Repetitive coronary artery occlusions induce release of growth factors into the myocardial interstitium

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Weihrauch, Dorothee, John Tessmer, David C. Warltier, and William M. Chilian. Repetitive coronary artery occlusions induce release of growth factors into the myocardial interstitium. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H969–H976, 1998.—Our objective was to delineate the temporal sequence of mitogenic activity in myocardial interstitial fluid (IF) during enhancement of collateral growth. Collateral development in chronically instrumented dogs was induced by eight 2-min coronary occlusions/day for 21 days. Collateralization was assessed by measurement of blood flow in the region distal to a total coronary occlusion. Myocardial IF was obtained periodically from an intramyocardial catheter, and mitogenic activity was assessed by proliferative response of cultured endothelial cells (EC) and vascular smooth muscle cells (VSMC) to the IF. Three experiments were conducted to test that the mitogenic activity is induced by protein growth factors: 1) protein digestion of the myocardial IF with Pronase-coupled latex beads; 2) heat inactivation (boiling) of the IF; and 3) neutralization of the mitogenic activity with antibodies for basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). Blood flow was reconstituted to baseline levels during occlusion after 3 wk of repetitive coronary occlusions. After initiation of occlusion the mitogenic activity of the myocardial IF on VSMC and EC increased up to days 12–14 and was reduced on days 19–23. Pronase treatment and heat inactivation blocked the mitogenic effect. Treatment with antibodies for bFGF and VEGF neutralized the proliferative response to the myocardial IF at specific times. bFGF antibody inhibited the mitogenic effect significantly on days 12–14. VEGF antibody neutralized the mitogenicity of the myocardial IF on day 7, days 12 and 13, and days 19 and 20 significantly. We conclude that myocardial IF harvested from ischemic myocardium is highly mitogenic up to 2 wk after initiation of repetitive coronary occlusions. After 3 wk of ischemia, the degree of mitogenic activity for VSMC and EC was decreased from peak levels. The antibodies could not neutralize the mitogenic effect of the myocardial IF during this time period. These results suggest that mitogens are expressed during various stages of collateral development in a time-dependent manner, that the mitogens are proteinaceous in nature, and that bFGF and VEGF are released into the myocardial IF.

myocardial ischemia; coronary collateral circulation; angiogenesis

THE CORONARY collateral circulation formed by interarterial anastomoses can provide delivery of perfusion to myocardium at risk after coronary artery occlusion. The native coronary collateral circulation is usually poorly developed and cannot provide adequate blood flow to sustain aerobic metabolism and normal contractile function. However, collateral vessels have the capacity to markedly expand their caliber and thus enhance conductance and deliver blood flow. Development of the collateral circulation has the potential to significantly ameliorate the pathological sequelae of coronary artery disease. Demonstrable collateral vessels have been found to limit electrophysiological changes, contractile dysfunction, and the extent of infarction in patients with coronary artery disease (14). There remains a paucity of information concerning the factors underlying the growth and maturation of coronary collateral vessels despite these apparent benefits.

Coronary collateralization involves both prominent expansion of preexisting vessels and neovascularization, and thus this process must involve vascular smooth muscle and endothelial cell mitosis (20). We hypothesized that the formation and/or release of mitogens is seminal to collateral development. Our goal was to delineate the temporal sequence of mitogenic activity in myocardial interstitial fluid during enhancement of collateral growth. We also proposed that the degree of mitogenic activity would be correlated with coronary collateralization assessed by measurement of myocardial blood flow in the region distal to a total coronary occlusion. A brief, repetitive coronary artery occlusion model was used in the present investigation for induction of collateral development in conscious, chronically instrumented dogs. This technique provides the advantage of careful monitoring of the time course of collateral angiogenesis as well as the release of endogenous growth factors into the interstitium.

MATERIALS AND METHODS

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. Furthermore, all conformed to the "Guiding Principles in the Care and Use of Animals" of the American Physiological Society and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

General Preparation

Mongrel dogs (n = 8 experimental and 5 sham) of either sex weighing 27 ± 1 (mean ± SE) kg were fasted overnight. Anesthesia was induced with propofol (50 mg/kg iv) and maintained with isoflurane (1.5–2.0%) in 100% oxygen via positive-pressure ventilation. A left thoracotomy was performed under sterile conditions, and heparin-filled catheters were implanted in the thoracic aorta and the right atrial appendage for measurement of arterial pressure and fluid administration, respectively. A heparin-filled catheter was also positioned in the left atrial appendage for measurement of left atrial pressure and administration of radioactive microspheres. A pneumatic occluder was placed around the
left anterior descending coronary artery (LAD) immediately distal to a Doppler flow velocity transducer.

