

Monoamine Oxidases Are Mediators of Endothelial Dysfunction in the Mouse Aorta

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Abstract—Monoamine oxidases (MAOs) generate H_2O_2 as a by-product of their catalytic cycle. Whether MAOs are mediators of endothelial dysfunction is unknown and was determined here in the angiotensin II and lipopolysaccharide-models of vascular dysfunction in mice. Quantitative real-time polymerase chain reaction revealed that mouse aortas contain enzymes involved in catecholamine generation and MAO-A and MAO-B mRNA. MAO-A and -B proteins could be detected by Western blot not only in mouse aortas but also in human umbilical vein endothelial cells. Ex vivo incubation of mouse aorta with recombinant MAO-A increased H_2O_2 formation and induced endothelial dysfunction that was attenuated by polyethylene glycol-catalase and MAO inhibitors. In vivo lipopolysaccharide (8 mg/kg IP overnight) or angiotensin II (1 mg/kg per day, 2 weeks, minipump) treatment induced vascular MAO-A and -B expressions and resulted in attenuated endothelium-dependent relaxation of the aorta in response to acetylcholine. MAO inhibitors reduced the lipopolysaccharide- and angiotensin II-induced aortic reactive oxygen species formation by 50% (ferrous oxidation xylenol orange assay) and partially normalized endothelium-dependent relaxation. MAO-A and MAO-B inhibitors had an additive effect; combined application completely restored endothelium-dependent relaxation. To determine how MAO-dependent H_2O_2 formation induces endothelial dysfunction, cyclic GMP was measured. Histamine stimulation of human umbilical vein endothelial cells to activate endothelial NO synthase resulted in an increase in cyclic GMP, which was almost abrogated by MAO-A exposure. MAO inhibition prevented this effect, suggesting that MAO-induced H_2O_2 formation is sufficient to attenuate endothelial NO release. Thus, MAO-A and MAO-B are both expressed in the mouse aorta, induced by in vivo lipopolysaccharide and angiotensin II treatment and contribute via the generation of H_2O_2 to endothelial dysfunction in vascular disease models. (*Hypertension*. 2013;62:140-146.) • [Online Data Supplement](#)

Key Words: endothelium ■ hypertension ■ monoamine oxidase ■ mouse models of disease ■ nitric oxide ■ nitric oxide synthase (endothelial) ■ oxidative stress

Reactive oxygen species (ROS) contribute to endothelial dysfunction by limiting NO availability and by inducing endothelial cell activation.¹ Several enzyme systems are considered as being responsible for the elevated vascular ROS production in endothelial dysfunction, including NADPH oxidases,² xanthine oxidase,³ electron leakage from the mitochondrial respiratory chain, cyclooxygenases,⁴ and the uncoupled endothelial NO synthase.⁵

Monoamine oxidases (MAOs) are another source of ROS but their relevance for vascular ROS formation has not gained much attention. Although MAOs in part mediate cardiac ischemia/reperfusion injury, it is not known whether MAO-dependent ROS production also contributes to vascular dysfunction and thus the development of endothelial dysfunction.⁶

MAOs are flavoenzymes that are located at the outer mitochondrial membrane, where they catalyze oxidative

deamination of amine neurotransmitters and vasoactive amines virtually in all mammalian tissues. Two isoforms with different tissue distribution, substrate affinities, and inhibitor sensitivities exist: MAO-B is the major enzyme in brain, whereas MAO-A is present not only in brain but also in other tissues, including the heart and vessels.⁷ By catalyzing the electron transfer from biogenic amines to molecular oxygen, aldehydes, ammonia, and hydrogen peroxide are formed as by-products.⁸

Although the substrate specificity of both MAO isoforms is similar, MAO-A has a higher affinity to serotonin and norepinephrine than MAO-B, which in turn prefers benzylamine and 2-phenylethylamine.⁹ Nevertheless, both enzymes degrade dopamine, tryptamine, and norepinephrine if present in sufficient concentration. In addition to being involved in a broad spectrum of mental and neurodegenerative diseases,¹⁰

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MAO-A-mediated H_2O_2 production has been shown to be relevant for ischemia–reperfusion injury of the kidney¹¹ and the heart. Moreover, MAO-A is thought to be involved in myocyte hypertrophy *ex vivo*^{12,13} and also in maladaptive myocardial hypertrophy and transition to heart failure *in vivo*.¹⁴

The unfavorable effects of MAO activation are antagonized by a couple of relatively selective MAO inhibitors, which are either irreversible, such as clorgyline for MAO-A and selegiline for MAO-B, or reversible, such as moclobemide for MAO-A and lazabemide for MAO-B, respectively.⁸ Systemic MAO inhibition leads to the accumulation of catecholamines with subsequent increase in sympathetic activity and hypertension.¹⁵ This aspect limits the use of MAO inhibitors in a vascular scenario, and therefore, MAO inhibitors have not been considered an approach to improve vascular function.

