Research progress in myocardial function and diseases related to muscarinic acetylcholine receptor (Review)

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Abstract. Muscarinic acetylcholine (ACh) receptors (also known as M receptors) are widely distributed in all organs and tissues of the body, mainly playing a role in cholinergic nerve conduction. There are five known subtypes of muscarinic ACh receptors, but their pharmacological mechanisms of action on myocardial function have remained to be clearly defined. Functional myocardial diseases and myocardial injuries, such as arrhythmia, myocardial ischemia, myocarditis and myocardial fibrosis, may be affected by muscarinic ACh receptors. This article reviews the research progress of the regulation of myocardial function by muscarinic ACh receptors and related diseases, with the aim of developing better strategies and providing references for further revealing and clarifying the signal transduction and mechanisms of muscarinic ACh receptors in cardiomyocytes, and finding potential myocardial protective drugs that act on muscarinic ACh receptors.

Contents

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1. Introduction

The muscarinic acetylcholine (ACh) receptor (M receptor or mAChR), also known as the muscarinic ACh receptor, is an important type of neurotransmitter receptor widely expressed in various tissues and organs of the human body. As a G protein-coupled membrane receptor, it regulates different intracellular signaling transduction mechanisms in the body (1), and has become a target for various chemical drugs (2,3). The cholinergic receptors can be divided into muscarinic ACh receptors and nicotinic ACh receptors (N receptor), and the majority of them in the heart are muscarinic ACh receptors. Therefore, studying the function and disease regulation of muscarinic ACh receptors in the heart is particularly important. Currently, there are a total of five subtypes of muscarinic ACh receptors, which are named M1-M5, and only three subtypes of muscarinic ACh receptors with biological functions have been discovered, which are M1, M2 and M3 (4). According to literature reports, these three functional muscarinic ACh receptors play an important role in the physiological and pathological regulation of the heart (5). However, researchers still have not fully clarified the myocardial function and pathophysiological mechanisms mediated by muscarinic ACh receptors. Known regulation of muscarinic ACh receptors in developing cardiomyopathy involves pathological changes such as myocardial infarction, myocardial fibrosis, cardiac hypertrophy, myocardial contraction and arrhythmia, as well as regulation of myocardial inflammation and non-nerve ACh systems (6).

These cardiomyopathies are commonly affected by myocardial apoptosis and necrosis, oxidative stress, mitochondrial dysfunction and autophagy, fission and biogenesis, etc. The exact mechanism by which muscarinic receptors affect these cardiomyopathies is still worthy of further in-depth research. For the present review, studies on recent research progress related to the impact of muscarinic ACh receptors on myocardial function were collected, aiming to discover the potential signaling transduction mechanism of muscarinic ACh receptors in the cardiomyocytes and providing a theoretical basis for further studying myocardial protective drugs that act on muscarinic ACh receptors.

2. The regulatory effects of muscarinic ACh receptors on myocardial contraction

The muscarinic ACh receptor is an important signal transduction mediator present on the membrane of cardiomyocytes, which regulates myocardial contraction (7-9). Over the years, the regulatory mechanism of the M2 muscarinic ACh receptor in cardiomyocytes has been widely reported. It has been pointed out that ACh, phthalimide-azo-iperoxo and naphthalimide-azo-iperoxo can reduce the myocardial contraction force and cardiac output by binding to the M2 muscarinic ACh receptor of cardiomyocytes (10,11). A study also found that bile acids have a new function of slowing down myocardial contraction through the M2 muscarinic ACh receptor (12). Another study reported that an agonist or inhibitor of the M2 muscarinic ACh receptor can regulate the contraction function of the heart by regulating Ras homolog family member A and Rac family small GTPase 1 through the long isoform of the regulator of G protein signaling 3 (13). On the contrary, M2 muscarinic ACh receptor inhibitors such as scopolamine, N-methylscopolamine and [(3R,4R)-3-(3cyclopropyl-1,2,4,oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane] (L-687,306), have been studied and shown to have the effect of increasing the heart rate and myocardial contraction in rats (14). The calcium channel in cardiomyocytes has long been considered an important pathway for regulating myocardial contraction. It has been reported that ACh can regulate calcium ions in cardiomyocytes, affecting the actomyosin interaction mechanism and the stretching of the sarcomere, thereby changing the myocardial contraction function (15). Furthermore, myocardial diseases are highly associated with the functional obstacle of ryanodine receptor (RyR2). It has been reported that the muscarinic ACh receptor can reduce the stimulation of the parasympathetic nervous system and calcium ion/calcium-dependent protein kinase II (CaMKII)-dependent reactivity, decrease the phosphorylation of RyR2 Ser-2814, lead to increased systolic calcium ion release and reduce the leakage of abnormal calcium ions, thereby improving calcium ion cycling efficiency (16). In addition, it has been proven that muscarinic ACh receptors can regulate the L-type calcium signal channel, CaMKII and the phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT)-neuronal nitric-oxide synthase signaling pathway involved in cardiomyocyte contraction function (17,18). In all, the muscarinic ACh receptor controls the homeostasis of calcium ion currents in cardiomyocytes. The energy metabolism of the heart is also one of the important functions affected by muscarinic ACh receptors. The response to M2 receptor activation could be observed when the agonist stimulates cyclic adenosine monophosphate (cAMP) production (19). There are relevant research reports that the non-selective muscarinic ACh receptor inhibitor atropine can enhance myocardial contraction by blocking cAMP-specific phosphodiesterase type 4 (20). The changes in cAMP affect protein kinase A-dependent phosphorylation targets, such as L-type calcium channels, which play an important role in regulating myocardial contractility (19). Ursolic acid could enhance muscarinic ACh receptor-mediated atrial natriuretic peptide secretion and regulate myocardial contraction (21). It has been reported that ACh can regulate the contractility of atrial myocardium by activating muscarinic receptors and regulating the kinetics of the actin myosin interaction in cardiomyocytes (15). The release of ACh between cardiomyocytes can be affected by neural and drug regulation (22,23). Muscarinic ACh receptors are also involved in the activation of atrial cyclooxygenase-2 and autoantibody mediates the positive/negative inotropic response to muscarinic agonists (24,25). In the heart muscle, the function of the muscarinic ACh receptor is closely related to the beating and stopping of the heart. It has been suggested that stimulating the muscarinic ACh receptor can lead to sudden cardiac arrest, while muscarinic ACh receptor inhibitors can effectively protect against this symptom (26). Age can regulate the activity of muscarinic ACh receptors on cardiomyocytes and ultimately affect myocardial contractility. As age increases, the activity of the muscarinic ACh receptor decreases, ultimately affecting myocardial contraction (27-29). The aforementioned research indicates that the muscarinic ACh receptor can regulate myocardial contraction of the heart, laying a foundation for the study of pathological effects of the muscarinic ACh receptor. The effects of muscarinic ACh receptors on myocardial contraction are listed in Table I and shown in Fig. 1.

3. The regulatory effects of muscarinic ACh receptors on myocardial infarction

The impact of the muscarinic ACh receptor on myocardial infarction is significant. Acute myocardial infarction is the risk disease with the highest mortality rate among patients with heart disease. The underlying pathogenesis and influencing factors have been the focus of medical research for numerous years (30). In myocardial infarction, the muscarinic ACh receptor can affect the onset and course of myocardial infarction. Meanwhile, the muscarinic ACh receptor in the cardiomyocytes can express increased expression in the conduction block caused by myocardial infarction (31). Some studies have pointed out that muscarinic ACh receptor inhibitors can worsen the condition of myocardial infarction by inhibiting the muscarinic ACh receptor on the cardiomyocyte (32). In basic and clinical research on myocardial infarction, it has been shown that the regulation of muscarinic ACh receptors can induce the occurrence of myocardial infarction, which in turn leads to heart failure (18,33). The mechanism of action of muscarinic ACh receptors mediating myocardial infarction is relatively complex, and it may involve multiple signaling pathways (34). Among them, the regulation of calcium channels is the main reason for triggering myocardial contraction and affecting the disease process of myocardial infarction. It

Table I. Effects of muscarinic ACh receptors on myocardial contraction.

