

## Review

# Pannexin-1 channels and their emerging functions in cardiovascular diseases

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## Abstract

Pannexin-1, Pannexin-2, and Pannexin-3 are three members of the Pannexin family of channel-forming glycoprotein. Their primary function is defined by their ability to form single-membrane channels. Pannexin-1 ubiquitously exists in many cells and organs throughout the body and is specially distributed in the circulatory system, while the expressions of Pannexin-2 and Pannexin-3 are mostly restricted to organs and tissues. Pannexin-1 oligomers have been shown to be functional single membrane channels that connect intracellular and extracellular compartments and are not intercellular channels in appositional membranes. The physiological functions of Pannexin-1 are to link to the adenosine triphosphate efflux that acts as a paracrine signal, and regulate cellular inflammasomes in a variety of cell types under physiological and pathophysiological conditions. However, there are still many functions to be explored. This review summarizes recent reports and discusses the role of Pannexin-1 in cardiovascular diseases, including ischemia, arrhythmia, cardiac fibrosis, and hypertension. Pannexin-1 has been suggested as an exciting, clinically relevant target in cardiovascular diseases.

**Key words:** Pannexin-1, ATP, arrhythmia, cardiac fibrosis

## Introduction

In 2000, Panchin *et al.* [1] first reported that Pannexin channels form integral membrane proteins in mammalian genomes. The Pannexin family consists of three members that are homologous to the invertebrate gap junction innexins and have more distant similarities in their membrane topologies and pharmacological sensitivities with the connexins [2,3]. Both Pannexins and connexins contain a 4-pass transmembrane sequence with a conserved intracellular N-terminus and a much longer, variable intracellular C-terminus. They share a similar protein structure [4]. Pannexin monomers oligomerize into large hexameric pores that may be opened by depolarization, increased extracellular K<sup>+</sup>, mechanical stimulation, N-methyl-D-aspartic acid (NMDA) receptor activation, intracellular Ca<sup>2+</sup>, and low oxygen and glucose conditions [5–8]. In this review, we will discuss the distribution of Pannexin-1 in the circulatory system, post-translational

modifications of Pannexin-1, function of the Pannexin-1, and the roles of Pannexin-1 in cardiovascular diseases, such as ischemia, arrhythmia, cardiac fibrosis, and hypertension.

## Distribution of the Pannexin Family

The Pannexin family consists of Pannexin-1, Pannexin-2, and Pannexin-3 [9]. The tissue distribution of Pannexin ranges from ubiquitous to very restrict areas depending on the paralog, and the distribution is often cell type-specific and/or developmentally regulated within some given tissues [10,11]. Pannexin-1 is ubiquitously expressed in human tissues, such as the brain, heart, lung, liver, small intestine, pancreas, spleen, colon, skeletal muscle, skin, testis, ovary, placenta, thymus, prostate, blood endothelium, and erythrocytes [12,13]. Pannexin-1 is also found to be expressed in the central

nervous system including the cerebellum, cortex, lens, retina, pyramidal cells, interneurons of the neocortex and hippocampus, substantia nigra, amygdala, olfactory bulb, neurons, and glial cells [14–17]. The expression of Pannexin-2 is more restricted to the central nervous system, including the cerebellum, cerebral cortex, occipital pole frontal lobe, medulla, temporal lobe, and putamen. However, low expression of Pannexin-2 is also found in thyroid, kidney, and liver tissues [18,19]. Pannexin-2 protein expression is further identified in the basal cells of the stria vascularis and spiral ganglion neurons of the rat cochlear system [20,21]. Pannexin-3 is found to be expressed in osteoblasts, synovial fibroblasts, whole joints of mouse paws, and cartilage from the inner ear. Pannexin-3 is also expressed in many cultured cell lines [22–24].

