Vascular Responses to α_1-Adrenergic Receptors in Small Rat Mesenteric Arteries Depend on Mitochondrial Reactive Oxygen Species

Li Hao, Tamiko Nishimura, Hua Wo, Carlos Fernandez-Patron

Background—Agonists of G-protein–coupled receptors (eg, adrenoceptors and angiotensin receptors) signal, at least in part, through matrix metalloproteinases (such as matrix metalloproteinase [MMP]-7) that transactivate the epidermal growth factor receptor (EGFR). Focusing on adrenoceptors, we examined whether the MMP-dependent signaling pathway depends on reactive oxygen species (ROS).

Methods and Results—In isolated rat mesenteric arteries, selective stimulation of α_1-adrenoceptors with phenylephrine induced MMP transactivation of the EGFR, mitochondrial ROS production (detected by MitoTrackerRed-CM-H_2XRos-fluorescence and dihydroethidium-fluorescence and high-performance liquid chromatography [HPLC]/MS assay) and vasoconstriction. Inhibition of the synthesis of either MMP-7 or EGFR with anti-sense or siRNA oligonucleotides, respectively, decreased mitochondrial ROS production in response to phenylephrine. Targeted mitochondrial ROS scavenging with MitoTrackerRed-CM-H_2XRos inhibited adrenergic vasoconstriction. Adrenoceptor-induced ROS increased mitochondrial membrane potential ($\Delta\psi$m), which was prevented by blockers of MMPs (GM6001, doxycycline), EGFR (AG1478), or complex I, all of which also prevented ROS production as well as vasoconstriction.

Conclusions—Production of mitochondrial ROS is a new event in the pathway by which vasoactive agonists that induce MMP transactivation of the EGFR modulate vascular tone. Moreover, our findings suggest a connection between agonist-induced activity of MMPs, the promotion of oxidative stress, enhanced vascular tone, and hypertrophy, which are all implicated in the development and progression of vascular disease. **(*Arterioscler Thromb Vasc Biol*. 2006;26:819-825.)**

Key Words: adrenergic ■ G-protein coupled receptor (GPCR) ■ matrix metalloproteinase (MMP) ■ growth factor receptor ■ signalling ■ mitochondria ■ reactive oxygen species (ROS) ■ membrane potential ■ vascular

The agonists of certain Gq-protein–coupled receptors (GPCRs), such as adrenoceptors and angiotensin receptors, are vasoconstrictors as well as growth factors, suggesting that vasoconstriction and growth may be signaled through common pathways. In line with this notion, we have recently proposed a mechanism whereby the maintenance of vascular tone may depend on transactivation (ie, tyrosine-phosphorylation) of growth factor receptors, such as the EGFR and PDGFR, by GPCR agonists.[1] Transactivation of the EGFR occurs, at least in some instances, through an extracellular pathway that depends on activity of extracellular matrix metalloproteinases (MMPs) and disintegrins,[2–4] which induce the shedding of growth factors (eg, heparin-binding epidermal growth factor, HB-EGF) and thereby EGFR transactivation. We have identified MMP-7 as a major HB-EGF sheddase responsible for EGFR transactivation in arteries.[1,5,6]

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Reactive oxygen species (ROS) are a newly emerging class of mediators involved in intracellular signaling of vascular tone and growth.[7,8] Recent research has implicated GPCR-induced ROS generation in angiotensin II-induced hypertension and angiotensin II-induced growth.[3,9,10] It was recently proposed that growth effects of adrenoceptors may also depend on ROS generation as well as EGFR transactivation by MMPs.[11,12]

Focusing on α_1-adrenoceptors as a model of GPCR linked to MMP-dependent EGFR transactivation, we now examine the hypothesis that maintenance of adrenergic vascular tone depends on generation of ROS downstream of MMPs and the EGFR.

Materials and Methods

Please see http://atvb.ahajournals.org.

Results

Maintenance of Adrenergic Vascular Tone Requires ROS

We first examined α_1-adrenoceptor signaling in small rat mesenteric arteries mounted on a microperfusion system and preconstricted with phenylephrine, a selective agonist of α_1-adrenoceptors. In this system, phenylephrine, when added to

