Original Contributions

Alteration of Endothelium-Dependent Hyperpolarizations in Porcine Coronary Arteries With Regenerated Endothelium

Catherine Thollon, Jean P. Bidouard, Christine Cambarrat, Isabelle Delescluse, Nicole Villeneuve, Paul M. Vanhoutte, Jean P. Vilaine

Abstract—The present study was designed to test the ability of regenerated endothelium to evoke endothelium-dependent hyperpolarizations. Hyperpolarizations induced by serotonin and bradykinin were compared in isolated porcine coronary arteries with native or regenerated endothelium, 4 weeks after balloon endothelial denudation. The experiments were performed in the presence of inhibitors of nitric oxide synthase (N\textsuperscript{G}-nitro-L-arginine) and cyclooxygenase (indomethacin). The transmembrane potential was measured using conventional glass microelectrodes. Smooth muscle cells from coronary arteries with regenerated endothelium were depolarized in comparison with control coronary arteries from the same hearts. Spontaneous membrane potential oscillations of small amplitude or spikes were observed in some of these arteries but never in arteries with native endothelium. In coronary arteries from control pigs, both serotonin and bradykinin induced concentration-dependent hyperpolarizations. In the presence of ketanserin, 10 \textmu mol/L serotonin induced a transient hyperpolarization in control coronary arteries. Four weeks after balloon denudation, the response to serotonin was normal in arteries with native endothelium, but the hyperpolarization was significantly lower in coronary arteries with regenerated endothelium. In control arteries, the endothelium-dependent hyperpolarization obtained with bradykinin (30 nmol/L) was reproducible. Four weeks after balloon denudation, comparable hyperpolarizations were obtained in coronary arteries with native endothelium. By contrast, in arteries with regenerated endothelium, the hyperpolarization to bradykinin became voltage-dependent. In the most depolarized cells, the hyperpolarization to bradykinin was augmented. The changes in resting membrane potential and the alteration in endothelium-dependent hyperpolarizations observed in the coronary arteries with regenerated endothelium may contribute to the reduced response to serotonin and the unchanged relaxation to bradykinin described previously. (Circ Res. 1999;84:371-377.)

Key Words: regenerated endothelium • endothelium-derived hyperpolarizing factor • serotonin • bradykinin

By releasing endothelium-derived relaxing factors (EDRFs) such as nitric oxide (NO),\textsuperscript{1} prostacyclin,\textsuperscript{2} and endothelium-derived hyperpolarizing factor (EDHF),\textsuperscript{3,4} the endothelium plays a key role in modulating the responses of the underlying vascular smooth muscle cells.\textsuperscript{5} All the major cardiovascular risk factors have been associated with endothelial dysfunction, which precedes clinically apparent vascular disease and its complications.\textsuperscript{6} Hypertension,\textsuperscript{7} diabetes mellitus,\textsuperscript{8} hypercholesterolemia,\textsuperscript{9} and atherosclerosis\textsuperscript{10} are associated with decreased responses to endothelium-dependent vasodilators. Experiments in animal models suggest that the occurrence of abnormal endothelium-dependent relaxations is an early event in the development of vascular disease.\textsuperscript{11-13} Because the turnover of endothelial cells is accelerated under most of these conditions,\textsuperscript{14} the functional alterations in the regenerated endothelium could play an important role in the genesis of coronary disease. Previous studies\textsuperscript{13} demonstrated that, in porcine coronary arteries with regenerated endothelium after balloon denudation, endothelium-dependent relaxations are normalized 8 days after the procedure. However, 4 weeks after denudation, the relaxations to UK 14,304 (a selective \(\alpha_{1}\)-adrenergic agonist), serotonin and aggregating platelets were reduced, whereas those induced by ADP, bradykinin, and the calcium ionophore A23187 were maintained. Because the decrease in endothelium-dependent relaxations seems restricted to stimuli that activate endothelial Gi-2 proteins,\textsuperscript{15,16} a selective Gi protein dysfunction seems likely.\textsuperscript{17-19} This selective endothelium impairment and the resulting marked dysfunction in the NO-EDRF pathway does not result from a change in the expression of Gi proteins but rather reflects their reduced function in regenerated endothelial cells.\textsuperscript{18} However, because EDHF contributes to the endothelium-dependent relaxations to bradykinin and A23187,\textsuperscript{20} an upregulation of the release of this factor rather than a selective Gi protein dysfunction may explain the better preservation of endothelium-dependent relaxations to these agonists. The present experiments were designed to test the ability of regenerated endothelium to...
evoke hyperpolarizations of the underlying smooth muscle of the porcine coronary artery.

