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REVIEW ARTICLE

Effect of taurine on ischemia–reperfusion injury

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Abstract Taurine is an abundant β-amino acid that regulates several events that dramatically influence the development of ischemia–reperfusion injury. One of these events is the extrusion of taurine and Na^+ from the cell via the taurine/Na^+ symport. The loss of Na^+ during the ischemia–reperfusion insult limits the amount of available Na^+ for Na^+/Ca^{2+} exchange, an important process in the development of Ca^{2+} overload and the activation of the mitochondrial permeability transition, a key process in ischemia–reperfusion mediated cell death. Taurine also prevents excessive generation of reactive oxygen species by the respiratory chain, an event that also limits the activation of the MPT. Because taurine is an osmoregulator, changes in taurine concentration trigger "osmotic preconditioning," a process that activates an Akt-dependent cytoprotective signaling pathway that inhibits MPT pore formation. These effects of taurine have clinical implications, as experimental evidence reveals potential promise of taurine therapy in preventing cardiac damage during bypass surgery, heart transplantation and myocardial infarction. Moreover, severe loss of taurine from the heart during an ischemia–reperfusion insult may increase the risk of ventricular remodeling and development of heart failure.

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Introduction

Taurine is an abundant amino acid found in very high concentration in the heart. Its putative physiological functions in mammalian cells include osmoregulation, antiinflammation, membrane stabilization, regulation of oxidative stress, ion transport modulation and regulation of mitochondrial protein synthesis. Several of these actions play central roles in ischemia–reperfusion injury. Taurine also has anti-apoptotic activity (Takatani et al. 2004a), although this effect has limited benefit to the ischemia-reperfused heart, as apoptosis only represents about 4 % of total cardiomyocyte death during an ischemia–reperfusion insult. Therefore, it is not surprising that taurine is a key determinant of ischemia–reperfusion injury. In a World Health Organization study, referred to as the Cardiovascular Diseases and Alimentary Comparison Study, an inverse association between population levels of taurine excretion and ischemic heart disease mortality was observed (Yamori et al. 2001). Furthermore, children contain lower myocardial taurine levels than infants, an effect associated with increased resistance to an ischemic cardioplegic insult (Imura et al. 2001). Taurine therapy has also been useful in reducing damage related to bypass and transplant surgery (Milei et al. 1992; Oriyanhan et al. 2005). These data reveal that both intracellular and extracellular taurine levels play important roles in the outcome of an ischemia–reperfusion insult. The present review examines the mechanism underlying the actions of taurine on the ischemia-reperfused heart and the clinical significance of the findings.

Role of calcium overload and oxidative stress in ischemia–reperfusion injury

Ischemia–reperfusion injury consists of both reversible and irreversible biochemical, structural and physiological changes that develop during the ischemia and reperfusion phases of an insult. Generally, the early period of an ischemic insult involves a series of reversible metabolic changes, with one of the early events in the ischemic insult being oxygen deficiency, which inhibits cytochrome c oxidase (complex IV) and restricts flux of reducing equivalents through the electron transport chain (Fig. 1). Consequently, respiratory chain complexes and electron acceptors upstream from cytochrome c oxidase accumulate reducing equivalents, decreasing the delivery of additional reducing equivalents to the electron transport chain, the entry of substrate into the citric acid cycle and the generation of ATP by the respiratory chain. The elevation in the mitochondrial NAD^+/NADH ratio also diminishes the rates of β-oxidation and flux through the citric acid cycle. Glycolysis and glycogenolysis initially rise, but because of elevations in the cytosolic NADH/NAD^+ ratio, pyruvate is preferentially converted to lactate. This series of events has a twofold effect. First, the size of the high energy phosphate pool plunges (Vary et al. 1979) and second, pH_i falls. Reperfusion of the heart following a short period of ischemia reverses these metabolic changes, rapidly restoring the size of the high energy phosphate pool, normalizing pH_i and mediating the recovery of contractile function.