A multiport catheter (0.8 mm OD, 0.04 mm ID) was implanted into the midmyocardium of the LAD perfusion territory to enable sampling of myocardial interstitial fluid. The catheter contained 48 25-gauge needle holes in a 2-cm segment. The thoracotomy incision was enclosed in layers, and pneumothorax was evacuated with a chest tube.

Regional Myocardial Perfusion

Carbonized plastic microspheres (15 ± 2 mm diameter, NEN, Boston, MA) labeled with 141Ce, 103Ru, 51Cr, or 95Nb were used to measure regional myocardial perfusion. Immediately before injection, the sphere suspension was ultrasonicated (Branson model 450, E/MC) for 15 min, and 2–3 × 10⁶ microspheres were administered into the left atrium as a bolus over 10 s and flushed with 10 ml of warm (37°C) sterile saline. A few seconds before the microsphere injection, a timed collection of reference arterial flow was initiated from the aortic catheter and withdrawn at a constant rate of 7 ml/min for 3 min.

Transmural tissue samples obtained from normal left circumflex coronary artery perfusion territory and the ischemic zone (LAD region) were selected for mapping of myocardial blood flow at the conclusion of each experiment. The tissue samples were subdivided into subepicardial, midmyocardial, and subendocardial layers of approximately equal thickness. Samples were weighed and placed in scintillation vials, and the activity of each isotope was determined. Similarly, the activity of each isotope in the reference blood flow sample was also assessed. Overlap among the nuclide emission spectra was eliminated by sequential correction. Tissue blood flow (ml·min⁻¹·g⁻¹) was calculated as Q_r × C_m/C_w, where Q_r is the rate of withdrawal of the reference blood flow sample (ml/min), C_m is the activity [in counts per minute (cpm)/g] of the myocardial tissue sample, and C_w is the activity (in cpm) of the reference blood flow sample.

Experimental Protocol

The myocardial interstitial fluid was collected daily from the indwelling intramyocardial catheter starting on the first postoperative day. After 1 wk of postoperative recovery, the LAD was repetitively occluded for 2 min once per hour, eight times each day, for 21 days in eight dogs. Samples of the myocardial interstitial fluid were obtained each morning before the onset of the eight daily occlusions. Three milliliters of isotonic saline were flushed into the catheter as 3 ml of the aspirate were withdrawn. Hemodynamics were monitored before, during, and after each occlusion. Five sham-operated dogs were instrumented identically but did not receive repetitive coronary occlusions (with the exception of 4 2-min occlusions at weekly intervals for measurement of coronary collateral blood flow by the microsphere technique). Myocardial interstitial fluid from sham dogs was obtained during the recovery period from surgery and during a subsequent 3-wk observation period.

The mitogenic properties of the myocardial interstitial fluid at various times were assessed by analyzing its effects on vascular smooth muscle or endothelial cell proliferation in vitro. The time points when the myocardial interstitial fluid was assessed for mitogenic activity included days 2–7 before and days 2, 6 and 7, 12–14, and 19–23 after initiation of repetitive coronary artery occlusions unless otherwise indicated.

Cell Culture Procedure

General conditions. Porcine pulmonary arterial vascular smooth muscle cells (VSMC) and porcine aortic endothelial cells (EC) were plated on 100-mm plastic culture dishes. The medium used for VSMC culture was Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS), L-glutamine, pyruvate, and antibiotic-antimycotic. M199 medium with 20% FBS, L-glutamine, sodium pyruvate, and antibiotic-antimycotic were utilized for EC culture. The cells were maintained in a humidified atmosphere at 37°C in the presence of 5% CO₂-20% O₂-75% N₂.

Cell proliferation. We hypothesized that the myocardial interstitial fluid would display mitogenic activity early after surgery secondary to inflammation/healing or after initiation of repetitive coronary artery occlusions previously demonstrated to enhance collateral perfusion (15). Mitogenic activity was assessed by the proliferative response of cells grown in culture to myocardial interstitial fluid samples. Cell proliferation was quantified by direct measurement of cell numbers using a hemacytometer. The cells were seeded in a 24-well plate with densities of 10,000 cells per well in DMEM containing 5% FBS overnight. On the following day, the medium was exchanged for 0.1% FBS in DMEM to growth-arrest the cells for 72 h. The cells were then incubated in a 10% solution of the myocardial interstitial fluid diluted in DMEM containing 0.1% FBS for 72 h. Other cultures were treated with 0.1 or 20% FBS to serve as growth-arrested negative or positive controls, respectively.

