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Monoamine oxidases as sources of oxidants in the heart

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Abstract

Oxidative stress can be generated at several sites within the mitochondria. Among these, monoamine oxidases (MAO) have been described as a prominent source. MAO are mitochondrial flavoenzymes responsible for the oxidative deamination of catecholamines, serotonin and biogenic amines, and during this process they generate H₂O₂ and aldehyde intermediates. The role of MAO in cardiovascular pathophysiology has only recently gathered some attention since it has been demonstrated that both H₂O₂ and aldehydes may target mitochondrial function and consequently affect function and viability of the myocardium. In the present review, we will discuss the role of MAO in catecholamine and serotonin clearance and cycling in relation to cardiac structure and function. The relevant contribution of each MAO isoform (MAO-A or -B) will be discussed in relation to mitochondrial dysfunction and myocardial injury. Finally, we will examine both beneficial effects of their pharmacological or genetic inhibition along with potential adverse effects observed at baseline in MAO knockout mice, as well as the deleterious effects following their over-expression specifically at cardiomyocyte level.

Keywords

monoamine oxidase; oxidative stress; mitochondrial dysfunction; heart failure; ischemia/reperfusion injury

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1 Introduction

Although it is generally accepted that mitochondria are the major source of reactive oxygen species (ROS) in cardiac myocytes [1–4], relevant issues, such as sites at which ROS formation occurs, control mechanisms, relationships between formation and removal reactions and the relative contribution of the various processes to the total accumulation of ROS are far from being elucidated conclusively. Mitochondria contain several enzymes that catalyze ROS formation either as the obligatory product or as the result of an occasional, possibly undesired, reaction.

1.1 Occasional ROS formation within mitochondria

This latter possibility is exemplified by the mitochondrial respiratory chain. A minor fraction of the electrons (about 0.1%) flowing through the transport chain is diverted causing the partial reduction of O₂ into superoxide [3]. This process occurs at the level of the first three complexes where flavins or quinones are able to act as single electron donors. In isolated mitochondria the relative contribution of sites I_F, I_Q, II_F and III_{Q_o} (the roman number indicates the complex and the letter specifies the involvement of flavin or quinone moieties) appears to depend on the substrate utilized [5]. The electron detour at these upstream sites is favored when flow is hampered downstream as a result of either protein alterations in respiratory complexes or inhibitory effects of toxicants.

Superoxide that does not cross the inner mitochondrial membrane is rapidly dismutated into the freely permeable H₂O₂ by Mn-superoxide dismutase (Mn-SOD). The finding that Mn-SOD deficient mice develop ROS toxicity and dilated cardiomyopathy [6], underlines the importance of ROS in this pathology and mitochondria as their source and target. This concept is further supported by the beneficial effects afforded by targeting catalase expression in mitochondria [7–10].

Besides superoxide generation by respiratory chain complexes, several other mitochondrial enzymes have been described as potential ROS producers. These include (but are not limited to) the flavin containing glycerol-3-phosphate-, proline- and dihydroorotate-dehydrogenase at the outer leaflet of the inner mitochondrial membrane, the electron transfer flavoprotein-ubiquinone (ETF:Q) oxidoreductase system of fatty acid β-oxidation within the inner mitochondrial membrane, and pyruvate- and 2-oxoglutarate dehydrogenase within the mitochondrial matrix [5]. All these enzymes and respiratory complexes normally catalyze reactions other than ROS formation, that are required for energy metabolism, cell function and viability maintenance. Their characterization as ROS forming enzymes has been carried out in isolated mitochondria by means of inhibitors or non-physiological procedures, such as glutathione depletion. Obviously, these approaches can hardly be adopted in living cells or tissues without jeopardizing a wide array of vital functions. This is a major caveat that eventually does not allow obtaining definite evidence that these potential ROS sources contribute to oxidative stress *in vivo*. If these processes were the only ones responsible for mitochondrial ROS formation, it would be actually impossible to demonstrate that these organelles are primarily involved in oxidative stress in living organs. In fact, oxidative changes within mitochondria could be just the result of alterations caused by ROS formed at other cellular sites. However, this is not the case because mitochondria contain other

enzymes that generate hydrogen peroxide (H_2O_2) as a direct and obligatory product. Inhibition of these enzymes does not affect other energy-linked functions and it provides convincing evidence of mitochondrial ROS formation *in vivo* and its role in pathophysiology of many organs including the cardiovascular system.

