Vascular Reactivity; GPCR Regulation

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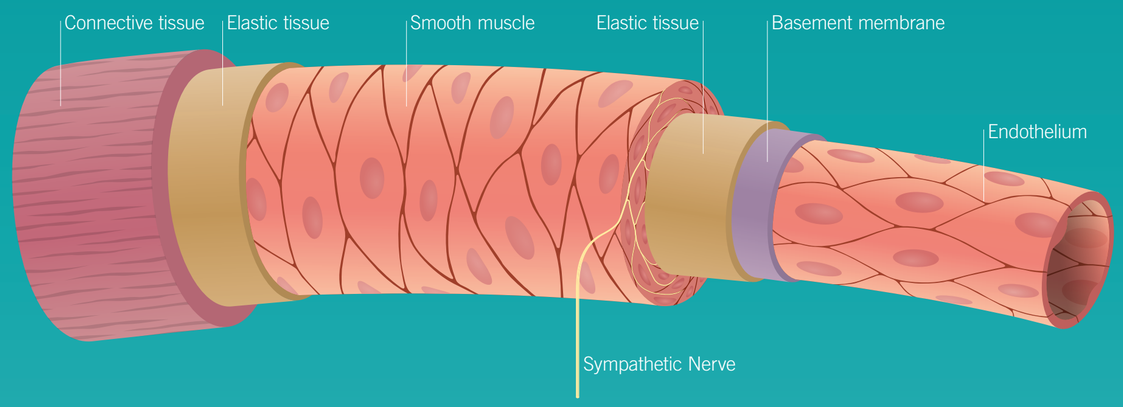
The Vascular System and Dysfunction

The heart is the first organ to develop in the embryo, with vessels developing within the third week and blood circulating after 30 days in humans, which is crucial for maintaining perfusion of the developing fetus. The adult vascular system comprising arteries, arterioles, venules and veins, is rich in receptors transducing a wide range of extracellular signals, vital for maintaining homeostasis. Blood vessels and in particular the small arterioles, are important contributors in setting peripheral resistance: contraction or dilatation in response to endogenous stimuli (including the chemical messengers listed) of a large proportion of vessels within the body will affect total peripheral resistance and blood pressure.

Dysfunction of the vascular endothelium contributes to shifting the balance from vasodilatation to vasoconstriction causing hypertension and is a key initiator of diseases such as atherosclerosis, stroke and heart failure. Cardiovascular disease remains a major cause of morbidity and mortality accounting for about 30% of all deaths globally, despite the widespread use of drugs that target crucial G-protein-coupled receptor (GPCR) systems, such as angiotensin AT_1 receptor antagonists and ß-blockers. This suggests that further targets remain to be identified and potentially could be derived from emerging transmitter systems or the remaining orphan receptors.



Vascular Endothelium, Perivascular Nerves and Smooth Muscle

The vascular endothelium is a single layer of epithelial cells that line every blood vessel in the body, and therefore is present in every organ that receives a blood supply. It has a mass that is comparable to other endocrine glands and an enormous surface area. The endothelium is no longer thought to be a simple barrier between the blood and smooth muscle, but a source of many locally acting vasoactive compounds such as endothelin-1 (ET-1) and nitric oxide (NO). Vascular reactivity is also modulated by the release of transmitters from nerves that travel with blood vessels to virtually all organs in the body, in addition to hormones circulating in the plasma.

Vascular smooth muscle comprises the bulk of the cell types within the vessel wall. They display remarkable plasticity in undergoing rapid phenotypic changes in response to mechanical, physiological or pathophysiological alterations in the local environment. The resulting shift from contractile to a de-differentiated, proliferating phenotype contributes to remodeling of the vascular wall. Many of these changes are mediated by GPCRs.

GPCRs

All GPCRs share seven transmembrane spanning domains. Vertebrate receptors can be divided into five major families based on protein sequence similarity and phylogenetic analysis: Rhodopsin Family (Class A), Secretin Family (Class B), Glutamate Family (Class C), Frizzled Family and Adhesion Family [1]. GPCRs modulating vascular reactivity predominantly belong to the first two of these families.

The aim of this poster is to highlight those GPCRs that on activation by their cognate ligands may function in one of three ways in blood vessels: as directly acting constrictors and dilators, or indirectly acting vasodilators. The schematic illustrated here, summarizes the predominant functional vasoactive responses of a generalized blood vessel to activation of specific GPCRs, with important caveats: the expression of receptors with their associated G-proteins and subsequent functional responses can vary among species, different vascular beds, and between arterial and venous or central and peripheral vessels. In addition, GPCRs can be up- or downregulated with disease, which dramatically alters the balance between constriction and dilatation. For example, in atherosclerosis ghrelin and thromboxane TP receptor densities are increased, with a downregulation of angiotensin AT_1 receptors in the medial smooth muscle layer of the human coronary artery [2].

Endogenous agonists include those that interact with established receptors and those that have been paired with previously designated orphan receptors [3,4], where a role in the control of vascular reactivity is emerging.