We also tested the hypothesis that the mitogenic activity of the myocardial interstitial fluid is caused by cytokines/growth factors that are predominantly proteins. Three experiments were conducted. First, a protein digestion of the myocardial interstitial fluid with Pronase (papain)-coupled latex beads (Pierce) was performed. Five parts of myocardial interstitial fluid were incubated overnight at 25°C with three parts of Pronase beads and two parts of 5× buffer containing 2 M HCl, 0.25 M CaCl₂, and 0.5 M boric acid at a pH of 6.2. The beads were centrifuged at 1,000 rpm for 3 min after the incubation period. The supernatant was collected and prepared for proliferation assays (as described above) to determine the activity of the Pronase-treated myocardial interstitial fluid. To test the efficiency of the Pronase beads on the degradation of the myocardial interstitial fluid, we increased the amount of beads 4 times (5 parts Pronase beads, 1.25 parts of the myocardial interstitial fluid, and 2.5 parts buffer), and VSMC were incubated with the digested myocardial interstitial fluid. Second, the myocardial interstitial fluid was boiled for 5 min to heat-inactivate proteins. The fluid was diluted and administered to growth-arrested VSMC and EC. The number of cells per well was determined after 72 h of incubation time, as described above. Third, the myocardial interstitial fluid was incubated with antibodies for basic fibroblast growth factor (bFGF, UBI) for VSMC and vascular endothelial growth factor (VEGF, Genzyme) for endothelial cells. The antibodies were used in a concentration of 10 µg/ml on VSMC and in a concentration of 10 μg/ml on EC in a 10% solution of the myocardial interstitial fluid in 0.1% FBS in DMEM. To demonstrate the efficacy of the inhibition and as control we evaluated the neutralization of mitogenic responses to recombinant bFGF and VEGF with the appropriate antibody. The concentration of the recombinant protein, which is the concentration needed for a 50% thymidine uptake according to manufacturer specifications, was 0.5 ng/ml for bFGF and 5 ng/ml for VEGF. The number of cells per well was determined after 72 h of incubation time as described above.
**Table 1.** Systemic hemodynamics and regional myocardial blood flow

<table>
<thead>
<tr>
<th></th>
<th>Sham Group</th>
<th>Occlusion Group</th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>115 ± 4</td>
<td>121 ± 4</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>87 ± 6</td>
<td>90 ± 10</td>
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<td>Myocardial blood flow, ml·min⁻¹·g⁻¹</td>
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<td></td>
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<tr>
<td><strong>Ischemic zone</strong></td>
<td></td>
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<tr>
<td>Subepicardium</td>
<td>0.24 ± 0.01</td>
<td>0.16 ± 0.50</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>0.07 ± 0.04</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Transmural</td>
<td>0.12 ± 0.03</td>
<td>0.10 ± 0.03</td>
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<tr>
<td><strong>Normal zone</strong></td>
<td></td>
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<tr>
<td>Subepicardium</td>
<td>0.90 ± 0.09</td>
<td>1.04 ± 0.09</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>1.22 ± 0.12</td>
<td>1.35 ± 0.12</td>
</tr>
<tr>
<td>Subendocardium</td>
<td>1.25 ± 0.09</td>
<td>1.36 ± 0.09</td>
</tr>
<tr>
<td>Transmural</td>
<td>1.13 ± 0.09</td>
<td>1.13 ± 0.09</td>
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Data are means ± SE. Significant differences: *P vs. corresponding sham in ischemic zone; †P < 0.05 vs. day 1.

**RESULTS**

**Hemodynamics**

Mean arterial pressure and heart rate in dogs exposed and not exposed (sham group) to chronic ischemia were similar at all time periods of measurement. Coronary collateral blood flow in the ischemic zone progressively increased to a level similar to that in the normal zone by 3 wk after the initiation of repetitive coronary artery occlusions. In contrast, flow to the ischemic zone did not increase during the 3-wk observation period in dogs not exposed to repetitive ischemic stimuli. Myocardial blood flow in the normal zone in the repetitive occlusion and sham groups over the 3-wk protocol remained unchanged (Table 1).

