Flow inhibits inward remodeling in cannulated porcine small coronary arteries

Adrian Pistea, Erik N. T. P. Bakker, Jos A. E. Spaan, and Ed VanBavel

Department of Medical Physics, Academic Medical Center, University of Amsterdam, The Netherlands

Submitted 2 March 2005; accepted in final form 12 July 2005

Pistea, Adrian, Erik N. T. P. Bakker, Jos A. E. Spaan, and Ed VanBavel. Flow inhibits inward remodeling in cannulated porcine small coronary arteries. Am J Physiol Heart Circ Physiol 289: H2632–H2640, 2005. First published July 15, 2005; doi:10.1152/ajpheart.00205.2005.—The mechanisms of flow-induced vascular remodeling are poorly understood, especially in the coronary microcirculation. We hypothesized that application of flow in small coronary arteries in organoid culture would cause a nitric oxide (NO)-mediated dilation and inhibit inward remodeling. We developed an organoid culture setup to drive a flow through cannulated arterioles at constant luminal pressure via a pressure gradient between the pipettes. Subepicardial porcine coronary arterioles with diameter at full dilatation and 60 mmHg (D0) of 168 ± 10 (SE) μm were cannulated. Vessels treated with Nω-nitro-arginine (L-NNA) to block NO production and untreated vessels were pressurized at 60 mmHg for 3 days with and without flow. Endothelium-dependent dilation to 10−6 M bradykinin was preserved in all groups. Tone was significantly less in vessels cultured under flow conditions in the last half of the culture period. Untreated and L-NNA-treated vessels regulated their diameter to yield shear stresses of 10.3 ± 2.1 and 14.0 ± 2.4 (SE) dyn/cm², respectively (not significantly different). Without L-NNA, passive pressure-diameter curves at the end of the culture period revealed inward remodeling in the control group [to 92.3 ± 1.3% of D0 (SE)] and no remodeling in the vessels cultured under flow conditions (100.2 ± 1.3% of D0); with L-NNA, the group subject to flow showed inward remodeling (92.1 ± 2.5% of D0). We conclude that pressurized coronary resistance arteries could be maintained in culture for several days with flow. Vessels cultured under flow conditions remained more dilated when NO synthesis was blocked. Inward remodeling occurred in vessels cultured under no-flow conditions and was inhibited by flow-dependent NO synthesis.

Adequate coronary perfusion depends on healthy main coronary arteries and sufficient caliber and good function of the coronary microvascular bed. After the advent of modern transcatheter and endovascular treatment of coronary stenosis, it became evident that small–resistance arteries also play a role in the pathophysiology of ischemic heart disease (17). Although much attention is given to endothelial dysfunction in the coronary microcirculation, very few studies directly address structural alterations that might occur as a consequence of such dysfunction. A recent clinical study indicated that, in the presence of an intermediate stenosis, hyperemic microvascular resistance is reduced compared with that in a reference vessel (38). This finding suggests that outward remodeling of resistance vessels was an adaptation to reduced coronary perfusion pressure; however, alternative interpretations are possible, and direct observations of coronary microvascular remodeling in mild stenosis are lacking. In contrast to these findings, in an experimental study with severe stenosis induced by arterial ligation, Hong et al. (19) reported inward hypertrophic remodeling of small arteries. Such remodeling with severe stenosis could be caused by paracrine signaling from the ischemic and failing myocardium or by an altered mechanical regimen, including reduced shear stress. Indeed, it has been established in vivo in several noncoronary beds that experimental flow reduction leads to inward remodeling (6, 25, 26).

Because it is difficult to observe the behavior of individual small coronary arteries in situ and to monitor local hemodynamic conditions in vivo, there is no direct evidence that flow has an effect on coronary microvascular caliber. An alternative approach is to mimic these hemodynamic conditions in isolated resistance vessels and follow the process of remodeling over days. The effect of flow on tone in isolated coronary arterioles has been studied in acute experiments (22, 23). However, the long-term effects of flow on coronary microvascular structure and remodeling have not been studied in isolated vessels.

We have developed an organoid culture system that allows us to preserve and study small cannulated resistance arteries under well-defined mechanical loading regimens. We recently observed a rapid, eutrophic inward remodeling of porcine coronary resistance vessels cultured at constant pressure in the absence of flow (1). In the present study, we extended this technology by applying luminal flow to the cultured vessels and studying the long-term behavior of tone in healthy vessels and vessels with endothelial dysfunction mimicked by the nitric oxide (NO) synthase (NOS) inhibitor Nω-nitro-arginine (L-NNA). Vessels under flow adapted their diameter to obtain physiological mean shear stresses (37) during culture in the absence and presence of L-NNA. However, flow inhibited inward remodeling by NO-dependent mechanisms.