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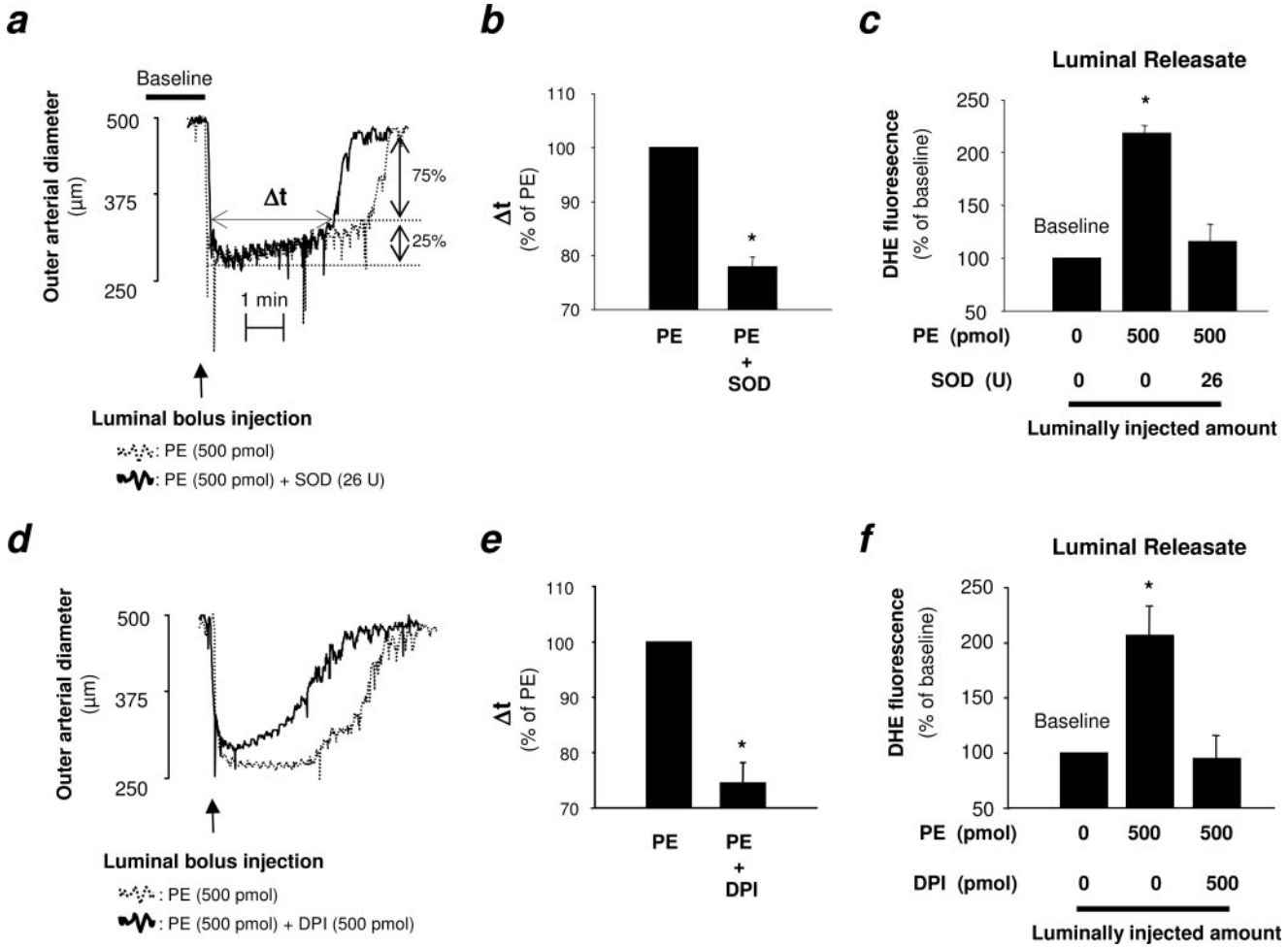

Figure 1. ROS production modulates vascular adrenergic tone. Small rat mesenteric artery mounted on the microperfusion system and studied at a constant flow rate of 2 μL/min. The arterial diameter was measured over the course of the entire experiment using a microscope coupled with a computerized video dimension analyzer system. Original traces showing the time-course of contractile response to a PE bolus (5 μL) injected in the line toward an artery, alone or together with either SOD (a) or DPI (d). Quantitative analysis of the duration (Δt) of PE-induced contractile responses alone versus in the presence of either SOD (b) or DPI (e). The duration of constriction was measured from the 2 time points at which the outer arterial diameter was 75% of maximal PE constriction. c and f, Quantitative analysis of ROS released luminally during PE-induced contractile responses. ROS in the luminal releasate were trapped by direct collection in a vial containing the ROS-sensitive fluorescent probe, DHE, followed by fluorescence analysis on a Typhoon imager. Traces and results (mean$\pm$SEM) are representative of 5 independent experiments. *$P\leq$0.05 vs PE (b,e) or vs baseline (c,f). SOD and DPI were studied in different arteries. Contractile responses to 2 consecutive injections of PE alone were nearly identical in magnitude and duration (please see Figure II).