1.2 Obligatory ROS formation within mitochondria

p66^{Shc} is a cytosolic adaptor protein that upon stress translocates to mitochondria where it catalyzes electron transfer from cytochrome c to oxygen [11], a process that can result in the formation of ROS. Indeed, ROS generation is reduced in cells lacking p66^{Shc} and in p66^{Shc-/-} mice, whose lifespan is increased by 30% [11–14] in a protected environment [15]. Furthermore, genetic deletion of p66^{Shc} protects against ischemia/reperfusion (I/R) injury in mice hearts [16] and brain [17] and diabetic complications such as cardiomyopathy, nephropathy, delayed wound healing, and endothelial dysfunction [18–21]. Nicotinamide adenine dinucleotide phosphate oxidase 4 (Nox4) is another ROS generating enzyme that localizes in the plasma membrane but also intracellularly, in the mitochondria, focal adhesions, nucleus, endoplasmic reticulum. Nox4 associates with p22^{phox} for its activation, and, unlike other Noxs, generates H_2O_2 in preference to superoxide [22]. Nox4/p22^{phox} appears to be constitutively active [23], although several studies have shown that Nox4 activity can be modulated by different stimuli [24–27]. Mice in which Nox4 is targeted in a cardiac-specific manner demonstrate that Nox4 is both protective and injurious in models of cardiac pressure overload [28, 29]. Furthermore, while certain studies reported Nox4 to be deleterious, contributing to mitochondrial dysfunction and several pathologies such as ischemic stroke, diabetic cardiomyopathy, vascular inflammation and remodeling [30–32], others concluded that Nox4 might be vascular-protective rather than vascular-damaging [33]. These controversies may stem from different genetic models in which Nox4 was either silenced or overexpressed, or they may reflect different roles and regulation under pathophysiological conditions. Either way, they warrant further investigation.

Another enzyme localized in the mitochondria is monoamine oxidase (MAO). Activation of this enzyme leads to H_2O_2 formation and has been shown to contribute to a number of neuronal disorders, such as Parkinson's or Alzheimer's disease, most likely due to formation of ROS responsible for oxidative damage to neurons [34]. Although MAO inhibitors are currently used in the clinic for treatment of neurodegenerative diseases, MAO role in cardiac pathophysiology has gained attention only recently. However, charting this territory is likely to be of major pathophysiological relevance because oxidative stress impairs functions in viable cardiac myocytes, leading to contractile failure. In this review we are going to focus mostly on their role in the heart and speculate on the potential use of these compounds for treating cardiovascular diseases.

1.3 Interaction among mitochondrial ROS sources

It is likely that an intense cross-talk exists between different ROS sources in the cell. This is supported by the observation that frequently, inhibition of single ROS source is able to completely abolish oxidative stress and the resulting damage. One way to explain this is to envision that there is an “amplification mechanism”, whereby a single ROS source is activated by an initial stress, starts to generate ROS and triggers other sites in the cell to start

producing free radicals leading therefore to oxidative stress. On the other hand, it should not be disregarded that there is significant “buffering” due to cellular antioxidant systems and that ROS formation or oxidative stress may become evident only after a certain threshold has been reached [35]. Either way, inhibition of a single ROS source is able to lower overall ROS levels and, in most cases, to prevent cellular structural and functional derangements. In this regard, it is worth mentioning that inhibitors of p66^{Shc} are not yet available, Nox inhibitors are not isoform-specific or approved for use in clinic, whereas it is inconceivable to think that electron transport chain inhibitors could be used in patients. On the contrary, MAO inhibitors are available and already used in the clinic for the treatment of mood disorders, Parkinson’s and Alzheimer’s disease [34, 36, 37]. Development of a new generation of reversible MAO-A inhibitors, such as moclobemide, makes it worthwhile investigating whether MAO inhibitors could also be used to treat cardiovascular pathologies.

Here, we will discuss the relevant contribution of MAO isoforms to myocardial injury and mitochondrial dysfunction. Next, we will examine the beneficial cardioprotective effects produced by either their pharmacological or genetic inhibition, along with the potential deleterious effects following their over-expression. Finally, we will discuss the role of MAOs in catecholamine and serotonin (5-HT) clearance in relation to cardiac structure and function.

2, MAO: structure, localization and function

Monoamine oxidases are flavoenzymes anchored to the outer mitochondrial membrane through a transmembrane helix located within the carboxyl-terminal domain. They are responsible for the oxidative deamination of neurotransmitters and dietary amines. MAOs exist in two isoforms, MAO-A and -B, distinguished by different substrate specificity and inhibitor sensitivity. These two enzymes present 70% homology in their primary sequence [38, 39] and both contain the obligatory cofactor flavin adenine dinucleotide (FAD) covalently bound through a thioether linkage to Cys406 in MAO-A and Cys397 in MAO-B [40, 41]. This flavin moiety is the only redox-dependent factor necessary for catalysis, since mutations of flavin-linking residues ablate enzymatic activity. Regardless of the similarities, the two isoforms differ considerably in the structures of their active sites opposite the flavin cofactor. In humans, MAO-A has a monopartite cavity, whereas MAO-B exhibits a bipartite cavity structure with an entrance and a substrate cavity. Ile199 functions as a conformational “gate” separating the two cavities. Although the crystal structure of human MAO-A is monomeric whereas MAO-B is dimeric, both enzymes are dimeric in their membrane-bound forms [42, 43].

Besides the covalent attachment of the FAD to the cysteine residues, nascent MAO polypeptidic chains undergo also other posttranslational modifications. The best-characterized processes are the removal of the initiator methionine in MAO-B (but not in MAO-A) and the acetylation of the N-terminus in both molecules (methionine for MAO-A and serine for MAO-B) [44–46]. In addition to the acetylation of the N-terminus, human, rat and mouse MAO-A and -B from several tissues have been shown to contain several acetylated lysine residues [47–49], although whether and how this modification affects their function and ability to generate ROS remains still unclear. Lastly, there are also several

serine residues in both MAO-A and -B that can be phosphorylated [50]. Among these, Ser209 phosphorylation within a putative p38 consensus motif results in the inhibition of MAO-A activity in neuronal cell lines [51]. This modification might be tissue-specific and so far no information is available on cardiac myocytes.

