HHS Public Access

Author manuscript

Biochem Soc Trans. Author manuscript; available in PMC 2016 April 07.

Published in final edited form as:

Biochem Soc Trans. 2015 June ; 43(3): 495–501. doi:10.1042/BST20150045.

Emerging concepts regarding pannexin 1 in the vasculature

Miranda E. Good[*], **Daniela Begandt**[*], **Leon J. DeLalio**[*,†], **Alexander S. Keller**[*,†], **Marie Billaud**[‡], and **Brant E. Isakson**[*,§,1]

[*]Robert M. Berne Cardiovascular Research Center, University of Virginia, Charlottesville, VA, U.S.A.

[†]Department of Pharmacology, University of Virginia, Charlottesville, VA, U.S.A.

[‡]Department of Cardiothoracic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, U.S.A.

[§]Department of Molecular Physiology and Biophysics, University of Virginia School of Medicine, Charlottesville, VA, U.S.A.

Abstract

Pannexin channels are newly discovered ATP release channels expressed throughout the body. Pannexin 1 (Panx1) channels have become of great interest as they appear to participate in a multitude of signalling cascades, including regulation of vascular function. Although numerous Panx1 pharmacological inhibitors have been discovered, these inhibitors are not specific for Panx1 and have additional effects on other proteins. Therefore, molecular tools, such as RNA interference and knockout animals, are needed to demonstrate the role of pannexins in various cellular functions. This review focuses on the known roles of Panx1 related to purinergic signalling in the vasculature focusing on post-translational modifications and channel gating mechanisms that may participate in the regulated release of ATP.

Keywords

endothelium; pannexin; pharmacology; smooth muscle; vasculature

Introduction

A key role for purinergic signalling in vascular function has been understood for well-over half of a century. What is not clear is the “how” related to *release* of nucleotides to induce the multiple purinergic cascades. This is especially important for drug-targeting of the purinergic signalling cascade. It had been postulated for some time that release of nucleotides is almost exclusively an exocytosis event. However, recent work has begun to demonstrate pannexin channels as another mean of ATP release from cells that is both highly regulatable and coupled to activation of receptors. This concept is important as it potentially allows for a concentration specific release of nucleotides both spatially and temporally. Although there are three pannexins (Panx1, 2, 3), Panx1 is the most ubiquitous.

[1]To whom correspondence should be addressed (brant@virginia.edu)..

This review focuses on what is currently known about Panx1 related to purinergic signalling in the vasculature, especially the resistance arteries.

On the importance of methodology and pharmacology in the study of pannexins

Since the initial discovery of Panx1 channels in 2003, great strides have been made in an effort to develop appropriate tools to study the role of these ATP-releasing channels in various cell types, tissue and organisms. From a pharmacological standpoint, Dahl et al. [1] defined Panx1 channels as a “sewer for drugs”, listing 37 drugs that have been reported to inhibit Panx1 channels in the past decade alone. The majority of these drugs are well-known blockers of other targets, such as organic anion transporters (probenecid), potassium channels (glibenclamide), chloride channel inhibitors (NPPB, DIDS), $P2X_7$ purinergic receptors (e.g. Brilliant Blue G, BzATP, suramin), gap junctions (flufenamic acid, 18α-glycyrrhetinic acid, carbenoxolone (CBX), connexin mimetic peptides, mefloquine) or inhibitors of the mitochondrial ATP efflux (bongkrekic acid, atractyloside) [1]. In an effort to uncover the role of Panx1 channels *in vitro* and *in vivo*, the scientific community mostly relied on CBX, mefloquine and probenecid. CBX has been used for over 25 years as a connexin-based channel blocker, but in 2005 it was one of the first agents shown to directly inhibit Panx1 currents [2]. In that study, the authors demonstrated a higher sensitivity of Panx1 channels to CBX compared with connexin-based channels, and this characteristic has been further used to help in differentiating between Panx1 and connexin-based channels. Similarly, mefloquine has shown to be more effective on Panx1 channels at low doses (~50 nM) while concentrations greater than 10 μM are required to inhibit connexin-based channels [3,4]. Nevertheless, these pharmacological properties have been assessed in overexpressing *in vitro* models, making it difficult to extrapolate the efficacy and specificity of these drugs in native cells or tissue, especially for tissues co-expressing connexins and pannexins such as the vasculature. On the other hand, probenecid does not affect connexin-based channels even at doses as high as 5 mM, while it effectively inhibits Panx1 channels with an IC50 of ~150 μM [5]. Probenecid was thus considered promising to use in the vasculature, but in reality, concentrations as high as 1–2 mM are required to observe an inhibitory effect in native cells or tissue, making the specificity of the drug questionable [5–8]. Of note, the efficacy of probenecid and CBX on Panx1 channel activity is severely impaired when other proteins are co-expressed, as shown with the potassium channel subunit Kvbeta3 [9]. This led to further question the influence of other proteins on the efficacy of those drugs in inhibiting Panx1 channels in native cells or tissue. With the growing need for more specific Panx1 inhibitors, and following the model of connexin mimetic peptides, the ^{10}Panx1 peptide was developed in 2006 by the Surprenant laboratory with the hope to specifically target Panx1 channels [10]. Unfortunately, it was later evidenced that the ^{10}Panx1 peptide also affects other targets such as connexin-based channels, and its effect can be replicated by other peptides of equivalent size, or by 1.5 kDa polyethylene glycol [11].