**Materials and Methods**

**Animals**

Sixty-three Large White pigs (male or female), 8 to 12 weeks of age, were used for the electrophysiological study. The animals were purchased from a commercial swine producer (Frenelles E.A.R.L., Boisemont, France) in accordance with the French Ministry of Agriculture. All experiments were performed in accordance with governmental and institutional guidelines for the use and care of animals.

**Coronary Endothelium Denudation**

Thirty-four pigs, 8 weeks old (mean weight 21 ± 0.5 kg), were anesthetized with an intramuscular injection of a mixture composed of tiletamine plus zolazepam (20 mg/kg) and atipamezole (50 μg/kg). Additional doses of anesthetic (sodium thiopental) were given intravenously as necessary. Animals were intubated with an endotracheal tube and mechanically ventilated with a respirator. Heparin (250 IU/kg) and lysin acetylsalicylate (10 mg/kg) were administrated intravenously to prevent thrombus formation and limit inflammation, respectively. Using aseptic surgical techniques, a percutaneous transluminal coronary angioplasty (PTCA) guide catheter (Cordis, model AR7E) was introduced via the femoral artery into the left or right coronary ostium, under fluoroscopic guidance (Radiodetect contrast product, x-ray imager, model BV25, Philips). A PTCA dilatation catheter (Cordis, balloon of 2 to 3 mm in diameter and 20 to 30 mm long) was then introduced into the chosen coronary artery through the guide catheter. The diameter of the balloon was adapted according to the size of the coronary artery. The endothelium was removed by inflating the balloon 3 times for 30 seconds. The pressure of inflation was adjusted so that the blood vessel was not overstretched (from 2 atm at the distal side to 8 atm at the proximal side). At the end of the surgical procedure, Terramycin (20 mg/kg) was given intramuscularly as an antibiotic prophylaxis. After recovery from anesthesia, the animals were housed in individual cages for 4 weeks. At the time of killing (12 weeks of age), the body weight of these animals was 32 ± 0.7 kg. The control animals (nonoperated) were killed at 10 weeks of age (n = 29, body weight 25 ± 0.8 kg).

**Membrane Potential Recording**

After anesthesia with tiletamine plus zolazepam (20 mg/kg, IM), the heart was removed and rapidly placed in an ice-cold oxygenated Krebs-Ringer solution. Then the left anterior descending, left circumflex, and right coronary arteries were dissected-free, cleaned of adherent fat and connective tissue, and maintained in an oxygenated Krebs-Ringer solution (20°C) and diluted in Krebs-Ringer solution to reach final concentrations of 10 mmol/L (H2O), and bradykinin (1 mmol/L, in H2O) were prepared as stock solutions (−20°C) and diluted in Krebs-Ringer solution to reach final concentrations. L-NA and serotonin were dissolved daily in H2O (10 mmol/L).

Membrane potential was measured with conventional glass microelectrodes (30 to 40 MΩ) filled with 3 mol/L KCl. The microelectrode was connected to an amplifier (model V180, Biological), and the membrane potential was monitored simultaneously on a digital storage oscilloscope (model 2211, Tektronix,) and a pen-chart recorder (model 3400, Gould). Criteria for considering the impalement successful were a sudden negative shift in potential at the cell penetration and a rapid return to the previous potential at the withdrawal of the microelectrode. A small potential deflection was often observed before abrupt penetration of the cell and on dislodgement of the microelectrode, probably as a result of crossing the endothelial cells.