the arterial bath (adventitia side) at concentrations between 1 and 10 μmol/L, results in a long-lasting ($\approx$2 hours) vasoconstriction, corresponding to a 30% to 40% reduction of the arterial diameter under baseline microperfusion conditions.[1] The luminal injection of boluses of 4 anti-oxidants: L-N-acetylcysteine, superoxide dismutase (SOD), MnTBAP (a cell permeable SOD mimetic), or diphenylene iodonium (DPI) (a nonselective inhibitor of many vascular oxidases including Nox, mitochondrial complex I, xanthine oxidase, and nitric oxide synthase)[7] resulted in dose-dependent vasodilatation (Figure Ia, available online at http://atvb.ahajournals.org). To measure ROS production during phenylephrine-induced vasoconstriction, we used dihydroethidium (DHE), which fluoresces red on oxidation by ROS, yielding 2 structurally distinct products, ethidium and oxyethidium (which is a specific product of the reaction of DHE with superoxide anion).[13,14] Luminal injection of a phenylephrine bolus resulted in vasoconstriction (Figure 1a and 1d, dotted lines; Figure II, available online at http://atvb.ahajournals.org), which was accompanied by a luminal release of ROS (as measured by an increase in DHE fluorescence in the collected luminal releasate) (Figure 1c and 1f). The coinjection of either SOD (to scavenge superoxide anion) or DPI (to inhibit vascular oxidases) with phenylephrine significantly decreased the duration (Δt), but not the magnitude, of phenylephrine-induced vasoconstriction (Figure 1a and 1d, solid lines; quantified in Figure 1b and 1e). Moreover, SOD and DPI decreased DHE fluorescence in the arterial releasates (Figure 1c and 1f). Analysis of DHE reaction products by liquid chromatography revealed an enhanced formation of oxyethidium versus ethidium in arteries stimulated with phenylephrine (Figure III, available online at http://atvb.ahajournals.org).

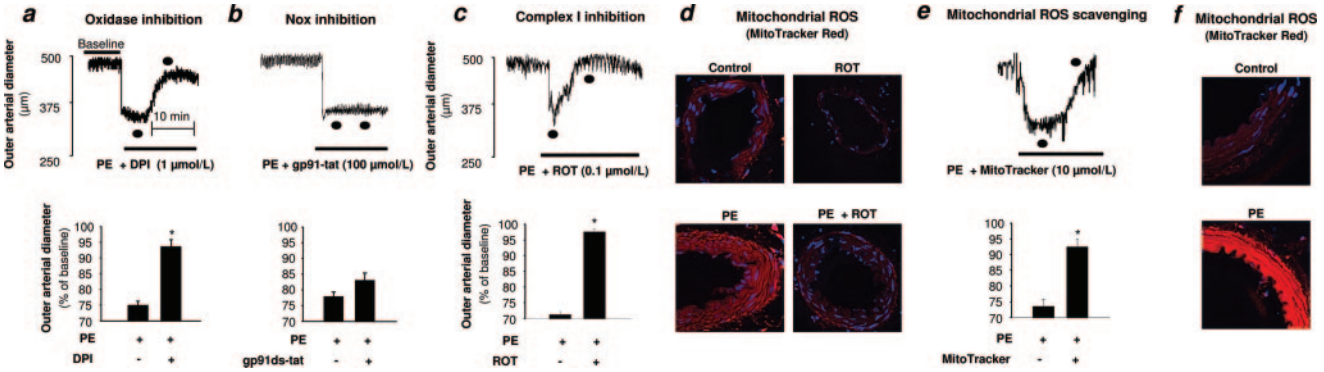

Figure 2. Adrenergic vascular tone requires generation of ROS at mitochondrial complex I. Small rat mesenteric artery mounted on the microperfusion system. PE was added to the bath (adventitia side) either alone or together with indicated drugs. Contractile responses were recorded and analyzed. Top panels, Original traces showing the time-course of arterial responses to PE in the presence of DPI (a), gp91ds-tat (b), rotenone (c) or reduced MitoTracker Red CM-H_2XRos (e). Bottom panels, Quantitative analysis of responses. d, Fluorescence laser scanning micrographs (40× objective, 2.5× microscope zoom) of cryosections of MitoTracker Red CM-H_2XRos–loaded arteries incubated for 10 minutes either in the absence (control) or presence of rotenone, followed by stimulation with PE for 45 minutes. f, MitoTracker Red CM-H_2XRos was added to the bath (adventitia side) of a microperfused artery either alone (control) or with PE. Contractile responses were recorded during 20 minutes. The artery was then fixed, cryosectioned, and analyzed by fluorescence laser scanning microscopy (40× objective, 2.5× microscope zoom). ●Representative time point when arterial diameter was acquired for quantitative analysis. Traces and results (mean±SEM) are representative of 4 to 5 independent experiments for every data point. *$P \leq 0.05$ vs PE.

