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G Protein Signaling and Vein Graft Intimal Hyperplasia

Reduction of Intimal Hyperplasia in Vein Grafts by a G$_{bg}$ Inhibitor Suggests a Major Role of G Protein Signaling in Lesion Development

Mark G. Davies, Tam T.T. Huynh, Gregory J. Fulton, Robert J. Lefkowitz, Einar Svendsen, Per-Otto Hagen, Walter J. Koch

Abstract—Vein grafting results in the development of intimal hyperplasia with accompanying changes in guanine nucleotide–binding (G) protein expression and function. Several serum mitogens that act through G protein–coupled receptors, such as lysophosphatidic acid, stimulate proliferative pathways that are dependent on the G protein $\beta\gamma$ subunit ($G_{b\gamma}$)–mediated activation of p21$^{ras}$. This study examines the role of $G_{b\gamma}$ signaling in intimal hyperplasia by targeting a gene encoding a specific $G_{b\gamma}$ inhibitor in an experimental rabbit vein graft model. This inhibitor, the carboxyl terminus of the $\beta$-adrenergic receptor kinase ($\beta$ARK$_{CT}$), contains a $G_{b\gamma}$-binding domain. Vein graft intimal hyperplasia was significantly reduced by 37% ($P<0.01$), and physiological studies demonstrated that the normal alterations in G protein coupling phenotypically seen in this model were blocked by $\beta$ARK$_{CT}$ treatment. Thus, it appears that $G_{b\gamma}$-mediated pathways play a major role in intimal hyperplasia and that targeting inhibitors of $G_{b\gamma}$ signaling offers novel intraoperative therapeutic modalities to inhibit the development of vein graft intimal hyperplasia and subsequent vein graft failure. (Arterioscler Thromb Vasc Biol. 1998;18:1275-1280.)

Key Words: vein grafts  ■  carboxyl terminus of $\beta$-adrenergic receptor kinase  ■  gene transfer  ■  G proteins  ■  intimal hyperplasia

Vein grafts are the most common conduit used for surgical revascularization procedures. However, these grafts are susceptible to the development of intimal hyperplasia and accelerated atherosclerosis, which result in failure rates of 20% to 40% at 5 years.$^{1,2}$ Intimal hyperplasia is a chronic structural lesion that develops after vein graft implantation and leads to luminal stenosis and occlusion.$^3$ It may be defined as abnormal migration and proliferation of vascular smooth muscle cells with associated deposition of extracellular connective tissue matrix. The development of intimal hyperplasia in experimental vein grafts is also associated with increased or novel expression of G proteins ($\alpha$ and $\beta\gamma$ subunits) in vivo, which occurs simultaneously with phenotypically altered contractile function.$^4$ Experimental vein grafts possess increased concentrations of the G protein $\alpha_\text{em}$, $\alpha_{e\gamma}$, $\alpha_{\gamma}$, and $\beta$ subunits.$^{4,5}$ $G_{\text{em}}$ is detectable in vein grafts that display intimal hyperplasia but not in the native jugular veins.$^{4,5}$ Regarding the physiology of vein grafts, contractile responses become sensitive to pertussis toxin (PTx), which differs from the PTx-insensitive contractile response in native veins.$^4$ PTx ADP ribosylates the $G_{\gamma}/G_{\alpha}$ subunits, causing ablation of signaling.$^6$ Thus, G proteins appear to play a critical role in the phenotypical alterations associated with vein grafts and intimal hyperplasia.

The signaling mechanisms triggering the proliferation and migration of vascular smooth muscle cells are not well understood, but G proteins could be involved because several growth factors that induce mitogenesis act through membrane-embedded, G protein–coupled receptors. On stimulation, G proteins dissociate to the $G_{\alpha}$ and $G_{\beta\gamma}$ subunits, which can both lead to cellular signaling events.$^{6,7}$ Several G protein–coupled mitogens, acting primarily through PTx-sensitive G proteins, have been shown to specifically trigger intracellular signaling events via $G_{\beta\gamma}$, leading to proliferation through activation of p21$^{ras}$ ($ras$) and subsequent activation of the p42 and p44 mitogen-activated protein (MAP) kinases.$^8$ The $\beta$-adrenergic receptor kinase 1 ($\beta$ARK-1) is a $G_{b\gamma}$-dependent cytosolic enzyme that phosphorylates its activated receptor substrate after translocation to the cell membrane and binding to the membrane-anchored $\beta$ subunit.$^9$ A peptide encoded by the carboxyl terminus of $\beta$ARK-1 ($\beta$ARK$_{CT}$) contains the specific $b\gamma$-binding domain of the enzyme.$^{10}$ It was previously shown that when cells are transfected with a $\beta$ARK$_{CT}$ plasmid or when peptides con-
taining the G$_{\beta\gamma}$-binding domain of βARK$_{CT}$ are introduced into the cells, several G$_{\beta\gamma}$-dependent processes are inhibited, including activation of ras and MAP kinase by several mitogens. In addition, it has also been demonstrated that the peptide encoded by βARK$_{CT}$ is specific for G$_{\beta\gamma}$ and does not directly alter G$_{\alpha}$-mediated responses. We hypothesized that blockade of the G$_{\beta\gamma}$ subunit would result in a reduction in intimal hyperplasia. This study examined the effect of βARK$_{CT}$ on the formation of vein graft intimal hyperplasia and phenotypical functional alterations.