It has now become evident that the most commonly used “Panx1 pharmacological inhibitors”, mefloquine, probenecid and ^{10}Panx1, have a wide range of effects in native cells

and tissue [12]. The interpretation of functional studies only using these drugs is thus rather difficult and a substantial number of publications solely based on pharmacological inhibitors targeting Panx1 have led to misleading conclusions with regards to the involvement of Panx1. To alleviate this, molecular tools such as RNA interference and knockout animal models have been used to greatly benefit the field in the understanding of the physiological and pathological relevance of the ubiquitous protein that is Panx1 [6,8,10,13–16]. However, it should be noted that cell-specific knockout animal models should be utilized when possible because a compensatory increase in Panx3 protein has been reported in the global $Panx1^{-/-}$ mouse ([17,18]). Given these considerations, the scientific community should be aware of the considerable challenges involved in the study of Panx1 channels with regards to the experimental conditions as well as the pharmacological tools. One should be cautious when drawing conclusions on the involvement of Panx1 channels by relying solely on "Panx1 pharmacological inhibitors" and complementary experiments using molecular tools such as siRNA targeting Panx1 (e.g. [6]) or cell-type specific knockout animals (e.g. [16]) should be implemented.

Post-translational modification of pannexins

The gating of pannexin channels by post-translational modification is an area of great biological interest. To date, much information has been gleaned about the numerous types of post-translational modifications that are found on pannexin channels, which include N-glycosylation [19–21] caspase cleavage [14,22], S-nitrosylation [23,24], and phosphorylation [16,19,25–27]. However, the mechanisms and stimuli that directly regulate pannexin channel activation and gating remain under intense investigation. Of all known pannexin post-translational modifications, S-nitrosylation and phosphorylation are indicated to play significant roles in the vasculature, largely since nitric oxide (NO) and kinase mediated signalling events strongly predominate constrictor/dilatator responses in veins and arteries [28,29].

The reversible post-translational attachment of NO moieties to cysteine residues, termed S-nitrosylation, has profound effects on protein function [12]. Previous reports suggest that ischemic conditions activate pannexins [15,30] and inhibitors of nitric oxide synthase (NOS) and antioxidants, such as DTT and GSH, can ameliorate channel activation [31]. In veins and arteries, NO acts as both a second messenger and a protein-modifying molecule [32]. Building on this concept, Panx1 was shown to be nitrosylated at cysteine 40 and 346 using a biotin switch assay in HEK (over-expressing) and endothelial (endogenous) cells [23]. Nitrosylation by the NO donor GSNO attenuated Panx1 channel activity and prevented release of ATP from cells [23]. Furthermore, site-specific mutagenesis of cysteine 40 and 346 to alanine residues conferred a constitutively open Panx1 channel [23], suggesting that S-nitrosylation of Panx1 may act as a negative regulatory mechanism in the vasculature. While it is still unknown which physiological stimuli directly regulate vascular S-nitrosylation events, it is alluring to think that a NO-dependent inactivation event exists for Panx1 channels to balance the Panx1 channel-dependent vasoconstriction in resistance arteries [6,16,33].

In addition to S-nitrosylation, the covalent addition of phosphate groups to proteins is a major regulator of protein function. While several phosphorylation sites for pannexins have been predicted, only indirect evidence exists to establish a possible mechanism for pannexin gating by specific kinases [25,26]. Current research implicates members of the Src family kinases (SFKs) as regulators of Panx1 channels. Panx1 channel activation in J778 macrophages was sensitive to both the SFK inhibitor PP2 and a small interfering peptide that targets a proline rich region on the $P2X_7$ carboxyl terminus (C-terminus) suggesting that Panx1 channel activation is mediated through $P2X_7$ receptors and SFKs [25]. Using a similar approach, Weilinger et al. [26] demonstrated that treatment with PP2 or a small interfering peptide against Panx1 tyrosine 308 prevents anoxia-induced Panx1 channel opening in rodent hippocampal brain slices. More recently, Billaud et al. demonstrated that pharmacological and genetic inhibition of an intracellular motif containing tyrosine 198 (Y198) prevents adrenergic-stimulated ATP release and vasoconstriction in resistance arteries [16]. These responses could be rescued in arteries from mice with Panx1 deleted specifically from the smooth muscle cells (SMCs) after transfection with wild-type Panx1, but not the tyrosine-containing motif mutant [16]. While the direct phosphorylation of Panx1 by SFK members has yet to be described, the reports by Weilinger et al. [26] and Billaud et al. [16] suggest that kinase phosphorylation events at Panx1 Y308 and Panx1 Y198, located within known SFK homology recognition sites (SH2 and SH3 respectively), could regulate Panx1 channel activity. Currently, the only indirect evidence for Panx1 phosphorylation is by Riquelme et al. [27] who demonstrated an increase in tyrosine and serine phosphorylation in electrically stimulated skeletal muscle. These and future investigations will help define post-translational mechanisms that regulate pannexin channel activity, as well as help identify new pathways that may contribute to the pathologies associated with vascular dysfunction.

Pannexin channel gating

Though noted for its permeability to ATP [34], Panx1 channels exhibit low ion selectivity and thus offers a broad, if transitory, window from the cytoplasm to the extracellular space [35,36]. A discrepancy has arisen regarding Panx1 channel conductance [37], which was originally reported at ~450–500 pS [34,35,38] but more recently reported on the order of 70–75 pS [39–41]. Panx1 channels are reported to be outwardly rectifying [34] with a pore size estimated at 17–21 Å (1 Å=0.1 nm) [42] (although the structure is not yet resolved) suggesting that Panx1 channels may have the potential to undermine the selective permeability of the cell membrane [36]. This large permeability is likely why Panx1 channels predominantly remain closed at physiologic voltages [34] and undergo extensive post-translational modification.