The second phase of an ischemic insult leads to an elevation in both $[Ca^{2+}]_i$ and reactive oxygen species (ROS) content (Murphy and Steenbergen 2008). The elevation in $[Ca^{2+}]_i$ during ischemia results from defects in excitation–contraction coupling (Fig. 2). Upon stimulation of the normal heart, Ca^{2+} enters the cardiomyocyte via the L-type Ca^{2+} channel. The rise in $[Ca^{2+}]_i$ triggers the release of Ca^{2+} from the sarcoplasmic reticulum, further elevating $[Ca^{2+}]_i$. Contraction is initiated by the interaction of Ca^{2+} with the muscle protein, troponin, which induces a conformational change allowing the interaction of myosin and actin. During diastole, $[Ca^{2+}]_i$ declines, as Ca^{2+} is pumped into the sarcoplasmic reticulum by a Ca^{2+} ATPase (SERCA2) and is extruded from the cell via the Na^+/Ca^{2+} exchanger. To maintain Ca^{2+} homeostasis, the uptake of Ca^{2+} via the L-type Ca^{2+} channel must be balanced by an equal extrusion of Ca^{2+} via the Na^+/Ca^{2+} exchanger. During ischemia, an imbalance develops between the influx and efflux of Ca^{2+}, resulting in excessive uptake of Ca^{2+} by the cardiomyocyte. In the ischemic-reperfused heart, the increase in $[Ca^{2+}]_i$ is directly related to the accumulation of H^+ and Na^+ (Fig. 2). The elevation in flux through the Na^+/H^+ exchanger leads to an extrusion of H^+ from the cell in exchange for Na^+. The resulting accumulation of Na^+ by the cardiomyocyte, leads to a shift in the direction of Ca^{2+} transport by the Na^+/Ca^{2+} exchanger leading to Ca^{2+} influx in exchange for Na^+ (Fig. 2). The net effect of these events is a significant elevation in $[Ca^{2+}]_i$. While the largest increase in $[Ca^{2+}]_i$ takes place upon reperfusion, $[Ca^{2+}]_i$ also rises modestly during the ischemic phase of the insult. Based on the cardioprotective actions of the Na^+/Ca^{2+} exchange inhibitors, it is now widely accepted that Ca^{2+} accumulation during both the ischemia and reperfusion phases of the insult contributes to ischemia–reperfusion injury (Karmazyn et al. 2005).

The other major event contributing to cell damage during an ischemia–reperfusion insult is the generation of reactive oxygen species (ROS). Because ROS generation is dependent upon tissue oxygenation, the rate of ROS generation is low during the ischemic insult (Robin et al. 2007). However, sufficient levels of ROS are formed during ischemia to cause some cellular damage. Indeed, the formation of lipid peroxidation products, such as protein adducts of 4-hydroxy-2-neonenal, rises to a new steady state level during ischemia (Eaton et al. 1999). Because antioxidants inhibit the production of the 4-hydroxy-2-nonenal protein adducts and protect the heart against ischemia–reperfusion injury, Eaton et al. (1999) concluded that the scavenging of 4-hydroxy-2-nonenal protects the ischemia-reperfused heart. The major sources of ROS during an ischemia–reperfusion insult are complexes I and III of the electron transport chain (Fig. 1). During the ischemic insult, oxygen deficiency restricts flux through the electron transport chain, resulting in an accumulation of reducing equivalents by electron carriers upstream from cytochrome c oxidase. Reperfusion causes a burst in oxygen consumption accompanied by a significant increase in ROS production, as electrons are diverted from the respiratory chain to oxygen, forming in the process superoxide. Several lines of evidence indicate that ischemia-mediated ROS generation is a major determinant of reperfusion injury. First, reversible inhibition of the electron transport chain during the ischemic phase of an ischemia–reperfusion insult protects the reperfused heart against excessive ROS generation and cell death (Chen et al. 2008, 2012). Second, ROS generated during an ischemic insult are capable of damaging the electron transport chain (Kowaltowski and Vercesi 1999; Lesnefsky et al. 2001). Third, although cellular levels of ROS in the ischemic heart reach a concentration capable of enhancing reoxygenation injury they are not sufficient to trigger cell death (Robin et al. 2007). Fourth, attenuation of oxidative stress during simulated ischemia diminishes oxidative stress and cell death during the metabolic burst accompanying reoxygenation (Robin et al. 2007; Eaton et al. 1999). These findings suggest that oxidative damage during ischemia increases

