected to a mean pressure of 80 mmHg and pressure pulsations of 60 mmHg peak-peak amplitude with sinusoidal waveform and 1.5 Hz frequency; St 80, vessels kept at 80 mmHg steady mean pressure without transvascular pressure pulsations; Osc 40, vessels subjected to a low mean pressure of 40 mmHg and pulsation of 75% peak-peak amplitude; St 40, arterioles kept at 40 mmHg mean pressure and no pressure pulsations; Amlo, amloidipine; EC, endothelial cell; IEL, internal elastic lamina; media, the media excluding the IEL; IM, total intima-media thickness; media matrix, percentage of the media occupied by collagen fibers between the smooth muscle cells; n SMC, no. of smooth muscle cell layers. Indicated are results for 6 conditions, with pooled results for groups that showed inward remodeling (St 40, Osc 40 and St 80) vs. the nonremodeled vessels (Osc 80 and Fresh) in the bottom 2 rows. For each vessel, ~10 measurements were made of each aspect. There were no significant differences in any of these characteristics between the various conditions (ANOVA) and between inwardly remodeling and nonremodeling groups (unpaired t-tests).

Fig. 8. Responsiveness to endothelin at start and end of culture with and without EC. □, Control, with EC; ●, 3 × 10⁻⁷ M verapamil; ▲, 10⁻⁷ M amloidipine; ◯, EC removed before first test; ○, EC removed and cultured in 10⁻⁷ M amloidipine. Calcium blockers were added after the initial test and washed out before the test on day 3. Arrows and thicker lines indicate the three vessels processed for electron microscopy (EM; Fig. 9); these 3 vessels were all from the same animal.

Fig. 9. Transmission electron microscopy of vessels after 3 days of culture.
Thus pulsatility appears to be a very critical parameter in determining vascular tone when continuously applied for days.

We have not tested the effect of amlodipine under all pressure profiles. Rather, we used the profile causing the strongest inward remodeling, steady 40 mmHg, and demonstrate that the presence of amlodipine reverses this to substantial outward remodeling. We believe that amlodipine would reverse inward remodeling under the other conditions also. This is particularly the case for the steady 80 mmHg condition that induced remodeling. Here, amlodipine-induced maximal dilation in combination with the high pressure would maintain the vessels at their largest diameter during culture. Inward remodeling could then only develop by active diameter reduction by processes other than tone. We have never seen this in dilated and pressurized vessels. Rather, remodeling in this model resembles a fixation of the state of vasoconstriction.

Amlodipine may induce release of NO (18). We intended to address this possibility by using EC-denuded vessels, but these experiments only demonstrated the detrimental consequences of culturing vessels without EC. Using rat cremaster vessels, we previously showed that the calcium blocker verapamil also inhibited inward remodeling (4). Therefore, we tend to believe that the direct SMC effect is crucial, but it could be enhanced by inducing NO release indeed.

Rhythmic variations of pressure induce pulsation of vascular diameter and consequently a capacitive flow. One could therefore argue that the associated shear stresses affect vessel behavior. However, these shear stresses are very low. A numerical calculation for worst-case estimates of vascular dimensions and diameter excursions reveals capacitive shears in the order of 1 dyne/cm² at both ends of the vessel; for symmetric cannula resistances, shear in the middle of the vessel is always zero. We therefore do not believe that capacitive shear is involved in the current findings. In vivo, capacitive shear stress occurs in coronary vessels as a result of rhythmic compression by the beating heart. Such shear stress is much higher because diameter oscillations here act on many vascular segments in series and consequently induce much larger volume displacements.

**Coronary Microvascular Remodeling**

The observed remodeling was not associated with substantial changes in ultrastructure. Thus a quantitation of EM images revealed no differences in intimal or medial thickness or number of SMC layers. Neointima formation, apoptosis, or other overt changes in structure were not observed. Total wall CSA, including the adventitia, determined from the video images, also remained unchanged. Together, these findings characterize the remodeling process as a gradual reorganization of the available cells and matrix around a smaller lumen. The process is not readily reversible as judged from the effect of three extra days of papaverine incubation (Fig. 2). To understand such remodeling, it should be realized that the passive pressure-diameter relation depends not only on the amount of fibers such as elastin, collagen in the wall, and the elastic properties of these fibers, but also on the way these fibers are connected. The observation that wall CSA remains constant during remodeling indicates that the amount of fibers remains essentially the same but that the available wall material is reconnected or rearranged, either around a larger lumen (in the case of amlodipine) or around a smaller lumen. We can, however, not fully exclude that small changes in wall composition occur in such “eutrophic” (i.e., without a change in CSAw) remodeling.

The pressure-diameter relations before and after culture allow the estimation of distensibility. The results of Fig. 3 show that inward remodeling is associated with a reduced distensibility. Such stiffening may be related to increased cross-linking of the matrix. Indeed, we recently found that the cross-linking enzyme tissue-type transglutaminase is causally involved in inward remodeling under a variety of in vivo and in vitro conditions (2).

