JPET Fast Forward. Published on December 12, 2006 as DOI: 10.1124/jpet.106.115329

JPET #115329

Title Page

Sirolimus Causes Relaxation of Human Vascular Smooth Muscle: a Novel Action of Sirolimus Mediated via K_{ATP} Channels

Srinivas Ghatta, Radhika R. Tunstall, Sohail Kareem, Mohamed Rahman,

and Stephen T. O'Rourke

Department of Pharmaceutical Sciences

North Dakota State University

Fargo, ND

(S.G., R.R.T., and S.T.O.)

and

Department of Internal Medicine

University of North Dakota

Grand Forks, ND

(S.K. and M.R.)

Running Title Page

Running Title: Sirolimus-Induced Vasodilation and K_{ATP} Channels

Address correspondence to: Dr. Stephen T. O'Rourke
Department of Pharmaceutical Sciences
North Dakota State University
Fargo, ND 58105-5055
U.S.A.
Tel: (701) 231-7836
Fax: (701) 231-7606
Email: stephen.orourke@ndsu.edu

Number of text pages: 21

Number of figures: 4

Number of tables: 0

Number of references: 31

Number of words in Abstract: 250

Number of words in Introduction: 249

Number of words in Discussion: 1,057

Abbreviations: BK_{Ca}, large conductance, calcium-activated potassium channel; EC_{50}, concentration necessary to produce 50% of the maximal response; E_{max}, maximal decrease in tension; FKBP12, FK506 binding protein; K_{ATP}, ATP-sensitive potassium channel; K_V, voltage-gated potassium channel; pD_2, -log (M) EC_{50}; SK_{Ca}, small conductance, calcium-activated potassium channel; U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$

Recommended Section Assignment: Cardiovascular

JPET Fast Forward. Published on December 12, 2006 as DOI: 10.1124/jpet.106.115329
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Little is known about the vasomotor effects of sirolimus and preliminary studies using animal models have provided conflicting results. The present study was designed to determine the effects of sirolimus on vasomotor tone in human blood vessels. Human radial artery segments were cut into rings, denuded of endothelium, and placed into organ chambers for isometric tension recording. Sirolimus (10^{-10} to 10^{-6} M) caused concentration-dependent relaxation of human arteries contracted with U46619 (10^{-8} M) (pD_2= 7.28 $\pm$ 0.1; E_{max}= 57 $\pm$ 6 %) or phenylephrine (10^{-6} M) (pD_2= 7.16 $\pm$ 0.4; E_{max}= 45 $\pm$ 9 %). Sirolimus-induced relaxation was unaffected by treatment with indomethacin (10^{-5} M) but was nearly abolished in tissues contracted by depolarization with elevated K^+ (60 mM). In U46619-contracted rings, the response to sirolimus was markedly inhibited in the presence of the specific K_{ATP} channel blocker, glyburide (10^{-6} M), but was unaffected by treatment with blockers of BK_{Ca}, (iberiotoxin, 10^{-7} M), SK_{Ca} (apamin, 10^{-6} M), or K_V (4-aminopyridine, 10^{-3} M). The K_{ATP} channel opener, aprikalim (10^{-7} to 10^{-5} M), caused concentration-dependent relaxations that were inhibited by glyburide (10^{-6} M) and abolished in tissues contracted with elevated K^+ (60 mM), thus confirming that K_{ATP}-channel opening causes relaxation of these arteries. These data suggest that sirolimus, at concentrations attained in vivo, causes relaxation of human arteries and this effect is mediated by opening of K_{ATP} channels in vascular smooth muscle. Reduced vasomotor tone is a heretofore unrecognized action of sirolimus that could potentially contribute to its efficacy in drug-eluting stents.

Introduction

Drug-eluting stents are being heralded as a major therapeutic advance in the treatment of obstructed blood vessels (Fattori and Piva, 2003). In particular, stents coated with polymers that gradually release sirolimus have generated considerable interest. Sirolimus is a potent immunosuppressant and antiproliferative agent, and restenosis rates are almost negligible with sirolimus-eluting stents (Morice et al., 2002; Sousa et al., 2003).

The cellular effects of sirolimus are mediated by binding of the drug to its cytosolic receptor, the FK506 binding protein (FKBP12), which results in blockade of cell cycle progression at the G1/S transition (Sehgal, 2003). The inhibitory effects of sirolimus on cell growth and migration in blood vessels are widely recognized and are believed to play a pivotal role in the remarkable efficacy of sirolimus in maintaining the patency of implanted stents (Marks, 2003; Asnaghi et al., 2004). Surprisingly, little is known about the effects of sirolimus on blood vessel function (i.e. vasodilation and vasoconstriction). This is particularly notable since sirolimus is released directly into the circulation (Suzuki et al., 2001; Hiatt et al., 2001), where it could potentially alter blood flow and tissue perfusion in regions distal to the site of stent placement. A limited number of preliminary studies have attempted to address this issue by using animal models, but the results are conflicting (Corbin et al., 1994; Milliard et al., 1998; Jeanmart et al., 2002; Gardiner et al., 2004). Hence, the present study was designed to determine the vasomotor effects of sirolimus in human blood vessels.

