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REVIEW

Contribution of apoptosis in myocardial reperfusion injury and loss of cardioprotection in diabetes mellitus

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Abstract Ischemic heart disease is one of the major causes of death worldwide. Ischemia is a condition in which blood flow of the myocardium declines, leading to cardiomyocyte death. However, reperfusion of ischemic regions decreases the rate of mortality, but it can also cause later complications. In a clinical setting, ischemic heart disease is always coincident with other co-morbidities such as diabetes. The risk of heart disease increases 2–3 times in diabetic patients. Apoptosis is considered to be one of the main pathophysiological mechanisms of myocardial ischemia–reperfusion injury. Diabetes can disrupt the anti-apoptotic intracellular signaling cascades involved in myocardial protection. Therefore, targeting these changes may be an effective cardioprotective approach in the diabetic myocardium against ischemia–reperfusion injury. In this article, we review the interaction of diabetes with the pathophysiology of myocardial ischemia–reperfusion injury, focusing on the contribution of apoptosis in this context, and then discuss the alterations of pro-apoptotic or anti-apoptotic pathways probably responsible for the loss of cardioprotection in diabetes.

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Introduction

Lessons from the diabetic heart

Diabetes mellitus is one of the most frequent risk factors of myocardial infarction (MI), and diabetic patients are more prone to cardiac ischemic dysfunction, including ischemia–reperfusion (IR) injury. In fact, more than 50 % of deaths among the diabetic population result from the consequences of coronary artery diseases [1, 2]. The mortality rate after an acute MI or coronary bypass surgery in diabetic patients is twice that of non-diabetic subjects. Progressive increase in the prevalence of diabetes worldwide makes it an important healthcare concern, and treatment of diabetic patients who suffer also from ischemic heart disease is a contemporary challenge [3].

Diabetic cardiomyopathy refers to a disease process which affects the myocardium in diabetic patients, causing a wide range of structural abnormalities eventually leading to left ventricular hypertrophy and diastolic and systolic dysfunction or a combination of these [4]. The concept of diabetic cardiomyopathy is based upon the idea that diabetes is the factor which leads to changes at the cellular level, leading to structural abnormalities.

Interaction of the diabetic state with therapeutic strategies, ischemic preconditioning (IPreC) and ischemic postconditioning (IPostC) (which are short repeated episodes of ischemia and reperfusion applied before the main ischemia or at the onset of reperfusion, respectively), has been studied in a very limited way, both in basic and clinical circumstances. Preclinical studies of myocardial protection

against IR injury by IPreC and IPostC in diabetic animal models are rather controversial and inconclusive [3]. The majority of studies have reported that diabetes attenuates the cardioprotective effects of preconditioning [5–8]. It is also reported that IPostC does not confer a cardioprotective effect in diabetic states. In this case, it has been reported that hyperglycemia [9] and experimentally induced metabolic syndrome [10] prevent the heart from being protected by postconditioning.

Hyperglycemia is associated with increased mortality after acute myocardial infarction in diabetic patients as well as in patients without diabetes mellitus [9]; however, the reasons for this increased risk are poorly understood. One possible explanation for the poor prognosis of diabetic patients after cardiovascular events is the loss of endogenous protective mechanisms (e.g., ischemic preconditioning), either by the resulting hyperglycemia or by diabetes itself. Mitochondrial dysfunction might be one reason for the failure to precondition the diabetic myocardium [11]. Furthermore, diabetes mellitus blocked the infarct size-reducing effect of late preconditioning, and hyperglycemia blocked ischemic preconditioning independently of plasma insulin concentrations and plasma osmolality [6, 11]. Blood glucose reduction by short-term insulin treatment was not able to restore cardioprotection in diabetic animals [11, 12]. These data show that hyperglycemia has detrimental effects on endogenous cardioprotective mechanisms.

Therefore, because of the complex pathophysiology and poor prognosis of diabetes in patients who have underlying ischemic heart disease, investigating the interaction of diabetes with IR injury and cardioprotective mechanisms is of particular interest, and reducing the outcomes of coronary artery disease using strategies that target IR injury would be particularly beneficial in this population.

Myocardial ischemia–reperfusion injury in healthy and diabetic conditions

Ischemic heart diseases are one of the major causes of death worldwide [13]. In general, ischemia is a condition in which blood flow and oxygen supply of the myocardium declines because of complete or partial occlusion of the vessels perfusing the myocardium that can lead to cardiomyocyte death [14]. During ischemia, non-aerobic metabolism occurs, leading to adenine nucleotide metabolism which results in an evacuation of the ATP reserves of the cell [14].

Restoration of blood flow to an ischemic heart is called myocardial reperfusion [14]. Despite the fact that the rapid blood reflow to ischemic regions (reperfusion) decreases the rate of mortality, it can also cause later complications like stiffness of heart walls, reduction of contractile function of the heart, increased permeability of blood vessels, and microcirculation disorders and arrhythmia which leads to tissue injury and even cell death [15, 16]. Thus, IR injury means tissue injury because of restoration of blood flow to a tissue after a period of ischemia or hypoxia [14]. Myocardial reperfusion injury mainly happens because of blood reflow to an ischemic zone [14]. These events result in a variety of reperfusion-based complications that are mentioned as reperfusion injury [16]. It has been shown that quick reperfusion causes pressure excess, myofibrillar extension and consequent edema, over-contraction of myocardium, and finally cardiomyocyte death [17].

Therefore, ischemia–reperfusion injury is a result of physical and biochemical disorders that occur mostly during transition from the ischemic period into the reperfusion phase. Mechanisms of IR injury are not fully understood, but numerous studies have shown that the over production of oxygen free radicals, cytosolic and mitochondrial Ca overload in the 1st minute of reperfusion, endothelial dysfunction, inflammatory reactions and neutrophil-endothelium interactions, myocardial necrosis, and apoptosis may be involved [16–19]. Of these factors, cardiomyocyte apoptosis is one of the important causes of myocardial infarction and IR injury. When apoptosis occurs during IR injury, it causes loss of cardiomyocyte volume and cardiac dysfunction. Therefore, prevention of apoptosis has attracted more attention as a new target for cardioprotection against IR injury [20].

On the other hand, ischemic heart diseases such as myocardial IR injury are caused by several risk factors and are always coincident with other disease like diabetes, hypertension, hyperlipidemia, heart failure, etc. Treatment of diabetic patients suffering from ischemic heart disease is considered to be an important medical issue, because complex pathophysiological factors and poor prognosis are involved in this context. Evaluation of the interaction of cellular mechanisms of diabetes with IR injury and cardioprotective mechanisms has attracted a lot of attention in recent years [3]. Diabetes mellitus is one of the major problems that is obviously increasing worldwide. Hyperglycemia induced by diabetes is often associated with complications such as cardiovascular disease, renal failure, retinopathy and neuropathy [21]. Hyperglycemia negatively affects cardiomyocyte structure, intracellular signaling and gene expression. Therefore, diabetes may interrupt the insulin-related transportation system of glucose in myocardium in the ischemic condition [22]. Studies have revealed that risk of myocardial infarction and heart disease increases 2–3 times in diabetic patients. As a result, diabetes is a pathologic state that disrupts the intracellular signaling cascade involved in myocardial protection [23].