**Protocol**

In all experiments, the response of the coronary arteries with regenerated endothelium was compared with that of control coronary arteries from the same heart. Because the same vascular preparation was never used for 2 consecutive experiments, the number of experiments performed corresponded to the number of vascular strips used. Furthermore, when unspecified, the number of experiments (n) corresponded to the number of animals. All experiments were performed in the presence of indomethacin (10 μmol/L) to inhibit cyclooxygenase and Nω-nitro-L-arginine (L-NA, 30 μmol/L) to inhibit NO synthase (NOS).

For the experiments with serotonin, ketanserin (10 μmol/L) was added to the physiological solution to inhibit the 5-hydroxytryptamine 2 (5-HT2) receptors present on the vascular smooth muscle of the porcine coronary artery.11 The membrane potential was measured at the end of the resting period (control values), after the incubation with different drugs and during the addition of agonists (serotonin or bradykinin).

**Drugs**

The following drugs were used: indomethacin, L-NA, ketanserin, 5-HT creatinine sulfate, and bradykinin (all from Sigma Chemical Co). Indomethacin (10 mmol/L, in ethanol), ketanserin (10 mmol/L, in H2O), and bradykinin (1 mmol/L, in H2O) were prepared as stock solutions (−20°C) and diluted in Krebs-Ringer solution to reach final concentrations. L-NA and serotonin were dissolved daily in H2O (10 mmol/L). Further dilutions were performed directly in Krebs-Ringer solution. Serotonin and bradykinin were added to the Krebs-Ringer solution to reach final concentrations of 10 μmol/L and 30 mmol/L, respectively, immediately before the application to the vascular preparation.

**Statistical Analysis**

Data are expressed as mean ± SEM from n experiments. For the experiments performed on coronary arteries from control animals, 1-way ANOVA with repeated measures was first carried out and followed by a Newman-Keuls test to analyze the effect of treatments. For the comparison between control coronary arteries from operated animals versus coronary arteries from control pigs, 2-way ANOVA with repeated measures on 1 factor followed by a Newman-Keuls test was performed. To compare the responses between the coronary arteries with regenerated endothelium to those with native endothelium from the same animals, Student t test for paired observations was used and followed with 2-way ANOVA with repeated measures on 2 factors. Differences were considered to be statistically significant at a value of P < 0.05.

**Results**

**Resting Membrane Potential**

In the control arteries taken from animals not subjected to a surgical procedure, the resting membrane potential of the coronary smooth muscle cells averaged −56.3 ± 1.7 mV (n = 15). Twenty-eight days after endothelium denudation, the resting membrane potential of smooth muscle cells from the coronary arteries with native endothelium was not significantly different from that observed in control animals (−57.1 ± 0.7 mV, n = 34). In coronary arteries with regenerated endothelium, before any pharmacological intervention, the smooth muscle cells were significantly less polarized than in the corresponding control arteries (−49.9 ± 1.2 mV; P ≤ 0.01, n = 34). Under control conditions, in 4 strips with regenerated endothelium, membrane potential oscillations (amplitude 4.9 ± 1.0 mV), with a spontaneous frequency of 6.5 ± 0.4/min, were recorded (Figure 1A). All impaired cells from these 4 vascular preparations showed similar fluctuations in resting membrane potential. Such oscillations were never observed in either coronary arteries from control animals or coronary arteries with native endothelium from pigs subjected to angioplasty 4 weeks earlier. When the
approximating 5 mV under all experimental conditions and slow depolarization on the first 20 minutes was observed, ie, vascular strips were superfused with the incubation solution, oscillations in resting membrane potential in coronary arteries with regenerated endothelium. Example of recordings obtained in 3 different coronary arteries. A, Membrane potential fluctuations of small amplitude (3 to 4 mV) and frequency (7/min) during the resting period (Krebs-Ringer solution) before any pharmacological intervention. B, Recordings of membrane potential in arteries with regenerated endothelium. Example of recordings obtained in 3 different coronary arteries. A, Membrane potential fluctuations of the same frequency as in panel A (7/min) but with higher amplitude (8 to 9 mV). 