Methods

Experimental Design
Forty New Zealand White rabbits underwent carotid interposition vein bypass grafting. Before grafting, veins were incubated in heparinized Ringer’s lactate (controls, n = 18) or plasmid solutions containing either βARK$_{CT}$ (n = 14, 190 µg/mL) or empty plasmid DNA (plasmid n = 8, 190 µg/mL) for 30 minutes at 37°C. These concentrations were shown to produce efficient transfection in vitro. Twenty-four vein grafts (controls n = 10, plasmid n = 6, and βARK$_{CT}$ n = 8) were harvested at 28 days by perfusion fixation. Intimal and medial dimensions of vein grafts were calculated by video morphometry. Sections were taken for scanning and transmission electron microscopy. Ten vein grafts (n = 5, control and βARK$_{CT}$) were analyzed for in vitro contractile responses to norepinephrine and phenotypical functional alterations.

DNA (plasmid n = 5, control and βARK$_{CT}$) was used as the negative control as previously described. Large- and small-scale plasmid preparations of pRK5 and pRK-βARK$_{CT}$ were purified on Qiagen columns (Qiagen, Inc) before vein graft gene transfer.

Gene transfer to the experimental vein grafts was done using our previously described plasmid, which contains cDNA encoding the bovine βARK$_{CT}$ (pRK-βARK$_{CT}$). This peptide contains the experimentally determined (Gln$^{546}$-Ser$^{670}$) G$_{\beta\gamma}$-binding domain. The empty pRK5 plasmid was used as the negative control as previously described. Large-scale plasmid preparations of pRK5 and pRK-βARK$_{CT}$ were purified on Qiagen columns (Qiagen, Inc) before vein graft gene transfer.

Analysis of βARK$_{CT}$ Transgene Expression

Three-day vein grafts were used for analysis of specific transgene expression. βARK$_{CT}$ mRNA expression was determined by standard methods of RT-PCR with an RT-PCR kit using TaqPlus DNA polymerase (Stratagene, Inc). Total RNA was first isolated using the single-step reagent RNAzol (Biotecx, Inc) and treated with DNase I to eliminate any possible plasmid contamination. A βARK$_{CT}$ primer set was used to specifically amplify βARK$_{CT}$ mRNA. The primers were as follows: sense primer (corresponding to the start of βARK$_{CT}$), 5’-GAATTCCGCACACATGGG-3’; and antisense primer (corresponding to the β globin–untranslated region linked to the end of the βARK$_{CT}$ cDNA), 5’-GGGAACAAAAAGGACCTTATAG-3’. This primer set amplifies a 670-bp fragment corresponding to βARK$_{CT}$ mRNA.

Operative Procedure

Anesthesia was induced and maintained with subcutaneously injected ketamine hydrochloride (60 mg/kg, Ketaset, Bristol Laboratories) and xylazine (6 mg/kg, Anased, Lloyd Laboratories). Antibiotic prophylaxis with 30 000 IU/kg of benzathine and procaine penicillin (Durapen, Vedco, Inc) was given intramuscularly at the time of induction. Surgery was performed using an operating microscope (JKH 1402, Edward Weck, Inc) under sterile conditions.

After exposure through a midline longitudinal neck incision, the right external jugular vein was identified, its branches were coagulated by diathermy at a distance from the vein to minimize injury, and the vein was then excised. After excision, the vein was incubated in a heparinized Ringer’s lactate solution (5 IU/mL, heparin, Elkins-Sinn, Inc) containing either βARK$_{CT}$ (n = 14, 190 µg/mL) or empty plasmid DNA (plasmid n = 8, 190 µg/mL) for 30 minutes at 37°C. Ex vivo, a cannula was placed atraumatically into a branch of the jugular vein, and the incubation solution was introduced once into the lumen of the vein without developing any distension pressure to ensure luminal and adventitial exposure. The right common carotid artery was identified and dissected, and both proximal and distal control was obtained. Heparin (200 IU/kg) was administered intravenously.