Methods

Tissue Preparation:

This study was approved by the Institutional Review Board at North Dakota State University. Informed consent was obtained from all patients from whom tissue samples were collected. Studies were conducted on unused segments of isolated human radial arteries obtained during coronary artery bypass graft surgeries performed at Meritcare Medical Center, Fargo, ND. Arterial segments were placed into cold physiological salt solution (composition (in mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, edetate calcium disodium 0.026, and glucose 11.1) and transported to the laboratory. The blood vessels were cleaned of adherent fat and connective tissue and cut into rings (3 to 4 mm in length). In order to study the effect of sirolimus directly on vascular smooth muscle, experiments were performed on endothelium-denuded preparations so as to avoid any potential confounding influence by the presence of endothelial cells. In all experiments, the endothelium was removed by inserting the tips of a pair of forceps into the lumen and gently rolling the tissues back and forth over filter paper soaked in physiological salt solution. The absence of intact endothelium was confirmed functionally by testing the ability of acetylcholine (10^{-6} M) to produce endothelium-dependent relaxation during contraction evoked by phenylephrine (10^{-7} M).

Organ chamber studies:

Arterial rings were suspended in water-jacketed organ chambers filled with 25 ml of physiological salt solution, which were aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37^{o}C (O'Rourke, 1996a,b). The rings were suspended by means of

two fine stainless steel wire clips passed through their lumen; one clip was anchored to the bottom of the organ chamber, while the other was connected to a strain-gauge force transducer for the purpose of measuring isometric force. The rings were placed individually at the optimal point of their length-tension relationship by progressively stretching them until the contractile response to KCl (20 mM) was maximal. The blood vessels were then allowed to equilibrate at their optimal length for one hour prior to exposure to vasoactive substances.

Vasodilator responses were determined in rings contracted with phenylephrine (10^{-6} M), U46619 (10^{-8} M), or KCl (60 mM). After the contractions reached a stable plateau, increasing concentrations of sirolimus (10^{-10} - 10^{-6} M), diltiazem (10^{-8} - 10^{-4} M) or aprikalim (10^{-7} - 10^{-5} M) were added to the organ chambers and relaxations were recorded. In some experiments, indomethacin (10^{-5} M) was added to the organ chamber 30 minutes prior to addition of the contractile agent in order to inhibit the production of vasoactive prostanoids. In other experiments, glyburide (10^{-6} M), iberiotoxin (10^{-7} M), apamin (10^{-6} M), or 4-aminopyridine (10^{-3} M) were added to the organ chamber 30 minutes prior to addition of the contractile agent in order to inhibit ATP-sensitive K channels (K_{ATP}), large conductance, calcium-activated K channels (BK_{Ca}), small conductance, calcium-activated K channels (SK_{Ca}), and voltage-gated K channels (K_v) respectively. Control and treated rings prepared from the same artery were studied in parallel.

Data Analysis

Relaxations are expressed as a percentage of the initial vasoconstrictor-induced tone. The maximal decrease in tension (E_{max}) and the concentration necessary to produce 50% of its own maximal response (EC_{50}) were determined. The EC_{50} values were converted to the negative logarithms and expressed as pD_2 (-log (M) EC_{50}). Results are expressed as mean $\pm$ S.E.M. and *n* refers to the number of patients from whom blood vessels were taken. Mean values were compared by Student's *t* test or analysis of variance. Values were considered to be significantly different when $P< 0.05$.

Drugs and Solutions

The following drugs were used: acetylcholine, 4-aminopyridine, diltiazem, glyburide, indomethacin, phenylephrine, (Sigma Chemical Co., St. Louis, MO); apamin, iberiotoxin (Tocris, Ellisville, MO); aprikalim (Rhone Poulenc Rorer, Alfortville, France); sirolimus (LC Laboratories, Woburn, MA); and U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$; Pharmacia & Upjohn, Kalamazoo, MI). Drug solutions were prepared daily, kept on ice, and protected from light until used. All drugs were dissolved initially in distilled water with the exception of aprikalim, which was dissolved in ethanol, glyburide, which was dissolved in 0.1 N NaOH, indomethacin, which was dissolved in an aqueous solution of sodium bicarbonate, and sirolimus, which was dissolved in dimethylsulfoxide, before further dilution in distilled water. Drugs were added to the organ chambers in volumes not greater than 0.2 ml. Drug concentrations are reported as final molar concentration in the organ chamber.

Results

Sirolimus (10^{-10} to 10^{-6} M) caused concentration-dependent relaxations in isolated human radial artery rings contracted with the thromboxane A_2-mimetic, U46619 (10^{-8} M) (Figure 1, upper panel). The pD_2 was 7.28 $\pm$ 0.1 and the maximal relaxation was 57 $\pm$ 6%. The inhibitory effect of sirolimus was not limited to U46619-induced contractions since sirolimus also caused concentration-dependent relaxations (pD_2= 7.16 $\pm$ 0.4, E_{max} = 45 $\pm$ 9%) in rings contracted with the α_1-adrenoceptor agonist, phenylephrine (10^{-6} M) (Figure 1, lower panel). These concentrations of sirolimus had no direct contractile or relaxant effects on unstimulated radial artery rings. Treatment of the rings with indomethacin (10^{-5} M) had no effect on the concentration-response curve to sirolimus (Figure 2), suggesting that cyclooxygenase metabolites of arachidonic acid play no role in the relaxations elicited by sirolimus.

