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P2X7 purinergic receptor: A potential target in heart diseases (Review)

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Abstract. The P2X7 purinergic receptor (P2X7R) is a non-selective cation channel activated by high levels of adenosine triphosphate that are commonly present in serious conditions. Activation of this purinergic receptor is closely related to the development of various disease states including inflammatory and neurodegenerative disorders, orthopedic diseases and types of cancer. Accumulating evidence has shown that the P2X7R plays a crucial role in the development of various heart diseases. For example, activation of P2X7Rs may alleviate myocardial ischemia-reperfusion injury by releasing endogenous cardiac protective substances. In contrast to these findings, activation of P2X7Rs can promote the development of acute myocardial infarction and myocarditis by inducing inflammatory responses. Activation of these receptors can also contribute to the development of different types of cardiomyopathies including diabetic cardiomyopathy, dilated cardiomyopathy and hypertrophic cardiomyopathy by inducing cardiac hypertrophy, fibrosis and apoptosis. Notably, inhibition of P2X7Rs can improve cardiac structure and function abnormalities following acute myocardial infarction, reduction of inflammatory responses following myocarditis and attenuation of the cardiomyopathy process. Furthermore, recent evidence has demonstrated that P2X7Rs are highly active in patients infected with coronavirus disease-2019 (COVID-19). Hyperactivation of P2X7Rs in COVID-19 may induce severe myocardial injury through the activation of several signaling pathways. The present study reviewed the important role of the P2X7R in cardiac dysfunctions and discusses its use as a possible new therapeutic approach for the prevention and treatment of several myocardial diseases.

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Key words: P2X7 purinergic receptor, myocardial infarction, myocarditis, cardiomyopathy, myocardial injury induced by coronavirus

Contents

1. Introduction

Cardiovascular diseases are the main cause of mortality globally, accounting for ~30% of annual worldwide mortalities (1). This number is expected to rise to ~40% by the year 2030 (2). The high mortality rate of cardiovascular diseases indicates that further assessments are required to understand the mechanisms underlying the development of these diseases and identify other pharmacological agents targeting protection against heart injuries. The P2X7 purinergic receptor (P2X7R) has been implicated in several signaling pathways and in the development of a variety of pathological conditions including chronic neuropathic pain, neurodegenerative diseases, such as multiple sclerosis, inflammatory disorders, orthopedic diseases, such as osteoporosis and cancerous diseases such as lung cancer (3-10). Studies have highlighted the role of the P2X7R in the development of heart diseases, such as acute myocardial infarction, myocardial ischemia-reperfusion injury and myocarditis (11-13). It is interesting to note that the importance of the P2X7R in heart injury mediated by the coronavirus disease-2019 (COVID-19) has been highlighted by various studies (14-16). These studies have revealed a potential new approach that positions the P2X7R as a prognostic cardiac biomarker and pharmacological target for the prevention and treatment of heart injuries, which may increase survival and improve the quality of life in patients with heart diseases. The present review article provided a brief overview of the properties of the P2X7R, its distribution in the heart and its pathological role in heart diseases, with a particular focus on acute myocardial infarction, myocardial ischemia-reperfusion injury, autoimmune myocarditis and various types of cardiomyopathies as well as myocardial injury induced by COVID-19.

2. P2X7R

P2X7R belongs to a family of purinergic receptors. This family is categorized into two main groups, namely the P1 and P2 receptors (17). P2 receptors are subdivided into P2X receptors (P2XRs), which are ligand-gated ion channels and P2Y receptors (P2YRs), which are G-protein coupled receptors (18,19). A total of seven P2XR subtypes (P2X1R-P2X7R) and eight P2YR subtypes (P2Y1R, P2Y2R, P2Y4R, P2YR6 and P2Y11R-P2Y14R) have been identified to date (20,21). All subtypes of P2XR are non-selective cation channels activated by exogenous adenosine triphosphate (ATP), which is triggered by the efflux of K^+ and the influx of Na^+ and Ca^{2+} (22). ATP is stored intracellularly in synaptic vesicles and is released from neuronal and non-neuronal cells in response to various stimuli such as hypoxia, pain, infection and inflammation (23,24). It is released into the extracellular space by vesicular exocytosis or pore-forming channels by pannexin 1 and connexin 43 (23-26). The release of ATP by vesicular exocytosis is a calcium-dependent release that is dependent on an increase in intracellular calcium concentration, whereas the ATP release through pore-forming channels is a calcium-independent release (27).