Eutrophic inward remodeling of resistance vessels occurs also in essential hypertension. It has been argued that such remodeling serves to reduce wall stress and that wall stress is a primary drive for inward remodeling (13). The current data, however, do not show this: wall stress is the highest in the Osc 80 group because of the higher pressure and the more dilated state during culture. Yet this group does not show inward remodeling. Rather than wall stress, the level of active tone correlated with inward remodeling. Thus the St 80 and amlodipine groups had roughly comparable wall tensions, but tone was present in only the St 80 vessels. In accordance, inward remodeling occurred in the St 80 group, whereas the vasodilated vessels in the amlodipine group developed outward remodeling. Also when vessels were compared at equal pressure profiles, remodeling correlated with tone rather than pressure. This was demonstrated by the second group, where amlodipine kept vessels vasodilated and reversed the inward remodeling to outward remodeling at identical pressure loads, i.e., a constant pressure of 40 mmHg. The correlation between tone and inward remodeling is in accordance with previous work from our laboratory on noncoronary vessels (4). Whether tone, the associated increase in SMC calcium, or some other parameter linked to tone is crucial for remodeling remains to be established.

It might be argued that EC deformation influences the behavior of the vessels to some extent, modulating the remodeling under the various interventions. However, we doubt that the occurrence and direction of remodeling depends on this: we previously compared rat cremaster vessels kept in culture at identical diameters, either through vasoconstriction or by lack of distention (pressure equal to a few mmHg). The latter vessels remained relaxed (4). In these experiments, endothelial deformation occurred to the same degree, but only the active vessels remodeled.

The results from the denuded vessels do not allow the drawing of conclusions on involvement of EC in differential inward remodeling between the groups. Rather, EC integrity appeared to be crucial for maintenance of wall structure and reactivity. Thus vessels were still responsive to endothelin immediately after denudation but had lost such reactivity after 3 days. Simultaneously, EM images revealed a highly disorganized media. It is not clear if other means of removing EC would have resulted in maintenance of reactivity. Direct mechanical removal has been found to be a good method in acute studies on small arteries in several laboratories. However, the long-term effect of such rubbing in vessels in culture has not been tested before.
Relevance for Coronary Artery Disease

We aimed to apply pressure profiles that approximate the local profiles in a healthy coronary bed in the presence of proximal coronary stenoses. The effect of stenosis clearly will depend on the stenosis degree, contractility of the heart, heart rate, and so on, making it impossible to give a generalized value for local pressure and pressure amplitude. Because of this uncertainty and variability, we have chosen to use three different profiles, with reduction of mean pressure and/or amplitude. Our choices were based on the following observations.

In the healthy heart, pressure in subendocardial vessels is lower than systemic because of resistance of the transmural vessels. The choice for 80 mmHg mean pressure was based on direct pressure recordings by Chilian (5). No data are available on the local microvascular pressure in the presence of mild or severe stenoses. However, during catheterization, pressure just distal to the stenosis was found to be 40–60 mmHg lower than systemic in stable angina (1, 15). Considering that local microvascular pressure will be still lower, 40 mmHg at this site seemed a reasonable choice to mimic stenoses.

Large transvascular pressure pulsations exist in the coronary circulation because of the contracting surrounding myocardium, resulting in volume changes and retrograde capacitive flows (7). These pulsations may also change in stenosis. Resistance of the stenosis and the compliance of the microvasculature together form a low-pass filter system that hinders the distal vasculature to retrogradely empty in systole. This would dampen the transvascular pressure pulsations. Indeed, subendocardial diameter pulsations were reduced in stenosis (10). Thus we believe that reducing mean pressure and/or pulsation amplitude approximates, at least in a qualitative sense, the changes in pressure profile distal to stenoses. In addition, the reduction in amplitude may also be relevant for myocardial hibernation and heart failure, where contractility is reduced.

The results show that the profiles St 40, Osc 40, and St 80, which all to some extent approximate the microvascular profile that could exist distal to stenosis, caused inward remodeling. In contrast, the Osc 80 profile, which more closely resembles that in a healthy coronary circulation, left vascular caliber unaltered.

Limited information is available on adaptation of the coronary microvascular structure distal to experimental stenoses. Mills and colleagues (11) found an increase in microvascular resistance distal to a coronary stenosis. Hong and coworkers (8) reported inward remodeling with intima hyperplasia of small intramyocardial coronary porcine arteries distal to a severe epicardial coronary artery stenosis. Saitoh and coworkers (14) found medial thickening of microvessels in a porcine model of large coronary endothelial injury. In contrast to these experimental models, patients with mild stenoses had a decreased dilated microvascular resistance, as determined using combined pressure-velocity wires during catheterization (17). This could possibly reflect compensating microvascular outward remodeling. Whether this relates to the use of calcium blockers by many of these patients is unclear. A detailed analysis of microvascular remodeling in coronary artery disease is still needed.

It is well appreciated that changes in local microvascular pressure form only one of the many aspects of coronary stenosis. Thus flow reserve clearly is also reduced, and microvessels distal to stenoses therefore lack periods of high shear stress. We recently showed that flow inhibits inward remodeling in these vessels in an NO-dependent manner (12), and further work should address the combined effects of flow reduction and pressure alteration in this model. Yet the current study directly shows severe consequences of pressure alterations on microvascular caliber, suggesting that pressure is a dominant factor. Vasodilator therapy reversed these structural changes to outward remodeling. Possibly, this adds to the beneficial effects of calcium blocker treatment. Full understanding of the causes and mechanisms of vascular remodeling and the possibilities to pharmacologically modulate these processes by vasodilators could provide new therapeutic options in coronary artery disease.

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