Role of ROS Production and Turnover in the Antioxidant Activity of Taurine

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Abbreviations

CAT	Catalase
DHLA	Dihydrolipoic acid
ETC	Electron transport chain
G6P	Glucose-6-phosphate
G6-PD	Glucose-6-phosphate dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
GST	Glutathione-S-transferase
LA	α-Lipoic acid
NNT	Nicotinamide nucleotide transydrogenase
PPP	Pentose phosphate pathway

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J. Marcinkiewicz, S.W. Schaffer (eds.), *Taurine 9*, Advances in Experimental Medicine and Biology 803, DOI 10.1007/978-3-319-15126-7_47

Prx3	Peroxiredoxin-3
RNS	Reactive nitrogen secies
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SR	Sarcoplasmic reticulum
Trx	Thioredoxin
TrxR	Thioredoxin reductase

1 Introduction

Taurine is a ubiquitous, semi-essential beta-amino acid found in very high concentration in the heart. One of its most widely recognized functions is its antioxidant activity (Pasantes-Morales and Cruz 1985; Zugno et al. 2007; Parvez et al. 2008; Cassol et al. 2010; Roy and Sill 2012). However, taurine does not function as a classical free radical scavenger, as its sulfur exists in a completely reduced state (sulfonic acid) and therefore is incapable of accepting more electrons (Arouma et al. 1988). Nonetheless, the beta-amino acid still is capable of functioning as an indirect antioxidant, either by diminishing the production of oxidants or by increasing the levels of the antioxidant defense system, which includes glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) (Tabassum et al. 2006; Das et al. 2010; Sevin et al. 2013; Taziki et al. 2013).

The antioxidant activity of taurine in the heart has been an area of considerable interest. The heart is dependent upon oxidative metabolism to generate ATP for muscle contraction. While the heart contains an abundant number of mitochondria for ATP generation, one untoward consequence of aerobic metabolism is the risk of excessive mitochondrial superoxide production, in which case the production of superoxide by the mitochondrial ETC exceeds the scavenging to reactive oxygen species (ROS) by the antioxidant defense enzymes, whose activity are generally low in the heart. In contrast to other members of the antioxidant defense system, the levels of the antioxidant, taurine, in the heart are extremely high, ranging from 3 mmol/kg in cow to 40 mmol/kg in mouse; levels in man are about 5 mmol/kg (Kocsis et al. 1976). These high intracellular levels of taurine require the accumulation of the amino acid from the blood. Inhibiting or knocking out the taurine transporter prevents both dietary and hepatically synthesized taurine from reaching the heart, causing intracellular levels of taurine to plunge and the heart to develop a cardiomyopathy (Novotny et al. 1991; Pion et al. 1992; Ito et al. 2008). It has been hypothesized that oxidative stress is responsible for the development of the taurine deficient cardiomyopathy. However, the mechanism underlying the antioxidant activity of taurine is an area of active research. The present review discusses the role of taurine in the regulation of oxidant production and turnover, as well as the effect of its antioxidant activity on contractile function.

2 Identity and Sources of Oxidants

Molecular oxygen is capable of accepting one, two or three electrons, forming in the process superoxide anion, hydrogen peroxide or hydroxyl radical, respectively. Superoxide is a weak oxidant but serves as a precursor of three highly reactive oxidants. In the presence of nitric oxide, superoxide is rapidly converted to peroxynitrite, a highly reactive oxidant belonging to a group of compounds referred to as reactive nitrogen species (RNS). Although the reaction between superoxide anion and nitric oxide is nonenzymatic, it is chemically favored, as it involves the reaction of the unpaired electron of nitric oxide with the unpaired electron of superoxide anion to form a product with paired electrons. Because of the limited availability of superoxide, the nonenzymatic peroxynitrite reaction competes with the superoxide dismutase-catalyzed formation of hydrogen peroxide, for superoxide anion. In the presence of modest to high levels of nitric oxide, the formation of peroxynitrite is favored over the dismutation of SOD. However, the conversion of superoxide anion to hydrogen peroxide proceeds when levels nitric oxide levels are either low or absent.