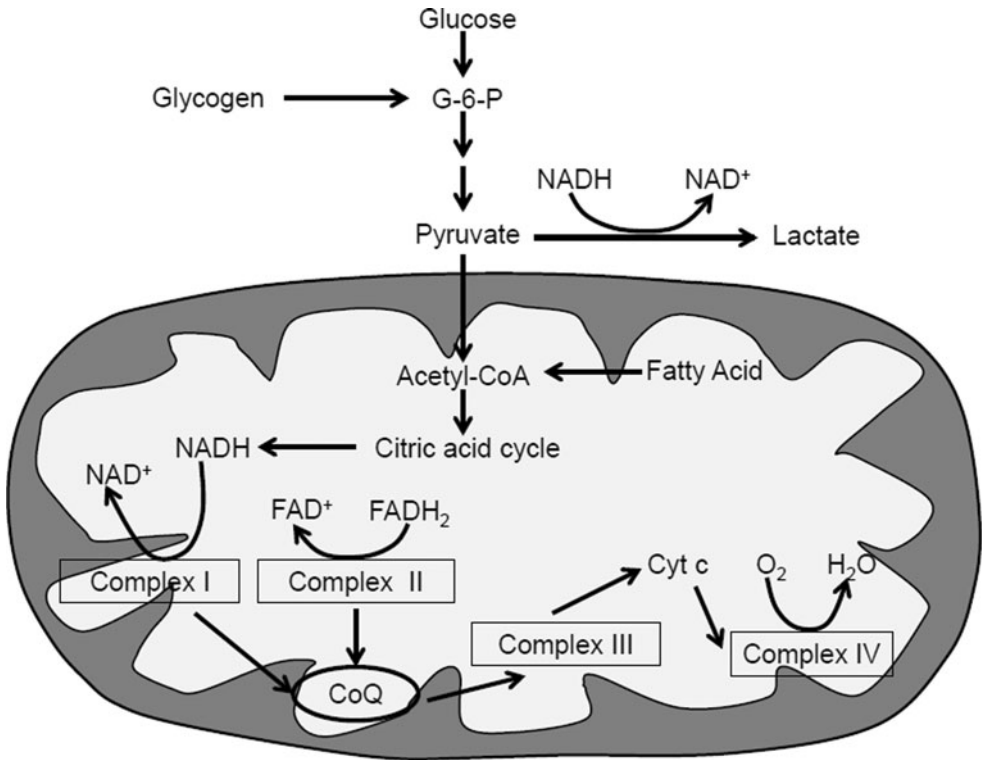

Fig. 1 Effect of ischemia on energy metabolism. During the initial phases of an ischemic insult, flux through the electron transport chain largely ceases, as oxygen deficiency prevents the reduction of oxygen to water by cytochrome c oxidase (complex IV). The formation of the bottleneck at cytochrome c oxidase leads to the accumulation of reducing equivalents by respiratory chain complexes upstream from complex IV and a rise in the NADH/NAD^+ and $FAD^+/FADH_2$ ratios. With the accumulation of reducing equivalents, metabolic pathways and energy metabolism within the mitochondria slow. In the cytosol, there is an initial increase in the rates of glycogenolysis and glycolysis. However, the mitochondrial defects and rise in the NADH/NAD^+ ratio limit the use of pyruvate by the citric acid cycle. The anaerobic pathways in the cytosol dominate, as the rise in the NADH/NAD^+ ratio causes the conversion of pyruvate to lactate, which in turn contributes to a decline in cellular pH_i. *G-6-P* glucose-6-phosphate

the capacity of the electron transport chain to generate damaging levels of ROS upon reperfusion (Chen et al. 2006, 2008; Robin et al. 2007). Although most studies have attributed mitochondrial damage during an ischemic insult to ROS generation by complexes I and III, ROS derived from NADPH oxidase during simulated ischemia contribute to oxidative damage in the perinuclear region of the cardiomyocyte (Hahn et al. 2011). In fact, inhibition of NADPH oxidase reduces the number of apoptotic cardiomyocytes during ischemia (Meischl et al. 2006).

The severity of ischemia–reperfusion injury is highly dependent upon the length of the ischemic phase of the insult, as reperfusion exhibits little benefit in cells severely compromised by the ischemic insult. However, in the absence of necrosis during the ischemic phase, reperfusion of the heart leads to significant restoration of contractile function. The "stunned myocardium" completely recovers contractile function during reperfusion, although the time required to repair the damage mediated by oxidative stress and calcium overload may exceed 24 h.

Cytoprotective pathways protect ischemia-reperfused heart

The mortality rate among patients suffering a myocardial infarction is high despite the use of optimal therapy. This observation has led to the search of interventions capable of protecting the heart during an ischemia–reperfusion insult. One such intervention is known as ischemic preconditioning, a phenomenon describing the beneficial effect of a short bout of ischemia/reperfusion prior to a more severe ischemia/reperfusion insult (Reimer et al. 1986). The brief period of ischemia/reperfusion triggers a signaling pathway that diminishes infarct size and improves recovery of contractile function arising from the severe insult (Yellon and Downey 2003). Interestingly, ischemic preconditioning is mimicked by treatment of the heart with several pharmacological cytoprotective agents, such as the opioids, bradykinin, adenosine and catecholamines (Costa et al. 2008). One of the key intermediate steps in the signaling pathway initiated by ischemic and