Panx1 channels open to varying extents in response to multiple stimuli [17,43]. However, the cytoplasmic C-terminus of Panx1 is critical to the maintenance of the channels closed state and may inhibit the Panx1 channel via a “ball-and-chain” mechanism, similar to that observed in other ion channels, whereby the C-terminus may enter the pore from the cytoplasmic side [22,44]. The well-described caspase cleavage of the C-terminus between amino acids 376 and 379 [14,22] renders Panx1 channels constitutively truncated and permanently active [14]. Among channels with similarly hypothesized ball-and-chain

mechanisms, Panx1 is the first known to undergo cleavage of the C-terminus as a method to permanently remove this channel inhibition mechanism [22].

Potentially important in the vasculature (e.g. in conduction pathways), a positive feedback loop of ATP-induced ATP release has been hypothesized, stemming from Panx1 channel release of ATP in response to mechanical stress [45]. Subsequent ATP activation of P2Y receptors on the same and/or adjacent cells could initiate a signalling cascade that releases intracellular Ca^{2+}, opening further Panx1 channels to propagate an extracellular ATP signal alongside an intracellular wave of Ca^{2+} and inositol triphosphate [45]. This possibility is reliant on Panx1 channels being sensitive to intracellular Ca^{2+}, for which there appears to be common knowledge but sparse physiological evidence.

Additionally, ATP-induced ATP release is also thought to be mediated through Panx1 channels and P2X receptors, which have been found to co-localize, where Panx1 channels open in response to ATP-gated $P2X_7$ receptor activation [10,46,47]. This positive feedback loop of ATP-induced ATP release may in turn be negatively regulated by extracellular ATP. Substituted cysteine accessibility data suggest that the first transmembrane domain and first extracellular loop (EL1) line the Panx1 channel pore [48]. At high extracellular concentrations, ATP is believed to inhibit Panx1 by interacting with arginine 75 on EL1, perhaps providing a steric obstruction at the extracellular end of the pore to block ATP passage through the open channel [36]. This could have critical importance in providing negative regulation to the purinergic receptor-activated positive feedback loop of ATP-induced Panx1 ATP release [49]. However, outside of evidence provided from non-vascular cell culture, it is currently not clear how this observation might relate to the intact vasculature.

Other important mechanisms controlling pannexin channel activation include membrane depolarization, decreased circulating oxygen levels and mechanical stimulation. Although there are reports of Panx1 channels being responsive to mechanical stimulation, such as cell swelling or stretching, it remains an active area of investigation whether this mechanism occurs *in vivo* [34,47,50]. It has also been shown that lowered blood pO_2 levels could open Panx1 channels on erythrocytes, releasing ATP to induce upstream vasodilation [51]. In addition, a key regulator in maintaining blood pO_2 levels, the carotid body, also releases ATP through Panx1 channels on type II cells, potentially further amplifying activation of the afferent nerves [52]. Oxygen-dependent activation of Panx1 has been demonstrated in erythrocytes and carotid bodies, both of which regulate peripheral resistance, but the impact of oxygen content on endothelial cells (ECs) or vascular smooth muscle cells (VSMCs) Panx1 channel activation is unknown. Lastly, membrane depolarization by activation of TRPM5 channels, with the addition of extracellular KCl, or application of a depolarizing voltage step has been shown to activate Panx1 channels *in vitro* [2,53,54]. However, KCl-induced vasoconstriction in isolated arteries was found to be independent of Panx1 channel activity indicating that the mechanisms for Panx1 channel activation are likely either cell type specific or altered *in vitro* [6].

Of note, the stimuli suggested to activate Panx1 channels (mechanical stimulation [34,47,50], increasing extracellular potassium concentration [54], rise in intracellular

calcium [45], membrane depolarization [2], increased intracellular redox potential [55], S-nitrosylation [23], oxygen deprivation [51,52]) could all be physiologically relevant to vascular physiology/pathophysiology. Hence, caution should be taken in the experimental design to study the role of Panx1 in the vascular wall, where vessel tension, potassium and calcium concentrations as well as oxygen tension should be monitored as close as possible to *in vivo* conditions. Such control over these parameters is crucial to accurately assess the role of Panx1 in the vasculature.

Pannexins in endothelial cells

Little is known about the role of Panx1 in ECs in health and disease. Most studies have focused on defining the expression patterns of pannexin isoforms in murine and rodent endothelium [56,57], while few studies have explored the signalling cascades that result in channel activation and subsequent ATP release [13,14]. Various stimuli like mechanical strain and EC agonists have been shown to trigger EC ATP release [6,13,58–59]. However, the role of pannexins, Panx1 in particular, in ECs remains understudied.

In ECs, pannexins are suggested to be involved in vasodilation and inflammatory cell adhesion [13,23]. Pannexin channels in inflammatory cells could play an important role during pathophysiological inflammatory conditions, such as participation in the secretion pathway of IL-1β [60] as well as transmission of a “find me” signal in early apoptotic cells [14]. Moreover, nucleotide release from ECs is demonstrated to have a vital role in regulating leucocyte adhesion to the vascular wall [61]. In human umbilical vein endothelial cells (HUVECs), pannexin channel-mediated ATP release is evoked by application of thrombin [13]. Prothrombin is cleaved to thrombin in injured vessels and then binds to the protease-activated receptor PAR-1 on ECs resulting in subsequent ATP release from these ECs reportedly due to increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) [62,63]. These results are in agreement with observations in *Xenopus* oocytes of Panx1 activation through increased $[Ca^{2+}]_i$ [45] suggesting a potential role for Panx1 in regulating thrombin-dependent vascular response.

Mechanical injury of ECs during surgical harvest of saphenous vein grafts has been shown to result in impaired endothelial and smooth muscle functions and intimal hyperplasia *ex vivo* [64]. Preparation-induced injury was shown to cause ATP release and activation of $P2X_7$ receptors and Panx1 channels, with downstream signalling of the $P2X_7$ receptor implicated in the development of intimal hyperplasia. However, inhibition of purinergic receptors or pannexin channels with periodate-oxidized adenosine triphosphate (oATP) and probenecid, respectively, restored EC function and prevented development of intimal hyperplasia [65]. These results further suggest that the pannexin-mediated ATP release within ECs could be a key regulator for physiologic and pathologic EC function.