P2X7R has distinctive features that differentiate it from the other P2XRs. One of these features is that it is activated by ATP with a half maximal effective concentration (EC_{50}) value in the range of 0.1-1 mM. This range is higher than that of the other P2XRs (EC_{50}, 1-10 μM) (28). Its activation requires a massive amount of extracellular ATP, which does not usually exist in normal cells (29). It seems likely that P2X7Rs have low activity levels under normal conditions (Fig. 1A). By contrast, during certain pathological conditions, including hypoxia, inflammation, pain, cellular damage and other stress conditions, high levels of extracellular ATP can activate P2X7Rs and subsequent multiple signaling pathways (30). Activation of P2X7Rs opens cationic channels that facilitate the flux of small cations, such as K^+, Na^+ and Ca^{2+}, resulting in the activation of several intracellular signaling pathways (31). These pathways include the activation of nucleotide-like receptor family pyrin domain member 3 (NLRP3) inflammasome, NF-κB, nuclear factor of activated T cells (NFAT), hypoxia-inducible factor 1-alpha (HIF-1α) and ERK1/2 as well as the formation of reactive oxygen species (ROS; Fig. 1B) (32,33). Furthermore, sustained activation of P2X7Rs opens large pores that facilitate the passage of large cations and organic dyes, such as choline and ethidium, respectively, which result in apoptotic cell death (34-36).

The NLRP3 inflammasome is the most notable response following P2X7R activation. The binding of ATP to P2X7Rs allows K^+ efflux and Na^+ and Ca^{2+} influx. Low levels of intracellular K^+ induce the configuration of the NLRP3 inflammasome with apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). This stimulates caspase-1 that cleaves the pro-inflammatory cytokines pro-IL-1β and pro-IL-18 to form IL-1β and IL-18, thus contributing to a series of inflammatory responses (37). These cytokines may induce profibrotic TGF-β1, resulting in fibrosis (38). Furthermore, the NLRP3 inflammasome with ASC activates caspase-1 that cleaves Gasdermin-D, which forms membrane pores and promotes the inflammatory cell death program (39). P2X7R induces inflammation by activation of NF-κB which increases several inflammatory cytokine genes, such as TNF-α and IL-1β (40). Similarly, P2X7R activation promotes NFAT function, which in turn leads to IL-2 inflammatory cytokine secretion, downregulation of glycogen synthase kinase activity and lymphocyte proliferation (32,41,42). HIF-1α is also a marked response following P2X7R activation. Ca^{2+} influx induced by P2X7R activation upregulates HIF-1α through phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway (43,44). This association between P2X7R and HIF-1α may promote angiogenesis by activating the vascular endothelial growth factor (VEGF) secretion (45,46). Furthermore, Ca^{2+} influx after P2X7R activation may induce cell proliferation and migration by enhancing ERK1/2 phosphorylation and activating NF-κB transcription, which in turn leads to the secretion of matrix metalloproteinases (MMPs) (47,48). In addition, the P2X7R activation may induce cell death by the formation of ROS (49). Taken together, the data indicate that P2X7R is possibly a starting point for the activation of several intracellular signaling pathways, which in turn cause inflammation, fibrosis, proliferation, angiogenesis and cell death. These multiple intracellular signaling pathways induced by the P2X7R activity indicate that P2X7Rs may represent an attractive target for the prevention and treatment of numerous pathological conditions.