In radial artery rings contracted by depolarization with KCl (60 mM), sirolimus-induced relaxation was nearly abolished (Figure 3, upper panel). By contrast, diltiazem (10^{-8} to 10^{-4} M), a voltage-dependent calcium channel blocker (O'Rourke et al., 2005), completely inhibited the contractile response to KCl in a concentration-dependent manner (Figure 3, lower panel). The pD_2 was 5.33 $\pm$ 0.1 and the maximal relaxation was 97 $\pm$ 3% for diltiazem-induced relaxation of rings contracted with KCl (60 mM).

Since the lack of effect of sirolimus in tissues contracted by depolarization with KCl suggested that sirolimus-induced relaxation is sensitive to changes in membrane potential, we tested whether or not the response to sirolimus is mediated via K channel activation. In rings contracted with U46619 (10^{-8} M), the selective K_{ATP}-channel blocker, glyburide (10^{-6} M) (Ashcroft and Ashcroft, 1990), significantly inhibited sirolimus-induced relaxation (Figure 4, upper panel), whereas selective blockers of other K channel

JPET Fast Forward. Published on December 12, 2006 as DOI: 10.1124/jpet.106.115329
This article has not been copyedited and formatted. The final version may differ from this version.

subtypes, including iberiotoxin (10^{-7} M), apamin (10^{-6} M), and 4-aminopyridine (10^{-3} M) were without effect on the response to sirolimus (n=5; data not shown). Aprikalim (10^{-7} to 10^{-5} M), a selective K_{ATP}-channel opener (Atwal, 1992), caused concentration-dependent relaxations of human radial artery rings contracted with U46619, and this response was also inhibited significantly in the presence of glyburide (10^{-6} M) (Figure 4, lower panel). Like sirolimus, aprikalim (10^{-8} to 10^{-4} M) failed to cause relaxation in rings contracted by depolarization with KCl (60 mM) (n=4; data not shown).

Discussion

In the present study, we demonstrate that sirolimus causes relaxation of isolated human arteries and that this response is likely mediated via the opening of K_{ATP} channels in vascular smooth muscle. The pharmacologic actions of vasodilators may be due either to a direct effect on vascular smooth muscle, or to an indirect effect mediated via the release of a relaxing factor(s) from endothelial cells (O'Rourke et al., 2005). The vasodilator effect of sirolimus in human radial arteries is not likely due to the release of an endothelium-derived relaxing factor, such as nitric oxide or endothelium-derived hyperpolarizing factor, since the experiments were performed on arterial rings in which the endothelium had been removed. Moreover, the cyclooxygenase inhibitor, indomethacin, had no effect on the response to sirolimus, thus ruling out a role for prostacyclin or other vasodilator prostaglandins in the observed response. These findings are consistent with a direct action of sirolimus on vascular smooth muscle cells to cause relaxation of human arteries.

The smooth muscle relaxant effect of sirolimus was not limited to contractile responses evoked by a single vasoconstrictor. Sirolimus was effective in relaxing arteries contracted with the thromboxane A_2-analog, U46619, and the α_1-adrenoceptor agonist, phenylephrine, suggesting that sirolimus may interfere with multiple receptor-mediated vasoconstrictor mechanisms. By contrast, sirolimus had little effect on contractions induced by elevated extracellular potassium. Potassium-induced contractions are the result of membrane depolarization, which causes increased calcium entry via voltage-operated calcium channels in vascular smooth muscle cells (O'Rourke et al., 2005). This mechanism was verified in human radial arteries by the experiments with diltiazem, a selective inhibitor of voltage-operated calcium channels that caused complete relaxation of potassium-induced contractions in a concentration-

dependent manner. The lack of effect of sirolimus under these same conditions suggests that the mechanism of action of sirolimus is dependent on membrane potential, but unlike diltiazem, does not involve direct blockade of extracellular calcium entry via voltage-operated calcium channels.

One mechanism by which sirolimus could elicit vascular smooth muscle relaxation is via activation of K channels, which results in smooth muscle relaxation that is dependent on membrane potential and is abolished in the presence of high concentrations of extracellular potassium (Hamilton et al., 1986; Cook et al., 1988). Several K channel subtypes are expressed in vascular smooth muscle cells (Brayden, 1996), including K_{ATP}, BK_{Ca} , SK_{Ca} , and K_v. Since glyburide, a potent and selective K_{ATP}-channel blocker (Ashcroft and Ashcroft, 1990), markedly inhibited sirolimus-induced relaxations it is likely that the mechanism of this response to sirolimus involves opening of K_{ATP}-channels. Moreover, the effect of sirolimus appears to be selective for K_{ATP}-channels, since blockers of BK_{Ca} (i.e. iberiotoxin) (Galvez et al., 1990), SK_{Ca} (i.e. apamin) (Banks et al., 1979), and K_v (i.e. 4-aminopyridine) (Hille, 2001) had no effect on the concentration-response curve to sirolimus. That opening of K_{ATP}-channels causes relaxation of human radial arteries is confirmed by the results with aprikalim (Atwal, 1992), a selective K_{ATP}-channel opener that caused concentration-dependent relaxations that were, like those to sirolimus, inhibited by glyburide and abolished in the presence of high concentrations of extracellular potassium. These results are in agreement with previous studies indicating a role for K_{ATP}-channels in regulating vasomotor tone in human arteries (Miura et al., 2003; Wareing et al., 2006).