Muscarinic receptor type	Target	Agonist/antagonist	CVDs	Publication year	(Refs.)
M2	Ca^{2+} channel	Ang-(1-7), ACh	Arrhythmia	2022	(9)
	Ca^{2+} channel	PAI	Arrhythmia	2019	(10)
	cAmp, I_K	Atropine	Dilated cardiomyopathy, ischemic cardiomyopathy, atrial tachyarrhythmias	2011	(25)
	Sympathetic nerve	Yridostigmine, donepezil	Heart failure, hypertension	2024	(23)
	RhoA, Racl, RGS3L	Carbachol	Heart failure	2022	(13)
	Parasympathetic nerve	Scopolamine, NMS, L-687306, arecoline	Bradycardia	2020	(14)
	Ca^{2+} ion channel	ACh	Atrial fibrillation	2022	(15)
	RyR2, PI3K	Carbachol	Heart failure	2020	(16)
	cAMP	Bile acid	Arrhythmias	2018	(12)
	cAMP	Carbachol, ACh	Cardiac arrest	2018	(26)
	CSPG4	Carbachol	Arrhythmia	2024	(27)
M3	L-type calcium channel, CaMKII	Tiotropium bromide	Myocardial infarction	2019	(18)
	Caspase-1, IL-1β	4-DAMP	Myocardial infarction, heart failure	2018	(29)
M1, M2	Parasympathetic nerve	Pirenzepine, atropine	Bradycardia	1997	(28)
M2, M3	RyR2	AF-DX116, J104129	Heart failure	2016	(17)
	Cyclo-oxygenase-2	Carbachol	Heart failure	2014	(24)
M1, M2, M3	PKA-dependent phosphorylation, L-type calcium channel	cAMP	Ischemia-reperfusion injury	2024	(19)
	Vagal afferent nerve	ACh	Heart failure	2020	(22)
	PDE4	Atropine	Tachycardia, arrhythmias	2017	(20)
	ANP secretion	Ursolic acid	Myocardial infarction	2014	(21)

ACh, acetylcholine; M, muscarinic ACh receptor; ACh, acetylcholine; cAMP, cyclic adenosine monophosphate; RGS3L, long isoform of the regulator of G protein signaling 3; CSPG4, chondroitin sulfate proteoglycan 4; RyR2, ryanodine receptor; PI3K, phosphoinositide 3-kinase; CaMKII, calcium ion/calcium-dependent protein kinase II; IL, interleukin; PDE4, phosphodiesterase 4; PKA, protein kinase A; RhoA, Ras homolog family member A; Rac1, Rac family small GTPase 1; ANP, atrial natriuretic peptide; PAI, phthalimide-azo-iperoxo; Ang-(1-7), angiotensin-(1-7); NMS, N-methylscopolamine; 4-DAMP, 4-diphenylacetoxy-N-methylpiperidine methiodide; L-687306, (3R,4R)-3-(3-cyclopropyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane; AF-DX116, 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,1 1-dihydro-6H-pyrido[2,3-b][1,4] benzodiazepin-6-one; J104129, (αR)-α-cyclopentyl-α-hydroxy-N-[1-(4-methyl-3-penten-1-yl)-4-piperidinyl]-benzeneacetamide fumarate.

has been shown that the receptor-interacting protein 3-induced activation of CaMKII in cardiomyocytes can trigger oxidative stress in cardiomyocytes, which has always been an important cause of myocardial infarction (35). This may be caused by affecting the opening of the mitochondrial permeability transition pore and myocardial necrosis. In response to the above mechanism of myocardial infarction injury, the pharmacological community has carried out corresponding research on the protective effects of muscarinic ACh receptors. Certain studies have reported that the agonist effect of drugs on muscarinic ACh receptors can alleviate myocardial infarction (36,37). Agitating muscarinic ACh receptors can protect

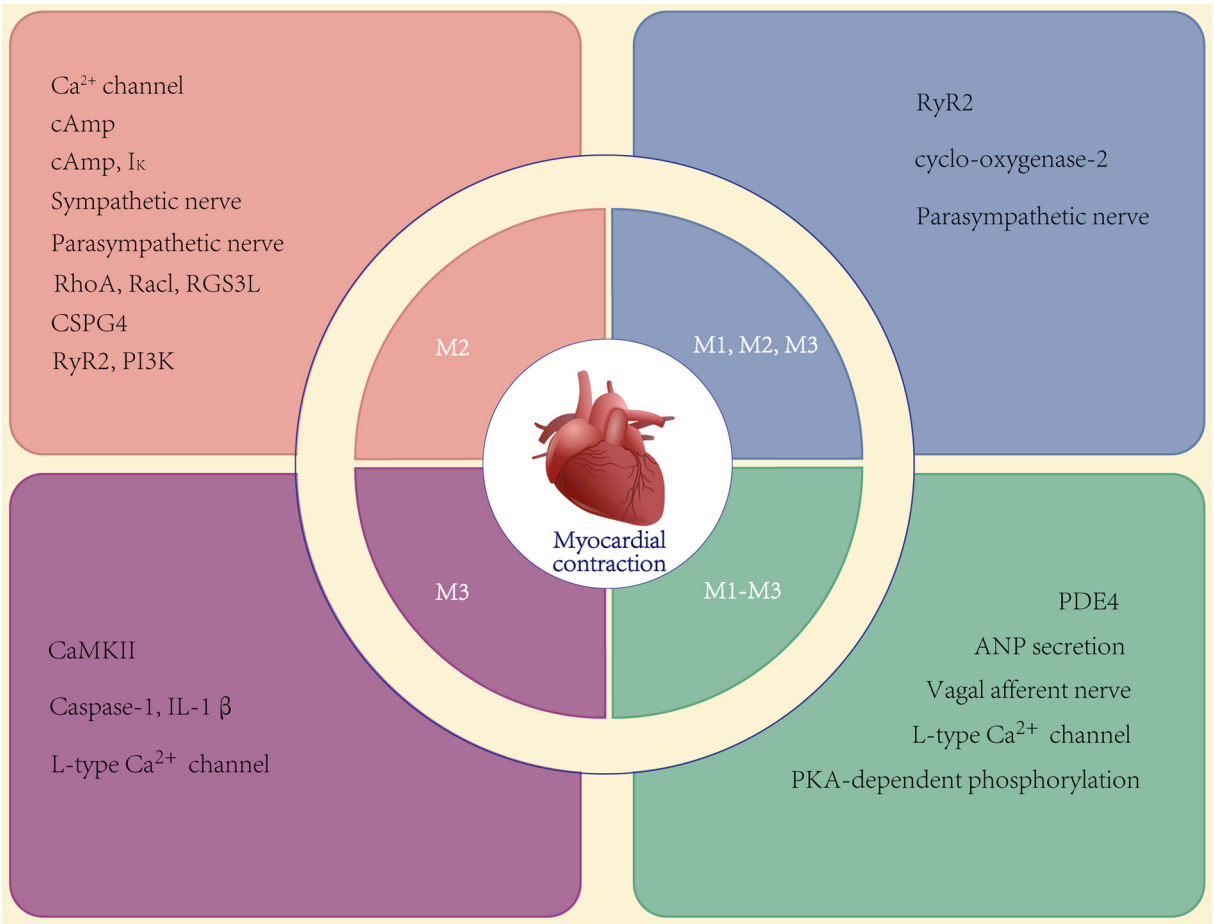

Figure 1. Regulatory effect of the muscarinic ACh receptor on myocardial contraction. The numerous cellular mechanisms include the activation of calcium and potassium channels, nerve and signal proteins, changes in biochemical indicators and changes in inflammatory factors, which may be involved in the contractile function of myocardium. M, muscarinic ACh receptor; ACh, acetylcholine; cAMP, cyclic adenosine monophosphate; RGS3L, long isoform of the regulator of G protein signaling 3; CSPG4, chondroitin sulfate proteoglycan 4; RyR2, ryanodine receptor; PI3K, phosphoinositide 3-kinase; CaMKII, calcium ion/calcium-dependent protein kinase II; IL, interleukin; PDE4, phosphodiesterase 4; PKA, protein kinase A.

against myocardial infarction through extracellular signal-regulated kinase 1/2 (ERK1/2)- and PI3K/AKT-mediated signaling pathways (38), and play a protective role in hypoxia and injury of cardiomyocytes (39). ACh can also alleviate the injury of cardiomyopathy by affecting adenosine 5'-monophosphate-activated protein kinase (AMPK) signaling and mitochondrial cristae reconstruction through interaction with muscarinic ACh receptors (40). Studies have shown that ACh can reduce the transient calcium amplitude of muscarinic ACh receptors to regulate the levels of calcium and iron, thereby alleviating oxidative stress injury (41). Vagal nerve stimulation experiments have shown that the increase in ACh levels can upregulate the expression of vascular endothelial growth factor A/B, and promote angiogenesis and protection, thereby playing a protective role in myocardial infarction (42,43). This result was later confirmed by the repair of the injured coronary artery and cardiomyocytes during infarction through the M2 muscarinic ACh receptor (44). Studies have shown that acetylcholinesterase (AChE) inhibitors can better protect against myocardial ischemia, myocardial infarction and heart failure than simply stimulating the release of ACh (45,46).

In recent years, the M3 muscarinic ACh receptor, as an important regulatory receptor in cardiomyocytes, has received widespread attention from the cardiovascular research community. Another study showed that inhibiting microRNA (miR)-376b-5p can affect the activation of the M3 muscarinic ACh receptor, thereby regulating downstream calcium signaling and reactive oxygen species-related cardioprotection pathways (47). Zhao *et al* (48) reported that agonist activation of the M3 muscarinic ACh receptor can alleviate myocardial injury caused by myocardial ischemia by regulating connexin 43 (Cx43) phosphorylation and cyclooxygenase-2 expression. Another study showed that chlorine-based choline can alleviate myocardial injury under the transverse aortic constriction myocardial ischemic model by agonist activation of the M3 muscarinic ACh receptor, while the M3 muscarinic ACh receptor inhibitor 4-diphenylacetoxy-N-methylpiperidine methiodide had the opposite effect (49,50). Inhibiting the M3 muscarinic ACh receptor was also observed to promote the influx of calcium ions into cells after myocardial ischemia, interfering with normal cellular energy metabolism and accelerating the death of cardiomyocytes (18). Wang *et al* (51) reported the signaling role of beta-catenin in the regulation of the downstream anti-apoptotic protein Bcl-2 mediated by the M3 muscarinic ACh receptor. Therefore, the regulatory role of the M3 muscarinic ACh receptor on myocardial infarction is relatively certain (Fig. 2).