recordings in panels b and c were obtained in 2 cells from the same vascular preparation. In these cells, some of the slow waves of depolarization lead to the generation of spikes with an amplitude in the range of 10 to 25 mV. vascular strips were superfused with the incubation solution, before the addition of agonists causing hyperpolarization, slow depolarization on the first 20 minutes was observed, ie, approximating 5 mV under all experimental conditions and all vascular tissues. Bradykinin or serotonin was given when the resting membrane potential had stabilized (Table). After the superfusion with the solution containing pharmacological inhibitors, the resting membrane potential was still significantly different between smooth muscle cells from coronary arteries with regenerated endothelium and native endothelium (Table). Some coronary arteries with regenerated endothelium (n=7) that had stable resting membrane potential under control conditions showed fluctuations of the membrane potential during incubation with the NOS blocker L-NA. Under these conditions, the amplitude of the oscillations was higher (6.4±1.8 mV, n=4) (Figure 1B) and could even generate spikes (Figure 1c), with an averaged maximal amplitude of 18.8±5.7 (n=3). The upstroke of these action potentials attained maximal levels, ranging between −15 and −20 mV and never showed an overshoot. The frequency of spontaneous fluctuations in membrane potential during exposure to L-NA was identical as under control conditions (7.6±0.8/min).

Hyperpolarization to Serotonin

In the presence of indomethacin (10 μmol/L), L-NA (30 μmol/L), and ketanserin (1 μmol/L), serotonin induced endothelium-dependent hyperpolarization in coronary strips from control animals. The response to serotonin was concentration-dependent (Figure 2) with maximal changes in membrane potential of −3.0±1.1 mV (n=6), −7.8±1.6 mV (n=7), and −13.2±1.9 mV (n=6), at 0.1, 1, and 10 μmol/L, respectively. These hyperpolarizations were transient. Thus, at 10 μmol/L serotonin, the hyperpolarization was maximal after 25.1±1.6 seconds. The membrane potential returned to control level within 1 minute (58.6±4.1 seconds, n=7).

Changes in Resting Membrane Potential During the Incubation Period Before the Application of Serotonin or Bradykinin

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Control Animals</th>
<th>Native</th>
<th>Regenerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NA+Indo+Ket Pre</td>
<td>−54.1±3.15</td>
<td>−56.8±1.82</td>
<td>−50.9±3.60</td>
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<td>Post −50.4±2.05</td>
<td>−51.3±1.70</td>
<td>−46.1±1.91</td>
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<tr>
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<td>...</td>
<td>−56.0±1.26</td>
<td>−49.4±1.75</td>
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<tr>
<td></td>
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<td>−49.1±1.33</td>
<td>−46.8±2.08</td>
</tr>
<tr>
<td>Ket Pre</td>
<td>...</td>
<td>−58.8±1.39</td>
<td>−51.8±1.24</td>
</tr>
<tr>
<td></td>
<td>Post ...</td>
<td>−51.4±1.29</td>
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</tr>
<tr>
<td>L-NA+Indo Pre</td>
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<td>−57.1±1.15</td>
<td>−49.0±1.71</td>
</tr>
<tr>
<td></td>
<td>Post −49.4±0.75</td>
<td>−49.9±0.69</td>
<td>−42.6±1.56</td>
</tr>
</tbody>
</table>