Hydrogen peroxide is a moderate oxidant capable of oxidizing electrophiles. In the presence of Fe^{2+}, superoxide is converted to a highly reactive oxidant, hydroxyl radical, a reaction that leads to the oxidation of Fe^{2+} to Fe^{3+}. This reaction, referred to as the Haber-Weiss reaction, is important in the heart because Fe^{2+} is prevalent, not only as free Fe^{2+}, but also in association with heme-containing proteins, such as myoglobin, cytochromes and oxidases/peroxidases. The t½ for hydoxyl radical in the cell is ~1 ns, indicating that it highly reactive, capable of readily transferring its unpaired electron to available electrophiles in its vicinity.

The heart is an anaerobic tissue that depends upon the availability of large amounts of ATP to drive contraction. In accordance with its ATP requirement, the major source of ROS in the heart is the mitochondrial electron transport chain (ETC), which supplies ATP for myocardial contraction. However, the ETC is also the major source of superoxide, which is formed when electrons divert from complexes I and III to the acceptor, oxygen. In normal mitochondria, electrons that enter the ETC at complexes I and II are passed on to ubiquinone which in turn passes the electrons on to complexes III and IV, the latter catalyzing the reduction of oxygen to water. This flow of electrons is closely coupled to the formation of a proton gradient, which is ultimately used to drive the biosynthesis of ATP. However, if flux of electrons through the ETC slows, electrons can be diverted away from the ETC to form superoxide anion. Conditions that lead to impaired ETC flux and the generation of superoxide by the ETC include ETC damage, impaired biosynthesis of ETC subunits, enhanced oxygen availability or excessive production of reducing equivalents.

Another major source of ROS in the heart is NADPH oxidase, an enzyme consisting of a central membrane-associated core surrounded by several cytosolic regulatory subunits (Nox1, -3, -4, -5, Duox1, -2, p47phox and p67phox). The classical isoform of NADPH oxidase is found in the neutrophil, where the enzyme serves as a source of ROS to attack foreign bodies. In the heart, NADPH oxidase initiates several signaling pathways in response to cellular stress (Jiang et al. 2011). Among the

targets of NADPH oxidase are apoptosis signal-regulating kinase-1, MAP kinase phosphatases, Akt and protein tyrosine phosphatases, all of which play a role in cell survival and cellular hypertrophy. Knocking out specific regulatory subunits of NADPH oxidase diminishes stress-induced responses, including cardiomyocyte hypertrophy, contractile dysfunction, ventricular remodeling and mortality in models of heart failure (Grieve et al. 2006; Doerries et al. 2007; Looi et al. 2008). Interestingly, two of the central neurohumoral factors, norepinephrine and angiotensin II, involved in the development of congestive heart failure, activate NADPH oxidase through a G-protein coupled pathway. Drugs that inhibit the actions of those two neurohumoral factors presently serve as the mainstay for the treatment of congestive heart failure.

The generation of ROS by xanthine oxidase has also been implicated in ischemia-reperfusion injury (Chambers et al. 1985). During ischemia, ATP is broken down to adenosine, which is subsequently converted to xanthine by the actions of adenosine deaminase, purine nucleotide phosphorylase and xanthine oxidase. The accumulation of xanthine by the ischemic heart results in the generation of ROS, as the conversion of xanthine to uric acid by xanthine oxidase results in the production of superoxide and hydrogen peroxide. Some investigators report that inhibition of xanthine oxidase protects the heart against ischemia-reperfusion injury (Chambers et al. 1985).

Inflammation is another major source of myocardial oxidative stress, as neutrophils are recruited to the heart in response to an ischemia/reperfusion insult, congestive heart failure, infective endocarditis and rheumatic heart disease. When activated during phagocytosis, neutrophils undergo a respiratory burst that leads to the generation of superoxide and other ROS, including hydrogen peroxide, hypochlorous acid, hydroxyl radical and single oxygen. These ROS are part of the armamentarium the neutrophil uses to protect the host against bacteria and other foreign invaders. However, in the diseased heart, the inflammatory response can damage normal tissue.