A proximal longitudinal arteriotomy was made, and one end of the reversed jugular vein was anastomosed to the artery in an end-to-side manner using a continuous 10-0 microvascular monofilament nylon suture (Ethilon, Ethicon, Inc, Somerville, NJ). The distal anastomosis was performed in a similar manner. Throughout the procedure, care was taken to avoid unnecessary instrumentation of the vein graft. The right common carotid artery was ligated and divided between the two anastomoses with 4-0 silk sutures, and the wound was closed in layers.

Morphology

Vein grafts were harvested 28 days after surgery. After isolation and systemic heparinization (200 IU/kg IV), the vein grafts were perfusion fixed in situ at 80 mm Hg with an initial infusion of HBSS (Gibco Laboratories, Life Technologies, Inc), followed by 2% glutaraldehyde made up in 0.1 mmol/L cacodylate buffer (pH 7.2) supplemented with 0.1 mmol/L sucrose to give an osmolality of ~300 mOsm. After 60 minutes, the specimen was removed and immersed in the glutaraldehyde fixative for an additional 24 hours. Cross sections from the middle portion of the vein graft were processed for light microscopy. With the use of standard histological procedures, each specimen was stained with a modified Masson’s trichrome and Verhoeff’s elastic tissue stain and dimensional analysis was performed by video morphometry (Innovision 150, American Innovation). The intima and media were delineated by identification of the demarcation between the crisscross orientation of the intimal hyperplastic smooth muscle cells and circular smooth muscle cells of the media, and the outer limit of the media was defined by the interface between the circular smooth muscle cells of the media and the connective tissue of the adventitia. The thickness of each layer was also determined. A ratio of the intimal and medial areas (intimal area/intimal+medial areas) and an index of luminal diameter to cross-sectional wall thickness (luminal diameter/cross-sectional wall thickness) were calculated.

In Vitro Contractile Studies

For these studies, the animals were anesthetized, the original incision was reopened, and the jugular vein and vein graft were isolated. The middle part of each vessel was sectioned in situ into four 5-mm segments and excised. These rings were suspended immediately from 2 stainless steel hooks in 5-mL organ baths containing oxygenated Krebs solution (122 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L MgCl$_2$, 2.5 mmol/L CaCl$_2$, 15.4 mmol/L NaHCO$_3$, 1.2 mmol/L KH$_2$PO$_4$, and 5.5 mmol/L glucose, maintained at 37°C and bubbled with a mixture of 95% O$_2$ and 5% CO$_2$). One hook was fixed to the bottom of the bath, and the other was connected to a force transducer (Myograph F-60, Narco Bio-Systems). The isometric tensions of the tissues were recorded on a multichannel polygraph (Physiograph Mk111-S, Narco Bio-Systems). The tissues were then placed under 0.5 g of tension and allowed to equilibrate in physiological Krebs solution for 1 hour. During the equilibration period, the Krebs solution was replaced every 15 minutes. After equilibration, the resting tension was adjusted to 0.25-g increments from 0.25 to 2.5 g, and the maximal response to a modified oxygenated Krebs solution (60 mmol/L KCl, 66.7 mmol/L NaCl, 1.2 mmol/L MgCl$_2$, 2.5 mmol/L CaCl$_2$, 15.4 mmol/L NaHCO$_3$, 1.2 mmol/L K$_2$PO$_4$, and 5.5 mmol/L glucose) was measured at each rested tension to establish a length-tension relation. On the basis of...
Transgene Expression
Successful transfection of the vein grafts was demonstrable 3 days after surgery. βARKCT mRNA was specifically amplified from DNase I–treated total RNA using RT-PCR from vein grafts treated with pRK-βARKCT, whereas control grafts treated with the empty pRK5 plasmid showed no transgene expression at 3 days (Figure 1). No pRK5 and pRK-βARKCT RNA was detectable at 5 and 7 days, suggesting transient expression of the vector (data not shown). Because the amount of tissue available was small, protein immunoblotting for βARKCT peptide expression was not possible.