The distribution of MAO in various tissues of different species has been investigated by use of specific inhibitors, immunohistochemistry, enzyme autoradiography and *in situ* hybridization [52–54]. MAO location has been intensively studied in the brain, where MAO-A has been prevalently found in noradrenergic and dopaminergic neurons. Conversely, MAO-B has been detected in cell bodies of serotonergic neurons, in histaminergic neurons and in glial cells [55–58]. In peripheral tissues, MAO-A has been found in placenta, fibroblasts, liver, intestine and thyroid gland, while platelets, liver, pancreatic islets and kidney contain mainly MAO-B [46, 59, 60]. Human cardiomyocytes contain both enzymes, although MAO-A appears to be the predominant isoform [61, 62].

The subcellular localization of MAOs appears to be variable depending on the tissue and that in some organs MAO may be localized in the norepinephrine storage particles [63]. The majority of MAO is associated with the outer mitochondrial membrane; fractions of the enzyme have, however, been described also in the nuclear envelope, endoplasmic reticulum and even in the plasma membrane. These findings remain controversial due to the lack of specificity of the methodologies employed and thus require more in-depth further investigation. In fact, more recent studies found MAO only in the outer mitochondrial membrane [60]. Interestingly, the use of TEMPO-substituted pargyline analogues (TEMPO or 2,2,6,6-tetramethyl-1-piperidinyloxy is a commonly used structure for making stable spin labels) and MAO inactivation by proteolysis allowed demonstrating that MAO-A and MAO-B are differently oriented in the outer mitochondrial membrane [64]. This finding might be of particular interest for designing novel subtype selective MAO inhibitors.

MAO-A catalyzes preferentially the oxidative deamination of norepinephrine and serotonin. Its activity is inhibited by low concentrations of clorgyline. Conversely, MAO-B has major affinity for phenylethylamine and benzylamine, and is inhibited by selegiline. Both isoforms catalyze the deamination of dopamine, tyramine, octopamine, 3-iodothyronamine and tryptamine and are inhibited by pargyline (for detailed review on MAO inhibitors and their clinical use please see [34, 36, 65]). The reaction of oxidative deamination occurs in several steps [66], ultimately resulting in the formation of the aldehyde from the corresponding amine, ammonia and H₂O₂. The aldehyde intermediate is rapidly metabolized to the corresponding acid by the action of aldehyde dehydrogenases (ALDH).

Although the roles of MAO in terminating the actions of neurotransmitters/dietary amines in central and peripheral nervous system and in the extraneuronal tissue have been extensively studied for decades, much less attention has been dedicated to the products of their activity. H₂O₂ is a ROS that could be toxic *per se* at high concentrations, or it could generate hydroxyl radical in the presence of Fe²⁺. Ammonia accumulation could also be toxic, although this has not been investigated yet in relation to MAO activity [67, 68]. Aldehyde intermediates are toxic for the biological systems [69, 70] and a decrease in aldehyde dehydrogenase activity, also due to increased oxidative stress [71], might further contribute

to the exacerbation of damage. Formation of these byproducts is a salient aspect of MAO biochemical and pharmacological profile that certainly warrants further attention.

3 MAO and neurotransmitters in the heart

MAO and serotonin

MAO substrates catecholamines and serotonin play a major role in the regulation of cardiac function. While catecholamines are mainly released by nerve endings, peripheral serotonin is produced and secreted by extra-neuronal cells. Most of the serotonin in the periphery is produced by intestinal enterochromaffin cells, stored in platelets and released after platelet activation during hemostasis or pathological thrombotic processes. However, serotonin can also be produced in the heart [72] by cardiac endothelial cells through transformation of 5-hydroxytryptophan into 5-HT by L-aromatic amino acid decarboxylase [73]. This finding suggested that serotonin released by activated platelets participates in cardiac injury/repair while that produced within the heart may play an important role in the regulation of physiological cardiac function. Accordingly, serotonin has been involved in a variety of cardiac pathophysiological processes, including ventricular hypertrophy [74], valve fibrosis [75] and I/R injury [76]. These cardiac effects of serotonin have been related to the stimulation of specific serotonin receptors [77, 78].

The availability and activity of cardiac serotonin depends not only on its release and/or production but also on its local degradation. Indeed, extracellular serotonin can be internalized into the cardiomyocytes through the plasma membrane serotonin transporter and metabolized by MAO-A [79]. In addition, targeted over-expression of MAO-A in mouse cardiomyocytes led to a significant decrease in cardiac serotonin, concomitant to the rise of its metabolite 5-hydroxyindoleacetic acid [80]. Thus, cardiac degradation of serotonin may be relevant for the regulation of the physiological and/or pathological effects of serotonin in the heart (Fig. 1).

MAO and norepinephrine

The myocardium is under the continuous influence of neurohormones, and of sympathomimetic amines in particular. Hence, their turnover and catabolism are likely to have an impact in acute and chronic cardiac conditions, particularly in presence of excessive neurohormonal stimulation, for instance during hypertension and/or early stages of congestive heart failure (CHF). Despite the high pathophysiological potential of abnormalities in catecholamine cycling/catabolism in cardiovascular diseases, investigation in this direction has never been burgeoning.