Recordings were performed before (pre) and after (post) the incubation with 10 μmol/L ketanserin alone (Ket), 10 μmol/L indomethacin and 10 μmol/L ketanserin (Indo+Ket), 30 μmol/L L-NA and 10 μmol/L indomethacin (L-NA+Indo), or 30 μmol/L L-NA, 10 μmol/L indomethacin, and 10 μmol/L ketanserin (L-NA+Indo+Ket). Data are mean±SEM for n experiments. When unspecified, n represents the number of animals per group. No statistical difference was observed between control coronary arteries from control and operated animals. Significant difference between native and regenerated endothelium. *P<.05; **P<.01.
Four weeks after balloon endothelial denudation, the hyperpolarization induced by serotonin at 10 μmol/L in the presence of indomethacin (10 μmol/L), L-NA (30 μmol/L), and ketanserin (10 μmol/L) was not significantly different from control in coronary arteries with native endothelium: -10.6±1.6 mV (n=8) versus -10.8±1.2 mV in coronary strips (n=7) from unoperated animals. In arteries with regenerated endothelium (Figure 3), the response to serotonin was significantly less than the corresponding control strips (-5.6±1.7 mV, Figure 5; P<0.05, n=8). Although the amplitude of the hyperpolarization was not influenced by the presence or absence of L-NA in control arteries, in arteries with regenerated endothelium, studied in the absence of L-NA, serotonin did not cause significant hyperpolarizations but rather evoked a small depolarization (Figure 3). Such depolarizations were not obtained in the presence of inhibitors of NOS, even in presence of spikes (Figure 3A) and also were never observed in coronary segments with native endothelium.

**Hyperpolarization to Bradykinin**

In control porcine coronary arteries, bradykinin evoked concentration-dependent hyperpolarizations (Figure 4) resistant to indomethacin (10 μmol/L) and L-NA (30 μmol/L). The threshold concentration to obtain endothelium-dependent hyperpolarization was 3 nmol/L. With higher concentrations, the amplitude of membrane potential changes increased and the time to peak of hyperpolarization decreased (not shown). The maximal value of hyperpolarization was obtained at 100 nmol/L. Because at the concentration of 30 nmol/L the amplitude of hyperpolarization was reproducible, albeit not maximal, this concentration was used for the evaluation of the endothelium-dependent hyperpolarization induced by the kinin in arteries with regenerated endothelium.

Four weeks after balloon endothelial denudation, the response to bradykinin (30 nmol/L) was not altered in coronary strips with native endothelium (Figure 5): -20.9±0.7 mV (n=16) versus -21.5±0.8 mV hyperpolarization in arteries from control animals (n=8). The temporal changes in membrane potential were not modified: the more hyperpolarized potential was reached after ~1 minute, and the hyperpolarization lasted longer than 3 minutes. In the corresponding coronary segments with regenerated endothelium, although the average change in membrane potential was not different with the control arteries, the amplitude of the hyperpolarization was not significantly different.
(-20.1 ± 1.6 mV, n = 16), the values of membrane potential before administration of bradykinin and that reached in its presence were significantly lower (Figure 5B). When examining the individual experiments, a high variability in the response to bradykinin was noticed in preparations with regenerated endothelium, showing identical, reduced, or increased hyperpolarizations (Figure 5A). The analysis of individual pairs of results showed that although similar responses were recorded in control arteries, the changes in membrane potential for segments with regenerated endothelium were related to the value of membrane potential immediately before the administration of the kinin (Figure 6). In the more depolarized cells, bradykinin evoked a larger hyperpolarization (up to -40 mV) (Figure 5A, bottom panel, and Figure 6).

**Discussion**

There are 2 main findings in the present study: (1) Four weeks after balloon endothelial denudation, the smooth muscle cells from porcine coronary arteries with regenerated endothelium showed intrinsic alterations of the resting membrane potential. (2) The changes in the endothelium-dependent hyperpolarizations of these cells may contribute to the reduced response to serotonin and the unchanged relaxation to bradykinin described previously.13,17,18

**Resting Membrane Potential in Arteries With Regenerated Endothelium**

The present study demonstrates that the smooth muscle cells from coronary arteries with regenerated endothelium are depolarized in comparison to those from the corresponding control arteries. The depolarization of these cells imply alterations of ionic conductance(s) implicated in the control of the resting membrane potential. This could be achieved by an increase in a depolarizing current (inward calcium or nonspecific currents and/or outward chloride current), a decrease in an outward repolarizing or hyperpolarizing potassium current, or both. Such alterations

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**Figure 4.** Concentration-dependent curve of the hyperpolarization induced by bradykinin in coronary arteries from 12 control pigs. Experiments (n=29 coronary artery strips) were performed in the presence of indomethacin (10 μmol/L) and L-NA (30 μmol/L) to block cyclooxygenase and NOS, respectively. Data are mean±SEM. EC50=7.21±1.06 nmol/L.