The molecular events underlying sirolimus-induced relaxation of human vascular smooth muscle cells remain to be elucidated. The pharmacologic data presented in the current study provide strong evidence for a role for K_{ATP}-channels in the smooth muscle-

relaxing effect of sirolimus; however, this interpretation requires confirmation by electrophysiological studies of the effects of sirolimus on K currents in human vascular smooth muscle. Nevertheless, the feasibility of this mechanism is supported by recent studies in neurons and cardiac myocytes, where sirolimus modulates K channel activity through binding to FKBP12, which may interact directly with K channels or associated proteins (Terashima et al., 1998; DuBell et al., 2000).

There are few reports at present of studies designed to investigate the effects of sirolimus on vasomotor tone. A direct effect of sirolimus on vasomotion was initially demonstrated in rat isolated aortic rings (Corbin et al., 1994), where acute in vitro administration of sirolimus reduced certain vasoconstrictor responses in an endothelium-dependent manner. Under in vivo conditions, however, intravenous injection of sirolimus into conscious rats caused a modest pressor response and decrease in regional blood flow (Gardiner et al., 2004), consistent with a vasoconstrictor effect. In light of the present results demonstrating that sirolimus causes endothelium-independent relaxation of human arteries, the importance of species differences and the need to assess the pharmacologic activity of vasoactive drugs in human tissues is readily apparent.

Chronic exposure to sirolimus may indirectly alter arterial diameter by causing endothelial dysfunction. In an *in vitro* porcine model, incubation of isolated coronary arteries with sirolimus for 48 hours caused impairment of endothelium-dependent relaxations evoked by serotonin and bradykinin (Jeanmart et al., 2002). In humans, implantation of sirolimus-eluting stents, but not bare metal stents, was associated with impaired endothelium-dependent vasodilation in response to exercise or acetylcholine in coronary arteries distal to the stent (Togni et al., 2005; Hofma et al., 2006). Coronary artery dilation by the endothelium-independent vasodilator, nitroglycerin, was not

altered. These data, though not directly comparable with the present results due to differences in experimental conditions, emphasize that the vasomotor effects of sirolimus are complex and may differ with regard to acute effects (i.e. vasodilation) versus long term effects (i.e. endothelial dysfunction).

In summary, sirolimus causes relaxation of human arterial smooth muscle in a manner consistent with the opening of K_{ATP}-channels. The smooth muscle-relaxing effect of sirolimus is observed at physiologically relevant concentrations, inasmuch as systemic whole blood levels of sirolimus on the order of ~10 nM may be achieved following stent implantation in coronary arteries (Suzuki et al., 2001; Yu et al., 2004) and oral administration of sirolimus to prevent restenosis (Guarda et al., 2004; Rodriguez et al., 2005). Although sirolimus concentrations of this magnitude are on the low end of the concentration-response relationship reported in the present study, it is likely that significantly higher local drug concentrations are attained in arteries located in close proximity to a drug-eluting stent, since the lipophilicity of sirolimus considerably enhances its uptake, distribution and retention in the arterial wall (Schreiber, 1991; Suzuki et al., 2001). Thus, relaxation of vascular smooth muscle is a heretofore unrecognized action of sirolimus that could potentially contribute to its efficacy in drug-eluting stents.

References

Ashcroft SJ and Ashcroft FM (1990) Properties and functions of ATP-sensitive K-channels. *Cell Signal* **2**:197-214.

Asnaghi L, Bruno P, Priulla M and Nicolin A (2004) mTOR: a protein kinase switching between life and death. *Pharmacol Res* **50**:545-549.

Atwal KS (1992) Modulation of potassium channels by organic molecules. *Med Res Rev* **12**:569-591.

Banks BE, Brown C, Burgess GM, Burnstock G, Claret M, Cocks TM and Jenkinson DH (1979) Apamin blocks certain neurotransmitter-induced increases in potassium permeability. *Nature* **282**:415-417.

Brayden JE (1996) Potassium channels in vascular smooth muscle. *Clin Exp Pharmacol Physiol* **23**:1069-1076.

Cook NS, Weir SW and Danzeisen MC (1988) Anti-vasoconstrictor effects of the K+ channel opener cromakalim on the rabbit aorta--comparison with the calcium antagonist isradipine. *Br J Pharmacol* **95**:741-752.

Corbin F, Blaise GA, Parent M, Chen H and Daloze PM (1994) Effect of rapamycin on rat aortic ring vasomotion. *J Cardiovasc Pharmacol* **24**:813-817.

DuBell WH, Lederer LW, Rogers TB (2000) K(+) currents responsible for repolarization in mouse ventricle and their modulation by FK-506 and rapamycin. *Am J Physiol Heart Circ Physiol* **278**:H886-897.

Fattori R and Piva T (2003) Drug-eluting stents in vascular intervention. *Lancet* **361**:247-249.

Galvez A, Gimenez-Gallego G, Reuben JP, Roy-Contancin L, Feigenbaum P, Kaczorowski GJ and Garcia ML (1990) Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion Buthus tamulus. *J Biol Chem* **265**:11083-11090.

Gardiner SM, March JE, Kemp PA, Fallgren B and Bennett T (2004) Regional haemodynamic effects of cyclosporine A, tacrolimus and sirolimus in conscious rats. *Br J Pharmacol* **141**:634-643.

Guarda E, Marchant E, Fajuri A, Martinez A, Moran S, Mendez M, Uriarte P, Valenzuela E and Lazen R (2004) Oral rapamycin to prevent human coronary stent restenosis: a pilot study. *Am Heart J* **148**:e9.

