SCIENTIFIC COMMUNIQUÉ V

CLINUVEL

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Following a series of Scientific Communiqués focused on the role of melanocortin signalling in melanogenesis, photoprotection and skin cancer, Scientific Communiqué V takes a closer look at melanocortins and their role in the regulation of the vascular system. In this Communiqué the explanatory language will be tailored to a non-academic audience.

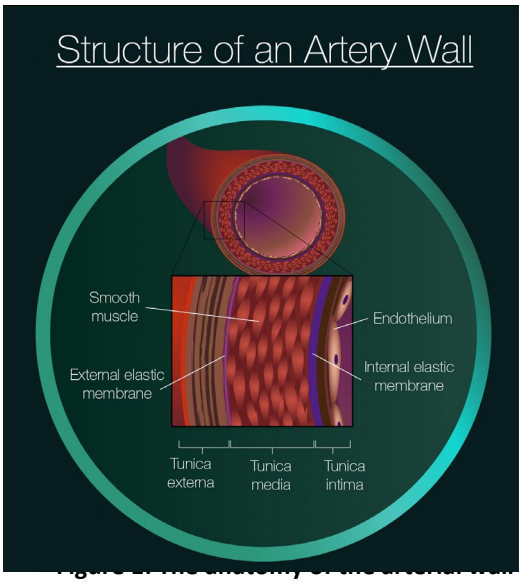
The vascular endothelium is a dynamic monolayer of specialised cells that line the walls of all blood vessels. Whilst previously assumed to function as only an inert barrier between the systematic blood and the smooth muscle within the vascular wall, the endothelium is now described as an endocrine *organ* with a multitude of indispensable roles in maintaining vascular health. Most notably, these cells release vasoactive signalling molecules that directly influence the tonal state of the blood vessels. Nitric oxide, or NO, is a potent endogenous vasodilatory-gas synthesised and released by the endothelium, causing the adjacent smooth muscle tissue (that constitutes the vascular walls) to relax. Endothelium dysfunction, and therefore a loss of NO production, is associated with chronic narrowing of the lumen of blood vessels (vasoconstriction). This may lead to a prolonged increase in vascular resistance or otherwise high blood pressure. A number of disorders, including atherosclerosis, hypertension, and diabetes are associated with the loss of NO signalling. In recent years, the ability to clinically manipulate the endothelium derived NO pathway has proven to be therapeutically relevant in preserving vascular health.

As understanding of melanocortin function in the endothelium has evolved, it has become clear that alpha-melanocyte stimulating hormone - and its analogues – may hold potential in addressing unmet medical needs and improve vascular wellbeing.

We will first discuss NO in relation to vascular health and disease, with a particular focus on NO's dominant role in controlling vascular tone. We will then evaluate in-depth the possible role of alpha-melanocyte stimulating hormone (α-MSH) and its analogues in promoting vascular homeostasis through its ability to manipulate NO availability.

The Vascular System

Blood vessels form the systemic circulatory system, which distributes blood throughout the body, critical for cell survival. This effectively supplies oxygen and nutrients to body tissues whilst serving as a removal system for respiratory waste. Additionally, the blood is an important regulator of body heat and pH, maintaining both at optimum levels for cellular function, as well as acting as a transport medium for cells including leukocytes and platelets.



The peripheral vascular system is one of a few determinates that ensure the appropriate regulation of blood flow. A single layer of endothelium sheathes the innermost layer (tunica intima) of the entire vascular system, providing a physical barrier between blood flow and the vascular tissue. With the apical surface of these cells in physical contact with the circulating blood, the endothelium is able to respond to a range of luminal stimuli including haemodynamic changes, or the presence of signalling peptides. This in turn causes an increase or decrease in the diameter of the vascular lumen through modifying vessel muscle contraction.

Critical in understanding the function of NO in vascular balance is its vasoactive properties within the endothelium. Synthesised by nitric oxide synthase (NOS), NO is a key signalling molecule expressed in almost all human cells modulating critical biological pathways that are fundamental to human survival. Its favourable pleiotropic actions on various physiological systems has since been demonstrated both *in vitro* and *in vivo*, including within the vasculature.

Following production, low concentrations of NO diffuse from the endothelium to the vascular smooth muscle, where NO consequently provokes muscle relaxation and inhibits excessive cell proliferation. Basal levels of NO also prevent endothelial apoptosis (cell death) and leukocyte adhesion, which would otherwise contribute to endothelium dysfunction and increased vascular permeability. It is thus apparent that benign concentrations of NO have a multitude of effects on regulating vascular health, with implications for many disorders.

