

Regulation of Ca<sup>2+</sup>-Dependent  
Vasoconstriction in Large and  
Small Arteries

**Yann Yoong Chan**

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The University of Adelaide

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# TABLE OF CONTENTS

<b>Thesis declaration</b> .....	i
<b>Author statements</b> .....	ii
<b>Acknowledgements</b> .....	vi
<b>Abbreviations</b> .....	vii
<b>List of figures</b> .....	x
<b>Conference presentations during candidature</b> .....	xiv
<b>Abstract</b> .....	xv
<b>A. Literature review</b>	
A1. Cardiovascular disease.....	1
A1.1. Pathophysiology of atheroma, thrombosis and vasospasm.....	1
A1.2. Acute coronary syndrome.....	2
A1.3. Myocardial infarction/ stroke.....	2
A1.4. Peripheral Artery Disease .....	3
A1.5. Microvascular disorder.....	4
A1.5.1. Hypertension.....	4
A1.5.2. Coronary microvascular disorders.....	5
A1.5.3. Cerebral vasospasm.....	6
A2. Mechanisms	
A2.1. Endothelial regulation of vascular tone.....	7
A2.1.1. Nitric oxide.....	7
A2.1.2. Prostacyclin.....	9
A2.1.3. Endothelial-derived hyperpolarising factor (EDHF).....	10
A2.2. Vascular smooth muscle (VSMC).....	10
A2.2.1. Regulation of contraction – myosin light chain kinase.....	11
A2.2.2. Ca <sup>2+</sup> sensitisation – myosin light chain phosphatase through RhoA/ Rho kinase and protein kinase C/ CPI-17 signalling pathways.....	11
A2.2.3. Premise for Ca <sup>2+</sup> -dependent and independent vasoconstriction...	12
A2.3. Ionic basis of Ca <sup>2+</sup> entry and regulation.....	15
A2.3.1. Voltage-gated Ca <sup>2+</sup> channels.....	18

---

---

A2.3.2. Ligand-gated/ Receptor-operated Ca <sup>2+</sup> channels.....	19
A2.3.2.1. Transient Receptor Potentiation channels.....	20
A2.3.2.2. Non-selective cation channels.....	21
A2.3.3. Store-operated Ca <sup>2+</sup> channels.....	21
A2.3.4. Intracellular Ca <sup>2+</sup> .....	22
2.3.4.1. Ryanodine receptors.....	23
2.3.4.2. Inositol Triphosphate Receptors.....	24
A2.3.5. Regulation of membrane potential.....	25
A2.3.6. Potassium channels.....	26
A2.3.6.1. Voltage-gated K <sup>+</sup> channels.....	27
A2.3.6.2. Ca <sup>2+</sup> -activated K <sup>+</sup> channels.....	28
A2.3.6.3. ATP-sensitive K <sup>+</sup> channels.....	30
A2.3.6.4. Inward-rectifier K <sup>+</sup> channels.....	31
A2.3.7. Chloride channels.....	31
A2.4. Ca <sup>2+</sup> extrusion pathways.....	32
A2.4.1. Extracellular Ca <sup>2+</sup> - PMCA.....	33
A2.4.2. Intracellular Ca <sup>2+</sup> - SERCA.....	33
A3. Current and potential treatments for managing vasoconstriction.....	34
A3.1. L-type Ca <sup>2+</sup> channel blockers.....	35
A3.2. Combined L-/T-type Ca <sup>2+</sup> channel blockers.....	36
A3.3. Statins.....	37
A4. Physiological-regulation of Ca <sup>2+</sup> entry mediated by GPCR in the vasculature.....	40
A4.1. Thromboxane A <sub>2</sub> .....	40
A4.2. Endothelin-1.....	42
A5. Aims.....	45