3. P2X7R in the heart

P2X7Rs are distributed widely throughout the body. In the heart, P2X7Rs are found in embryonic stem cell-derived cardiomyocytes and their activation can increase the expression levels of several cardiac-specific genes, such as α-myosin heavy chain (α-MHC) and α-actinin (50). They are also expressed in epicardium-derived cells, indicating that they play roles in embryonic cardiac growth and development (51). P2X7Rs are mainly present in the sinoatrial node, right atrium and left ventricular. In the rat heart, P2X7R is highly expressed in the right atrium and left ventricular, while in the human heart it is highly expressed in the right atrium (52). Furthermore, these receptors are present in cardiac muscle cells notably atrial cardiomyocytes and other cardiac cells, such as cardiac endothelial cells and cardiac fibroblasts (53,54). The wide distribution of the P2X7R in cardiac cells suggests that this receptor has a crucial role in several cardiac pathological conditions, which are discussed in detail below.

P2X7R in acute myocardial infarction. Acute myocardial infarction (AMI), commonly called a heart attack, is a serious condition characterized by sustained ischemia and reduced blood flow to the heart muscle, resulting in an accelerated death of heart muscle cells (55). Several cellular and molecular changes occur following myocardial infarction, which can be categorized into the inflammatory, proliferative and maturation phases. Firstly, the inflammatory phase (the early phase within 0-4 days) is characterized by the release of ROS and pro-inflammatory mediators as well as fibrin deposition and necrosis of cardiomyocytes. Secondly, the proliferative phase (within ~1-2 weeks) is characterized by extracellular matrix deposition, myofibroblast differentiation, angiogenesis and the formation of tissue granules. Thirdly, the maturation phase

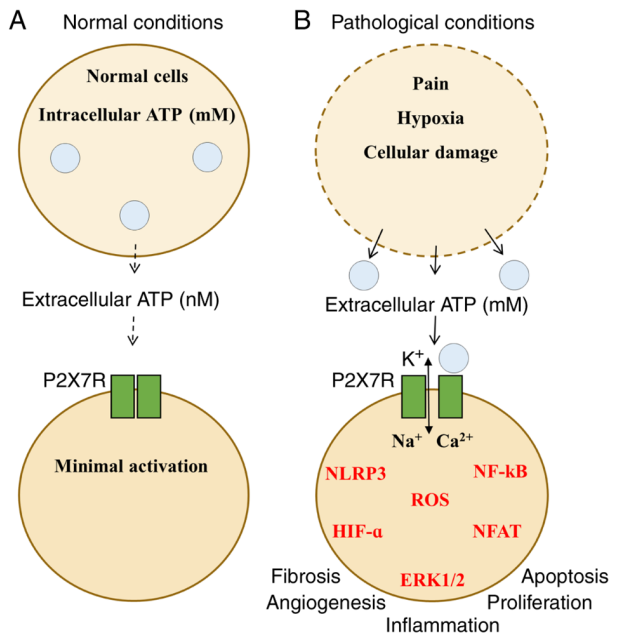

Figure 1. Activation of P2X7R. ATP is a physiological agonist of P2X7Rs. (A) Under normal conditions, the intracellular ATP concentration is present at high levels, within the mM range (32,87) and the extracellular ATP concentration is present at low levels, within the nM range (88). This concentration of extracellular ATP does not activate the P2X7 purinergic receptor, indicating that P2X7Rs have negligible activity under normal conditions. (B) Under pathological conditions, such as hypoxia, inflammation and cellular damage, the extracellular ATP concentration can increase and subsequently activate the P2X7 purinergic receptor and several signaling pathways including the NLRP3 inflammasome, the NF-κB, NFAT, HIF-1α, ERK1/2 and ROS. P2X7R, P2X7 purinergic receptor; ATP, adenosine triphosphate; NLRP3, nucleotide like receptor family pyrin domain member 3 inflammasome; NFAT, nuclear factor of activated T cells; HIF-1α, hypoxia-inducible factor 1-alpha; ROS, reactive oxygen species.

(from weeks to months), is characterized by apoptosis of myofibroblasts and the formation of a mature scar (56-58).