Nitric Oxide Synthesis in the Endothelium

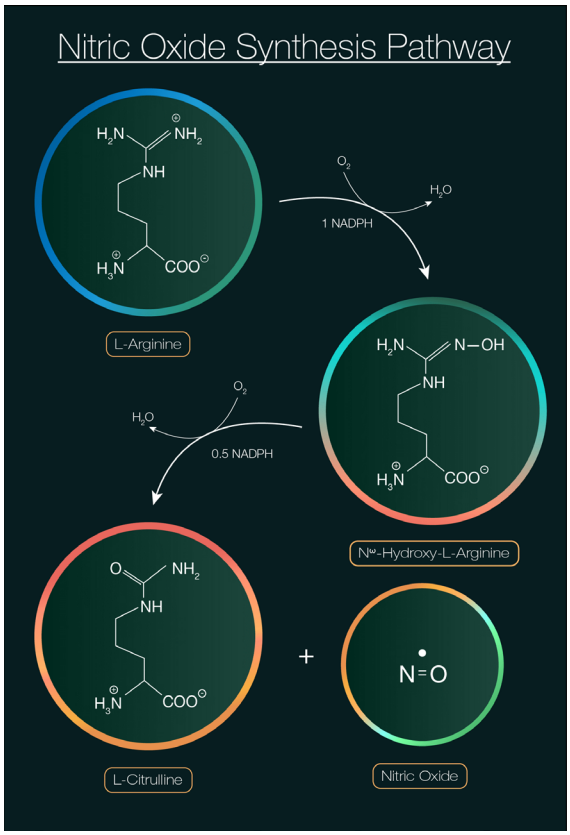


Figure 2: Synthesis of nitrous oxide

We now look closer at the chemistry of NO, starting with its production. NOS belongs to a family of endogenous enzymes which regulate the production of NO. L-arginine, an amino acid and the precursor of NO, is found in excess within the cytosol of the endothelial cells. This molecule undergoes hydroxylation when bound to NOS to form the intermediate molecule NG-hydroxy-L-arginine. This reaction is quickly followed by NOS mediated oxidation, generating L-citrulline and NO **(Figure 2)**. NO diffuses from cells to their target tissues, initiating various signalling pathways. In the case of endothelium mediated vasodilation, NO diffuses to the smooth muscle layer, stimulating the NO-cyclic guanosine cyclic monophosphate (cGMP) pathway.

Three isoforms of NOS have been identified in mammals: neuronal NOS (nNOS or NOS I), inducible NOS (iNOS or NOS II) and endothelium NOS (eNOS or NOS III), named after the respective cell types within which they were originally identified. The endothelial cells express both the eNOS and iNOS isoforms. However, the activity of each synthase is regulated through alternative pathways; unlike iNOS, eNOS is constitutively expressed in the membrane of the endothelium.

All NOS isoforms exist as homodimers, constituted of two identical monomers. Each monomer has a functional C- terminal reductase and an N-terminal oxygenase domain, however in its monomer form, NOS remains non-functional. NOS contains four prosthetic groups bound to either the reductase or oxygenase terminals: haem (Fe), flavin adenine dinucleotide (FAD), tetrahydrobiopterin (BH4) and flavin mononucleotide (FMN). Combined, the binding of L-arginine, NADPH and calcium-CaM alongside the four prosthetic groups enable NOS assembly as an active dimer **(Figure 3)**. In its active form NOS is able to efficiently synthesise NO.

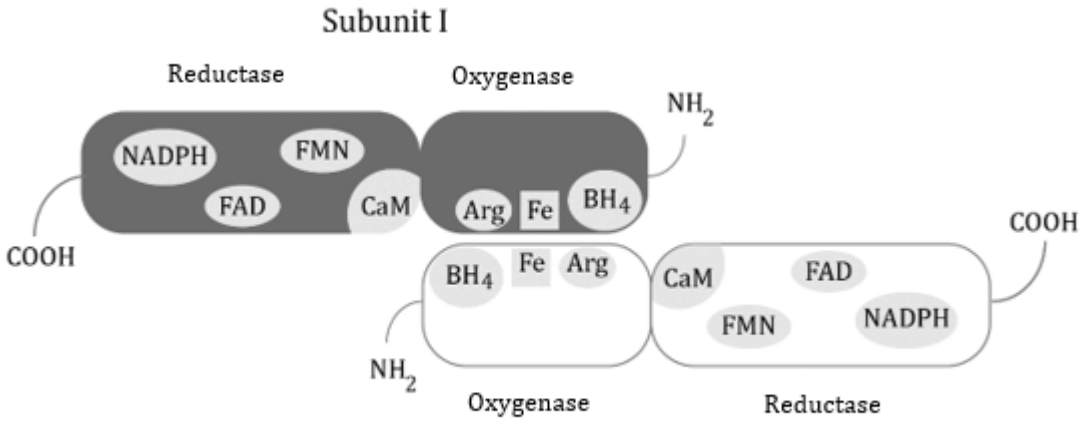


Figure 3: Nitric oxide synthase homodimer

As previously described, the vascular endothelium dynamically responds to a variety of intrinsic and extrinsic stimuli in the blood, including vasodilatory agonists. Bradykinin and acetylcholine

are two prime examples of endothelium vasodilatory molecules that work to activate endothelial signalling. These agonists directly bind to G-protein coupled receptors located on the plasma membrane of the endothelial cells, which stimulates a cascade of internal events, leading to the upregulation of a secondary messenger known as inositol trisphosphate (IP3). IP3 binds to its respective receptor on the membrane on the endoplasmic reticulum (ER), the main internal store of calcium ions in non-muscular cells, accounting for 75% of the cell's reservoir. The binding of IP3 causes a conformational change of the receptor, enabling ions to pass from the ER into the cytosol of the endothelial cells. Recent evidence has illustrated crosstalk between the ER and mitochondria. Following the release of calcium ions from the ER, mitochondrial mCIC receptors additionally pump out calcium ions residing in the mitochondria to enhance calcium ion levels in the cytosol of the endothelial cells. The intracellular concentration of calcium ions in the endothelium play a crucial function in eNOS activation.

eNOS activity is strictly regulated. Under physiological conditions, eNOS is anchored intracellularly to invaginations in the endothelium membrane. These invaginations, also referred to as caveolae, are a specialised subset of lipid rafts located along the plasma membrane of the endothelial cells, which act to localise eNOS to clusters of target receptors nearby. eNOS is made inactive during conditions of low intracellular calcium ion concentrations through its association with cavolin-1. Cavolin-1 is oligomeric integral membrane protein that holds eNOS in close contact with the caveolae membrane. However, in doing so, it renders eNOS inactive.