Mitochondrial Involvement in Adrenergic Vascular Tone

To measure the relative contribution to adrenergic tone of 2 major vascular oxidases, NAD(P)H oxidase (Nox) and mitochondrial complex I, we examined the contractile response to phenylephrine when added to the arterial superfusion bath either alone or together with inhibitors of these enzymes (Figure 2). In the presence of DPI (a blocker of both mitochondrial complex I and Nox), although phenylephrine caused vasoconstriction, arteries could not maintain the tone and relaxed in a time-dependent fashion (Figure 2a). In contrast, phenylephrine vasoconstriction was not affected by gp91ds-tat (10 to 100 μmol/L), a selective inhibitor of Nox2 (Figure 2b). This membrane permeable peptide contains a "decoy sequence," which inhibits assembly of active Nox by preventing the interaction of membrane-bound glycoprotein, gp91phox, with the cytosolic Nox 2 subunit, p47phox.[15] Further, the Nox inhibitor apocynin (up to 100 μmol/L) did not affect the contractile response to phenylephrine. Interestingly, in the presence of rotenone (100 nmol/L), a blocker of mitochondrial complex I, although phenylephrine caused vasoconstriction, arteries did not maintain the tone and relaxed in a time-dependent manner (Figure 2c). Further, the injection of boluses of rotenone (from 50 to 500 pmol) in the line toward a phenylephrine preconstricted microperfused artery resulted in dose-dependent vasodilatation (Figure IV, available online at http://atvb.ahajournals.org). Similar to rotenone, antimycin A (1 μmol/L, a complex III inhibitor), cyanide (1 mmol/L, a blocker of complex IV), and oligomycin (1 μmol/L, a complex V ATP synthase inhibitor) all opposed adrenergic vascular tone (data not shown).

To confirm that mitochondria are a major source of the ROS made in response to α_1-adrenoceptors, we used a mitochondria-selective ROS scavenger and fluorescent probe, MitoTracker Red CM-H_2XRos. This reduced form of Mitotracker Red does not fluoresce until it has been oxidized by ROS after uptake into the mitochondrial matrix of respiring cells, and has recently been used to demonstrate ROS generation in mitochondria during cold-induced vasoconstriction.[16] In MitoTracker Red CM-H_2XRos–loaded arteries, phenylephrine resulted in a dramatic increase in red fluorescence, demonstrating that α_1-adrenoceptor stimulation triggers ROS generation in mitochondria of arteries (Figure 2d; PE). However, in the presence of rotenone, adrenergic stimulation did not increase mitochondrial ROS production (Figure 2d; PE+ROT). If adrenergic vasoconstriction depended on mitochondrial-derived ROS, then mitochondrial-specific ROS scavenging by MitoTracker Red CM-H_2XRos should inhibit adrenergic vasoconstriction, mimicking the vascular tone effects of rotenone. In the presence of MitoTracker Red CM-H_2XRos, phenylephrine resulted in vasoconstriction but the arteries could not maintain the tone and relaxed to their baseline diameters (Figure 2e). Moreover, laser scanning microscopy analysis of sections made from these same arteries revealed that fluorescence of MitoTracker Red CM-H_2XRos was brighter in arteries stimulated with phenylephrine (Figure 2f; PE) versus nonstimulated arteries (Figure 2f; control).

To examine the generality of these findings, we studied high K^+-induced vasoconstriction, which depends on smooth muscle cell depolarization and, in contrast to phenylephrine, does not involve GPCR activation. Interestingly, we observed that both rotenone and MitoTracker Red CM-H_2XRos inhibited vasoconstriction induced by high K^+ (Figure Va, Vb, and Vd, available online at http://atvb.ahajournals.org). Arteries treated with KCl also exhibited an increase in MitoTracker Red CM-H_2XRos fluorescence, which was prevented by rotenone (Figure Vc, Ve).

Adrenoceptors Modulate ROS and Vascular Tone Via MMPs

We have previously proposed that adrenergic-dependent vasoconstriction depends on activity of MMPs, such as MMP-7, which mediate the adrenoceptor–EGFR transactivation path-

