20-HETE Relaxes Bovine Coronary Arteries Through the Release of Prostacyclin

Phillip F Pratt, John R Falck, Komandla M Reddy, Jason B. Kunian, William B Campbell

Abstract—Neutrophils respond to ischemic injury by infiltrating the myocardium via the vascular wall. During this process, neutrophils are activated and release inflammatory mediators. Some of these mediators are metabolites of arachidonic acid. We have reported that neutrophils metabolize arachidonic acid to 20-HETE, a cytochrome P_{450} metabolite. We investigated the effects of 20-HETE on coronary vascular tone by examining 20-HETE-induced changes in isometric tension in bovine coronary artery rings precontracted with the thromboxane-mimetic, U46619. 20-HETE relaxed precontracted coronary rings in a concentration-dependent manner [EC_{50} of 3 × 10^{-7} mol/L]. Pretreatment with indomethacin, a cyclooxygenase inhibitor, shifted the concentration-response curve to the right [EC_{50} of 1 × 10^{-6} mol/L]. Maximal relaxations were not affected. This suggested that 20-HETE-induced relaxations were, in part, dependent on the cyclooxygenase pathway. Relaxations to 20-HETE were not significantly changed in endothelium-denuded rings. To determine whether metabolism of 20-HETE to a vasoactive compound might explain the relaxations caused by 20-HETE, rings of coronary artery were incubated with [^{3}H] 20-HETE. The incubation buffer was extracted and the [^{3}H] products resolved on reverse-phase HPLC. Both denuded and intact arteries failed to metabolize [^{3}H] 20-HETE. To investigate whether 20-HETE-induced relaxations were related to release of prostacyclin, we measured the release of 6-keto PGF_{1α} from bovine coronary arteries. 20-HETE (1 × 10^{-6} mol/L) stimulated an increase in 6-keto PGF_{1α} in intact vessels (908 ± 138 pg/mL versus 1402 ± 157 pg/mL, basal versus stimulated). Thus, 20-HETE-induced relaxations are due, in part, to the stimulation of the release of the dilatory prostanoid, prostacyclin.

(Hypertension. 1998;31(part 2):237-241.)

Key Words: neutrophils • cytochrome P_{450} • coronary artery • prostacyclin • endothelium • cyclooxygenase

The interaction between vascular endothelial cells and neutrophils is implicated as a major component of the mechanism of myocardial ischemia reperfusion injury. During reperfusion of a formerly ischemic myocardial region, neutrophils localize to damaged tissue and adhere to endothelial cells. After they adhere to the endothelium and are activated, neutrophils release several agents capable of causing cellular damage beyond that incurred during the period of ischemia. Because neutrophils are presumed to be responsible for most damage that occurs after the onset of reperfusion, reperfusing damaged tissue with blood devoid of leukocytes reduces the amount of tissue damage. However, because neutrophils also have recuperative effects, completely irradiating neutrophils may be a less effective therapy than targeting only the potentially destructive compounds they release.

Among the compounds released by activated neutrophils during reperfusion are arachidonic acid and its metabolites. Increased phospholipase activity during ischemia and ATP depletion liberates arachidonic acid from phospholipid stores, subsequently making arachidonic acid metabolism more efficient during the period after reperfusion. Neutrophil metabolize arachidonic acid using all three major enzymes involved in its metabolism: cyclooxygenase, cytochrome P_{450}, and lipoxygenase. Arachidonic acid metabolites from all three enzymatic pathways are known to be vasoactive. Leukotrienes, derived from the lipoxygenase pathway, act as either vasoconstrictors or vasodilators, depending on the vascular tissue being examined. Prostaglandin I_{2} (PGI_{2}), or prostacyclin, is a product of the cyclooxygenase pathway in coronary vascular endothelium and is a known coronary vasodilator. The vascular reactivity of prostacyclin provides therapeutic effects against coronary artery thrombotic characteristics of myocardial ischemia and the “no-reflow” phenomenon. A variety of cytochrome P_{450} metabolites affect vascular tone, including the ω-hydroxylation product 20-HETE.