Another prototypic hypertensive agent is angiotensin II (Ang II). Interestingly, potential interactions of Ang II and MAOs have been reported in the central nervous system. In coculture systems of hypothalamic and brain stem neurons, Ang II stimulated MAO activity, but the underlying mechanism was not studied.¹⁶ More recently, it was reported that inhibition of the renin–angiotensin system decreases cardiac MAO activity.¹⁷ Whether there is an interaction between Ang II and MAO in vessels is not known. Ang II is well known to activate and induce other sources of ROS like vascular NADPH oxidases,¹⁸ and Ang II also increases vascular xanthine oxidase activity.¹⁹ Moreover, there is even evidence that Ang II increases superoxide production in mitochondria of endothelial cells.²⁰ By these mechanisms, Ang II is a well-characterized inducer of endothelial dysfunction. Given these different, well-established sources of ROS, it is hard to predict whether MAO and the subsequent ROS formation by the enzyme have a role in Ang II–induced vascular dysfunction.

In the present study, we tested whether MAOs are a source of vascular ROS production in healthy and diseased vessels and whether the enzymes contribute to the development of endothelial dysfunction.

Materials and Methods

Endothelial function was determined by organ chamber experiments, gene expression was determined by quantitative real-time polymerase chain reaction, and protein expression by Western blot. Cyclic GMP (cGMP) was determined by radio immuno assay. For further details, please see the online-only Data Supplement.

Results

Ex Vivo MAO-A Incubation Attenuates Endothelium-Dependent Relaxation by Forming H_2O_2

Although MAO-A is a known source of H_2O_2 , it is unknown whether the enzyme contributes to the development of endothelial dysfunction. To address this aspect, murine lung endothelial cells and mouse aortic rings were incubated with a commercially available preparation of MAO (MAO-A, Promega, 0.2–1 mU/mL for 12 hours). As demonstrated by Western blot analysis in endothelial cells, MAO incubation increased the enzyme content of the cell preparation (Figure 1A). Endothelium-dependent relaxation of vascular segments incubated with MAO was significantly attenuated. This effect was prevented

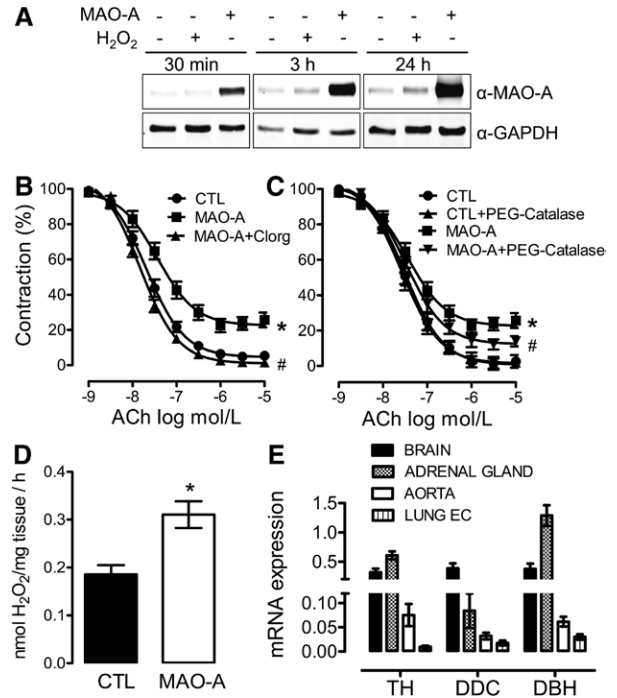


Figure 1. Effects of *ex vivo* monoamine oxidase (MAO-A) on vascular function. **A**, Western blot analysis of MAO-A of mouse lung endothelial cells incubated with MAO-A (0.2 mU/mL) or H_2O_2 (30 μ mol/L) for the durations indicated. GAPDH served as loading control (CTL). **B** and **C**, Acetylcholine-induced relaxation in phenylephrine-precontracted mouse aortic segments with (MAO-A) and without (CTL) preincubation with MAO-A (1 mU/mL, 12 h) in the presence or absence of clorgyline (Clorg, 10 μ mol/L, **B**) or polyethylene glycol (PEG)-catalase (100 U/mL, **C**), $n=6$. **D**, Aortic H_2O_2 formation determined by ferrous oxidation xylenol orange assay, $n=6$. **E**, Quantitative real-time polymerase chain reaction for tyrosine hydroxylase (TH), DOPA decarboxylase (DDC), and dopamine B hydroxylase (DBH) relative to the housekeeping gene *EEF2 α* in the murine tissues indicated (EC indicates murine endothelial cells), $n=4$. * $P<0.05$ MAO-A vs CTL; # $P<0.05$ with and without Clorg and PEG-catalase, respectively.

