Cyclophilin A Is a Secreted Growth Factor Induced by Oxidative Stress

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Abstract—Reactive oxygen species have been implicated in the pathogenesis of atherosclerosis, hypertension, and restenosis, in part by promoting vascular smooth muscle cell (VSMC) growth. Many VSMC growth factors are secreted by VSMC and act in an autocrine manner. Here we demonstrate that cyclophilin A (CyPA), a member of the immunophilin family, is secreted by VSMCs in response to oxidative stress and mediates extracellular signal–regulated kinase (ERK1/2) activation and VSMC growth by reactive oxygen species. Human recombinant CyPA can mimic the effects of secreted CyPA to stimulate ERK1/2 and cell growth. The peptidyl-prolyl isomerase activity is required for ERK1/2 activation by CyPA. In vivo, CyPA expression and secretion are increased by oxidative stress and vascular injury. These findings are the first to identify CyPA as a secreted redox-sensitive mediator, establish CyPA as a VSMC growth factor, and suggest an important role for CyPA and enzymes with peptidyl-prolyl isomerase activity in the pathogenesis of vascular diseases. (Circ Res. 2000;87:789–796.)

Key Words: oxidative stress n cyclophilin n secretion n mitogen-activated protein kinase n smooth muscle cells

Reactive oxygen species (ROS) have been implicated in the pathogenesis of atherosclerosis, hypertension, and restenosis, in part by promoting vascular smooth muscle cell (VSMC) growth.1–4 We have previously reported that ROS stimulate VSMC growth and DNA synthesis.5 This proliferation was associated with stimulation of protein kinases, especially the extracellular signal–regulated kinases (ERK1/2, also termed p42/44 mitogen-activated protein kinases [MAPKs]).4 ERK1/2 are stimulated by growth factors and cytokines and play pivotal roles in cell growth and differentiation.6,7 Activation of ERK1/2 by ROS generators, such as the naphthoquinolinedione LY83583, menadione, and xanthine/xanthine oxidase as well as H2O2, was biphasic; an early peak of ERK1/2 activity was present at 5 to 10 minutes, whereas a delayed ERK1/2 activation appeared at 2 hours.8 A similar biphasic activation of ERK1/2 has been reported for mitogens such as fibroblast growth factor.9 Recently, the delayed ERK1/2 activation has been reported to be mediated by different mechanisms than the early ERK1/2 activation and to be critical for cell cycle progression and cell proliferation.9,10

Increasing evidence suggests that secretion of growth factors in response to VSMC agonists mediates their mitogenic activity. For example, epiregulin, an epidermal growth factor–related growth factor, is a potent VSMC-secreted mitogen whose expression is regulated by angiotensin II, endothelin-1, and thrombin.11 These same agonists also stimulate secretion of other growth factors, including platelet-derived growth factor12,13 and transforming growth factor-β.14 However, no factors have been identified as mediators of VSMC proliferation in response to ROS.

We hypothesized that in response to ROS, VSMCs may secrete factors that participate in autocrine and paracrine growth mechanisms by stimulating ERK1/2 activity. In the present study, we report that cyclophilin A (CyPA) is an important secreted oxidative stress-induced factor, because it is secreted from rat VSMC in response to ROS and from fibroblasts transfected with mox1 [a superoxide generating homolog of the phagocyte NAD(P)H oxidase catalytic subunit]. Furthermore, we show that secreted CyPA stimulates ERK1/2, increases DNA synthesis, and inhibits nitric oxide–induced apoptosis in VSMCs. Finally, we demonstrate that immunostaining of CyPA is dramatically increased in balloon-injured rat carotid, with a time course that parallels neointima formation. These results suggest an important role for CyPA in the pathogenesis of vascular diseases.

Materials and Methods

Cell Culture

VSMCs were isolated from 200 to 250 g male Harlan Sprague-Dawley rats and maintained in 10% calf serum and DMEM, as previously described.15 Passage 5 to 14 VSMCs at 70% to 80% confluence in 150-mm or 35-mm dishes were growth-arrested by incubation in 0.1% calf serum and DMEM for 48 hours before use.

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Mox1-transfected NIH3T3 cells were prepared and grown as described previously.16

Preparation of Conditioned Medium
VSMCs were washed 3 times with HBSS (in mmol/L): NaCl 130, KCl 5, CaCl2 1.5, MgCl2 1, HEPES 20, and pH 7.4), and equilibrated in HBSS for 1 hour. Cells were then exposed to 1 μmol/L LY83583 (Calbiochem) or HBSS only for 2 hours. Conditioned medium from LY83583-stimulated cells (LY-CM) or control medium from HBSS-incubated cells (Ctl-CM) was then collected and centrifuged at 800g at 4°C for 10 minutes to remove cell debris. LY-CM or Ctl-CM was concentrated 100-fold by using a Centricon Plus-80 filter (Millipore Inc) to yield concentrated LY-CM or concentrated Ctl-CM.