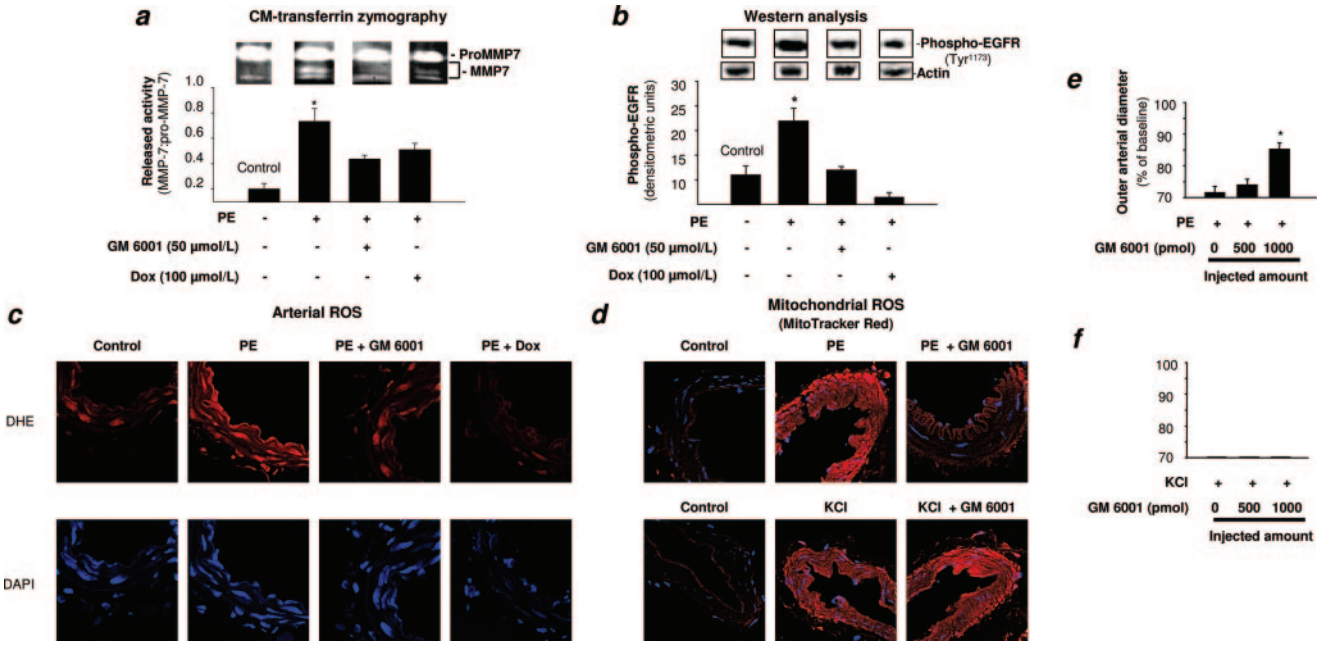

Figure 3. Adrenoceptor-dependent ROS generation requires MMP activity. Small mesenteric arteries were pre-incubated in the absence or presence of GM 6001 or doxycycline (for 10 minutes) and then stimulated with PE for 45 minutes. a, MMP-7 activity in arterial releasates assessed by CM-transferrin zymography. b, Western analysis of arterial lysates with phospho-EGFR–specific antibodies. Results (mean±SEM) are representative of 4 to 5 independent experiments. *$P \leq 0.05$ vs control. c, Fluorescence laser scanning micrographs (40× objective, 2.5× microscope zoom) of sections of isolated DHE-loaded arteries incubated in the absence (control) or presence of GM 6001 or doxycycline (for 10 minutes) followed by stimulation with PE for 45 minutes. d, Fluorescence laser scanning micrographs (40× objective, 2.5× microscope zoom) of cryosections of MitoTracker Red CM-H_2XRos–loaded arteries incubated in the absence (control) or presence of GM 6001 (for 10 minutes) followed by stimulation with PE or KCl for 45 minutes. Quantitative analyses of arterial responses to bolus injections of GM 6001 into microperfused arteries pre-constricted with either PE (e) or KCl (f). Arterial diameter was determined at time points after bolus injections (as in Figure I). Results (mean±SEM) are representative of 4 to 5 independent experiments for every data point. *$P \leq 0.05$ vs PE.

way.[1] To examine whether activity of MMPs also modulates ROS production, we treated arteries with phenylephrine in the absence or presence of 2 unrelated MMP inhibitors (GM 6001 and doxycycline) and assessed ROS generation using both DHE (to detect intracellular ROS generation and accordingly also mitochondrial ROS) and reduced MitoTracker Red CM-H_2XRos (to specifically detect ROS generation in mitochondria). Both MMP inhibitors blocked MMP-7 activation (determined by CM–transferrin zymography) and EGFR transactivation (determined by Western blot with phospho-EGFR–specific antibody) (Figure 3a and 3b), and prevented phenylephrine-induced ROS generation (Figure 3c and 3d). Further, analysis of DHE reaction products by liquid chromatography revealed that MMP blockade with GM 6001 inhibited the enhanced formation of oxyethidium versus ethidium in arteries stimulated with phenylephrine (Figure III). Similar to phenylephrine, high K^+ increased mitochondrial ROS generation, but blockade of MMPs with GM 6001 failed to decrease this (Figure 3d). The inhibition of MMPs with GM 6001 promoted vasodilatation of phenylephrine, but not high K^+, preconstricted arteries (Figure 3e and 3f).