Calmodulin (CaM), a ubiquitously expressed cytosolic acid protein, is also present within the endothelial cells. CaM contains two pairs of calcium binding helix loop helix motifs, otherwise known as EF hands. In conditions of high intracellular calcium ion concentrations, induced by IP3, calcium ions are able to bind to CaM's EF hands. In turn, a conformational change in CaM's structure is instigated, exposing CaM's hydrophobic binding regions. Calcium bound CaM can now displace cavolin-1 and thus interact with eNOS via these exposed binding regions. CaM-eNOS interaction facilitates NO synthesis as CaM enables the transfer of electrons from the reductase to oxygenase domains of eNOS during L-arginine's conversion to NO **(Figure 3)**.

eNOS activity can also be altered through post-transcriptional modifications, most notably through phosphorylation of the protein's amino acid residues. These modifications occur downstream of alternative transduction pathways however they have the ability to either increase or dampen NO production. AKT-mediated phosphorylation of the serine-1179 residue is associated with greater NO generation as it reduces eNOS-CaM dissociation. AKT is a serine/threonine-specific protein kinase, which is upregulated in the endothelium when shear stress or mechanical distension has been detected in the lumen of the vessel: a consequence of high blood pressure. In contrast, threonine-495 phosphorylation increases eNOS sensitivity to the negative regulators of NO production. This modification has been associated with diabetes (hyperglycaemia). Synthesised NO is now able to diffuse out of the cytosol of the endothelium where it instigates its signalling capabilities on target cells.

Decreased bioavailability of NO is highly influenced by a range of factors. Polymorphisms in the eNOS gene can result in the translation of an enzyme with a reduced function. As a result, the production of NO is impaired. Alternatively, post translational modifications of eNOS that favour its inactive form will also contribute to poor NO synthesis. Atherogenic risk factors, such as hypertension, diabetes and smoking have all been shown to initiate endothelium signalling pathways which downregulate eNOS activity and/or expression. It is of no surprise that these circumstances lead to vascular damage. Therapies aimed to increase NO levels may therefore work to promote vascular wellbeing and help prevent against the progression of vascular diseases.

NO actions in the vasculature

There are three major types of blood vessels: arteries, veins, and capillaries. While arteries and veins share a similar anatomy (each arranged into three distinct layers) the relative ratios of these layers vary considerably; reflecting the heterogeneous role of the vessels (**Figure 4)**. In both cases, an abundance of smooth muscle tissue is located in the middle layer (tunica media) of vessel walls, alongside elastic fibres and connective tissue. The smooth muscle cells, or myocytes, allow the vessels to contract and relax thus increasing or decreasing vascular resistance. These specialised cells contain many of the motor proteins actin and myosin, which collectively form thick and thin filaments, respectively. Muscle contraction is dependent on the binding of these two filaments with one another, otherwise known as cross bridge formation. The interaction is followed by opposing sliding, instigated by myosin, which can be described as the 'power stroke' movement. It is these filaments that act as the contractile machinery within the muscle tissue (**Figure 5**). It must be noted that contractive activity relies on a high cytosolic concentration of calcium ions. Vasoconstrictive agonists such as endothelium-1 and angiotensin positively regulate the open state of native calcium ion channels present on the plasma membrane of the smooth muscle. This ensures calcium ion concentrations remain high as ions continuously flux into the cell from the extracellular space. The activity of kinase proteins, known as myosin light chain kinases (MLCKs), are rendered active by increasing concentrations of calcium ions. MLCKs directly phosphorylates myosin causing a conformational change of the protein, enabling the thick myosin filaments to interact and 'drag' the thin filaments across their length. The thin actin filaments are anchored to dense bodies that, in turn, are bound to the myocyte cell membrane (the sarcolemma). As sliding occurs, tension is generated as the thin filaments pull the dense bodies [and thus the sarcolemma] inwards. It is this spatial reorganisation during filament sliding that enables the shortening of the cell which corresponds to a contractile phenotype. A reduction in the diameter of the vessel lumen is seen, associated with an increase in blood pressure and vascular resistance. Smooth muscle contraction is unique as the myocytes can retain their contractile state for prolonged periods of time, which evokes slow sustained contraction of the vessel walls (latch-bridge hypothesis). Relaxation only occurs when contractile stimuli are absent or if a dilatory signal is able to inhibit the actions of vasoconstriction. In this environment, the filaments remain in an unbound and immobile condition. Consequently, luminal diameters increase as the vessels dilate causing blood pressure to fall.