Apoptosis is involved in the pathophysiology of myocardial reperfusion injury in healthy and diabetic conditions

Lessons from apoptosis

Apoptosis is a process that needs energy, and which occurs through chromatin accumulation, DNA fragmentation and apoptotic body formation with no changes in membrane stability and no inflammatory response [24]. Under physiological conditions, apoptosis eliminates damaged or unnecessary cells and keeps balance between cell proliferation and cell death. However, in the IR condition it can induce cardiomyocyte death and reduction of cardiac function [25]. Previous studies have revealed that myocardial ischemia and reperfusion causes apoptotic cell death, but it does not take place until reperfusion follows ischemia [26]. The importance of apoptosis in cell death following reperfusion injury has been shown well in animal models. Although reperfusion provides oxygen and glucose essential for the life of cells, it can supply energy for the apoptosis process, as well [27]. In isolated heart, P53 mRNA increases during reperfusion, while after ischemia, P53 is distributed sparsely. This indicates that ischemia by itself is not sufficient to induce apoptosis. The absence of apoptosis during ischemia indicates that cell ATP deposits are depleted during ischemia and in turn it prevents apoptosis occurrence [28].

On the other hand, apoptosis in body organs would occur because of diabetes [29]. Several studies have shown that diabetes causes myocardial apoptosis in human and animal models [30]. Myocardial apoptosis in the diabetic condition may cause greater cardiac dysfunction and dysrhythmia [31]. Additionally, diabetic mice treated with insulin have shown decreases in blood glucose level and also prevention of myocardial apoptosis, 3 days after insulin administration. To sum up, myocardial apoptosis is directly related to diabetic pathogenesis [30]. Mitochondria have an important role in diabetes, cardioprotection, as well as IR injury [32, 33]. Under different pro-apoptotic conditions like oxidative stress elevation in diabetes, mitochondria take part in the occurrence of apoptosis. The relation between ROS generation and cytochrome-c release and consequent activation of caspase 3 in diabetes and hyperglycemia, indicates that ROS is generated from high levels of glucose and causes apoptosis initiation. Partial inhibition of hyperglycemia by insulin supplements almost prevents myocardial structural disorders and myocyte death. Therefore, apoptosis is an important cause of diabetic cardiomyopathy which itself is created as a result of abnormal cellular metabolism and gene expression in the primary stages of hyperglycemia [33]. IR induces apoptosis in multiple ways, including increase in BAX level, decrease in Bcl-2 level, activation of TNF-α or FAS receptors, activation of caspases, activation of the P_{53} pathway, cytochrome-c and C-JUN kinase, and activation of neutrophils or macrophages [34]. Apoptosis has two main pathways leading to activation of caspases, including extrinsic and intrinsic pathways. In the extrinsic pathway, cell surface receptors participate in caspase 8 activation; in the intrinsic pathway, however, the mitochondria are involved in activation of caspases [35, 36]. All apoptotic pathways converge on the activation of caspases (Fig. 1).

Initiation of the extrinsic apoptotic pathway involved in IR is through TNF receptor-linked signals. This pathway causes activation of FADD by recruiting membrane-attached death receptors like FAS. FADD forms a complex with procaspase 8, which in turn activates caspase 8 and finally procaspase 3. Nevertheless, activation of the intrinsic pathway of apoptosis occurs earlier than the extrinsic pathway [37]. Mitochondria have an essential role in the controlling of cell death in most vertebrates; the mitochondrial pathway is the main pathway of apoptosis [38]. In the heart, mitochondria form 30 % of cardiomyocyte volume and provide more than 90 % of ATP essential for cardiac function [39]. During myocardial ischemia, reduction or loss of blood flow to the heart prevents oxygen, glucose and fatty acids supply to the myocardium. It also causes intracellular Ca overload, reduction in intracellular pH, and finally mitochondrial dysfunction [40].

Mitochondrial permeability transition pores and ATP-sensitive potassium channels

The mitochondrial permeability transition pore (MPTP) is a non-selective large conductance channel in the mitochondrial inner membrane, for which several proteins have been suggested to be the subunits. A change in the role of mitochondria from ATP generators to apoptosis inducers takes place by opening of the MPTP in the inner membrane of mitochondria. MPTP is normally closed, but under specific conditions of cell stress, such as reperfusion, it would be opened [41, 42]. This means that under physiologically normal conditions, the mitochondrial inner membrane is impermeable to all agents except for some ions and metabolites. However, under stress insults, opening of the MPTP in the inner membrane of mitochondria leads to the generation of osmotic colloidal pressure in mitochondria and mitochondrial inflation and finally rupture of the mitochondrial outer membrane, leaving the mitochondrial inner membrane intact and non-damaged because of cristae [41]. Important parameters involved in mitochondrial dysfunction during reperfusion are reduction of mitochondrial membrane potential, loss of ATP generating ability, overproduction of ROS and consequent opening of MPTP and release of pro-apoptotic

