Vessel Plus

Review

Exploitation of K_ATP channels for cardiac surgery

AlleaBelle Bradshaw[1] (iD)**, Jennifer S. Lawton[2]** (iD)

[1]Department of Surgery, MedStar Georgetown University Hospital, Washington, DC 20007, USA.
[2]Division of Cardiac Surgery, Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

Correspondence to: Jennifer S. Lawton, Division of Cardiac Surgery, Department of Surgery, Johns Hopkins University School of Medicine, 1800 Orleans St., Zayed 7107, Baltimore, MD 21287, USA. E-mail: jlawton4@jhmi.edu

Abstract

The many ways in which ATP-sensitive potassium (K_ATP) channels can be exploited for human benefit have expanded over recent decades. Especially since the early 2000s, research has improved our understanding of the components and mechanisms of K_ATP channels. They have the potential to have a prominent role in cardiac surgery. Pharmacologic and non-pharmacologic activation of K_ATP channels has been shown to be both cardioprotective and neuroprotective in early basic science and clinical studies. However, many questions remain unanswered and require further study, necessitating further basic science work and large human clinical trials. This review discusses the history and recent progress in the research relating to the use of K_ATP channels for cardiac surgery.

Keywords: K_ATP channels, cardioprotection, neuroprotection, cardiac surgery, diazoxide

INTRODUCTION

ATP-sensitive potassium (K_ATP) channels are present in many different tissues in humans, including cardiac muscle[1], vascular smooth muscle[2,3], lymphatics[4,5], liver[6,7], and pancreas[8,9]. These channels are clinically important because malfunction can lead to a variety of pathologies[10], and because they are pharmacologic targets of therapeutics[11,12]. Agents targeting these channels are already used to treat and even cure some diseases (neonatal diabetes and congenital hyperinsulinism are effectively treated with K_ATP channel inhibitors and K_ATP channel openers, respectively)[13,14], yet there is potential for further exploitation of K_ATP channels to benefit patients[15]. The goal of studying these channels is to gain information that will allow the development of new pharmacologic agents aimed at improving cardiac and neurologic outcomes after cardiac surgery.

Pathology associated with K_{ATP} channels can result from decreased or increased function, depending on the specific mutation and subunit affected. Loss of function of K_{ATP} channels results in hyperinsulinism and vascular hypercontractility[16,17], whereas gain of function leads to prolonged PR intervals, arrhythmias, and hypotension[2,3,18-20]. The pathology of Cantu syndrome (hypertrichosis-osteochondrodysplasia-cardiomegaly syndrome) results from genetic gain of function of K_{ATP} channel subunits, resulting in abnormalities including cardiac enlargement and ventricular hypertrophy, pericardial effusion, pulmonary hypertension, and hypertrichosis[11,21]. Understanding the abnormalities in Cantu syndrome is informative with regard to the non-pathologic role of K_{ATP} channels in human physiology[22]. Some pharmacologic agents that activate K_{ATP} channels may cause some of the same changes as those seen in Cantu syndrome, and this modulation of these channels has the potential to be beneficial[11,23,24].

The body of literature on the nature and role of K_{ATP} channels in various tissues has grown steadily over recent decades. A subset of literature focuses on the role of these channels in cardiac disease[25]. Activation of K_{ATP} channels is involved in cardioprotection or preservation of the myocardium during stress, such as the global ischemia imposed during cardiac surgery[26]. The channels are similarly involved in the protection of the neural cortex and spinal cord[27-29]. Previous authors have reviewed the early basic science of K_{ATP} channels[30-33]. In this review, we highlight recent evidence with a focus on the role of these channels in cardioprotection and neuroprotection during cardiac surgery.

STRUCTURE AND FUNCTION OF K_{ATP} CHANNELS

Cardiac myocytes contain two K_{ATP} channel entities: one located in the *sarcolemmal* membrane (*s*K_{ATP}) and a proposed *mitochondrial* channel (*mito*K_{ATP}). The structure and function of sK_{ATP} and mitoK_{ATP} appear to be related but with important differences[34-36]. The well-characterized sK_{ATP} channel has been cloned and is present at a very high density in the heart (2,000-3,000/myocyte)[37]. The sK_{ATP} channel is a hetero-octamer of four pore-forming polypeptides of the inwardly rectifying K^+ channel family Kir6.x and four sulfonylurea receptor subunits of the superfamily of ATP-binding cassette transporters[38-40]. The sulfonylurea receptor (SUR) subunit represents the site for blockade by sulfonylureas and stimulation by potassium channel openers and adenosine diphosphate (ADP). The Kir6.x subunit is the location for inhibition by ATP[38,40].

The mitoK_{ATP} channel may be primarily responsible for the cardioprotection provided by K_{ATP} channel modulation[26,41], but the sK_{ATP} channel is better characterized and may also be involved[42]. The physiological role of the sK_{ATP} channel in cardiac tissue involves modulating cellular function in response to stress such as metabolic inhibition[25,43]. Specifically, the channel is opened in response to stress and closed (inhibited) in the presence of ATP[44]. The function of the mitoK_{ATP} channel appears to involve organelle response to stress, with effects on both the volume and the function of the mitochondria[45-47].

The focus of this review is the role function of K_{ATP} channels in the myocardium. However, K_{ATP} channels are present in cardiac muscle, skeletal muscle, and smooth muscle, and their function in smooth muscle is worth noting in this discussion about the cardiovascular effects of K_{ATP} channels[48]. Diazoxide is a K_{ATP} channel opener that has been found to cause substantial arterial and arteriolar dilatation[49,50]. This agent was first used clinically as an antihypertensive agent before its potential as a cardioprotective agent was identified[51]. The side effects of diazoxide have limited its clinical use for hypertension, though it is still used clinically primarily to treat hypoglycemia[52-55]. The role of K_{ATP} channels in causing hypotension is primarily attributed to the vascular smooth muscle cells[2,3]; however, these channels are also found in vascular endothelial cells, and the action in endothelial cells may contribute to the effects on vascular tone[56,57].

Some progress has been made in understanding the molecular identity of the mitoK$_{ATP}$ channel[45], but the quest to definitively characterize these channels continues. One of the challenges that has limited progress towards better understanding mitoK$_{ATP}$ channels has been that K$_{ATP}$ channel openers and blockers are both nonspecific; therefore, it is difficult to differentiate effects on sarcolemmal versus mitochondrial channels[24,58]. The determination of the implicated subunits and the structural identification of a mitoK$_{ATP}$ channel would allow the prevention of undesirable side effects of nonspecific channel openers and inhibitors and allow for directed cardiac targeting for clinical use. By utilizing the genetic deletion of known sarcolemmal K$_{ATP}$ channel subunits, it may be possible to indirectly identify subunits of a mitochondrial K$_{ATP}$ channel involved in cardioprotection or neuroprotection[41,45,59].

Novel mitochondrial proteins encoded by CCDC51 and ABCB8 genes, with unknown function, have been implicated as proteins involved in a mitoK$_{ATP}$ channel[45]. Known potential sarcolemmal K$_{ATP}$ channel subunits (Kir6.1, Kir6.2, Kir1.1, SUR1) and non-K$_{ATP}$ channel proteins (ROMK) have been systematically evaluated using pharmacologic blockade, genetic deletion, or genetic alteration (gain of function) in multiple mouse models to identify potential mitoK$_{ATP}$ channel proteins involved in diazoxide cardioprotection[41,59-66]. However, no subunit has thus far been definitively implicated in cardioprotection by diazoxide. The characterization of the specific molecular profile of mitoK$_{ATP}$ channels is a challenge that requires further research[36,67].