by cotreatment with the MAO-A inhibitor clorgyline, although the compound had no effect on responses in control segments (Figure 1B). To elucidate whether the attenuation was a consequence of MAO-dependent H_2O_2 formation, experiments were repeated after incubation with polyethylene glycol–catalase. Indeed, polyethylene glycol–catalase in part prevented the attenuation of acetylcholine-induced relaxation (Figure 1C). Accordingly, after *ex vivo* MAO treatment, vascular H_2O_2 formation increased by 60% as determined by ferrous oxidation xylenol orange (FOX) assay (Figure 1D). These findings indicate that MAO could contribute to vascular ROS formation even without supplementation of catecholamines, suggesting that sufficient amount of substrates are produced within the vessel. Therefore, expression of enzymes involved in catecholamine biosynthesis was determined by real-time polymerase chain reaction. Although expressed to a much lower degree than in murine adrenal glands and brain, tyrosine hydroxylase, DOPA decarboxylase, and dopamine β -hydroxylase were readily detectable in the mouse aorta. Importantly, expression was also present in lung endothelial cells excluding that adventitial nerves are the only vascular source of catecholamine generating enzymes (Figure 1E). Collectively, these data demonstrate

that MAO can be a mediator of endothelial dysfunction and that vessels generate sufficient amounts of catecholamines to facilitate MAO-dependent H_2O_2 production.

Vascular Expression of MAO Increases in Response to Ang II and Lipopolysaccharide

Because MAO inhibition had no effect on vascular function in control vessels, we wondered whether MAO could be a mediator of endothelial dysfunction in situations of increased vascular oxidative stress, that is, after a potential induction of the enzyme. To address this aspect, the expressions of MAO-A and MAO-B were determined by real-time polymerase chain reaction and Western blot. Under resting conditions, MAO-A, as judged from the computed threshold (ct) value difference of the real-time polymerase chain reaction as well as the intensity of the staining in the Western blot, was greatly more abundant than MAO-B. Ex vivo incubation of mouse aortic segments in organic culture with Ang II (100 nmol/L) or lipopolysaccharide (LPS; 1 μ g/mL) increased the expression of both homologues by \approx 2-fold (Figure 2A and 2B). Similar responses were observed after removal of the endothelium (Figure S1A in the

online-only Data Supplement). Also in cultured lung endothelial cells, Ang II induced a significant increase in MAO-A and MAO-B expressions, whereas LPS had little effect (Figure S1B). Interestingly, in these cells, also incubation with H_2O_2 (30 μ mol/L) resulted in an increase of endothelial MAO-A protein, potentially suggesting that MAO-A is generally induced in situations of oxidative stress (Figure 1A). To determine whether mRNA induction also translates into changes in protein expression, immunohistochemistry (Figure 2C) and Western blot analyses (Figure 2D) were performed. A pronounced increase in vascular MAO-A expression was readily observed with both techniques, with most prominent changes occurring in the medial layer of the vessel. For MAO-B, potentially as consequence of the lower abundance of the protein, the available antibodies yielded numerous bands on Western blot analyses. Thus, although it could be concluded that MAO-B protein expression also increased in response to Ang II and LPS, histological analysis was infeasible because of the significant non-specific staining of the available antibodies.