3 Effect of Taurine on ROS Production

The primary mechanism underlying the antioxidant activity of taurine appears to be linked to a conjugation reaction between taurine and the wobble uridine of $tRNA^{Leu(UUR)}$ forming the product 5-taurinomethyluridine-$tRNA^{Leu(UUR)}$. This post-translational conjugation reaction is important because it substantially strengthens the interaction between the UUG codon of leucine mRNA and the AAU anticodon of $tRNA^{Leu(UUR)}$. Thus, the decoding of UUG in the presence of conjugation-free $tRNA^{Leu(UUR)}$ is severely diminished, resulting in reduced expression of UUG dependent mitochondria encoded proteins. The most UUG dependent proteins are subunits of ETC complex I, therefore, in the taurine deficient heart the activity of complex I is dramatically reduced. The resulting decrease in ETC flux renders the mitochondria susceptible to superoxide production, as electrons are diverted away from complex I to the acceptor oxygen, forming superoxide anion. Recently, we found that taurine deficient cardiomyocytes and fibroblasts are oxidatively stressed, an effect reversed by taurine treatment (Jong et al. 2012). These data support the

view that taurine acts as an antioxidant, primarily by reducing the generation of superoxide by the ETC.

Li et al. (2009) have argued that the antioxidant activity of taurine also extends to NADPH oxidase, an important source of cytosolic ROS in the cardiomyocyte. They found addition of norepinephrine to the incubation medium of adult cardiomyocytes leads to cellular apoptosis, an effect they attributed to NADPH oxidase-mediated activation of calpain. Taurine treatment not only inhibited the activation of NADPH oxidase but also ROS-mediated activation of calpain and apoptosis. In their study, NADPH oxidase activity was defined as diphenyleneiodonium-sensitive activity. However, diphenyleneiodonium not only inhibits NADPH oxidase but also NADH ubiquinone oxidoreductase, an enzyme involved in the generation of superoxide by complex I of the mitochondrial ETC (Li and Trush 1998). Because taurine improves the status of complex I, it is logical to assume that the mitochondrial actions of taurine contribute to the reported inhibition of NADPH oxidase and norepinephrine-mediated apoptosis. Miao et al. (2013) also reported that taurine diminishes the expression of NADPH oxidase subunits in bacterial-induced mastitis. However, in the presence of an inflammatory response, taurine is capable of diminishing ROS production through the formation of taurine chloramine, which inhibits both the inflammatory response and NADPH oxidase activity through a reduction in the phosphorylation of p47phox and its association with the core subunits of NADPH oxidase. It is important to recognize that taurine might also influence NADPH oxidase activity by modulating NADPH content. It has been shown that taurine deficiency leads to an increase in reducing equivalents, which elevates the $NADH/NAD^+$ and $NADPH/NADP^+$ ratios (Mozaffari et al. 1986). Taurine deficiency also decreases glucose-6-phosphate (G6P) content, which is a substrate for G6P dehydrogenase (G6-PD), a major source of NADPH in the heart (Mozaffari et al. 1986). Thus, more work is warranted to investigate a possible link between NADPH oxidase deactivation and taurine exposure.

Oxidative stress is a major cause of injury in the ischemic-reperfused heart (Murphy and Steenbergen 2008). During the ischemia phase of an ischemia-reperfusion insult, ROS contributes to oxidative damage within the mitochondria that renders the heart susceptible to a burst of ROS generation upon reperfusion. Ueno et al. (2007) found that taurine treatment protects the heart against reperfusion injury, which appears to be largely caused by the generation of superoxide by the mitochondria. By minimizing damage to the ETC, taurine should protect the heart against excessive mitochondrial ROS generation (Schaffer et al. 2014). However, there is also evidence that the generation of superoxide by xanthine oxidase also contributes to oxidative damage during reperfusion (Chambers et al. 1985). According to Das and Sil (2012), taurine is capable of reducing xanthine oxidase activity in diabetic kidney. Although the mechanism underlying the reduction in xanthine oxidase activity was not examined, it is possible that taurine, by reducing mitochondrial ROS generation, minimizes oxidative activation of xanthine oxidase and the production of more ROS.

One of the most important functions of taurine is the neutralization of hypochlorous acid and the formation of taurine chloramine, which serves as an important anti-inflammatory agent (Marcinkiewicz and Kontny 2014). These reactions play a central role in the antioxidant activity of taurine.