K$_{ATP}$ CHANNELS AND CARDIOPROTECTION

K$_{ATP}$ channels provide endogenous myocardial protection via coupling of cell membrane potential to myocardial metabolism, since these channels are inhibited by ATP and open during times of metabolic stress[44]. Pharmacologic opening of K$_{ATP}$ channels mimics ischemic preconditioning (IPC) in multiple animal models[68-72]. Pharmacologic or non-pharmacologic opening of K$_{ATP}$ channels with diazoxide affords protection to isolated myocytes when the channels are opened before the onset of stress, but not if administered after the onset of stress[73]. In an isolated heart model, opening K$_{ATP}$ channels before ischemia and during early ischemia but not upon reperfusion facilitated cardioprotection[74]. The requirement that these channels are targeted prior to stress is critical because it limits the usefulness of targeting these channels for cardioprotection to situations such as cardiac surgery where a known subsequent ischemic insult occurs[75]. Diazoxide given throughout an ischemic episode led to maximal protection in human atrial trabeculae, suggesting that diazoxide could be a helpful additive to cardioplegia[76].

Cardiac surgery is necessary to treat various cardiac diseases, but the surgery paradoxically causes injury to myocardium that is often already compromised. Contributors to myocardial injury during cardiac surgery include exposure to hypothermic hyperkalemic cardioplegia[77], an imposed global ischemic period, and an already compromised myocardium. The clinical manifestation of the myocardial injury after cardiac surgery is myocardial stunning (MS). Myocardial stunning is defined as myocardial dysfunction despite the resumption of blood flow[78], similar to the clinical consequences following global ischemia for cardiac surgery when flow is restored. MS is defined clinically as the need for inotropic support after surgery for > 24 h but < 72 h, and it affects almost 70% of patients after cardiac surgery[79]. This injury itself is reversible, by definition, but it is associated with worse patient outcomes.

Early studies in canines demonstrated reduced myocardial function after brief myocardial ischemia[80], indicating that patients undergoing cardiac ischemia required for heart surgery would have a decrease in myocardial function following reperfusion. The search for methods to protect the myocardium from such insults has been ongoing since cardiac surgery began in the 1950s. Many pharmacologic and nonpharmacologic methods have been identified with varying degrees of efficacy and clinical potential[81].

The use of carefully designed cardioplegia solutions to facilitate cardiac arrest during surgery has been the primary method of protection from global ischemia, but the cardioplegia itself contributes to myocyte injury[82]. The use of K_{ATP} channel openers may offer protection against such injury. Advancements in understanding how myocardial K_{ATP} channels are involved in cardioprotection have occurred over the last several decades. [Table 1] This research has progressed towards safely using K_{ATP} channel openers for the benefit of human patients with cardiac disease, though they are not yet used clinically for this purpose.

In 1983, Noma demonstrated that the K_{ATP} channel was involved in the regulation of energy metabolism in cardiac cells[1]. For the past 40 years since Noma's study was published, there have been ongoing efforts to understand and utilize K_{ATP} channels for patients with cardiac pathology. Murry *et al.* published data demonstrating non-pharmacologic cardioprotection with IPC in dogs, which was a catalyst for subsequent work exploring the mechanism of IPC[83]. In 1992, Gross *et al.* demonstrated that the benefits of preconditioning in dogs were abolished by blocking the K_{ATP} channel pharmacologically[84,85]. The link between cardioprotection and K_{ATP} channels was then established, and led to further studies seeking to exploit the K_{ATP} channel for pharmacologic cardioprotection[86].

In the mid- to late-1990s, many experiments were performed on isolated animal hearts in a Langendorff apparatus model to further explore the potential of pharmacologic cardioprotection with different K_{ATP} channel openers. The Langendorff perfused heart model refers to a procedure that is useful for experiments in which the goal is to evaluate heart function in response to medications or ischemia-reperfusion. The method involves using an isolated heart perfused via the aorta and, thus, the coronary arteries[87-89]. Such models can utilize physiologic crystalloid perfusion or blood perfusion to more closely mimic clinical situations[90]. Pinacidil and aprikalim are two K_{ATP} channel openers that were used in early studies to assess cardioprotection, and both were found to be protective during cardiac ischemia. The benefit of pinacidil was compared to St. Thomas' solution (a commonly used hyperkalemic cardioplegia solution[91]); Pinacidil provided better postischemic recovery in isolated hearts after ischemia[90,92,93]. K_{ATP} channel openers were found to be effective in crystalloid hypothermic, hyperkalemic cardioplegic solutions, in blood cardioplegia, and in the acutely injured heart[90,93,94]. The cardioprotective effect of pinacidil when added to hypothermic depolarizing cardioplegia was lost with a K_{ATP} blocker, suggesting that the benefit involved K_{ATP} channels[94].

Multiple K_{ATP} channel openers have been studied for cardioprotection, and each has a different pharmacology. Pinacidil was used in many early studies evaluating K_{ATP} channel openers and cardioprotection, but many researchers have since focused on diazoxide due to its proposed specificity for mitoK_{ATP} channels, unlike most other K_{ATP} channel openers[34]. Garlid found that diazoxide has a 2,000-fold greater affinity for mitoK_{ATP} compared to the sarcolemmal K_{ATP} channel[95]. However, some of the other potassium channel openers including cromakalim, nicorandil, and pinacidil may still have some role in some role in cardioprotection[96]. Comparisons of cromakalim and diazoxide suggested that diazoxide has less effect on cardiac action potential duration than some other potassium channel openers[97]. The shortening of the cardiac action potential has been proposed as a mechanism of cardioprotection by K_{ATP} channel openers[98].

THE MOLECULAR MECHANISM OF CARDIOPROTECTION AFFORDED BY K_{ATP} CHANNEL ACTIVATION IS UNKNOWN

The understanding of K_{ATP} channels on mitochondrial membranes developed in the 1990s after they were characterized by Inoue *et al.* in 1991[99]. The interest in the mitoK_{ATP} channel for cardioprotection was proposed to be based on responses of mitochondria to stress. As previously stated, mitoK_{ATP} channels open during stress, which facilitates increased mitochondrial volume and reduced calcium overload, both of which are beneficial for mitochondrial function and overall cellular adaptation[45,100].