Hamilton TC, Weir SW and Weston AH (1986) Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br J Pharmacol* **88**:103-111.

Hiatt BL, Carter AJ and Yeung AC (2001) The drug-eluting stent: is it the Holy Grail? *Rev Cardiovasc Med* **2**:190-196.

Hille B (2001) *Ion Channels of Excitable Membranes*. Sinauer Assoc., Sunderland, MA.

Hofma SH, van der Giessen WJ, van Dalen BM, Lemos PA, McFadden EP, Sianos G, Ligthart JM, van Essen D, de Feyter PJ and Serruys PW (2006) Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. *Eur Heart J* **27**:166-170.

Jeanmart H, Malo O, Carrier M, Nickner C, Desjardins N and Perrault LP (2002) Comparative study of cyclosporine and tacrolimus vs newer

immunosuppressants mycophenolate mofetil and rapamycin on coronary endothelial function. *J Heart Lung Transplant* **21**:990-998.

Marks AR (2003) Rapamycin: signaling in vascular smooth muscle. *Transplant Proc* **35**:231S-233S.

Milliard S, Silva A, Blaise G, Chen H, Xu D, Qi S and Daloze P (1998) Rapamycin's effect on vasomotion in the rat. *Transplant Proc* **30**:1036-1038.

Miura H, Wachtel RE, Loberiza FR Jr, Saito T, Miura M, Nicolosi AC and Gutterman DD (2003) Diabetes mellitus impairs vasodilation to hypoxia in human coronary arterioles: reduced activity of ATP-sensitive potassium channels. *Circ Res* **92**:151–158.

Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnar F and Falotico R (2002) A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med* **346**:1773-1780.

O'Rourke ST (1996a) Effects of potassium channel blockers on resting tone in isolated coronary arteries. *J Cardiovasc Pharmacol* **27**:636-642.

O'Rourke ST (1996b) K_{ATP} channel activation mediates nicorandil-induced relaxation of nitrate-tolerant coronary arteries. *J Cardiovasc Pharmacol* **27**:831-837.

O'Rourke ST, Vanhoutte PM, and Miller VM (2005) Biology of Blood Vessels: Vascular Pharmacology., in *Vascular Medicine: A Companion to Braunwald's Heart Disease* (M.A. Creager VJD, and J. Loscalzo ed) pp 71-98, Saunders Elsevier, Philadelphia.

Rodriguez AE, Rodriguez Alemparte M, Vigo CF, Fernandez Pereira C, Llaurado C, Vetcher D, Pocovi A and Ambrose J (2005) Role of oral rapamycin to prevent restenosis in patients with de novo lesions undergoing coronary stenting: results of the Argentina single centre study (ORAR trial). *Heart* **91**:1433-1437.

Schreiber SL (1991) Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* **251**:283-287.

Sehgal SN (2003) Sirolimus: its discovery, biological properties, and mechanism of action. *Transplant Proc* **35**:7S-14S.

Sousa JE, Costa MA, Abizaid A, Sousa AG, Feres F, Mattos LA, Centemero M, Maldonado G, Abizaid AS, Pinto I, Falotico R, Jaeger J, Popma JJ and Serruys PW (2003) Sirolimus-eluting stent for the treatment of in-stent restenosis: a quantitative coronary angiography and three-dimensional intravascular ultrasound study. *Circulation* **107**:24-27.

Suzuki T, Kopia G, Hayashi S, Bailey LR, Llanos G, Wilensky R, Klugherz BD, Papandreou G, Narayan P, Leon MB, Yeung AC, Tio F, Tsao PS, Falotico R and Carter AJ (2001) Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation* **104**:1188-1193.

Terashima A, Nakai M, Hashimoto T, Kawamata T, Taniguchi T, Yasuda M, Maeda K and Tanaka C (1998) Single-channel activity of the Ca2+-dependent K+ channel is modulated by FK506 and rapamycin. *Brain Res* **786**:255-258.

Togni M, Windecker S, Cocchia R, Wenaweser P, Cook S, Billinger M, Meier B and Hess OM (2005) Sirolimus-eluting stents associated with paradoxic coronary vasoconstriction. *J Am Coll Cardiol* **46**:231-236.

Wareing M, Bai X, Seghier F, Turner CM, Greenwood SL, Baker PN, Taggart MJ, and Fyfe GK (2006) Expression and function of potassium channels in the human placental vasculature. *Am J Physiol Regul Integr Comp Physiol* **291**:R437-46.

Yu MY, Gao RL, Jiang J, Cheng SJ, Yuan JQ, Wang CN, Zheng JG, Meng L and Zi ZJ (2004) Pharmacokinetics of rapamycin-eluting stents in miniswine coronary model. *Chin Med J (Engl)* **117**:1459-1463.

Footnotes

This work was supported by a Grant-in-Aid from the American Heart Association to S.T.O.