Intimal Hyperplasia
All animals survived to 28 days, and all grafts were patent at harvest. The jugular vein of the rabbit consists of a single layer of endothelial cells and 1 or 2 layers of smooth muscle cells in a loose, connective tissue matrix (histological data not shown). Microscopically, the luminal surfaces of the vein grafts from each group were covered by a layer of intact endothelial cells, beneath which lay a hyperplastic intima with the smooth muscle cells arranged in a crisscross pattern with little extracellular matrix (Figure 2). The medial smooth muscle cells in the grafts from each group appeared slender, were arranged in a circular pattern, and contained a greater amount of extracellular matrix, suggestive of medial hyper trophy. At 28 days, there was a significant (36%) reduction in intimal thickness in βARKCT vein grafts (45±4 μm, n=8) compared with either plasmid (69±3 μm, n=6) or control (70±4 μm, n=10) vein grafts without a significant change in medial thickness. Results of dimensional analysis of the control and treated groups are shown in Table 1. There was a 52% decrease in intimal area (Table 1), whereas the medial area was unchanged in the βARKCT–treated vein grafts compared with the plasmid-treated grafts (Table 1). The intimal ratio was significantly reduced in the βARKCT vein

### Results

The EC50 value, the concentration for the half-maximal response, for each agonist in each ring was calculated by logistic analysis and is expressed as −log[EC50]. All data are presented as mean±SEM, and statistical differences between groups were tested by ANOVA with post hoc Tukey-Kramer multiple-comparison tests (functional studies) and with a Kruskal-Wallis nonparametric ANOVA with post hoc Dunn’s multiple-comparison tests (morphometric data).

#### Data and Statistical Analyses

The EC50 value, the concentration for the half-maximal response, for each agonist in each ring was calculated by logistic analysis and is expressed as −log[EC50]. All data are presented as mean±SEM, and statistical differences between groups were tested by ANOVA with post hoc Tukey-Kramer multiple-comparison tests (functional studies) and with a Kruskal-Wallis nonparametric ANOVA with post hoc Dunn’s multiple-comparison tests (morphometric data).

#### Table 1. Dimensional Analysis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Plasmid</th>
<th>βARKCT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen, mm²</td>
<td>20.5±1.5</td>
<td>28.6±4.01</td>
<td>16.6±2.33*</td>
<td>0.02</td>
</tr>
<tr>
<td>Intima, mm²</td>
<td>1.14±0.09</td>
<td>1.29±0.12</td>
<td>0.62±0.03*</td>
<td>0.01</td>
</tr>
<tr>
<td>Media, mm²</td>
<td>1.08±0.11</td>
<td>1.29±0.17</td>
<td>1.12±0.10</td>
<td>0.18</td>
</tr>
<tr>
<td>Intimal ratio</td>
<td>0.52±0.02</td>
<td>0.54±0.02</td>
<td>0.36±0.02†</td>
<td>0.02</td>
</tr>
<tr>
<td>Luminal index</td>
<td>39.4±2.6</td>
<td>44.2±3.1</td>
<td>37.8±3.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Area of the lumen and the intimal and medial layers from control (n=10), plasmid treated (n=6), and βARKCT treated (n=8) grafts. The intimal ratio (intimal area/intimal + medial area) and luminal index (luminal diameter/cross-sectional wall thickness) are also shown. Values are mean±SEM. Statistical analysis was done by Kruskal-Wallis nonparametric ANOVA with post hoc Dunn’s multiple-comparison tests.

†P<0.05 vs plasmid.

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grafts ($P<0.01$, $0.36\pm0.02$, mean$\pm$SEM) compared with either the plasmid ($0.54\pm0.02$) or control ($0.52\pm0.02$) vein grafts. The luminal area of the $\beta$ARK CT-treated vein grafts was 41% less than the plasmid-treated vein grafts, whereas the luminal indices were not significantly different for the control, plasmid, and $\beta$ARK CT vein grafts, suggesting that there has been no negative modulation of remodeling in the vein grafts.

### Contractile Function of Experimental Vein Grafts

Control and $\beta$ARK CT-treated vein grafts responded with concentration-dependent contractions to the agonists norepinephrine and serotonin. In the presence of PTx at concentrations sufficient to produce 100% ADP ribosylation of G proteins, the contractile responses in control vein grafts to norepinephrine ($P<0.01$) and serotonin ($P<0.01$) were significantly reduced compared with untreated control vein grafts (Table 2). This is the typical functional alteration seen in experimental vein grafts, because native veins do not have a PTx-sensitive component in their contractile responses to these G protein–coupled agonists. In contrast, the responses of the $\beta$ARK CT-treated vein grafts to norepinephrine and serotonin were unchanged in the presence of PTx, indicating the loss of a $G_{\text{ia}}$ component (Table 2).