Table 1. Published research demonstrating or relating to the cardioprotective effects of K_{ATP} channel openers

Reference (author, year)	Model	Findings
Noma, 1983[1]	Mammal myocytes	The K_{ATP} channel appeared important for regulation of cellular energy metabolism in cardiac cells
Murry *et al.*, 1986[83]	Canine model	Intermittent episodes of ischemia protected the myocardium by delaying cell death when the myocardium was later subjected to ischemia
Grover *et al.*, 1990[71]	Rat heart, Langendorff; canine model	Nicorandil resulted in improved contractility via indirect action, but cromakalim was directly cardioprotective
Tseng *et al.*, 1990[37]	Canine myocytes	Pinacidil increased efflux through K_{ATP} channels, and this was modulated by enzymatic reaction
Gross *et al.*, 1992[84]	Canine model	Benefits of preconditioning in dogs were abolished by blocking the K_{ATP} channel
Auchampach *et al.*, 1992[68]	Canine model	Blocking the K_{ATP} channel prevented benefits of IPC on infarct size after prolonged coronary occlusion
Galiñanes *et al.*, 1992[70]	Rat heart, Langendorff	The K_{ATP} channel opener lemakalim had anti-ischemic effects, but not in combination with high K^+ cardioplegia
Shigematsu *et al.*, 1995[69]	Guinea pig ventricles	Ischemia causes activation of K_{ATP} channels, which contributes of recovery of contraction after reperfusion
Inagaki *et al.*, 1996[38]	Isolated proteins from rat and mouse	A family of SUR receptors determines the function of K_{ATP} channels
Lawton *et al.*, 1996[93]	Rabbit heart, Langendorff	Pinacidil provided better postischemic recovery compared with controls
Lawton *et al.*, 1996[92]	Rabbit heart, Langendorff	Pinacidil and aprikalim are comparable to St. Thomas' solution for cardioprotection
Garlid *et al.*, 1997[26]	Rat hearts, Langendorff	Diazoxide was significantly more potent than sarcolemmal K_{ATP} at opening mitoK_{ATP}, implicating a role for mitoK_{ATP} in cardioprotection
Shyng *et al.*, 1997[40]	Genetically modified cells	The K_{ATP} channel pore is octameric or tetradimeric in structure. Each includes four Kir6.2 subunits and SUR1 subunits
Lawton *et al.*, 1997[94]	Rabbit heart, Langendorff	Pinacidil was cardioprotective, and this cardioprotection was lost with a K_{ATP} blocker
Lawton *et al.*, 1998[90]	Rabbit heart, Langendorff	Pinacidil was comparative to warm blood cardioplegia for systolic recovery
Nakai *et al.*, 2001[101]	Rabbit heart, Langendorff	Diazoxide and IPC were both cardioprotective. Effects were lost with a mitoK_{ATP} channel blocker
Forbes *et al.*, 2001[114]	Rat heart, Langendorff	Adding either diazoxide or pinacidil caused increased ROS, and this is blocked when 5-hydroxydecanoate or antioxidant is added
Murata *et al.*, 2001[104]	Rabbit myocytes	Attenuation of mitochondrial Ca^{2+} overload, because of partial mitochondrial depolarization by mitoK_{ATP} channels, provided cardioprotection
Tsuchida *et al.*, 2001[74]	Rabbit heart, Langendorff	Opening K_{ATP} channels before ischemia and during early ischemia, but not upon reperfusion, was important for cardioprotection
Carroll *et al.*, 2001[115]	Human atrial myocytes	Diazoxide causes preconditioning via mitochondrial swelling and free radical production
Ockaili *et al.*, 2001[121]	Rabbit model	3-nitropropionic acid, a mitochondrial SDH inhibitor, has anti-ischemic effects due to mitoK_{ATP} channel opening
McCully *et al.*, 2002[131]	Swine model	Adding diazoxide to cardioplegia decreased myocardial apoptosis and mitochondrial damage
Krenz *et al.*, 2002[112]	Rat vascular smooth muscle cells	K_{ATP} channel opening by diazoxide or pinacidil led to ROS production from mitochondria
Hanley *et al.*, 2002[119]	Swine heart mitochondria	Succinate oxidation and SDH activity were inhibited by diazoxide, suggesting a cardioprotective mechanism other than K_{ATP} channel activation
Wang *et al.*, 2003[137]	Humans	Preconditioning with diazoxide was protective compared to cardioplegia alone, resulting in better hemodynamic recovery
Suzuki *et al.*, 2003[109]	Mouse heart, Langendorff	Diazoxide enhanced actional potential shortening during ischemia by activating sarcolemmal K_{ATP} channels

Oldenburg *et al.*, 2003[116]	Mouse heart proteins	The K_{ATP} channel opener P1075 opened mitoK_{ATP} channels and was cardioprotective via this action
Dzeja *et al.*, 2003[120]	Rat heart mitochondria; rat heart, Langendorff	Production of ROS and degradation of nucleotides were reduced by diazoxide, resulting in cellular protection
Uchiyama *et al.*, 2003[127]	Rat heart, Langendorff	Pharmacological preconditioning involved protein kinase C, and this involves adenosine, mitoK_{ATP} activation, and nitric oxide
Rousou *et al.*, 2004[100]	Rabbit heart, Langendorff	Diazoxide added to cardioplegia was cardioprotective via preservation of mitochondrial physiology
Wakahara *et al.*, 2004[86]	Rat heart, Langendorff	The mitoK_{ATP} channel blocker and antioxidant abolished postischemic recovery of contractile function by diazoxide but not by IPC
Ardehali *et al.*, 2004[111]	Rat liver mitochondria	The mitoK_{ATP} channel composition includes SDH as a component
Eaton *et al.*, 2005[125]	Rat heart, Langendorff	IPC and diazoxide appeared to have protective effects via a mechanism involving reactive oxygen species generation before ischemia onset
Mizutani *et al.*, 2005[77]	Rabbit myocytes	Cellular swelling was detrimental for cardiac contractility, and diazoxide attenuated swelling
Busija *et al.*, 2005[122]	Piglet mitochondria	ROS production was increased by diazoxide, and this was likely via inhibition of SDH
Deja *et al.*, 2006[76]	Human atrial trabeculae	Diazoxide given throughout an ischemic episode led to maximal protection, suggesting diazoxide could be a helpful additive to cardioplegia
Yonemochi *et al.*, 2006[130]	Rat myocytes	The mitoK_{ATP} channels acted as a trigger and mediator of cardioprotection through $\Delta\Psi$m loss
Prasad *et al.*, 2006[61]	Mouse heart, Langendorff	The sarcolemmal K_{ATP} channel appeared necessary for exaggerated swelling and reduced contractility after cardioplegia
Mizutani *et al.*, 2006[82]	Rabbit myocytes	Diazoxide abolished the swelling and reduced contractility caused by St. Thomas' cardioplegia
Kim *et al.*, 2006[128]	Rat myocytes	Diazoxide cardioprotection appeared to occur via protein kinase C pathway
Al-Dadah *et al.*, 2007[106]	Rabbit myocytes	The attenuation of both swelling and reduced contractility by diazoxide was unchanged by adding K_{ATP} blockers
Flagg *et al.*, 2008[44]	Mouse myocytes	Atrial and ventricular K_{ATP} channels have fundamentally different structures, including a difference in SUR1
Wojtovich *et al.*, 2008[110]	Rat heart, Langendorff; rat mitochondria	MitoK_{ATP} activation in IPC may involve complex II inhibition by malonate
Deja *et al*, 2009[138]	Humans	Adding diazoxide to cardioplegia improved myocardial protection
Sellitto *et al.*, 2010[41]	Mouse myocytes	Diazoxide did not open the ventricular sarcolemmal K_{ATP} channel. The mechanism involved a pathway involving SUR1
Zhang *et al.*, 2011[108]	Mouse myocytes	HMR 1098, a K_{ATP} channel blocker, is nonspecific. Methods such as genetic deletion are needed to confirm channel subunit involvement in cardioprotection
Maffit *et al.*, 2012[107]	Human myocytes	Diazoxide lessened swelling with or without inhibition of K_{ATP} channel
Anastacio *et al.*, 2013[60]	Mouse mitochondria	The benefit of diazoxide during stress involves inhibition of SDH and possibly opening of mitoK_{ATP}
Anastacio *et al.*, 2013[103]	Mouse mitochondria	Inhibition of mitoK_{ATP} channels with 5-HD reduced mitochondrial volume during stress, indicating a role for mitoK_{ATP} channel with diazoxide
Anastacio *et al.*, 2013[124]	Mouse mitochondria	The cardioprotection of diazoxide was independent of the SUR1 subunit of the K_{ATP} channel, though it may occur via inhibition of the SDH enzyme complex
Garlid *et al.*, 2013[117]	Rat heart mitochondria	The mitochondrial ROS involved in cardioprotection by IPC and diazoxide appeared to be hydroxyl radical (HO·)
Janjua *et al.*, 2014[73]	Mouse ventricular myocytes, human atrial myocytes	Diazoxide was only cardioprotective if administered at the onset of stress and not if administered after onset of stress
Henn *et al.*, 2015[62]	Mouse ventricular mitochondria	The subunits Kir1.1, Kir3.1, and Kir3.4 all could possibly be involved in cardioprotection caused by diazoxide
Henn *et al.*, 2015[63]	Mouse myocytes	The Kir6.1 subunit improved myocyte tolerance to stress, but the mechanism was unknown
Henn *et al.*, 2016[65]	Mouse myocytes	The negative effects of cardioplegia were decreased in Kir6.1GOF myocytes