Materials and Methods

Organoid culture. Hearts were obtained from female Yorkshire farm pigs (20–25 kg) as previously described (1). Experiments were approved by the local committee on animal experimentation of the Academic Medical Center. Small subepicardial arteries [168 ± 10 (SE) μm] were excised from the left ventricle and mounted on pipettes in an organoid culture setup as described previously (1). Cannulated arteries were kept for 3 days at 37°C in Leibovitz culture medium containing ciprofloxacin (20 μg/ml; Ciproxin 100, Bayer, Mijdrecht, The Netherlands) and a combination of penicillin G sodium (100 U/ml), streptomycin sulfate (100 μg/ml), and amphotericin B (0.25 μg/ml; Antibiotic-Antimycotic, Gibco, Paisley, UK). Bovine serum albumin (1 g/100 ml) was added to the perfusion fluid. Vessels were cultured under no-flow (shear stress <1 dyn/cm²) and flow (generated by a pressure gradient; Fig. 1) conditions. In a second series of experiments, the NOS inhibitor L-NNA (10−4 M) was added...
to the culture medium in the perfusion and incubation solution. The incubation fluid was refreshed daily.

Work protocol. On day 0, vessels were mounted on cannulas, pressurized at 60 mmHg, carefully checked for leaks, and tested for smooth muscle cell (SMC) function (contraction to 10^{-5} M endothelin) and endothelium-dependent dilation to 10^{-7} M bradykinin in the continuous presence of endothelin. Failure to respond to endothelin or bradykinin, indicating possible damage during dissection and cannulation, occurred in four vessels, which were discarded. After both drugs were washed out for 20 min, papaverine (5 × 10^{-5} M) was added to achieve full passive dilation. Pressure was lowered to minimum (10 mmHg) and, after stable dilation was achieved, increased from 10 to 120 mmHg in 10-mmHg steps. Inner diameter was recorded at the end of each pressure step. After this passive pressure-diameter relation was recorded, pressure was returned to 60 mmHg and the incubation fluid was refreshed. All vessels recovered myogenic tone. A 10-mmHg pressure gradient between the reservoirs of the flow setup was used to start flow. No flow was present in the control setup.

Endothelin was used only at the start of the experiment. Because the vessels developed basal tone, endothelium-dependent dilation to bradykinin was tested on days 1–3 without further preconstriction. At 20 min before the bradykinin test, flow was stopped; after bradykinin was washed out for 20 min and the incubation fluid was refreshed, flow was resumed. On day 3, after dilation to bradykinin was assessed, papaverine was added, and the passive pressure-diameter relation was again recorded. When bradykinin, endothelin, or papaverine was applied, incubation was stopped and the drug was administered directly to the 2-ml vessel chamber. Washout was accomplished by restarting incubation with fresh medium and discarding the first 40 ml of outflow before recirculation was established. During culture, the incubation fluid reservoir contained ≥20 ml of culture medium.

Flow and shear stress calculation. Intravascular fluid was recirculated via a roller pump triggered by a level sensor (Fig. 1). Each “on” period of the pump resulted in pumping 12.7 μl of fluid from the downstream to the upstream reservoir. Dividing this volume by the period of time between two consecutive “on” periods of the pump yielded the mean value of flow in that interval, with a time resolution of ∼2 min. This method is theoretically sensitive to possible small leaks in the vessel and evaporation from the downstream reservoir. On the basis of the observation that upstream reservoir volume did not change over the course of 3 days within a resolution of 1 ml, leakage and evaporation were estimated to affect the flow signal by <5%. Shear stress (τ) was computed using the calculated value of flow (Q) and the recorded value of inner diameter (d) according to the following formula

$$\tau = \frac{32\dot{Q}\eta}{\pi d^3}$$

where η is viscosity (0.001 Ns/m²). Flow and shear stress were computed offline after the experiment was completed.

Data recording. Inner diameter was continuously monitored through an inverted microscope with attached video camera and recorded (sample frequency = 2 Hz) using self-constructed software. Pressure and pump activity signals were also continuously recorded at 2 Hz.