Arteries and cultured rat vascular smooth muscle cells (VSMCs) treated with MMP-7 antisense or EGFR siRNA oligonucleotides to decrease the expression of these mediators showed decreased mitochondrial ROS generation (Figures VI and VII, available online at http://atvb.ahajournals.org).

The catecholamine norepinephrine (10 μmol/L), which binds to α-and β-adrenoceptors and thus transactivates the EGFR in vascular smooth muscle, also triggered mitochondrial ROS generation, which was prevented by EGFR siRNA (Figure VII) and by MMP inhibition with GM6001 (Figure VIII, available online at http://atvb.ahajournals.org).

Adrenoceptors Modulate Mitochondrial Membrane Potential ($\Delta\Psi$m) Via an MMP-Dependent Pathway

Similar to arteries, the stimulation of α_1-adrenoceptors in VSMCs increased ROS generation in mitochondria, as measured using DHE (Figure 4a; PE) and MitoTracker Red (Figure 4b; PE). DHE fluorescence was abolished by MnTBAP, suggesting that superoxide anion was responsible for intracellular DHE oxidation (Figure 4a; PE+MnTBAP).

In addition to increasing mitochondrial ROS production, the stimulation of α_1-adrenoceptors also increased mitochondrial membrane potential ($\Delta\Psi$m), as indicated by a decrease in the ratio of green to red fluorescence of live VSMCs loaded with the potentiometric dye JC-1 (Figure 4c and 4d; PE). This increase in $\Delta\psi$m was detected as early as 2 minutes after stimulation and lasted for at least 10 minutes (Figure IX, available online at http://atvb.ahajournals.org), a time-course that parallels EGFR transactivation by phenylephrine in these cells (data not shown). The α_1-adrenoceptor–induced increases in both ROS generation and $\Delta\psi$m were prevented by the inhibition of MMPs (with GM 6001), the EGFR (with AG