Makepeace et al., 2018[134]	Mouse heart, Langendorff	Adding diazoxide to cardioplegia provided improved recovery after ischemia
Paggio et al., 2019[45]	HeLa cells with plasmids encoding K_{ATP} proteins and mouse models	Over- or under-expression of mitoK_{ATP} is detrimental. Cardioprotection by diazoxide is lost when mitoK_{ATP} is suppressed
Suarez-Pierre et al., 2020[135]	Swine model	Diazoxide preserved systolic and diastolic ventricular function after ischemia in a large animal model
Ahmad et al., 2021[126]	Mouse myocytes	S-nitrosating agent and diazoxide are cardioprotective individually, but the beneficial effect was lost when they were combined
Velez et al., 2022[136]	Swine model	Diazoxide reduced myocardial stunning and facilitated separation from cardiopulmonary bypass
Wang et al., 2023[59]	Mouse myocytes and hearts	Neither Kir1.1 (ROMK) nor SUR1 were involved in the mechanism of cardioprotection by diazoxide

K_{ATP}: Adenosine triphosphate-sensitive potassium channels; mitoK_{ATP}: mitochondrial adenosine triphosphate-sensitive potassium channels; IPC: ischemic preconditioning; 5-HD: 5-hydroxydecanoate; SDH: succinate dehydrogenase; SUR1: sulfonylurea sensitive regulatory subunit 1; STAT3: signal transducer and activator of transcription 3; SUR 1: sulfonylurea sensitive regulatory subunit 1; ROMK: renal outer medullary potassium.

Diazoxide and perhaps other K_{ATP} channel openers were initially suggested to be cardioprotective via a mechanism involving a mitoK_{ATP} channel rather than a sK_{ATP} channel in the few years after the mitoK_{ATP} channels were characterized[26,33]. Efforts to define the role of both sarcolemmal and mitochondrial channels involved in cardioprotection were begun. Pharmacologic cardioprotection and non-pharmacologic cardioprotection using IPC were compared: both were cardioprotective, and the cardioprotection initially appeared to be inhibited in the presence of a selective mitoK_{ATP} channel blocker, 5-hydroxydecanoate[101]. Additionally, when a mitoK_{ATP} channel blocker was added to isolated hearts with either diazoxide or IPC, this abolished the improvement in contractility after ischemia that had been afforded by diazoxide but not by IPC, suggesting that a mitoK_{ATP} was critical for diazoxide's mechanism but not for IPC[86]. In line with these findings, a later study using genetic deletion found that diazoxide's cardioprotection was not due to action at a sK_{ATP} channel[41].

Ultimately, the molecular mechanism of action at a proposed mitoK_{ATP} channel remains unknown. Potential mechanisms largely focus on the mitochondria and include K_{ATP} channel-related and non-related mechanisms[42]. It is also possible that the effects of diazoxide and other K_{ATP} channel openers can be attributed to a combination of mechanisms[102].

POTENTIAL MECHANISMS OF CARDIOPROTECTION (K_{ATP} CHANNEL AND CHANNEL-INDEPENDENT)
Mitochondrial and cellular volume alteration
Both cellular and mitochondrial volume alterations have been investigated in the search for mechanisms of cardioprotection relating to K_{ATP} channels and their openers. While opening sK_{ATP} channels results in K^+ efflux from the cell[30], opening of a mitoK_{ATP} channel results in K^+ influx from the cytosol into the mitochondria and mitochondrial swelling, suggesting a potential basis for mitoK_{ATP}-dependent changes in mitochondrial and cellular volume alteration during to stress[32,46,67]. Interestingly, opening the mitoK_{ATP} channel was associated with changes in mitochondrial matrix volume, calcium concentration, and

respiration[100]. Diazoxide facilitated mitochondrial swelling in ischemia[103] (whereas mitochondria otherwise tend to contract in this setting) and reduced mitochondrial calcium accumulation, which are beneficial for mitochondrial function[100]. In animal myocytes undergoing ischemia and reperfusion, pharmacologic opening of mitoK_{ATP} channels was associated with decreased matrix calcium overload because of mitochondrial membrane depolarization[104].

The mechanisms and effects of cellular swelling observed in ischemic myocytes secondary to stress have been studied for several decades[105], and the prevention of swelling has been suggested to be a potential mechanism of K_{ATP} cardioprotection. Studies in the 2010s elucidated relationships between cellular volume changes during stress and myocyte contractility. Stresses that caused cellular swelling (hypoosmotic stress; exposure to hyperkalemic, hypothermic cardioplegia; or exposure to metabolic inhibition) resulted in reduced contractility that was prevented with the addition of K_{ATP} channel opener diazoxide[77,82,106]. Hyperosmotic stress resulted in myocyte shrinkage and improved contractility, and this inverse relationship between function and myocyte size suggested a cellular mechanism for myocardial stunning[77]. Thus, a proposed cellular model of myocyte stunning was created to test mechanisms of diazoxide cardioprotection[77].

Interestingly, in myocytes, an open sK_{ATP} channel appeared necessary for the observed cellular swelling and reduced contractility after exposure to hypothermic, hyperkalemic cardioplegia[61]. An interplay between cellular volume and mitochondrial volume may be important in cardioprotection afforded by K_{ATP} channel openers.

Pharmacologic channel blockers have also been utilized to determine potential mechanisms of cardioprotection and implicated channel subunits. The attenuation of both swelling and reduced contractility by diazoxide was unchanged by sarcolemmal or mitochondrial K_{ATP} channel blockers, contradicting other work with channel blockers[106-108]. This led to the proposal that pharmacologic channel blockers are nonspecific and only definitive methods such as genetic deletion could confirm channel subunit involvement in cardioprotection.