Electron microscopy. After the experiment, some vessels were fixed at 60 mmHg and during full dilation in McDowell’s fixative (29) and refrigerated until further postfixation with 1% osmium tetroxide (Electron Microscopy Sciences) and 1% lanthanum nitrate (Sigma) in water for 1 h at room temperature followed by 1% aqueous uranyl acetate for 1 h. Vessels were then embedded in plastic and cut for transmission electron microscopy.

Data analysis. Values are means ± SE. One-way ANOVA was used to assess differences between groups. If only two groups were compared, an unpaired Student’s t-test was used. If repeated measurements were performed within groups, statistical significance was assessed using the general linear model for repeated measurements (GLM-RM) with SPSS version 12.0.1 for Windows.

RESULTS

Vascular function. We cultured 34 cannulated vessels, of which 3 were discarded (because of failure to maintain tone or for technical reasons). Maximal inner diameters at day 0 and 60 mmHg were not significantly different between groups (P = 0.445 by 1-way ANOVA): 171 ± 27 (SE) μm (no flow, no
Most of the vessels (84%, 26 of 31) developed rhythmic vasomotion (Fig. 4, A–D). Vasomotion was not always regular; when a clear regular pattern could be recorded, we analyzed frequency of vasomotion (Fig. 4E). Vasomotion frequency was significantly lowered by L-NNa (P = 0.025 by GLM-RM), whereas flow had no significant effect (P = 0.099 by GLM-RM).

The endothelium-dependent response to bradykinin was preserved during the culture period and was >80% in all groups (Fig. 5). Amplitude of the dilatory response to bradykinin was not significantly dependent on day of culture, presence of flow during culture, and presence of L-NNa (P = 0.747 by 3-way ANOVA). However, there was a marked difference in the dynamics of the dilation, demonstrating the effectiveness of L-NNa in the present setting. The duration of the dilatory response to bradykinin was much longer in the absence of L-NNa. To quantify this, we measured the response at the end of 3 days of culture, i.e., the time needed for spontaneous return to the active diameter corresponding to the half-maximal dilation \([t_{D0.5}]\) during 5 min of continuous exposure to bradykinin. In the absence of L-NNa, 10 of 17 vessels had not returned to active diameter corresponding to half-maximal dilation \((D_{0.5})\) after 5 min \([t_{D0.5}] = 227.1 \pm 24.8\) (SE) s, \(n = 17\), data pooled for flow and no-flow vessels. In contrast, in the presence of L-NNa, all vessels returned to \(D_{0.5}\) within 2 min \([t_{D0.5}] = 41.4 \pm 7.9\) s, \(n = 14\), which is significantly faster than in the absence of L-NNa (\(P < 0.0001\), unpaired t-test).

Shear stress depends on diameter and flow, the latter resulting from the imposed pressure gradient, cannula resistances, and vessel resistance. The theoretical relation between diameter and stress for a given pressure gradient (\(\Delta P\)) is given by

\[
\tau = \frac{32\Delta P \eta d}{\pi d^2 R_p + 128\eta L}
\]

where \(\eta\) is fluid viscosity, \(R_p\) is the sum of cannula resistances, and \(L\) is length of the vessel. This relation is based on Poiseuille’s resistance of the cannulas and vessel; the loss of kinetic energy density when fluid leaves the cannula tip is on the order of 0.01 mmHg and, therefore, is negligible. Figure 6 shows this relation for one vessel. In this setting, shear stress would be small for a highly narrowed vessel, where flow is strongly reduced because of vessel resistance, or for a very large vessel, where flow is limited by the cannula resistances. For each experiment, a characteristic theoretical peak shear...
stress \((\tau_{\text{peak}})\) and associated vessel diameter \((d_{\text{peak}})\) are found. In this example, the vessel operated at diameters larger than \(d_{\text{peak}}\) \((d_{\text{peak}} = 40 \mu m\) and \(\tau_{\text{peak}} = 100 \text{ dyn/cm}^2\)) resulting in a reduction of shear stress on widening. This vessel settled at a shear stress of 15.5 dyn/cm\(^2\) by the end of the experiment.