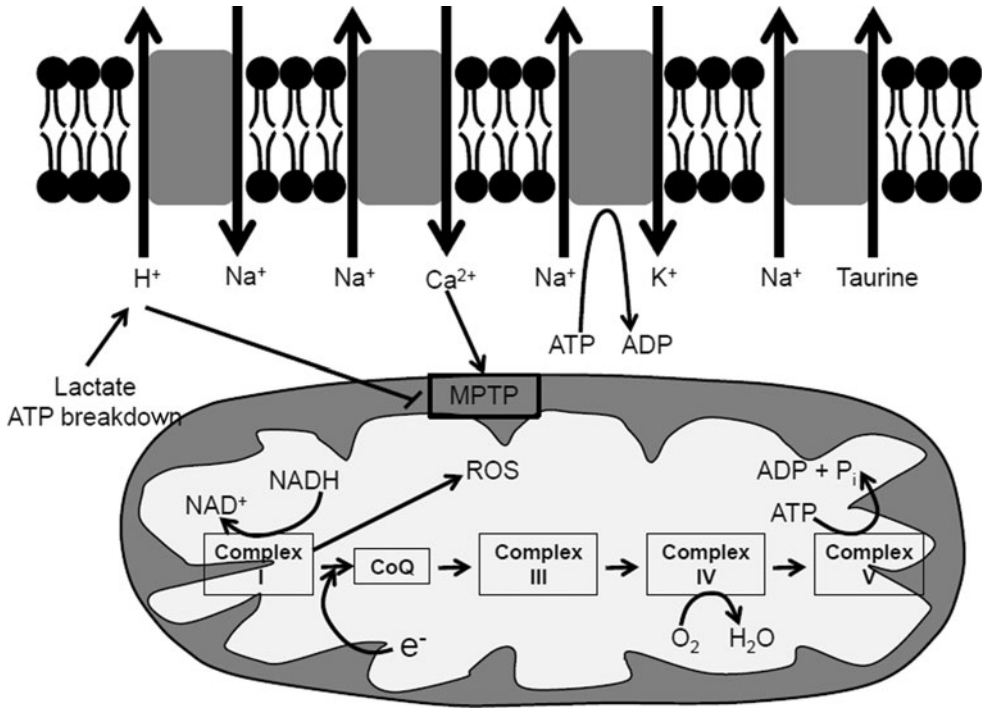

Fig. 2 Calcium overload triggers mitochondrial permeability transition. During ischemia the breakdown of ATP and the accumulation of lactate reduce pH_i. As $[H^+]_i$ concentration increases, the Na^+/H^+ exchanger facilitates the entry of Na^+ into the cell in exchange for H^+. Inhibition of the Na^+/K^+ ATPase also contributes to the increase in $[Na^+]_i$. Stimulation of the Na^+/Ca^{2+} exchanger promotes the cellular uptake of Ca^{2+} in exchange for Na^+, leading to Ca^{2+} overload. Although elevated $[Ca^{2+}]_i$ is a major initiator of the mitochondrial permeability transition (MPT) during an ischemia–reperfusion insult, low pH_i prevents the activation of the MPT during the ischemic phase of the insult. Upon reperfusion, pH_i rises and the MPT is initiated. However, the rise in $[Ca^{2+}]_i$ and initiation of the MPT is attenuated by another event, the loss of taurine and $[Na^+]_i$ from the cell, which reduces the amount of Na^+ available for Na^+/Ca^{2+} exchange

pharmacological preconditioning is the activation of protein kinase C-ε (Yellon and Downey 2003). Among other actions protein kinase C-ε prevents the activation of the mitochondrial permeability transition (MPT), a phenomenon that permeabilizes the mitochondrial membrane, leading to inhibition of oxidative phosphorylation and the release of pro-apoptotic factors from the mitochondria (Rasola and Bernardi 2011). Because calcium and oxidative stress act synergistically to induce the MPT, it is not surprising that calcium antagonists and antioxidants protect the heart against initiation of the MPT. Interestingly, although ischemic preconditioning diminishes the degree of oxidative stress and calcium overload in the ischemia-reperfused heart (Quarrie et al. 2011; Waldenstrom et al. 2012; Zhu et al. 2007), initiation of the cytoprotective signaling pathway appears to be the primary basis for the cardioprotection mediated by preconditioning (Ferdinandy et al. 2007). Indeed, other conditions that activate protein kinase C-ε, such as hyperglycemia, also precondition the cardiomyocyte (Schaffer et al. 2000).

Another cytoprotective pathway involved in preconditioning results in the activation of Akt, a kinase that phosphorylates several proteins, including glycogen synthase kinase-3β (GSK-3β). In contrast to most kinases, GSK-3β is constitutively active but is inactivated by phosphorylation. In its active, unphosphorylated state, GSK-3β favors the open state of the mitochondrial permeability transition (MPT) pore (Juhaszova et al. 2004; Das and Steenbergen 2012). On the other hand, phosphorylation-mediated inhibition of GSK-3β is associated with enhanced cardiomyocyte survival, cardiac hypertrophy and improved contractile function in pressure-overloaded hearts (Sussman et al. 2011). A host of agents, including hormones, cytokines, integrins and nutrients, exert their anti-apoptotic action largely through the activation of Akt. One of the most important of these agents is insulin, which protects the ischemic heart by initiating a cytoprotective signaling pathway that leads to the phosphorylation and activation of Akt. However, ischemic preconditioning also inactivates GSK-3β (Das and Steenbergen 2012). In some preconditioning pathways, both Akt and PKC-ε contribute to cardioprotection (Costa et al. 2008). Nonetheless, most scientists believe that irrespective of the initiator of the preconditioning pathways, cytoprotection results from the inhibition of the MPT.