While the mitoK_{ATP} channel became a focus of cardioprotection, there was still evidence suggesting a role for the sK_{ATP} channel. A study using the Langendorff model found that diazoxide's cardioprotection was provided via sK_{ATP} channels rather than mitoK_{ATP} channels[109]. However, work using whole-cell voltage clamping of ventricular myocytes determined that diazoxide does not open sarcolemmal K_{ATP} channels; this led the investigation of diazoxide cardioprotection away from the sK_{ATP} channel[41].

Mitochondrial respiration

There are several potential K_{ATP}-independent mechanisms of diazoxide cardioprotection at the mitochondrial level including the inhibition of succinate dehydrogenase (SDH), increased reactive oxygen species signaling, and protein kinase C activation[42,110-117]. While the majority of studies have implicated K_{ATP} channels, some have emphasized that diazoxide and other openers have important K_{ATP} channel-independent actions, and therefore cardioprotective mechanisms remain unclear[42,118].

K_{ATP} channel opener diazoxide is a known inhibitor of mitochondrial enzyme complex II, SDH, a component of the electron transport chain[119,120]. Consistent with this idea, other SDH inhibitors have been found to be cardioprotective, and reversal of SDH and succinate accumulation is the primary driver of mitochondrial reactive oxygen species (ROS) production that underlies ischemia/reperfusion injury[110,121-123]. The exact nature of the interaction between SDH and mitoK_{ATP} is unknown. In myocyte

experiments, diazoxide cardioprotection required inhibition of SDH, and the role of K_{ATP} channel activity was not clear[60]. Diazoxide inhibited SDH in the presence of mitoK_{ATP} channel inhibitor 5-hydroxydecanoate, and SDH inhibition alone did not lead to an increase in mitochondrial volume (a surrogate for mitoK_{ATP} activity)[124]. These findings suggested that SDH is not directly upstream of a mitoK_{ATP} channel.

Reactive oxygen species mimic IPC (attributed to activity at a K_{ATP} channel) and antioxidants block IPC[114,115]. The role of ROS was investigated as a potential cardioprotective mechanism of diazoxide[67]. Diazoxide and pinacidil increased ROS in cardiomyocytes, and this increase was blocked with the co-administration of 5-hydrodecanoate or an antioxidant, supporting the hypothesis that ROS are involved in cardioprotection facilitated by mitoK_{ATP} channels[114]. In isolated perfused rat hearts, ROS generation prior to ischemia onset contributed to the cardioprotection of both IPC and diazoxide[125]. In animal models, glutathione (an antioxidant) administered before ischemia prevented cardioprotection by diazoxide, via prevention of inhibition of SDH or the inhibition of ROS formation[60]. Similarly, a mitochondrial-targeted antioxidant that inhibits mitochondrial enzyme complex I MitoSNO (given at reperfusion) reduced cardioprotection by diazoxide, suggesting an interplay at the mitochondrial level [Figure 1][126]. Data published in 2019 provided further evidence that mitoK_{ATP} is important for redox homeostasis by showing that diazoxide results in increased ROS in wild-type mice, but not in cells lacking proposed mitoK_{ATP} channel subunits[45].

Some have suggested that K_{ATP} cardioprotection and IPC involve the activation of protein kinase C (PKC) (specifically PKC-€) and may be blocked by PKC inhibition or genetic deletion[112,127,128]. Diazoxide has also been implicated in the translocation of PKC-€ from the cytosol to the mitochondria as a mechanism of cardioprotection[128].

Others have evaluated the role of apoptosis in cardioprotection via the exploitation of K_{ATP} channels. In a myocyte model, diazoxide and pinacidil protected rat ventricular myocytes against apoptosis[129]. Another myocyte study found that diazoxide was protective against apoptosis, although protection depended on the timing of treatment[130]. The cardioprotection associated with diazoxide in a swine model was found to result from decreased myocyte apoptosis and mitochondrial damage[131]. Recent reviews have also discussed the inhibition of apoptosis as a potential mechanism of K_{ATP} channel modulation in cardioprotection[132].

TRANSLATIONAL AND HUMAN STUDIES USING K_{ATP} CHANNEL OPENERS FOR CARDIOPROTECTION

The studies performed in the 2000s and early 2010s led to knowledge of the basic mechanisms of K_{ATP} channels within cells and organelles, providing the framework for understanding how K_{ATP} openers are beneficial for cardioprotection, elucidating their mechanisms of action. Over recent years, researchers have then turned to the potential role of these channels as pharmacologic targets in human patients. To facilitate the understanding of potassium channel openers at tissue and organism levels, isolated heart models and intact large animal models that mimic conditions of myocardial ischemia during cardiac surgery have been developed to test the systemic hemodynamic effects associated with K_{ATP} channel opener diazoxide.

An early study in 2005 comparing diazoxide to control in a porcine model found that diazoxide did not provide cardioprotection (infarct size and systolic function) after myocardial ischemia[133]. The authors acknowledged that their results were incongruent with others' findings and postulated that this could be due to preconditioning effects of anesthetics or an incorrect dose of diazoxide. Recent studies have been more promising. In an isolated mouse heart model, adding diazoxide to cardioplegia led to improved diastolic
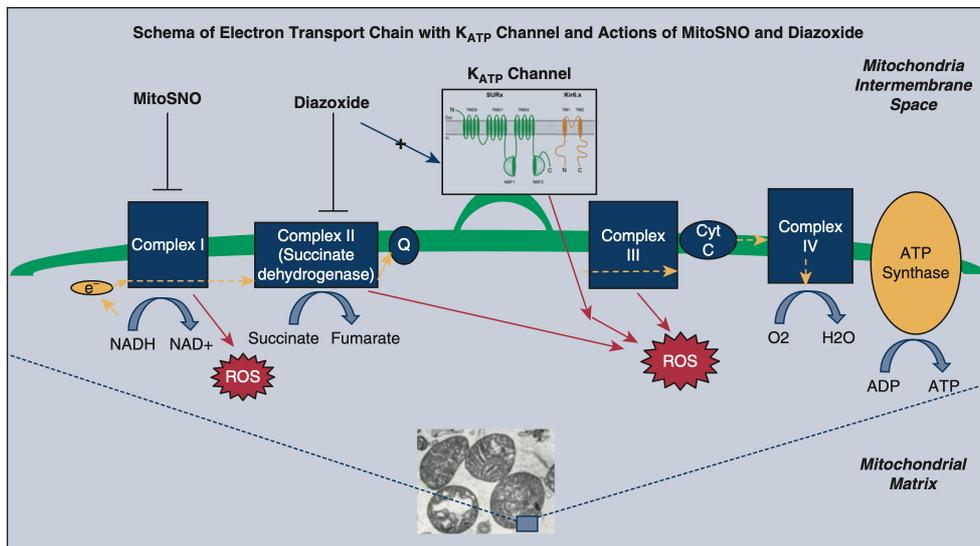
Figure 1. Electron transport chain with K_{ATP} channel and actions of MitoSNO and diazoxide. The schema simplifies the activity of the inner membrane of the mitochondrion, including the proposed K_{ATP} channel and electron transport chain. At the bottom of the figure, a photo taken by electron microscopy from the Lawton laboratory, of individual mitochondria. In the schema, diazoxide is depicted as having an inhibitory effect on Complex II (succinate dehydrogenase) while activating the K_{ATP} channel. The cardioprotection by diazoxide may occur due to either of the mechanisms or another mechanism. MitoSNO has an inhibitory effect on Complex I, which prevents SDH accumulation, and this is thought to be the cardioprotective mechanism of MitoSNO. While each is cardioprotective via these mechanisms, these two agents have a synergistic negative effect[126]. This figure is used with permission from Elsevier (obtained September 4, 2023, license number 5621920353581)[126]. MitoSNO: Mitochondria-targeted S-nitrosating agent; NADH: nicotinamide adenine dinucleotide, reduced form; NADþ: nicotinamide adenine dinucleotide, oxidized form; ROS: reactive oxygen species; ATP: adenosine triphosphate; ADP: adenosine diphosphate; Cyt C: cytochrome C.