For the flow vessels, the applied pressure gradients and cannula resistances \([458 \pm 16\) \((\text{SE})\) mmHg ml\(^{-1}\) min\(^{-1}\)\] resulted in \(\tau_{\text{peak}}\) of 82 ± 14 dyn/cm\(^2\) (without l-NNa) and 72 ± 4 dyn/cm\(^2\) (with l-NNa, \(P = 0.517\)) at \(d_{\text{peak}}\) of 31 ± 2 \(\mu m\) (without l-NNa) and 34 ± 1 \(\mu m\) (with l-NNa). Vessels operated at diameters below or above \(d_{\text{peak}}\), but all settled at diameters above \(d_{\text{peak}}\) by day 3 \((3.1 \pm 0.3 \times d_{\text{peak}}\) without l-NNa and 2.8 ± 0.2 \(\times d_{\text{peak}}\) with l-NNa, \(P = 0.424\) by unpaired \(t\)-test). Actual shear stress values were very irregular on day 1 but reached a remarkably stable level in the last day of culture (Fig. 6B), which was not significantly different in the presence and absence of l-NNa: 14.0 ± 2.4 and 10.3 ± 2.1 dyn/cm\(^2\), respectively. These relatively constant shear stress values were obtained despite substantial differences in flow between individual experiments.

Vascular structure and remodeling. Figure 7 shows the passive pressure-diameter relations at the beginning and end of the 3 days of culture. Inward remodeling, i.e., a downward shift in these relations, was observed in the no-flow vessels, regardless of the presence or absence of l-NNa. Thus, on day 3, diameter at maximum dilation at 60 mmHg was 92.3 ± 1.3 and 94.2 ± 1.2% of \(D_0\) without with l-NNa, respectively. In the presence of flow but in the absence of l-NNa, inward remodeling was inhibited \((\text{day 3 diameter was 100.2 ± 1.3}\% \text{ of } D_0)\). In the presence of l-NNa, inward remodeling occurred also in the vessels with flow, with day 3 diameter reaching 92.1 ± 2.5% of \(D_0\) (Fig. 6). In all groups, wall cross-sectional area did not vary significantly (data not shown); thus, if present, inward remodeling was eutrophic.

To obtain a qualitative impression of the ultrastructure of the vessels, six vessels were processed for electron microscopy. Vascular smooth muscle phenotype was well preserved during culture, as indicated by electron microscopy (Fig. 8). Thus SMC remained elongated. The majority of SMC demonstrated contractile elements. No signs of replication or apoptosis were observed. The number of SMC layers was on the order of three to four. Although we did not extensively quantify ultrastructure, no obvious difference in the number of layers was observed between the groups. No signs of neointima formation were observed, whereas endothelial coverage was preserved in all groups.

**DISCUSSION**

This is the first study to assess in vitro remodeling of small arteries cultured in the presence of flow. The main finding of this study is that cannulated small coronary arteries in organoid culture were able to regulate shear stress when subjected to a
constant pressure gradient. This was not affected by blockade of endothelial NOS (eNOS). However, in the absence of eNOS blockade, shear stress was regulated by tone only, whereas in the presence of eNOS blockade, inward remodeling also occurred. In vessels without flow, inward remodeling occurred irrespective of the presence of L-NNA.

Critique of the method. Because it is difficult to access small coronary arteries in vivo, the organoid culture setup is very useful for study of the long-term behavior of these vessels in a well-defined and well-controlled environment. We considered using a pump, rather than a pressure gradient, to drive the flow through the vessels. However, this method has two concerns: 1) in terms of practicality, we found it very difficult to maintain vessels at forced flows; occasional moments of deep constriction resulted in very high pressures, which were needed to maintain flow, causing damage and even dissociation of the vessel from the cannulas; and 2) from a physiological point of view, in a flow-driven system, any vasoconstriction would lead to an increase in shear stress, and deep tone would lead to extreme shear stress. Our organoid culture method allows the vessels to determine their own shear stress: on constriction, shear stress would first increase and then decrease when vessel resistance becomes significant (Fig. 6). These events resemble the mechanical load experienced by a single vessel within a vascular network (16). A disadvantage of the present approach is that flow becomes variable. Interestingly, the vessels adapted tone and structure to yield a shear stress with much less variation between experiments than the variation in flow. Thus, although the flow variation was not intended, it helped us observe the ability of the vessels to regulate shear stress.