**Figure 5.** Hyperpolarization induced by bradykinin (BK, 30 nmol/L) in coronary arteries with regenerated endothelium. All coronary arteries with regenerated endothelium were compared with control arteries from the same heart. Experiments were performed in the presence of indomethacin (10 μmol/L) and L-NA (30 μmol/L). A. Illustration of the heterogeneity of responses to bradykinin. B. Maximal values of hyperpolarization induced by bradykinin. Data are mean±SEM from 16 experiments in coronary arteries from operated animals and 8 experiments in coronary arteries from 5 control pigs. Significant difference from the control coronary arteries with native endothelium. **P<0.001.

**Figure 6.** Voltage dependence of the hyperpolarization induced by bradykinin in porcine coronary arteries with regenerated endothelium. All experiments were performed in the presence of indomethacin (10 μmol/L) and L-NA (30 μmol/L).
could include changes in the number of active channels, variations in their open probability, or differences in their regulation. Obviously, alterations in ionic homeostasis could also change the driving force for ions and thus affect the ionic current. Whatever the cause of this relative depolarization, the change in resting membrane potential in these coronary arteries is relevant, because it could modify the myogenic tone of these vessels. This interpretation is confirmed by the observation of spontaneous electrical activities in some arteries with regenerated endothelium. Such membrane potential instability was not observed in the corresponding control arteries. The resting membrane potential of arterial smooth muscle cells is generally stable except in the microvasculature; in the cerebral microcirculation, spontaneous contractions have been associated with an unstable membrane potential of smooth muscle cells. The membrane potential of smooth muscle cells in rhythmically constricting arteries is also significantly less negative than that of quiescent ones. In the porcine coronary artery, cell membrane potential oscillations, spikes, and associated spontaneous contractions have been observed, under marked depolarization with tetrabutylammonium, a potassium channel blocker. The membrane potential oscillations obtained in the present study may be partly the result of the depolarized state of the smooth muscle cells. Indeed, action potentials can be generated only within a certain range of cell membrane potential (≈−40 to −20 mV). Furthermore, rhythmic spontaneous activity has been demonstrated in human coronary arteries and with higher frequency in vessels from older patients or those with cardiovascular diseases and atherosclerotic changes. Hence, the depolarization of smooth muscle cells, observed 4 weeks after balloon denudation, may be a key factor in the development of alterations in vasomotion of these coronary arteries.

Previous studies have shown, in the same porcine model, a decreased response to serotonin while the relaxation to bradykinin remained normal, despite the reduction in the EDRF-NO pathway. Therefore, the endothelium-dependent hyperpolarizations induced by both agonists were compared in porcine coronary arteries 4 weeks after denudation and in control arteries.

**Endothelium-Dependent Hyperpolarization Induced by Serotonin and Bradykinin**

In the coronary arteries from control pigs, both serotonin and bradykinin induced concentration-dependent hyperpolarizations that are resistant to indomethacin and L-NA. The hyperpolarizations observed in the present study during exposure to bradykinin are in agreement with previous observations. Under the experimental conditions imposed, bradykinin given at 30 nmol/L as a single concentration induced an endothelium-dependent hyperpolarization of smooth muscle cells of ≈−20 mV. This level of hyperpolarization was slightly higher than that observed when concentrations of bradykinin were applied in a cumulative manner. The temporal aspects of the membrane hyperpolarization were also in agreement with previous reports. On the contrary, the endothelium-dependent hyperpolarization induced by serotonin was transient, and it was observed at higher concentrations. A similar response to serotonin has been reported in the same preparation. In preliminary studies, it was noted that the response to serotonin is labile, in that many factors can influence the amplitude of the hyperpolarization: (1) rapidity of dissection of coronary arteries after heart excision; (2) stretch of the blood vessels during dissection; (3) a long period before the beginning of the experiments (the response was markedly reduced after 8 hours); and (4) previous exposure to serotonin even after a long washout (desensitization).