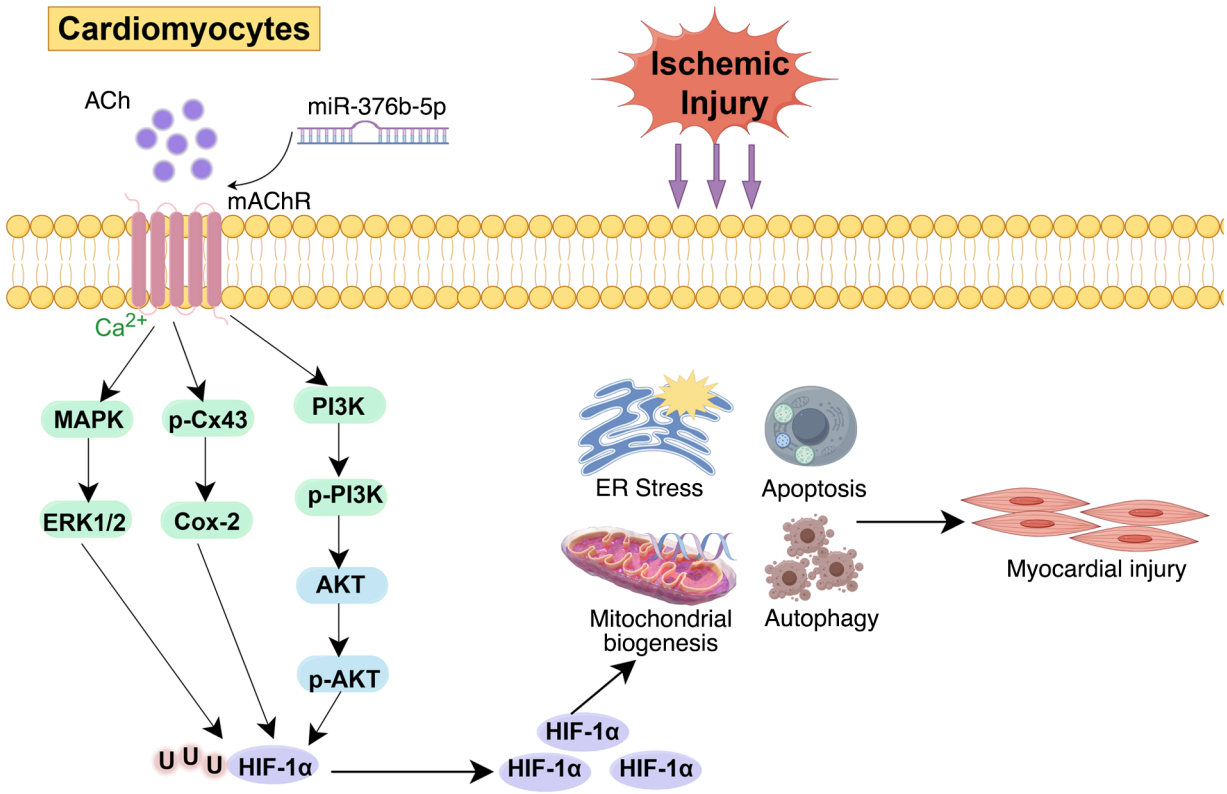

Figure 2. Regulatory mechanisms of muscarinic ACh receptor on myocardial infarction. ACh and miR-376b-5p can act on muscarinic receptors, affect HIF-1α through MAPK/ERK1/2, Cx43/Cox2 and PI3K/Akt, and affect cardiomyocyte apoptosis, autophagy, endoplasmic reticulum stress and mitochondrial biogenesis, and ultimately affect myocardial infarction. ACh, acetylcholine; M, muscarinic acetylcholine receptor; mAChR, muscarinic ACh receptor; MAPK, phospho-p38 mitogen-activated protein kinase; ERK1/2, extracellular signal-regulated kinase 1/2; Cx43, connexin 43; Cox-2, cyclooxygenase-2; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; HIF-1α, hypoxia inducible factor-1α; ER, endoplasmic reticulum; miR, microRNA; p-AKT, phosphorylated AKT.

4. Effects of muscarinic ACh receptors on myocardial ischemia and reperfusion injury

Existing studies have shown that ischemia/reperfusion injury is closely related to the regulation of muscarinic ACh receptors (Fig. 3). According to relevant research reports, various anti-muscarinic ACh receptor drugs, including ipratropium, tiotropium bromide, atropine and adriamycin, have been reported to increase the incidence of ischemic/reperfusion myocardial injury by regulating the muscarinic ACh receptor, resulting in cardiac toxicity (18,52,53). ACh can play a protective role in ischemic reperfusion injury by stimulating the muscarinic ACh receptor. Therefore, exploring the protective mechanism of the muscarinic ACh receptor against ischemic reperfusion injury is becoming a new research hotspot in the field of myocardial ischemia/reperfusion injury. There is evidence that by stimulating the muscarinic ACh receptor on the heart, it can have a significant protective effect on the mouse myocardial ischemia-reperfusion injury model, reducing the area of myocardial injury caused by ischemia-reperfusion (34). It was also shown that stimulation of the myocardial muscarinic ACh receptor can cause the release of ACh mediated by the muscarinic ACh receptor, resulting in a response of ischemic preconditioning, which can protect against myocardial ischemia-reperfusion injury (54). Donepezil and other cholinergic receptor agonists can protect against cell apoptosis after myocardial ischemia-reperfusion injury by regulating the level of phosphorylated (p)-Cx43 (ser368) and the balance of mitochondrial activity and autophagy (55). ACh can also alleviate endoplasmic reticulum stress in myocardial cells and increase cell viability (56), blocking mitochondrial unfolding protein, thereby reducing myocardial cell apoptosis induced by hypoxia/reoxygenation (57). Existing studies have shown that vagus nerve stimulation activating the muscarinic ACh receptor can reduce mitochondrial function through the M3 muscarinic ACh receptor/CaMK kinase b/AMPK signaling pathway to protect against myocardial ischemic injury (58). The mechanism of this ischemic myocardial injury may be myocardial cell apoptosis and related metabolic dysfunction. Experiments with catestatin binding to the M2 muscarinic ACh receptor indicate that the M2 muscarinic ACh receptor can play a myocardial protective role by regulating the ERK1/2 and PI3K/AKT signaling pathways in cells (38).

5. Effects of muscarinic ACh receptors on myocardial fibrosis

Myocardial fibrosis can occur in various cardiovascular diseases and has been considered one of the most common conditions in cardiomyopathy, the pathogenesis of which remains to be fully elucidated. Although cardiac fibroblasts are the main cells that constitute heart tissue, the mechanism of muscarinic ACh receptor-induced myocardial fibrosis still requires further research (Fig. 4). In addition, relevant neural studies have shown that stimulation of the vagus nerve, which is the same as the parasympathetic nerve, and exogenous