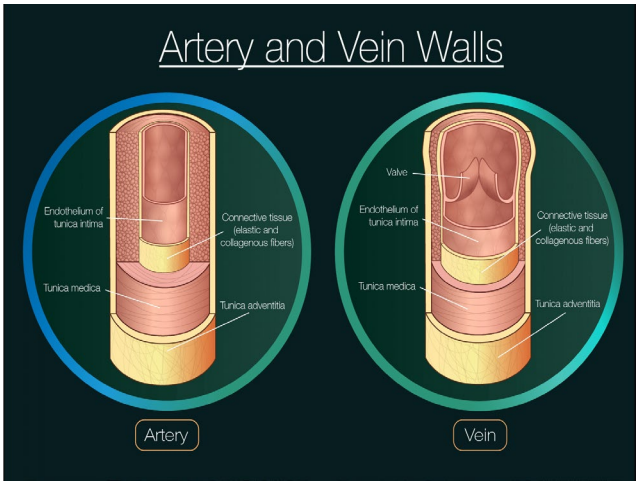


Figure 4: The structure of the arteries and veins. The structure of these blood vessels can be divided into 3 layers, or tunicas. The intima is the innermost layer composed of a layer of endothelial cells surrounded by a basement membrane. The tunica media (or middle layer) is composed of elastin and smooth muscle cells, which control the luminal diameter of the vessel. The adventitia (or outermost layer) provides structure and shape to the vessel and is composed of connective tissue. The structure of capillaries (not shown) diverge from that of the arteries and veins as they are composed of only an intima layer to allow for efficient gas and solute exchange.

NO promotes vasodilation through stimulation of the NO- cyclic guanosine monophosphate (cGMP) pathway. Diffusing down from the tunica intima, NO enters the sarcoplasm and activates

the intracellular enzyme, soluble guanylyl cyclase (sGC). Once bound, NO is stabilised and sGC is able to trigger a cascade of signalling events, including the activation of cyclic guanosine monophosphate (cGMP). Myosin phosphatase (MCLP), a downstream effector of sGC-cGMP signalling, opposes MLCK as it dephosphorylates myosin. cGMP simultaneously reduces intracellular calcium ion concentrations within the cytoplasm, further inhibiting MLCK's actions **(Figure 6).** Resultantly, smooth muscle cells relax as filament sliding is inhibited.