The highest possible shear stress in the setup ($\tau_{\text{max}}$) varied slightly for the individual vessels because of vessel length, cannula resistances, and applied pressure gradients. However, $\tau_{\text{max}}$ was always >40 dyn/cm² and was above reported in vivo values for vessels of this diameter (37). Thus, in the present

Fig. 6. Shear stress. A: shear stress and diameter recorded in 1 experiment plotted with theoretical values of shear stress as a function of diameter (formula 2). At this constant pressure gradient and cannula resistance, the vessel can achieve any value of shear stress between 0 and maximum shear stress by active constriction and dilation. Shear stress on day 3 varied slightly at –15.5 dyn/cm². B: shear stress values were highly irregular on day 1 and then settled between 10 and 20 dyn/cm².

Fig. 7. Passive pressure-diameter curves on days 0 and 3. A significant downward shift (inward remodeling) occurred in all groups, except “with flow no L-NNA.”
Fig. 8. Electron microscopy. Vessels were fixed at 60 mmHg on day 0 (A and B) and day 3 (C–J) at low (A, C, E, G, and I; scale bar, 10 μm) and high (B, D, F, H, and J; scale bar, 2 μm) magnification. C and D: vessel cultured without flow and without L-NNA. E and F: vessel cultured under flow, without L-NNA. G and H: vessel cultured without flow, with L-NNA. I and J: vessel cultured with flow and with L-NNA. L, lumen; EC, endothelial cell; SMC, smooth muscle cell; Fbl, fibroblast; IEL, internal elastic lamina; Col, collagen fibers; EJ, endothelial junction; Adv, adventitia; Med, media; d, inner diameter measured at fixation by video microscopy. There was no difference in major structures of the vessel wall between day 0 and day 3: smooth muscle cells preserved their contractile phenotype and endothelial coverage was preserved. Some discontinuities in the endothelium were observed “with flow with L-NNA.”
providing a better block. Our best evidence for effectiveness of concentrations are closer intracellularly than in the medium, with completely, block eNOS activity. The concentrations available inside the cell are influenced by the limited rate of the cationic amino acid transporter (CAT1) and the relative degradation rates of L-arginine and the blocker. Because L-arginine is eventually converted but L-NNA is not, we speculate that the concentrations are closer intracellularly than in the medium, providing a better block. Our best evidence for effectiveness of L-NNA is, in the long term, the clear effect on inward remodeling of flow vessels and, in the short term, the altered dynamics of the response to bradykinin. Endothelium-dependent dilation induced by many pharmacological stimuli, including bradykinin, is believed to be a combination of a fast-acting endothelium-derived hyperpolarizing factor effect and a slower NO-dependent effect secondary to eNOS activation (3, 8, 10). Indeed, we found a markedly more transient response to bradykinin in the presence of L-NNA.

In developing the culture system, we have established that papaverine alone at $5 \times 10^{-5}$ M resulted in the same amount of dilation as papaverine in a calcium-free solution, and on no occasion were vessels observed to dilate beyond the level induced by this concentration of papaverine. Thus we are confident that the diameter in the presence of papaverine reflects the fully dilated state. We did not wish to use calcium-free solutions because of their effect on vessel viability and, possibly, ultrastructure.

We have used electron microscopy to demonstrate intact ultrastructure of the cultured vessels. Indeed, good endothelial coverage was present, no neointima formation was observed, and contractile phenotype of SMC was preserved. The nuclei of SMC lacked the invaginations observed by Wamhoff et al. (39) in larger coronary arteries. One could suspect that this reflects a fundamental difference between large and small arteries, with consequences for function. However, the presence of these clefts in nuclei of large vessels seems heterogeneous (9), whereas these features have also been reported in small vessels (12, 34). On the basis of the study of sleek and Duling (34) on small arteries, we suspect that invaginations in the nuclei are caused by the change in geometry when arteries constrict. Absence of nuclear clefts in our study could therefore be caused by fixation at full dilation and under pressure. More work is needed to establish whether such invaginations depend on the state of contraction and whether function is affected, e.g., by the effect on calcium handling. In any case, there seems to be no evidence for a direct role in the remodeling process.