Address for reprint requests: Dr. Stephen T. O'Rourke, Department of Pharmaceutical Sciences, North Dakota State University, PO Box 5055, Fargo, ND 58105-5055

Email: stephen.orourke@ndsu.edu

[1]Current address: Dr. Srinivas Ghatta, UW 2531, RI-CEDD, GlaxoSmithKline, 709 Swedeland Rd, King of Prussia, PA 19406

Legends for Figures

Figure 1: Relaxation response to sirolimus in isolated human radial arteries. Radial arterial rings were contracted with either U46619 (10^{-8} M) (upper panel), which produced contractions averaging 2.46 $\pm$ 0.3 g, or phenylephrine (10^{-6} M) (lower panel), which produced contractions averaging 2.20 $\pm$ 0.7 g. After the contractile response reached a stable plateau, increasing concentrations of sirolimus were added to the tissues. Each point represents the mean $\pm$ SEM (n=5-12).

Figure 2: Sirolimus-induced relaxation of isolated human radial arteries in the absence (open symbols) or presence (filled symbols) of indomethacin. Radial arterial rings were incubated with or without indomethacin (10^{-5} M) for 30 min prior to contraction with U46619 (10^{-8} M), followed by addition of increasing concentrations of sirolimus to the tissues. The data are expressed as a percentage of the U46619-induced increase in tension, which averaged 2.38 $\pm$ 0.5 g in control rings and did not differ significantly in rings incubated with indomethacin. Each point represents the mean $\pm$ SEM (n=5).

Figure 3: Response of isolated human radial arteries to sirolimus (upper panel) and diltiazem (lower panel). Radial arterial rings were contracted with KCl (60 mM) prior to addition of increasing concentrations of either sirolimus or diltiazem to the tissues. The data are expressed as a percentage of the KCl-induced increase in tension, which averaged 4.75 $\pm$ 1.2 g. Each point represents the mean $\pm$ SEM (n=5).

Figure 4: Response of isolated human radial arteries to sirolimus (upper panel) and aprikalim (lower panel). Radial arterial rings were incubated with (filled bars) or without (open bars) glyburide (10^{-6} M) for 30 min prior to contraction with U46619 (10^{-8} M). After the contractile response reached a stable plateau, increasing concentrations of either sirolimus or aprikalim were added to the tissues. The data are expressed as a percentage of the U46619-induced increase in tension, which averaged 2.97 $\pm$ 0.3 g in control rings and did not differ significantly in rings incubated with glyburide. Each bar represents the mean $\pm$ SEM (n=5). *denotes a statistically significant difference ($P<0.05$) between responses obtained in the absence and presence of glyburide.

JPET Fast Forward. Published on December 12, 2006 as DOI: 10.1124/jpet.106.115329
This article has not been copyedited and formatted. The final version may differ from this version.