Immunodepletion
Concentrated conditioned medium was incubated with rabbit anti-CyPA polyclonal antibody (1:100 dilution [Biomol Research Laboratories]) or an equal amount of normal rabbit serum for 22 hours and then incubated with protein A-agarose (Life Technologies) for 2 hours on a roller system at 4°C. The supernatants and control medium were applied to stimulate VSMCs, and ERK1/2 activity was analyzed by Western blot.

Rat Carotid Injury and Immunohistochemistry
Male Sprague-Dawley rats (350 to 400 g [Charles River Laboratories, Wilmington, Mass]) were used. The surgical procedures and immunohistochemistry were performed as previously described.17 Paraffin-embedded rat carotids in 5-μm sections were deparaffinized and boiled for 10 minutes in 10 mmol/L citrate buffer, pH 6, and then blocked with 5% normal goat serum for 30 minutes.17 Rabbit polyclonal antibody for cyclophilin A (1:1000 dilution [Biomol Research Laboratories]) and rat adsorbed biotinylated goat anti-rabbit secondary antibody (1:250 dilution [Vector]) were used, followed by 30-minute incubation in Vectastain ABC-AP (Vector) and 25-minute development in the nonquenching fluorescent alkaline phosphatase substrate Vector Red.

Other Techniques
Western blot analysis,18,19 DNA synthesis,20 MTT assay for cell viability,21,22 and determination of apoptosis23 were performed as previously described.

Statistical Analysis
Data are presented as mean±SD for all experiments that were performed at least 3 times. Significant differences were determined by Student’s t test (P<0.05).

Results
Cyclophilin A Is Secreted and Involved in Regulation of ERK1/2 Activation by Oxidative Stress
Previously we showed that brief exposure of VSMCs to LY83583, which generates O2 –, stimulated ERK1/2 with peak at 10 minutes and return to baseline at 30 minutes.4 In this new series of experiments, cells were studied for up to 6 hours after LY83583 exposure. A second peak of ERK1/2 activation was apparent at 2 hours, with return to baseline by 6 hours (Figure 1A). To confirm that biphasic ERK1/2 was attributable to ROS generation by LY83583, the effects of several antioxidants were studied. Both MnTBAP (a cell-permeable SOD mimetic [Calbiochem]) and catalase (from human erythrocytes [Calbiochem]) blocked early-phase ERK activation induced by LY83583 (Figure 1B). Catalase, but not MnTBAP, blocked late-phase ERK activation in response to LY83583 (Figure 1B). In addition, we observed that both Tiron (a superoxide scavenger [Sigma]) and diphenylene iodonium (DPI) (an inhibitor of the NADPH oxidase [Sigma]) blocked this biphasic ERK activation (Figure 1C). Because LY83583-induced O2 – generation peaked at 15 minutes and returned to baseline by 2 hours, as measured by lucigenin chemiluminescence,6 these results suggest that the late activation of ERK1/2 by LY83583 may be attributable to secretion of factors into the medium. To test this hypothesis, conditioned medium was prepared by exposing VSMCs to 1 μmol/L LY83583 for 2 hours in HBSS at 37°C. As a control, cells were exposed to vehicle alone. Then conditioned medium was added to naive growth-arrested VSMCs. Cells exposed to LY-CM, but not Ctl-CM, showed a significant increase in ERK1/2 activity, with a peak (8.9±0.9-fold) at 10 minutes (Figures 1D and 1E). The magnitude of ERK1/2 activation by LY-CM was equivalent to that observed in cells exposed to LY83583 for 2 hours. In addition, all tested antioxidants did not affect LY-CM–induced ERK1/2 activation (Figure 1F). These results indicate that a factor was secreted into the medium by VSMCs exposed to oxidative stress, which we term SOXF for secreted oxidative stress–induced factor.