A strong relationship has been noted between P2X7R expression and AMI. P2X7Rs are upregulated in an experimental *in vivo* AMI model (13,59,60). A clinical study confirmed that P2X7R mRNA expression was upregulated in patients with AMI (61), suggesting that P2X7Rs may be used as predictive biomarkers in AMI. Previous studies have shown that the upregulation of P2X7R expression in AMI is commonly accompanied by an enhanced inflammatory response. For example, overexpression of P2X7R following myocardial infarction increases the NLRP3 inflammasome and release the proinflammatory cytokine IL-1β (59). Furthermore, activation of P2X7Rs aggravated AMI injury in an animal model resulting in increased ROS levels and vasopressin activity (60). Taken together, these studies indicate that the P2X7R plays a key role in the pathogenesis of myocardial infarction.

The inhibition of P2X7Rs may enhance cardiac function and improve survival following AMI. Gao *et al* (13) indicated that inhibition of P2X7Rs with short hairpin RNA attenuated sympathetic hyperinnervation by suppressing nerve growth factor levels. This study further indicated that inhibition of P2X7Rs ameliorated inflammatory infiltration by suppressing NF-κB activation and improved cardiac dysfunction by inhibiting the AKT/ERK1/2 signaling pathways (13). Furthermore, Mezzaroma *et al* (62) demonstrated that inhibition of P2X7Rs with small interfering RNA prevents caspase-1 activity and ameliorates cardiac remodeling. The role of P2X7R in AMI was confirmed by using specific P2X7R antagonists. It was shown that the P2X7R antagonist pyridoxalphosphate-6-azophenyl-2',4-disulfonic acid hindered the formation of the ASC/cryopyrin inflammasome and reduced infarct size as well as cell death following AMI (62). In addition, the application of the P2X7R antagonist Brilliant Blue G (BBG) attenuated sympathetic hyperactivity and cardiac dysfunction by reducing oxidative stress as well as vasopressinergic cell activation in AMI rats (60). These studies indicate that P2X7R is a possible candidate for the treatment of myocardial infarction, particularly during the early inflammatory phase. The effect of P2X7Rs in the late phases of myocardial infarction remains to be elucidated. Therefore, further studies are required to verify whether the P2X7R plays a key role during the proliferation and maturation phases following myocardial infarction.

P2X7R in myocardial ischemia-reperfusion injury. Myocardial ischemia/reperfusion (I/R) injury is a complex condition characterized by the restoration of blood flow to the ischemic heart muscle (63). P2X7R expression is upregulated in the experimental model of myocardial I/R injury (64,65). There is controversy regarding the ability of P2X7R to exacerbate or reduce myocardial I/R injury. A previous study indicated that the P2X7R promoted cardiac damage by inducing inflammatory responses in myocardial I/R injury. In an animal model of myocardial I/R injury, overexpression of the P2X7R was shown to increase the activity of NF-κB and release several inflammatory cytokines, such as IL-6, IL-8, IL-10 and TNF-α (64). However, it is still unknown whether P2X7R inhibitors can preserve heart function in response to myocardial I/R injury. Therefore, further assessment studies on the effect of the P2X7R-induced inflammatory response in myocardial I/R injury are recommended.

In contrast to these findings, other studies have shown that the activation of the P2X7R can alleviate myocardial I/R injury by stimulating the release of endogenous cardioprotectants. During myocardial I/R injury, it was demonstrated that pannexin-1 interacts with P2X7Rs to form a channel. The activation of this channel is responsible for the release of the cardioprotectants adenosine and sphingosine 1-phosphate (11). These cardioprotectants can attenuate mitochondrial damage, prevent myocardial apoptosis and improve myocardial I/R injury via the activation of the PI3K/AKT signaling pathway (66). This was confirmed by using pannexin-1 and P2X7R antagonists in an animal model. Vessey *et al* (66) indicated that the pannexin-1 antagonist carbenoxolone and the P2X7R antagonist BBG increases infarct size and blocks cardioprotection in a rat experimental myocardial I/R injury model. Furthermore, overexpression of the P2X7R following myocardial ischemia increases ERK1/2 phosphorylation, suggesting that the P2X7R may prevent myocardial apoptosis and attenuate cardiomyocyte injury in response to I/R (65,67). Considering all this evidence, the activation of P2X7R seems to be beneficial in myocardial I/R injury, suggesting that the P2X7R agonist is a possible target for the prevention of myocardial I/R injury.