A limitation of this study is that arterial wall morphology, including area of intima, media, and adventitia, and composition of the wall, including cellularity and matrix content, were not quantified. Thus, although electron microscopy revealed no gross changes in cellularity or matrix content, we have not quantified these properties by extensive morphometry on electron- or light-microscopy sections. We cannot therefore rule out that modest changes might have occurred; future detailed analysis is required, including determination of intima, media, and adventitia area. Yet, on the basis of our video-microscopy measurements of inner and outer diameter, inward remodeling occurred without a change in total wall cross-sectional area. This pattern of remodeling was previously found in this model (2, 5) and is also seen in essential hypertension (31). Such eutrophic inward remodeling reflects a reorganization of the material around a smaller luminal diameter that is especially apparent at full vasodilation. This is possible because the passive diameter of a vessel depends not only on the composition of the wall (e.g., amount or percentage of collagen), but also on the way this material is organized by, for example, cross-links. We recently identified a role for the cross-linking activity of transglutaminases in vivo and in vitro inward remodeling in various small vessels, including porcine small coronary arteries (1). Further steps are needed to assess whether suppression of inward remodeling by flow in the present study involves an inhibition of transglutaminase activity. In any case, the eutrophic remodeling in resistance vessels is clearly different from the remodeling processes in large vessels, where cellular proliferation and matrix synthesis are documented under many conditions.

Interpretation of results. Our in vitro approach shows that inward remodeling in the absence of flow or after blockade of eNOS can occur in the absence of blood-borne cells or chemokines and that inhibition of inward remodeling by a relatively low level of shear stress does not require these blood-derived elements. We did not test whether it is possible to induce outward remodeling by very high levels of shear stress. At least in the case of arteriogenesis, a form of very marked flow-induced outward remodeling, participation of monocyte recruitment in the remodeling response is well described (33). We previously observed outward remodeling after long-term vasodilator therapy in cultured porcine coronary vessels (35). This indicates that such remodeling does not require blood-borne elements.

The vessels achieved relatively constant levels of shear stress toward the end of the experiment. Because applied flows were quite variable, these results suggest that shear stress is the driving force for diameter adjustments and that shear stress is regulated. However, we did not systematically vary flow in individual vessels to test whether shear stress remains constant despite variations in flow. The final levels of shear stress in the presence of L-NNA were not significantly different from those in the absence of L-NNA. There was a tendency toward higher shear stress in the presence of L-NNA, and this difference may have become statistically significant for larger sample sizes. It seems likely that NO plays a role in shear stress regulation, but there are other compensating or additional mechanisms for regulating shear stress in conditions of NO deficiency. In coronary vessels, production of hydrogen peroxide (21, 27), prostaglandins (7), and P-450 metabolites (30) in response to shear has been reported. The best evidence for additional mechanisms from our experiments is that shear stress was regulated by tone only in the absence of L-NNA, but by a combination of inward remodeling and tone in the presence of L-NNA. Moreover, the endothelium-dependent vasodilatory response to bradykinin was present with or without L-NNA, consistent with the findings of Hein et al. (18). Resolution of the nature of such additional mechanisms in the present setting requires future experimentation. In any case, the results demonstrate that inward remodeling can occur in the presence of a
healthy endothelium, because bradykinin-induced dilation remained maximal also in the vessels that remodeled and because the ultrastructure remained intact. We previously showed that inward remodeling can be prevented by sustained application of vasodilators other than flow (35). However, the influence of flow is continuously present, and it is important to realize that the NO-dependent component of flow is a major determinant of vascular structure.

In vivo experiments show that flow is a major determinant of vascular caliber. Thus, in microvascular networks, vascular diameter and flow are often related by approximately a third-power relation, indicating that shear stress dominates the size of individual segments within such networks (16). Remodeling induced by flow interventions is directly demonstrated by in vivo experiments where flow is reduced or increased in resistance or conductance vessels by sutures or artificial arteriovenous fistulas (25).

Full studies of long-term effects of flow on tone and structure in isolated vessels have not been published previously. The present results show that NO appears not to play a significant role in the level of tone at the applied shear stress of \( \sim 15 \text{ dyn/cm}^2 \). Interestingly, NO does play a role in inhibition of inward remodeling by flow, because vessels in the presence, but not the absence, of L-NAA demonstrated inward remodeling in the presence of flow. These observations are supported by preliminary data from Looft-Wilson et al. (28) showing that L-NAA does not affect long-lasting vascular tone but does affect inward remodeling. Thus the involvement of NO in flow-induced dilation and inhibition of inward remodeling diverge. It was not our purpose to elucidate the cellular events associated with NO-induced suppression of inward remodeling. However, we recently showed that transglutaminases play a key role in inward remodeling of coronary vessels (1). These enzymes are inhibited by direct nitrosylation by NO (24). Whether this mechanism is involved in the present experiments remains to be determined.