MAO Is a Mediator of Endothelial Dysfunction After Ang II Treatment

To address the pathophysiological relevance of Ang II-mediated MAO induction, mouse aortic segments were studied after in vivo treatment with Ang II (1 mg/kg per day, 2 weeks by minipump). Similar as for the ex vivo stimulation, this approach resulted in an induction of MAO-A and MAO-B (Figure 3A). Importantly, determination of H_2O_2 formation in the isolated mouse aorta indicated that \approx 50% of the Ang II-induced H_2O_2 formation was sensitive to MAO inhibition (Figure 3B). Accordingly, endothelium-dependent relaxation in response to acetylcholine was attenuated after in vivo Ang II treatment and incubation with inhibitors for MAO-A, as well as MAO-B in part restored normal endothelium-dependent relaxation (Figure 3C–3F). Coincubation with both inhibitors had an additive effect and completely normalized endothelium-dependent relaxation. Given the well-established role of endothelial NO synthase (eNOS) uncoupling and Nox activation in response to Ang II, these findings were unexpected and point toward a potential antioxidant or nonspecific effect of MAO inhibition. By 2 different assays for H_2O_2 (Amplex red and FOX assay), however, no significant antioxidant effect of the MAO inhibitors was observed (Figure S2A). Moreover, MAO inhibition had no effect on xanthine oxidase-driven H_2O_2 formation (Figure S2B); also Nox activity in cells overexpressing active Nox1, Nox2, and Nox4, respectively, was not affected by MAO inhibition (data not shown). Collectively, these observations identify MAO-A and B as mediators of endothelial dysfunction.

MAO Is a Mediator of Endothelial Dysfunction After LPS Treatment

To further establish the principle of MAO-mediated endothelial dysfunction, we sought for a second independent pathway and studied vascular responses to in vivo LPS treatment.²¹ Similar to the response to Ang II, LPS treatment resulted in endothelial dysfunction and increased H_2O_2 formation (Figure 4A and 4B). MAO inhibition attenuated vascular H_2O_2 formation (Figure 4B) and partially restored normal responses

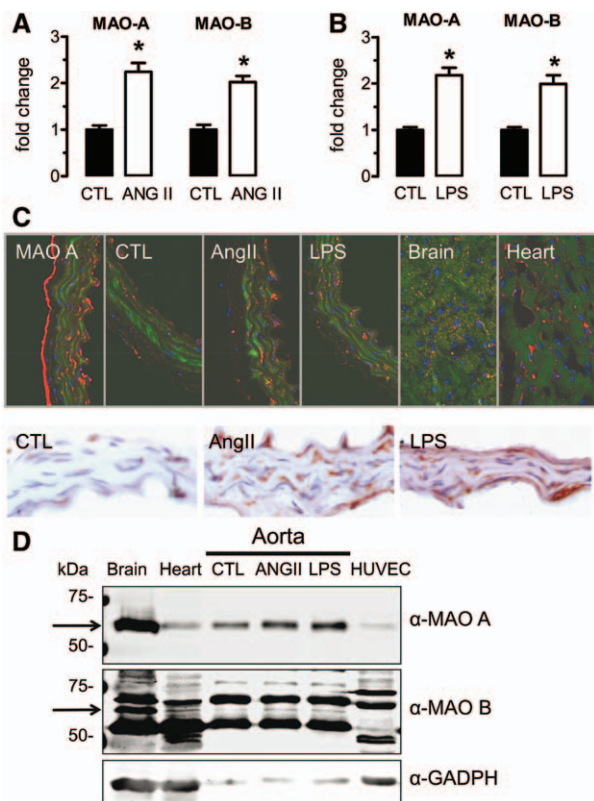


Figure 2. Vascular monoamine oxidase (MAO) expression. Mouse aortic segments were incubated (24 h, organ culture) with solvent (CTL), MAO-A (1 mU/mL), angiotensin II (Ang II; 100 nmol/L), and lipopolysaccharide (LPS; 1 μ g/mL). In some experiments, murine brain and heart served as positive controls. **A** and **B**, Quantitative real-time polymerase chain reaction for MAO-A (**A**) and MAO-B (**B**) expression. House keeping gene *EEF2 α* , $n > 10$, $*P < 0.05$. **C**, Immunofluorescence and immunohistochemistry. Red and brown, anti-MAO-A antibody; green, anti-smooth muscle α -actin; blue, DAPI. **D**, Western blot analysis for the proteins indicated. GADPH served as loading control, numbers next to the blots indicate the size of the molecular weight markers. HUVEC indicates human umbilical vein endothelial cells.