4 Identity and Sources of Antioxidant Defense System

SOD, an enzyme that catalyzes the conversion of superoxide to hydrogen peroxide, exists as several isoforms. In humans, there are three isoforms; CuZnSOD which is present in the cytosol, Mn-SOD which is mitochondrial and contains manganese at the active site, and extracellular SOD (EC-SOD) (Valko et al. 2006). Mn-SOD is one of the most effective antioxidant enzymes, capable of eliminating superoxide anion in the matrix or on the inner side of the inner mitochondrial membrane (Turrens 2003). Surai et al. (1999) investigated the antioxidant profile of various tissues in newly hatched chick and found that the heart contains higher Mn-SOD activity than other tissues. The SOD content of neonates is lower than that of adults and can be further reduced by hypoxia although glucose treatment restores activity to nearly control levels in the presence of hypoxia (Anju et al. 2009). On the other hand, treatment with 100 % oxygen following a hypoxic insult causes a further increase in ROS levels, an effect associated with the induction of SOD. The combination of glucose, epinephrine and oxygen also decreases SOD activity.

CAT catalyzes the conversion of hydrogen peroxide to water. It is a membrane bound enzyme found in the peroxisomes and in heart mitochondria but not in the mitochondria of other tissues (Dhalla et al. 2000; Turrens 2003). Although the activity of CAT is low in the myocardium, it plays an important role in protecting the heart from ischemia-reperfusion insults (Dhalla et al. 2000). Because the active site of CAT binds two moles of hydrogen peroxide, the reaction does not proceed at low levels of hydrogen peroxide. The enzyme is phosphorylated and stimulated by non-receptor tyrosine kinases, c-Abl and Arg, which increase CAT activity at low hydrogen peroxide concentrations (Rhee et al. 2005). It has also been shown that CAT activity is suppressed under hypoxic conditions in the neonatal rat heart. This suppression is ameliorated by glucose supplementation, but if oxygen and epinephrine treatment is combined, the ameliorative effect of glucose is abolished, an effect analogous to that of SOD (Anju et al. 2009).

Vitamin C (ascorbic acid) is a powerful non-enzymatic antioxidant that protects membranes against oxidation. It functions in an aqueous environment, such as in lungs and lens. Almost 99.9 % of vitamin C exists as $AscH^-$, which can react with free radicals to produce semidihydroascorbate radical, which is surprisingly rather nonreactive (Valko et al. 2006).

Vitamin E, which is present in eight different forms, functions as an antioxidant in hydrophobic environments. The major membrane-bound antioxidant in humans is α-tocopherol, which is highly effective against the process of lipid peroxidation. A synergistic reaction involving vitamin C and α-tocopherol results in the oxidation of α-tocopherol to an α-tocopherol radical and the reduction of ascorbic acid (Valko et al. 2006). The α-tocopherol radical and the reduced form of ascorbic acid can also couple with α-lipoic acid and glutathione (Fig. 1). Both vitamins C and E are found in the cytoplasm of the heart. Although there is evidence that vitamin E protects the ischemic heart, some epidemiological data have yielded contradictory results, revealing the need of further experimentation in human subjects.

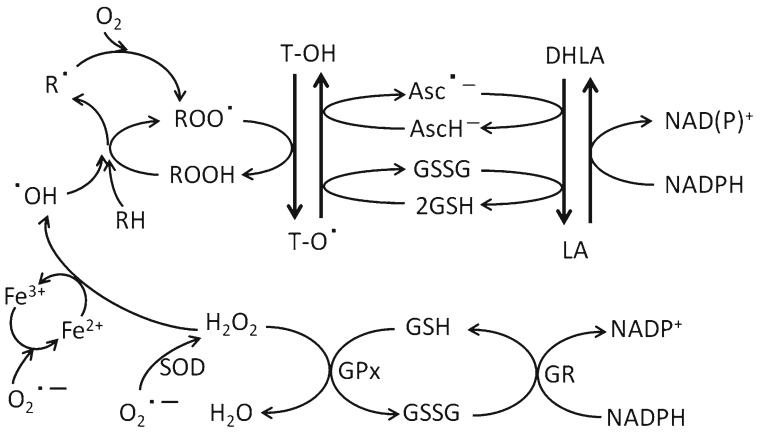

Fig. 1 Major antioxidant defense mechanisms. Superoxide anion ($O_2^{\cdot-}$) is converted to hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD). H_2O_2 in turn is converted to H_2O by glutathione reductase (GPx) in a reaction that is coupled to the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase (GR) reduces GSSG to GSH using NADPH as the reducing agent. Alternatively, H_2O_2 can be reduced to hydroxyl radical ($OH^{\cdot}$) via the Haber-Weiss reaction. Hydroxyl radical is extremely reactive and extracts unpaired electrons from a fatty acid (RH) or a lipid radical ($R^{\cdot}$), leading to the production of a lipid peroxy radical ($ROO^{\cdot}$). Vitamin E (T-OH) reduces the peroxy radical to a hydroperoxide but is converted to a vitamin E radical in the process. Active vitamin E is restored by the reduction of the radical by either GSH or ascorbic acid ($AscH^{-}$). Dihydrolipoic acid (DHLA) is capable of reducing GSSG and Asc^{*-}