Mitochondrial permeability transition

Evidence that irreversible opening of the MPT pore is the terminal event in ischemia–reperfusion injury has generated considerable interest in the structure and actions of the MPT pore. It has been known for several years that the MPT pore mediates mitochondrial permeabilization, which promotes mitochondrial swelling, collapse of the mitochondrial membrane potential, uncoupling of oxidative phosphorylation, ATP depletion, and cell death. The MPT pore was originally thought to be a nonspecific pore that spanned the mitochondrial membrane, allowing the movement of solutes less than $\sim$1.5 kDa in size across the membrane (Baines 2009). Although there has been considerable debate regarding the composition of the MPT pore (Baines 2009; Halestrap et al. 2007; Kokoszka et al. 2004; Woodfield et al. 1998), it is generally accepted that Bcl-2 and GSK-3β are regulators of the MPT pore (Das and Steenbergen 2012). According to Chen et al. (2001) overexpression of Bcl-2 renders the heart resistant to ischemia–reperfusion injury, an effect possibly related to the inhibition of ATP transport/turnover and the actions of pro-apoptotic factors (Imahashi et al. 2004). Bcl-2 also diminishes the degree of acidosis, which in turn restricts the rise in $[Ca^{2+}]_i$ in the ischemia-reperfused heart. Interestingly, inhibition of GSK-3β also slows the turnover of ATP while altering mitochondrial adenine nucleotide transport and the phosphorylation state of the voltage-dependent anion channel (VDAC), which serves as either a regulator or an active component of the MPT pore (Das et al. 2008). Interestingly, one of the kinases capable of phosphorylating VDAC is PKC-ε (Baines et al. 2003). VDAC can also undergo S-nitrosylation during ischemic preconditioning although the effects of VDAC S-nitrosylation on ischemia–reperfusion injury remain to be established (Kohr et al. 2011).

Ischemia is accompanied by depletion of ATP, an elevation in $[Ca^{2+}]_i$, and enhanced ROS production (Babsky et al. 2002; Quarrie et al. 2011). Although low pH_i restrains MPT pore formation during ischemia, ATP loss, elevated $[Ca^{2+}]_i$ and ROS generation facilitate pore formation (Halestrap et al. 2007; Murphy and Steenbergen 2008). However, low pH_i is a dominant regulator of the MPT pore, as it inhibits MPT pore formation despite the favorable status of ATP, ROS levels and $[Ca^{2+}]_i$. Upon reperfusion, the favorable events dominate, as pH_i is augmented, ROS generation is elevated and $[Ca^{2+}]_i$ is increased. In the reperfused heart, only stimulation of cytoprotective pathways, addition of antioxidants, administration of Ca^{2+} overload antagonists and MPT pore modulators are capable of overcoming the actions of the initiators of the MPT pore. Hence, the cytoprotective pathways and antioxidants represent effective therapy to protect the heart against reperfusion injury. However, it is likely that the use of agents opposing the opening of the MPT pore may be even more effective therapy than initiators of the cytoprotective pathways. Yet, despite the recent focus on the structure and function of the MPT pore, the development of more effective therapy awaits further study of the MPT pore.

Alteration in ischemia–reperfusion injury by taurine treatment and depletion

Taurine regulates several events that influence the outcome of an ischemia–reperfusion event, such as high energy phosphate metabolism, inflammation, maintenance of ion homeostasis and osmoregulation. This has provided the impetus for the study of taurine treatment and loss on ischemia–reperfusion injury. It is noteworthy that both taurine treatment and taurine deficiency protect the heart against an ischemia–reperfusion insult, a finding that underscores the multiplicity of taurine's actions. Indeed, the mechanisms involved in the cardioprotective effect of taurine treatment are very different from those involved in the effects of taurine depletion.

Loss of taurine from the ischemia-reperfused heart

During ischemia and hypoxia, cells accumulate osmotically active metabolites and ions that raise cardiomyocyte osmolality (Steenbergen et al. 1985). Because the cell membrane is vulnerable to excessive cell stretching, cell swelling initiates a compensatory protective mechanism, known as the regulatory volume decrease. As part of the regulatory volume decrease, both taurine and Na^+ are extruded from the cell. However, one of the enzymes involved in the extrusion of Na^+ during the regulatory volume decrease is the Na^+/K^+ ATPase, which remains depressed during ischemia. Thus, $[Na^+]_i$ continues to rise during the ischemic insult, as both the reduction in Na^+/K^+ ATPase activity and the influx of Na^+ via the Na^+/H^+ exchanger lead to Na^+ retention (Schaffer et al. 2002). However, as $[Na^+]_i$ approaches $\sim$20 mM, the efflux of Na^+ and taurine via the Na^+/taurine symporter becomes thermodynamically favorable (Suleiman et al. 1992). Although only modest amounts of taurine are lost from the heart during the ischemic insult, massive amounts of taurine leave the heart upon reperfusion. This massive efflux of taurine has been documented for various models of ischemia/hypoxia as well as for different cell types (Crass and Lombardini 1977; Kavianipour et al. 2003; Kramer et al. 1981; Schaffer et al. 2002; Song et al. 1998; Suleiman et al. 1997). Song et al. (1998) have proposed that three mechanisms exist for the release of taurine from the anoxic-reperfused heart: (a) release through

volume-activated ion channels, (b) phospholipase-mediated leakage across the cell membrane and (c) reversal of the Na^+-dependent transporter (Suleiman et al. 1992). Which of the three mechanisms is responsible for the extrusion of taurine from the ischemia-reperfused heart is pathologically important. While irreversible taurine loss is a measure of heart damage, reversible taurine loss can benefit the ischemic heart by promoting Na^+ efflux and reducing hyperosmotic stress. Interestingly, the amount of taurine released during bypass surgery is less when cold crystalloid medium is used during the procedure than if a blood cardioplegic solution is employed, indicating that taurine release is complex and can be regulated (Suleiman et al. 1997).