### Distribution of Pannexin-1 in the Circulatory System

Pannexin-1 has been found throughout the pulmonary and systemic arterial system. Pannexin-1 is expressed in the epithelium and smooth muscle of smaller arteries and arterioles [25]. Locovei *et al.* [26] reported that Pannexin-1 is expressed in the smooth muscle cells of the middle cerebral artery and in human erythrocytes. In addition, high expression levels of Pannexin-1 have been found in human skeletal muscle and heart samples [25]. However, Kienitz *et al.* [27] found that Pannexin-1 levels are actually relatively low in the heart, and they also found that the protein exists primarily in an un-glycosylated state [27]. Pannexin-1 expression and glycosylation are significantly increased when the heart is subject to an ischemic insult [28], or the myocytes are cultured [27].

### Post-translational Modifications of Pannexin-1

Currently, glycosylation is the primarily identified post-translational modification of Pannexin-1 [29–31]. Glycosylation of the Pannexin-1 isoforms leads to a migration shift on the sodium dodecyl sulfate gel with bands representing three different glycosylation states [32]. The Cys346 residue plays a vital role in regulating the Pannexin-1 opening, because its mutation by site-directed mutagenesis leads to a constitutively leaky channel. When the Cys346 residue is mutated, Pannexin-1 is hypoglycosylated, resulting in impaired gating of the channel [33].

Caspase-3 cleavage is another type of post-translational modification of Pannexin-1 [34]. The caspase-3 cleavage site is found in human Pannexin-1 at amino acids 376–379 (DVVD) [35,36]. Sandilos *et al.* [37] revealed that cleavage of the C-terminus at the caspase-3-specific cleavage site induces a constitutive opening of the Pannexin-1 channel during apoptosis [37]. The C-terminus is necessary for the regulation of Pannexin-1 channel opening or closing.

### Functions of the Pannexin-1 Channel

After its initial discovery, Pannexin-1 protein has been thought to form gap junctions that are localized to specialized cell–cell appositional areas containing tens to thousands of closely packed intercellular channels spanning the two plasma membranes [38]. Pannexin-1 has been hypothesized to form intercellular gap junctions between adjacent cells, and evidence from oocyte expression initially supported this notion [39]. However, further studies on the structure and function of Pannexin-1 in many cultured cell models and in some organs revealed that Pannexin-1 does not form gap junctions but appears to form single-pass membrane channels that connect the intracellular and

extracellular compartments. Pannexin-1 oligomeric structures embed in a single plasma membrane when it opens, providing a conduction pathway that links the cytosol to the extracellular space [40].

Evidence from those cultured cell models and organs supports that Pannexin-1 is a transmembrane protein that forms a channel between the cytosol and extracellular space. The role for Pannexin-1 oligomers is different from that of connexin oligomers that form intercellular gap junctions between adjacent cells. The key difference is likely due to the mechanism whereby Pannexin-1 is highly glycosylated on its extracellular loops, which may impede docking with Pannexin-1 channels on the neighboring cells [41–44]. Recent evidence has suggested that the physiological function of Pannexin-1 channels may be involved in the efflux of adenosine triphosphate (ATP) that acts as a paracrine signal, and regulates cellular inflammasomes in a variety of cells and in HIV replication, etc. [45–50].

### Efflux of ATP

The roles of Pannexin-1 channels linked to efflux of ATP have been investigated in several types of cells, including astrocytes, glial cells, and neurons in the central nervous system [51–56], as well as in airway epithelia [48], T-cells [57–59], taste cells [60], keratinocytes [61], circulating erythrocytes, vascular smooth muscle cells, endothelial cells [62], and urothelial cells [63]. Thrombin could stimulate ATP release through Pannexin-1 channels in human umbilical vein endothelial cells [49]. Some connexin isoforms such as connexins 26, 32, 37, and 43 have also been shown to transport ATP molecules [64,65]. However, connexin and Pannexin-1 have quite distinguishable physiological features, particularly their ability to form full or partial channels *in vivo* and in the spectrum of binding partners [66].