Thioredoxin (Trx) is a small multifunctional, disulfide-containing protein, which is found at levels 100–10,000-fold less than those of GSH. Trx can undergo a coupled redox reaction, in which two sulfhydryl groups of Trx are converted to a disulfide unit while a disulfide bridge of a protein is reduced to two sulfhydryls. Thioredoxin reductase (TrxR) uses NADPH to catalyze the reduction of oxidized Trx to its reduced form (Valko et al. 2006). Both Trx and TrxR have isoforms; Trx1 and TrxR1 are localized to the cytosol while Trx2 and TrxR2 are found in the mitochondria. TrxR2 is a FAD-containing selenoenzyme that reduces the disulfide form of Trx2 using matrix NADPH (Hurd et al. 2005). Trx2 activity resembles that of peroxiredoxin-3 (Prx3), which is present exclusively in the mitochondria and rapidly reacts with hydrogen peroxide. In addition to detoxifying peroxynitrite, it scavenges as much as 90 % of the available hydrogen peroxide. It has been suggested that mitochondrial oxidative stress plays an important role in the development of heart failure. Hence, mitochondrial specific antioxidants reduce the risk of heart failure by scavenging damaging ROS (Marí et al. 2013; Murphy 2012; Tsutsui et al. 2009).

LA is a disulfide derivative of octanoic acid, also called thioctic acid. Because it is both water and fat-soluble, it is distributed in both cellular membranes and the cytosol. It is rapidly converted to its reduced dithiol form, dihydrolipoic acid (DHLA). Both LA and DHLA are strong antioxidants that function as scavengers of

ROS, regenerators of other antioxidants, chelators of redox metals and activators of oxidized proteins (Valko et al. 2006). The oxidation of DHLA is coupled to the reduction of oxidized glutathione (GSSG) and ascorbate, which in turn reduces α-tocopherol radical to regenerated vitamin E (Fig. 1). Recently, LA was found to attenuate diabetes-associated upregulation of p22phox and p47phox expression, leading to a reduction in NADPH-induced ROS generation. Several studies have reported that LA is cardioprotective, but further studies are required to establish its effectiveness in the heart relative to that of the other antioxidants (Ghibu et al. 2009).

Carotenoids are pigments found in plants and microorganisms. Their conjugated double-bonds play an important role in their antioxidant activity, which involves the protection of lipids against peroxidative damage (Valko et al. 2006). Lycopene, one of the most abundant dietary carotenoids, protects against congestive heart failure even at relatively low levels (Lennie et al. 2013). Vitamin A, also one of the carotenoids, is reported to significantly reduce isoproterenol-induced myocardial injury, presumably by diminishing oxidative stress and stabilizing membranes (Pipaliya and Vaghasiya 2012). β-carotene (pro-vitamin A), a carotenoid contained in human diet, fruits and colored vegetables, is present in the cytoplasm of the heart. It reportedly increases the GSH/GSSG ratio in the heart and liver of rats and to prevent collagen biosynthesis and fibrosis (Novo et al. 2013). Although many epidemiologic studies have suggested that higher plasma β-carotene content is associated with a lower risk of heart disease, several clinical trials have claimed that β-carotene supplementation has either no or a negative effect on the risk of heart disease (Voutilainen et al. 2006). It is likely that β-carotene consumed in the diet benefits the heart, while high levels of β-carotene used in clinical trials might have an adverse effect.

Polyphenols are classified into four categories, based on the number of phenolic rings and structural moieties (phenolic acids, flavonoids, stilbenes and lignans). For example, flavonoids contain a diphenylpropane moiety consisting of two aromatic rings linked through three carbons that together usually form an oxygenated heterocyclic compound. Flavonoids are commonly synthesized by plants as second metabolites and constitute the most important single group of polyphenols. They are subclassified into six groups: flavonols, flavones, isoflavones, flavanones, anthocyanidis and flavonols. The antioxidant activity of polyphenols resides in their ability to induce antioxidant enzymes, such as SOD, CAT, GST and Gpx. Polyphenols protect the heart by inhibiting ischemia/reperfusion injury, hyperlipidemia, hypertension, inflammation, atherosclerosis and age-related changes (Khurana et al. 2013; Valko et al. 2006). They also chelate trace metals, which presumably contributes to their antioxidant activity (Malireddy et al. 2012).

