Isoprostanes constrict human radial artery by stimulation of thromboxane receptors, Ca^{2+} release, and RhoA activation

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Objectives: Radial artery vasospasm remains a potential cause of early graft failure after coronary bypass graft surgery, despite pretreatment with α-adrenergic or calcium channel blockers. We examined the roles of isoprostanes and prostanoid receptors selective for thromboxane A_2 in the vasoconstriction of human radial arteries.

Methods: Human radial arterial segments were pretreated intraoperatively with verapamil/papaverine or nitroglycerine/phenoxybenzamine, or not treated. In the laboratory, we measured isometric contractions in ring segments, vasoconstriction in pressurized segments, and changes in $[Ca^{2+}]$ and K^+ currents in single cells.

Results: Although phenoxybenzamine eliminated adrenergic responses, the isoprostane 15-F_{2t}-IsoP and 2 closely related E-ring molecules (15-E_{1t}-IsoP and 15-E_{2t}-IsoP) still evoked powerful contractions; 15-E_{2t}-IsoP was approximately 10-fold more potent than the other 2 agents. Responses were mediated through thromboxane receptors because they were sensitive to ICI-192605. Furthermore, they were sensitive to the Rho-kinase inhibitors Y-27632 or H-1152 (both 10^{-5} mol/L) or to cyclopiazonic acid (which depletes the internal Ca^{2+} pool), but not to nifedipine. In single cells, 15-E_{2t}-IsoP elevated $[Ca^{2+}]_i$ and suppressed K^+ current.

Conclusions: Isoprostanes accumulate after coronary artery bypass graft surgery, yet none of the currently available antispasm treatments for radial artery grafts is effective against isoprostane-induced vasoconstriction. It is imperative that more specific treatment strategies be developed. We found that isoprostane responses in radial arteries are mediated by prostanoid receptors selective for thromboxane A_2 with activation of Rho-kinase and release of Ca^{2+}. Pretreatment of radial artery grafts with Rho-associated kinase inhibitors may potentially reduce postoperative graft spasm. Clinical studies to test this are indicated.

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The long-term benefits of arterial conduits in coronary artery bypass graft (CABG) surgery are well established.[1,2] Radial artery (RA) grafts have been used frequently because of their versatile applications, easy handling, and relatively simple harvesting with low incidences of postoperative complications.[3-5] However, the relatively more muscular nature of the RA grafts makes them more susceptible to mechanical and pharmacologic stimulations,[6] which usually result in vasoconstriction (although the actual postsurgical incidence of this is unclear). In its most severe form, the entire RA graft can close off as the result of graft spasm. The precise mechanism of RA graft spasm is still not well understood: The strategies developed to treat it have largely been empirical and based on hypothetic extrapolations from other tissues, and have not succeeded in preventing RA spasm com-

Abbreviations and Acronyms

CABG = coronary artery bypass graft
KCl = potassium chloride
NE = norepinephrine
RA = radial artery
ROCK = Rho-associated kinase
TP receptors = prostanoid receptors selective for thromboxane A_2

pletely. Therefore, there is a need to better understand the mechanisms underlying perioperative RA spasm.

The CABG operation is essentially an ischemia-reperfusion injury that is known to set the stage for the production of free radicals that attack membrane lipids, leading in part to the production of metabolites known as isoprostanes.[7] The latter can be produced while the lipids are in free form, as well as when they are still esterified within the membrane (to be released even weeks later by phospholipases). In fact, isoprostanes are widely used as markers of oxidative stress. Several groups have now documented their accumulation in the blood after CABG.[8-10] However, our group and many others have shown isoprostanes to be powerfully bioactive;[7,11] for example, they are powerful vasoconstrictor agents acting through prostanoid receptors selective for thromboxane A_2 (TP receptors). Here, we compared the responsiveness of human radial arteries with 3 different isoprostanes and used a variety of pharmacologic tools to characterize the signaling pathway(s) by which they act. We examined the effects of several different isoprostanes on tension and vessel diameter in whole tissues, as well as cytosolic $[Ca^{2+}]$ and K^+ currents in single cells. Altogether, we document powerful excitatory effects of the isoprostanes on the human RA. Concurrently, we considered the relative efficacies of 2 different pharmacologic strategies currently used within the operative suite aimed at preventing vasospasm after CABG surgery.