20-HETE, derived from arachidonic acid, is released from activated neutrophils and contributes to vascular tone in a number of organ systems. Because 20-HETE can be metabolized in much the same way as arachidonic acid, a number of the effects of 20-HETE are attributed to metabolism by the cyclooxygenase pathway. 20-HETE constucts rabbit aorta and mesentery, cortical, and renal arteries only in the presence of cyclooxygenase activity and the vascular endothelium, suggesting that cyclooxygenase metabolism of 20-HETE is involved.
20-HETE takes place in endothelial cells. However, cyclooxygenase activity is not limited to the endothelium. Whereas cyclooxygenase is required for 20-HETE to contract rat aortic rings, the vasoconstrictor effect is only slightly reduced by removal of the endothelium. Several of the actions of 20-HETE, however, are independent of cyclooxygenase. Calcium renal arcuate arteries exposed to 20-HETE contract similarly in the presence and absence of a cyclooxygenase inhibitor, suggesting an alternate mechanism that could include cyclooxygenase-independent 20-HETE metabolism or direct action of 20-HETE on smooth muscle cells, endothelial cells, or both. Indeed, Zou and coworkers reported that 20-HETE is an endogenous inhibitor of the large conductance calcium-activated potassium channel in rat renal arteriolar smooth muscle cells.

Because 20-HETE is released from activated neutrophils and contributes to vascular tone in various tissues, we decided to examine a possible role for 20-HETE in the regulation of coronary vascular tone. Using isolated bovine coronary artery rings, we measured 20-HETE-induced changes in isometric tension under basal and precontracted conditions. We found that 20-HETE was an effective and endothelium-independent vasodilator, suggesting an alternate mechanism that could explain part of the vasodilatory effects of 20-HETE.

Methods

Vascular Reactivity

Bovine hearts (2 to 4 kg) were obtained from the local slaughterhouse. The epicardial left anterior descending coronary artery was dissected, cleaned of adhering fat and connective tissue, and placed in a Krebs bicarbonate solution containing the following (in mmol/L): NaCl (119), KCl (5), NaHCO3 (24), KH2PO4 (1.2), glucose (11), EDTA (0.02), and CaCl2 (3.2). The vessels were cut into rings and care was taken to avoid damaging the endothelium. In some vessels, the endothelium was deliberately removed by gently rubbing the lumen with forceps. The rings were suspended on a pair of stainless steel hooks in a 15-mL water-jacketed organ chamber. One hook was anchored to a steel rod and the other was attached to a force transducer (model FT-03C, Grass Instruments). Tension was recorded on a Grass model 7D polygraph (Grass Instruments). The organ chamber was filled with Krebs bicarbonate solution that was bubbled with 95% O2/5% CO2 and maintained at 37°C. The vessels were challenged with repeated exposures to 20 mmol/L KCl and progressively increased in basal tension to determine the optimal resting tension. This tension was found to be 2 g for vessels of 2-mm diameter. After the vessels equilibrated for 15 hours, KCl (40 mmol/L) was added until reproducible contractions were obtained. The thromboxane-mimetic U46619 (2 X 10^-4 mol/L) was added to increase basal tone to approximately 50% to 70% of KCl induced contractions. U46619 was selected to contract the vessels because it gave reproducible, sustained contractions that did not fade with time. Cumulative concentrations of 20-HETE (10^-10 to 10^-6 mol/L) were added to the organ chambers, and maximal effect was determined. To establish the mechanisms of relaxation, the same vessels were treated with indomethacin (10^-5 mol/L) or the endothelium was removed by physical rubbing. The presence of endothelium was determined by addition of methacholine (10^-5 mol/L) to precontracted vessels. Results were expressed as percent relaxation (relative to the U46619 contraction) with 100% relaxation representing the basal, pre-U46619 tension, which was 2 g.

Release of Prostaglandin I2 by Bovine Coronary Arteries

Vessels were dissected from bovine hearts as described previously and cut into rings (2- to 4-mm diameter). 6-Keto PGF1α was measured in single rings in the absence of tension. Rings were placed in 1 mL of HEPES buffer and maintained at 37°C for 10 minutes in the presence of 10^-3 mol/L of U46619 to mimic the organ chamber conditions. 20-HETE (10^-7 and 10^-6 mol/L) was then added and the incubation continued for 15 minutes. At the end of the incubation period, the media was decanted and examined for production of PGI2 by radioimmunoassay of its stable metabolite 6-keto PGF1α as previously described. 20-HETE in concentrations of 1 X 10^-6 and 1 X 10^-5 mol/L did not cross-react with the 6-keto PGF1α antibody or alter the displacement of [3H] 6-keto PGF1α by 6-keto PGF1α (data not shown).