Although an EDRF has been demonstrated to be NO, the nature of EDHF is still unknown. It could be a cytochrome P450 metabolite, derived from arachidonic acid. When considering differences between hyperpolarizations induced by bradykinin and serotonin in the present study or those provoked by bradykinin and A23187 compared with that of thrombin, one cannot exclude the existence of several EDHFs. These hyperpolarizations, resistant to indomethacin and L-NA, differ by their amplitude and their kinetics. The fact that in the presence of regenerated endothelium, the transient response to serotonin was reduced while the sustained hyperpolarization induced by bradykinin could be increased would be also in favor of the existence of 2 different mechanisms. It is generally accepted that EDHF increases a potassium conductance leading to the hyperpolarization of the smooth muscle cells. Because all potassium channels implicated in the control of resting membrane potential of smooth muscle cells are voltage-dependent, the depolarization of smooth muscle cells in coronary arteries with regenerated endothelium would be expected to influence the response to EDHF. In the vascular strips with regenerated endothelium, the response to bradykinin was correlated with the value of membrane potential before the administration of the kinin. The maximal opening of potassium channels would lead the membrane to reach potential values close to the equilibrium potential for potassium. Therefore, in preparations with regenerated endothelium, the depolarization of smooth muscle cells induced an increase in the response to bradykinin, illustrating an augmentation of the EDHF pathway in the most altered cells. This result correlates well with previous experiments showing a maintained relaxation to bradykinin in coronary strips with regenerated endothelium whereas it was blunted under depolarizing conditions. Therefore, the lack of impairment of the bradykinin-evoked relaxation, despite a reduction in the NO pathway, could be the result of a greater contribution of the EDHF pathway. Furthermore, although an altered relaxation to serotonin was described in the same experimental model, the present study demonstrates a reduction in the hyperpolarization induced by serotonin. The fact that relaxations to serotonin in porcine coronary arteries are almost completely inhibited by the presence of NOS inhibitors is in favor of a poor participation of the EDHF pathway to the response. This interpretation is supported by the weak hyperpolarizations obtained at high concentrations of serotonin in the present study. Because this small endothelium-dependent hyperpolarization induced by serotonin is decreased 4 weeks after

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**Endothelium-Dependent Hyperpolarization**

**Induced by Serotonin and Bradykinin**

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balloon denudation, no compensatory effect of EDHF for the reduced response to this agonist is plausible. On the contrary, depolarizations were observed that were not blocked by the presence of a high concentration of ketanserin, excluding the implication of 5-HT1 receptor on the vascular smooth muscle. These depolarizations may have curtailed the endothelium-dependent hyperpolarizations. Shimokawa et al13 have previously shown an increase in the contraction to serotonin in the same model and have suggested that the regenerated endothelium releases endothelium-derived contracting factor in response to the monoamine.

The present findings are in favor of a minor implication of EDHF in the endothelium-dependent response of porcine coronary artery to serotonin, but the participation of EDHF in the relaxation response to bradykinin is more important. After balloon endothelial denudation, the endothelium-dependent hyperpolarization induced by serotonin is markedly reduced in the presence of regenerated endothelium, whereas that generated by bradykinin is maintained or even increased in coronary arteries showing an alteration of resting membrane potential. Because in coronary arteries with regenerated endothelium the EDRF-NO pathway is markedly reduced,17,18 the present results help to explain the better preservation of endothelium-dependent relaxations to bradykinin in this model.

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