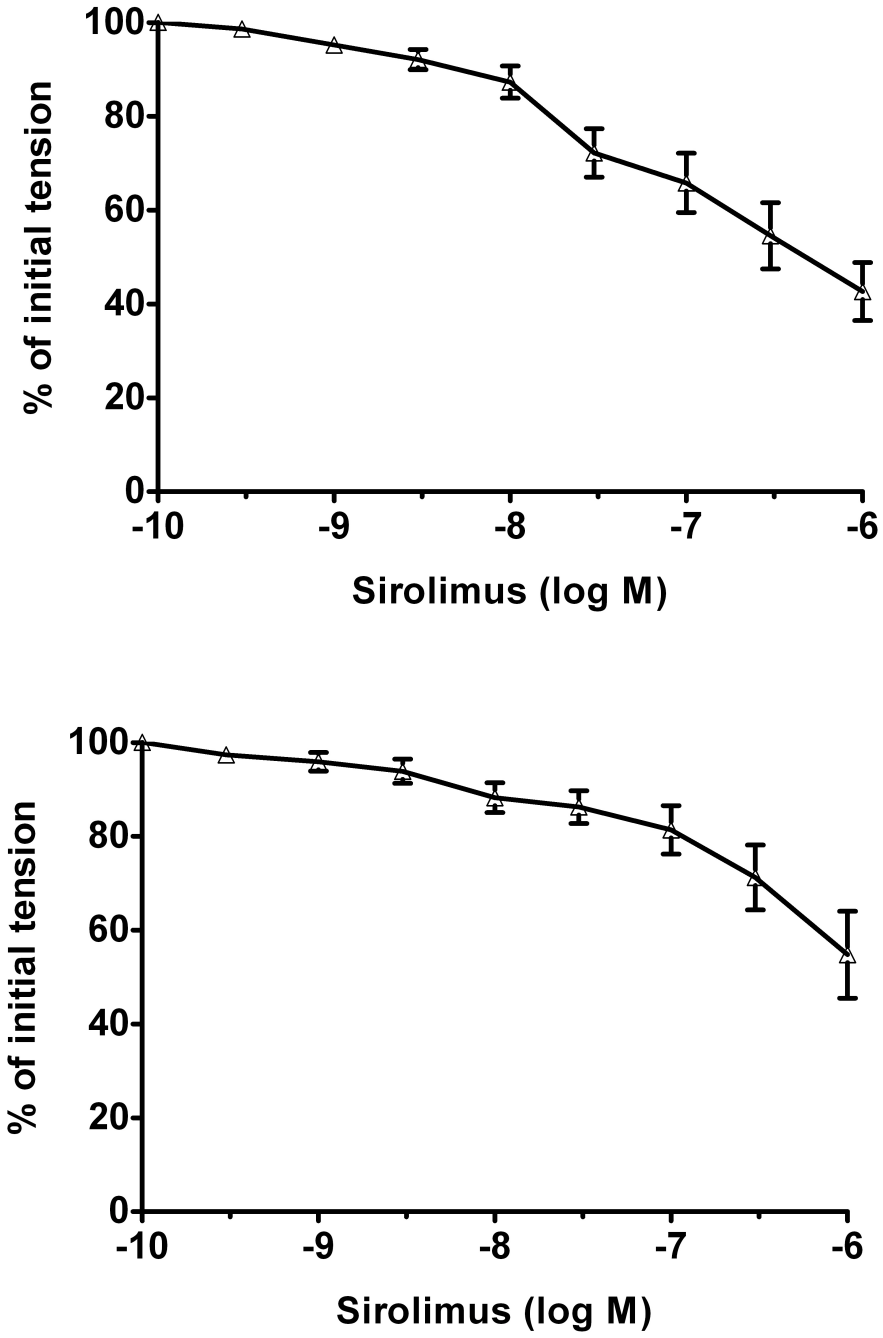


Figure 1

JPET Fast Forward. Published on December 12, 2006 as DOI: 10.1124/jpet.106.115329
This article has not been copyedited and formatted. The final version may differ from this version.

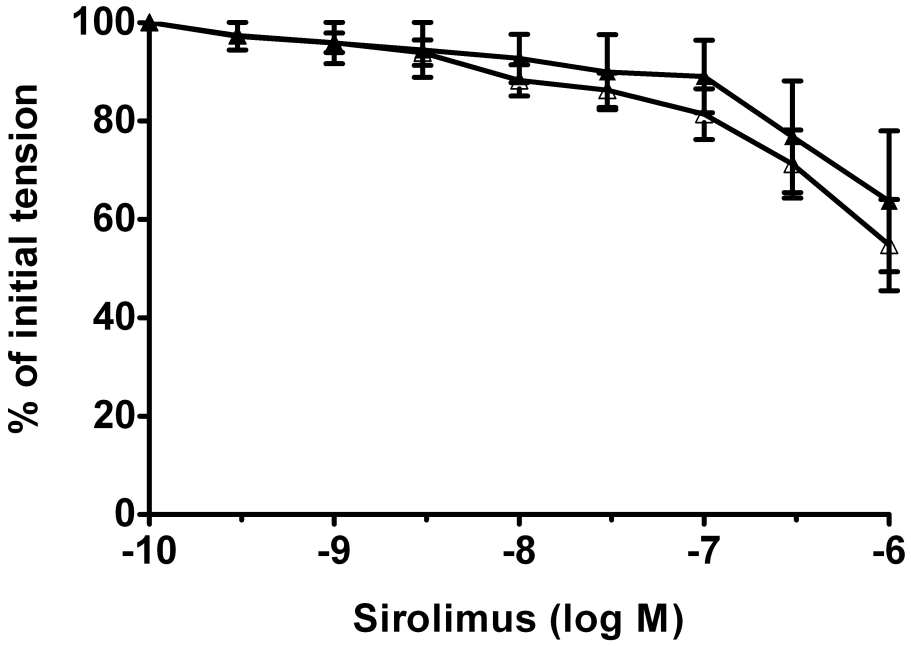
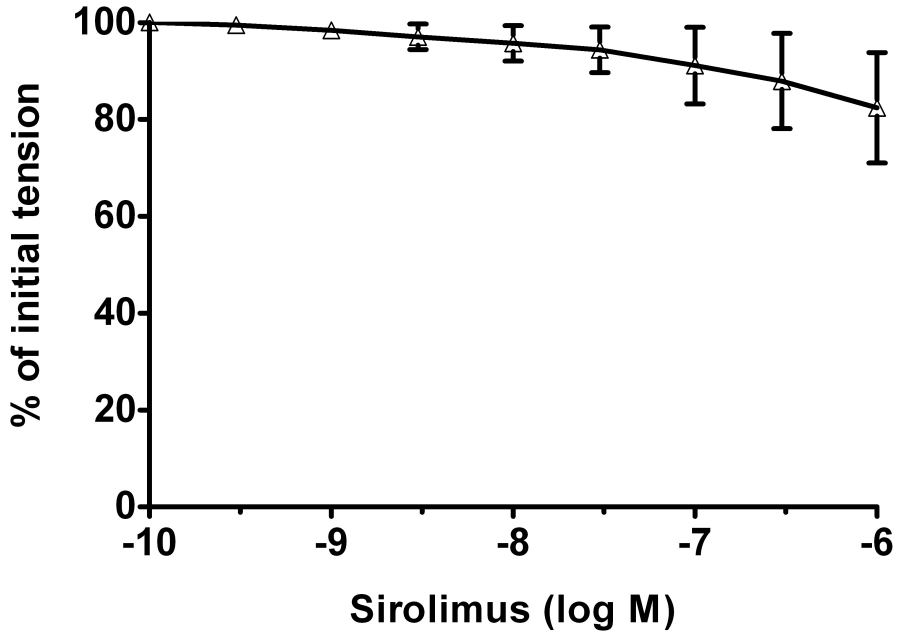


Figure 2

JPET Fast Forward. Published on December 12, 2006 as DOI: 10.1124/jpet.106.115329
This article has not been copyedited and formatted. The final version may differ from this version.