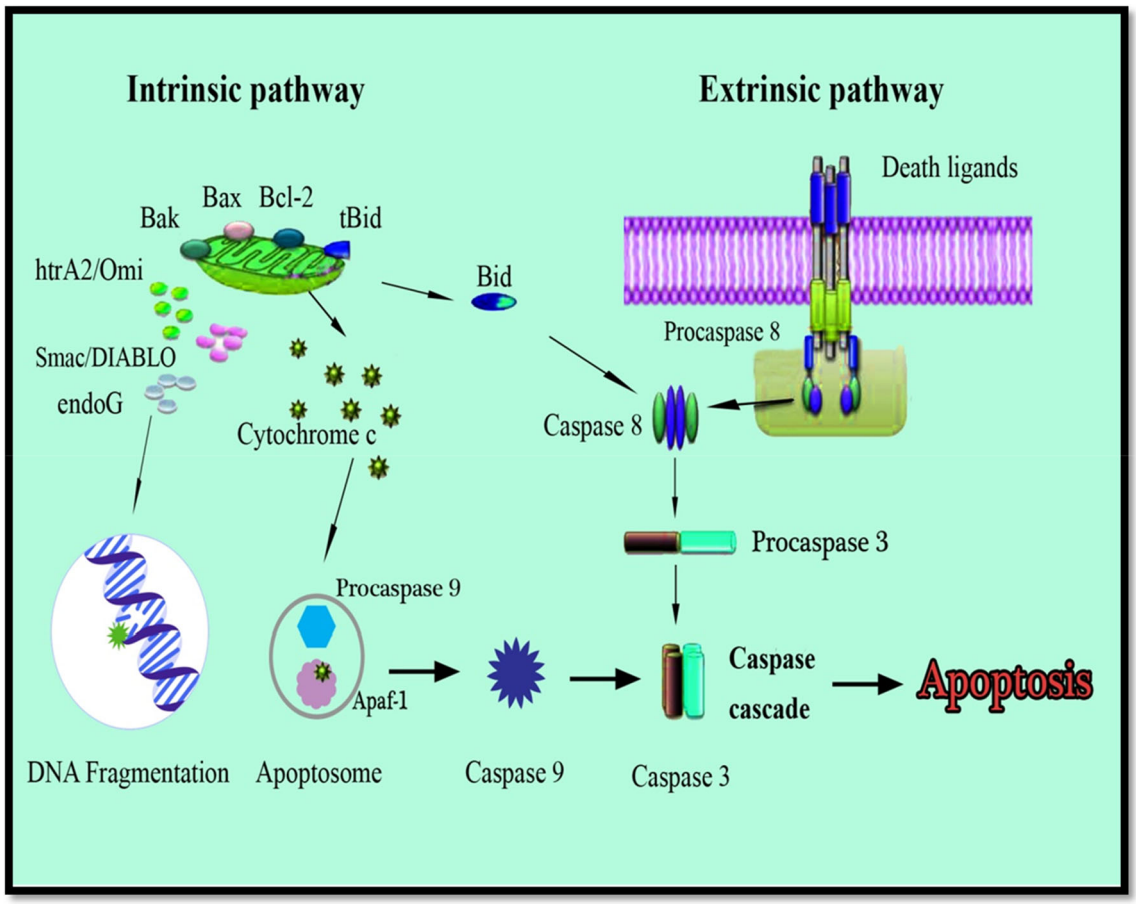

Fig. 1 Extrinsic and intrinsic pathways of apoptosis and their cross-talk through the cleavage of the BH3 only protein BID (for more information, see the text)

signaling factors that cause activation of caspase signaling cascades [25].

Researchers have also revealed that mitochondrial ATP-sensitive potassium (mitoK$_{ATP}$) channels in the outer membrane of the mitochondrion play a protective role in primary stages of apoptosis by maintaining the integrity of the mitochondrion [18, 43]. The mitoK$_{ATP}$ channel regulates the mitochondrial matrix volume. It has been shown that activation of mitoK$_{ATP}$ by diasoxide decreases the severity of IR injury [44]. Activation of mitoK$_{ATP}$ also causes reduction of both mitochondrial membrane potential and Ca uptake by mitochondria during ischemia. It has been suggested that MPTP and the mitoK$_{ATP}$ channel are two end effectors of cardioprotective pathways [45], both of which are dysfunctional during diabetes [18, 46] (see below and Fig. 2).

Pro-apoptotic mediators

On the mitochondrial surface, mitochondrial outer membrane permeability (MOMP) and MPTP are regulated by means of two different classes of the Bcl-2 family. Anti-apoptotic members include Bcl-2, Bcl-xl, Bcl-w, Mcl-1, and A$_1$/BFI-1, which inhibit release of mitochondrial apoptogenic factors following cell death triggers. Pro-apoptotic members are BID, BAX, BAK, BCL-Xs, BAD, BID, BIK, BIM, Hrk, and BOK, which may play important roles in cell death during reperfusion injury. BID and BAX are mainly located in the cytosol; following death stimuli, they transfer into mitochondria and cause cytochrome-c release [25, 47].

Bcl-2 family members make the mitochondrial outer membrane more permeable to release the pro-apoptotic mediators from the inter-membrane space of mitochondria to the cytosol. The released pro-apoptotic factors include cytochrome-c, smac/DIABLO, htrA$_2$/Omi protease and endoG. Smac/DIABLO and htrA$_2$/Omi proteases cause activation of caspases by prevention of caspase-inhibiting protein production. The endoG transfers into the nucleus and mediates DNA fragmentation [48].

Apoptosis regulation is highly related to the proportion of anti-apoptotic proteins to pro-apoptotic ones. BAX homodimers are involved in formation and opening of MPTP, while Bcl-2 prevents pore formation. This implies that

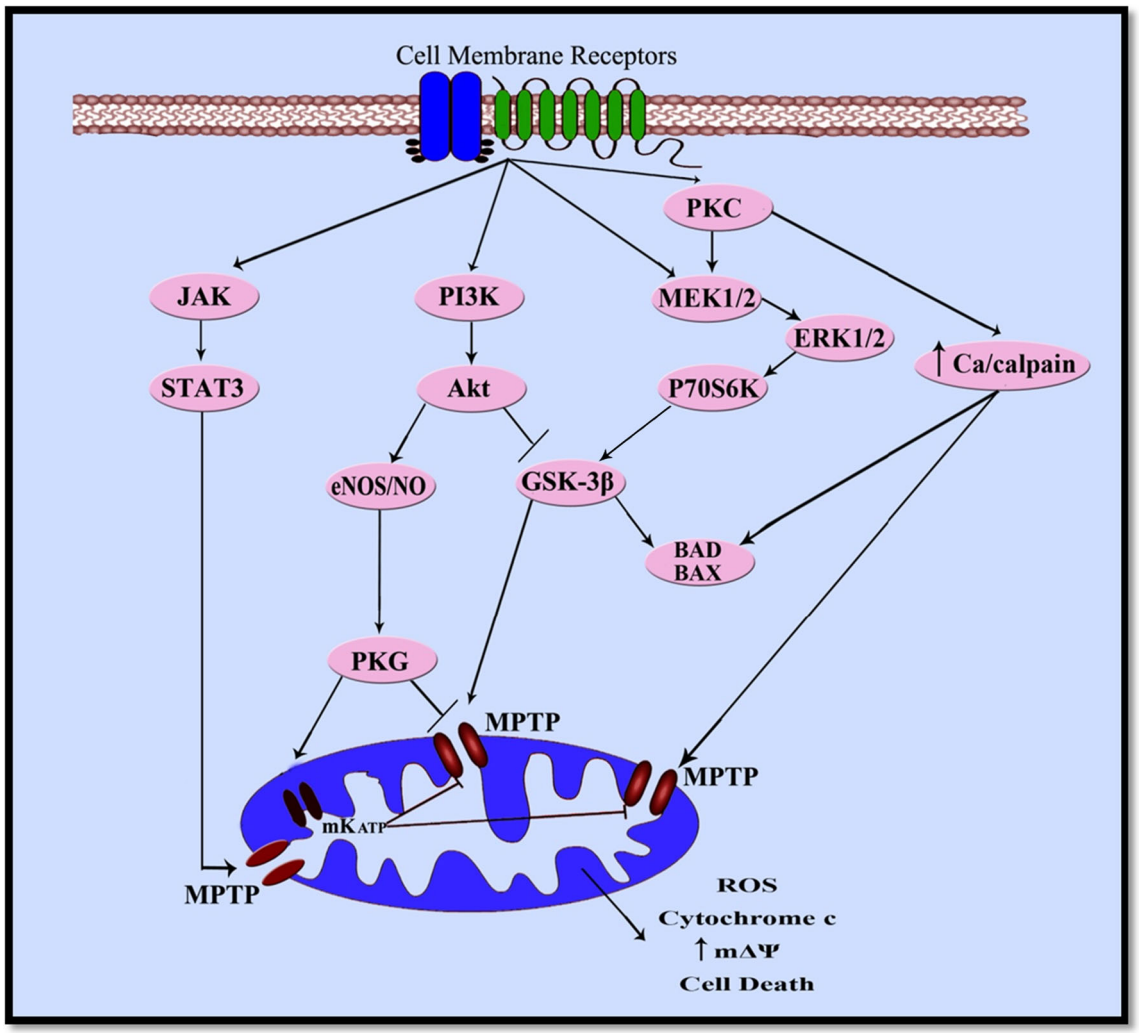

Fig. 2 Signaling pathways converging on and affecting MPTP opening and mitochondrial K_{ATP} channels. *Arrows* indicate activation; *crossed-end lines* indicate inhibition (see the text for more details)