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<b>B. ET-1 signalling, is it time to think beyond traditional Ca<sup>2+</sup> channel antagonists: is there a role for acute blockade of vascular SR Ca<sup>2+</sup> release and Rho kinase?</b>	46
B1. Introduction.....	47
B2. Methods	
B2.1. Drugs and reagents.....	49
B2.2. Isolated vessel preparation.....	49
B2.3. Functional myography.....	50
B2.4. Western blotting.....	51
B2.5. Cell isolation.....	53
B2.6. Patch clamping.....	54
B2.7. Data analysis.....	55
B3. Results	
B3.1. Extracellular Ca <sup>2+</sup> entry via L-type Ca <sup>2+</sup> channels (Ca <sub>v</sub> 1.2) is required for ET-1-mediated vasoconstriction.....	56
B3.2. Ca <sup>2+</sup> sensitisation is an important mediator of ET-1-mediated vasoconstriction.....	60
B3.3. SR Ca <sup>2+</sup> release contributes significantly to ET-1-mediated vasoconstriction.....	63
B3.4. Na <sup>+</sup> and Cl <sup>-</sup> are not required to initiate ET-1-mediated vasoconstriction.....	64
B4. Discussion.....	65
<b>C. Na<sup>+</sup> entry mediates TxA<sub>2</sub> receptor-dependent Ca<sub>v</sub>1.2 channel activation in rat mesenteric but K<sup>+</sup> flux mediates vasoconstriction in rat caudal artery</b>	69
C1. Introduction.....	70
C2. Methods	
C2.1. Drugs and reagents.....	72
C2.2. Animal and tissue preparation.....	72
C2.3. Functional myography.....	73

---

---

C2.3.1. Selectivity of SKF 96365.....	73
C2.3.2. Ionic substitution of Na <sup>+</sup> with membrane impermeant NMDG <sup>+</sup> .....	74
C2.3.3. BK <sub>Ca</sub> channels.....	74
C2.3.4. SR Ca <sup>2+</sup> depletion using CPA.....	74
C2.3.5. Cl <sup>-</sup> channels.....	75
C2.4. Cell isolation.....	75
C2.5. Patch clamping.....	75
C2.6. Data analysis.....	76
C3. Results	
C3.1. Can we effectively use SKF96365 as a selective NSCC inhibitor?.....	78
C3.2. Extracellular Na <sup>+</sup> entry through NSCC is required for the activation of L-type Ca <sup>2+</sup> channels in TxA <sub>2</sub> receptor-mediated vasoconstriction in rat mesenteric but not caudal arteries.....	81
C3.3. The role of BK <sub>Ca</sub> channels in the <u>activation</u> of L-type Ca <sup>2+</sup> channels in TxA <sub>2</sub> receptor-dependent vasoconstriction.....	83
C3.4. SR Ca <sup>2+</sup> release was not involved in TxA <sub>2</sub> receptor-mediated vasoconstriction.....	85
C3.5. Cl <sup>-</sup> channels contributed to the activation of L-type Ca <sup>2+</sup> channels in TxA <sub>2</sub> receptor-mediated vasoconstriction.....	87
C4. Discussion.....	89
<b>D. The acute vasomotor responses of simvastatin and its molecular mechanisms</b>	<b>93</b>
D1. Introduction.....	94
D2. Methods	
D2.1. Drugs and reagents.....	97
D2.2. Animal and tissue preparation.....	97
D2.3. Functional myography.....	97
D2.4. Cell isolation.....	98
D2.5. Patch clamping.....	99

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---

D2.6. Western blotting.....	100
D2.7. Data analysis.....	102
D3. Results	
D3.1. Short-term, 30-minute simvastatin treatment attenuated TxA <sub>2</sub> receptor-mediated vasoconstriction but not TxA <sub>2</sub> receptor-mediated MYPT phosphorylation in isolated rat caudal arteries.....	103
D3.2. Short-term 30-minute simvastatin treatment significantly attenuated TxA <sub>2</sub> receptor-mediated vasoconstriction in isolated rat mesenteric arteries, independent of endothelial NO or prostacyclin (PGI) synthesis.....	105
D3.3. Treatment with short-term (30 minutes), high dose simvastatin did not attenuate the Thr855 or Thr697 phosphorylation state of MYPT from isolated rat mesenteric arteries stimulated by a TxA <sub>2</sub> receptor agonist.....	107
D3.4. 30-minute, high dose simvastatin treatment increased phosphorylation of Ser-1177 site of eNOS in isolated rat mesenteric arterial segments stimulated by a TxA <sub>2</sub> receptor agonist.....	109
D3.5. Voltage-dependent Ca <sub>v</sub> 1.2 current was inhibited by simvastatin.....	111
D3.6. Short-term (30 minutes) high dose simvastatin attenuated the contractile response to high K <sup>+</sup> -mediated depolarisation.....	113
D4.	
Discussion.....	114
<b>E. General Discussion</b>	
E1. Summary.....	118
E2. Study limitations.....	122
E3. Clinical implications.....	123
<b>References</b> .....	124

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# DECLARATION

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Signed,

Yann Yoong Chan

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Statements of authorship appear in the print copy of  
the thesis held in the University of Adelaide Library.