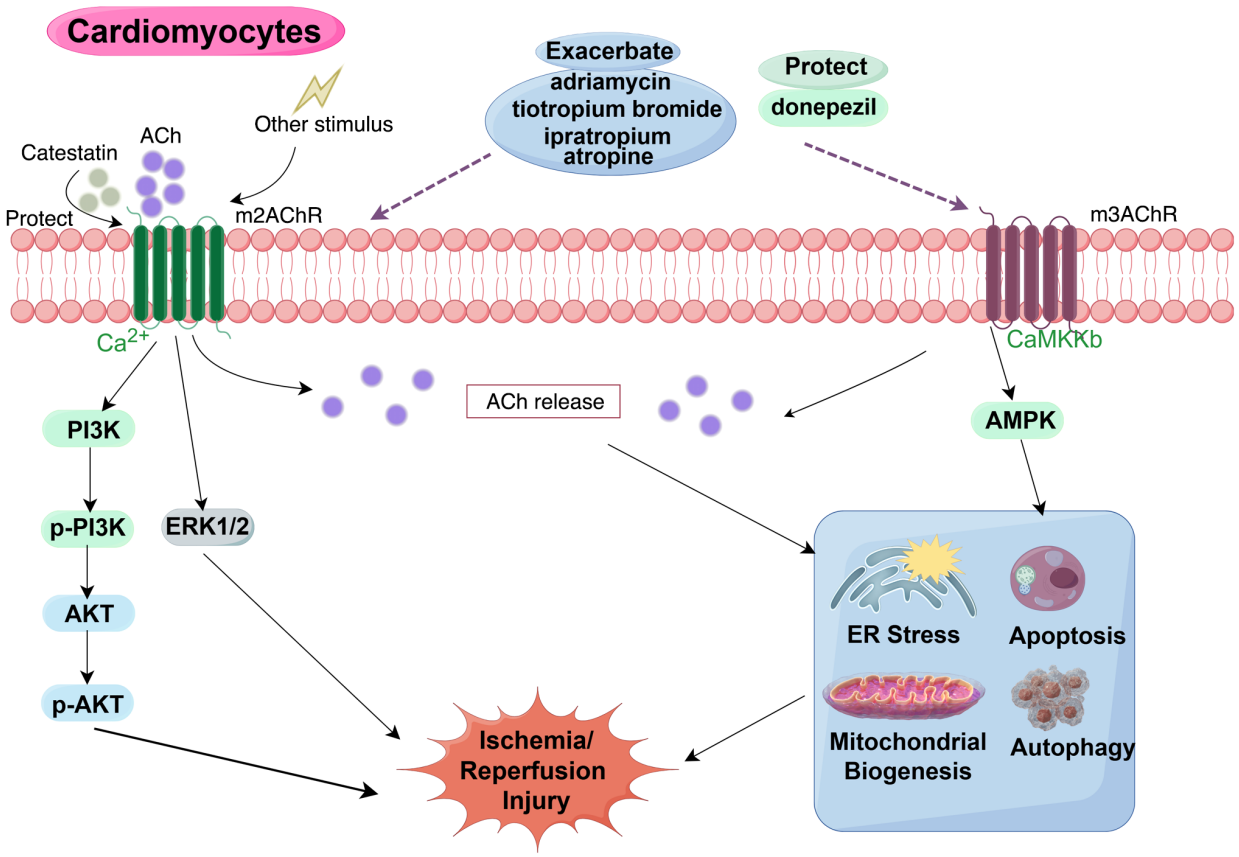

Figure 3. Role of muscarinic ACh receptor in myocardial ischemia and reperfusion injury. Ischemia/reperfusion injury may be regulated by drugs and stimuli through m2AChR receptors on PI3K/Akt and ERK1/2 pathways. Meanwhile, ischemia/reperfusion injury may be affected by ACh and drugs through m3AChR on cardiomyocyte apoptosis, autophagy, ER stress and mitochondrial biogenesis regulated by the AMPK pathway. ACh, acetylcholine; mAChR, muscarinic ACh receptor; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; p-AKT, phosphorylated AKT; ERK1/2, extracellular signal-regulated kinase 1/2; CaMKKb, calmodulin-dependent protein kinase kinase b; ER, endoplasmic reticulum.

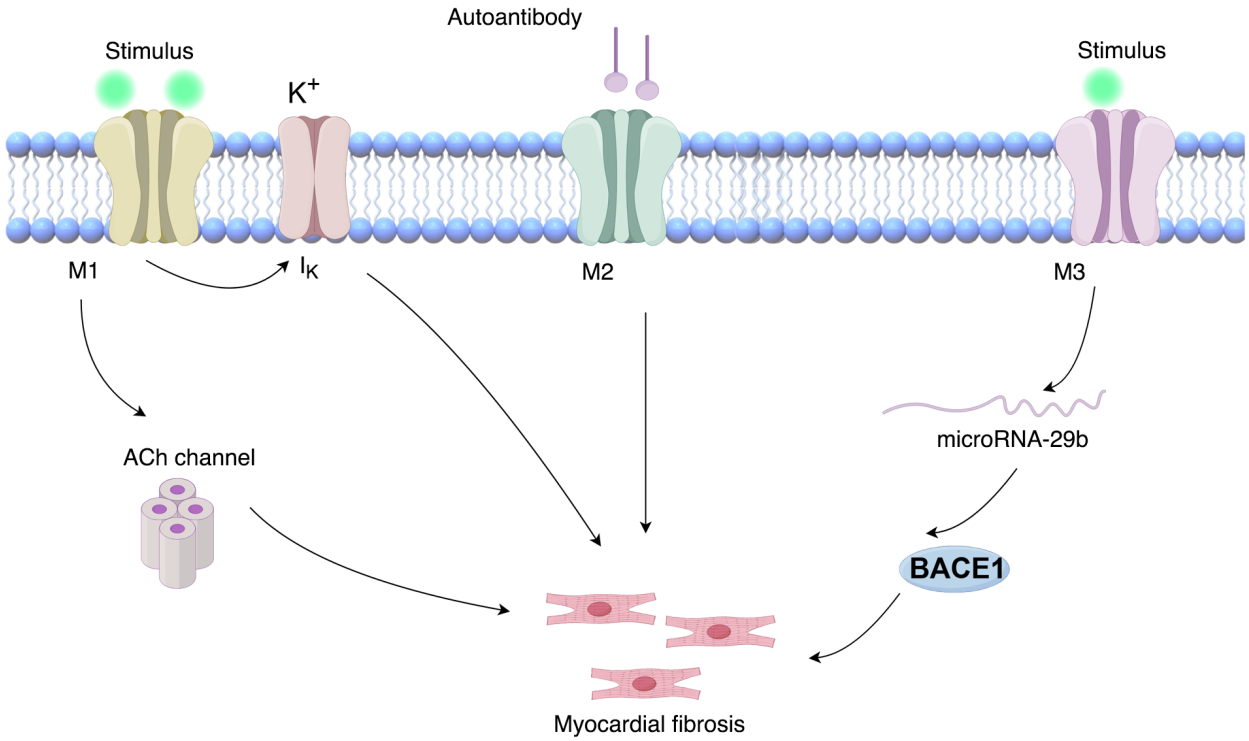

Figure 4. Mechanism of muscarinic ACh receptor in myocardial fibrosis. Stimulus and autoantibodies may affect myocardial fibrosis through ACh and potassium channels of M1, M2 and M3 receptor-mediated microRNA-29b/BACE1. M, muscarinic ACh receptor; ACh, acetylcholine; BACE1, beta-site app cleaving enzyme 1.

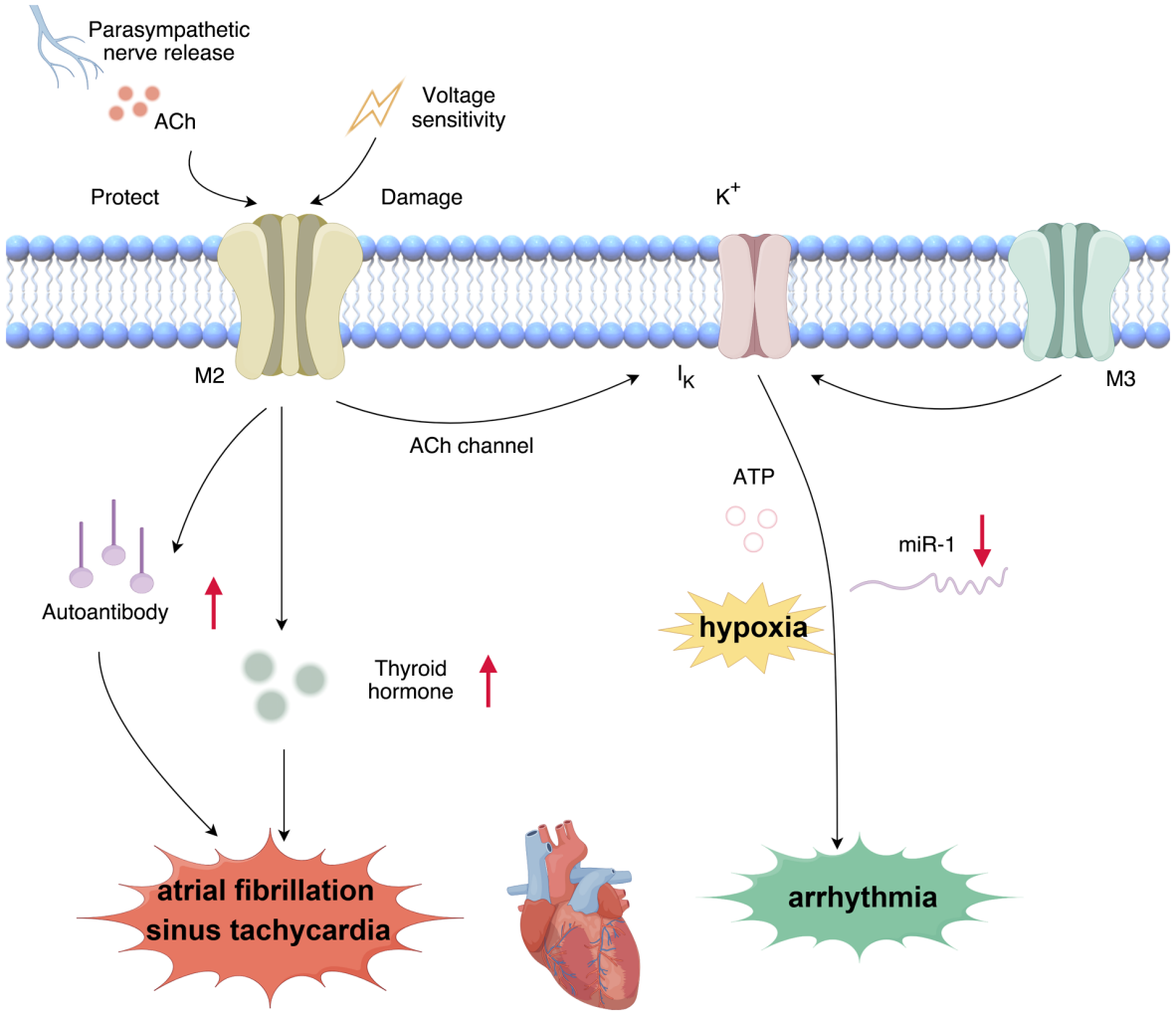

Figure 5. Effect of muscarinic ACh receptor on arrhythmia. ACh may protect against atrial fibrillation and sinus tachycardia caused by the increase of autoantibodies and thyroid hormones through M2 receptors induced by voltage. Both M2 and M3 could regulate cardiomyocyte ATP, miR-1 and hypoxia through potassium ion channels to affect arrhythmia. M, muscarinic ACh receptor; ACh, acetylcholine; miR, microRNA.