these proteins apply their effects by MPTP regulation [47]. It has been observed that over-expression of Bcl-2 reduces IR injury. Furthermore, Bcl-2 decreases acidification and ATP deprivation during ischemia. This phenomenon may be the first stage of cardioprotection by Bcl-2 in the IR condition [49]. The anti-apoptotic role of Bcl-2 in the myocardium has been proved by the fact that genetic correction of the myocardium by the Bcl-2 anti-apoptotic gene protects the myocardium during ischemia–reperfusion. It is also evident that increases in the proportion of Bcl-2 to BAX can prevent myocardial apoptosis progress after ischemia and reperfusion [50, 51]. Bcl-2 acts as a scavenger of free radicals in myocardial and neural tissues and in this way it can play a protective role against IR injury. It has also been confirmed that the relation between Bcl-2 and calcium pumps in mitochondrial membrane, endoplasmic reticulum and nuclear membranes prevents Ca excess during IR. In general, the important role of Bcl-2 is regulation of MPTP activity and prevention of mitochondrial Ca overload that results in reduced cell damage induced by apoptotic or necrotic pathways [52]. Cell survival in the presence of a death stimulus like IR is determined by Bcl-2 proteins signaling through induction or inhibition of MOMP [53]. IR causes activation of the caspase cascade, leading to caspase 3 activation [54]. Activation of caspases is also due to cytochrome-c release. Cytochrome-c is released through outer membrane channels, namely mitochondrial apoptosis-induced channels (MACs). MAC activity that has been observed before mitochondrial depolarization is strictly under the control of Bcl-2 proteins, and can be the initiator of apoptotic mediator release from mitochondria in order to induce cell death. BAX oligomers have been known to be components of these channels [53]. This shows that both intrinsic and extrinsic pathways of apoptosis are activated in diabetes [55]. Two pathways of apoptosis in diabetes can have cross-talk; BID breaks down

and changes to t-BID with caspase 8. T-BID can cause inhibition of Bcl-2 proteins and also activation of BAK and BAX [56]. The experiments have revealed that caspase 3 activity is increased by caspases 8 and 9 during diabetes [55]. The intrinsic pathway of apoptosis also has cross-talk with caspases, pro-apoptotic members of Bcl-2 family, cytochrome-c, and apoptosis inhibitory factor (AIF) [57] (Fig. 1).

Signaling pathways leading to MPTP

MPTP opening during reperfusion is controlled by several out-mitochondrial kinases (Fig. 2). One important kinase in this group is glycogen synthase kinase-3beta (GSK-3β). GSK-3β, as an important regulator of cell function, has essential roles in diabetes, inflammation, cancer, Alzheimer's disease and ischemic insults. Additionally, it acts as a negative regulator in myocardial hypertrophy and a key enzyme in response to myocardial ischemia–reperfusion injury. It has been observed that phosphorylation of GSK-3β decreases the myocardial infarcted zone size through prevention of MPTP opening [58, 59]. GSK-3β activity is regulated via phosphorylation by other proteins. In undamaged cells, GSK-3β is active and causes phosphorylation of different substrates. GSK-3β phosphorylation occurs through the activation of different protein kinases including PI3K/Akt, P90RSK, PKA, PKC, P70S6K, and some isoforms of MAPK which all inactivate it, leading to an anti-apoptotic effect [44]. The phosphorylation of GSK-3β prevents MPTP opening, leading to a cardioprotection phenotype [17]. As discussed below, the phosphorylated levels of GSK-3β are reduced in both types of diabetes, as a result of failure in the activation of its upstream activators and kinases. Therefore, the active and dephosphorylated forms of GSK-3β would be high in diabetic conditions, which may lead to further opening of MPTP during IR injury in diabetic conditions. In addition, the activity of other kinases like PI3K/Akt, ERK1/2, JAK/STAT and PKC are negatively affected by diabetes (see below).

Mitogen activated protein kinases (MAPK) take part in different cell functions like growth and proliferation [60, 61]. Three major classes of MAP kinases that have been studied widely in the heart are ERK1/ERK2, JNK2, JNK1, and P38 kinases, which have two (α and β) isoforms. The alpha isoform acts as a pro-apoptotic factor, and the β isoform acts as an anti-apoptotic factor in ventricular myocytes. A fourth member of the MAP kinase family, named BMK_1 or ERK_5, has been recently recognized in cardiac tissue. Activation of ERK_1/ERK_2 and BMK_1 causes protection against apoptosis under ischemic conditions [62]. In IR conditions, ERK_1/ERK_2 are activated in response to the stimulation of tyrosine kinase and G-protein-coupled receptors and can mediate cell protection [63]. It has also been reported that ERK primes the GSK-3β for phosphorylation and inactivation by P70STK [44].

The phosphatidyl inositol signaling pathway (PI3K/Akt pathway) is a negative feedback regulator or a compensatory mechanism that decreases both the inflammatory and apoptotic responses induced by death inducers: thus in IR injury it can cause myocardial protection against apoptosis [64]. This pathway becomes active in response to activation of a wide range of receptors like growth factors and G-protein-coupled receptors. This pathway plays its role by phosphorylation (or inactivation) of different substrates like GSK-3β, apoptotic proteins including BAD, BAX, BIM, P53, caspases, and PKC [24]. It has also been proved that the PI3K/Akt signaling pathway prevents cardiomyocyte apoptosis and IR injury through modulation of TLR_4 signaling [65] (Fig. 2).

Diabetes increases oxidative stress and intracellular Ca concentration because of hyperglycemia, and MPTP opening occurs due to oxidative stress and Ca overload during diabetes [66]. Cytochrome-c release from mitochondria has been observed both in diabetic myocardium and in cultured myoblasts with hyperglycemic medium, and it has been proved that mitochondrial dysfunction in diabetic tissues is one of the causes of diabetic damage [33]. Studies have revealed that both types of diabetes may induce apoptosis through several pathways. According to studies on STZ-induced type 1 diabetic animals, myocardial apoptosis was significantly increased compared to the control group and the mitochondria have an important effect on apoptosis in myocardial cells, because the mitochondrial cytochrome-c release causes caspase-3 activation and then cell death [33]. It has been reported that the intracellular protein kinases and signaling pathways are affected by diabetic situations (Fig. 3). Although some studies have demonstrated that an increase in GSK-3β activity was not observed in type-1 diabetes models [23], the other studies showed that GSK-3β activity is also increased in the primary stages after streptozocin injection, leading to apoptosis [30]. Activation of caspase 3 in GSK-3β signaling causes myocardial apoptosis and it shows that GSK-3β has an important role in myocardial apoptosis in diabetic conditions [67]. Furthermore, it has been observed that caspase-3 activity increases in db/db mice, as a model of type 2 diabetes. Generally, both internal and external apoptosis pathways are involved in type 2 diabetes, resulting in increased myocardial apoptosis [55]. In transgenic rats (GK diabetic rats) a defect in AMPK activity occurs, resulting in cell death and cardiac dysfunction [58]. It has also been shown that the PI3K/Akt signaling pathway is impaired in type 2 diabetic rats by mechanisms that are related to endoplasmic reticulum stress. As a result, active GSK-3β protein levels and the opening of the MPTP are increased in diabetic circumstances. In addition, in