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## ABBREVIATIONS

9-AC	9-anthracenecarboxylic acid
AA	Arachidonic acid
ACE	Angiotensin converting enzyme
ACS	Acute Coronary Syndrome
BH <sub>4</sub>	Tetrahydrobiopterin
BK <sub>Ca</sub>	Ca <sup>2+</sup> -activated K <sup>+</sup> channels
[Ca <sup>2+</sup> ] <sub>cyt</sub>	Cytosolic Ca <sup>2+</sup> concentration
CaM	Calmodulin
CCB	Ca <sup>2+</sup> channel blocker
CFTR	Cystic fibrosis transmembrane regulator
cGMP	Cyclic guanosine monophosphate
COX	Cyclooxygenase
CPA	Cyclopiazonic acid
CPI-17	17kDa C kinase-activated phosphatase inhibitor
CRAC	Ca <sup>2+</sup> -release activated Ca <sup>2+</sup> channel
CSFP	Coronary slow flow phenomenon
DAG	Diacylglycerol
DFP	Diisopropyl fluorophosphate
DTT	1,4-dithiothreitol
ECL	Enhanced chemi-luminescence
EDH	Endothelial-derived hyperpolarisation
EDHF	Endothelial-derived hyperpolarising factor
EGTA	ethylene glycol-bis(2-aminoethyl ether)- <i>N,N,N',N'</i> -tetraacetic acid
E <sub>K</sub>	K <sup>+</sup> equilibrium potential
E <sub>m</sub>	Membrane potential
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
GABA	Gamma-aminobutyric acid
GPCR	G protein-coupled receptor
HMG-CoA	3-hydroxy-3-methyl-glutaryl CoA
I <sub>CRAC</sub>	Ca <sup>2+</sup> -release activated Ca <sup>2+</sup> current

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IgG	Immunoglobulin G
IP <sub>3</sub>	Inositol trisphosphate
IP <sub>3</sub> R	Inositol trisphosphate receptor
K <sub>ATP</sub>	ATP-sensitive K <sup>+</sup> channels
K <sub>IR</sub>	Inward-rectifier K <sup>+</sup> channels
K <sub>v</sub>	Voltage-gated K <sup>+</sup> channels
L-NAME	N <sub>ω</sub> -nitro-L-arginine methyl ester hydrochloride
MI	Myocardial infarction
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MYPT	Myosin phosphatase targeting unit
NMDG <sup>+</sup>	N-methyl-D-glucamine
NO	Nitric oxide
NOS	Nitric oxide synthase
PAD	Peripheral artery disease
PI3K	Phosphoinositide 3-kinase
PIP <sub>2</sub>	Phosphatidyl-inositol-bisphosphate
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
PKG	Protein kinase G
PLC	Phospholipase C
PMCA	Plasma membrane Ca <sup>2+</sup> ATPase
ROCC	Receptor-operated Ca <sup>2+</sup> channel
ROK	Rho-associated kinase
RyR	Ryanodine receptor
SCID	Severe Combined Immunodeficiency
SDS	Sodium dodecyl sulfate
SEM	Standard error of mean
Ser	Serine
SERCA	Sarco-endoplasmic reticular Ca <sup>2+</sup> ATPase
SOCC	Store-operated Ca <sup>2+</sup> channel
SOCE	Store-operated Ca <sup>2+</sup> entry
SR	Sarcoplasmic reticulum

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STIM	Stromal-interacting molecule
TBS	Tris-buffer saline
TBS-T	Tris-buffer saline – Tween 20
TCA	Trichloroacetic acid
TEA	Tetraethylammonium
Thr	Threonine
TRP	Transient receptor potential channel
TRPC	Transient receptor potential canonical channel
TRPV	Transient receptor potential vanilloid-related channel
TxA <sub>2</sub>	Thromboxane A <sub>2</sub>
TXS	TxA <sub>2</sub> synthase
U46619	9,11-Dideoxy-11 $\alpha$ ,9 $\alpha$ -epoxymethanoprostaglandin F <sub>2<math>\alpha</math></sub>
VGCC	Voltage-gated Ca <sup>2+</sup> channel
VSMC	Vascular smooth muscle cell