Vessels cultured in the presence of flow maintained lower levels of tone during the last days of culture (Fig. 3). However, at the start of the experiments, the onset of flow did not immediately cause dilation, as was expected on the basis of studies by Sorop et al. (36) and Kuo et al. (23). Diameter was larger in the vessels with flow than in the no-flow vessels only after the first night. Also, when flow was stopped on the following days, no vasoconstriction was observed for \( \geq 20 \) min. This could be due to the rather low level of applied shear. Indeed, in one vessel, initial shear to 30 dyn/cm\(^2\) did cause vasodilation. Perhaps, in the present study, initial tests before the onset of flow changed the sensitivity to shear. These tests included the use of 1) endothelin, to test for reactivity and preconstrict the vessel for testing bradykinin sensitivity, and 2) papaverine, to determine the initial passive pressure-diameter relation. The use of endothelin, in particular, might have interfered with flow-induced dilation (32). Although endothelin was applied for only 15 min on day 0 and then washed out, its effects could have been long lasting (11). It remains to be established why vessels in this study, although they have less tone when cultured under flow, respond so unexpectedly to acute changes in flow at the same shear stresses and whether the use of endothelin at the beginning of the experiment plays a role.

Rhythmic vasomotion occurred in 26 of the 31 vessels. The five vessels in which vasomotion did not occur were incubated in the absence of L-NAA. These five vessels had remarkably deep tone. Frequency of vasomotion was lower in the presence of L-NAA. This effect of L-NAA shows that the endothelial cells are involved in modulating vasomotion frequency, even though the oscillator is believed to be intrinsic to the SMC (13–15). Although we previously showed that deep tone is a major drive for inward remodeling (2, 5), we also observed that the duration of vasomotion is positively correlated with inward remodeling in rat cremaster arterioles (4). Similarly, in the present study, some positive correlation was found (data not shown). The present study does not allow resolution of the relative influence of tone and vasomotion on remodeling under the various conditions. Mechanistically, the high-calcium oscillations during vasomotion might activate enzyme systems, such as transglutaminases, which are reported to be involved in inward remodeling (1).

The amount of inward remodeling observed in the no-flow vessels was less than that reported by Bakker et al. (2) in rat skeletal muscle. This might be explained by the milder pre remodeling regimen. Because fetal calf serum has been reported to induce a strong remodeling response, we used bovine serum albumin in the perfusion fluid. Bovine serum albumin minimized the number of unknown chemical stimuli in the system. Endothelial reactivity of these porcine vessels remained adequate in the absence of the serum, whereas the serum was required by the rat vessels for maintenance if proper reactivity (2).

Microvascular remodeling in coronary artery disease. Coronary artery disease comprises not only proximal stenosis but possibly also impaired function of the microvascular bed. Structural changes in the microvascular bed in coronary artery disease are very poorly defined. Using pressure and velocity measurements during catheterization, Verhoeven et al. (38) quantified minimal coronary microvascular resistance in patients with moderately severe stenosis, characterized by normal autoregulation at rest. These authors observed a decrease in microvascular resistance distal to the stenosis compared with healthy perfusion areas. These results suggest that stenosis has induced a compensating outward remodeling in the microcirculation. However, alternative interpretations are possible, and direct observations of coronary microvascular remodeling in mild stenosis are lacking.

In contrast, direct observations of the coronary microvascular bed in experimental severe stenosis reveal inward remodeling (19). The present study shows that in healthy vessels a relatively modest level of shear is sufficient to prevent inward remodeling, suggesting that such remodeling could occur only in severe stenosis. However, in microvascular endothelial dysfunction or NO deficiency, simulated in the present study by L-NAA, inward remodeling could occur in the presence of shear. A possible mechanism for inward remodeling under NO deficiency is matrix cross-linking by the tissue-type transglutaminase (1). This enzyme is directly inhibited by low concentrations of NO through nitrosylation (24). Further work is required to quantify structural changes of the coronary microvasculature under various degrees of stenosis and endothelial dysfunction and to further assess the role of structural adaptations to shear stress.
FLOW INHIBITS INWARD REMODELING IN SMALL CORONARY ARTERIES

ACKNOWLEDGMENTS

We appreciate the expertise of Titia Rolf in performing histology and electron microscopy. We are grateful to Sjak Verschuren for his technical contribution in building the experimental setup.

GRANTS

E. N. T. P. Bakker was supported by The Netherlands Heart Foundation Grant NHS2001D038.