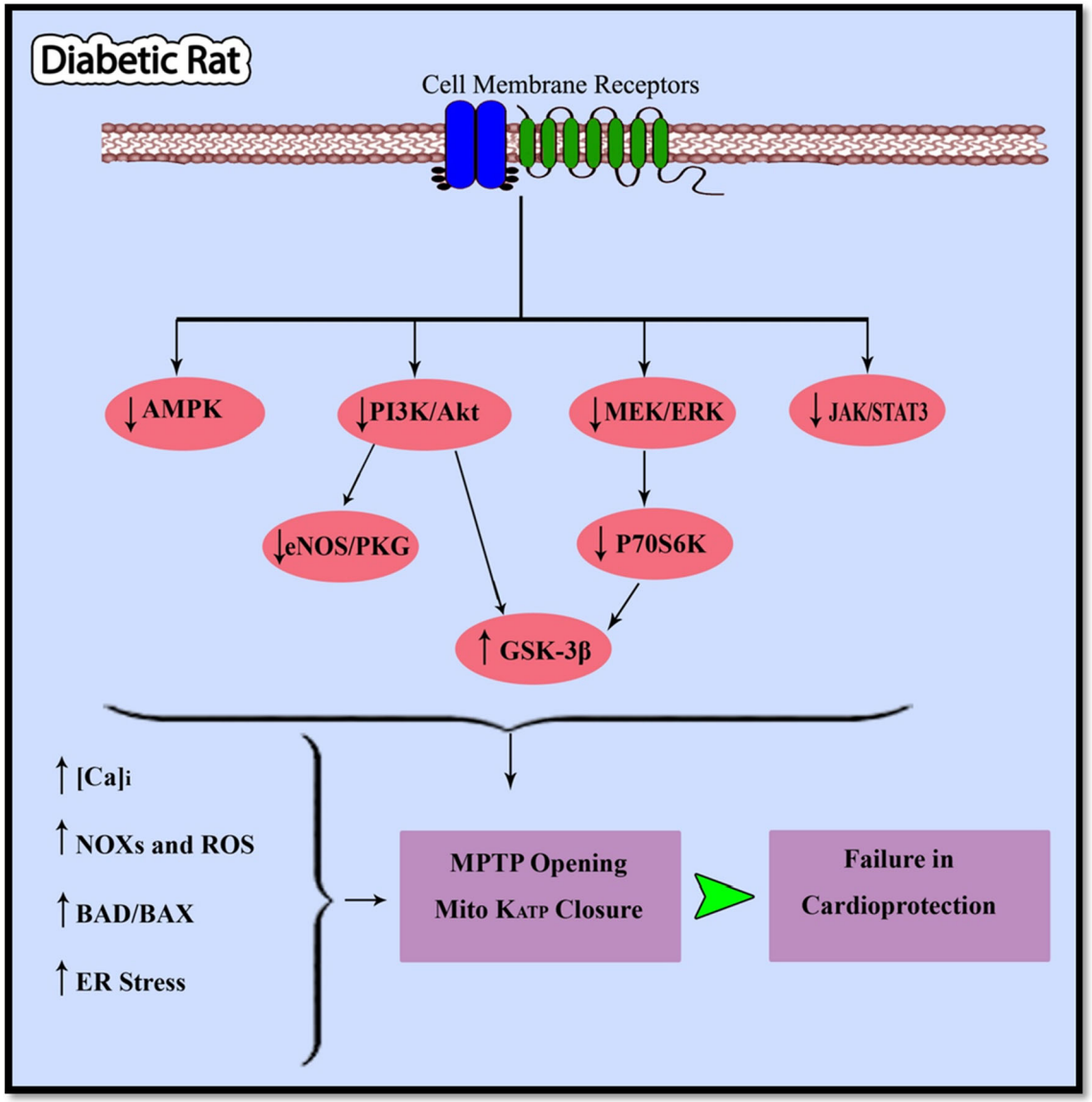

Fig. 3 Alterations of signaling pathways leading to the failure of cardioprotection in diabetic rat (for more information, please see the text)

obese rats, diabetes increases ROS production and PKC activation, leading to the induction of apoptosis through the increased activity of GSK-3β and the amount of NADPH [23].

Previous studies have shown that phosphorylation of PI3K/Akt, ERK_1/ERK_2, $JAK/STAT_3$ and $MEK_1/_2$ by pharmacological agents during the 1st minute of reperfusion after ischemia can prevent myocardial apoptosis [68]. Diabetes may inactivate these intracellular signaling kinases, or reduce their activity, by which it can increase the cardiac ischemic injuries and inhibit cardioprotection (Fig. 3). Activation of PI3K/Akt and ERK_1/ERK_2 during reperfusion induces the reperfusion induced salvage kinase (RISK) pathway [69]. The RISK pathway is used to describe the activation of survival kinases by protective agonists such as adenosine, erythropoietin, anesthetics and insulin. Administration of these agonists at the onset of reperfusion causes rapid activation of PI3K and ERK and finally cardioprotection through inhibition of MPTP opening [44].

There is also proof that cardiac ischemia increases mitochondrial Ca concentration and causes Ca-dependent cell death. When Ca uptake by mitochondria increases during ischemia (which is also seen in diabetic situations because of increased intracellular Ca concentrations), water enters into mitochondria due to osmotic pressure and causes mitochondrial matrix expansion and inner membrane potential reduction. These alterations reduce the internal driving force and of Ca current toward mitochondria during IR [70, 71]. Calpain (a Ca-dependent protein) is a mediator

responsible for the impaired cardiac contractile force during ischemic injury through proteolysis of structural proteins. Studies have revealed that calpain causes BAX fragmentation and its transformation into an 18-kDa BID and also BID fragmentation and production of its active form, α, which causes cytochrome-c release. Calpain also causes Bcl-xl fragmentation and changes it into a pro-apoptotic form. It is evident that calpain activity appears in the first 15 min of reperfusion. Calpain is controlled by an inhibitor named calpastatin. It has been recently reported that the inhibitory action of calpastatin decreases in the ischemic heart [72].

It is also worth noting that β1 and β2 adrenoreceptors (AR) are both expressed in the myocardium, and they have differential effects on apoptosis. Stimulation of β1-AR has pro-apoptotic effect whereas β2-AR stimulation is anti-apoptotic [73]. β1-stimulated apoptosis is mediated by activation of PKA and Ca entry from voltage-dependent Ca channels. β2-AR stimulation leads to increased PI3K activity and its downstream target, Akt/PKB. Inhibition of Gi coupling to PI3K or PI3K itself prevents the protective effect of β2-AR stimulation [74]. Beta-AR-stimulated apoptosis involves the mitochondrial pathway. Inhibition of mitochondrial transition pore opening or caspase activation decreases β-AR-stimulated apoptosis. Reactive oxygen species production is also involved in this process, since superoxide dismutase/catalase mimetics or catalase over-expression prevent β-AR-stimulated apoptosis [75]. Evidence from experimental models of diabetes reveals a decreased inotropic response to β-AR stimulation [76] which is due to reduction in the number of myocardial β-ARs and defects in β-AR signaling [77].