Vascular Reactivity

We examined the effects of 20-HETE under basal and precontracted conditions. Under 2 g of basal tone, neither the ethanol vehicle nor 20-HETE had an effect on coronary smooth muscle tone. When the vessels were...
A Vehicle Control

B 20-HETE

C 20-HETE + Indomethacin

D 20-HETE + Bradykinin

Figure 1. Effects of 20-HETE on bovine coronary arteries. Original tracings depict the effects of ethanol vehicle (A) 20-HETE under basal (B) and U46619-precontracted (C) conditions, and the effects of indomethacin (D) on 20-HETE-induced relaxations of precontracted bovine coronary arteries

precontracted with U46619, 20-HETE produced concentration-dependent relaxations with maximal relaxations occurring at $1 \times 10^{-5}$ mol/L and an EC$_{50}$ of $3 \times 10^{-7}$ mol/L (Figs 1C and 2). Endothelial integrity was tested at the end of the experiment by challenging U46619-precontracted vessels with methacholine ($1 \times 10^{-6}$ mol/L). A vasorelaxant response was assumed to indicate presence of an intact endothelium. Pretreatment with the cyclooxygenase inhibitor indomethacin ($1 \times 10^{-5}$ mol/L) shifted the 20-HETE concentration-response curve to the right (EC$_{50}$ of $1 \times 10^{-4}$ mol/L) with no change in maximal relaxations (Figs 1D and 2). Removing the endothelium had little effect on 20-HETE-induced relaxation with maximal relaxation again occurring at $10^{-5}$ mol/L and the EC$_{50}$ remaining at $3 \times 10^{-7}$ mol/L (Fig 3).

Figure 2. 20-HETE-induced relaxations of bovine coronary arteries are inhibited by indomethacin. Bovine coronary artery rings ($2$-mm diameter) were contracted with U46619 ($10^{-8}$ mol/L) before the addition of increasing concentrations of 20-HETE to the organ chambers. Some vessels were pretreated with indomethacin ($10^{-5}$ mol/L) for $10$ minutes before precontraction with U46619. Summarized data are expressed as percent relaxations (mean ± SEM).

Figure 3. Effects of endothelium removal on 20-HETE-induced relaxations of bovine coronary arteries. Bovine coronary artery rings, intact and denuded, were contracted with U46619 ($10^{-8}$ mol/L) before the addition of increasing concentrations of 20-HETE to the organ chambers. Summarized data are expressed as percent relaxations (mean ± SEM).

Metabolism of [3H] 20-HETE by Bovine Coronary Arteries

To determine whether the relaxations to 20-HETE were mediated by its cyclooxygenase-dependent metabolism to a vasodilatory 20-hydroxy-prostaglandin, [3H] 20-HETE was incubated with rings of intact and denuded vessels in the absence of tissue. The media were extracted, and the extract was analyzed by RP-HPLC. [3H] 20-HETE eluted in fractions 95 to 105. We failed to detect radio-labeled metabolites of [3H] 20-HETE in either intact or denuded vessels that were not present in the tissue-free control incubation (Fig 4). Similar results were obtained for incubations of [3H] 20-HETE with cultured bovine coronary artery endothelial cells (data not shown).

Release of Prostaglandin I$_2$ by Bovine Coronary Arteries

To determine whether the indomethacin-sensitive portion of the 20-HETE concentration-response curve for relaxation could be caused by the stimulated release of prostacyclin, we measured the release of its stable metabolite, 6-keto PGF$_{1\alpha}$, from coronary artery rings stimulated with 20-HETE. The basal release was $908 \pm 138$ pg/mL. It was increased in a concentration-related manner by 20-HETE. 20-HETE (1 $\times$ $10^{-6}$ mol/L) produced a significant increase above basal (Fig 5).

Discussion

It has been well documented that neutrophils infiltrate areas of damaged myocardium during ischemia-reperfusion injury. Once activated, neutrophils release free radicals, proteases, and metabolites of arachidonic acid. We have previously demonstrated that canine neutrophils produce 20-HETE because 20-HETE has been reported to act as both a vasoconstrictor and a vasodilator. Therefore, we intended to examine the effects of 20-HETE on bovine coronary arteries.
Coronary Vascular Effects of 20-HETE

By measuring changes in isometric tension under basal and U46619-induced contraction.

In contrast to previous studies by Escalante and coworkers in rat aortic rings, we found that 20-HETE had no contractile effects on bovine coronary arteries under basal or precontracted conditions. Instead, 20-HETE produced a concentration-related decrease in isometric tension under precontracted conditions. A portion of these relaxations in precontracted vessels was dependent on cyclooxygenase activity, as indomethacin attenuated the relaxations to 20-HETE. Other investigators have also reported a dependency on cyclooxygenase activity in mediating a portion of 20-HETE's effects. For example, Schwartzman and coworkers demonstrated that 20-HETE-induced contractions of rat aorta were mediated by a 20-hydroxy-prostaglandin-endoperoxide. Carroll et al also reported an indomethacin-sensitive action of 20-HETE in an isolated perfused rabbit kidney model. In their study, 20-HETE produced a profound decrease in renal perfusion pressure that was abolished by indomethacin.