EC function can also be controlled by perivascular nerves releasing neurotransmitters that bind to specific receptors on the EC surface, such as acetylcholine and calcitonin gene-related peptide (CGRP) [66–68]. Recently, Gaete et al. [69] suggested that the neuropeptide and strong vasodilator CGRP activates Panx1 channels in mesenteric resistance vessels and primary cultures of mesenteric ECs. $CGRP_{8–37}$, a CGRP receptor antagonist, and

probenecid, a Panx1 channel blocker, inhibited pannexin channel activation as measured by ethidium uptake [69]. Furthermore, CGRP treatment resulted in decreased Panx1 and eNOS expression [69]. This observation could explain the endothelial dysfunction observed during pathological conditions associated with inflammation through the reduction in eNOS levels [70–75].

Pannexins in vascular smooth muscle cells

Pannexin expression in VSMCs is limited to arteries less than 300 μm in diameter in mice [57]. These small arteries contribute significantly to the regulation of peripheral resistance; however, the mechanisms controlling peripheral resistance are not fully understood. ATP release in the cardiovascular system is known to participate in physiological and pathological conditions such as hypoxia-induced vasodilation, α1D-adrenergic receptor (α1D-AR) mediated vasoconstriction and hypertension [32]. Multiple non-vascular SMCs release ATP, such as SMCs from the colon and bladder, both of which express Panx1 [76–79]. Only recently have ATP-releasing pannexin channels emerged as regulators of vascular function, with recent evidence revealing an important and vital role for Panx1 expression in VSMCs of small systemic arteries [6,16]. Systemic arteries are highly innervated by sympathetic nerves that release norepinephrine (NE), which then activates α1D-ARs, expressed on the adjacent VSMCs, and results in vasoconstriction [80,81]. Interestingly, this vasoconstriction is dependent upon activation of the purinergic receptors, where blockade of purinergic receptors with either a non-specific antagonist, suramin, or a P2Y subtype specific inhibitor, reactive blue-2, significantly blunted adrenergic vasoconstriction [6]. In addition, degradation of extracellular ATP, the purinergic receptor agonist, with an ectonucleotidase, apyrase, profoundly reduced adrenergic mediated vasoconstriction [6]. Together these data implicate the need for regulated ATP release from the VSMCs. Indeed, inhibition of Panx1 with non-specific and specific inhibitors or genetic deletion of VSMC Panx1 resulted in significant reduction in vasoconstriction following stimulation with phenylephrine (PE), an α1-AR agonist, revealing a clear function for Panx1 in VSMC vasoconstriction [6].

The functional link between α1D-ARs and Panx1 channels was further explored using mimetic peptides against Panx1, two peptides against the cytoplasmic tail region and two peptides against the intracellular loop region [16]. Application of these peptides and further experiments using point mutations in a cell culture model resulted in the discovery of a necessary amino acid motif (YLK at amino acids 198–200) in the intracellular loop region of Panx1 for Panx1 channel-dependent ATP release following α1D-AR stimulation [16]. Interestingly, vasoconstriction via alternative mechanisms, such as endothelin-1 or serotonin, is not dependent upon Panx1 mediated ATP release [16]. Together these data strongly implicate a role for Panx1-dependent regulation in adrenergic driven vasoconstriction.

Due to the role of Panx1 channels during vasoconstriction of isolated resistance arteries, blood pressure was examined to determine if Panx1-dependent vasoconstriction affected peripheral resistance and, therefore, overall mean arterial pressure (MAP). Mice lacking VSMC Panx1 are indeed hypotensive suggesting the regulation of peripheral resistance is dependent on Panx1 expression in VSMCs [16]. In agreement with an adrenergic-dependent

mechanism, the drop in blood pressure is more profound during the night when mice are more active and thus during greater sympathetic activity [16]. Injection of the intracellular loop mimetic peptide, which blocked PE-stimulated vasoconstriction in isolated arteries by possibly targeting the YLK motif, also caused a significant drop in MAP in C57Bl6 mice [16]. In congruence with this observation, hypotension is observed when guinea pigs are treated with mefloquine, a potent Panx1 channel inhibitor [3,82]. Additionally, hypotension is listed as a side effect for mefloquine, an FDA approved drug used to prevent and treat malaria. These data indicate that Panx1 expression in VSMCs of resistance arteries is vital for the development of adrenergic vasoconstriction and overall peripheral resistance.

Future directions

Pannexin channels represent an exciting and new area of investigation in the vasculature, with cell biology and biochemistry providing important insight into function. Although the pharmacology remains problematic, molecular tools such as siRNA, shRNA and KO animal models have led to stronger conclusions about the function of Panx1.

Acknowledgements

Due to condensed nature of this review, we apologize for not citing every paper on pannexins and the vasculature. We thank Angela K. Best for critical feedback.

Funding This work was supported by the National Institutes of Health [grant numbers HL088554 and HL120840 (to B.E.I.)]; and the National Institutes of Health [training grant number HL007284 (to L.J.D. and M.E.G.)].