### Regulation of cellular inflammasomes

In addition to forming the channels for efflux of ATP, Pannexin-1 also plays an important role in inflammatory responses. Pannexin-1 proteins mediate the ‘find me’ signal released by apoptotic cells to recruit phagocytes to clear the dying cells [35]. Pelegrin and Surprenant [67] showed the evidence that Pannexin-1 channels are involved in the secretion pathway of pro-inflammatory cytokines such as interleukin-1 $\beta$ . In the immune system, Pannexin-1 channels have been shown to be involved in recruiting the inflammasome [68] and in the release of the pro-inflammatory cytokine interleukin-1 $\beta$  from macrophages [69].

### Other functions

In addition, Pannexin-1 plays an important role in the central nervous system. Several studies in the brain have shown that Pannexin-1 is involved in augmenting glutamatergic synaptic signals in the hippocampus and in pathophysiological states, such as neuronal death during stroke and dysfunction during seizure-like conditions [70–74]. Pannexin-1 also participates in paracrine communication between astrocytes and adjacent cells, and has been associated with ischemia-induced neuronal death and epileptic seizures [54,75].

Recently, studies have shown that Pannexin-1 can regulate the function of vascular tone because of its expression in endothelial cells. Compared with connexins, Pannexin-1 channels are insensitive to physiological levels of extracellular calcium because of its faster pore opening kinetics, larger unitary conductance, and weaker voltage gating. Pannexin-1 may be involved in the cell signaling cascade downstream of the P2Y/P2X purinergic,  $\alpha 1/\alpha 2$ -adrenergic, transient receptor potential vanilloid, and NMDA receptors. The activity of Pannexin-1 channels may be regulated by those surface receptors [76].

## Pannexin-1 in Cardiovascular Diseases

Several studies have supported the finding that Pannexin-1 is coupled to several types of ligand-gated ionotropic and metabotropic receptors. In addition, Pannexin-1 may contribute to a large diversity of circulatory functions and may play an important role in cardiovascular diseases. Therefore, it is an intriguing challenge to explore the role of Pannexin-1 in cardiovascular diseases, such as ischemia, arrhythmia, cardiac fibrosis, and hypertension.

### Pannexin-1 channels and ischemia

In an ischemic state, the opening of Pannexin-1 channels leads to an increase in membrane permeability. Pannexin-1 channels open under oxygen stimulation and glucose deprivation *in vitro*, mimicking stroke pathological conditions [71]. During ischemia, NO production is enhanced, which induces changes in redox potential. Zhang *et al.* [72] reported that Pannexin-1 activity is enhanced in the presence of NO donors. Therefore, there is a potential for nitric oxide-dependent regulation of Pannexin-1 channel function [77]. Pannexin-1/P2X7 forms channels that are responsible for the release of cardioprotectants induced by ischemic pre- and post-conditioning [78]. Carbenoxolone and mefloquine, two Pannexin-1 channel inhibitors, block both ischemic pre-conditioning and ischemic post-conditioning by inhibiting the release of cardioprotectants [78]. Pre-conditioning-isolated perfused rat hearts with ATP minimize infarct size and result in the recovery of left ventricular developed pressure. Post-conditioning with ATP after ischemia during reperfusion also has protective effect. Both carbenoxolone and mefloquine block the heart protection of ATP pre- and post-conditioning, indicating that ATP protection is evoked via the opening of Pannexin-1 channels [79]. Carbenoxolone and mefloquine also block the release of the endogenous cardioprotectants S1P and adenosine when they are added after the index ischemia during full reperfusion. These data suggest that ischemic pre-conditioning has a component that requires the release of cardioprotectants via Pannexin-1/P2X channels [80].

### Pannexin-1 channels and arrhythmia

Pannexin-1 has been found to express itself and form large ion channels in *Xenopus* oocytes and mammalian cells [27]. Endogenous large conductance channels and those related to the expression of Pannexin-1 share key pharmacological properties. After being cultured for 4 days, large conductance channel activity can no longer be detected in myocytes but can be rescued by adenoviral gene transfer of Pannexin-1. In isolated cardiac myocytes, Pannexin-1 forms a large conductance channel that can be activated by  $Ca^{2+}$  release from the sarcoplasmic reticulum. These data demonstrate that Pannexin-1 forms a large conductance channel in cardiac myocytes. Sporadic openings of single Pannexin-1 channels in the absence of  $Ca^{2+}$  release can trigger action potentials, suggesting that Pannexin-1 channels potentially promote arrhythmogenic activities [27].