### Electron Microscopy of Vein Grafts

Scanning electron microscopy of both control vein grafts and vein grafts transfected with empty plasmid showed the luminal surface to be lined with sharply outlined endothelial...

**TABLE 2. Sensitivity of Contractile Responses**

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine</th>
<th>Norepinephrine</th>
<th>Serotonin</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With PTx</td>
<td>With PTx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.00$\pm0.09$</td>
<td>5.16$\pm0.09^*$</td>
<td>6.34$\pm0.10$</td>
<td>5.54$\pm0.26^*$</td>
</tr>
<tr>
<td>$\beta$ARK CT</td>
<td>5.91$\pm0.19$</td>
<td>5.81$\pm0.18$</td>
<td>6.57$\pm0.10$</td>
<td>6.55$\pm0.13$</td>
</tr>
</tbody>
</table>

Data are mean$\pm$SEM. Contractile sensitivity is shown as $-\log EC_{50}$. $^*P<0.01$ compared with corresponding vein graft not treated with PTx (ANOVA). There was no difference in the maximal contractile responses to the two agonists in the different preparations when they were normalized to their responses to the 60-mmol/L KCl solution.

**Figure 3.** A, Scanning electron micrograph from a vein graft transfected with plasmid (pRK5). Endothelial cells are sharply outlined with well-defined cell borders. Stomata are small and few (arrowheads). Some polymorphonuclear neutrophils can be seen (arrow). Magnification $\times640$. B, Scanning electron micrograph from a vein graft transfected with $\beta$ARK CT. Endothelial cell junctions are sharply outlined. Some well-defined junctional stomata are shown (arrows). The morphological features are similar to those of the plasmid-transfected vein graft. One single, somewhat deformed polymorphonuclear neutrophil is in the upper left corner. Original magnification $\times640$.  

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cells with well-defined cell borders (Figure 3A). Occasional junctional stomata were noted. Transmission electron microscopy of these vein grafts confirmed the presence of well-formed endothelial cells, beneath which were well-developed smooth muscle cells of both contractile (cytoplasm predominantly filled with contractile filaments) and synthetic (cytoplasm filled with synthetic organelles) phenotypes in a loose, connective tissue matrix. No inflammatory cells or evidence of apoptosis was identified in these grafts. Scanning electron microscopy of vein grafts transfected with βARK_{Ct} showed a similar picture for the control and plasmid-transfected vein grafts with well-preserved, normal-appearing endothelial cells with occasional stomata at their junctions on the luminal surface (Figure 3B). Transmission electron microscopy showed an ultrastructural pattern similar to that of the control and plasmid-transfected vein grafts.

**Discussion**

This study revealed 3 novel findings in the area of surgical and gene-transfer manipulations of vein grafts. First, we showed for the first time that plasmid DNA can be transiently transferred to an experimental vein graft. Second, we found that the transfection of a G_{bg} inhibitor (βARK_{Ct}) results in a significant in vivo biological effect (ie, reduction in intimal hyperplasia up to 28 days). Finally, βARK_{Ct} transfection resulted in alterations in functional coupling of G_{a} subunits to vascular agonists.

There is considerable interest in modulating the development of vein graft intimal hyperplasia by molecular means. To date, transfection of vein grafts with adenovirus has been demonstrated to have limited efficacy. LeClerc et al17 showed that β-galactosidase can be introduced into the endothelium of vein grafts at the time of operation but that the viral transfection is short-lived and is lost by 7 days. Chen et al18 demonstrated that the vascular cell adhesion molecule can be transfected into porcine vein grafts at the time of implantation but, again, with limited duration of transfection. Mann et al19 demonstrated that liposome-delivered antisense oligonucleotide can achieve effective delivery of anti-cyclin/cdk2, with a significant decrease in intimal hyperplasia, but there was an increase in medial hypertrophy compared with controls. We have demonstrated that pluronic gel delivery of c-myc anti-sense oligonucleotide will decrease vein graft intimal hyperplasia without changing the medial response.20 This study is the first to demonstrate plasmid transfection into a vein graft with a sustained biological effect. Efficient transfer of DNA was determined by βARK_{Ct} mRNA amplification in 3-day-old vein grafts. Only the vein grafts treated with the βARK_{Ct} plasmid showed transgene expression. DNase I was added to the isolated total RNA to eliminate any possibility of plasmid contamination.