function following a period of global ischemia[134]. Two subsequent studies were conducted in swine models. In the first, swine treated with hypothermic, hyperkalemic cardioplegia with diazoxide (single dose) prior to a 2-h global ischemic period were found to have improved systolic and diastolic ventricular function compared to cardioplegia alone [Figure 2][135]. In the second, swine underwent 30 min of occlusion of the left anterior descending artery prior to 2 h of global ischemia protected with cardioplegia or cardioplegia with diazoxide (dosed every 20 min) [Figure 3][136]. Compared to cardioplegia alone, animals that received diazoxide had decreased myocardial stunning and shortened time to separate from cardiopulmonary bypass [Figure 4][136]. These studies provided some of the most convincing preclinical data to date that diazoxide will be beneficial as an additive to cardioplegia in humans undergoing cardiac surgery requiring global ischemia. It is important to acknowledge the limitations of the translational models that have been widely used to study K_{ATP} channels and cardioprotection. These models may not provide sufficient confidence to translate to human pathophysiology. These limitations highlight the importance of randomized clinical trials in humans before widespread adoption.

Two small, randomized trials in humans have investigated the cardioprotective effects of diazoxide in humans[137,138]. Wang *et al.* (2003) randomized 40 patients undergoing coronary artery bypass grafting to receive either 1.5 mg/kg diazoxide infusion or placebo *intravenously* prior to undergoing global ischemia for cardiac surgery. They found improved hemodynamic recovery after surgery in patients who received diazoxide, though they noted that further studies were needed to determine an optimal dosing protocol[137]. Deja *et al.* (2009) randomized 40 patients to receive *intermittent warm blood cardioplegia* that was supplemented with 100 µmol/L diazoxide or placebo prior to global ischemia for cardiac surgery[138]. They found that patients treated with diazoxide required less inotropic support and had higher cardiac indices after global ischemia[138]. Both human studies were small, involved low-risk patients, and were primarily
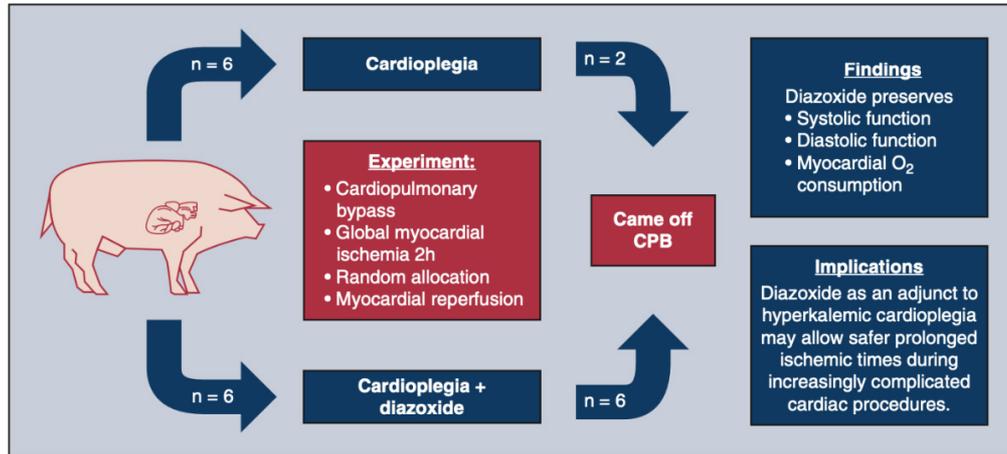
Figure 2. Summary of the benefits with use of K_{ATP} channel opener diazoxide in a translational swine model of hypothermic cardioplegic arrest and prolonged global ischemia similar to a prolonged global ischemia model for clinical cardiac surgery. Swine undergo cardiopulmonary bypass and prolonged global ischemia of the heart via placement of aortic cross clamp (120 min) with one dose of cardioplegia, similar to clinical cardiopulmonary bypass. Animals are randomized to one of two groups: cardioplegia alone or cardioplegia plus diazoxide. Animals who underwent cardioplegia plus diazoxide in this model had preserved systolic and diastolic function, suggesting that adding diazoxide to cardioplegia may result in improved outcomes[135]. This model simulates the clinical situation of a patient presenting to the operating room for a prolonged global ischemic period for cardiac surgery protected with one dose of cardioplegia[135]. This figure is used with permission from Elsevier (obtained September 4, 2023, license number 5621921258491)[135]. CPB: Cardiopulmonary bypass.
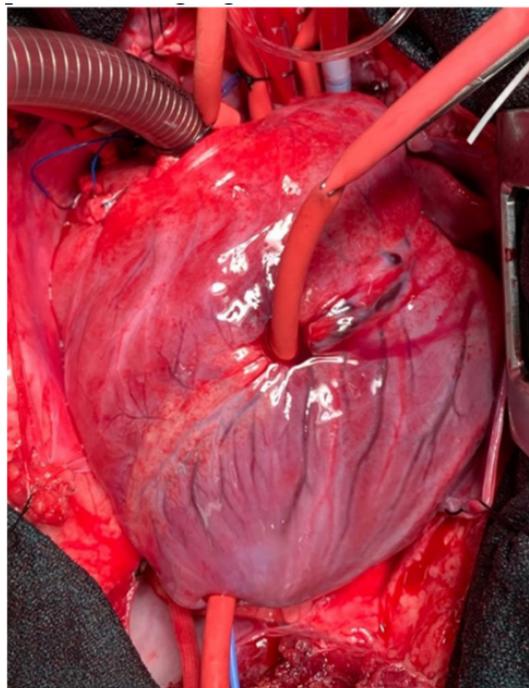