Purification of SOXF from conditioned medium by sequential chromatography suggested that proteins of the cyclophilin family might act as a SOXF.21 CyPA is an abundant cytosolic protein that is the main target of the immunosuppressive drug cyclosporine A (CsA). Recently, CyPA has been reported to be secreted (for example, from lipopolysaccharide-stimulated macrophages).24 To prove that CyPA is a SOXF, Western blot analysis was performed with antibodies specific for CyPA (both the polyclonal CyPA antibody and monoclonal antibody mAb7F1 yielded similar results). CyPA was detected in LY-CM but not Ctl-CM (Figure 2A). The secretion of CyPA is specific, because no α-actin or c-raf-1 was present in LY-CM (Figure 2A), which are abundant cytoskeletal and cytosolic proteins in VSMCs, respectively. In addition, catalase, Tiron, and DPI (but not MnTBAP) inhibited the secretion of CyPA (Figure 2B). H2O2 also stimulated CyPA secretion from VSMCs (Figure 2B). These results showed that CyPA is secreted from VSMCs in response to oxidative stress and is a candidate SOXF. To estimate the concentration of CyPA present in LY-CM, Western blot analysis was performed using known concentrations of human recombinant CyPA (hrCyPA) as a standard. The intensity of Western blot immunoreactivity analyzed by densitometry was linear in the range used (least squares regression yielded the following equation: y = 4104.5x + 105.5, R2=0.98). On the basis of this comparison, the CyPA concentration in conditioned medium is ~5 nmol/L.

To determine the extent to which secreted CyPA contributes to ERK1/2 activation by LY83583, CyPA in concentrated LY-CM was immunodepleted with anti-CyPA antibody (Figure 2C). Stimulation of VSMC by concentrated LY-CM increased ERK1/2 activity by 7.4±1.8-fold (Figure 2D). Immunodepletion of CyPA significantly inhibited ERK1/2 activation (51±12% decrease, P<0.01, n=4), whereas immunodepletion with preimmune serum had no significant effect (Figure 2D). These findings indicate that
CyPA is a SOXF that accounts, in part, for the late-phase ERK1/2 activation stimulated by LY83583.

To strengthen the link between oxidative stress, CyPA expression and secretion, and cell growth, we studied cells stably transfected with mox1. In comparison with cells transfected with vector alone (NEF2), mox1-transfected cells (YA28) produce significantly greater amounts of Ohs and grow faster and to a greater cell density. The antioxidant N-acetyl cysteine (NAC) inhibited the growth of the mox1-transfected cells. The expression of CyPA was approximately 2-fold increased in YA28 cells (Figure 3A). However, the greatest difference was a 10-fold increase in the level of CyPA secreted by YA28 cells compared with NEF2 cells (Figure 3B). Importantly, the secretion of CyPA was completely inhibited by treating cells with 20 mmol/L NAC, consistent with ROS as an essential mediator for CyPA secretion (Figure 3B).

To determine additionally the ability of CyPA to stimulate ERK1/2 activation in VSMC, hrCyPA was studied. The preparation of hrCyPA used for these studies was highly purified, as shown by silver stain analysis, which revealed that >95% of total protein migrated at a molecular weight of 18 kDa, consistent with CyPA (Figure 4A). hrCyPA stimulated ERK1/2 activation in VSMC in a concentration-dependent manner, with an EC50 of 0.25 nmol/L (Figure 4B). Stimulation of ERK1/2 was not attributable to contamination by lipopolysaccharide, because heat treatment of hrCyPA abrogated its stimulating activity. The time course for ERK1/2 activation by 10 nmol/L hrCyPA (Figure 4C) was similar to that observed with conditioned medium (Figure 1F), with peak at 10 minutes.

**Peptidyl-Prolyl Isomerase Activity Is Required for CyPA-Induced ERK1/2 Activation**

CyPA is a member of the immunophilin family, which possess peptidyl-prolyl isomerase (PPIase) activity. CsA is an immunosuppressive drug that strongly inhibits the PPIase activity of CyPA. To investigate whether PPIase activity is required for CyPA-induced ERK1/2 activation, hrCyPA was incubated with CsA for 30 minutes at 4°C and then applied to VSMC. CsA inhibited hrCyPA-induced ERK1/2 activation (Figure 5A) but had no effect on Angiotensin II–induced ERK1/2 activation (Figure 5B). Importantly, CsA significantly inhibited ERK1/2 activation by LY-CM, indicating that cyclophilins present in LY-CM are biologically active (Figure 5C). Taken together, these results indicate that PPIase activity is involved in ERK1/2 activation by CyPA.
Cyclophilin A Stimulates VSMC Growth and Protects VSMC Against Apoptosis

To measure the potential growth promoting effects of CyPA secreted from VSMC, we studied the effects of LY-CM and hrCyPA on VSMC DNA synthesis. LY-CM significantly stimulated DNA synthesis in VSMC (3-fold increase versus 0.1% serum) assayed by [3 H]thymidine incorporation (Figure 6A). In contrast, Ctl-CM had no mitogenic activity (Figure 6A). In addition, 10 nmol/L hrCyPA also significantly stimulated DNA synthesis (2-fold increase versus 0.1% serum, Figure 6B). Thus, CyPA contributes significantly to the growth promoting activity present in LY-CM.