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## LIST OF FIGURES

Figure A1. Illustration of developed tension in vascular smooth muscle as a consequence of changing the balance between $\text{Ca}^{2+}$ -CaM MLCK and MLCP activity.....	14
Figure A2. Composite of the subcellular signalling mechanisms involved in various agonist-mediated vasoconstriction.....	17
Figure A3. The synthesis of cholesterol and isoprene moieties via the mevalonate pathway and how statins can inhibit the production of the isoprenes.....	39
Figure B1. Voltage-dependent $\text{Ca}_v1.2$ current was potentiated by the addition of ET-1.....	57
Figure B2. The voltage- and ET-1-dependent $\text{Ca}_v1.2$ current was attenuated in the presence of the L-type CCB, nifedipine in isolated rat mesenteric VSMCs.....	58
Figure B3. Blocking $\text{Ca}_v1.2$ channels did not completely inhibit ET-1-mediated vasoconstriction in rat mesenteric arteries.....	59
Figure B4. $\text{Ca}^{2+}$ sensitisation contributed to ET-1-mediated vasoconstriction in	

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rat mesenteric arteries.....	61
Figure B5. Depletion of the SR $Ca^{2+}$ store attenuated ET-1-mediated vasoconstriction in rat mesenteric arteries.....	63
Figure B6. $Na^+$ and $Cl^-$ entry were not required for ET-1-mediated vasoconstriction in rat mesenteric arteries.....	64
Figure C1. SKF 96365 attenuated $TxA_2$ receptor-mediated vasoconstriction in rat mesenteric and caudal arteries.....	79
Figure C2. The amplitude of $Ca_v1.2$ current was significantly reduced following channel blockade using 1 $\mu M$ SKF 96365 in isolated rat mesenteric VSMCs, independent from NSCC.....	80
Figure C3. Blocking plasma membrane $Na^+$ entry attenuated $TxA_2$ receptor-mediated vasoconstriction in rat mesenteric but not caudal arteries.....	82
Figure C4. Inhibition of the $BK_{Ca}$ channels attenuated $TxA_2$ receptor-dependent vasoconstriction in rat caudal but not mesenteric arteries.....	84
Figure C5. SR $Ca^{2+}$ store was not involved in thromboxane $A_2$ receptor-mediated vasoconstriction in both rat (A) mesenteric and (B) caudal arteries.....	86

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Figure C6. Inhibition of the Cl <sup>-</sup> channels significantly attenuated the TxA <sub>2</sub> receptor-mediated vasoconstriction in rat mesenteric but not caudal arteries.....	88
Figure D1. Short-term simvastatin treatment significantly attenuated TxA <sub>2</sub> receptor-mediated vasoconstriction but not Thr855 phosphorylation of MYPT in isolated rat caudal arteries.....	104
Figure D2. Short-term, 30-minute high dose simvastatin treatment significantly attenuated TxA <sub>2</sub> receptor-mediated vasoconstriction in isolated rat mesenteric arteries.....	106
Figure D3. Short-term, high dose simvastatin treatment did not attenuate the Thr855 or Thr697 phosphorylation of MYPT in isolated rat mesenteric arteries.....	108
Figure D4. Short-term 30-minute, high dose simvastatin treatment activated eNOS via increased Ser-1177 phosphorylation in rat mesenteric arteries.....	110
Figure D5. Voltage-dependent Ca <sub>v</sub> 1.2 current was significantly attenuated in the presence of simvastatin in isolated rat mesenteric VSMCs.....	112
Figure D6. Short-term, 30-minute high dose simvastatin inhibited high K <sup>+</sup> -mediated vasoconstriction in rat mesenteric arteries.....	113

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Figure D7. Temporal profile for the anti-atherogenic, anti-inflammatory and  
vasodilatory properties of statin therapy..... 116

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# CONFERENCE PRESENTATIONS

## International

2013

- Congress of the International Union of Physiological Sciences (IUPS),  
Birmingham, UK

## National

2013

- Faculty of Health Sciences Postgraduate Research Conference, Adelaide  
AUSTRALIA

2012

- The Queen Elizabeth Hospital Research Day, Adelaide AUSTRALIA
- Cardiac Society Australasian and New Zealand Annual Scientific Meeting  
(CSANZ), Brisbane AUSTRALIA

2010

- Cardiac Society Australasian and New Zealand Annual Scientific Meeting  
(CSANZ), Adelaide AUSTRALIA
- Cardiovascular Research Forum, Adelaide AUSTRALIA
- Joint conference of Australian Neuroscience Society and Australian  
Physiological Society, Sydney AUSTRALIA