Impairment of norepinephrine neuronal reuptake and concomitant down-regulation of the β -adrenergic system are well documented in human and experimental CHF [81–83], contributing to the loss of systolic performance in this syndrome. In normal hearts, 92% of the norepinephrine released by sympathetic nerves is recaptured by norepinephrine transporter (NET), 4% is removed by extra-neuronal uptake (through the extra-neuronal monoamine transporter, EMT), and the remaining 4% enters the circulation (Fig. 1). However, NET function declines in CHF contributing to norepinephrine “spillover”, used as a prognostic marker and a therapeutic index in these subjects [82, 84, 85]. Consequently, the

extra-neuronal/cardiomyocyte uptake almost doubles in CHF patients [85], leading to enhanced MAO and catechol-*O*-methyl-transferase (COMT) activity. MAO-A activity is central to this pathogenetic mechanism since, in pressure overloaded hearts, both the pharmacological and genetic ablation of MAO-A activity prevent cardiac norepinephrine depletion, restoring NET expression levels and left ventricle (LV) function to control values. Our findings are fully in keeping with previous evidence showing that systemic infusion of MAO inhibitors increases the number of NET recognition sites through higher norepinephrine availability [86]. Regardless, these findings hint at the intriguing possibility that MAO-A inhibition may lead to increased (or preserved) catecholamine neuronal re-uptake and recycling rather than neosynthesis. This “gearing down” mechanism is strategic in conditions such as exercise in which the maximal rate of catecholamine synthesis (not exceeding 4 fold) cannot match the increased demand (>10 fold) for catecholamine release at the neuro-effector junction [69]. Envisioning a similar situation in human heart failure is plausible because failing heart is a “catecholamine depleted” one [87, 88], an effect that is due in part to deficient neuronal re-uptake (for review, see [89]) and impaired catecholamine neosynthesis, owing to the progressive loss of functional sympathetic fibers [90].

MAO and dopamine

Dopamine is preferentially catabolized by MAO-B, isoform very abundant in species such as mice and humans [61, 91]. Indeed, genetic ablation of MAO-B is accompanied by a rise in cardiac dopamine levels, up-regulation of extracellular signal-regulated kinases (ERK1/2) and lower apoptosis rates after pressure overload [92]. These data fit nicely with previous observations concerning the involvement of mitogen-activated protein kinase kinase 1 (MEK1)-ERK1/2 signaling cascade in maintenance of physiological hypertrophy and preserved cardiac function in stressed hearts [93].

It is tempting to speculate that increased ERK1/2 activity is related to the higher dopamine content in MAO-B^{-/-} mice. Dopamine receptors (D₁₋₄) are expressed in mouse hearts (our unpublished observations), and dopamine levels are elevated in the absence of MAO-B. Therefore, the link between ERK1/2 and dopamine might be supported by the activation of D₂ receptors that have been shown to trigger ERK1/2 activation in neuronal and non-neuronal tissue [94]. Thus, endogenous dopamine might be involved in the governance of the plasticity of the adult heart, particularly in myocardial response/protection against chronic stress. Notably, dopamine is still widely used to treat heart failure, particularly after cardiac surgery in infants. The latter use is seemingly due to the fact that newborn myocardium is richer in D₁ and β₂-adrenergic receptor (AR) mRNA levels as compared to the adult one [95].

4 MAOs in cardiovascular diseases

4.1 MAO inactivation

4.1.1 Baseline phenotypical outcomes of MAO deletion—Deletion of MAO-A and MAO-B genes has proven their important roles in neurotransmitter metabolism and behavior. MAO-A^{-/-} mice have elevated brain levels of serotonin, norepinephrine and, to a lesser extent, dopamine [96–98], whereas only 2-phenylethylamine is increased in MAO-

B^{-/-} mice [99]. Interestingly, all monoamines were increased in the brains of combined MAO-A/B^{-/-} mice, to a much greater extent than those observed in either MAO-A^{-/-} or MAO-B^{-/-} mice [100]. This suggests that, in the absence of one isoenzyme, the other can partially overtake its catalytic role [46]. For an in-depth discussion on behavioral outcomes due to MAO deficiency we refer the readers to an excellent review by Bortolato and Shih [46].

The effects of MAO deletion have been characterized also in the cardiovascular setting. Mice lacking MAO-A activity show higher cardiac levels of serotonin, norepinephrine and epinephrine [88, 101] (and unpublished data). This increase in catecholamine levels has its structural and functional consequences on the heart. Lairez and coworkers [101] have shown that MAO-A^{-/-} mice display cardiomyocyte hypertrophy and LV dilation at baseline, although LV dysfunction was absent and no hemodynamic alterations were observed. This effect can most likely be attributed to elevated cardiac 5-HT levels and hyperactivation of 5-HT_{2A} receptors observed in MAO-A^{-/-} mice. Another line of hypomorphic MAO-A mutants (MAO-A^{neo}) resulting in expression of truncated non-functional variant of MAO-A^{neo} transcript, displays a slightly different phenotype at baseline [88]. Despite the increase in cardiac catecholamine levels, these mice do not show any structural differences but reveal some hemodynamic differences. For instance, LV systolic pressure, dP/dt_{max}, and dP/dt_{min} (indexes of contractility and relaxation, respectively) are all lower in MAO-A^{neo} compared to wild type (WT) littermates. Contractile function assessed by preload-recrutable stroke work index was also lower in MAO-A^{neo} mice, so these differences were potentially related to loading changes. However, chamber volume and ejection fraction were similar between the two strains. Regardless of the differences between the two genetic models, it is likely that adaptive changes occur in the MAO-A gene deletion mouse models because these are global and constitutive transgenic mice. Further supporting this contention is the fact that 6 weeks treatment of the mice with MAO-A inhibitor clorgyline does not have any effects on basal cardiac structure or function as assessed by histological and hemodynamic measurements [88].