5 Glutathione-Linked ROS Scavenging System

GSH is a tripeptide, thiol-containing, multifunctional intracellular non-enzymatic antioxidant. It is found in fairly high concentrations throughout the cell, including the cytosol (1–11 mM), the nucleus (3–15 mM) and the mitochondria (5–11 mM).

During its reaction with other radicals, GSH is oxidized into a thiyl radical; two thiyl radicals can then dimerize into GSSG. Thus, the GSH/GSSG ratio is a good marker of oxidative stress. Although GSH is a reducing agent, GSSG can react with a protein sulfhydryl group to form a protein–glutathione-mixed disulfide.

The redox state of GSH is determined in part by nonenzymatic reactions involving proteins, ROS and vitamins. It not only serves as a scavenger of hydroxyl radicals and singlet oxygen but is also capable of regenerating vitamins C and E and ensuring the proper sulfhydryl content of proteins (Fig. 1).

Several key enzymes also alter the redox state of glutathione. One of those enzymes is GPx, which exists in two forms. One of the forms is a selenium-independent GST while the other is a selenium-dependent GPx (Valko et al. 2006). Hydrogen peroxide, which is formed during the dismutation of superoxide anion by SOD, is largely decomposed by GPx (Turrens 2003). The GPx reaction uses GSH to neutralize either hydrogen peroxide or organic peroxides (ROOH), with the former converted to water and the later to alcohol. In the process, GSH is oxidized to GSSG (Oka et al. 2012). By comparison, GST catalyzes the destruction of organic peroxides but not that of hydrogen peroxide (Turrens 2004). There are several isoforms of GPx, the major isoform being GPx1, which is localized mainly in the cytosol where it scavenges hydrogen peroxide. GPx4, which is attached to the inner mitochondrial membrane facing the matrix, readily neutralizes lipid hydroperoxides and is therefore recognized for its ability to protect membranes against oxidative damage. The mitochondria, which are a major source of ROS, contain significant levels of both GSH and GPx (Marí et al. 2013; Murphy 2012). Interestingly, the heart of newly hatched chicks contains high GPx levels. GPx is thought to contain much higher hydrogen peroxide scavenging activity compared with catalase or SOD in rat heart (Dhalla et al. 2000; Tsutsui et al. 2006). The heart from newly hatched chicks contains higher GPx dependence than other tissues (Surai et al. 1999).

Glutathione reductase (GR) couples the reduction of GSSG to the oxidation of NADPH. In the cytosol, the GR reaction is dependent upon the availability of NADPH formed by two reactions of the pentose phosphate pathway (PPP). The first step, which is rate limiting, results in the generation of NADPH as G6P is oxidized to 6-phosphogluconic acid. Because the PPP is not very active in the heart, the competition between G6-PD of the PPP and phosphohexose isomerase of the glycolytic pathway for available G6P is a major determinant of NADPH generation by the PPP. Also regulating G6-PD activity is the content of NADPH, which feedback inhibits the formation of more NADPH, and GSSG, which reverses the effects of NADPH (Zimmer 1992).

6 Antioxidant Defense System of the Heart

Mitochondria are plentiful in the heart, therefore, the heart requires an active antioxidant system to maintain the balance between ROS production, which is very active in heart, and ROS neutralization, which involves the antioxidant enzymes and the GSH redox system. The antioxidant enzymes located in the mitochondria

include Mn-SOD, CAT, GPx and GR. However, a central role in the maintenance of redox balance falls on the NADPH-dependent enzymes (GR, Trx, peroxiredoxin III, and glutaredoxin). NADPH in the mitochondria is mainly supplied by three pathways: $NADP^+$-dependent isocitrate dehydrogenase, malic enzyme and nicotinamide nucleotide transhydrogenase (NNT), with NNT responsible for more than 50 % of mitochondrial NADPH formation. NNT catalyzes the reduction of $NADP^+$ to NADPH, a half reaction coupled to the oxidation of NADH to NAD^+ (Yin et al. 2012; Garcia et al. 2010). Also important is the mitochondrial GSH:GSSG ratio, which is normally greater than 100:1. When ROS production overwhelms the antioxidant defense system, GSH is oxidized to GSSG, although there is no transporter to export GSSG out of the mitochondria. Hence, to maintain the redox state, GSSG is used in the glutathionylation of proteins. It is known that complexes I and IV, aconitase, and pyruvate dehydrogenase can undergo glutathionylation, a reaction that decreases their activities. However, glutathionylation of cardiac proteins has been implicated in several cardiovascular diseases (Pastore and Piemonte 2013).