**Proliferation of VSMC and EC Before Repetitive Coronary Occlusions**

The proliferative response of VSMC and EC to the myocardial interstitial fluid during 1 wk of recovery after surgery before the initiation of repetitive coronary occlusions is shown in Fig. 1. The mitogenic activity of the myocardial interstitial fluid was prominent during the first several days after surgery. The treatment with myocardial interstitial fluid produced greater than doubling of VSMC number. This pronounced mitogenic effect appeared to wane at day 6 and was absent by day 7, i.e., the myocardial interstitial fluid did not produce cell proliferation above baseline (0.1% FBS treatment). Similar results were obtained with EC.

**Proliferation of VSMC and EC To Myocardial Interstitial Fluid Obtained From Repetitive Coronary Occlusion and Sham Groups**

The proliferative response of VSMC to the myocardial interstitial fluid obtained from dogs after initiation of brief, repetitive coronary artery occlusions is summarized in Fig. 2. The mitogenic effect was pronounced at the earliest time points and peaked at days 12–14 after the onset of repetitive occlusions. VSMC proliferation...
on days 19–23 waned from the peak response. Myocardial interstitial fluid obtained from sham dogs did not induce proliferation greater than that observed in the growth-arrested (0.1% FBS) controls.

The mitogenic effect of the myocardial interstitial fluid on EC was less than that observed with VSMC. The peak proliferative response of EC to the myocardial interstitial fluid was a 58% increase in cell number compared with a 155% increase for VSMC. The myocardial interstitial fluid of sham dogs did not cause proliferation above that observed in growth-arrested controls (Fig. 3).

Effects of Pronase Treatment on the Proliferative Properties of Myocardial Interstitial Fluid

The proteolytic digestion of the myocardial interstitial fluid with Pronase-coupled latex beads resulted in a dramatic decrease of the mitogenic response of VSMC (Fig. 4) and EC (Fig. 5). The effect of Pronase was dose dependent in that the higher concentration of Pronase completely inhibited increased proliferation caused by the myocardial interstitial fluid, whereas the lower concentration only partially blocked the response.

Effects of Heat Inactivation on the Proliferative Properties of Myocardial Interstitial Fluid

Heat inactivation of the myocardial interstitial fluid obtained on day 2 and days 5 and 6 caused a total inhibition of the proliferation of VSMC. In contrast, at two later time points, heat inactivation either attenuated (days 11 and 15) or had no effect (days 19–21) on VSMC proliferation (Fig. 6).

In contrast, the mitogenic effect of the myocardial interstitial fluid on EC was abolished by heat inactivation at all time points (Fig. 7).
Neutralization of the Proliferative Properties of Myocardial Interstitial Fluid on VSMC and EC With Antibodies to bFGF and VEGF

The proliferative response of VSMC to the myocardial interstitial fluid was significantly inhibited by the antibody to bFGF on days 12–14. This effect waned at days 18 and 19. The antibody had only a small or no effect at the early time points (Fig. 8). The neutralization of the proliferative response of EC to the myocardial interstitial fluid was most pronounced on day 7, days 12 and 13, and days 19 and 20. The VEGF antibody had no effect on days 21–23 (Fig. 9). Recombinant bFGF and VEGF were each significantly inhibited by the specific antibody (Figs. 8 and 9). We verified specificity of the inhibitory action of the antibodies by examining if mitogenic actions of other growth factors were attenuated. Within this context, anti-bFGF did not affect acidic fibroblast growth factor (aFGF)-induced proliferation and anti-VEGF did not affect that induced by platelet-derived growth factor (PDGF)-AA. However, mitogenic actions of PDGF-BB were significantly attenuated by anti-VEGF, suggesting some cross-reactivity to growth factors in the same family.

DISCUSSION

This investigation is the first to report the vascular cell proliferative activity of the myocardial interstitial fluid obtained from myocardium of chronically instrumented dogs exposed to repetitive episodes of ischemia. The results indicate that after a 1-wk recovery from surgery 1) the myocardial interstitial fluid obtained from myocardium not exposed to ischemia does not induce proliferation of VSMC and EC; 2) the myocardial interstitial fluid obtained from dogs sustaining repetitive episodes of ischemia causes marked proliferative activity of VSMC and EC; and 3) pronase digestion of myocardial interstitial fluid significantly reduced mitogenic effect on VSMC and EC at all time points (Fig. 5). Pronase digestion reduced mitogenic effect of myocardial interstitial fluid on EC significantly at all time points (*P < 0.05 vs. corresponding control).