Atrial fibrillation is the most common sustained arrhythmia. Patients with atrial fibrillation are at risk of thrombus formation. Atrial inflammation is vital to atrial fibrillation initiation and progression. The mechanical stretch of atrial myocytes induces macrophage migration by ATP released through Pannexin-1 channels. Carbenoxolone, a Pannexin-1 blocker, inhibits this inflammatory change *in vivo*. In murine macrophages co-cultured with HL-1 murine atrial myocytes-derived cells, mechanical stretch stimulates atrial myocytes and induces macrophage migration, while carbenoxolone inhibits this

enhanced migration. Mechanical stretch of atrial myocytes also induces transient increase of the extracellular ATP level, which is inhibited by carbenoxolone. siRNA knockdown of Pannexin-1 reduces extracellular ATP levels and inhibits macrophage migration. Daily carbenoxolone administration was found to significantly inhibit macrophage infiltration in the atrium [81].

### Pannexin-1 channels and cardiac fibrosis

Cardiac fibrosis is one of the causes of heart failure which leads to the impairment of cardiac function. The main characteristic of cardiac fibrosis is the excessive deposition of extracellular matrix protein. The signaling pathways of angiotensin II (Ang II) and transforming growth factor (TGF)- $\beta$  are involved in the process of cardiac fibrosis. Ang II and TGF- $\beta$  can cause cardiac fibrosis, shorten the effective refractory period, and reduce the conduction velocity. Nishida *et al.* [82] found that mechanical stretch in cardiac myocytes induces the expression of the heterotrimeric G12 family G protein ( $G\alpha_{12/13}$ ), which activates the expressions of the fibrogenic genes TGF- $\beta$ , connective tissue growth factor, periostin, and angiotensin-converting enzyme. The activation of these fibrogenic genes through  $G\alpha_{12/13}$  is initiated by ATP and uridine diphosphate released from Pannexin-1 channels. They further indicated that inhibitory polypeptides of  $G\alpha_{12/13}$  in cardiomyocytes inhibit the levels of those fibrogenic genes and suppress mechanical stretch-induced fibrosis. Inhibiting the nucleotide-stimulated P2Y6 receptor also suppresses the expressions of fibrogenic genes and cardiac fibrosis. These results indicate that Pannexin-1 and extracellular ATP, which work as upstream mediators of Ang II and TGF- $\beta$ , trigger fibrosis in mechanical stretch-induced cardiac fibrosis [82].

Myocardial infarction is commonly followed by cardiac fibrosis, which is linked to arrhythmia and sudden cardiac death. Induced ischemia rapidly increases the glycosylation of Pannexin-1, resulting in increased trafficking to the plasma membrane. ATP release through Pannexin-1 channels participates in the process of subsequent cardiac fibrosis following myocardial infarction. Cellular stress increases ATP release through myocyte Pannexin-1 channels. Increased ATP level leads to fibroblast transformation to the activated myofibroblast phenotype through the MAPK and p53 signaling pathways, both of which are involved in the development of cardiac fibrosis. ATP released from Pannexin-1 channels acts as a paracrine signal in cardiac myocytes during ischemia and results in the profibrotic responses to ischemic cardiac injury [28].

In atherosclerosis, plaque inflammation can activate the release of cytokines and leads to the degradation of the fibrous cap. These events result in a weak plaque, which can potentially rupture and release its contents into the circulation [83]. Pinheiro *et al.* [50] found that histamine increases the level of intracellular  $Ca^{2+}$ . The increased intracellular  $Ca^{2+}$  enhances the release of ATP via Pannexin-1 channels. Furthermore, the release of ATP activates P2 receptors, promotes proliferation of fibroblasts, and increases collagen production. Fibroblasts are the principle cell type of vascular adventitia. Therefore, increased proliferation of fibroblasts plays a crucial role in atherosclerotic lesion progression and eventual rupture. This evidence indicates that Pannexin-1 channels, ATP, and P2 receptors are involved in fibroblast proliferation and plaque destabilization [84].