MAO-B^{-/-} mice instead show higher cardiac dopamine content compared to their WT littermates, and also norepinephrine and epinephrine, although to a lesser extent compared to MAO-A^{neo} mice [92] (and unpublished observations). At baseline, echocardiography did not show any apparent morphological difference between WT and MAO-B^{-/-} mice. However, a more thorough examination via pressure-volume loops analysis revealed that these null mice have slightly reduced contractility/relaxation at baseline and β-adrenergic desensitization, although fractional shortening and ejection fraction were not significantly different when compared to WT mice [92]. As in the case of MAO-A^{-/-} or mutant mice, it is likely that these changes are due to excessive catecholamine build-up in these mice from birth. This issue could be better evaluated using conditional cardiomyocyte-specific MAO-A^{-/-} or -B^{-/-} mice, but these are currently unavailable.

4.1.2 MAO deletion in cardiovascular disease—MAO knockout and mutant mouse models have proven useful for the evaluation of the MAO role and contribution to cardiovascular disease. *In vitro* studies using adult mouse cardiomyocytes isolated from both WT and MAO-A^{neo} mice, showed that NE acts in part independently of α- or β-AR.

Norepinephrine-induced hypertrophy is partially mediated by MAO-A generated ROS and stimulation of MAO-A activity, independently of receptor activation, is sufficient to trigger myocyte hypertrophy [88].

The important role of MAO in the transition from compensated hypertrophy to heart failure and I/R injury was confirmed in *in vivo* studies. MAO-A deficient mice are protected from I/R injury due to lower ceramide/sphingosine 1-phosphate ratio, identified as a critical event in MAO-A-mediated cardiac cell apoptosis [102]. Despite the better characterized role of MAO-A in experimental heart failure, the potential contribution of MAO-B to this syndrome should not be disregarded. The relative expression of each MAO isoform varies greatly across different tissues and species. For instance, MAO-A is the predominant isoform in the rat heart, with little or none of the MAO-B activity, whereas exactly the opposite is true in mice [61, 91]. Recent studies investigated the role of both isoforms employing genetic models [88, 92]. In a mouse model of CHF induced by transverse aortic constriction (TAC), both MAO-A^{neo} and MAO-B^{-/-} mice displayed reduced cardiac oxidative stress, LV remodeling and apoptosis. Furthermore, absence of MAO activity in those hearts completely prevented LV dilation/pump failure, favoring instead the maintenance of stable concentric hypertrophy in chronically stressed hearts. Thus, genetic ablation of either MAO-A or -B activity benefited TAC hearts by reducing ROS burden and countering LV remodeling, mitochondrial dysfunction and cardiomyocyte apoptosis. On the other hand, catecholamine cycling was also improved in mice lacking MAO-A or MAO-B activity. Indeed, both norepinephrine and dopamine degradation is increased in failing hearts due to MAO activation, and its inhibition leads to improved neuronal pools and availability of these catecholamines.

At variance with previously mentioned findings, a study by Lairez and colleagues [101] observed exacerbated ventricular hypertrophy in MAO-A^{-/-} mice subjected to aortic banding, attributing this effect to the hyperactivation of 5-HT_{2A} receptors in MAO-A^{-/-} mice. As mentioned previously, these knockout mice already showed some adaptive mechanisms at baseline that were further exacerbated by aortic banding. Differences in the genetic model could underlie this apparent contradiction. Indeed, other studies have shown that MAO-A^{-/-} animals show behavioral and morphological alterations distinct from those of MAO-A^{neo} mice [98]. Furthermore, the severity of aortic banding might also explain the differences because banding of the ascendant aorta (Lairez' study) results in a less acute model of CHF as compared to the banding of transverse aorta (Kaludercic' study). In fact, experimental protocol in the first study involved only compensated hypertrophy and the observations were not extended long enough to detect LV dilation and failure. Conversely, the latter study showed that both MAO isoforms play a major role in the transition from compensated hypertrophy to heart failure.

Setting aside these methodological issues, there is no doubt that MAO-A expression and/or activity is a major contributing factor to the development of pathologic hypertrophy and heart failure, at least in rodents. This contention is further supported by the observation that cardiomyocyte-specific over-expression of MAO-A leads to cardiomyocyte necrosis and heart failure whereas pharmacological inhibition of MAO is equally effective in preventing

the occurrence of CHF and avoids potential adaptive changes at baseline [80, 88]. It remains to be determined whether myocyte-specific gene deletion would yield the same results.

