Regulation of Ca^{2+}-Dependent Vasoconstriction in Large and Small Arteries

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ABBREVIATIONS

9-AC	9-anthracenecarboxylic acid
AA	Arachidonic acid
ACE	Angiotensin converting enzyme
ACS	Acute Coronary Syndrome
BH_4	Tetrahydrobiopterin
BK_{Ca}	Ca^{2+}-activated K^+ channels
$[Ca^{2+}]_{cyt}$	Cytosolic Ca^{2+} concentration
CaM	Calmodulin
CCB	Ca^{2+} 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^{2+}-release activated Ca^{2+} 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-aminoethlyether)-*N,N,N',N'*-tetraacetic acid
E_K	K^+ equilibrium potential
E_m	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_{CRAC}	Ca^{2+}-release activated Ca^{2+} current

IgG	Immunoglobulin G
IP_3	Inositol trisphosphate
IP_3R	Inositol trisphosphate receptor
K_{ATP}	ATP-sensitive K^+ channels
K_{IR}	Inward-rectifier K^+ channels
K_v	Voltage-gated K^+ channels
L-NAME	N_ω-nitro-L-arginine methyl ester hydrochloride
MI	Myocardial infarction
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MYPT	Myosin phosphatase targeting unit
$NMDG^+$	N-methyl-D-glucamine
NO	Nitric oxide
NOS	Nitric oxide synthase
PAD	Peripheral artery disease
PI3K	Phosphoinositide 3-kinase
PIP_2	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^{2+} ATPase
ROCC	Receptor-operated Ca^{2+} 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^{2+} ATPase
SOCC	Store-operated Ca^{2+} channel
SOCE	Store-operated Ca^{2+} entry
SR	Sarcoplasmic reticulum

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_2	Thromboxane A_2
TXS	TxA_2 synthase
U46619	9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$
VGCC	Voltage-gated Ca^{2+} channel
VSMC	Vascular smooth muscle cell

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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

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 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 Ca^{2+} release from the sarcoplasmic reticulum (SR) and 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 Ca^{2+} release contributed significantly to ET-1-dependent vasoconstriction. Using a fixed Ca^{2+} concentration coupled with biochemical analysis, we also demonstrated the contribution of RhoA/ Rho kinase-dependent 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 Na^{+} entry and 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 A_2 (TxA_2). In the context of TxA_2 receptor-mediated vasoconstriction, there remains a gap in our understanding as to how TxA_2 causes membrane

depolarisation and subsequent activation of L-type Ca^{2+} channels. Using functional vascular myography, we have identified that extracellular Na^{+} entry through NSCC and Cl^{-} channels are required for the activation of L-type Ca^{2+} channels in TxA_2-dependent vasoconstriction in the small rat mesenteric arteries; whilst K^{+} fluxes through the BK_{Ca} channels activates the L-type Ca^{2+} channels in 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 TxA_2 mimetic, U46619. We demonstrated in functional experiments that short-term 30-minute, high dose simvastatin treatment attenuated 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 TxA_2 stimulation in rat mesenteric arteries. Perhaps more interestingly, the voltage-dependent L-type, $Ca_v1.2$ current was inhibited by simvastatin in a TxA_2 receptor-mediated fashion, consistent with our functional data which showed that simvastatin significantly attenuated the contractile response to high K^{+} depolarisation.

Taken together, these data provide both evidence and rationale for the development and short-term use of SR 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 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.