Class A

The superfamily of seven-transmembrane spanning GPCRs that belong to Class A is the largest and most diverse family of membrane-spanning proteins. Receptors in Class A are the major target for about one third of the currently available drugs. In the most recent list published by the IUPHAR/BPS Guide to Pharmacology, [1] 197 non-sensory receptors in Class A are reported to interact with established transmitters or chemical messengers that include many vasoactive compounds.

Following completion of 99% of the human genome, bioinformatics has been applied to identify most, if not all, of the remaining genes that potentially encode as yet unliganded 'orphan' receptors in this family. In addition to the established receptors in Class A where the ligand is known, a further 89 orphan GPCRs have been proposed to exist in this category [1]. These orphan receptors continue to be expressed in cell lines and paired with ligands by screening libraries of existing or novel compounds isolated from tissue [3,4]. Many new transmitters are emerging and several, including apelin, kisspeptin and chemerin [5,6,7], display potent activity in the human cardiovascular system. Activation of Class A receptors can result in vasoconstriction or vasodilatation either directly or via release of endothelium-derived relaxing factors.

Emerging Vasoconstrictors		Emerging Vasodilators	
Agonist	**Receptor**	**Agonist**	**Receptor**
CC Chemokines	CCR5	Apelin	Apelin
Chemerin	CMKLR1	Ghrelin	Ghrelin
Kisspeptin	Kisspeptin	Nociceptin	NOP
Sphingosine-1-phosphate	$SIP_{1\text{-}3}$	Relaxin	RXFP1

Class B

The second group of GPCRs, Class B [8], comprises 15 receptors two of which form dimers with receptor accessory proteins [receptor activity modifying proteins (RAMPs)] to produce additional distinct functional receptor complexes. To date, most members of this family are activated by 30–50 amino acid peptides. These principally function in the vasculature as directly acting vasodilators, including the established transmitters, adrenomedullin, calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP), as well as emerging systems such as the urocortins.

Emerging Vasodilators	
Agonist	**Receptor**
Urocortins I, II, III	CRF_2

Class C

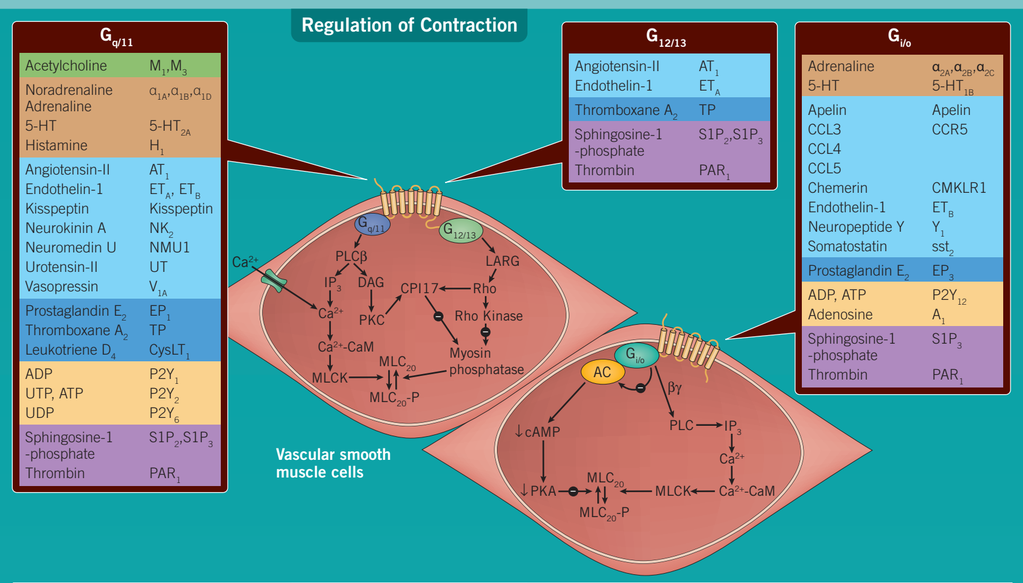
Intriguingly, the 15 Class C metabotropic receptors activated by ligands, including GABA and glutamate, can be localized presynaptically to perivascular nerves but are not widely expressed in the vascular endothelium or smooth muscle.

Frizzled Family

NC-IUPHAR has classified a further eleven GPCRs as belonging to a separate class of the frizzled and smoothened family [1]. Frizzled receptors are activated by a family of cysteine-rich glycosylated Wnt protein ligands. Receptors are widely expressed in the vascular system (as well as other organs) and have been implicated in remodeling, including cell proliferation, differentiation and apoptosis, but a role in vascular reactivity has not been reported.

Adhesion Family

Some of the 33 receptors, designated orphans, in this family may play a role in the development of the cardiovascular system or angiogenesis, but a role in vascular reactivity has not been reported.