7 Effect of Taurine on Antioxidant Defense System

Most of the studies examining the link between taurine and the antioxidant defense system have focused on the liver, which is a unique tissue because taurine is both synthesized in the liver and used by the liver to conjugate bile acids and $tRNA^{Leu(UUR)}$. Oxidative stress has been commonly associated with reductions in the levels and activities of SOD, CAT, GPx, GST, GSH, GR and G6-PD in the liver, an effect prevented by taurine treatment (Das et al. 2010; Devi and Anuradha 2010; Hagar 2004; Pushpakiran et al. 2004; Tabassum et al. 2006; Taziki et al. 2013). These findings are consistent with the view that oxidative stress damages the antioxidant enzymes. By minimizing the degree of oxidative stress, taurine prevents the decline in activity of these antioxidant enzymes.

As in the liver, taurine treatment of the oxidatively-stressed heart is associated with reversal of ROS-mediated reductions in antioxidant enzyme activity (Pushpakiran et al. 2004; Shiny et al. 2005; Sahin et al. 2011; Yang et al. 2013). In a related study, Pansani et al. (2012) showed that taurine deficiency is associated with a decrease in the activities of myocardial GPx and CAT. Although most reports administer large amounts of taurine to oxidatively-stressed animals, it is relevant that β-alanine-mediated taurine deficiency promotes the decline in GPx and CAT, suggesting that intracellular taurine levels might regulate the activity of the antioxidant defense system. However, in a study in which taurine levels were examined, there was no correlation between changes in the activity of the antioxidant enzymes and taurine levels (Anand et al. 2011). Nonetheless, the preponderance of evidence suggests that taurine treatment is capable of reducing the degree of oxidative damage to the antioxidant enzymes. Remaining to be determined is whether physiological levels of taurine modulate the activity of the antioxidant defense system. Moreover, the possibility that taurine might alter the expression of the antioxidant defense enzymes should be considered.

8 Effect of ROS and Taurine on Contractile Function

It is widely recognized that ROS diminish contractile function, a process thought to contribute to both acute cardiac injury and chronic development of heart failure. In this section, we focus on the primary mechanisms involved in ROS-mediated acute cardiac injury and contractile dysfunction. The role of ROS in the development of cardiac hypertrophy and congestive heart failure has been the topic of a recent review article by Ito et al. (2014).

Maximal force of contraction depends upon the viability of the muscle proteins, the rate of ATP biosynthesis and proper handling of Ca^{2+}. Although the most important action of taurine in the heart appears to be the maintenance of normal ETC function, moderate depletion of taurine does not alter cellular ATP levels (Mozaffari et al. 1986). However, impaired ETC function often leads to excessive mitochondrial ROS production, which can contribute to Ca^{2+} mishandling by the heart. Therefore, the present section is limited to the regulation of Ca^{2+} movement and contractile function by ROS and taurine.

The rate of cardiac contraction is normally determined by the rate at which the sinoatrial nodal pacemaker cells generate their stimulatory impulses. When these impulses reach the ventricle, they activate Na^{+} channels to cause depolarization of the cardiomyocyte. The resulting change in membrane potential activates the voltage-dependent L-type Ca^{2+} channels situated on the cell membrane. Once activated, these channels remain open for a short period of time, during which Ca^{2+} enters the cell. Satoh and Sperelakis (1993) have reported that exposure of isolated chick cardiomyocytes to medium containing 10^{-7} M Ca^{2+} and 20 mM taurine inhibits Ca^{2+} transport by the L-type Ca^{2+} channel, however, they did not examine the effect of physiological concentrations of extracellular taurine (normally ~50 μM) at physiological concentrations of plasma Ca^{2+} (normally ~2.5 mM). There is also some evidence that ROS can inhibit L-type Ca^{2+} channel activity, however, taurine lacks free radical scavenging activity and would be incapable of directly reducing the levels of both hydroxyl radical and hydrogen peroxide (Zima and Blatter 2006). Thus, the L-type Ca^{2+} channel is unlikely to be a site of taurine action.