Activation of both G protein–coupled and protein tyrosine kinase–coupled receptors results in a sequential cascade of phosphorylation reactions that converge at the MAP kinase step in the phosphorylation cascade.7,21–23 Intraoperative transfection of vein grafts with βARK_{Ct} resulted in a 36% reduction in intimal hyperplasia at 28 days. The marked decrease in intimal hyperplasia appears to be the result of modulation of G protein signaling activity in the vein graft smooth muscle cells. These findings suggest that G_{bg} is critical in the proliferation of smooth muscle cells required for intimal hyperplasia formation. This is a significant finding, because most of the attention is given to tyrosine kinase receptor–activating growth factors, such as platelet-derived growth factor, fibroblast growth factor, and insulin. However, in light of our findings, the role of activated G protein–coupled receptors, which subsequently leads to G_{bg}-mediated mitogenesis, should be further defined. This hypothesis is supported by our previous findings that G_{i}-coupled receptors, such as lysophosphatidic acid, α_{2a}-adrenergic, and M2 muscarinic cholinergic receptors, activate the ras–MAP kinase pathway through G_{bg}, whereas G_{a_{i}}-coupled and G_{a_{b}}-coupled receptors do not.8,24 In addition, we have also shown that a tyrosine kinase growth factor, insulin growth factor 1, can activate the ras–MAP kinase pathway through G_{bg}.25 Other agents that can couple and activate G_{a_{i}} include thrombin and angiotensin II (both of which can also couple to G_{a_{q}}). Both of these agents have been implicated in stimulating growth. We cannot directly state that G_{bg} inhibits MAP kinase activity, because we have not been able, to date, to demonstrate significant MAP kinase activity at early time points in either control or transfected vein grafts. Thus, several candidate potential G protein–coupled receptors exist that might direct vein graft intimal hyperplasia and whose signaling would be inhibited by the βARK_{Ct}.

One difference in our approach to inhibiting vascular smooth muscle proliferation is that it is targeted to a specific pathway. Approaches such as antisense oligonucleotides19,26 or herpesvirus thymidine kinase gene delivery are nonselective inhibitors of mitogenesis and may affect other cell types.27 βARK_{Ct} targets 1 common pathway that is apparently shared by several mitogens. Our findings suggest a critical role for G_{bg}, signaling in intimal hyperplasia.

Functional responses of vascular smooth muscle cells to vasoactive agonists may be representative of “stimulus-response coupling” and may broadly define the status of many of the common extracellular signals, surface receptor systems, and intracellular regulatory mechanisms present. The increases in G proteins after vein grafting1 are associated with the development of enhanced PTx-sensitive contractile responses that are known to be coupled to Go proteins.28,29 Native veins do not have PTx-sensitive contractile responses. Although βARK_{Ct} does not affect G_{a_{i}}-mediated events, intraoperative transfection with βARK_{Ct} abolished the sensitivity of the contractile responses of the vein graft to PTx. This suggests that the contractile responses in the βARK_{Ct}-transfected vein grafts are mediated predominantly by PTx-insensitive G_{a_{b}} subunits (ie, G_{a_{b}} which is more like native veins). The mechanism of G protein–coupling change is not known, and additional experiments will be required to determine whether this is a property of specific G_{bg} inhibition or another mechanism, possibly dealing with the loss of vascular smooth muscle “buildup.” Nonetheless, our results indicate that transfection of vein grafts with a plasmid containing a G_{bg} inhibitor produces a significant biological effect with the potential to have a large impact in the surgical vein graft arena.

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Conclusions
This study shows, for the first time, that plasmid-directed local gene transfer can be achieved with a biological effect. In addition, this is the first demonstration of \( \beta ARK_{CT} \)-mediated inhibition of G\textsubscript{i} function in vivo and suggests that targeting G protein pathways may offer new intraoperative therapeutic modalities to reduce the development of vein graft intimal hyperplasia and subsequent vein graft failure.

Acknowledgments
This study was supported by the US Public Health Service [grants HL15448 (P.-O.H.) and TW04810 (R.T.L.)]. Gregory J. Fulton held a Trinity College Dublin Postgraduate Scholarship. Einar Svendsen was supported by the Blix Family Foundation for Medical Research and the Astri and Edvard Riisens Legacy. The technical assistance of Bergen) is greatly appreciated. Microsutures were a gift of L. Barber, E. Tomhave, and C. Skaer (Duke University) and

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