Previous studies demonstrated that 20-HETE may induce vasodilator and vasoconstrictor effects within the same preparation. The vasodilator effects were partially blocked by indomethacin, indicating a role for cyclooxygenase metabolites in mediating a portion of the 20-HETE-induced vasodilatation. Indomethacin, a cyclooxygenase inhibitor, attenuated 20-HETE-induced coronary vasodilation, suggesting that at least a portion of the 20-HETE-induced vasodilatation was mediated by a cyclooxygenase metabolite. Because 20-HETE is produced and released by neutrophils, and because 20-HETE relaxations are attenuated by the cyclooxygenase inhibitor, indomethacin, our first belief was that 20-HETE, derived from neutrophils, was metabolized to a vasoreactive compound by cyclooxygenase in endothelial cells, smooth muscle cells, or both. However, when vessels were incubated with [3H] 20-HETE, no polar metabolites were detected.

Because 20-HETE is not metabolized itself, it is possible that 20-HETE stimulates the release of arachidonic acid in endothelial cells, smooth muscle cells, or both and that the free arachidonic acid is metabolized by cyclooxygenase to a vasoreactive eicosanoid such as prostacyclin. Our results from incubating bovine coronary arteries with 20-HETE indicate that bovine coronary arteries produce prostacyclin in response to increasing concentrations of 20-HETE. It is unclear whether there is a 20-HETE receptor located on smooth muscle cells, endothelial cells, or both and whether, once it is activated, this receptor increases phospholipase activity, and subsequently the release of free arachidonic acid, or displaces arachidonic acid from membrane lipids. Although a 20-HETE receptor has yet to be characterized, precedent does exist for arachidonic acid metabolite receptors. The prostacyclin analog, Ciloprost, was used to characterize a prostacyclin receptor in bovine coronary artery. These mechanisms could act individually or in conjunction to mediate the vasodilatory effects of 20-HETE.

Regardless of the mechanism involved in mediating 20-HETE-induced relaxations, we know that 20-HETE causes U46619-induced precontracted bovine coronary arteries to relax and that bovine coronary arteries release prostacyclin when exposed to 20-HETE. Furthermore, the change in prostacyclin concentration induced by 10^{-5} mol/L 20-HETE reflects a 1 to 3 nmol/L concentration of prostacyclin in the buffer. This concentration of prostacyclin has previously been demonstrated to produce approximately 30% relaxation of precontracted bovine coronary arteries. This is the same amount of...
Neutrophils are known to infiltrate damaged myocardial tissue during ischemia–reperfusion injury. Neutrophil-induced injury that occurs during reperfusion of a formerly ischemic myocardial region apparently results from neutrophil localization and adhesion to tissue damaged by the period of ischemia. Microvascular plugging, referred to as the "no-reflow" phenomenon, may contribute to myocardial injury that occurs during reperfusion. In fact, neutrophil depletion has been shown to reduce infarct size in these models. However, neutrophils, once activated, release many substances, and it is not clear that the damage that occurs during ischemia–reperfusion injury is mediated by arachidonic acid metabolites. Because prostacyclin release is often associated with a reduction in coronary thrombosis, it is possible that 20-HETE acts to counter the effects of neutrophil- or platelet-induced coronary obstruction by releasing prostacyclin. It is also possible that 20-HETE directly alters neutrophil accumulation. Evidence for this comes from a previous study in which we demonstrated that 20-HETE inhibits A23187-induced aggregation of neutrophils.

In summary, our results indicate that 20-HETE is a potent vasodilator of bovine coronary arteries, a portion of this vasodilation is attenuated by the cyclooxygenase inhibitor, indomethacin. Furthermore, 20-HETE is not metabolized by bovine coronary arteries. Instead, the indomethacin-sensitive relaxations to 20-HETE are mediated by the release of the vasodilatory prostanoid, prostacyclin.

Acknowledgments
The authors express their appreciation to Ms. Gretchen Barg for her secretarial assistance. These studies were supported by Grants HL51055 and GM-31278 from the National Heart, Lung, and Blood Institute of the National Institutes of Health.

References
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Hypertension. 1998;31:237-241
doi: 10.1161/01.HYP.31.1.237

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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