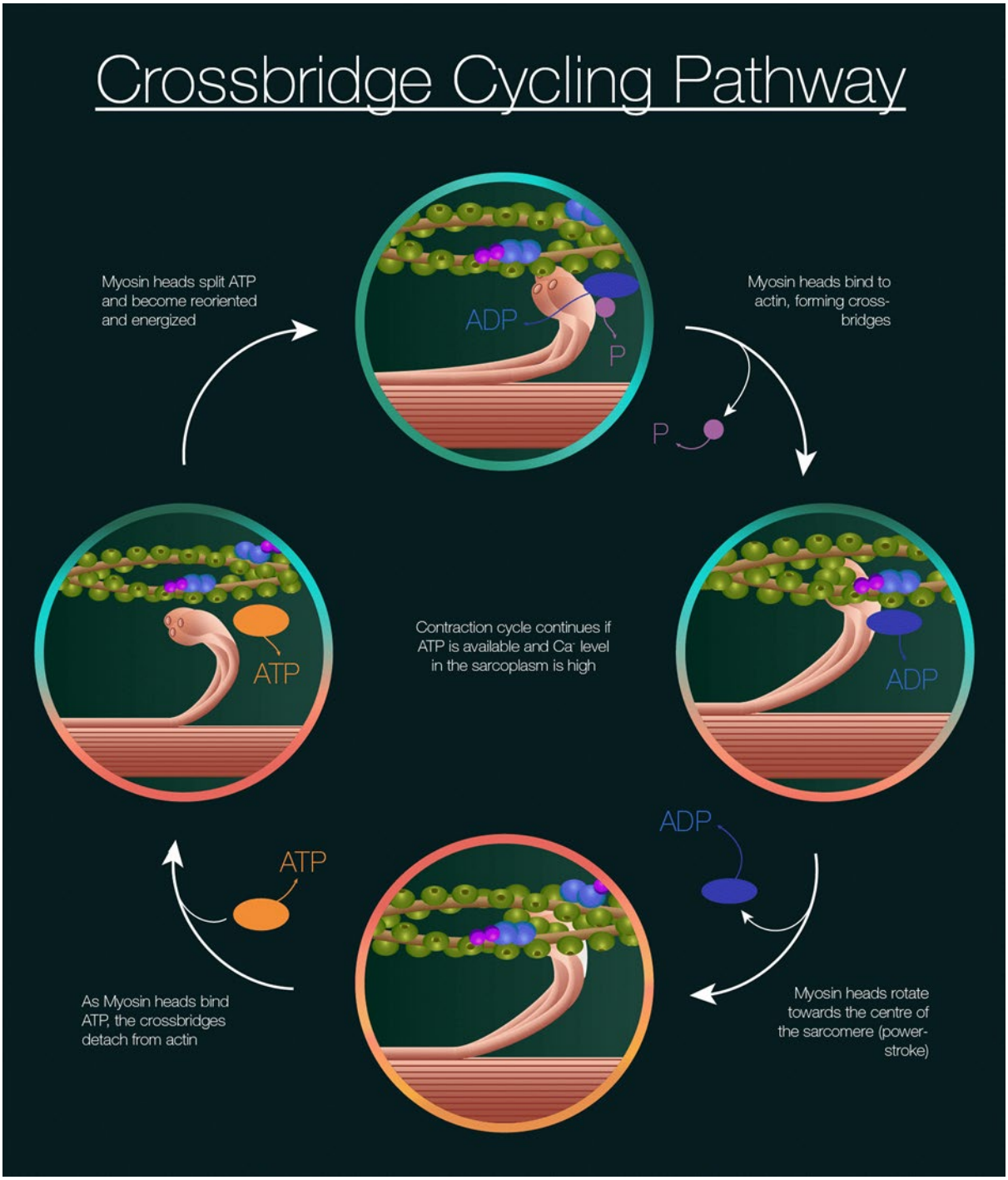


Figure 5: The contraction cycle within the vascular wall

Myosin-actin cross bridge cycling: The thick filaments are composed of numerous myosin proteins, with the head of the myosin proteins protruding from the filament. Each myosin head is equipped with an ATPase and an ATP molecule bound. After phosphorylation (i.e. MLCK), ATPase converts the bound ATP to ADP + an inorganic phosphate, Pi. The myosin heads are now in a high energy state and bind to the actin proteins that form the thin filaments (cross bridge formation), releasing Pi. The resultant release of ADP, following the release of Pi, causes a conformational change of the myosin head, initiating the 'power stroke'. As the thick filament moves courtesy of individual myosin units, the thin actin filament is pulled towards the centre of the cell (muscle cell contraction). As ATP binds to the myosin, the protein detaches actin (muscle cell relaxation).

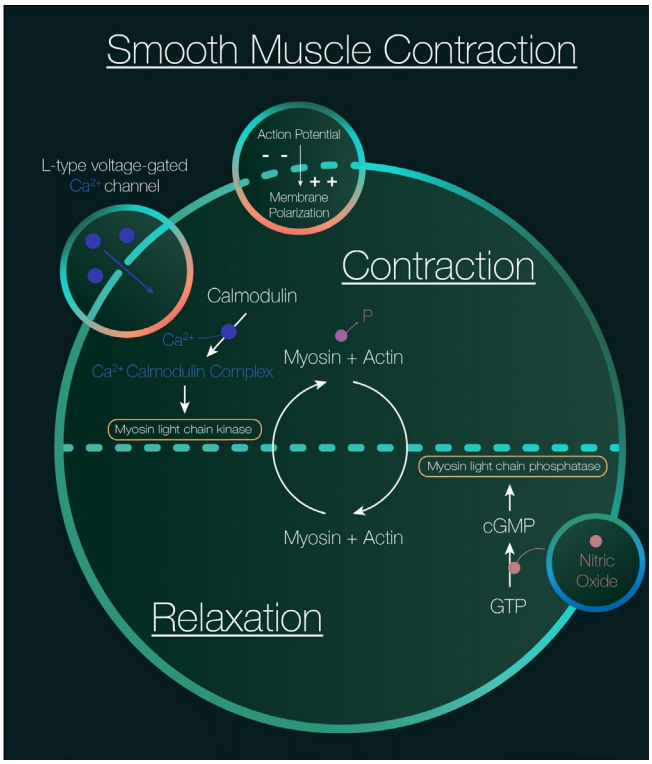


Figure 6: Smooth muscle contraction and relaxation

Calcium ion channels on the myocyte cell membrane open in response to vasoconstrictive mediators including endothelin-1, angiotensin and membrane depolarisation from sympathetic nerve stimulation. Calcium ions influx from the extracellular space into the cytosol of the cell. An organelle known as the sarcoplasmic reticulum (SER) acts as the myocytes' internal store of calcium ions and are additionally released to amplify calcium mediated responses within the cell. These ions are able to bind to resident CaM and the calcium- CaM complex is able to stimulate MLCK activity. Through a process of phosphorylation, MLCK causes a conformational change of myosin filament heads. MLCK increases myosin ATPase activity and endorses myosin-actin interaction (cross bridge formation). ATP hydrolysis changes the angle of the myosin heads so that during filament sliding, maximum movement is achieved. NO diffuses into the myocyte and upon entering the sarcoplasm, NO binds and activates sGC. sGC is catalyses guanosine 5'-triphosphate (GTP) to cGMP. The synthesis of cGMP activates cGMP-dependent protein kinases (PKG). PKG phosphorylates MCLP which counteracts the action of MLCK by causing myosin dephosphorylation. PKG also phosphorylates calcium ion channels (IP3R and voltage gated calcium channels) to reduce intracellular calcium availability. Calcium ATPase pump calcium ions out of the cell and back into the SER. Resultantly, myosin-actin sliding is prohibited.

While a combination of both vascular contraction and relaxation is critical in maintaining vascular tone and in turn controlling the velocity and volume of blood carried to target tissues, loss of this intricate balance is followed by abnormal blood perfusion and eventual cell death. Chronic hypertension is a classic clinical manifestation seen during aberrant myocyte contraction and loss of NO expression. The raised blood pressure increases hydrostatic pressures within the microvasculature, resulting in increased fluid filtration from the lumen of the vasculature to the surrounding tissue. The mechanical force of the blood additionally damages the surrounding tunica intima of the blood vessel. The lubricant like glycocalyx layer on the surface of the endothelium is damaged, exposing receptor sites to circulating leukocytes and leading to enhanced endothelium dysfunction as an inflammatory response in the vessels is initiated.