Fig. 4. Proliferation rate of VSMC is reduced at all time points after treatment with Pronase-coupled latex beads (*P < 0.05 vs. corresponding control).

Fig. 5. Pronase digestion reduced mitogenic effect of myocardial interstitial fluid on EC significantly at all time points (*P < 0.05 vs. corresponding control).

Fig. 6. Proliferation of VSMC was significantly reduced by heat inactivation of myocardial interstitial fluid on day 2, days 5 and 6, and days 11 and 15 after initiation of repetitive occlusions (*P < 0.05 vs. corresponding control). On days 19–21 after onset of ischemic stimuli, heat inactivation did not neutralize proliferative effect of myocardial interstitial fluid.
tion of VSMC and EC; 3) the proliferative effect of the myocardial interstitial fluid is abolished by Pronase or heat inactivation, suggesting that the mitogen(s) are likely to be proteins; 4) the mitogenic activity is neutralized by antibodies for bFGF and VEGF at certain time points; and 5) the appearance of mitogen(s) in the myocardial interstitial fluid is associated with dramatic development of the coronary collateral circulation. Another important finding of this study was that the myocardial interstitial fluid produced proliferation of vascular cells during the recovery period after instrumentation, but this effect waned by 1 wk after the surgery. Taken together, the results support the concept that coronary angiogenesis in response to brief episodes of myocardial ischemia is caused by vascular cell mitogens released into the myocardial interstitium and that the expression of mitogens, especially bFGF and VEGF, varies over time during progression of collateral development.

Repetitive Coronary Artery Occlusion Model and Collateral Development

Critical to this concept are several issues, including the model of coronary angiogenesis and collateral development, growth factors involved in coronary angiogenesis, and physiological implications of the data.

Repetitive episodes of myocardial ischemia of a certain minimal duration are required to induce development of a functional collateral circulation in the dog (9, 15). Other models have been used to induce coronary collateral growth (Ameroid constriction and micovascular embolization), and although each has its strengths and limitations, we elected to use repetitive total coronary artery occlusions because of the predictability and precise time of the initiating signal (first occlusion) (5) and standardization of ischemic stimulus between animals (total occlusion). A 2-min coronary artery occlusion performed one time per hour, eight times per day, for 3 wk results in coronary collateralization, indicated by maintenance of normal resting tissue flow and contractile function in the ischemic zone during sustained total coronary artery occlusion (8). This model allows precise quantification of the duration, time course, and severity of the ischemic stimulus, reproducibly induces collateral formation (5, 8), and enables continuous monitoring of the influence of endogenously released growth factors during collateral development. Moreover, the 2-min duration of coronary occlusion avoids myocardial stunning and tissue necrosis (5, 15, 17, 25, 26). Finally, accelerated growth of coronary collaterals (above that occurring with 8 occlusions each day) is not observed if a 2-min occlusion is performed hourly 24 times each day (8).

We elected to study cell proliferation in vitro because it allows the study of one process, i.e., effects of growth factors on vascular cell proliferation. We do not have
information of other important aspects of angiogenesis, such as tissue remodeling associated with protease expression. The measurement of cell proliferation by determining the cell number is a direct way to quantify mitogenic activity. It is, however, labor intensive. Thymidine and bromodeoxyuridine reveal information about DNA synthesis but can provide misleading results because the incorporation and phosphorylation of the thymidine may be disturbed by permeability barriers and/or certain kinases (1).

A limitation of the methodology used in this investigation relates to the analysis of the chemical nature of the growth factors by heat denaturation. The structure of some proteins protects against heat inactivation, which may provide a partial explanation as to why the mitogenicity of myocardial interstitial fluid cannot be eliminated at all time points (18). We believe that the growth factors are proteins because the mitogenic activity of the myocardial interstitial fluid can be inhibited at all time points with Pronase, a proteolytic enzyme with broad substrate specificity (13). Neutralization of the mitogenic activity of the myocardial interstitial fluid with antibodies for growth factors, which play an important role during angiogenesis, was an approach utilized to elucidate the biological activity of bFGF and VEGF in the myocardial interstitial fluid. We selected antibodies for the growth factors bFGF and VEGF because the presence of these growth factors in VSMC and EC is well established in the literature (11, 22, 24). Our findings imply that bFGF and VEGF are two mitogens responsible for VSMC and EC proliferation, respectively. We are compelled to mention a caveat for these experiments. The specificity of the antibodies and possible cross-reactivity is not well established in the canine. Thus we cannot conclude with conviction the precise roles for bFGF and VEGF in collateral development.