Indirectly Acting Vasodilators

In addition to regulating coagulation and thrombus formation, the endothelium contributes to the control of vascular tone by responding to a range of physical factors, such as shear stress, and chemical messengers to release endothelium-derived relaxing factors, including NO, endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (PGI_2) [9]. In many cases, the chemical message is transduced by GPCRs that are expressed on the surface of endothelial cells and stimulate the release of relaxing factors, causing the underlying smooth muscle to dilate. Receptor subtypes for the same transmitter can be expressed on both endothelial and smooth muscle cells. In many cases, these endothelial cell receptors (such as endothelin ET_B receptors) might act as a feedback mechanism to oppose vasoconstrictor responses mediated through a different subtype of the same receptor expressed on the underlying smooth muscle (e.g. endothelin ET_A receptor) [10]. In contrast a single receptor, such as the apelin receptor, whilst expressed on vascular smooth muscle, mediates its predominant vasodilator effect via receptors on endothelial cells linked to release of endothelium-derived relaxing factors [5].

Directly Acting Vasoconstrictors

The sympathetic nervous system, principally through the release of noradrenaline from perivascular nerves, activates smooth muscle α-adrenoceptors to tonically constrict the vasculature. Other locally generated vasoconstrictors include the peptide angiotensin II, 5-HT released from platelets and histamine from mast cells.

The endothelium is also the source of locally acting endothelium-derived contracting factors such as ET-1, thromboxane A_2 and urotensin-II, mediators that can oppose the actions of endothelium-derived vasodilators to maintain basal tone [9]. Smooth muscle GPCRs also respond to eicosanoids and nucleotides released locally from the endothelium or perivascular nerves, in addition to circulating constrictor hormones including adrenaline.

Directly Acting Vasodilators

Chemical messengers circulating in the plasma or locally released mediators from endothelial cells or perivascular nerves might also function as directly acting vasodilators by interacting with GPCRs of both Class A and Class B expressed on smooth muscle. Despite a beneficial role in promoting vasodilatation, receptors in Class B have proved challenging targets for the development of orally active small molecule drugs with high affinity and good pharmacokinetic properties. A small number of CGRP receptor antagonists have been evaluated in early phase clinical trials for migraine [8] but have not yet successfully made it to market.

Products available from Tocris

5-HT Receptors
CP 94253, Ketanserin, MDL 100907, NBOH-2C-CN, SB 224289, TCB-2

Adenosine A_{2A} Receptors
CGS 21680, Istradefylline, LUF 5834, PSB 0777, SCH 58261, ZM 241385

Adrenergic Receptors
Atipamezole, CGP 20712, CL 316243, ICI 118,551, Imiloxan, Isoproterenol

Angiotensin Receptors
Losartan potassium, Novokinin, PD 123319, Telmisartan

Chemokine CC Receptors
DAPTA, Maraviroc

Apelin Receptors
[Pyr1]-Apelin-13, Apelin-17 (human, bovine), Apelin-36 (human), Apelin-36 (rat, mouse), ML 221, ML 233

Bradykinin Receptors
[Phe8Ψ(CH-NH)-Arg^9]-Bradykinin, Bradykinin, HOE 140, R 715, Sar-[D-Phe^8]-des-Arg^9-Bradykinin, WIN 64338

Endothelin Receptors
BQ 788, BQ-123, BQ-3020, FR 139317, IRL-1620, IRL-2500

Ghrelin Receptors
[D-Lys^3]-GHRP-6, Ghrelin (human), Ghrelin (rat), K-(D-1-Nal)-FwLL-NH_2, MK 0677, YIL 781

Kisspeptin Receptor
Kisspeptin 10 (human), Kisspeptin 10 (rat), Kisspeptin 234, Metastin (human)

NOP Receptors
BAN ORL 24, (±)-J 113397, JTC 801, Nociceptin, SCH 221510, UFP-101

Prostanoid Receptors
Alprostadil, Epoprostenol, Prostaglandin E_2, Prostaglandin $F2_α$, Ro 1138452, U 46619

Protease-Activated Receptors
2-Furoyl-LIGRLO-amide, RWJ 56110, SCH 79797, SLIGKV-NH_2, TRAP-6

Purinergic Receptors
AR-C 118925XX, ATP disodium salt, (+)-Clopidogrel hydrogen sulfate, MRS 2365, MRS 2905, SAR 216471

Urotensin-II Receptors
(±)-AC 7954, SB 657510, SB 706375, Urotensin II (human)

Abbreviations
AA, arachidonic acid; **AC**, adenylyl cyclase; **Ca^{2+}**, calcium ions; **CaM**, calmodulin; **cAMP**, cyclic adenosine monophosphate; **cGMP**, cyclic guanosine monophosphate; **CRF**, corticotropin releasing factor; **DAG**, diacylglycerol; **eNOS**, endothelial nitric oxide synthase; **G**, G-protein; **GC**, guanylyl cyclase; **IP3**, inositol (1,4,5)-trisphosphate; **K^+**, potassium ions; **LARG**, leukemia-associated Rho GEF; **MLC20**, 20-kDa regulatory light chain of myosin; **MLCK**, myosin light chain kinase; **NOP**, N/OFQ peptide receptor; **PKA**, protein kinase A; **PKC**, protein kinase C; **PLA2**, phospholipase A_2; **PLC-β**, phospholipase C β isoform.

References and further reading
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