Another anti-apoptotic protein kinase, adenosine monophosphate kinase (AMPK) is also involved in glucose and fatty acid (FA) homeostasis and in body metabolism control. It has been shown that activation of this kinase is a strong route for protection of the myocardium from IR injury [78]. Modulation of AMPK activity in the diabetic heart improved cardiac function and prevented the diabetic heart from IR injury. During IR injury, AMPK is activated quickly and stimulates glucose uptake and glycolysis during ischemia in order to supply the ATP for cardiac function. AMPK, as a cardiovascular protective kinase, can phosphorylate the intracellular proteins and prevent MPTP opening. It also causes GSK-3β phosphorylation and prevention of mitochondrial ROS generation [79].

Insulin resistance and its association with cardioprotection

Diabetes mellitus has been classified into 2 types. Type 1 diabetes is characterized by autoimmune destruction of pancreatic beta cells and reduction in insulin production. But the main characteristic of type 2 diabetes is insulin resistance [80]. Insulin resistance means a deficiency in glucose and lipid metabolism. Insulin resistance and endothelial dysfunction are risk factors for cardiovascular disease [81]. As discussed earlier, diabetes increases heart disease as the cause of diabetic cardiomyopathy. The pathogenesis of diabetic cardiomyopathy is multi-factorial, but changes in energy metabolism, such as glucose and fatty acid metabolism are considered to be helpful mechanisms [80].

Insulin is an anabolic hormone that helps glucose oxidation and therefore provides ATP [81]. Insulin has direct effects on the cardiac FA metabolism, can directly induce glucose uptake through GLUT4 translocation, and indirectly causes the inhibition of lipolysis in adipose tissue [80]. In normal physiological conditions, insulin regulates glucose metabolism through the PI3K/Akt pathway. Insulin binds to its receptor and causes the phosphorylation of IRS. These substrates have SH2 domains and can provide sites for other signaling protein molecules such as PI3K signaling molecules. Activated PI3K leads to the production of PIP2 and PIP3, and Akt will eventually be phosphorylated. Then, activated Akt can also phosphorylate GSK3β and inactivate it [82, 83]. In general, several factors such as bradykinin, cardiotrophin-1, insulin and TGFβ-1 have cardioprotective effects through ERK, PI3K signaling pathways [84]. It has been shown that insulin has a direct effect on the reduction of IR injury through cell-survival signaling pathways. Insulin has anti-apoptotic effects through the activation of cell-survival pathways. Tyrosine kinase, PI3K and S6K signaling are essential to these cardioprotective effects of insulin [85]. In addition, insulin induces NO production through the PI3K/Akt pathway and eNOS phosphorylation, thereby reducing apoptosis and protecting the heart against IR injury [86].

As we discussed, under pathophysiological conditions such as type 2 diabetes, insulin resistance occurs [81]. A 2-fold increase in GSK-3β activity has been proved in an insulin resistant condition compared with the normal condition. This means that hyperinsulinemia and hyperglycemia leads to activation of GSK-3β and will be followed by inhibition of insulin signaling in the myocardium. Thus, activation of GSK-3β plays an important role in diabetic cardiomyopathy [82]. It has been demonstrated that deficiency in the Akt and then eNOS activity lead to insulin resistance and thus cause myocardial injury [86]. Insulin resistance can lead to impaired mitochondrial function by reducing myocardial AMPK activity, which decreases FA oxidation [82]. Because of insulin cardioprotective activity, we can conclude that insulin can be used as a therapeutic target to increase myocardial strength against IR-induced apoptosis [85].

NADPH oxidases (NOXs), endoplasmic reticulum stress and their association with cardioprotection

Oxidative stress is involved in the physiopathology of myocardial IR injury. Low levels of oxidants can cause myocardial protection, but high levels of ROS can be harmful and cause the death of cardiomyocytes [87]. ROS induces apoptosis through mitochondrial depolarization and increases the duration of the opening of MPTP. Therefore, reducing oxidative stress is an important factor in reducing myocardial IR injury [88]. Although many sources of ROS have been discovered, evidence has shown NADPH oxidase (NOX) to be the main producer of ROS during hypoxia or ischemia [87]. The NOX family is composed of 7 members, including NOX1–NOX5, Duox1 and Duox2 that result in the transmission of electrons from biological membranes to produce ROS. Among the sources of ROS, NOXs 1, 2, and 4 are expressed in cardiac tissue. NOX2 produces large amounts of ROS, leading to myocardial damage, but NOX1 produces fewer amounts of ROS and regulates intracellular pathways. The difference between ROS production by NOX1 and NOX2 can activate different intracellular signaling pathways [87]. In addition, NOX2 and NOX4 contribute to the opening of MPTP during reperfusion [89].

ROS produced by NOX1 and NOX2 inhibits ERK1/2 phosphorylation. NOX2 elimination induces STAT3 phosphorylation in the SAFE (survivor activating factor enhancement) signaling pathway of cardioprotection, while NOX1 elimination increases Akt phosphorylation in the RISK signaling pathway of cardioprotection [87]. It has been found that NOXs also play an important role in diabetic cardiomyopathy. As we noted, the phosphorylation of Akt and STAT3 is significantly reduced in the diabetic myocardium. Increased myocardial oxidative stress and reduced myocardial antioxidant capacity, due to deficiencies in the GSK-3β, Akt and STAT3 signaling pathways, can make a diabetic heart prone to IR injury-induced apoptosis [90]. Some studies have shown that NOX2 and NOX4 expression increases in type 1 diabetes, but type 2 diabetes increases NOX4 expression in the myocardium, resulting in increased oxidative stress; although, some studies have shown that diabetes has no effect on NOX4 expression [91]. Thus, the oxidative stress and apoptosis during IR injury in the diabetic condition can be prevented by treatments targeting the activation of Akt and STAT3 signaling [90].

Endoplasmic reticulum (ER) is a central organelle in the cell and has an important role in lipid biosynthesis, calcium homeostasis, protein folding and maturation [92]. ER stress may lead to apoptosis through three pathways. The first apoptotic pathway involves the JNK pathway in which activation of caspases occurs through the mitochondrial pathway or through Apaf-1. The second apoptotic pathway involves the activation of caspase 12 in rodents. Caspase 12 translocates from the ER to the cytosol and directly breaks down procaspase 3 without the involvement of the mitochondria. Among the factors that lead to caspase 12 activation in ER-stressed cells are m-calpain and caspase 7. The third apoptotic pathway is mediated by activating the transcription of CHOP/GADD153, which inhibits Bcl2 and Bcl-xl expression, and thus produces ROS [92]. The role of ER stress in diabetic cardiomyopathy has been reported recently. Since apoptosis plays an important role in diabetic cardiomyopathy and ER stress also leads to apoptosis under diabetic conditions, ER stress is an important factor in the pathogenesis of diabetic cardiomyopathy [92]. In addition, oxidative stress as an ER stress activator, has an important role in diabetic cardiomyopathy.

Adenosine monophosphate kinase, as mentioned above, is an important sensor of cellular energy and regulates lipid and glucose metabolism through up-regulation of related genes. It has been proved that there is cross-talk between AMPK and ER stress. Many studies have shown that ER stress interferes with insulin effects and causes insulin resistance and diabetes [93]. Because of the important role of ER stress in the development of diabetic cardiomyopathy, ER stress inhibitors may be a new target for drug discovery in diabetic cardiomyopathy [92].