Abbreviations

α1D-AR	α1D-adrenergic receptor
CGRP	calcitonin gene-related peptide
CBX	carbenoxolone
EC	endothelial cell
EL1	first extracellular loop
HUVEC	human umbilical vein endothelial cell
MAP	mean arterial pressure
NO	nitric oxide
NOS	nitric oxide synthase
Panx1	pannexin1
PE	phenylephrine
SFK	Src family kinase
SMC	smooth muscle cell
VSMC	vascular smooth muscle cells

References

1. Dahl G, Qiu F, Wang J. The bizarre pharmacology of the ATP release channel pannexin1. Neuropharmacology. 2013; 75:583–593. CrossRef PubMed. [PubMed: 23499662]
2. Bruzzone R, Barbe MT, Jakob NJ, Monyer H. Pharmacological properties of homomeric and heteromeric pannexin hemichannels expressed in Xenopus oocytes. J. Neurochem. 2005; 92:1033–1043. CrossRef PubMed. [PubMed: 15715654]
3. Iglesias R, Spray DC, Scemes E. Mefloquine blockade of Pannexin1 currents: resolution of a conflict. Cell Commun. Adhes. 2009; 16:131–137. CrossRef PubMed. [PubMed: 20218915]
4. Cruikshank SJ, Hopperstad M, Younger M, Connors BW, Spray DC, Srinivas M. Potent block of Cx36 and Cx50 gap junction channels by mefloquine. Proc. Natl. Acad. Sci. U.S.A. 2004; 101:12364–12369. CrossRef PubMed. [PubMed: 15297615]
5. Silverman W, Locovei S, Dahl G. Probenecid, a gout remedy, inhibits pannexin 1 channels. Am. J. Physiol. Cell Physiol. 2008; 295:C761–C767. CrossRef PubMed. [PubMed: 18596212]
6. Billaud M, Lohman AW, Straub AC, Looft-Wilson R, Johnstone SR, Araj CA, Best AK, Chekeni FB, Ravichandran KS, Penuela S, et al. Pannexin1 regulates alpha1-adrenergic receptor-mediated vasoconstriction. Circ. Res. 2011; 109:80–85. CrossRef PubMed. [PubMed: 21546608]
7. Xia J, Lim JC, Lu W, Beckel JM, Macarak EJ, Laties AM, Mitchell CH. Neurons respond directly to mechanical deformation with pannexin-mediated ATP release and autostimulation of P2X7 receptors. J. Physiol. 2012; 590:2285–2304. CrossRef PubMed. [PubMed: 22411013]
8. Ransford GA, Fregien N, Qiu F, Dahl G, Conner GE, Salathe M. Pannexin 1 contributes to ATP release in airway epithelia. Am. J. Respir. Cell Mol. Biol. 2009; 41:525–534. CrossRef PubMed. [PubMed: 19213873]
9. Bunse S, Locovei S, Schmidt M, Qiu F, Zoidl G, Dahl G, Dermietzel R. The potassium channel subunit Kvbeta3 interacts with pannexin 1 and attenuates its sensitivity to changes in redox potentials. FEBS J. 2009; 276:6258–6270. CrossRef PubMed. [PubMed: 19780818]
10. Pelegrin P, Surprenant A. Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. EMBO J. 2006; 25:5071–5082. CrossRef PubMed. [PubMed: 17036048]
11. Wang J, Ma M, Locovei S, Keane RW, Dahl G. Modulation of membrane channel currents by gap junction protein mimetic peptides: size matters. Am. J. Physiol. Cell Physiol. 2007; 293:C1112–C1119. CrossRef PubMed. [PubMed: 17652431]
12. Billaud M, Lohman AW, Johnstone SR, Biwer LA, Mutchler S, Isakson BE. Regulation of cellular communication by signaling microdomains in the blood vessel wall. Pharmacol. Rev. 2014; 66:513–569. CrossRef PubMed. [PubMed: 24671377]
13. Godecke S, Roderigo C, Rose CR, Rauch BH, Godecke A, Schrader J. Thrombin-induced ATP release from human umbilical vein endothelial cells. Am. J. Physiol. Cell Physiol. 2012; 302:C915–C923. CrossRef PubMed. [PubMed: 22159088]
14. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, Armstrong AJ, Penuela S, Laird DW, Salvesen GS, et al. Pannexin 1 channels mediate `find-me' signal release and membrane permeability during apoptosis. Nature. 2010; 467:863–867. CrossRef PubMed. [PubMed: 20944749]
15. Bargiotas P, Krenz A, Hormuzdi SG, Ridder DA, Herb A, Barakat W, Penuela S, von Engelhardt J, Monyer H, Schwaninger M. Pannexins in ischemia-induced neurodegeneration. Proc. Natl. Acad. Sci. U.S.A. 2011; 108:20772–20777. CrossRef PubMed. [PubMed: 22147915]
16. Billaud M, Chiu YH, Lohman AW, Parpaite T, Butcher JT, Mutchler SM, DeLalio L, Artamonov MV, Sandilos JK, Best AK, et al. A molecular signature in the pannexin1 intracellular loop confers channel activation by the α1 adrenoreceptor in smooth muscle cells. Sci. Signal. 2015; 8:ra17. CrossRef PubMed. [PubMed: 25690012]
17. Lohman AW, Isakson BE. Differentiating connexin hemichannels and pannexin channels in cellular ATP release. FEBS Lett. 2014; 588:1379–1388. CrossRef PubMed. [PubMed: 24548565]
18. Penuela S, Kelly JJ, Churko JM, Barr KJ, Berger AC, Laird DW. Panx1 regulates cellular properties of keratinocytes and dermal fibroblasts in skin development and wound healing. J. Invest. Dermatol. 2014; 134:2026–2035. [PubMed: 24522432]

