The Molecular Basis of Small Vessel Constriction in Endothelin-1 Models and Peripheral Arterial Disease

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List of figures and tables

List of common abbreviations

5HT	5,hydroxytryptamine / serotonin
ABI	ankle brachial index
ADMA	asymmetric dimethylarginine
ADP	adenosine diphosphate
ANOVA	analysis of variance
APS	ammonium persulfate
ARB	angiotensin II receptor blocker
ATP	adenosine triphosphate
BH_4	tetrahydrobiopterin
cGMP	cyclic guanosine monophosphate
COX	cyclooxygenase
CRP	c-reactive protein
CSFP	coronary slow flow phenomenon
CT	computer tomography
DAG	diacylglycerol
DFP	diisopropylfluorophosphate
DTT	dithiothreitol
EC	effective concentration
ECL	enhanced chemiluminescent
EDHF	endothelial-derived hyperpolarising factor
EDIN	epidermal cell differentiation inhibitor
eNOS	endothelial nitric oxide synthase
ET-1	endothelin-1
GDI	guanine nucleotide dissociation inhibitor
GDP	guanosine diphosphate
GEF	guanine-nucleotide exchange factors
GF109203X	bisindolylmaleimide
GTP	guanosine triphosphate
HRP	horse radish peroxidase
IgG	immunoglobulin G
ILK	integrin-linked kinase
IMA	Internal mammary artery
iNOS	inducible nitric oxide synthase
IP_3	inositol triphosphate
L_0	optimum resting length
LAD	left anterior descending coronary artery
LC_{20}	20kDa regulatory light chains of myosin
L-NAME	N^g-nitro-L-arginine methyl ester
MLCK	myosin light chain kinase
MYPT	myosin phosphatase targeting subunit
NO	nitric oxide
nNOS	neuronal nitric oxide synthase
PAD	peripheral artery disease
PDBu	phorbol 12,13-dibutyrate
PDE	phosphodiesterase
PE	phenylephrine
PGI_2	prostacyclin

PI3K	phosphatidylinositol 3-kinase
PIP_2	phosphatidylinositol 4,5-biphosphate
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PKG	protein kinase G
ROK	Rho-associated kinase
SAH	subarachnoid haemorrhage
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SR	sarcoplasmic reticulum
SSRIs	selective serotonin re-uptake inhibitors
TBS-T	Tris buffered saline – Tween 20
TCA	trichloroacetic acid
TEMED	N,N,N',N'-tetramethylethylenediamine
TPI	toe pressure index
ZIPK	zipper interacting protein kinase

Thesis Abstract

Peripheral artery disease (PAD) affects 20% of people over the age of 65 years and the prevalence increases with age. Predominately affecting the lower limbs, PAD causes chronic ischaemic leg pain, reduced quality of life, and increased risk of death by heart attack and stroke. It is well established vascular disease is a large vessel, atherothrombotic disorder, however the importance of vasospasm and/or increased vascular tone is less well recognized, particularly in the microvasculature. Current vasodilatory medical therapies have focused on extracellular Ca^{2+} entry or specific receptor blockade. Targeting subcellular enzymes to attenuate general agonist-mediated vasoconstriction has not yet been implemented. Although specific agonists, such as endothelin-1 (ET-1), have been implicated in PAD, whether chronic exposure to these vasoconstrictors causes altered receptor profiles of circulating hormones have not been identified. The direct contractile responses of diseased human microvessels to specific agonists also remain unclear.

We used 1) a rat model to identify the acute temporal activation of PKC and ROK during rapid and sustained ET-1-mediated vasoconstriction 2) a rat model of chronically elevated ET-1-meditated vasoconstriction to identify altered receptor profiles to specific agonists and 3) human subcutaneous arteries from patients with PAD to identify their functional and biochemical properties compared to age-matched non-PAD patients.

We report that PKC and ROK inhibition in large caudal and small mesenteric rat arteries are effective in attenuating acute ET-1-mediated vasoconstriction in

both the rapid and sustained phases of constriction. Chronically elevated ET-1 in a healthy rat model blunts the acute contractile response to the thromboxane A_2 mimetic, U46619 but does not change the vascular response to exogenously added ET-1, the α_1-adrenergic agonist, phenylephrine and serotonin receptor activation. In human subcutaneous arteries we identified an increased maximum contractile response to serotonergic and α_1-adrenergic receptor activation in PAD vs non-PAD patients, while vascular responses to K^+-mediated activation of voltage-gated Ca^{2+} channels, thromboxane A_2, and ET-1 receptor activation were unchanged. Altered vascular reactivity was independent from the abundance and Ser1177-dependent and Thr855-dependent activation state of eNOS and myosin phosphatase, respectively. We identified, patients with PAD have more $5HT_{2A}$ receptors than patients with no symptomatic PAD, suggesting a possible mechanism for increased contractile responses to serotonin receptor activation.

These data suggest 1) subcellular targets that block the inhibition of myosin phosphatase may be valuable in attenuating the vasoconstrictor response to several agonists, and provide additional benefit to specific receptor blockade, 2) while elevated ET-1 is a strong marker of vascular disease, it may have less direct impact on vascular reactivity, 3) decreased contractile responses to thromboxane A_2 following chronic ET-1 infusion is most likely caused by down regulation of thromboxane A_2 receptors, which could have important implications for patients on antiplatelet agents, 4) blockade of enhanced serotonin and α_1-adrenergic vasoconstriction may be beneficial in improving subcutaneous microvascular blood flow in patients with PAD