Figure 3. Photograph of porcine heart in the Lawton laboratory following cannulation for cardiopulmonary bypass, placement of left ventricular pressure catheter, and placement of left anterior descending tourniquet for regional ischemia prior to a longer global ischemia in a model simulating clinical cardiopulmonary bypass with an acutely injured heart. Regional ischemia is evident in this porcine heart after occlusion of the left anterior descending coronary artery for 30 min for regional ischemia. Subsequently, the heart will be exposed to two hours of global ischemia protected with cardioplegia with or without diazoxide, followed by reperfusion[136]. This model simulates the clinical situation of a patient presenting to the operating room with ongoing ischemia prior to a coronary revascularization procedure with prolonged global ischemia[136]. This figure is used with permission from Elsevier (obtained December 12, 2023, license number 5686380805849)[136].
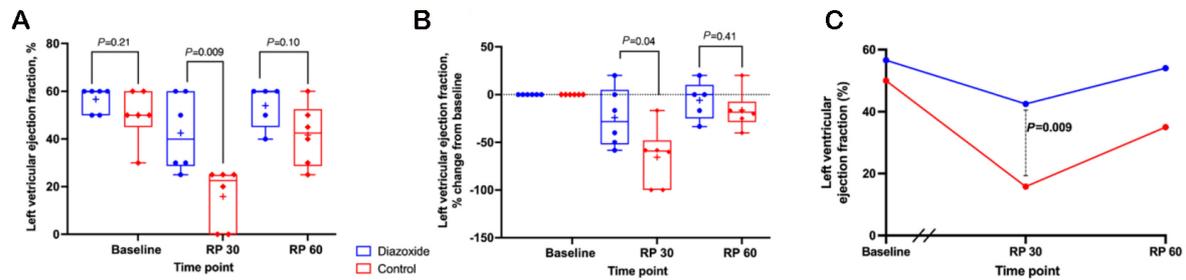
Figure 4. K_{ATP} channel opener diazoxide was associated with improved left ventricular ejection fraction at 30 min after reperfusion in a porcine model of 30 min of regional ischemia followed by 2 h of global ischemia, simulating clinical cardiopulmonary bypass in the acutely injured heart prior to cardiac surgery. Using a translational porcine model of regional ischemia (30 min) followed by 2 h of global ischemia, animals were randomized to one of two groups: cardioplegia alone (red, $n = 6$) or cardioplegia plus diazoxide (blue, $n = 6$). Each dot represents an individual animal ($n = 6$ in each group). The lower and upper borders of each box represent the lower and upper quartile, respectively. The middle line represents the median, and the cross represents the mean. Left ventricular ejection fraction was measured at baseline, before regional and global ischemia, and throughout 1 h of reperfusion by transesophageal echocardiography. Statistical comparisons using Student's t test. (A) Left ventricular ejection fraction represented as a percentage. (B) Left ventricular ejection fraction represented as percent change from baseline. (C) LVEF represented over time[136]. This figure was previously published under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution with appropriate citation and without modification[136]. K_{ATP}: Adenosine triphosphate - sensitive potassium; LVEF: left ventricular ejection fraction; RP 30: reperfusion for 30 min; RP 60: reperfusion for 60 min.

aimed at demonstrating feasibility and safety, which were achieved. Neither human study evaluated a K_{ATP} channel opener in hypothermic, hyperkalemic cardioplegia prior to global ischemia. This is an important next step, as normothermic, hyperkalemic cardioplegia (in contrast to hypothermic) has not been implicated in myocyte swelling and reduced contractility following stress[82]. Additional studies are needed with K_{ATP} channel openers in hypothermic, hyperkalemic cardioplegia to demonstrate feasibility and, subsequently, safety and efficacy prior to widespread human use.

THE ROLE OF K_{ATP} CHANNELS IN NEUROPROTECTION

K_{ATP} channels may also have a role in neuroprotection during cardiac surgery that involves deep hypothermic circulatory arrest for aortic replacement, congenital heart surgery, pulmonary endarterectomy, repair of thoracoabdominal aneurysms, or other complex procedures. The primary methods of neuroprotection for such surgery include: deep hypothermic circulatory arrest (DHCA), antegrade cerebral perfusion, and retrograde cerebral perfusion[139]. Similar to cardioprotection for cardiac surgery, methods of neuroprotection are imperfect. A recent study of patients undergoing aortic arch surgery with HCA and unilateral ACP had a 4.8% incidence of permanent neurological dysfunction[140]. A wide variety of pharmacologic agents have been studied to improve neurologic outcomes after cardiac surgery[141,142]. Among the agents that may be useful in these efforts is K_{ATP} channel opener diazoxide[27,143].

Several studies in the late 1990s explored the role of K_{ATP} channels in neuroprotection [Table 2]. K_{ATP} channel openers have been demonstrated to depolarize hippocampal mitochondria, the area of greatest injury in DHCA[144]. K_{ATP} channels were neuroprotective during hypoxia in rat neocortical tissues[145], and diazoxide preserved neuronal-vascular function after cerebral ischemia in a swine model[146]. Diazoxide was an effective form of pharmacologic preconditioning in canine models, as evidenced by both improved clinical neurologic scores and histopathology compared to controls[28,147]. Similarly, diazoxide pretreatment resulted in improved neurologic outcomes in a spinal cord injury model, and the suggested mechanism was K_{ATP} channel activity[29]. MitoK$_{ATP}$ channel openers have also been demonstrated to inhibit apoptosis in neurons by preserving mitochondrial inner membrane potential[148].

Table 2. Published research demonstrating or relating to the importance of the K_{ATP} channel in neuroprotection

Reference (author, year)	Model	Findings
Garcia de Arriba *et al.*, 1999[145]	Rat neocortical tissue	ATP-dependent potassium channels were neuroprotective during hypoxia in rat neocortical tissues
Domoki *et al.*, 1999[146]	Swine model	Diazoxide preserved neuronal-vascular function after cerebral ischemia in newborn pigs
Debska *et al.*, 2001[144]	Rat hippocampal tissue	Potassium channel openers depolarized hippocampal mitochondria
Shake *et al.*, 2001[28]	Canine model	Intravenous diazoxide was neuroprotective in dogs undergoing cardiopulmonary bypass
Caparrelli *et al.*, 2002[29]	Rabbit model	Diazoxide pretreatment resulted in improved neurologic outcomes, and the mechanism appeared to be due to the K_{ATP} channel activity
Teshima *et al.*, 2003[148]	Cerebellar neurons	MitoK$_{ATP}$ channel openers inhibited apoptosis by preserving mitochondrial inner membrane potential
Barreiro *et al.*, 2006[147]	Canine model	Pretreatment with diazoxide plus HCA led to improved neurologic outcomes versus HCA alone
Yamanaka *et al.*, 2018[151]	Mouse model	Diazoxide and EPO were synergistically protective of the spinal cord after ischemia with upregulation of a common receptor
Yamanaka *et al.*, 2019[152]	Mouse model	Diazoxide and EPO were synergistically protective of the spinal cord after ischemia via upregulation of NGF
Yamanaka *et al.*, 2019[150]	Mouse model	Oral diazoxide preserved motor function in spinal cord ischemia-reperfusion injury by the STAT3 pathway
Ikeno *et al.*, 2023[153]	Mouse model	Direct and indirect activation of mitoK$_{ATP}$ channels were involved pharmacological spinal cord protection with motor function preservation

EPO: Erythropoietin; NGF: nerve growth factor; STAT3: signal transducer and activator of transcription; HCA: hypothermic circulatory arrest.

The mechanism of diazoxide for neuroprotection is similar to one of many proposed for its cardioprotective action. Diazoxide is a K_{ATP} channel opener that inhibits complex II and maintains mitochondrial membrane potential. This prevents mitochondrial dysfunction and cell death following DHCA and injury due to N-methyl-D-aspartate (NMDA) excitotoxicity via unique pathways and could be utilized to produce synergistic benefit with NMDA blockade during cardiac surgery with DHCA [Figure 5][149].