19. Penuela S, Bhalla R, Gong XQ, Cowan KN, Celetti SJ, Cowan BJ, Bai D, Shao Q, Laird DW. Pannexin 1 and pannexin 3 are glycoproteins that exhibit many distinct characteristics from the connexin family of gap junction proteins. J. Cell Sci. 2007; 120:3772–3783. CrossRef PubMed. [PubMed: 17925379]
20. Penuela S, Bhalla R, Nag K, Laird DW. Glycosylation regulates pannexin intermixing and cellular localization. Mol. Biol. Cell. 2009; 20:4313–4323. CrossRef PubMed. [PubMed: 19692571]
21. Boassa D, Ambrosi C, Qiu F, Dahl G, Gaietta G, Sosinsky G. Pannexin1 channels contain a glycosylation site that targets the hexamer to the plasma membrane. J. Biol. Chem. 2007; 282:31733–31743. CrossRef PubMed. [PubMed: 17715132]
22. Sandilos JK, Chiu YH, Chekeni FB, Armstrong AJ, Walk SF, Ravichandran KS, Bayliss DA. Pannexin 1, an ATP release channel, is activated by caspase cleavage of its pore-associated C-terminal autoinhibitory region. J. Biol. Chem. 2012; 287:11303–11311. CrossRef PubMed. [PubMed: 22311983]
23. Lohman AW, Weaver JL, Billaud M, Sandilos JK, Griffiths R, Straub AC, Penuela S, Leitinger N, Laird DW, Bayliss DA, et al. S-nitrosylation inhibits pannexin 1 channel function. J. Biol. Chem. 2012; 287:39602–39612. CrossRef PubMed. [PubMed: 23033481]
24. Bunse S, Schmidt M, Prochnow N, Zoidl G, Dermietzel R. Intracellular cysteine 346 is essentially involved in regulating Panx1 channel activity. J. Biol. Chem. 2010; 285:38444–38452. CrossRef PubMed. [PubMed: 20829356]
25. Iglesias R, Locovei S, Roque A, Alberto AP, Dahl G, Spray DC, Scemes E. P2X7 receptor-Pannexin1 complex: pharmacology and signaling. Am. J. Physiol. Cell Physiol. 2008; 295:C752–C760. CrossRef PubMed. [PubMed: 18596211]
26. Weilinger NL, Tang PL, Thompson RJ. Anoxia-induced NMDA receptor activation opens pannexin channels via Src family kinases. J. Neurosci. 2012; 32:12579–12588. CrossRef PubMed. [PubMed: 22956847]
27. Riquelme MA, Cea LA, Vega JL, Boric MP, Monyer H, Bennett MV, Frank M, Willecke K, Saez JC. The ATP required for potentiation of skeletal muscle contraction is released via pannexin hemichannels. Neuropharmacology. 2013; 75:594–603. CrossRef PubMed. [PubMed: 23583931]
28. Cooke JP, Dzau VJ. Nitric oxide synthase: role in the genesis of vascular disease. Annu. Rev. Med. 1997; 48:489–509. CrossRef PubMed. [PubMed: 9046979]
29. Lincoln TM, Dey N, Sellak H. Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. J. Appl. Physiol. 2001; 91:1421–1430. 1985. PubMed. [PubMed: 11509544]
30. Thompson RJ, Zhou N, MacVicar BA. Ischemia opens neuronal gap junction hemichannels. Science. 2006; 312:924–927. CrossRef PubMed. [PubMed: 16690868]
31. Zhang L, Deng T, Sun Y, Liu K, Yang Y, Zheng X. Role for nitric oxide in permeability of hippocampal neuronal hemichannels during oxygen glucose deprivation. J. Neurosci. Res. 2008; 86:2281–2291. CrossRef PubMed. [PubMed: 18381763]
32. Lohman AW, Billaud M, Isakson BE. Mechanisms of ATP release and signalling in the blood vessel wall. Cardiovasc. Res. 2012; 95:269–280. CrossRef PubMed. [PubMed: 22678409]
33. Nielsen MS. Sympathetic vasoconstriction takes an unexpected pannexin detour. Sci. Signal. 2015; 8 fs4 PubMed.
34. Bao L, Locovei S, Dahl G. Pannexin membrane channels are mechanosensitive conduits for ATP. FEBS Lett. 2004; 572:65–68. CrossRef PubMed. [PubMed: 15304325]
35. Locovei S, Bao L, Dahl G. Pannexin 1 in erythrocytes: function without a gap. Proc. Natl. Acad. Sci. U.S.A. 2006; 103:7655–7659. CrossRef PubMed. [PubMed: 16682648]
36. Qiu F, Dahl G. A permeant regulating its permeation pore: inhibition of pannexin 1 channels by ATP. Am. J. Physiol. Cell Physiol. 2009; 296:C250–C255. CrossRef PubMed. [PubMed: 18945939]
37. Sandilos JK, Bayliss DA. Physiological mechanisms for the modulation of pannexin 1 channel activity. J. Physiol. 2012; 590:6257–6266. CrossRef PubMed. [PubMed: 23070703]
38. Iglesias R, Spray DC, Scemes E. Mefloquine blockade of Pannexin1 currents: Resolution of a conflict. Cell Commun. Adhes. 2009; 16:131–137. CrossRef PubMed. [PubMed: 20218915]