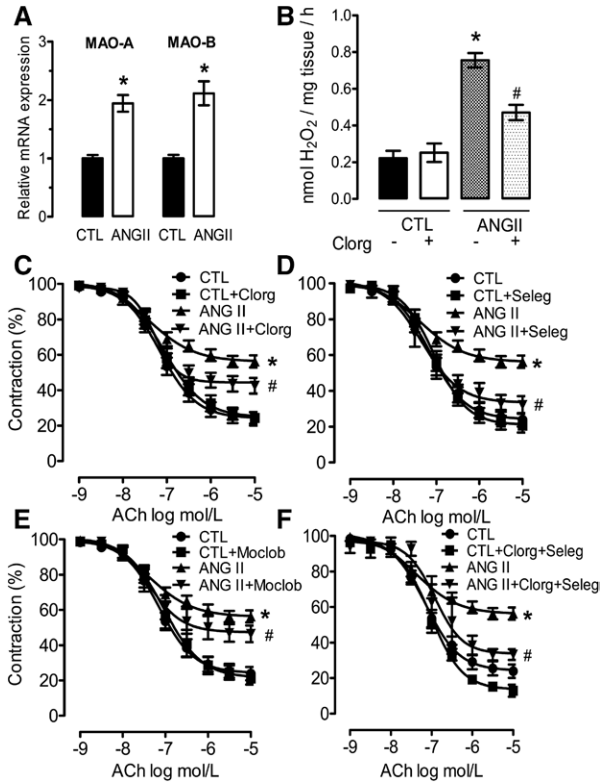


Figure 3. Role of monoamine oxidase (MAO) in angiotensin II (Ang II)-induced endothelial dysfunction. Aortic segments from mice with and without in vivo treatment with Ang II (1 mg/kg per day, 2 wk). **A**, Quantitative real-time polymerase chain reaction for the genes indicated relative to EEF 2 α , n=12, * P <0.05, * P <0.05 control (CTL) vs Ang II. **B**, H₂O₂ formation by FOX assay in the presence of absence of the MAO-A inhibitor clorgyline (Clorg, 10 μ mol/L) n=6, * P <0.05 with and without Ang II treatment, # P <0.05 with and without Clorg. **C–F**, Acetylcholine (ACh)-induced endothelium-dependent relaxation, n=6, * P <0.05 with and without Ang II treatment, # P <0.05 with and without the inhibitor indicated. Clorg (10 μ mol/L), irreversible MAO-A inhibitor, selegiline (Seleg; 10 μ mol/L), irreversible MAO-B inhibitor, moclobemide (Moclob; 10 μ mol/L, reversible MAO-A inhibitor).

to acetylcholine after LPS treatment (Figure 4C–4F). These data may indicate that MAO is of a general importance in the control of endothelium-dependent relaxation in vascular disease conditions.

Vascular MAO Induction Is Mediated by Nuclear Factor Kappa B but Not NADPH Oxidase

Because little is known regarding the mechanisms leading to MAO induction in the vasculature, a restricted expression screen was performed using pharmacological inhibitors and Ang II as well as LPS ex vivo incubation in isolated aortic segments. Expression control of MAO-A and MAO-B was similar. The induction of both enzymes was insensitive to mitogen-activated protein kinase inhibitors, protein kinase C inhibition, JAK2 (Janus kinase-2) inhibition, or blockade of mitochondria or elements of the respiratory chain. In contrast, compounds that interfere with nuclear factor κ B signaling and the phosphatidylinositol kinase inhibitor wortmannin blocked Ang II and LPS-stimulated MAO-A and MAO-B induction (Figure S3). Importantly, it was noted previously that phosphatidylinositol kinase inhibitors prevent nuclear factor κ B