stimulation of the M3 muscarinic ACh receptor, can alleviate the process of myocardial fibrosis via the miR-29b/beta-site app cleaving enzyme 1 axis both *in vivo* and in *in vitro* experimental models (49,59). An *in vitro* experimental study showed that inhibiting ACh levels can induce the formation of cardiac fibroblasts (60). A previous clinical study has shown that the amount of M2 muscarinic ACh receptor autoantibody can be used as a suitable detection method for judging the severity of the pathological and physiological conditions of patients with left atrial fibrosis (61). In addition, recent experimental results of a human mechanistic study also showed that the level of M2 muscarinic ACh receptor autoantibody in the serum of patients with atrial fibrosis was significantly higher than that in the non-atrial fibrosis group, and immunohistochemical analysis and western blot analysis of left atrial appendage tissue suggested that M2 muscarinic ACh receptor is closely related to the process of myocardial fibrosis (62). All of the above evidence indicated that the muscarinic ACh receptor has an important connection with the onset, diagnosis and treatment of myocardial fibrosis. Another study showed that the M1 muscarinic ACh receptor is significantly upregulated in patients with chronic atrial fibrillation and can affect atrial fibrillation by regulating the $I_{K,ACh}$ channel in human atrial myocytes (63).

6. Effects of muscarinic ACh receptors on arrhythmia

Recently, it has been shown that arrhythmia is related to the regulation of muscarinic ACh receptors (Fig. 5). Activation of the muscarinic ACh receptor can reduce heart rate and even cause bradycardia (64). Studies have confirmed that blocking the parasympathetic nerve in the heart is an important factor in inducing ventricular arrhythmia and increasing myocardial energy consumption (65). In the study of arrhythmia, the regulatory mechanism of I_K channels has recently attracted attention. Studies have shown that agonists of muscarinic ACh receptors, including ACh, can alleviate the symptoms of cardiac arrhythmia without affecting the contractility of the heart (66). The mechanism includes hypoxia and regulation of I_K-ATP (67-69). Atrial fibrillation is one of the important symptoms in patients with arrhythmia, and it is closely related to the regulatory effect of muscarinic ACh receptors on heart function. Therefore, numerous scholars have conducted in-depth research on the association between atrial fibrillation and myocardial muscarinic ACh receptors. It has been shown that inhibiting the release of ACh can effectively inhibit the effect of atrial fibrillation in patients after cardiac surgery (70). The specific manifestation showed that M2 muscarinic ACh

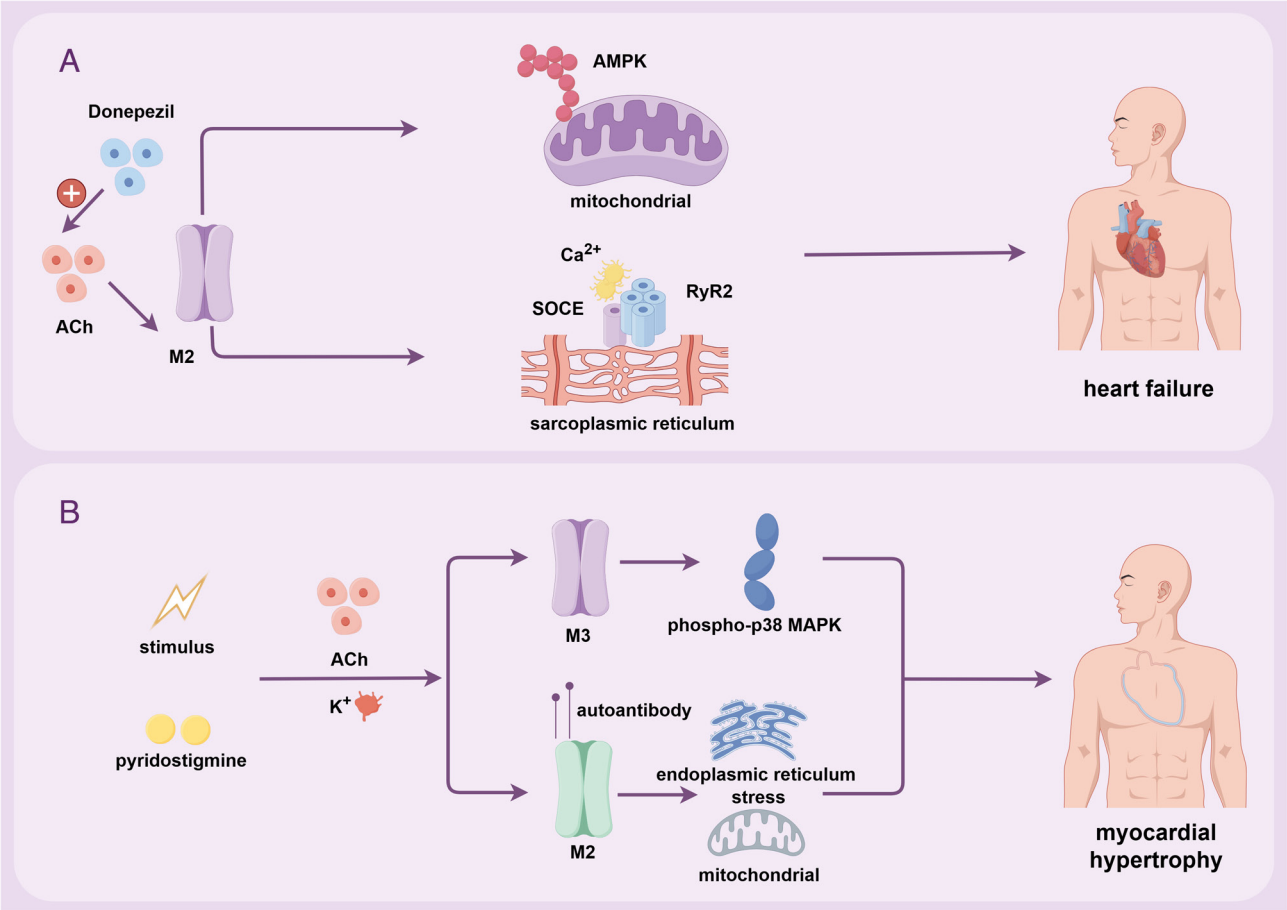

Figure 6. Effect of muscarinic ACh receptor on heart failure and myocardial hypertrophy. (A) Effect of muscarinic ACh receptor on heart failure. (B) Effect of muscarinic ACh receptor on myocardial hypertrophy. M, muscarinic ACh receptor; ACh, acetylcholine; AMPK, adenosine 5'-monophosphate-activated protein kinase; SOCE, store-operated calcium entry; RyR2, ryanodine receptor; MAPK, mitogen-activated protein kinase.

receptors could induce the release of autoantibodies and thyroid hormones, promoting the susceptibility to atrial fibrillation and sinus tachycardia (27,71). Researchers have found that the level of autoantibodies to M2 muscarinic ACh receptors in patients with atrial fibrillation shows a significant increase (62). Another study reported that changes in the voltage sensitivity of M2 muscarinic ACh receptors can lead to heart diseases such as atrial fibrillation and sinus tachycardia (72). Furthermore, M3 muscarinic ACh receptor overexpression was observed to reduce the incidence and mortality of arrhythmia after myocardial ischemia-reperfusion injury. This effect may be mediated by downregulating the expression of arrhythmogenic miR-1 and increasing the inward rectifier potassium current, which may be a new anti-arrhythmic strategy for diagnosis (73). Other studies have shown that the release of ACh triggered by parasympathetic nerve cells can increase the sensitivity of animals to arrhythmia and increase the heart rate (74-76).