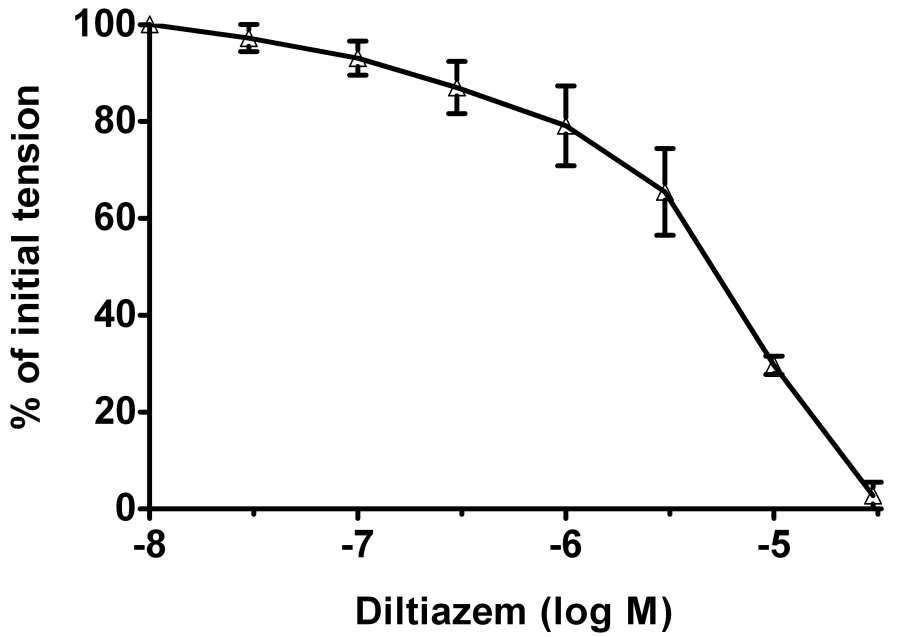


Figure 3

JPET Fast Forward. Published on December 12, 2006 as DOI: 10.1124/jpet.106.115329
This article has not been copyedited and formatted. The final version may differ from this version.

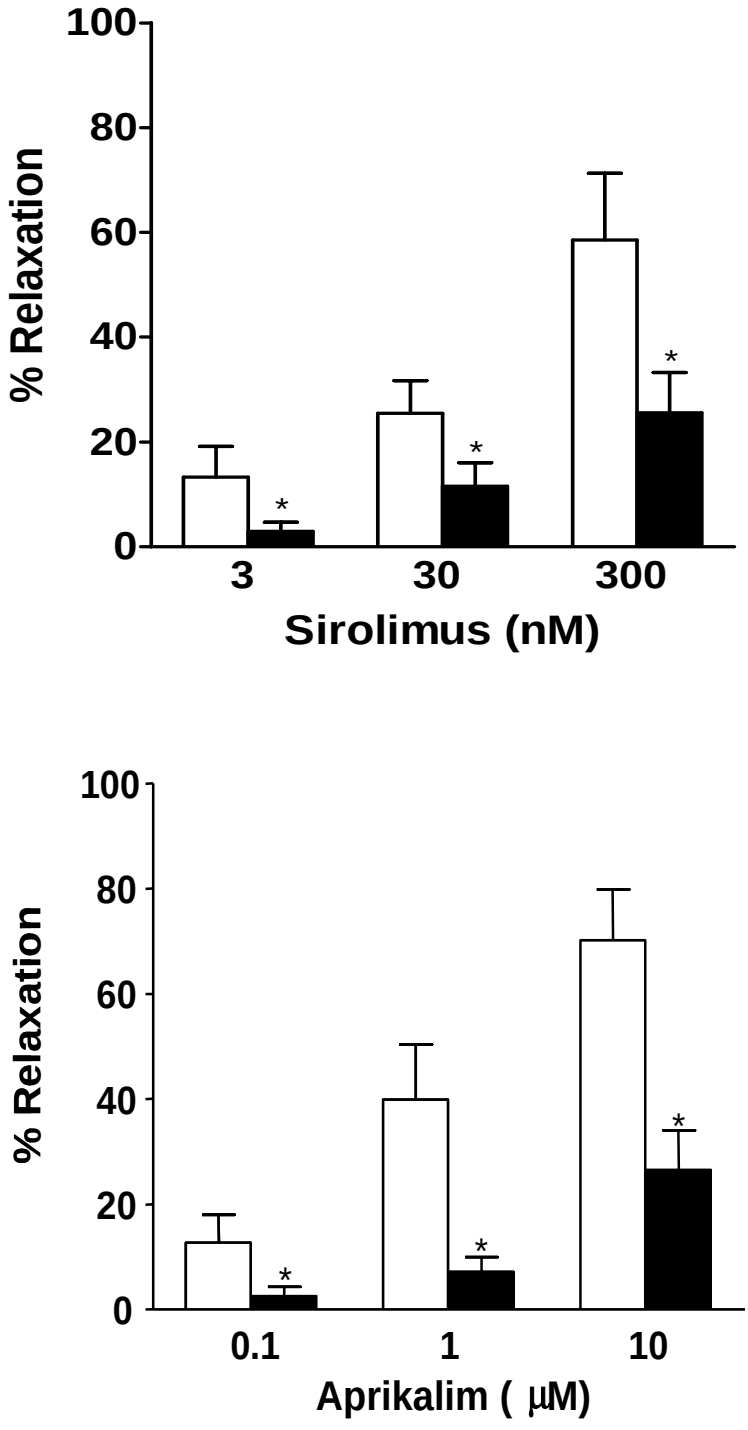


Figure 4