Materials and Methods

Preparation of Arterial Tissues

All experimental procedures were approved by the ethics committee of St Joseph's Healthcare and Hamilton Health Sciences.

RA tissues were harvested as pedicles (with veins and periarterial fat) using the traditional open technique and treated by immersion for 30 minutes at room temperature in Ringer's lactate containing either verapamil plus papaverine (3.25 and 0.25 mg/mL, respectively) or phenoxybenzamine plus nitroglycerine (2.0 and 0.2 mg/mL, respectively). A third set of tissues were set aside without undergoing any such pretreatment (control). RA tissues that were not used in the surgical procedure were then transported on ice to the laboratory where the loosely adherent adipose and connective tissues were removed and the arterial vessels were cut into ring segments approximately 3 to 4 mm long, and then used immediately or stored overnight for use the following day.

Tissue Bath Technique

Radial arterial rings were mounted in standard 2.5-mL muscle baths containing Krebs–Ringer buffer (see below for composition); a stretch of 1 g was imposed perpendicularly to the axis of the lumen, and isometric contractions were recorded as we have described.[12] These were equilibrated for 1 hour by challenging with 60 mmol/L KCl 3 times, after which responsiveness to adrenergic stimuli and/or to U-46619 (a thromboxane A_2 analogue) was assessed. Where indicated, some tissues were left in the muscle baths overnight (at 37°C and with continuous bubbling) for the same assessment the following day.

Video-monitored Perfusion System

Radial arterial segments were kept overnight in Dulbecco's Modified Eagle Medium at 37°C. The segments were cannulated at both ends and mounted in a video-monitored perfusion system (Living Systems Instrumentation Inc, Burlington, Vt). The artery was bathed in an 8-mL chamber containing Krebs–Ringer buffer (continuously bubbled) on the stage of an inverted microscope and superfused externally at a rate of 2 mL/min by Krebs–Ringer buffer (at 37°C). Distal and proximal pressures were monitored, and the mean arterial pressure was controlled distally to 85 mm Hg by a pressure servocontrol system. Images were acquired using a digital camera (30 frames/sec) and passed through a video dimension analyzer that derives overall vessel diameter on a frame-by-frame basis. Arteries were equilibrated for 1 hour in Krebs-Ringer's buffer (37°C), replaced at 15-minute intervals, challenged twice with 60 mmol/L KCl, and then challenged with isoprostanes 15-F_{2t}-IsoP and 15-E_{2t}-IsoP before and after treatment with pharmacologic blockers.

Single Cell Studies

Individual myocytes were dissociated from human RA rings using collagenase, elastase, and papain/dithiothreitol, as we described previously.[13,14] Cytosolic concentrations of $[Ca^{2+}]_i$ were recorded using a custom-built confocal microscope, and membrane currents were recorded using the standard patch-clamp electrophysiologic technique, both as described previously.[13-16]

Solutions and Chemicals

Krebs–Ringer buffer contained 116 mmol/L of NaCl, 4.2 mmol/L of KCl, 2.5 mmol/L of $CaCl_2$, 1.6 mmol/L of NaH_2PO_4, 1.2 mmol/L of $MgSO_4$, 22 mmol/L of $NaHCO_3$, and 11 mmol/L of D-glucose, bubbled to maintain the pH at 7.4. L-NNA (10^{-4} mol/L) and indomethacin (10^{-5} mol/L) were also added to prevent the generation of nitric oxide and cyclo-oxygenase metabolites of arachidonic acid, respectively. Chemicals were obtained from Sigma Chemical Company (St Louis, Mo), except for U46619 (Cayman Chemical Company; Ann Arbor, Mich), ICI 192605 (Tocris; Ellisville, Mo), H-1152 (Calbiochem; distributed by VWR Canlab, Mississauga, Ontario), and fluo-4 AM and pluronic acid (Molecular Probes; distributed by Invitrogen Canada, Burlington, Ontario). Pharmacologic tools were prepared in distilled water (norepinephrine [NE]; phenylephrine; phentolamine, H-1152), eth-