NO's mechanism of action extends from solely achieving *dilatory responses* within the blood vessels. NO has been demonstrated to also have anti-adhesion and anti-inflammatory properties. Inflammation is a critical protective mechanism mediated by leukocytes and platelets. It aids the body to fight against pathogens, remove cellular debris and is an integral component of the wound healing process. However, persistent or abnormal inflammatory responses are damaging and lead to disease propagation. Leukocytes are found within the blood, and upon detection of proinflammatory mediators such as cytokines (immunomodulating agents), these cells are recruited to the site of injury, eliciting immune responses. During recruitment, leukocytes must adhere and emigrate across the vascular endothelium to reach its target site. To facilitate this, the endothelium upregulates its expression of cell adhesion molecules, known as selectins and integrins. Leukocytes, expressing their complementary ligands, are able to bind to the endothelium via these adhesion molecules and release proteases to assist transmigration across

endothelial junctions to reach the site of damage. When this is occurring in the capillaries, increased vascular permeability ensues. If this process is prolonged, components found exclusively in the blood flood the extracellular matrix, disrupting fluid homeostasis. Leukocyte infiltration in the walls of the arteries and veins is associated with vascular wall damage as leukocytes release cytokines, which in turn degrade the extracellular matrix of the vessel. NO is one of several molecules that, at basal levels, downregulates endothelial adhesion molecule expression. This prevents excessive inflammatory responses through the inhibition of additional leukocyte recruitment and binding, especially when this response is no longer beneficial to health. When NO bioavailability has been diminished, this can lead to inappropriate expression of selectins and integrins on the endothelium wall. The leukocyte-mediated endothelium degradation deteriorates endothelial barrier function which not only increases vascular permeability but further acts as a positive regulator of reduced NO synthesis as more endothelial cells are damaged and undergo apoptosis in extreme cases.

Furthermore, low levels of NO inhibits platelet aggregation. NO is also released into the blood where it enters the cytosol of circulating platelets. Through the canonical NO-cGMP signalling pathway, NO decreases intracellular calcium concentrations, which corresponds to the downregulation of platelet surface receptors including P-selectin. Expression of these molecules is necessary for platelets to interact with one another, generating a clotting response. The central function of platelets is to prohibit haemorrhage from sites of vascular injury. NO's downregulation of thromboxane A2 and P-selectin prevents aberrant blood coagulation which would otherwise disturb normal blood flow. Inappropriate platelet activation and aggregation also contributes to ischemia as clot formation may cause vessel occlusion.

A change in vascular composition can further contribute to complications in maintaining blood pressure and tissue perfusion. With the structure of arteries and veins specific to their function, disruption or abnormalities within their structures will result in the decreased functional activity of the vessels. Diminished function is associated with vascular dysfunction and thus disease. In atherosclerosis, vascular wall stiffness occurs. The vessels lose their ability to effectively regulate blood flow as they can no longer dynamically respond to stimuli competently. Smooth muscle proliferation and migration into the intima is one element causing vessel rigidity in atherosclerosis. This is a typical injury response in atherogenesis and contributes to atherosclerosis progression. Proliferation and migration play pivotal roles in pathogenic vascular remodelling as vessel wall hypertrophy is seen and the balance between the tunica components is lost. NO has a suggested function in inducing proliferative arrest. Effectors of cGMP have demonstrated downregulation of the epidermal growth factor (EGFR) signalling pathway which is associated with cell cycle progression to S phase; the DNA replication phase. Further evidence has shown NO may also modify cell cycle progression independent of cGMP as well. NO stalls smooth muscle cells in the G0/G1 phase, inhibiting DNA replication and eventually cell division.

It is clear that a deficit of eNOS derived NO signalling corresponds to a variety of diseases that lead to poor vascular health. Emerging evidence has shown α-melanocyte stimulating hormone (α-MSH) and its analogues to be positive regulators of NO. This suggests a potential role for α-MSH in vascular diseases whose aetiology results from NO deficiencies.

Alpha -MSH and NO

The G-protein coupled melanocortin receptor MC1R (see SCIENTIFIC COMMUNIQUÉ I) is expressed by various tissues including endothelial cells. As previously stated, it has been suggested that α-MSH promotes endothelium dependent vasodilation through its ability to stimulate the NO-cGMP pathway in underlying smooth muscle cells.

In 2012, Gatti et al demonstrated the administration of α-MSH increased the expression of eNOS enzymes in the basilar artery of the rat. The results from this study were further validated by Rinne et al (2013) who determined α-MSH administration to cultured human endothelial cells increased eNOS activation through transcriptional regulation and post-transcriptional phosphorylation of this enzyme. *In vivo* examination of a cyclic α-MSH analogue in mice suggested MC1R downstream signalling has a dominant role in post-transcriptional modification of eNOS. While these results are promising, the mechanism in which MC1R signalling results in increased eNOS activity is yet to be determined and requires further investigation.