Effect of taurine treatment on ischemia–reperfusion injury

The first study examining the effect of taurine on ischemia–reperfusion injury employed a model of myocardial stunning (Kramer et al. 1981). In that study, working hearts were subjected to low flow, global ischemia in which the treated and untreated hearts were perfused with buffer containing or lacking 10 mM taurine, respectively. In relation to the untreated group, the taurine treated hearts showed no improvement in the recovery of cardiac work or the amount of nucleotides/nucleosides and creatine kinase released from the stunned myocardium. However, the hearts subjected to prolonged periods of ischemia lost more taurine and experienced greater stunning (reversible contractile dysfunction). Although these findings showed that taurine therapy did not affect the severity of stunning, they imply that intracellular taurine content may determine the degree of stunning, possibly by regulating a known regulator of myocardial stunning, such as ROS generation.

Using a model of low flow ischemia, Chahine and Feng (1998) found that taurine (10 mM) treatment reduced the extent of oxidative stress and diminished the incidence of myocardial arrhythmias. Unfortunately, the authors did not indicate if the reperfused hearts experienced reversible or irreversible tissue damage. A year later, Oz et al. (1999) examined the effect of variations in perfusate calcium (2.5 vs. 1.25 mM calcium), the vascular-dependent Ca^{2+} antagonist nifedipine, taurine and the combination of the three variables on ischemia–reperfusion injury. Although they reported a beneficial effect of taurine treatment on the ischemia-reperfused heart, the observation that taurine treatment led to more than 100 % recovery of contractile function was left unexplained. A more intriguing study was carried out by Hanna et al. (2004), who found that addition of 0.1 mM taurine to the drinking water for 6 months protected the perfused heart against ROS generation during an ischemia–reperfusion insult. However, the authors did not determine if intracellular taurine levels rose or if taurine altered some other process in the heart. A more complete study was performed by Ueno et al. (2007), who examined hearts subjected to global ischemia for 20 min followed by reperfusion for 1 h. Some hearts were treated for 10 min with buffer containing 10 mM taurine prior to the ischemic insult while the other taurine treatment group was exposed to buffer containing 10 mM taurine during the first 10 min of reperfusion. Although exposure of the heart prior to ischemia significantly reduced creatine kinase release and lipid peroxidation product formation, it only modestly improved contractile function. The most potent effect of taurine was observed in hearts treated with taurine containing buffer during the early phase of reperfusion, a time dominated by both Ca^{2+} accumulation and ROS formation. There have also been a number of studies reporting protective effects of taurine therapy by hearts or cultured cardiomyocytes subjected to a hypoxia-reoxygenation insult, a model associated with significant oxidative stress (Franconi et al. 1985; Sawamura et al. 1986; Takahashi et al. 2003). Taurine therapy has also proved beneficial in clinical conditions, such as bypass surgery and heart transplant (see "Clinical significance of taurine-mediated protection against ischemia–reperfusion injury").

Mechanisms underlying beneficial effect of taurine treatment

During an ischemia–reperfusion insult, both Ca^{2+} overload and oxidative stress contribute to the opening of the MPT pore (Halestrap et al. 2007). Therefore, it is significant that taurine treatment diminishes the degree of oxidative stress in the reperfused heart. According to Hanna et al. (2004), addition of taurine to the drinking water of rodents 6 months prior to an ischemia–reperfusion insult protects the heart against ROS generation. Moreover, Ueno et al. (2007) found that hearts perfused with buffer containing 10 mM taurine exhibit improved recovery of contractile function and diminished oxidative stress. Although neither study examined the effect of taurine treatment on intracellular and mitochondrial taurine content, the law of mass action argues that elevations in extracellular taurine concentration should diminish myocardial taurine release by transporters or channels located on the cell membrane. Recently, Jong et al. (2012) reported that taurine depletion leads to increased mitochondrial ROS generation by the isolated neonatal cardiomyocyte. Indeed, maintenance of the mitochondrial taurine pool is a crucial determinant of mitochondrial function, as the taurine pool provides substrate for one of the posttranslational modifications of $tRNA^{Leu(UUR)}$ (Suzuki et al. 2002). While taurine modified $tRNA^{Leu(UUR)}$ readily decodes UUG codons, a translational defect develops in cells harboring unmodified

$tRNA^{Leu(UUR)}$ tRNA (Kirino et al. 2004; Yasukawa et al. 2002). The mitochondria encoded proteins, which serve as both subunits and regulators of respiratory chain complex assembly, are essential for normal respiratory chain function. In accordance with this idea, Jong et al. (2012) found that mitochondrial respiration is reduced while mitochondrial superoxide generation is enhanced in the taurine-deficient cell. Thus, it is logical to assume that taurine treatment maintains normal levels of taurine in the mitochondria of the reperfused heart, ensuring a more efficient flow of electrons through the respiratory chain and minimizing mitochondrial ROS generation, which is the major cause of reperfusion injury.