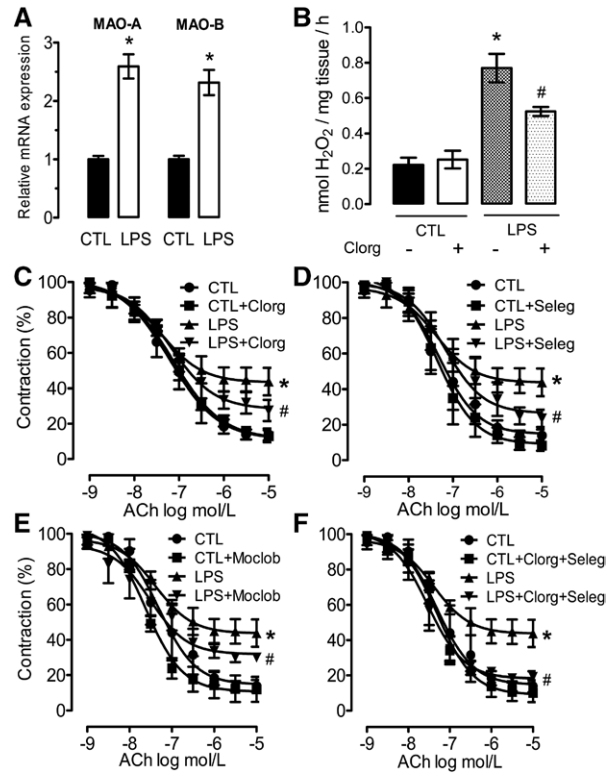


Figure 4. Role of monoamine oxidase (MAO) in lipopolysaccharide (LPS)-induced endothelial dysfunction. Aortic segments from mice with and without in vivo treatment with LPS (8 mg/kg, IP, overnight). **A**, Quantitative real-time polymerase chain reaction for the genes indicated relative to EEF 2 α , n=12, * P <0.05, * P <0.05 control (CTL) vs LPS. **B**, H₂O₂ formation by FOX assay in the presence of absence of the MAO-A inhibitor clorgyline (Clorg, 10 μ mol/L) n=6, * P <0.05 with and without LPS treatment, # P <0.05 with and without Clorg. **C–F**, Acetylcholine (ACh)-induced endothelium-dependent relaxation, n=6, * P <0.05 with and without LPS treatment, # P <0.05 with and without the inhibitor indicated. Clorg (10 μ mol/L), irreversible MAO-A inhibitor, selegiline (Seleg; 10 μ mol/L), irreversible MAO-B inhibitor, moclobemide (Moclob; 10 μ mol/L, reversible MAO-A inhibitor).

signaling,²² suggesting that this pathway is most relevant for MAO induction. No evidence for a role of NADPH oxidases in MAO induction was observed. The nonspecific Nox inhibitor apocynin had no effect on MAO induction (Figure S3), and Ang II-stimulated MAO-A and MAO-B induction was readily detectable in the aorta of Nox1, and 4 triple knockout mice (MAO-A: 1.9 \pm 0.2-fold; MAO-B: 1.8 \pm 0.2-fold; n=3).

MAO-A Limits Endothelial cGMP Accumulation

In the present concept of endothelial dysfunction, the production of O₂⁻ and the subsequent reaction with NO is the starting point of a vicious cycle. In this, a continuous production of peroxynitrite leads to an ever increasing eNOS uncoupling and thus attenuation of NO formation and further increases in O₂⁻ release. Importantly, MAO, as a flavine dehydrogenase, can entertain only 2 electron transfer and thus does not produce significant amounts of O₂⁻. Therefore, we tested whether MAO limits endothelial NO release. Because direct NO measurements are of questionable reliability (NO electrode, nitrite accumulation), endothelial cGMP was determined as a biomarker of NO generation. These experiments were performed in human umbilical vein

endothelial cells because cultured murine lung endothelial cells contain very little guanylyl cyclase. Importantly, upon stimulation with histamine and thus increases in endothelial calcium level and subsequently eNOS activity, cGMP levels more than doubled in human umbilical vein endothelial cells. This effect was not observed after MAO incubation. Cotreatment with MAO inhibitors, however, restored histamine-induced cGMP accumulation. This demonstrates that MAO activity and not contaminants possibly contained in the commercial enzyme preparation block endothelial cGMP accumulation (Figure 5). Thus, MAO-derived products directly limit endothelial NO formation.

Discussion

In the present study, we focused on the role of MAOs for the development of endothelial dysfunction. We detected MAO expression in murine vessels and found the enzymes to be induced in response to Ang II and LPS by pathways involving phosphatidylinositol kinase and nuclear factor κ B. Blockade of endogenous MAO activity reduced the vascular formation of H_2O_2 and partially restored normal endothelium-dependent relaxation in vessels pre-exposed to Ang II or LPS. MAO-A limited endothelial cGMP accumulation, suggesting that products of the enzyme attenuate endothelial NO formation.

ROS-induced endothelial dysfunction is a well-established pathomechanism, but usually this phenomenon is linked to increased formation of O_2^- , but not H_2O_2 . In fact, only O_2^- can scavenge NO and thereby give rise to the formation of peroxynitrite, which is a prerequisite for eNOS uncoupling.⁵ In contrast, there is ample evidence to suggest that vascular H_2O_2 formation might even be beneficial for the vascular system. H_2O_2 induces and activates eNOS²³. H_2O_2 is also an endothelium-derived hyperpolarizing factor in many vessels and can directly oxidize and thus activate protein kinase $G\alpha$.²⁴ Moreover, the fact that the NADPH oxidase Nox4 has rather beneficial effects in the cardiovascular system was linked to the ability of the enzyme to produce H_2O_2 and not O_2^- as the other Nox isoforms do.²⁵