P2X7R in autoimmune myocarditis. Autoimmune myocarditis is an inflammatory disease of the myocardium characterized by the infiltration of inflammatory monocytes, macrophages and $CD4^+$ helper T cells into the myocardium and consequently fibrosis and necrosis (68-70). P2X7R expression is upregulated in ~50% of the experimental models of autoimmune myocarditis, which leads to $CD4^+$ helper T cell and macrophage infiltration (12). A previous study using a mouse model of autoimmune cardiomyopathy indicated that the wild-type mice demonstrated increases IL-1β and IL-17 cytokine production. However, the levels of these cytokines are decreased in $P2X7R^{-/-}$mice (71). In addition, treatment of mice with autoimmune myocarditis mice with the P2X7R antagonist A740003 improves their cardiac function by inhibiting $CD4^+$ helper T cell and macrophage infiltration (12). These studies indicated that the P2X7R is a possible target for the treatment of autoimmune myocarditis.

P2X7R in cardiomyopathies. Cardiomyopathy is a disorder of the cardiac muscle characterized by structural and functional changes of cardiomyocytes (72). A total of two types of cardiomyopathies have been identified, primary or secondary. Primary cardiomyopathies can be divided into three main classes as follows: Genetic (hypertrophic cardiomyopathy), mixed-genetic and non-genetic (dilated cardiomyopathy) and acquired (inflammatory cardiomyopathy). Secondary cardiomyopathies are usually associated with a variety of systemic disorders (diabetes) and toxicity of specific drugs (chemotherapy) (72). Cardiac fibrosis is a key feature of various cardiomyopathies and is defined as an excess deposition of the extracellular matrix including type I collagen by cardiac fibroblasts (73). It is notable that activation of P2X7Rs by the P2X7R agonist BzATP elevates the protein expression of profibrotic markers, including TGF-β1, connective tissue growth factor (CTGF) and α-smooth muscle actin (α-SMA) in neonatal rat cardiac fibroblasts (67). This indicates that that P2X7Rs participate in the development of cardiac fibrosis (74).

An association between P2X7 expression and cardiomyopathy has been shown since overexpression of this receptor has been observed in various forms of cardiomyopathy. It has been demonstrated that P2X7Rs are upregulated in mouse models of experimental diabetes (68) and experimental dilated cardiomyopathies (75). Overexpression of P2X7Rs in animal cardiomyopathy models is commonly accompanied by cardiac hypertrophy, fibrosis and apoptosis. For example, overexpression of P2X7R in a mouse model of diabetic cardiomyopathy increases cardiac hypertrophy markers, such as atrial natriuretic peptide and β-myosin heavy chain, fibrosis markers, such as collagen I and TGF-β1 and apoptosis markers, such as caspase 3 and Bax (75). A genetic study demonstrated an association between P2X7R expression and cardiomyopathy. In humans, the single nucleotide polymorphism of P2X7R (E186K) that results in loss of function is associated with hypertrophic cardiomyopathy (76).

Inhibition of P2X7R can attenuate cardiac fibrosis in cardiomyopathies. Cardiac fibrosis markers including collagen I, CTGF, α-SMA and TGF-β1 are reduced in a P2X7R knockdown model. This was supported by using P2X7R antagonists in an animal model of cardiomyopathy. The application of the P2X7R antagonist BBG attenuated cardiac fibrosis by inhibiting the NLRP3/IL-1β signaling pathway (74). The application of the P2X7R antagonist A438079 ameliorates cardiac hypertrophy, fibrosis and apoptosis by inhibiting the PKCβ/ERK signaling pathway (75). These data demonstrate that P2X7Rs have a potential role in the prevention and/or treatment of various forms of cardiomyopathy.