Taurine treatment is also associated with a form of "osmotic preconditioning", a term coined to describe the cardioprotective phenomenon mediated by high concentrations of extracellular mannitol and taurine depletion (Pastukh et al. 2005). Among other effects, osmotic stress increases the phosphorylation and activation of Akt and initiates an Akt-dependent cytoprotective pathway. Taurine treatment also activates Akt, an effect blocked by inhibition of the Akt phosphorylating enzyme, PI 3-kinase (Takatani et al. 2004b). As seen in Fig. 3, the Akt-dependent signaling pathway activates PKC-ε, which inhibits formation of the MPT pore. Thus, like other forms of preconditioning, taurine treatment not only diminishes ROS generation but also reduces the impact of ROS on MPT pore formation.

Countering the beneficial effects of taurine treatment on Akt signaling and mitochondrial respiratory function are declines in the efflux of taurine and Na^+ from the ischemia-reperfused heart (Suleiman et al. 1992; Schaffer et al. 2002; Waldenstrom et al. 2012). In the untreated, reperfused heart, the loss of taurine and Na^+ from the cell restricts the amount of Ca^{2+} entering the cell via the Na^+/Ca^{2+} exchanger (Fig. 2). However, any increase in the extracellular levels of taurine (as in the taurine treated, reperfused heart) reduces the taurine gradient across the cell membrane, thereby restricting cellular taurine and Na^+ efflux via the Na^+/taurine symporter. Although there is some evidence that taurine may regulate the activity of some Ca^{2+} transporters (Satoh and Minoru 1997), Schaffer et al. (2002) found that the dominant effect of taurine treatment on $[Ca^{2+}]_i$ in cardiomyocytes subjected to simulated ischemia is the alteration in $[Na^+]_i$.

Effect of taurine depletion on ischemia–reperfusion injury

Reductions in intracellular taurine content also alter ischemia–reperfusion injury. According to Allo et al. (1997), taurine-deficient hearts subjected to 30 min of regional ischemia followed by 2 h of reperfusion exhibit a significant decline in infarct size/area at risk, with infarct size and intracellular taurine levels exhibiting a linear relationship. The effect of taurine depletion is completely reversible, as the increase in infarct size is completely prevented by restoration of the intracellular taurine pool prior to the ischemia–reperfusion insult. Another interesting observation from the study was that despite reductions in infarct size, the taurine-deficient hearts showed no improvement in contractile function, presumably because taurine is a key determinant of contractile function (Ito et al. 2008; Novotny et al. 1991). In a follow-up study, Schaffer et al. (2002) reported that taurine-deficient cardiomyocytes are also resistant to hypoxia-mediated apoptosis.

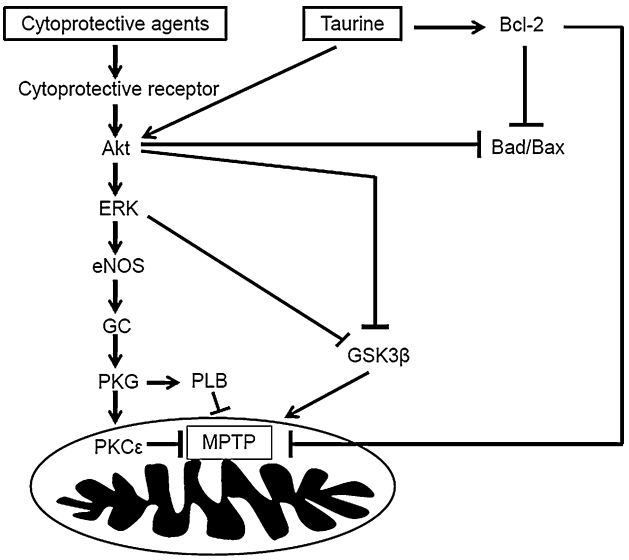

Fig. 3 Cytoprotective pathway that blocks mitochondrial permeability transition. Shown is one of the cytoprotective pathways initiated by pharmacological and ischemic preconditioning that prevents the activation of the MPT. In this pathway, a cytoprotective agent interacts with its receptor, promoting the phosphorylation and activation of Akt. Taurine treatment and depletion also lead to the activation of Akt. Downstream from Akt is eNOS (endothelial nitric oxide synthase), which activates guanylate cyclase, which in turn stimulates protein kinase G (PKG). Two targets of PKG are phospholamban (PLB) and PKC-ε, which inhibit the formation of the MPT pore (MPTP). The MPT is also inhibited by Bcl-2, a cytoprotective agent that is upregulated by taurine deficiency. Activation of the MPT is also mediated by GSK3β, which is under regulation by the inhibitory actions of Akt and ERK