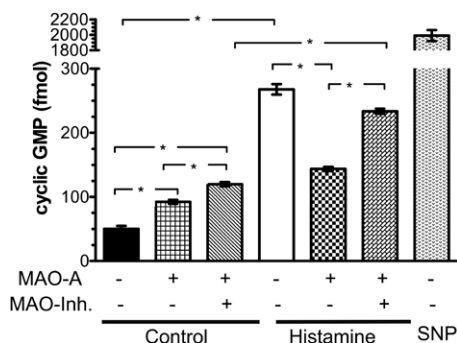


Figure 5. Monoamine oxidase (MAO-A) limits endothelial cyclic GMP (cGMP) accumulation. Quantitative determination of cGMP levels as a measure of NO availability in human umbilical vein endothelial cells in the presence of the phosphodiesterase inhibitor isobutylmethyl xanthine (0.5 mmol/L) and stimulation with either MAO-A alone (0.2 mU/mL) or MAO-A (0.2 mU/mL) with the MAO inhibitor combination of selegiline (10 μ mol/L) and cloglyline (10 μ mol/L) for 30 minutes and if indicated, additionally with histamine (1 μ mol/L) for 5 minutes, $n=6$, $*P<0.05$, $*P<0.05$. Inh indicates MAO-A inhibitors selegiline and cloglyline; and SNP, sodium nitroprusside dihydrate (30 μ mol/L).

Nevertheless, beyond a certain concentration, ≈ 30 μ mol/L in the extracellular space, H_2O_2 promotes inflammatory activation, induces endothelial dysfunction, and may even promote induction of apoptosis. Two important mechanisms may underlie this effect: glutathionylation of eNOS²⁶ and proline-rich tyrosine kinase 2 (PYK2)-mediated phosphorylation of eNOS on Tyr657.²⁷ PYK2 is known to be activated by H_2O_2 through thiol oxidation,²⁸ and the subsequent PYK2-mediated tyrosine phosphorylation of eNOS seems to transfer the enzyme into an insoluble, inactivate state.²⁷ Previously, it was noted that this pathway is stimulated by H_2O_2 and activated by NADPH oxidases in response to Ang II. Because MAO-A incubation limited endothelial cGMP accumulation, it is appealing to speculate that thiol oxidation is also operative in the present scenario and mediates endothelial dysfunction. Indeed, PYK2 inhibition in part prevented the effect of MAO-A on endothelium-dependent relaxation (Sturza, unpublished observation, 2012). Obviously, these findings are mechanistically interesting, as they were, however, obtained with ex vivo MAO incubation, their physiological relevance is questionable. Nevertheless, because MAO inhibition in models of ROS-induced endothelial dysfunction improved endothelium-dependent relaxation in the present study, we suggest that a similar mechanism is operative under physiological conditions.

Polyethylene glycol-catalase failed to completely prevent the effects of MAO on vascular function. A possible explanation could be that the large molecule did not sufficiently penetrate the vessel but additional mechanisms might also be operative. Because we have excluded that other important vascular sources of ROS are nonspecifically inhibited by MAO blockers and because the compounds used in the present study had only a minor antioxidant effect, it is tempting to speculate that MAO by a second mechanism might promote development of endothelial dysfunction. A potential explanation is the degradation of β -receptor stimulating catecholamines at the endothelial surface. Adrenaline by increasing endothelial cAMP promotes endothelial NO formation by a protein kinase A-mediated phosphorylation on eNOS-Ser 1177.²⁹ Moreover, a cross talk between cAMP and cGMP on the level of phosphodiesterases is well established.³⁰ Thus, removal of locally produced catecholamines by MAOs has the potential to impair endothelium-dependent relaxation. There is, however, a second fascinating alternative to explain potential deleterious effects of MAOs on the endothelium, which is by the formation of NH_3 . NH_3 is known to be a mediator of cerebral vascular dysfunction in hepatic encephalopathy. Ammonia stimulates the formation of ROS by cerebral endothelial cells,³¹ and reduction of ammonia levels in a liver failure model improved endothelial function.³²

For the formation of H_2O_2 and ammonia, MAOs have to be supplied with substrate. Previous studies have demonstrated that the increased vascular formation of ROS after supplementation with serotonin was in part MAO mediated.³³ It was, however, not studied whether this effect occurs without supplementation of substrate, that is, whether endogenous vascular catecholamines are sufficient to fuel MAOs. Adventitial nerves produce norepinephrine, and platelets positioned at the endothelial surface could serve as a source of serotonin. Moreover, vascular cells express serotonin

transporters and are known to store significant amounts of this biogenic amine.³⁴ Another less well studied alternative could be the direct production of catecholamine by vascular cells. Although this was not directly tested and was not a focus of the present study, at least the expression of several enzymes involved in catecholamine synthesis could be detected by real-time polymerase chain reaction albeit at lower levels than in the adrenal medulla. The present observation that MAO inhibition restores endothelial function in situations of increased MAO expression, therefore, could be interpreted as support for sufficient autonomous production or storage of yet to be defined MAO substrates in diseased vessels.