Brandes' group recently demonstrated the important role of MAOs in vasculature, showing that MAO expression in mouse aortas was induced through phosphatidylinositide 3-kinase and NF κ B pathway following angiotensin II and lipopolysaccharide treatment and greatly contributed to vascular formation of H₂O₂ [35]. Indeed, pharmacological inhibition of both MAO-A with clorgyline or moclobemide and MAO-B with selegiline completely prevented this increase in oxidative stress and restored normal endothelium-dependent relaxation in vessels. Furthermore, these Authors found that MAO-A limited endothelial cyclic guanosine monophosphate accumulation, suggesting that MAO-generated species limit endothelial nitric oxide formation. Although this is not the first study to highlight the involvement of MAOs in vascular formation of ROS [103, 104], Sturza and colleagues demonstrated that endogenous vascular catecholamines are sufficient to fuel MAO activity, even without supplementing exogenous substrate. The evidence that vascular cells express several enzymes involved in catecholamine synthesis supports this. On the other hand, these observations do not exclude the possibility that there may be still unidentified MAO substrates whose catabolism may be enhanced in pathological states.

4.2 MAO over-expression

As detailed above, MAO-A was identified as a major source of ROS in the heart. As such, any increase in MAO-A activity could lead to enhanced generation of ROS and potential ROS-mediated damage. MAO-A activity can be augmented by means of increased substrate availability, such as serotonin or norepinephrine in the heart. Indeed, hyperactivity of sympathetic drive is evidenced by increased plasma norepinephrine and epinephrine, elevated sympathetic outflow, and heightened norepinephrine spillover in human CHF [105]. Concerning serotonin, one study reported a significant rise in platelet and plasma 5-HT concentrations in CHF [106]. On the other hand, enhanced generation of ROS by MAO-A could result from the enhanced expression of this enzyme at the transcriptional or post-transcriptional level. Such MAO-A up-regulation has been demonstrated in the aging heart, a situation frequently associated with heart failure [107]. In order to better understand whether this increase occurred in cardiac cells, we recently isolated cardiomyocytes from young, middle-age and old rats to measure MAO-A and MAO-B activities independently from other cell types present in the heart. Interestingly, we found that senescent cardiomyocytes displayed strong enhancement of MAO-A activity (95-fold between age of 2-month and 23 month) [80]. Since cardiac aging is a major risk factor for heart failure, we also evaluated the expression of MAO-A in a model of heart failure induced by aortic banding in the rat. We demonstrated that MAO-A activity was enhanced in cardiac hypertrophy and failure in this model [80]. In addition, some studies based on transcriptomic or proteomic analysis identified MAO-A as one of the most up-regulated proteins in different models of rat heart failure induced by volume overload [108], pressure overload [109] or myocardial infarction [110, 111]. At present, the mechanisms involved in MAO-A up-regulation in the heart are unknown.

In order to investigate the consequences of an increase in cardiac MAO-A activity on heart function, we recently developed *in vitro* and *in vivo* models of MAO-A over-expression [80]. *In vivo*, mice with cardiac-specific over-expression of MAO-A (MAO-A Tg) had enhanced levels of H₂O₂ in the heart and displayed enhanced staining with 8-oxo-2'-deoxyguanosine, a marker of DNA oxidation, which was more prominent in mitochondria. Moreover, norepinephrine and 5-HT concentrations were decreased in the hearts of MAO-A Tg mice, with concomitant increase in 5-hydroxyindoleacetic acid and dihydroxyphenylglycol. Thus, enhancing MAO-A in cardiomyocytes was able to impact global norepinephrine and 5-HT content in the heart. We demonstrated that MAO-A up-regulation was responsible for structural and functional damage of mitochondria accompanied by decreased adenosine triphosphate (ATP) concentrations and mitochondrial biogenesis. Histological analysis of cardiac sections of MAO-A Tg mice demonstrated important cardiomyocyte drop-out (about 50% of cardiomyocytes) between 6 and 12-weeks of age, together with reactive hypertrophy of residual cardiomyocytes and cardiac fibrosis. Progressive dilatation of the heart was measured by echocardiography, and led to heart failure and premature death at about 30 weeks. By combining *in vitro* adenoviral transduction of MAO-A and *in vivo* over-expression in the heart, we found that p53 participated in mitochondrial damage and necrosis linked to MAO-A/H₂O₂ pathway. Altogether, we provided evidence for the first time that an increase in MAO-A expression could drive spontaneous mitochondrial damage and myocardial degeneration in heart failure and aging [80]. A similar observation has been made in the brain where MAO-B expression is increased during aging. Interestingly, over-expression of MAO-B in astrocytes resulted in oxidative stress, dopaminergic neuron degeneration and a phenotype that mimicked Parkinson disease in mice [112, 113]. Altogether, these studies constitute a direct proof of the deleterious effect of MAO in the generation of ROS and cell death. They provide new models for studying the mechanisms by which pathological conditions occur in relation with monoamine oxidase activation.