Cardiac fibroblasts and their association with cardioprotection

Cardiac fibroblasts (CFs) contribute to myocardial structure and function, surround cardiac myocytes, and are responsible for the production of extracellular matrix proteins [94]. CFs are involved in myocardial extracellular matrix production and growth factor secretion. Myocardial IR injury may also induce activation of pro-apoptotic elements which finally lead to CFs apoptosis [95]. CFs cause cardioprotective effects by producing some factors such as IL-33 and FGF1/2 after myocardial ischemia and reperfusion. It has been reported that TIMP-1, which is secreted from CFs, can protect cardiomyocytes from IR injury by mediating ERK1/2 and PI3K/Akt signaling pathways [96].

On the other hand, it is known that the diabetic condition significantly reduces the number of CFs. The cause of decrease in the number of CFs in the diabetic heart is due to apoptosis in CFs and defects in collagen synthesis [55]. Physiological concentrations of insulin cause collagen synthesis and DNA stimulation in CFs. Inhibition of PI3K/Akt in insulin-resistant and diabetic circumstances inhibits insulin-mediated fibroblast DNA synthesis. This suggests that insulin increases cardiomyocyte growth and increased DNA synthesis in CFs by different intracellular mechanisms. Hyperglycemia has no effect on cardiomyocyte

protein synthesis, but instead stimulates the expression of fibronectin and TGFβ1 in CFs. Thus, it has been shown that hyperglycemia and insulin-resistance increase TGFβ1 expression in cardiac fibroblasts and leads to myocardial dysfunction [97].

The role of apoptosis in the failure of ischemic preconditioning and ischemic postconditioning in treatment of diabetic heart

It is important to understand whether the induction of apoptosis during diabetes reduces the efficacy of therapeutic interventions (IPreC and IPostC) in protecting the diabetic heart against ischemia–reperfusion injury. It seems that the apoptotic changes caused by diabetes, in addition to IR-induced apoptotic alterations, intensifies the problem. Diabetes often causes modification in myocardial response to IR and its protective interventions, IPostC and IPreC by impairing intracellular signaling pathways and mediators responsible for cell resistance against cell death. Therefore, diabetic patients have poor prognosis after acute myocardial infarction [98].

In several studies it has been shown that IPreC causes protection against myocardial infarction by inducing PI3K/Akt, PKC, and JAK/STAT pathways. Among these pathways, activation of the PI3K/Akt pathway has an important role in myocardial protection from IR injury. This pathway causes myocardial protection through different substrates like anti-apoptotic proteins, eNOS and PKC. In recent studies on GSK-3β that is phosphorylated by Akt and MAPK/ERK, researchers have found that IPreC and IPostC induce their cardioprotective effects by phosphorylation and inactivation of GSK-3β, leading to the inhibition of MPTP [99, 100] (Fig. 4).

Pre-clinical and clinical studies have revealed that the cardioprotective effects of IPreC are diminished both in human and animal models with a diabetic condition. According to the primary data, effectiveness of IPostC is also decreased in diabetic rats [3, 32, 59]. Diabetic patients exhibit a higher incidence of congestive heart failure and sudden cardiac death, and the post-MI prognosis is also worse in this population.

The mechanisms of attenuation of the cardioprotection effect of IPreC and IPostC during diabetes are not well understood. During diabetes, some abnormalities in the physiology of the myocardium, including diabetes-induced cardiomyopathy and myocardial dysfunction, arrhythmias, reduced blood flow and metabolic disturbances develop, and these alterations may interact, to some extent, with protective interventions. Furthermore, sub-cellular changes in diabetic tissues can play important roles in this case. In diabetic circumstances, hyperglycemia, increased production of fatty acids, excessive generation of free radicals and

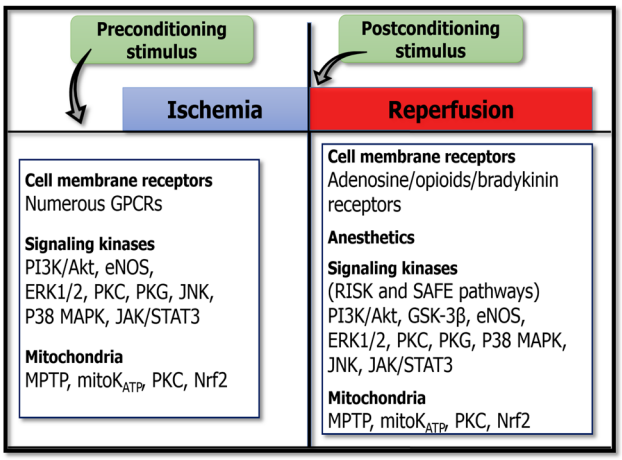

Fig. 4 Overview of the cardioprotection mechanisms by conditioning strategies

lipid peroxidation, impaired production of nitric oxide, impaired activation of survival protein kinases and mitochondrial dysfunction, as well as other abnormalities could all be candidate mechanisms for the interaction of diabetes with cardioprotection in IR injury [2, 4]. In addition, coronary blood flow to an ischemic myocardium is adversely affected during diabetes and hyperglycemia. Hyperglycemia decreases coronary collateral blood flow, impairs coronary microcirculatory responses to ischemia, and causes endothelial dysfunction, in part by increasing inflammatory mediators and reactive oxygen species and decreasing nitric oxide availability [2]. In addition, the diabetic myocardium exhibits a variety of abnormalities in sarcolemmal ion transport, including depression of Na–H and Na–Ca exchange processes and inhibition of Ca ATPase and Na–K ATPase. Sarcoplasmic reticular function also appears to be defective in the diabetic myocardium, with depressed ATP-dependent Ca transport and Ca-stimulated ATPase activity via a decreased ATP synthesis and electron transport chain in the mitochondria. Thus, the altered function of mitochondria may lead to the dysfunction of various ion channels and exchange processes, and this could play a role in apoptotic processes in myocardial IR injury [83]. In some studies, it has been proved that diabetes in its primary stages may cause increased myocardial resistance against IR injury, but in the developed stages of diabetes this resistance diminishes and the heart becomes more vulnerable to IR injury [101]. It has been reported that in the first 2 weeks of diabetes the heart is more resistant against IR injury, which is attributed to VEGF activation, eNOS over-expression, NO generation and the shortage of oxidative stress; however, cardiac protection after 6–8 weeks has not been observed [3].

According to the experimental reports, a two-fold increase in GSK-3β activity has been proved in the diabetic

condition compared with the normal condition. Increased insulin resistance could be the main reason for myocardial IR injury in the type-2 diabetic condition. Hyperglycemia and hyperinsulinemia hyper-activate GSK-3β. Activated GSK-3β can prevent insulin signaling and glucose consumption through IRS-1 phosphorylation [98]. Since the STAT-3 signaling pathway plays an important role in the cardioprotection induced by IPreC and IPostC, STAT-3 down-regulation may be one of the mechanisms that inhibits the protective effects of conditioning in diabetic rats [98] (Fig. 3). GSK-3β activity drives MPTP opening in response to increased ROS and Ca concentration, which are features of diabetic states, but conditioning significantly increases the threshold of MPTP opening in rat heart's mitochondria [102]. It has been proved that the cardioprotective effect of conditioning is reduced 6 weeks after induction of diabetes: the main factor responsible for myocardial ischemia–reperfusion injury is MPTP opening [103]. MPTP opening is one of the main underlying mechanisms in diabetes-induced apoptosis.