Because of local pools and an inhomogeneous distribution of target molecules, ROS signaling is compartmentalized.³⁵ This aspect is of relevance because mean tissue-wide concentrations of ROS may differ greatly from those present in hot spots of ROS signaling. On the cellular level, the present study seems to suggest that vascular MAO is predominantly expressed in smooth muscle cells with fewer enzyme being present in the endothelium. Indeed, the total vascular mRNA expression of MAO was not affected by endothelial denudation. Moreover, cultured endothelial cells expressed significantly lower amounts of MAO and responded less well to MAO-inducing stimuli as compared with the whole vessel. Nevertheless, exposure of murine lung endothelial cells to H₂O₂, which stimulates nuclear factor κB signaling, was sufficient to increase MAO protein expression as detected by Western blot. On the cellular level, MAO is expressed at the outer mitochondrial membrane and thus is another source of mitochondrial ROS in addition to the respiratory chain and p66shc.⁶ In this context, it should, however, be mentioned that an intense cross talk between the different ROS generators within mitochondria but also between the mitochondrion and other cellular compartments is present. It seems that cellular stimulation with ROS in general triggers a uniform ROS formation by several sources,³⁶ and interfering with a single ROS generator therefore often lowers the ROS formation by the other systems.³⁶ Although this concept seems rather theoretical, in the present study, we observed that 50% of the increased ROS formation in response to Ang II or LPS treatment was sensitive to MAO inhibition even if it was previously reported that NADPH oxidase, NOS uncoupling, or xanthine oxidase are the sources of ROS under these conditions. An alternative interpretation of the finding that virtually any form of ROS generator contributes to total vascular ROS formation in disease conditions is the threshold hypothesis. It assumes that increased ROS levels become only apparent once the antioxidant defense, built up by peroxiredoxin and glutathione peroxidase has been exhausted. Obviously, in such a model, inhibition of an individual source of ROS may lower the total detectable ROS level again below the postulated threshold level of the antioxidant capacity.

An important limitation of the present study is that we based a significant part of our observations on pharmacological MAO inhibition. Although the compounds used here had relatively little antioxidative effects and did not inhibit the closely related xanthine oxidase, we cannot completely exclude that off-target effects of the MAO inhibitors contribute to that normalization of endothelial function. Indeed, we

restricted our functional analysis to isolated vessels and did not perform in vivo MAO inhibition or used MAO knockout mice. Obviously, such strategies and animals are available, but systemic MAO inhibition in the disease models used in the present study is not suited to address the role of MAO in endothelial function. Catecholamine production or signaling is increased in septic shock³⁷ and also after LPS and Ang II treatments, conditions that at least sensitize the sympathetic nervous system.³⁸ Because MAOs are a main pathway of catecholamine degradation, the MAO inhibitor-induced accumulation of these vasoactive compounds will alter the blood pressure response present in the model of vascular dysfunction used here and interfere with the development of endothelial dysfunction.¹⁵ Accordingly, an appropriate analysis of individual MAO enzymes in the vascular system would require tissue-specific, inducible genetic deletion models, which have not been generated, yet.

Perspectives

With the present study, we have established MAOs as novel mediators of endothelial dysfunction in the mouse LPS and Ang II model. MAO-A and MAO-B are both expressed in the vascular system and are both induced in response to LPS and Ang II. MAO-mediated endothelial dysfunction is in part mediated by H₂O₂ and limits endothelial cGMP accumulation. Inhibition of MAOs restored normal endothelial function in diseased vessels ex vivo. Despite this, MAO inhibitors are not suited to attenuate vascular dysfunction because the global increase in catecholamine levels resulting from MAO inhibition will lead to severe side effects like hypertension. With novel therapeutic strategies like local drug delivery or targeted siRNA approaches, this problem might be overcome and then MAOs could become suitable targets to improve vascular function.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Monoamine oxidases (MAOs) are identified as a mediator of endothelial dysfunction.
- The enzymes are induced in vascular inflammation and hypertension.
- Inhibition of MAOs ex vivo normalized endothelial function in the murine angiotensin II and lipopolysaccharide model.

What Is Relevant?

- MAOs are enzymes involved in the metabolism of catecholamines, which generate H₂O₂ and ammonium as by-products.

- MAOs are thought to contribute to neuronal and cardiac injury during ischemia reperfusion. Their function in vascular disease is unknown.

Summary

We identified MAOs as novel mediators of endothelial dysfunction. Targeted inhibition of MAOs might be used to treat vascular dysfunction in hypertension and inflammation.