5 Mitochondria as a selective target of MAO-mediated injury

Mitochondrial dysfunction is frequently associated with oxidative stress, cell death and cardiac damage under several cardiovascular disease conditions. Considering that MAOs are located in the mitochondria, one might envision that exacerbated MAO activity might lead to damage of these organelles. A recent study by Villeneuve and colleagues [80] showed that cardiac-specific overexpression of MAO-A was accompanied by ultrastructural defects of cardiac mitochondria, ATP depletion and ultimately led to cardiomyocyte necrosis and heart failure. Furthermore, MAO-A over-expression in neonatal cardiomyocytes mimicked these results triggering oxidative stress-dependent p53 activation, leading to peroxisome proliferator-activated receptor-gamma coactivator 1 α down-regulation, mitochondrial impairment, and cardiomyocyte necrosis (Fig. 2). Subsequently, we demonstrated the existence of a direct link between MAO activation, mitochondrial ROS formation and mitochondrial dysfunction [92]. Indeed, analyzing ROS formation after MAO activation in a spatiotemporal manner via the genetically-encoded redox sensitive fluorescent probe HyPer enabled us to target it specifically to mitochondria or cytosol. We observed that H₂O₂ formation occurs much earlier at the mitochondrial level rather than in the cytosol. This is an

important finding since it reiterates the issue that mitochondria are “early targets” of endogenously produced oxidative stress that leads to mitochondrial dysfunction. Indeed, MAO activation was associated with the loss of mitochondrial membrane potential, unveiled after incubation with oligomycin. This suggests that ATP synthase was working in the reverse mode, i.e. hydrolyzing glycolytically generated ATP and compensating in this way for the dysfunctional respiratory chain [114].

Other so far neglected but potentially interesting by-products of MAO activity are aldehydes generated from amines. These aldehydes are toxic species [115] that need to be converted to less harmful metabolites. Thus, MAO is functionally coupled with a NAD(P)⁺-dependent ALDH, which oxidizes the aldehyde to the corresponding carboxylic acid. Alternatively, depending on the location and the intracellular conditions, aldehydes can be reduced to alcohols or glycols by aldehyde reductase or alcohol dehydrogenase [46]. In the heart, these aldehydes are normally rapidly inactivated and transformed into corresponding acids by ALDH2, the most abundant ALDH isoform expressed in this tissue and localized in the mitochondria [116]. Previous studies have shown the ALDH2 activity is inhibited in conditions of high oxidative stress and that stimulation of its activity reduces myocardial I/R injury in rats [71]. Aldehydes induce inactivation of a number of macromolecules including the proteasome, the electron transport chain in the mitochondria, as well as inactivation of ALDH2 itself [117]. At present, there is only indirect evidence suggesting that ALDH2 activity might be reduced in heart failure, whereas aldehyde formation might be enhanced because malondialdehyde levels are increased in this condition. On the other hand, we recently showed that aldehydes generated by amine catabolism via MAO play a major role in the MAO-mediated mitochondrial dysfunction in cardiac myocytes [92]. In fact, dopamine incubation of siRNA-treated cardiomyocytes against ALDH2 led to high ROS production and accumulation of 3,4-dihydroxyphenylacetaldehyde, an aldehyde deriving from dopamine that is known to be very reactive and a potent neurotoxin [118], resulting ultimately in the loss of mitochondrial membrane potential. Whether degradation of other MAO substrates, such as norepinephrine or serotonin, yields toxic aldehydes that target mitochondrial function still warrants further investigation.

Altogether, this evidence lends support to the contention that mitochondria are early targets not only of extrinsic or endogenously produced ROS, but also of MAO-derived aldehyde intermediates that further fuel mitochondrial and myocardial damage (Fig. 2).

6 Conclusions

Evidence available so far suggests that MAO inhibition is beneficial for treatment of cardiovascular pathologies. From a translational point of view a major hurdle in accepting the use of MAO inhibitors in clinic is the possible occurrence of the so-called “cheese-effect”. In fact, ingestion of food rich in tyramine, such as wine and cheese, has been found to cause hypertensive crises in patients treated with irreversible MAO-A inhibitors. The introduction of a new generation of reversible MAO inhibitors appears to avoid this adverse effect. In fact, a very recent retrospective analysis reported that severe adverse hemodynamic events, such as hypertension and tachycardia, do not occur more frequently in users of both the irreversible MAO inhibitor tranylcypromine and the reversible MAO-A

inhibitor moclobemide compared to nonusers [119]. In this investigation, cardiac contractility was not included as an endpoint. Hence, whether taking MAO (-A or -B) inhibitors improves cardiac function in patients with CHF remains a fascinating and potentially important pragmatic question to ask in future studies.

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Abbreviations

5-HT	serotonin
β-AR	β-adrenergic receptor
ALDH	aldehyde dehydrogenase
ATP	adenosine triphosphate
CHF	congestive heart failure
COMT	catechol- <i>O</i> -methyl-transferase
EMT	extraneuronal monoamine transporter
ERK1/2	extracellular signal-regulated kinases
FAD	flavin adenine dinucleotide
H₂O₂	hydrogen peroxide
I/R	ischemia/reperfusion
LV	left ventricle
MAO	monoamine oxidase
NET	norepinephrine transporter
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
Nox	NADPH (nicotinamide adenine dinucleotide phosphate) oxidase
ROS	reactive oxygen species
SOD	superoxide dismutase
TAC	transverse aortic constriction
WT	wild type

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Highlights

- Monoamine oxidases are a major source of H₂O₂ within the mitochondria.
- Their expression and activity is increased in several pathological conditions.
- Monoamine oxidase inhibition prevents oxidative stress and cardiac dysfunction.
- Monoamine oxidase-generated H₂O₂ and aldehydes can induce mitochondrial dysfunction.