7. Effects of muscarinic ACh receptors on heart failure and myocardial hypertrophy

An increasing number of studies have shown that stimulating the muscarinic ACh receptor through drugs or neural transmission may become an important means of treating heart failure (77) (Fig. 6). Certain studies have shown that the continuous excitation of muscarinic ACh receptor by ACh can release calcium ions through the RyR2-mediated calcium store of the sarcoplasmic reticulum in cardiomyocytes, and the regulation of calcium signals related to store-operated calcium entry can alleviate heart failure (78,79). Another study has shown that ACh can serve as a key compensatory mediator in the development of heart failure in mice by stimulating the M2 muscarinic ACh receptor of cardiomyocytes to alleviate the occurrence and development of ventricular remodeling and heart failure (80,81). The drug donepezil, which has the function of AChE inhibitor, can increase ACh in rat hearts and play a long-term protective effect on heart failure (82). Research data show that increasing ACh by using central or peripheral AChE inhibitors can effectively improve heart failure and the heart's autonomic nervous imbalance and hemodynamic changes in patients with hypertension (83). ACh can regulate the effect of AMPK on mitochondrial cristae remodeling by stimulating the muscarinic ACh receptor, thereby alleviating the hypertrophy of cardiomyocytes induced by palmitic acid (40).

Myocardial hypertrophy is a common clinical cardiomyopathy, which is also closely related to the induction of muscarinic ACh receptor (84). One of the reasons for myocardial hypertrophy may be related to the decrease in K^+ repolarization associated with the M3 muscarinic ACh receptor, which leads to the harmful myocardial remodeling. Overexpression of M3 muscarinic ACh receptor in cardiomyocytes can alleviate the

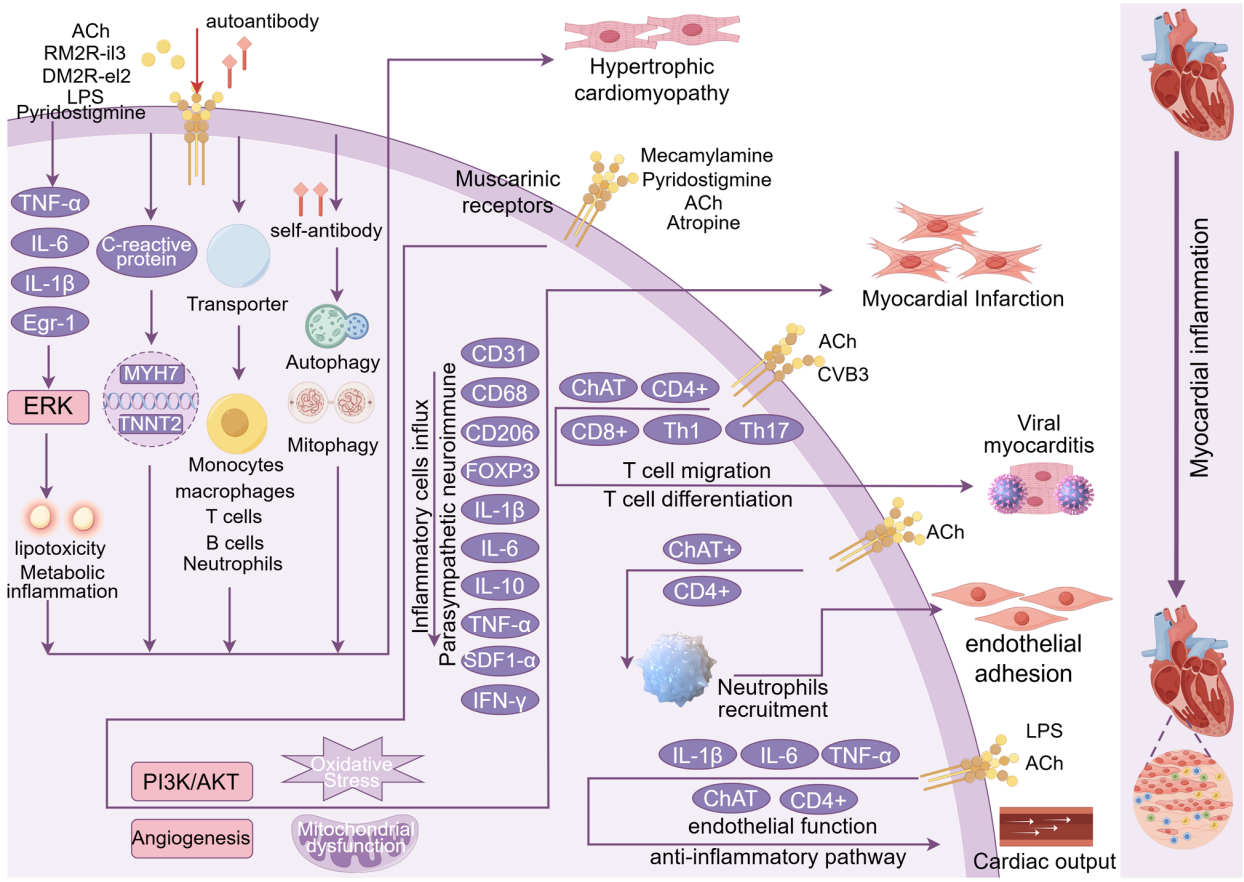

Figure 7. Role of muscarinic ACh receptor on myocardial inflammation. Drugs, chemicals and biological mediators may act on muscarinic ACh receptors to regulate various immune responses, affecting viral myocarditis, cardiac output and endothelial adhesion. The inflammatory responses could affect the regulation of myocardial injury through the ERK, PI3K/AKT, oxidative stress, mitochondrial function and angiogenesis pathways on the regulation of myocardial injury. M, muscarinic ACh receptor; ACh, acetylcholine; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; Egr-1, early growth response-1; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; CD, cluster of differentiation; FOXP3, forkhead box p3; SDF-1α, stromal cell-derived factor-lα; IFN-γ, interferon-γ; ChAT, acetylcholine transferase; Th, helper T cell; DM2R-el2, donor-immunized second extracellular loop of M2R; RM2R-il3, recipient third intracellular loop of M2R; MYH7, myosin heavy chain 7; TNNT2, troponin T2; CVB3, Coxsackievirus B3.

adverse myocardial remodeling (85). Wang *et al* (86) found that cardiac hypertrophy can play a protective role by inhibiting the P38/p-p38 mitogen-activated protein kinase signaling pathway and blocking the M3 muscarinic ACh receptor. The role of M2 muscarinic ACh receptor in regulating myocardial hypertrophy is also relatively obvious. Studies show that aerobic exercise can improve myocardial hypertrophy by regulating M2 muscarinic ACh receptors, affecting mitochondrial quality control and endoplasmic reticulum stress in cardiomyocytes (87). It has been shown that autoantibodies to M2 muscarinic ACh receptor can also cause myocardial hypertrophy in rabbits (88). The AChE inhibitor pyridostigmine can improve myocardial hypertrophy by prolonging the duration of ACh on the M2 muscarinic ACh receptor. These results were confirmed by echocardiography, immunofluorescence and precipitation methods (89). Intermittent excessive activation of M2 muscarinic ACh receptors can also exacerbate myocardial hypertrophy through oxidative stress (90).

8. Effects of muscarinic ACh receptors on myocardial inflammation

The regulation of the inflammatory response by the muscarinic ACh receptor has also been widely studied (Fig. 7). The role of the muscarinic ACh receptor in regulating myocardial inflammation is mainly achieved by affecting neural transmission and changes in lymphocytes. Studies have shown that, compared to the normal group, myocardial inflammation confirmed by enzyme-linked immunosorbent assay is closely related to the increase in the level of self-antibodies mediated by the M2 muscarinic ACh receptor (91), and the autoimmune antibodies show a role in heart function damage and myocardial inflammation (92). Through stimulating cholinergic neurons, a study showed good anti-inflammatory effects in a heart model of Wistar rats through immunohistochemistry and cytokine measurement (93). In addition, a study suggested that the inhibition of AChE by bromocriptine enhances the function of ACh, thereby preventing inflammatory autonomic dysfunction (94). The muscarinic ACh receptor can also regulate the inflammatory immune mediators of myocardial cells, such as the anti-inflammatory effect of T lymphocytes (95). Cox *et al* (96) reported that infection can promote the increase in the expression of ACh transferase (ChAT) in CD4+T and CD8+T cells, thereby enhancing the immune response. Furthermore, the release of ACh and ChAT+ B lymphocytes has been shown to inhibit the activation of macrophages (97). In the viral myocarditis mouse model, the anti-inflammatory pathway of ACh may be achieved through the differentiation of

Table II. Mechanisms of muscarinic ACh receptors in myocardial inflammation.