Early studies on neuroprotection focused on the brain cortex, but more contemporary work evaluated K_{ATP} channels and protection of the spinal cord, which is applicable to cardiac surgical procedures on the descending aorta that have a known complication of paraplegia from spinal ischemia. Diazoxide attenuated spinal cord ischemia-reperfusion injury, and motor function after spinal cord ischemia was improved in a mouse model with diazoxide compared to controls, an action that appeared to be associated with expression of the signaling transducer and activator of transcription (STAT) 3 pathway[150]. These results have been confirmed by several others that have demonstrated the potential of diazoxide to reduce spinal cord injury following ischemia[151-153].

Further work is needed to characterize the mechanisms involved in neuroprotection provided by diazoxide and its potential use in cardiac surgery.

FUTURE DIRECTIONS

Future work will further advance the understanding of the function and potential of K_{ATP} channels for the benefit of patients undergoing cardiac surgery. Perhaps one of the most anticipated future endeavors is a large clinical trial using diazoxide as an additive to cardioplegia. While the use of diazoxide for
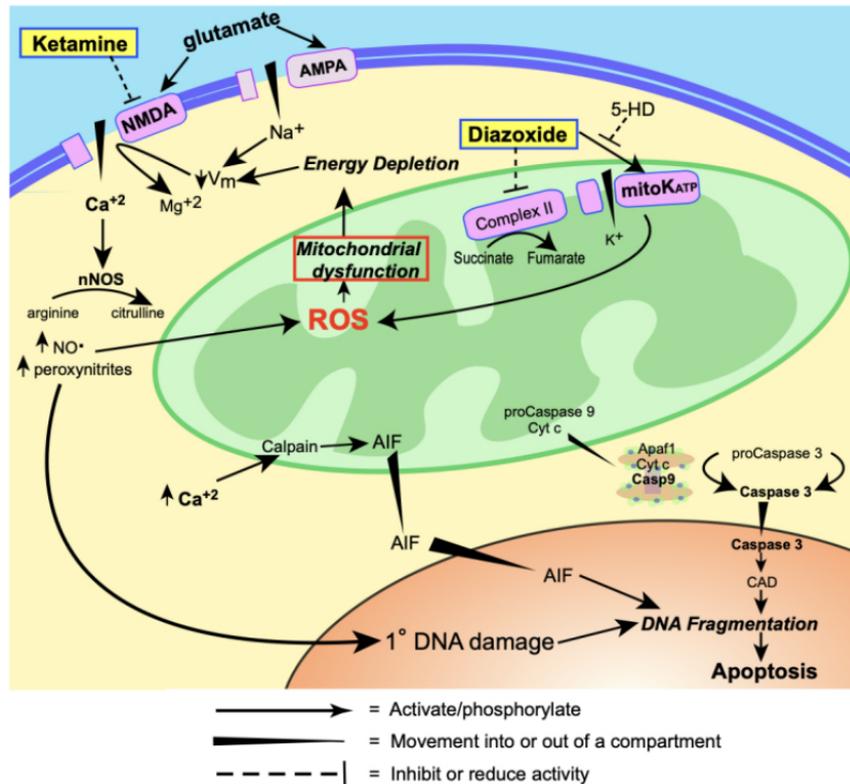
Figure 5. Mechanistic depiction of neuron mitochondria with ketamine, glutamate, diazoxide, and other second messengers and potential mechanisms of injury during hypothermic circulatory arrest. This picture depicts a neuron with intracellular, intramitochondrial, and nuclear potential mechanisms of injury during hypothermic circulatory arrest. Both ketamine and diazoxide have potential neuroprotective effects. Ketamine is depicted here as affecting the NMDA receptors and subsequent second messengers within cytosol. Diazoxide is depicted as acting on the mitochondrial membrane at the mitoK$_{ATP}$ channel and Complex II. Both pathways eventually affect ROS within the mitochondria. Downstream effects of both pathways are also thought to involve mechanisms within the nucleus that lead to DNA changes and apoptosis. Exploration of positive synergism of these agents is one option for future directions in neuroprotection research[149]. This figure was created by Mary Ann Wilson, PhD, and is used with her permission. A version of this figure was also previously published[149]. This figure is used with permission from Elsevier (obtained December 7, 2023, license number 5683710387270). ROS: Reactive oxygen species; MitoK$_{ATP}$: mitochondrial K$_{ATP}$ channel; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA: N-methyl-D-aspartate; Cyt C: cytochrome C; DNA: deoxyribonucleic acid; NO: nitric oxide free radical.

cardioprotection in cardiac surgery patients has been demonstrated as safe and feasible[137,138], more data are needed in high-risk human patients and with the use of hypothermic, hyperkalemic blood cardioplegia.

Important fundamental questions remain about K$_{ATP}$ channels. One unanswered question was made apparent in a recent study in which diazoxide was combined with a mitochondria-targeted S-nitrosating agent (mitoSNO). Each of these agents was known to be cardioprotective individually. However, the cardioprotective mechanism of diazoxide was found to be exclusive of the mechanism of MitoSNO; when combined, these agents were not beneficial[126]. The authors concluded that using nitric oxide donors could be detrimental in the presence of diazoxide, and this requires further investigation. The idea of exploring the positive and negative synergism of diazoxide with various other pharmacological agents should be further studied in both cardioprotection and neuroprotection. It will be critical to know which agents might enhance or negate the beneficial effects of diazoxide or other K$_{ATP}$ agents.

Identification of the potential components of K_{ATP} channels and their roles in cardioprotection and neuroprotection, as well as non-channel mechanisms of action of pharmacologic channel openers, will require ongoing investigation. The latest work on the components of K_{ATP} channels in cardioprotection found that two specific subunits - ROMK and SUR1 - are not implicated in myocardial protection[59]. The subunits implicated in cardioprotection and neuroprotection remain to be determined. Answering this could lead to specific and targeted methods to exploit this channel pharmacologically to protect both the heart and the brain during cardiac surgery.

SUMMARY

This article reviews the background of K_{ATP} channels in the context of their potential use for myocardial protection and neuroprotection, summarizes recent data supporting their use, and outlines future directions for the role of these agents in cardiac surgery. Since it was first understood that K_{ATP} channels were present in cardiac cells and that they mimic IPC, research has advanced towards the goal of developing pharmacologic agents that could target these channels to improve outcomes for patients after cardiac surgery. Progress has been made using: genetic deletion to characterize the potential implicated protein subunits of the involved channels; pharmacological research and single cell voltage clamping to differentiate agents that result in channel activation; translational animal studies to demonstrate feasibility, safety, and potential benefit and small human randomized trials to demonstrate feasibility. Recently, efforts have focused specifically on a diazoxide because of its specificity for mitoK_{ATP} channels, and this agent shows promise for being able to facilitate cardioprotection and neuroprotection for patients. Continued investigation of K_{ATP} channels and their precise molecular mechanism of action will lead and support a broader understanding of the potential ways to target these channels to improve the lives of human patients.

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Author's contributions
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Both authors declared that there are no conflicts of interest.

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Not applicable

Consent for publication
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