### Pannexin-1 channels and hypertension

Pannexin-1 is expressed in the pulmonary and systemic arterial wall, both in endothelial cells and in smooth muscle cells [25,45].

Pannexin-1 expression is higher in smooth muscle cells from smaller arteries that play a more direct role in peripheral resistance, thus exerting a greater effect on blood pressure compared with larger arteries [25,45]. Therefore, the Pannexin-1/ATP signaling pathway is speculated to participate in the regulation of vascular tone and blood pressure [25,45,85–88].

Smooth muscle cells, which contribute to peripheral resistance, are highly innervated by the sympathetic nervous system [89]. Endogenous catecholamines, such as epinephrine and norepinephrine, are released from the sympathetic nerve fibers and contract the vessel smooth muscle via the  $\alpha$ 1-adrenergic receptor [90]. Billaud *et al.* [91] demonstrated that the presence of Pannexin-1 at the plasma membrane of the smooth muscle cells forms the thoracodorsal arterial wall [91]. Phenylephrine, an  $\alpha$ 1-adrenergic receptor agonist, induces the contractile response of pressurized thoracodorsal arteries [62]. Probenecid and mefloquine, two Pannexin-1 inhibitors, significantly inhibit the contractile response of thoracodorsal arteries induced by phenylephrine [91]. Pannexin-1 siRNA also has similar effect as its inhibitors. Increase of the amount of Pannexin-1 in the smooth muscle leads to enhanced contractility of thoracodorsal arteries induced by phenylephrine. The degree of constriction in response to phenylephrine has been shown to be correlated with the amount of Pannexin-1 in the smooth muscle cells [91]. They concluded that Pannexin-1 plays an important role in the regulation of vascular tone.

Ang II signaling contributes to the excitation of the carotid body in chronic heart failure and chronic or intermittent hypoxia. Ang II activates the Pannexin-1 current in type II cells of the carotid body that is reversibly abolished by the Pannexin-1 inhibitor carbenoxolone and Ang II receptor antagonist losartan. ATP can also activate Pannexin-1 currents in type II cells, resulting in synergistic effects with Ang II. The Pannexin-1 current is inhibited by BAPTA-AM, suggesting that intracellular  $Ca^{2+}$  signaling contributed to the Pannexin-1 channel opening. It is plausible that paracrine stimulation of type II cells by Ang II contributes to enhancing carotid body excitability [92]. Ang II mobilizes intracellular calcium and activates Pannexin-1 channels in the rat carotid body.

## Perspectives

The Pannexin-1 channel, purinergic receptor, and extracellular ATP are important modulators of many cellular events and hold great potential in understanding and treating those cardiovascular disorders. The Pannexin-1 channel plays a cardioprotective role in the ischemia process and is responsible for the release of cardioprotectants induced by ischemic pre- and post-conditioning. However, the activation of the Pannexin-1 channels has been linked to the efflux of ATP and contributes to the development of arrhythmia, cardiac fibrosis, and hypertension. The inhibitors of the Pannexin-1 channel may be used to treat arrhythmia, cardiac fibrosis, and hypertension, while the activator of Pannexin-1 would be useful to protect against cardiac ischemia diseases. It is crucial to understand the contribution of Pannexin-1 channels and purinergic receptors in those physiological and pathological conditions, which will help to improve therapeutic approaches and invent new drugs for clinical use.

The cardiovascular diseases discussed in this review contribute to a large number of fatalities worldwide. Although much progress has been made in the treatments of these diseases, there are still many strategies that need to be further explored. As the investigations concerning Pannexin-1 channels and purinergic receptors go on, new therapeutics for these disorders will soon be developed.

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