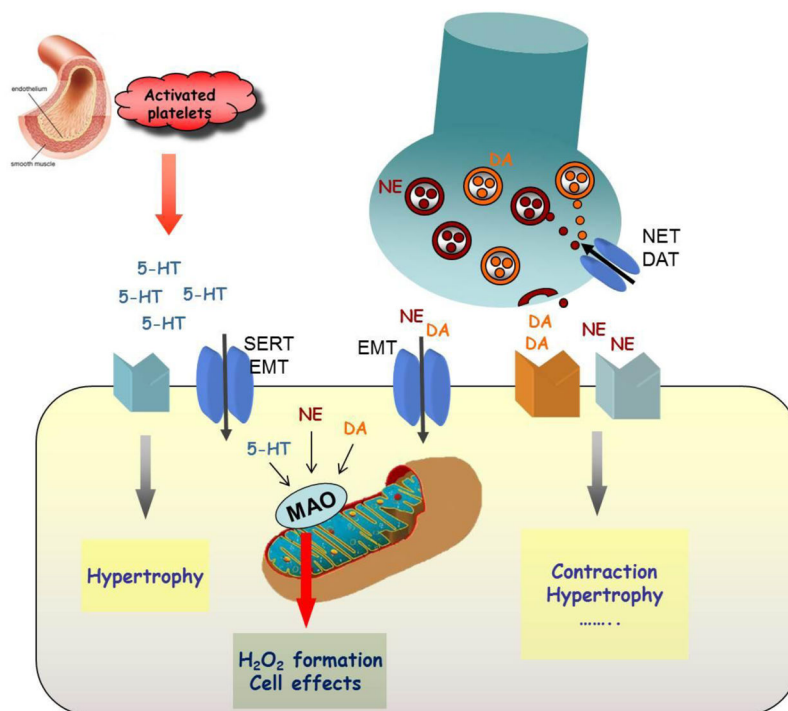


Figure 1. Possible effects of serotonin or catecholamines on cardiac myocytes

Serotonin (5-HT), released from the activated platelets, and catecholamines (NE, DA) released from the intracardiac nerves, interact with their receptors present at the level of the sarcolemma to exert their effects. Once this interaction is over, the majority of the neurotransmitter is rapidly re-uptaken through the transporter present in the membrane of the nerve terminal (NET, DAT) and only small percent escapes into the circulation or is uptaken through the extraneuronal monoamine transporter (EMT), present in the cardiomyocyte membrane. Once in the cytoplasm, these neurotransmitters are degraded by monoamine oxidases (MAO) and generate H₂O₂ that, in turn, might affect cellular processes even in the physiological conditions.

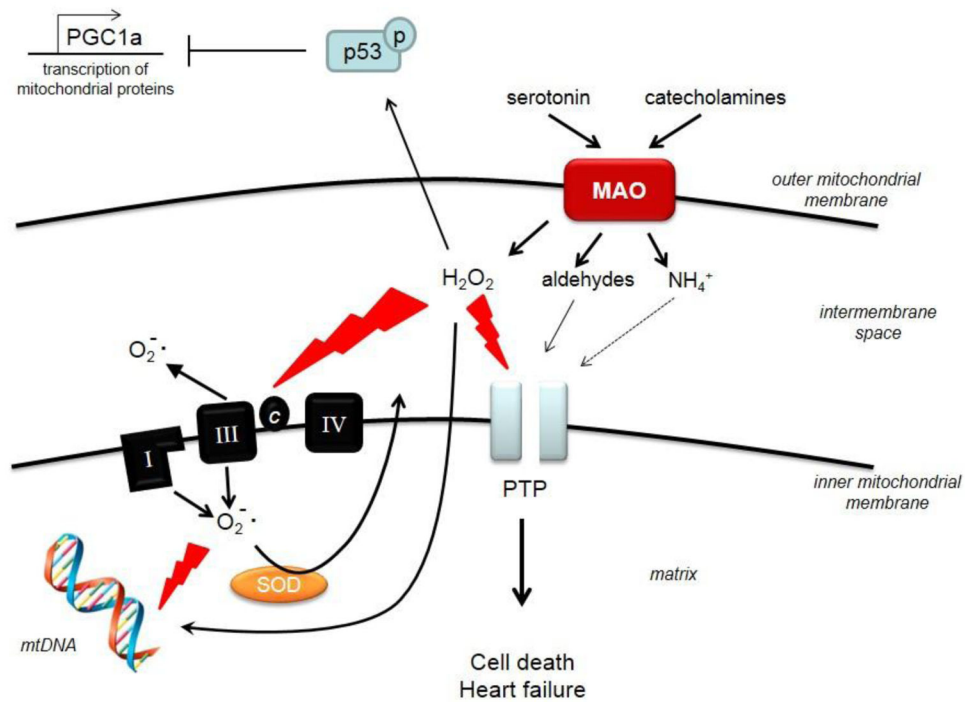


Figure 2. Deleterious effects of MAO activation on mitochondrial function

Up-regulation of monoamine oxidase activity (MAO) due to higher substrate availability results in enhanced formation of H₂O₂ and aldehyde intermediates that can directly affect the transfer of the electrons across the respiratory chain and the opening of the permeability transition pore (PTP), leading to cardiomyocyte death, oxidative damage of mitochondrial DNA (mtDNA) and heart failure. Furthermore, MAO-generated oxidative stress triggers p53 activation that, in turn, leads to down-regulation of peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α), a master regulator of mitochondrial biogenesis.