Rinne et al (2015) showed that a loss of function mutation in the MC1R gene corresponded to the impairment of the endothelium's ability to induce vasodilation. Disease phenotypes, including early onset of atherosclerosis and vascular stiffness (linked with an inability to regulate vascular tone), were reported, which were associated with a significant increase in smooth muscle cell proliferation. At low levels, NO is effective as an inhibitor of cell proliferation through its proficiency in inducing cell cycle arrest. Coupled with NO functionality as a vasodilator, this further describes a second mechanism in which upregulating NO expression via the MC1R pathway provides vascular protection. α-MSH's protective role in vascular disease may further extend from solely inducing vasodilation through to its ability to manage excessive smooth muscle proliferation and thus prevent vessel stiffness.

Work has also focused on the role of the antioxidant manganese-dependent superoxide dismutase (Mn-SOD), a reactive oxygen species (ROS) scavenger, expressed broadly within cells following the administration of α-MSH analogues and what impact this may have on vascular homeostasis. Studies have shown increased expression of Mn-SOD in cells after the administration of an α-MSH analogue. The main source of ROS in the endothelium comes from the incomplete reduction of oxygen molecules during respiration, leading to the production of the superoxide anion (O_2^-). As a free radical, O_2^- is unstable. NO and O_2^- can react with one another to form peroxynitrite ($ONOO^-$), a highly damaging oxidant and nitrating agent. $ONOO^-$ is able to oxidise the structural components of the endothelial cells, including lipids and proteins, causing organelle and cell membrane damage. This process damages the endothelium, placing these cells under extreme stress.

Not only does O_2^- reduce NO bioavailability, preventing NO from diffusing to its target sites and causing injury to the endothelium itself, but $ONOO^-$ induced damage leads to reduce NO production as it oxidises BH4, an eNOS prosthetic group **(Figure 3)**. This destabilises the eNOS dimer, declaring it inactive. α-MSH's ability to increase the expression of Mn-SOD, therefore suggests a third therapeutic explanation of the protective effects of α-MSH in vascular conditions. Mn-SOD inhibits O_2^- reacting with NO and other molecules through catalysing the dismutation of superoxide into oxygen and hydrogen peroxide. This in turn prevents oxidation and nitration of endothelium components that would otherwise have led to endothelium impairment and eNOS inactivation.

Collectively these studies have exhibited a clinical application for α-MSH in maintaining vascular homeostasis. Through its favourable regulation of eNOS via post-transcriptional modifications and preventing ONOO- generation, α-MSH increases the bioavailability of NO in the endothelial cells of the vasculature. Sufficient concentrations of NO can therefore stimulate vasoprotective changes in diseases who origins result from a loss of NO production or availability.

SUMMARY

In this Scientific Communiqué V we have discussed the anatomy of the human vascular system, its function, the role of nitrous oxide, its mechanism in contraction and relaxation through smooth muscle within the vascular wall and last the possible role of melanocortins within this system.

Ms Lauren Herbert, Communications Associate

GLOSSARY

Acetylcholine	A neurotransmitter that functions to propagate nerve impulses across the neuromuscular junction between a nerve and a muscle.
Actin	An abundant protein that forms actin filaments in all eukaryotic cells.
ADP	Adenosine 5' diphosphate, a nucleotide produced by the hydrolysis of the terminal phosphate of ATP. Regenerates ATP when phosphorylated by an energy generating process.
Angiotensin	A peptide hormone that causes vasoconstriction.
Atherosclerosis	A process of progressive thickening and hardening of the walls of arteries as a result of fat deposits on their inner lining.
ATP	Adenosine 5' triphosphate, nucleoside triphosphate composed of adenine, ribose and three phosphate groups. The principal carrier of chemical energy in a cell. The terminal phosphate groups are highly reactive, their hydrolysis or transfer takes place with the release of a large amount of free energy.
ATPase	An enzyme that catalyses the hydrolysis of ATP.
Basement membrane	Also referred to as the basal lamina, a thin mat of extracellular matrix that separate the epithelial sheets, and many other cell types from connective tissue.
BH4	Tetrahydrobiopterin (THB), a co-factor of NOS.
Bradykinin	A nonapeptide that causes vasodilation.
Calmodulin	Ubiquitous intracellular Ca^{2+} binding protein that undergoes a large conformational change when it binds to Ca^{2+}, allowing it to regulate the activity of many target proteins.

cAMP	Cyclic AMP is a nucleotide that is generated from ATP by adenylyl cyclase in response to various extracellular signals. It is a small signalling molecule that activates cAMP dependent proteins such as protein kinase A.
cGMP	Cyclic GMP, a nucleotide that is generated from GTP by guanylyl cyclase in response to extracellular signals.
Cytosol	Contents of the main compartment of the cytoplasm, excluding membrane bound organelles such as the endoplasmic reticulum and mitochondria.
Cytosolic acid	A proton donor found in the cytosol of a cell.
Dense body	Each thin (actin) filament is anchored to this structure which exerts a pull on the membrane when the fibre contracts.
Endocrine	Relating to hormones that are secreted into the blood
Endoplasmic reticulum (ER)	Labyrinthine membrane bound compartment in the cytoplasm of eukaryotic cells, where lipids are synthesised, and membrane bound proteins and secretory proteins are made.
Endothelial cells	Flattened cell type that forms a sheet (the endothelium).
Endothelium	A layer of flattened cells (endothelial cells) that line the blood vessels and lymph vessels of the body.
Endothelin-1	A peptide hormone with diverse biological actions including vasoconstriction.
eNOS	Endothelium nitric oxide synthase, constitutively expressed in the endothelium synthesising NO.
FAD	Flavin adenine dinucleotide, functions one of the prosthetic group within NOS. Enables the transfer of electrons within the NOS homodimer.
FMN	Flavin mononucleotide, functions as prosthetic group of various oxidoreductases including NOS. Enables the transfer of electrons within the NOS homodimer.
Free radical	Atom or molecule which is extremely reactive by virtue of it having at least one unpaired electron.
Glycocalyx layer	A carbohydrate-rich layer lining the vascular endothelium.
Gs protein	Stimulatory G protein that, when activated, activates the enzyme adenylyl cyclase and thus stimulates the production of cyclic AMP
GTP	Guanosine 5’ triphosphate, a nucleoside triphosphate produced by the phosphorylation of GDP (guanosine diphosphate). Like ATP, it releases a large amount of free energy on hydrolysis of its terminal phosphate group. Has a specialised role in microtubule assembly, protein synthesis and cell signalling.
Helix loop helix	DNA binding structural motif present in many gene regulatory proteins.