Mechanism underlying the beneficial effect of taurine depletion

Jong et al. (2011) found that resistance of taurine-deficient cardiomyocytes to hypoxia-mediated apoptosis is related to decreased MPT pore formation. A probable explanation for the preconditioning-like effect of taurine depletion is the initiation of the Akt-dependent cytoprotective pathway, an

effect blocked by the inhibition of PI 3-kinase (Pastukh et al. 2005). However, taurine depletion also elevates cellular levels of Bcl-2 (Pastukh et al. 2005), a cytoprotective agent that not only blocks apoptosis but also protects the ischemic heart by reducing ATP turnover, preventing cytosolic acidification during ischemia and regulating the actions of VDAC, a regulator of component of the MPT pore (Imahashi et al. 2004). Because similar effects are mediated by high concentrations of mannitol and taurine treatment, taurine depletion also appears to initiate "osmotic preconditioning" (Pastukh et al. 2005).

Another cytoprotective mechanism of taurine depletion is attenuation of ischemia-mediated elevations in $[Ca^{2+}]_i$ (Schaffer et al. 2002), an effect shared by ischemic preconditioning. Schaffer et al. (2002) found that taurine depletion results in a net decrease in $[Na^+]_i$. Moreover, during simulated ischemia, the rise in $[Na^+]_i$ and $[Ca^{2+}]_i$ is significantly diminished in the taurine depleted cardiomyocyte. Thus, prevention of Ca^{2+} overload is the likely mechanism underlying attenuation of ischemia-mediated apoptosis in the taurine depleted cardiomyocyte.

Countering the beneficial effects of taurine depletion on Akt signaling, Bcl-2 content and ischemia-mediated $[Ca^{2+}]_i$ are elevations in ROS generation and reductions in ATP production. Jong et al. (2012) recently found that maintenance of a large mitochondrial taurine pool is required for normal electron transport chain activity. This pool serves as a source of substrate for the posttranslational conversion of the wobble position uridine of $tRNA^{Leu(UUR)}$ to 5-taurinomethyluridine, a reaction essential for efficient decoding of UUG (Kirino et al. 2004). According to Jong et al. (2012), the mitochondria encoded protein most affected by taurine depletion is ND6, whose mRNA contains 8 UUG codons. ND6 is not only a subunit of complex I of the respiratory chain, but is essential for the assembly and activity of complex I (Bai and Attardi 1998). By diminishing the levels of 5-taurinomethyluridine, taurine depletion decreases the efficiency of respiratory chain thereby favoring ROS generation, an effect exaggerated during ischemia–reperfusion.

Clinical significance of taurine-mediated protection against ischemia–reperfusion injury

Four recent studies show that taurine therapy may have clinical applications related to cardiac transplantation, bypass surgery and ischemia–reperfusion injury, conditions in which the heart undergoes periods of ischemia and reperfusion. First, Milei et al. (1992) found that patients receiving a rapid intravenous infusion of 5 g of taurine before bypass surgery exhibit fewer necrotic cells and less lipid peroxidation damage after completion of the procedure than patients infused with medium lacking taurine. Second, Oriyanhan et al. (2005) reported that arrested hearts stored in St. Thomas' cardioplegic solution containing 10 mM taurine are more resistant to storage-induced ischemic injury than arrested hearts stored for 6 h in cold cardioplegic solution lacking taurine. Third, in a related study, Sahin et al. (2011) found that taurine feeding (200 mg/kg/day) diminishes elevations in oxidative stress, inflammation and swelling during 5 h of cold isotonic storage. Fourth, Ueno et al. (2007) found that taurine treatment at the time of reperfusion protects the ischemic heart against reperfusion injury, including contractile dysfunction, creatine kinase release and lipid peroxidation. Taurine is a naturally occurring substance with little toxicity. Moreover, taurine therapy improves several areas of clinical concern in the ischemia-reperfused heart, including high energy phosphate generation, oxidative stress, inflammation and osmotic stress. Therefore, further studies to maximize the beneficial actions of taurine in the clinical setting are warranted.

In contrast to taurine treatment, taurine depletion is a poor strategy for protecting the ischemia-reperfused heart. Krieg et al. (2004) found that the augmented release of taurine from the ischemic heart is not the mechanism underlying preconditioning. Moreover, despite the reduction in infarct size mediated by taurine-deficient hearts, taurine deficiency does not improve the recovery of contractile function following an ischemia–reperfusion insult, an effect likely related to the important role of taurine in maintaining normal respiratory chain function. However, the massive amount of taurine lost from the heart during ischemia may have important clinical ramifications. Not only does taurine depletion adversely affect contractile function, but it leads to impaired ATP biosynthesis and oxidative stress. Thus, severe loss of taurine from an ischemia-reperfused heart might increase the risk of ventricular remodeling and future development of congestive heart failure.

Conflict of interest The authors declare that there are no conflicts of interest.

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