# Statin Mediated Vasodilation in the Vasculature

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*Dedicated to the memory of John McEachern*

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Signed,

Scott Copley

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**THESIS ABSTRACT**

Clinical trials have established the efficacy and safety of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) in lowering cardiovascular morbidity and mortality in patients with and without coronary artery disease. Traditionally, the beneficial effects of statins have been ascribed entirely to their ability to lower serum cholesterol. Evidence indicates, however, that statins may exert cholesterol-independent or "pleiotropic" effects.

Statins may modulate vascular reactivity via the inhibition the RhoA/Rho-kinase pathway in both the vascular endothelium and the underlying vascular smooth muscle. To examine this hypothesis, we coupled the measurement of isometric force in isolated rat caudal artery segments with molecular analysis of the downstream targets of Rho-kinase in both the endothelium and smooth muscle.

We report that clinical concentrations of pravastatin inhibit $\alpha_1$-adrenoreceptor mediated vascular contraction through an endothelial-dependent mechanism. Our results suggest that this is mediated by an increase in P[Ser1177]eNOS phosphorylation, consistent with increased eNOS activation, increased nitric oxide production and the inhibition of the RhoA/Rho-kinase pathway in the vascular endothelium.

In the context of Thromboxane$A_2$ ($TxA_2$) receptor-mediated contraction we report that acute high dose simvastatin administration causes a robust reduction in contraction. We describe a concomitant increase eNOS Ser1177 phosphorylation, suggesting activation of eNOS and increased NO production, however, experiments in which

eNOS was inhibited suggest that this mechanism does not account for the majority of relaxation. Perhaps more importantly, we report the increased activation of smooth muscle myosin phosphatase that may account for simvastatin-mediated relaxation in this preparation.

Extending these results to a chronic setting we examined the consequence of 7-day statin administration on rats. Using non-invasive tail cuff we demonstrate reductions in the systolic blood pressure of healthy rats treated with clinically relevant doses of simvastatin for 7 days. Using a perfused isolated heart model we report reduced $TxA_2$-receptor mediated coronary perfusion pressure in hearts isolated from these animals and a reduction in $TxA_2$-receptor mediated contraction in isolated blood vessels. Western blot analysis revealed an increase in the expression of endothelial nitric oxide synthase (eNOS) that was concomitant with these effects. Additional administration of high dose simvastatin further reduced $TxA_2$-receptor mediated contraction via disinhibition of smooth muscle myosin phosphatase.

These results suggest that statins may be a viable treatment option to effect acute vasodilatation in patients with normal cholesterol levels but with abnormal vasomotor reactivity and/or endothelial dysfunction.

## COMMON ABREVIATIONS

| | |
|---|---|
| $[Ca^{2+}]_{cyt}$ | cytosolic $Ca^{2+}$ concentration |
| 5-HT | serotonin |
| AA | arachidonic acid |
| ACh | acetylcholine |
| BP | blood pressure |
| cAMP | cyclic guanosine monophosphate |
| cGMP | cyclic adenosine monophospate |
| CAD | coronary artery disease |
| CHD | coronary heart disease |
| COX | cyclooxygenase |
| CPI-17 | PKC-potentiated inhibitory protein of 17 kDa |
| CRP | C-reactive protein |
| CSFP | coronary slow flow phenomenon |
| DAG | diacylglycerol |
| EC | effective concentration |
| ECL | enhanced chemiluminescence |
| EDHR | endothelial derived hyperpolarizing |
| EET | epoxyeicosatrienoic acid |
| Emax | maximal contraction factor |
| EDRF | endothelial derived relaxing factor |
| HMG-CoA | 3-hydroxy-3-methyl-glutaryl-CoA reductase |
| eNOS | endothelial nitric oxide synthase |
| ET-1 | endothelin-1 |
| FH | familial hypercholesterolemia |
| FPP | farnesyl pyrophosphate |
| GGPP | geranylgeranyl pyrophosphate |
| GSNO | S-nitrosoglutathione |
| GTP | guanosine triphosphate |
| HDL | high density lipoprotein |
| HR | heart rate |
| HUVEC | human umbilical vein endothelial cell |
| IgG | immunoglobulin G |

| | |
|---|---|
| ILK | integrin linked kinase |
| $IP_3$ | inositol trisphosphate |
| L-NAME | $N_\omega$-Nitro-L-arginine methyl ester |
| $LC_{20}$ | 20 kDa light chains of myosin |
| LDL | low density lipoprotein |
| MAP | mean arterial pressure |
| MI | myocardial infarction |
| MLCK | myosin light chain kinase |
| MLCP | myosin light chain phosphatase |
| MS | multiple sclerosis |
| MYPT1 | myosin phosphatase targeting subunit |
| NO | nitric oxide |
| NOS | nitric oxide synthase |
| OD | optical density |
| P | statistical probability |
| PCI | percutaneous coronary intervention |
| $PGI_2$ | prostacyclin |
| PI3K | phosphatidylinositol 3-kinase |
| PKA | protein kinase A |
| PKC | protein kinase C |
| PKG | protein kinase G |
| PLC | phospholipase C |
| PP1c | protein phosphatase type 1 |
| RA | rheumatoid arthritis |
| ROK | Rho associated kinase (Rho-kinase) |
| ROS | reactive oxygen species |
| S.E.M | standard error of the mean |
| SDS | sodium dodecyl sulfate |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| Ser | serine |
| SR | sarcoplasmic reticulum |
| TBS | tris buffered saline |
| TBS-T | tris buffered saline – Tween 20 |
| TCA | trichloroacetic acid |

| | |
|---|---|
| Thr | threonine |
| TPR | total peripheral resistance |
| TRPC | transient receptor potential channel |
| $TxA_2$ | thromboxane $A_2$ |
| VSM | vascular smooth muscle |
| ZIPK | zipper interacting protein kinase |