Hydroxylation	A chemical process that introduces a hydroxyl group (-OH) into an organic compound.
iNOS	Inducible nitric oxide synthase, a small molecule that has various roles in cellular functions and acts via a cGMP-mediated signal transduction pathway. It is upregulated under pathophysiological conditions within the vascular endothelium, synthesising NO.
IP3	Insositol 1,4,5 triphosphate, a small intracellular signalling molecule produced during the activation of the inositol phospholipid pathway. Acts to release Ca2+ from the endoplasmic reticulum.
(Protein) Kinase	An enzyme that catalyses the addition of phosphate groups of ATP to one or more amino acid molecules.
PKG	cGMP-dependent protein kinase or Protein Kinase G, A serine/threonine kinase that's activated by cGMP.
Leukocyte	A general name for all the nucleated blood cells lacking haemoglobin. May also be referred to as white blood cell.
Lumen	The inside space of a tubular structure, such as an artery or intestine.
Mitochondria	Membrane bound organelle that carries out oxidative phosphorylation and produces the majority of ATP in eukaryotic cells.
Motor protein	Protein that uses energy derived from nucleoside triphosphate hydrolysis to propel itself along a linear track (i.e. a protein filament).
Myocytes	Cell type specialised for contraction.
Myosin	A large class of motor proteins that move along actin filaments
MLCK	Myosin light chain kinase, a calcium/calmodulin-dependent serine/threonine kinase.
MLCP	Myosin light chain phosphatase, a serine/threonine-specific protein phosphatase.
NADPH	Reduced nicotinamide adenine dinucleotide, an electron donor.
Neutrophil	While blood cell that is specialised for the uptake of particulate material by phagocytosis. Enters tissues that become infected or inflamed.
NO	Nitric oxide, a gaseous signalling molecule that is widely used in cell-cell communication.
NOS	Nitric oxide synthase, an enzyme that synthesises nitric oxide by the deamination of arginine.
nNOS	Neuronal nitric oxide synthase is constitutively expressed in neurones and produces NO in nervous tissue in both the central and peripheral nervous systems.
Oxidation	Loss of electrons from an atom.

Phosphatase	An enzyme that catalyses the hydrolytic removal of phosphate groups from a molecule.
Platelets	Cell fragment lacking a nucleus, that is found abundantly in the blood stream. Helps initiating blood clotting in injured blood vessels.
Polymorphic	A gene with two or more alleles that co-exist at a high frequency in a population.
Prostaglandins	A class of unsaturated fatty acids that are involved in the contraction of smooth muscle, the control of inflammation and body temperature, and many other physiological functions.
Prosthetic group	A non-protein group forming part of or combined with a protein.
ROS	Reactive oxygen species are chemically reactive chemical species containing oxygen, such as the superoxide anion or hydrogen peroxide.
Sarcoplasmic reticulum (SER)	A special type of smooth endoplasmic reticulum found in smooth and striated muscle fibres whose function is to store and release calcium ions.
Selectin	A family of protein cell adhesion molecules (or CAMs) that mediate transient Ca2+ dependent cell-cell adhesion in the bloodstream.
Serine/threonine kinase	An enzyme that phosphorylates specific proteins on serine or threonine's (amino acids).
Shear stress	The frictional force generated by blood flow in the endothelium, that is, the force that the blood flow exerts on the vessel wall
Signalling cascade	Sequence of linked intracellular reactions, typically involving multiple amplified steps in a relay chain, triggered by an activated cell surface receptor.
sGC	Soluble guanylyl cyclase is the only known receptor for nitric oxide, NO. It is soluble, i.e. found within the cytoplasm of cells. Activation of the receptor leads to the synthesis of the second messenger cyclic guanosine monophosphate (cGMP).
Thromboxane	A substance formed from prostaglandin precursors in platelets that promotes the constriction of blood vessels and aids blood clotting.
Vasoconstriction	The narrowing or constriction of the blood vessels, reducing the size of the blood vessel opening (lumen). Blood flow is reduced, and blood pressure increases.
Vasodilation	The dilation, or widening, of blood vessels, increasing the size of the vessel opening (lumen). Vasodilation causes increased blood flow through the blood vessels and decreased blood pressure.

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