Sarcolemmal K_{ATP} channels in cardiomyocytes may help the heart to adapt to stress. Moreover, opening of mitochondrial K_{ATP} channels has an important role in the mechanisms of cardioprotection and anti-apoptotic processes [3, 104] (Fig. 2). Diabetes causes disorders in organelles of myocytes like the sarcolemma, sarcoplasmic reticulum and mitochondria, which are related to several abnormal states in energy metabolism, reduction in the activity of Na–H and Na–Ca exchanges, decreases in sarcoplasmic reticulum Ca and Na–K pump activity. Diabetes changes the function of myocardial and vascular K_{ATP} channels and further causes reduction in the number of K_{ATP} channels: in this way it can reduce the cardioprotective effects of IPreC and IPostC [105].

NO has an important role in the conditioning mechanism [12, 46, 106]. NO metabolism is impaired in diabetes [8] and coronary NO production is also decreased after aloxan-induced diabetes in dogs. Hyperglycemia has direct effects on K_{ATP} channels, eNOS reduction and diminution of PKC activity that leads to decreases in the protective effects of protective strategies in diabetic animals. One of the causes of poor prognosis in diabetic patients with MI is a decrease in the protective effects of conditioning, due to reduced eNOS activity [12]. Diabetes as well as IR cause increased ROS production and NO reduction because of peroxynitrite generation in the heart. Furthermore, under normal conditions, NO production induced by eNOS and iNOS increases survival hemeoxygenase-1 (HO-1) expression in tissues, but with high levels of peroxynitrite in the heart (as seen in diabetes), HO-1 expression is impaired. Therefore, STZ-induced diabetes in rats decreases the HO-1 level in the myocardium during ischemia, which in turn causes increased myocardial inflammatory responses to IR injury [107].

Conclusion and future directions

In this review we discussed what apoptotic changes induced by diabetes may be added to the apoptotic alterations of IR injury that intensify the severity of tissue injury, and what happens during diabetes that reduces the efficacy of therapeutic interventions (IPostC and IPreC) to protect the heart against IR injury. Treatment of diabetic patients suffering from ischemic heart disease is considered to be an important issue, because of its complex pathophysiological features and poor prognosis. Diabetes may increase the intensity of myocardial apoptotic damage induced under the conditions of IR injury. Evaluation of the cellular mechanisms of diabetes and their interaction with IR injury and cardioprotective mechanisms have attracted a lot of attention. Apoptosis is one of the main mechanisms of this interaction. Diabetes can attenuate the cardioprotective effects of preconditioning and postconditioning in IR hearts through impeding the anti-apoptotic signals of cardioprotection. In this case, it is warranted to identify other possible molecular mechanisms responsible for diabetes interventions with cardioprotection, other than those reviewed in this article.

Of these mechanisms, one important one would be autophagy. Both autophagy and apoptosis are necessary for cell survival and are involved in various diseases such as diabetes and cardiovascular disease. Recently, cross-talk between apoptosis and autophagy has been the subject of many investigations, because of the molecular connections between these two signaling pathways [108, 109] (Fig. 5).

Autophagy as an intracellular defense factor involves the removal of damaged organelles. It has been demonstrated that increased autophagy is associated with reduced apoptosis in IR injury [110, 111]. The insulin signaling pathway may lead to autophagy inhibition through the activation of PI3K/Akt and AMPK/mTOR signaling. Thus, defects in insulin secretion (type 1 diabetes) or insulin resistance (type 2 diabetes) may alter the autophagy-induced modulation of IR injury [112]. However, little information is available on the pathophysiological role of autophagy in the pathogenesis of diabetic cardiomyopathy and IR injury. Therefore, further studies are needed to understand how the molecular mechanisms of autophagy can help or worsen cell survival or cell death in myocardial IR injury in healthy or diabetic circumstances.

Because of the cross-talk between apoptosis, autophagy and functions of organelles such as ER and mitochondria, autophagy is considered to be a new therapeutic target in the treatment of diabetes and ischemic diseases. On the

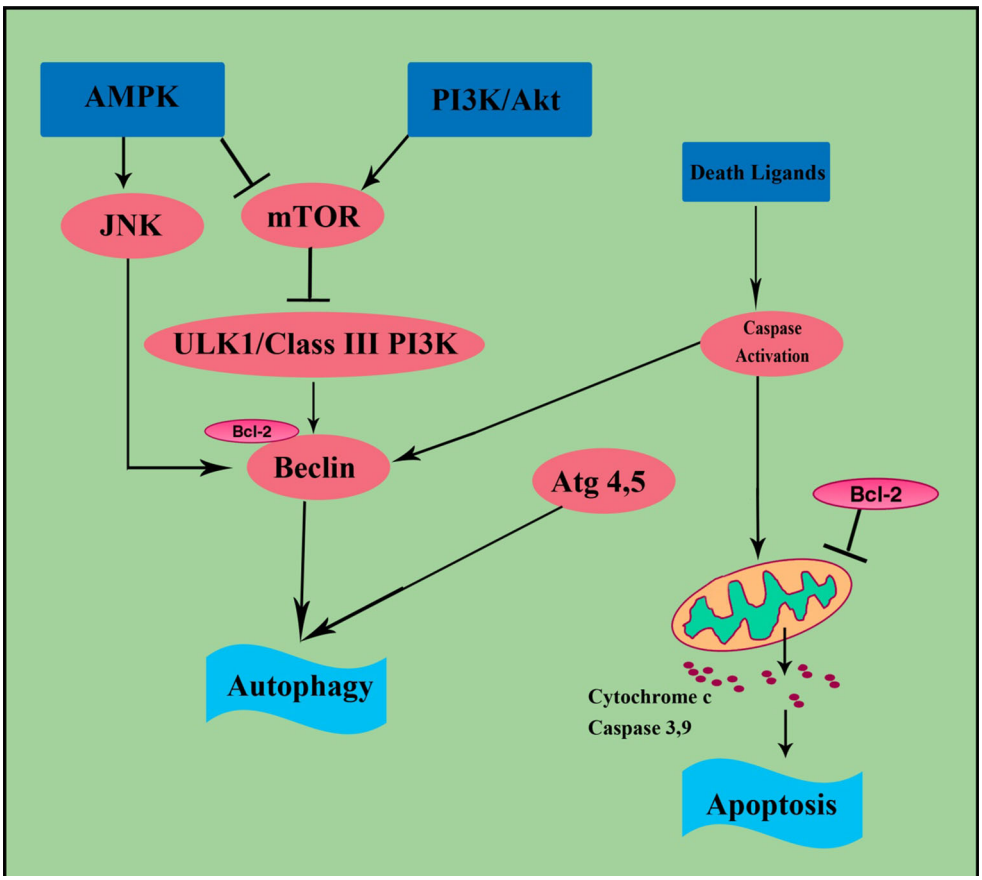

Fig. 5 An example of cross-talk between autophagy and apoptosis. Activation of AMPK restores autophagy but inhibits apoptosis

other hand, targeting the interfering mechanisms of diabetes with IR injury and verifying their crucial effectors in future studies may lead us to find out promising approaches to the treatment of diabetic hearts with IR injury.

Conflict of interest None.

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