Function	Stimulus	Species	Target	Mechanism	Year	(Refs.)
Hypertrophic cardiomyopathy	M2 autoantibody	*Homo sapiens*	C-reactive protein, high-sensitivity C-reactive protein	MYH7 and TNNT2 genetic heterogeneity	2020	(91)
	RM2R-il3, DM2R-el2	Mouse	Self-antibodies	Autophagy and mitophagy	2018	(92)
	ACh, LPS	Mouse	Vesicular ACh transporter	Monocytes/ macrophages, T cells, B cells, neutrophils	2021	(103)
	Pyridostigmine, ACh	Mouse	TNF-α, IL-6, IL-1β, ERK, Egr-1	Metabolic inflammation, cardiac lipotoxicity	2018, 2023	(100,101)
MI	Pyridostigmine bromide	Rat	CD68, CD206, FOXP3, IL-1β, IL-6, IL-10, TNF-α	Parasympathetic neuro-immune, oxidative stress	2017	(93)
	Pyridostigmine bromide	Rat	IFN-γ, IL-6, IL-1β, IL-10, TNF-α	Mitochondrial dysfunction, inflammatory cell influx, angiogenesis	2019	(94)
	ACh, mecamylamine, atropine	Rat	TNF-α, IL-6, IL-1β, CD31, SDF-1α	PI3K/AKT	2023	(99)
Viral myocarditis	ACh	Mouse	ChAT, CD4+, CD8+ T cells	T-cell migration	2019	(96)
	CVB3	Mouse	CD4+ T cells, Th1, Th17 cells	CD4+ T-cell differentiation	2018	(98)
Endothelial cell adhesion	ACh	Mouse	CD4+ T-cells, ChAT+ B cells	Recruitment of neutrophils	2013	(97)
Cardiac output	LPS	Rat	IL-1β, IL-6, TNF-α	Cholinergic anti-inflammatory pathway	2018	(104)
	ACh	*Homo sapiens*	ChAT, CD4+ T cells	Vascular endothelial function	2023	(105)

ChAT, choline acetyltransferase; ACh, acetylcholine; LPS, lipopolysaccharide; M, muscarinic ACh receptor; MI, myocardial infarction; ACh, acetylcholine; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; Egr-1, early growth response-1; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; CD, cluster of differentiation; FOXP3, forkhead box p3; SDF-1α, stromal cell-derived factor-lα; IFN-γ, interferon-γ; ChAT, acetylcholine transferase; Th, helper T cell; DM2R-el2, donor-immunized second extracellular loop of M2R; RM2R-il3, recipient third intracellular loop of M2R; MYH7, myosin heavy chain 7; TNNT2, troponin T2; CVB3, coxsackievirus B3.

CD4+ T cells and the regulation of the expression of Th1 and Th17 cytokines (98). This may reveal the current regulatory mechanism of myocardial inflammation, such as myocardial cell lesions and cell infiltration symptoms. Exogenous ACh stimulation may activate the PI3K/AKT pathway (99), and the ERK/early growth response-1 pathway (100,101), thereby alleviating inflammation and oxidation. Similarly, it has been found that a small population of ChAT+ natural killer cells, which are, however, transcriptionally distinct, have immune protective effects in a mouse model (102). The stimulation of the muscarinic ACh receptor can also alleviate cardiac inflammation through the muscarinic ACh and nicotine receptors. Recently, the regulatory effect of the non-neuronal cholinergic system on myocardial inflammation has also been reported (97). A study suggested that the non-neuronal cholinergic system can regulate the activation and inhibition of inflammatory cells, such as the number of macrophages and Forkhead box protein P3+ T cells in myocardium through the muscarinic ACh receptor (103). However, studies have indicated that, although the non-neuronal cholinergic system can regulate the release of ACh and produce the effect of stimulating the muscarinic ACh receptor, it was not found

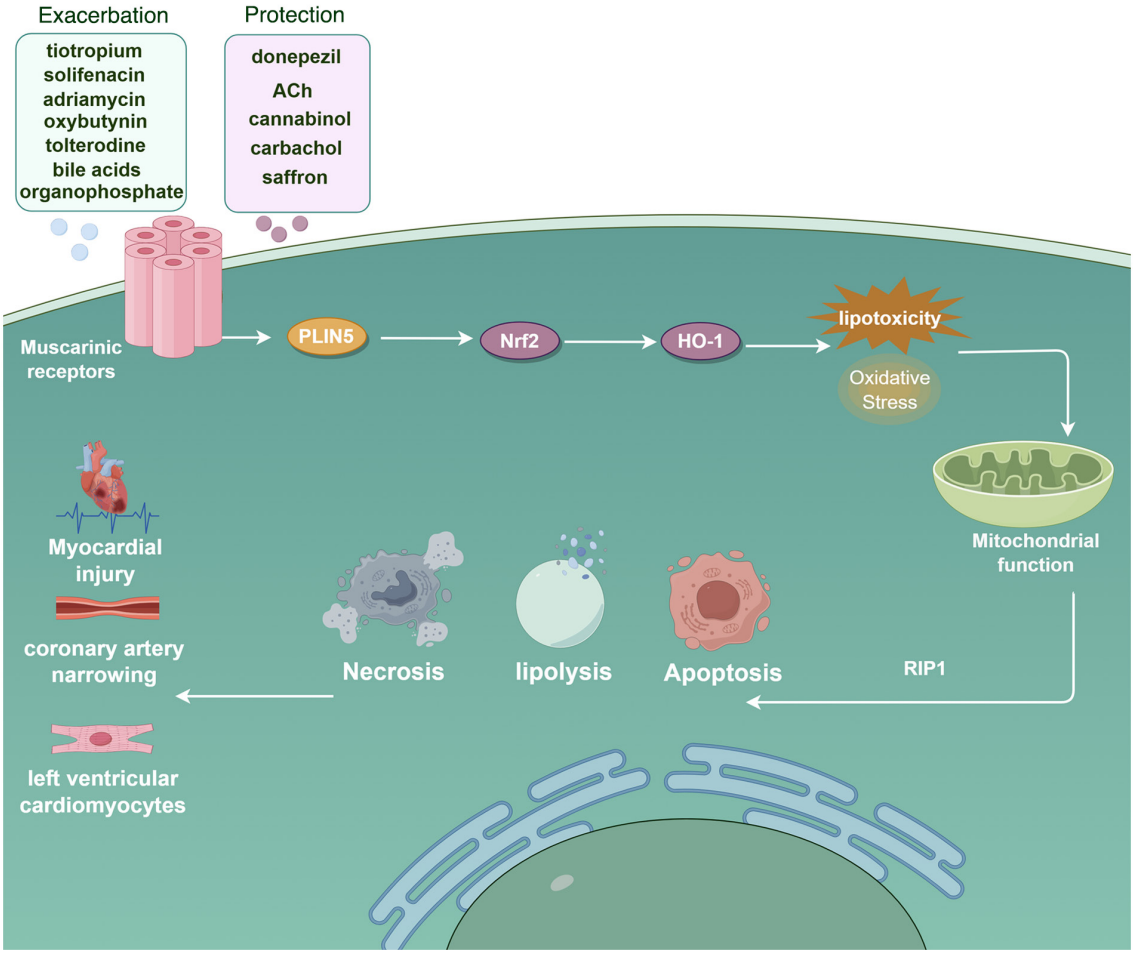

Figure 8. Muscarinic ACh receptors and regulation of cardiotoxicity. Numerous drugs and chemicals may act on muscarinic ACh receptors and affect the lipid toxicity and oxidative stress of cardiomyocytes through PLIN5, Nrf2 and HO-1. Subsequently, it affects cell apoptosis, necrosis and lipolysis by changing mitochondrial function and RIP1. These mechanisms ultimately affect the state of the coronary artery, myocardial cells and myocardial injury. ACh, acetylcholine; PLIN5, perilipin 5; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme-oxygenase-1; RIP1, receptor-interacting protein kinase 1.

to improve or exacerbate the myocardial inflammatory response (104,105). Therefore, whether the non-neuronal cholinergic system can play a role in the myocardial inflammatory response still requires further research. All of the roles of myocardial inflammation through the regulation of muscarinic ACh receptors are listed in Table II.

9. Study on the muscarinic ACh receptors and cardiotoxic effects

Long-term clinical studies have found that numerous anti-muscarinic ACh receptor drugs have cardiotoxic effects on the heart (32,106). Long-acting muscarinic ACh inhibitors such as tiotropium and other tracheal dilating drugs have been reported to exacerbate myocardial injury (107,108) and increase the incidence rate of acute coronary syndrome (109). The use of muscarinic ACh receptor inhibitors such as oxybutynin, solifenacin and tolterodine, which are used to treat hair loss, has been found to be associated with the incidence rate of myocardial disease (110). Adriamycin has long been considered to have cardiotoxic effects, but its mechanism of toxicity in the body has remained elusive. In recent years, studies on its regulation of the muscarinic ACh receptor in cardiomyocytes have been ongoing. Research suggests that the activation of the muscarinic ACh receptor can protect against myocardial injury and cardiomyocyte apoptosis caused by adriamycin, mediated by the nuclear factor erythroid 2-related factor 2/heme-oxygenase-1 pathway (111). It has been shown that donepezil can protect against oxidative stress and mitochondrial function deficiency, reduce the apoptotic ratio of Bax/Bcl-2 and cleaved-caspase 3/caspase 3, and protect against receptor-interacting protein kinase 1-mediated necrosis of cardiomyocytes caused by adriamycin by activating the muscarinic ACh receptor (112). Another study indicated that saffron can exert antioxidant properties and inhibit the apoptosis pathway of myocardial cells through the M2 muscarinic ACh receptors, thereby preventing myocardial toxicity caused by organophosphate poisoning (113). Furthermore, increased ACh under parasympathetic stimulation can inhibit the programmed death of cardiomyocytes, protecting against adriamycin-induced myocardial toxicity (114). In addition, high concentrations of bile acids were shown to produce cardiotoxic effects by acting on the M2 muscarinic ACh receptor (12). ACh can promote lipolysis and activate mitochondrial interactions through perilipin 5, effectively inhibiting cardiomyocyte apoptosis and lipotoxicity (115). The release of large amounts of carbachol stimulates ACh to activate the muscarinic ACh receptor, leading to coronary artery narrowing and changes in