Although inhibition of the L-type Ca^{2+} channel abolishes myocardial contractile function, the amount of Ca^{2+} entering the cell via the L-type Ca^{2+} channel is insufficient to maximally stimulate contraction. Indeed, L-type current is a minor source of Ca^{2+} compared to the next step in the Ca^{2+} cycle, the release of Ca^{2+} from the intracellular Ca^{2+} storage vesicles of the sarcoplasmic reticulum (SR). The size of the SR Ca^{2+} stores ensures an important role for the SR in both normal contraction and heart failure. ROS are capable of oxidizing key cysteine residues of the SR channels involved in Ca^{2+} release, known as the Ca^{2+}-gated SR Ca^{2+} channels. Modification of these channels contributes to the development of heart failure by promoting Ca^{2+} leakage from the SR Ca^{2+} storage vesicles (Zima and Blatter 2006; Terentyev et al. 2008). Thus, the antioxidant activity of taurine might improve contractile function by preventing SR Ca^{2+} leakage although high extracellular taurine (20 mM) does not directly influence SR Ca^{2+} leakage from skinned skeletal muscle

fibers (Bakker and Berg 2002). Thus, the SR Ca^{2+} release channels (ryanodine channels) are unlikely to be major sites of taurine action.

In contrast to the ryanodine receptor, taurine has a significant influence on the SR Ca^{2+} ATPase (Steele et al. 1990; Bakker and Berg 2002). The SR vesicles are surrounded by a medium rich in taurine (5–30 mM), therefore, the effects of taurine on Ca^{2+} uptake by the SR Ca^{2+} pump are physiologically important. Steele et al. (1990) found that at a submaximal concentration of Ca^{2+} (0.12 μM) taurine exposure (over a concentration range of 0.01–40 mM) increased Ca^{2+} loading of the SR, thereby promoting an increase in caffeine-induced contracture of the skinned rat heart. On the other hand, 30 mM taurine reduced the amplitude of caffeine-induced contracture of skinned rat heart bathing in medium containing a comparatively high Ca^{2+} concentration (0.47 μM). Thus, in the hypodynamic heart exposed to low concentrations of Ca^{2+}, physiological concentrations of taurine increase contractile function by facilitating Ca^{2+} loading of the SR. However, in the Ca^{2+} overloaded heart, taurine decreases contractile function. The mechanism underlying this action of taurine has not been established. However, recently we found that taurine depletion leads to a decline in SR Ca^{2+} ATPase activity and in the phosphorylation state of phospholamban, both effects that are consistent with the actions of taurine on SR Ca^{2+} uptake. It remains to be determined if ROS contribute to the reduction in the phosphorylation state of phospholamban and in SR Ca^{2+} ATPase activity. We have found that β-alanine-mediated taurine loss is associated with prolongation of cardiomyocyte Ca^{2+} transients, delayed relaxation of the taurine deficient heart and an elevation in cardiomyocyte ROS content (Schaffer et al. 2000; Jong et al. 2012). Although it has been established that ROS inhibit SR Ca^{2+} ATPase activity (Zima and Blatter 2006), a key experiment has not been performed, namely, determining the effect of antioxidant therapy on myocardial relaxation and ROS content.

9 Conclusion

One of the most important actions of taurine is its antioxidant activity. It has been documented that taurine is not a characteristic scavenger of ROS. Instead, several indirect mechanisms contribute to its antioxidant activity, including reducing ROS production by complex I of the ETC, limiting the activation of xanthine oxidase and interfering with ROS-producing inflammatory reactions. Taurine treatment also elevates the levels of the antioxidant defense system, largely by preventing the loss of the antioxidant enzymes through oxidative damage.

The development of a taurine deficient cardiomyopathy can be traced to a decline in the antioxidant activity of the heart. Not only does the accumulation of ROS in the taurine deficient heart cause cardiomyocyte death, but it leads to impaired handling of Ca^{2+}. Consequently, both systolic and diastolic function of the heart are impaired, as SR Ca^{2+} pump activity is reduced.

Acknowledgements This work was funded by a grant from Taisho Pharmaceutical Co.

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