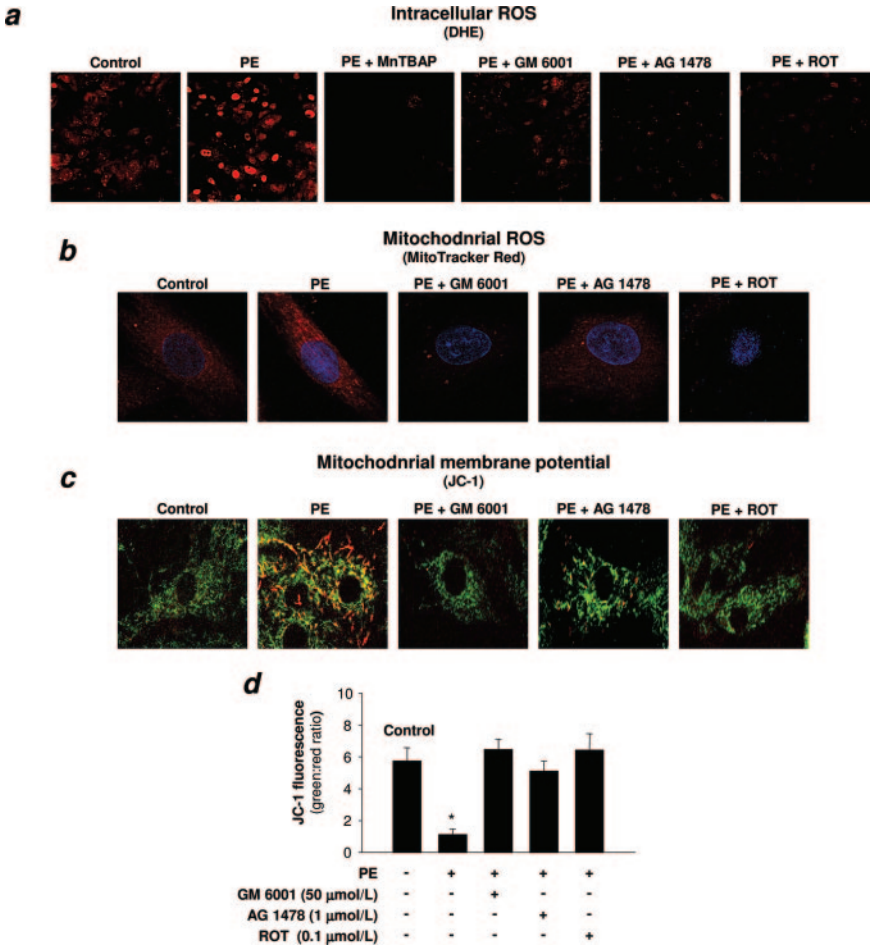

Figure 4. Adrenergic signaling increases mitochondrial membrane potential: involvement of MMPs, the EGFR, and complex I-derived ROS. Primary rat VSMCs plated on glass-bottom tissue culture dishes were starved for 24 hours and loaded with DHE, MitoTracker Red CM-H_2XRos, or JC-1 for 20 minutes. Loaded cells were then incubated for 10 minutes in the absence or presence of indicated inhibitors, followed by stimulation with PE for 10 minutes. a, Detection of intracellular ROS generation. DHE oxidation products fluoresce brightly red (40× objective). b, Detection of mitochondrial ROS generation. Reduced MitoTracker Red CM-H_2XRos is targeted to mitochondria where it must be oxidized to form a red fluorescent product (63× objective, 3.2× microscope zoom). c, The response to phenylephrine involves an increase in $\Delta\Psi$m, which is opposed by blockade of MMPs (PE+GM 6001), EGFR (PE+AG 1478), or complex I (PE+ROT) as detected using JC-1, a potentiometric dye that fluoresces green in mitochondria with membrane potentials $<\approx$150 mV and fluoresces red at high (>150 mV) membrane potentials (40× objective, 3× digital zoom). d, Quantification of JC-1 green to red fluorescence 5 minutes after PE stimulation. Results are representative of 3 independent experiments. *$P\leq0.05$ vs control. For time-lapse micrographs of PE-induced increases in membrane potential, see Figure IX.

1478), or complex I (with rotenone) (Figure 4a to 4d). Mitochondrial depolarization with cyanide chlorophenylhydrazone (CCCP) also opposed the phenylephrine-induced increase in $\Delta\Psi$m (Figure Xa, available online at http://atvb.ahajournals.org) and vascular tone (Figure Xb).

A Role of Nitric Oxide in ROS Mediation of Adrenergic Signaling?

A purported mechanism for the biological actions of ROS involves the reaction with endogenous nitric oxide (NO) to form peroxynitrite, thus reducing NO bioavailability and, thereby, NO-dependent vasodilatation.[17] We reasoned that if the signaling of vascular tone by ROS depended primarily on decreasing NO-dependent vasodilatation, then levels of protein nitrotyrosine, a marker of peroxynitrite, would be significantly increased by phenylephrine. However, Western analysis did not reveal any significant change in protein nitrotyrosine staining on stimulation of arterial adrenoceptors with phenylephrine (data not shown). Using NO-selective fluorescent probe (DAF-2), we observed no significant changes in levels of NO released luminally on stimulation of arterial adrenoceptors by luminal infusions of phenylephrine. If the vasoconstrictor effect of ROS depended on decreasing NO-dependent vasodilatation, we would also expect a loss of the vasodilatory effects of complex I inhibition by rotenone when performed in the presence of the NO synthesis inhibitor L-NAME, because NO would not be present to effectuate the vasodilatation. However, the vasodilatory effects of bolus injections of rotenone (50 to 500 pmol) were preserved in the presence of L-NAME (750 μmol/L), mimicking the effects of GM 6001, which were also unchanged in the presence of L-NAME (data not shown). In endothelium-denuded mesenteric arteries, the constrictor activity of phenylephrine was preserved and α_1-adrenergic signaling triggered mitochondrial ROS generation, as detected using MitoTracker Red CM-H_2XRos. Targeted mitochondrial ROS scavenging with MitoTracker Red CM-H_2XRos inhibited the maintenance of adrenergic constriction in endothelium-denuded arteries. The only difference we observed between endothelium-denuded and intact arteries was that low concentrations of rotenone (0.1 or 1 μmol/L) did not inhibit vascular tone, whereas a higher concentration of rotenone (10 μmol/L) as well as antimycin A (1 μmol/L, a complex III inhibitor) and CCCP (1 μmol/L, a mitochondrial uncoupler) did (data not shown).

Discussion

This study shows that α_1-adrenergic vascular tone in small rat mesenteric arteries depends on mitochondrial ROS generation and the subsequent increase in $\Delta\Psi$m downstream of MMPs and the EGFR.

Previously, mitochondrial-derived ROS have also been implicated in α_2-adrenergic vasoconstriction of rat tail arterioles in response to cold-induced and hypoxia-induced pulmonary vasoconstriction.[18–21] Other studies have shown a

major role for Nox in the vascular tone effects of angiotensin II.[10,22,23] Although it is unclear why the relative contributions of mitochondrial versus nonmitochondrial ROS sources to transactivation signaling varies, published and current results demonstrate that ROS are key signaling messengers in the vascular wall. We also observed that both receptor-dependent (ie, adrenergic) and receptor-independent (ie, high K^+) signaling stimulated ROS generation in mitochondria, although the pathways for this are distinct; only the adrenergic pathway uses MMPs to trigger mitochondrial ROS generation and to maintain vascular tone.