39. Chiu YH, Ravichandran KS, Bayliss DA. Intrinsic properties and regulation of Pannexin 1 channel. Channels (Austin). 2014; 8:103–109. CrossRef PubMed. [PubMed: 24419036]
40. Ma W, Compan V, Zheng W, Martin E, North RA, Verkhratsky A, Surprenant A. Pannexin 1 forms an anion-selective channel. Pflugers Arch. 2012; 463:585–592. CrossRef PubMed. [PubMed: 22311122]
41. Romanov RA, Bystrova MF, Rogachevskaya OA, Sadovnikov VB, Shestopalov VI, Kolesnikov SS. The ATP permeability of pannexin 1 channels in a heterologous system and in mammalian taste cells is dispensable. J. Cell Sci. 2012; 125:5514–5523. CrossRef PubMed. [PubMed: 22956545]
42. Ambrosi C, Gassmann O, Pranskevich JN, Boassa D, Smock A, Wang J, Dahl G, Steinem C, Sosinsky GE. Pannexin1 and Pannexin2 channels show quaternary similarities to connexons and different oligomerization numbers from each other. J. Biol. Chem. 2010; 285:24420–24431. CrossRef PubMed. [PubMed: 20516070]
43. Bond SR, Naus CC. The pannexins: past and present. Front. Physiol. 2014; 5:58. CrossRef PubMed. [PubMed: 24600404]
44. Dourado M, Wong E, Hackos DH. Pannexin-1 is blocked by its C-terminus through a delocalized non-specific interaction surface. PloS One. 2014; 9:e99596. CrossRef PubMed. [PubMed: 24911976]
45. Locovei S, Wang J, Dahl G. Activation of pannexin 1 channels by ATP through P2Y receptors and by cytoplasmic calcium. FEBS Lett. 2006; 580:239–244. CrossRef PubMed. [PubMed: 16364313]
46. Locovei S, Scemes E, Qiu F, Spray DC, Dahl G. Pannexin1 is part of the pore forming unit of the P2X7 receptor death complex. FEBS Lett. 2007; 581:483–488. CrossRef PubMed. [PubMed: 17240370]
47. Negoro H, Urban-Maldonado M, Liou LS, Spray DC, Thi MM, Suadicani SO. Pannexin 1 channels play essential roles in urothelial mechanotransduction and intercellular signaling. PloS One. 2014; 9:e106269. CrossRef PubMed. [PubMed: 25170954]
48. Wang J, Dahl G. SCAM analysis of Panx1 suggests a peculiar pore structure. J. Gen. Physiol. 2010; 136:515–527. CrossRef PubMed. [PubMed: 20937692]
49. Qiu F, Wang J, Dahl G. Alanine substitution scanning of pannexin1 reveals amino acid residues mediating ATP sensitivity. Purinergic Signal. 2012; 8:81–90. CrossRef PubMed. [PubMed: 21987098]
50. Beckel JM, Argall AJ, Lim JC, Xia J, Lu W, Coffey EE, Macarak EJ, Shahidullah M, Delamere NA, Zode GS, et al. Mechanosensitive release of adenosine 5′-triphosphate through pannexin channels and mechanosensitive upregulation of pannexin channels in optic nerve head astrocytes: a mechanism for purinergic involvement in chronic strain. Glia. 2014; 62:1486–1501. CrossRef PubMed. [PubMed: 24839011]
51. Sridharan M, Adderley SP, Bowles EA, Egan TM, Stephenson AH, Ellsworth ML, Sprague RS. Pannexin 1 is the conduit for low oxygen tension-induced ATP release from human erythrocytes. Am. J. Physiol. Heart Circ. Physiol. 2010; 299:H1146–H1152. CrossRef PubMed. [PubMed: 20622111]
52. Zhang M, Piskuric NA, Vollmer C, Nurse CA. P2Y2 receptor activation opens pannexin-1 channels in rat carotid body type II cells: potential role in amplifying the neurotransmitter ATP. J. Physiol. 2012; 590:4335–4350. CrossRef PubMed. [PubMed: 22733659]
53. Huang YA, Roper SD. Intracellular Ca(2+) and TRPM5-mediated membrane depolarization produce ATP secretion from taste receptor cells. J. Physiol. 2010; 588:2343–2350. CrossRef PubMed. [PubMed: 20498227]
54. Silverman WR, de Rivero Vaccari JP, Locovei S, Qiu F, Carlsson SK, Scemes E, Keane RW, Dahl G. The pannexin 1 channel activates the inflammasome in neurons and astrocytes. J. Biol. Chem. 2009; 284:18143–18151. CrossRef PubMed. [PubMed: 19416975]
55. Retamal MA. Connexin and Pannexin hemichannels are regulated by redox potential. Front. Physiol. 2014; 5:80. PubMed. [PubMed: 24611056]
56. Burns AR, Phillips SC, Sokoya EM. Pannexin protein expression in the rat middle cerebral artery. J. Vasc. Res. 2012; 49:101–110. CrossRef PubMed. [PubMed: 22301733]