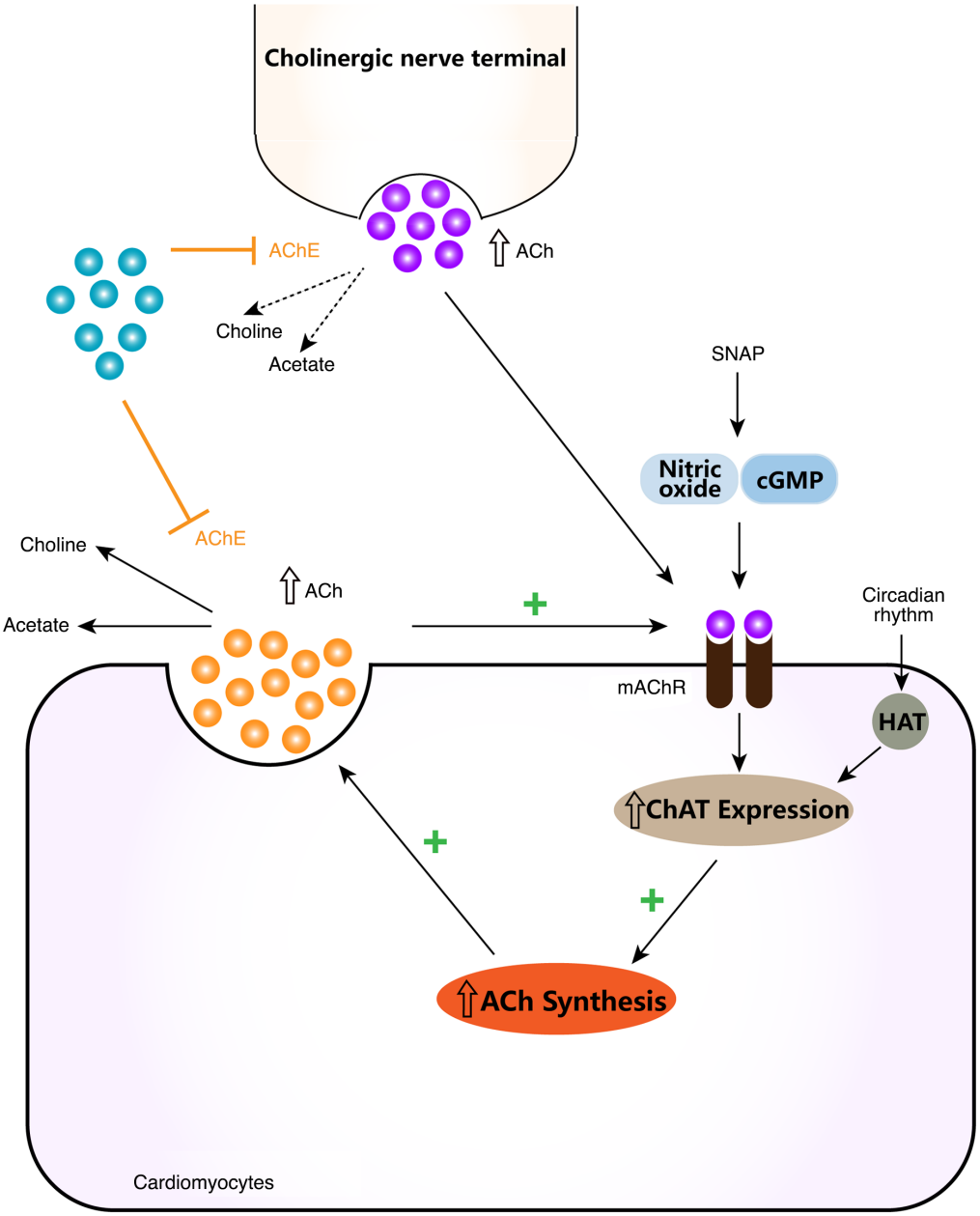

Figure 9. Mechanisms of muscarinic ACh receptors in the non-neuronal cholinergic system of cardiomyocytes. The cardioprotective mechanism of non-neuronal cholinergic system of cardiomyocytes is based on increasing the production of ACh. This includes stimulation of cholinergic neurons to electrically activate cholinergic neurons to produce and release ACh. Another possibility may be the pharmacological method of prolonging the effect of ACh by using AChE inhibitors. SNAP could activate muscarinic ACh receptor by transmitting nitric oxide and cGMP to cardiomyocytes, thereby increasing the synthesis and expression of ChAT and ACh. Finally, the circadian rhythm of the heart affects the synthesis and expression of ChAT and ACh by regulating HAT. mAChR, muscarinic ACh receptor; ACh, acetylcholine; AChE, acetylcholinesterase; SNAP, S-nitroso-N-acetyl-DL-penicillamine; cGMP, cyclic guanosine monophosphate; HAT, histone acetyltransferase; ChAT, ACh transferase.

the structure of left ventricular cardiomyocytes in rats, causing myocardial toxicity, while long-term treatment with cannabinol can significantly inhibit this toxic effect (116). A schematic illustrating the mechanisms of drugs acting on muscarinic ACh receptors with cardiotoxic effects is provided in Fig. 8.

10. Effects of muscarinic ACh receptors on the non-neuronal cholinergic system (NNCS)

With the continuous deepening of the research on muscarinic ACh receptors, a unique cholinergic regulatory factor production and circulatory system was discovered in recent years, which has attracted widespread attention from pharmacologists. The degradation of ACh in the body by AChE also maintains it at an appropriate level, which may be due to the regulatory function of the non-neural cholinergic system (NNCS) (117). In the heart, this is a self-regulating cholinergic synthesis and secretion system of cardiomyocytes, and except for nerve cells, almost all components of the neural ACh system are retained. The NNCS may contain components such as ChAT, ACh, nicotinic/muscarinic ACh receptor, high-affinity choline uptake and cholinesterase. They can participate in cell proliferation, migration, differentiation, cell barrier formation, programmed cell death,

hypoxia/reoxygenation injury and other cellular functions and metabolic processes (118-120). The acute promyelocytic leukemia cell line NB-4 can regulate the expression of M3 muscarinic ACh receptors under the stimulation of choline, thus exerting its anti-inflammatory effects *in vivo* (121). ChAT-transgenic cells have been confirmed to have the potential of systemic anti-inflammatory response, which can alleviate inflammatory lesions (122,123). Cardiomyocytes increase the expression of AChE through the activation of muscarinic ACh receptors and ChAT gene transcription, and then synthesize ACh. Both RNA interference experiments of ChAT gene and M-receptor inhibitor competition experiments have proved this (124). Further research showed that, through the regulation of the NNCS in cardiomyocytes, the expression of ChAT is higher in female hearts than in males. The circadian rhythm has an important impact on histone acetyltransferase activity, thereby regulating the expression of ChAT (125). The newly discovered S-nitroso-N-acetyl-DL-penicillamine can produce nitric oxide and cyclic guanosine monophosphate, activate the expression of the AChE gene and the function of muscarinic ACh receptors, and have a protective effect against myocardial injury (126). The muscarinic ACh receptors on the NNCS are presented in Fig. 9. Although numerous relevant research achievements have been made in this direction in recent years, there are still many gaps and deficiencies in the study of NNCS in cardiomyocytes that need to be filled. In the future, this may provide a new diagnostic and therapeutic method for myocardial infarction and inflammatory protection.

11. Conclusion

The muscarinic ACh receptor and cardiac myocytes are both involved in the regulation of heart function. Current research on the myocardial function and diseases related to the muscarinic ACh receptor shows that its range of action is closely related to numerous heart diseases and physiological functions, but its potential mechanism of action is still being continuously explored. So far, the function and structure of the muscarinic ACh receptor have been successively excavated by the research community, but the regulatory role it plays in the function of cardiomyocytes, as well as its physiological and pathological mechanisms of action, still remain to be fully elucidated. This article summarized the research progress on myocardial contraction and related diseases mediated by muscarinic ACh receptors. It was found that muscarinic ACh receptors can play important roles in regulating calcium homeostasis, mitochondrial dysfunction, cardiomyocyte apoptosis and autophagy, inflammatory cells and mediators, providing potential new research directions for the development of cardiomyopathy drugs in the future. With the introduction of the new concept of the myocardial NNCS, the research on the muscarinic ACh receptor in the field of myocardial function and related diseases shows further extensive prospects and value. These findings will provide new theoretical evidence for muscarinic ACh receptors as potential targets for myocardial protective drugs.

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Availability of data and materials

Not applicable.

Authors' contributions

CS was involved in writing the original draft, data curation and conceptualization of the study. QZ contributed to writing the original draft and was responsible for methodology and visualization. ZS and YL were involved in reviewing and editing the manuscript. SC contributed to the review and editing of the manuscript and was responsible for the supervision and conceptualization of the study. Data authentication is not applicable. All authors have 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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