Another important aspect of our results is that we sampled myocardial interstitial fluid from the midmyocardium, but collateral growth occurs not only in intramyocardial vessels but also in arteries in the subepicardium. This prompts the question, Would a growth factor produced in the midmyocardium or subendocardium (where the ischemic insult is the greatest) have access to the subepicardium and potentially produce collateral growth? We believe that this could occur because Hanley et al. (6) reported that intramyocardial injections of adenosine were recovered in a chamber sutured on the epicardial surface. Thus there is ready movement of materials from the midmyocardium to the epicardium. Moreover, it has been recently reported that bFGF is found in the pericardial fluid of patients with angina (6). Taken together, these results suggest that intramyocardial production of mitogens, i.e., the location we are sampling for mitogenic activity, could influence the growth of vessels on the epicardial surface.

Growth Factors Involved in Coronary Angiogenesis

The present investigation strongly suggests that ischemic myocardium selectively releases growth factors/mitogens that induce coronary collateral growth. This study only allowed partial resolution of which growth factors are involved. Several growth factors have been proposed in coronary collateral growth. The presence of aFGF and bFGF in normal hearts of various mammals has been well established (4, 11, 16, 19, 22, 24). However, the role for bFGF as a mitogen elaborated from ischemic myocardium has been complicated by observations suggesting that the expression of this mitogen does not increase during coronary collateralization (22). A role for aFGF has been proposed but only as the result of infiltration of macrophages (which contain the message) into the ischemic region (22). However, a role for bFGF was supported by observations that bFGF increased the rate and magnitude of collateral growth in a canine model (11, 24).

VEGF has been implicated in coronary collateral development by some reports, but previous findings are complicated by the observations that administration of this mitogen does not augment angiogenesis in a model of coronary collateral development (24). VEGF has been suggested to play an important role in angiogenesis (2, 12). This is supported by the demonstration that VEGF mRNA increased 10- to 20-fold in ischemic porcine myocardium distal to an ameroid constrictor compared with the normal zone (2). Furthermore, Banai et al. (2) showed that administration of VEGF could promote angiogenesis in skeletal muscle.

Another group of mitogens/cytokines, which may be involved in coronary collateralization, are the insulin-like growth factors (IGF). These agents have been shown to be active not only in embryonic and neonatal growth but also in tissue renewal and repair throughout adulthood. This is especially evident when tissues must adapt to specific demands (e.g., cardiac and renal hypertrophy) and during regeneration of blood vessels and wound repair (21). Kluge et al. (10) reported an enhancement of transcription of IGF-1 in the pig after 72 h of myocardial ischemia and suggested a role of IGF-1 in angiogenic processes. In the aggregate, the many disparate results and a number of potential candidates preclude conclusions about the role of a specific mitogen in coronary angiogenesis at this time.

Physiological Implications

Our data demonstrate that the myocardial interstitial fluid harvested from ischemic myocardium is highly mitogenic for up to 2 wk after the initiation of repetitive coronary occlusions. After 3 wk of ischemia, the degree of mitogenic activity for VSMC and EC was decreased from peak levels. This occurred simultaneously with the recruitment of additional coronary collateral blood flow and subsequent reduction in the intensity of the ischemic stimulus.

Not a single growth factor is responsible for coronary angiogenesis. In fact, the present results show the contention that multiple mitogens are expressed during various stages of collateral development. Pronase treatment reduced the proliferation of VSMC and EC in response to the myocardial interstitial fluid at all time points, but heat did not abolish the mitogenic effect of
the myocardial interstitial fluid on VSMC on days 11 and 15 and days 19–21 after initiation of repetitive coronary artery occlusions. Neutralization with VEGF abolished the proliferation of EC at day 7, days 12 and 13, and days 19 and 20. In contrast the bFGF antibody had a significant effect only after 12–14 days following initiation of repetitive occlusions. Thus, at certain time points, different growth factors like bFGF and VEGF may have been activated and were responsible for angiogenic processes. Further studies are required to elucidate the temporal relationships of their expression and the synergy of different growth factors in response to the intensity of the ischemic stimulus.

In summary, the present results indicate that brief episodes of myocardial ischemia induce the release of protein growth factors/cytokines into the myocardial interstitium that promote coronary collateral growth. Our results further suggest that bFGF and VEGF are both involved in collateral growth and that the role of each growth factor appears to vary during the time course of collateral growth.

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