Involvement of the P2X7R in heart injury mediated by COVID-19. COVID-19 is a respiratory viral infection characterized by the excessive and sustained production of inflammatory cytokines, the so-called cytokine storm (15,77). This disease can cause serious injuries in various organ systems, including the cardiovascular system. Viral myocarditis is the most common form of heart injury mediated by COVID-19 (78). Arrhythmia and myocardial infarction have also been reported as heart injuries in patients with COVID-19 (79,80). There is growing evidence that demonstrates the importance of purinergic receptors, particularly the P2X7R, in COVID-19 (81). It is important to note that the P2X7R is hyperactivated in patients infected with the COVID-19 viral strain. This hyperactivity can cause myocardial injury by the activation of several intracellular signaling pathways. The first possible pathway is the cytokine storm. During this viral infection, the P2X7R is stimulated by high levels of ATP resulting in NLRP3 inflammasome activation and considerable inflammatory cytokine production. These cytokines contribute to the cardiac inflammatory responses that may cause AMI, viral myocarditis and arrhythmia (14,81). The second possible pathway involves the angiotensin-converting enzyme (ACE) II. During COVID-19 infection, the P2X7R triggers pathways associated with the action of the renin-angiotensin-aldosterone system (RAAS) (81). The main effector molecule in the RAAS is angiotensin II, which causes vasoconstriction, cardiac hypertrophy and apoptosis. This molecule is upregulated in several pathological conditions including cardiovascular diseases. In fact, ACE generates angiotensin II from angiotensin I, while ACE2 inhibits the activity of angiotensin II by transferring it to angiotensin 1-7. Therefore, ACE2 has a cardioprotective effect against myocardial injury (82). It has been demonstrated that during the COVID-19 viral infection, the virus enters human cells by binding to ACE2 resulting in the downregulation of the ACE2 signaling pathway, which can potentially cause myocardial injury (83-85). Another possible pathway is that of VEGF. Hyperactivity of P2X7Rs following COVID-19 has been shown to increase VEGF production (15). This may stimulate angiogenesis in cardiac cells which in turn leads to cardiac hypertrophy. Collectively, these studies indicate that the P2X7R plays a major role in myocardial injury caused by COVID-19. It can be hypothesized that inhibition of P2X7R is a promising therapeutic target for the prevention or treatment of cardiac injuries in patients with COVID-19.

4. Conclusion

In conclusion, the aforementioned studies have shown that the P2X7R plays an essential role in the development of several heart diseases. The majority of the studies have demonstrated that the activation of the P2X7R promotes the development of AMI, autoimmune myocarditis and various types of cardiomyopathies including diabetic cardiomyopathy, dilated

cardiomyopathy and hypertrophic cardiomyopathy. Activation of the P2X7R during COVID-19 infection may also enhance the process of myocardial injury via the activation of several intracellular signaling pathways. Based on this evidence, it is likely that the P2X7R can be used as a prognostic indicator for the detection of various heart diseases. Overall, P2X7R inhibitors appear to be a promising therapeutic target for the prevention or treatment of heart diseases, as these inhibitors have been shown to have primarily anti-inflammatory effects against AMI and myocarditis, as well as antifibrotic and anti-apoptotic effects in the case of cardiomyopathies. This view is supported by a recent review that draws attention to purinergic receptors as therapeutic targets for the treatment of cardiovascular disease (86). However, it is important to note the efficacy of P2X7R inhibitors during the progression of heart disease when these inhibitors are used in clinical cardiology, as they may not have the same beneficial effects in different types of heart disease. This has been observed in experimental models of myocardial infarction, the use of P2X7R inhibitors may enhance cardiac function and significantly improve survival in the early inflammatory phase following myocardial infarction (13,60,62). On the other hand, the use of P2X7R inhibitors may increase infarct size and abrogate the cardioprotective effects of adenosine and sphingosine 1-phosphate in myocardial I/R injury (66). To develop a complete profile of P2X7R in the setting of heart diseases, additional experimental studies are required to elucidate the effects of P2X7R inhibitors on other common cellular and molecular features associated with heart diseases. For example, the effects of P2X7R inhibitors can be examined on cardiac fibroblast proliferation, myofibroblast differentiation, angiogenesis and scar formation following myocardial infarction. In addition, further studies are required to demonstrate the effect of P2X7R inhibitors on the COVID-19 signaling pathways associated with myocardial injury.

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Authors' contributions

AD conducted the literature research, wrote the manuscript and designed the figure. AA, TA and NA contributed to the writing and revisions of the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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