Based on our previous work implicating MMPs in EGFR transactivation,[1] we investigated whether the EGFR was a component of the signaling pathway leading from adrenoceptors to the mitochondrial ROS generator. In VSMCs and arteries, the stimulation of adrenoceptors resulted in EGFR transactivation (ie, increased EGFR-Tyr^{1173}-phosphorylation). However, GM 6001 and doxycycline, 2 unrelated MMP inhibitors, significantly decreased EGFR transactivation and ROS generation. Blockade of the EGFR with AG 1478 also abrogated α_1-adrenoceptor–mediated ROS generation in mitochondria. We confirmed these findings using anti-sense or siRNA approaches to decrease the synthesis of MMP-7 or EGFR, respectively, and thus their individual contributions to α_1-adrenergic signaling. Interestingly, our studies targeting MMP-7 suggest that it is not primarily activated by ROS (unpublished results). Altogether, these findings support a novel essential role for MMPs upstream of ROS generation, as opposed to the widely held notion that ROS are upstream activators of MMPs.[24]

Catecholamines such as norepinephrine signal via alpha and beta adrenoceptors to transactivate the EGFR.[11,12,25] Consistently, we observed that norepinephrine-stimulated mitochondrial ROS generation was inhibited by blocking the transactivation pathway at the level of MMPs or the EGFR. It is tempting to speculate that agonist-induced MMP-transactivation of the EGFR to generate ROS is a mechanism shared by agonists of many vasoactive GPCRs, not just adrenoceptors.

Blockade of mitochondrial complex I with rotenone caused a potent vasodilation of intact arteries, even in the presence of L-NAME to inhibit NO synthesis, suggesting that NO is not required for rotenone-induced vasodilation. Interestingly, rotenone was unable to cause vasodilation in endothelium-denuded arteries, even though production of mitochondrial ROS was maintained in these vessels following phenylephrine stimulation. In contrast, blockade of another mitochondrial ROS generator (complex III) or mitochondrial ROS scavenging with MitoTracker Red CM-H_2XRos caused vasodilation of endothelium-denuded as well as intact arteries. Therefore, we suggest that acute vascular injury (eg, as caused by endothelial damage) may alter the relative contributions of complexes I versus III to ROS generation and to maintenance of vascular tone, likely at the level of the connection between the EGFR and mitochondria in vascular smooth muscle. Studies to address this hypothesis are ongoing.

A form of ROS involved in vascular signaling via α_1-adrenoceptors is superoxide anion, because signaling was inhibited by the SOD mimetic MnTBAP and this inhibition was temporally concerted with a decrease in ROS generation and release in both arteries and VSMCs. Further, analysis of DHE reaction products in arteries using liquid chromatography revealed enhanced formation of oxyethidium after adrenergic stimulation. How and why superoxide anion, a highly reactive charged species, is released from the vascular wall is unclear. However, adrenergic stimulation may acutely increase production of superoxide (and probably other ROS) to an extent that cannot be prevented by endogenous scavenging systems (such as SOD).

Our findings suggest that maintenance of vascular tone by adrenoceptors depends on mitochondrial ROS, which directly modulate mitochondrial function. Adrenergic stimulation increased $\Delta\Psi m$, and this increase was blocked by any inhibitor affecting our model adrenoceptor→MMP→EGFR→ROS pathway. Moreover, the mitochondrial respiratory chain uncoupler, CCCP, which increases the conductance of the mitochondrial membrane to protons, also opposed adrenergic tone. Further studies may reveal a central role for ATP downstream of mitochondrial ROS and $\Delta\Psi m$, because the observed hyperpolarization of the mitochondrial membrane in response to adrenergic agonists would likely result in an increase in ATP production. Intriguingly, the complex V blocker oligomycin, at a concentration (1 μmol/L) that fully inhibits mitochondrial ATP synthesis in VSMCs, completely opposed adrenergic tone in our system, identical to the mitochondrial respiration uncoupler CCCP (unpublished result). Studies are underway to establish how an adrenoceptor-induced increase in $\Delta\Psi m$ sustains vascular tone, the role of ATP synthesis in vascular adrenergic signaling, and whether pharmacological manipulation of $\Delta\Psi m$ in vascular smooth muscle has therapeutic potential for decreasing vascular tone in hypertension.

Taken together, current findings suggest a connection between agonist-induced activity of MMPs, the promotion of oxidative stress, enhanced vascular tone and hypertrophy, which are all implicated in the development and progression of hypertensive disorders.[7,26] Such a mechanism could explain, at least in part, why these apparently unrelated events tend to concur in the setting of vascular disease.

Acknowledgments

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