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## ABSTRACT

Vasospasm, of coronary, cerebral or peripheral circulation, is associated with myocardial infarction, stroke and claudication. Many forms of vasospasm are well characterised and can be effectively managed using therapies including nitrates and L-type  $\text{Ca}^{2+}$  channel blockers. However, some forms of vasoconstriction are less well managed by these therapies. Evidence also indicates that endothelin-1 (ET-1) can potentiate vasoconstriction elicited by other agonists. Consequently, an elevation in local and/or circulating ET-1, for example inflammation associated with acute coronary syndrome, has prompted us to explore the extent to which  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR) and  $\text{Ca}^{2+}$  sensitisation contribute to acute ET-1-mediated vasoconstriction. To examine this hypothesis, we coupled functional measurement of the isometric tension in isolated rat mesenteric arterial segments with biochemical analysis of the downstream target of Rho kinase, myosin phosphatase (MYPT). We identified that SR  $\text{Ca}^{2+}$  release contributed significantly to ET-1-dependent vasoconstriction. Using a fixed  $\text{Ca}^{2+}$  concentration coupled with biochemical analysis, we also demonstrated the contribution of RhoA/ Rho kinase-dependent  $\text{Ca}^{2+}$  sensitisation, consistent with an increased phosphorylation state at Threonine-855 site of MYPT in an ET-1-dependent vasoconstriction in the microvasculature. Extracellular  $\text{Na}^+$  entry and  $\text{Cl}^-$ , on the other hand, are not required to mediate ET-1-mediated contractile response.

The inflammatory milieu not only contains ET-1, but also platelet-derived thromboxane  $\text{A}_2$  ( $\text{TxA}_2$ ). In the context of  $\text{TxA}_2$  receptor-mediated vasoconstriction, there remains a gap in our understanding as to how  $\text{TxA}_2$  causes membrane

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depolarisation and subsequent activation of L-type  $\text{Ca}^{2+}$  channels. Using functional vascular myography, we have identified that extracellular  $\text{Na}^+$  entry through NSCC and  $\text{Cl}^-$  channels are required for the activation of L-type  $\text{Ca}^{2+}$  channels in  $\text{TxA}_2$ -dependent vasoconstriction in the small rat mesenteric arteries; whilst  $\text{K}^+$  fluxes through the  $\text{BK}_{\text{Ca}}$  channels activates the L-type  $\text{Ca}^{2+}$  channels in  $\text{TxA}_2$ -dependent vasoconstriction in large rat caudal arterial preparation.

In addition, we examined the relative efficacy of short-term, 30-minute high dose simvastatin administration in attenuating vasoconstriction in rat caudal and mesenteric arteries, challenged with the  $\text{TxA}_2$  mimetic, U46619. We demonstrated in functional experiments that short-term 30-minute, high dose simvastatin treatment attenuated  $\text{TxA}_2$  receptor-mediated vasoconstriction in both rat caudal and mesenteric arteries, with greater efficacy in the small arteries. Specifically, this reduction was endothelium-independent in the mesenteric arterial preparation. However, an increase in the phosphorylation state of eNOS suggested that there may be a tonic nitric oxide release and simvastatin may have the capacity to mediate an increase in the phosphorylation of Serine-1177 eNOS independently. In addition, biochemical western blot analysis revealed that the Threonine-855 phosphorylation state of MYPT was not influenced by 30-minute simvastatin treatment in both arterial preparations. Similarly, Threonine-697 phosphorylation state of MYPT also remained unaffected by either simvastatin treatment or  $\text{TxA}_2$  stimulation in rat mesenteric arteries. Perhaps more interestingly, the voltage-dependent L-type,  $\text{Ca}_v1.2$  current was inhibited by simvastatin in a  $\text{TxA}_2$  receptor-mediated fashion, consistent with our functional data which showed that simvastatin significantly attenuated the contractile response to high  $\text{K}^+$  depolarisation.

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Taken together, these data provide both evidence and rationale for the development and short-term use of SR  $\text{Ca}^{2+}$  release blockade therapy in addition to the implementation of Rho kinase inhibitor for patients with acute vasospastic disorders, who are resistant to conventional nitrate and L-type CCB therapies. Our data also outlined several vasomotor mechanisms including the L-type  $\text{Ca}^{2+}$  channel blockade and increase in eNOS activity, independent from the statin's cholesterol lowering property, which may be responsible for the benefit of short-term statin therapy in reducing mortality following cardiac intervention.