57. Lohman AW, Billaud M, Straub AC, Johnstone SR, Best AK, Lee M, Barr K, Penuela S, Laird DW, Isakson BE. Expression of pannexin isoforms in the systemic murine arterial network. J. Vasc. Res. 2012; 49:405–416. CrossRef PubMed. [PubMed: 22739252]
58. Bodin P, Bailey D, Burnstock G. Increased flow-induced ATP release from isolated vascular endothelial cells but not smooth muscle cells. Br. J. Pharmacol. 1991; 103:1203–1205. CrossRef PubMed. [PubMed: 1652343]
59. Bodin P, Burnstock G. ATP-stimulated release of ATP by human endothelial cells. J. Cardiovasc. Pharmacol. 1996; 27:872–875. CrossRef PubMed. [PubMed: 8761855]
60. Pelegrin P, Surprenant A. The P2X(7) receptor-pannexin connection to dye uptake and IL-1beta release. Purinergic Signal. 2009; 5:129–137. CrossRef PubMed. [PubMed: 19212823]
61. Eun SY, Park SW, Lee JH, Chang KC, Kim HJ. P2Y(2)R activation by nucleotides released from oxLDL-treated endothelial cells (ECs) mediates the interaction between ECs and immune cells through RAGE expression and reactive oxygen species production. Free Radic. Biol. Med. 2014; 69:157–166. CrossRef PubMed. [PubMed: 24486339]
62. Gruenhagen JA, Yeung ES. Investigation of G protein-initiated, Ca2+-dependent release of ATP from endothelial cells. Biochim. Biophys. Acta. 2004; 1693:135–146. CrossRef PubMed. [PubMed: 15313015]
63. Schwiebert LM, Rice WC, Kudlow BA, Taylor AL, Schwiebert EM. Extracellular ATP signaling and P2X nucleotide receptors in monolayers of primary human vascular endothelial cells. Am. J. Physiol. Cell Physiol. 2002; 282:C289–C301. CrossRef PubMed. [PubMed: 11788340]
64. LoGerfo FW, Quist WC, Cantelmo NL, Haudenschild CC. Integrity of vein grafts as a function of initial intimal and medial preservation. Circulation. 1983; 68:II117–II124. PubMed. [PubMed: 6872181]
65. Voskresensky IV, Wise ES, Hocking KM, Li FD, Osgood MJ, Komalavilas P, Brophy C, Cheung-Flynn J. Brilliant blue FCF as an alternative dye for saphenous vein graft marking: effect on conduit function. JAMA Surg. 2014; 149:1176–1181. CrossRef PubMed. [PubMed: 25251505]
66. Burnstock G. Local mechanisms of blood flow control by perivascular nerves and endothelium. J. Hypertens. Suppl. 1990; 8:S95–S106. CrossRef PubMed. [PubMed: 1982771]
67. Rubino A, Burnstock G. Capsaicin-sensitive sensory-motor neurotransmission in the peripheral control of cardiovascular function. Cardiovasc. Res. 1996; 31:467–479. CrossRef PubMed. [PubMed: 8689638]
68. Tsuru H, Tanimitsu N, Hirai T. Role of perivascular sympathetic nerves and regional differences in the features of sympathetic innervation of the vascular system. Jpn. J. Pharmacol. 2002; 88:9–13. CrossRef PubMed. [PubMed: 11855682]
69. Gaete PS, Lillo MA, Figueroa XF. Functional role of connexins and pannexins in the interaction between vascular and nervous system. J. Cell. Physiol. 2014; 229:1336–1345. CrossRef PubMed. [PubMed: 24446239]
70. Wang X, Wu Z, Tang Y, Fiscus RR, Han C. Rapid nitric oxide- and prostaglandin-dependent release of calcitonin gene-related peptide (CGRP) triggered by endotoxin in rat mesenteric arterial bed. Br. J. Pharmacol. 1996; 118:2164–2170. CrossRef PubMed. [PubMed: 8864557]
71. Sorkin LS, Xiao WH, Wagner R, Myers RR. Tumour necrosis factor-alpha induces ectopic activity in nociceptive primary afferent fibres. Neuroscience. 1997; 81:255–262. CrossRef PubMed. [PubMed: 9300418]
72. Brain SD, Grant AD. Vascular actions of calcitonin gene-related peptide and adrenomedullin. Physiol. Rev. 2004; 84:903–934. CrossRef PubMed. [PubMed: 15269340]
73. Yoshizumi M, Perrella MA, Burnett JC Jr, Lee ME. Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. Circ. Res. 1993; 73:205–209. CrossRef PubMed. [PubMed: 7685252]
74. Huang AL, Vita JA. Effects of systemic inflammation on endothelium-dependent vasodilation. Trends Cardiovasc. Med. 2006; 16:15–20. CrossRef PubMed. [PubMed: 16387625]
75. Ding J, Song D, Ye X, Liu SF. A pivotal role of endothelial-specific NF-kappaB signaling in the pathogenesis of septic shock and septic vascular dysfunction. J. Immunol. 2009; 183:4031–4038. CrossRef PubMed. [PubMed: 19692637]

76. Diezmos EF, Sandow SL, Markus I, Shevy Perera D, Lubowski DZ, King DW, Bertrand PP, Liu L. Expression and localization of pannexin-1 hemichannels in human colon in health and disease. Neurogastroenterol. Motil. 2013; 25:e395–e405. CrossRef PubMed. [PubMed: 23594276]

77. Timoteo MA, Carneiro I, Silva I, Noronha-Matos JB, Ferreirinha F, Silva-Ramos M, Correia-de-Sa P. ATP released via pannexin-1 hemichannels mediates bladder overactivity triggered by urothelial P2Y6 receptors. Biochem. Pharmacol. 2014; 87:371–379. CrossRef PubMed. [PubMed: 24269631]

78. Cheng Y, Mansfield KJ, Sandow SL, Sadananda P, Burcher E, Moore KH. Porcine bladder urothelial, myofibroblast, and detrusor muscle cells: characterization and ATP release. Front. Pharmacol. 2011; 2:27. CrossRef PubMed. [PubMed: 21713125]

79. Katsuragi T, Tamesue S, Sato C, Sato Y, Furukawa T. ATP release by angiotensin II from segments and cultured smooth muscle cells of guinea-pig taenia coli. Naunyn-Schmiedeberg's Arch. Pharmacol. 1996; 354:796–799. CrossRef. [PubMed: 8971742]

80. Tanoue A, Nasa Y, Koshimizu T, Shinoura H, Oshikawa S, Kawai T, Sunada S, Takeo S, Tsujimoto G. The alpha(1D)-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. J. Clin. Invest. 2002; 109:765–775. CrossRef PubMed. [PubMed: 11901185]

81. Jackson WF, Boerman EM, Lange EJ, Lundback SS, Cohen KD. Smooth muscle alpha1D-adrenoceptors mediate phenylephrine-induced vasoconstriction and increases in endothelial cell Ca2+ in hamster cremaster arterioles. Br. J. Pharmacol. 2008; 155:514–524. CrossRef PubMed. [PubMed: 18604236]

82. Coker SJ, Batey AJ, Lightbown ID, Diaz ME, Eisner DA. Effects of mefloquine on cardiac contractility and electrical activity in vivo, in isolated cardiac preparations, and in single ventricular myocytes. Br. J. Pharmacol. 2000; 129:323–330. CrossRef PubMed. [PubMed: 10694239]