Figure 2. CyPA is secreted specifically by VSMCs treated with LY83583 and is involved in late ERK1/2 activation. CyPA in LY-CM was analyzed by Western blot using antibodies to CyPA (A, top panel) and then reprobed with antibodies to α-actin and c-Raf-1. B, CyPA in conditioned mediums from VSMCs treated with LY83583 plus MnTBP or catalase, Tiron, DPI, or 200 μmol/L H2O2 were analyzed. C, Concentrated LY-CM was treated with anti-CyPA antibody, normal rabbit serum (NRS) to immunodeplete CyPA. The supernatants were then subjected to Western blot assay for CyPA. D, VSMCs were stimulated with LY-CM, LY-CM immunodepleted with anti-CyPA antibody, or LY-CM immunodepleted with normal rabbit serum for 10 minutes. A representative Western blot for ERK1/2 activity and ERK2 protein levels is shown.

Figure 3. CyPA expression and secretion are increased in mox1 overexpressing cells. A, CyPA in cell lysate from NEF2 and YA28 (mox1-transfected) cell lines was analyzed by Western blot. B, Level of CyPA secreted was measured in conditioned medium from NEF2 and YA28 cells incubated with HBSS for 2 hours and YA28 cells with HBSS plus 20 mmol/L NAC for 2 hours.

Figure 4. hrCyPA activates ERK1/2 in VSMC. A, hrCyPA was analyzed on SDS-PAGE followed by silver stain. B and C, VSMCs were treated with the indicated concentrations of hrCyPA for 10 minutes (B) or for the indicated time with 10 nmol/L hrCyPA (C). Results of Western blot for ERK1/2 activity and ERK1/2 protein levels are representative and are mean±SD of 4 separate experiments.

hrCyPA on VSMC DNA synthesis. LY-CM significantly stimulated DNA synthesis in VSMC (3-fold increase versus 0.1% serum) assayed by [3 H]thymidine incorporation (Figure 6A). In contrast, Ctl-CM had no mitogenic activity (Figure 6A). In addition, 10 nmol/L hrCyPA also significantly stimulated DNA synthesis (2-fold increase versus 0.1% serum, Figure 6B). Thus, CyPA contributes significantly to the growth promoting activity present in LY-CM.
To determine whether CyPA prevents VSMC apoptosis, we used sodium nitroprusside (SNP), which was shown to induce VSMC apoptosis. \(^{25,26}\) Incubating VSMC with 1 mmol/L SNP for 24 hours decreased cell viability to 19.4% of control, measured with a modified MTT assay (Figure 7A). Addition of 10 nmol/L hrCyPA in the presence of 1 mmol/L SNP blocked apoptosis, with cell viability returning to 47% of control (Figure 7A). In response to 0.5 mmol/L SNP for 24 hours, \(10.3\%\) of VSMCs were apoptotic as measured by nuclear morphology after DAPI staining (Figure 7B), consistent with previous reports. \(^{25, 26}\) Addition of either LY-CM or CyPA significantly inhibited apoptosis induced by 0.5 mmol/L SNP, with decreases of 91% and 55%, respectively (Figure 7C).

### Cyclophilin A Expression and Secretion Are Increased In Vivo by Vascular Injury

Oxidative stress has been shown to mediate many of the changes that lead to vascular lesion formation in vivo. \(^1\) Because the rat carotid balloon injury model is associated with ROS generation and neointima formation develops largely as a consequence of VSMC proliferation, \(^{27,28}\) we examined the time course of CyPA expression in this model. Morphological analysis demonstrated formation of a neointima within 1 week after injury (Figure 8). Immunoreactive CyPA was present at low level in sections of sham-operated arteries (Figure 8, top panel). By 24 hours after balloon injury, abundant CyPA immunoreactivity was present throughout the vessel, with particularly strong staining in the adventitia (Figure 8, second panel). By 4 days, the majority of the CyPA staining was localized to the first cells forming the neointima and the most luminal medial VSMC layers (Figure 8, third panel). After 1 week of injury, when a substantial neointima had formed, CyPA immunoreactivity was highest in the neointima and most luminal medial VSMC (Figure 8, bottom panel). No staining was observed in sections incubated with normal rabbit serum (data not shown).

### Discussion

The present study is the first to identify cyclophilin A (CyPA) as a secreted oxidative stress-induced factor and characterizes novel extracellular functions for CyPA as a growth and survival factor. Specifically, we show that CyPA is a secreted redox-sensitive mediator that stimulates ERK1/2 activity, promotes VSMC proliferation, inhibits VSMC apoptosis, and exhibits increased expression and secretion in the presence of sustained intracellular ROS generation and after vascular injury. The PPIase activity of CyPA is required for these actions. Thus, these findings indicate an important role for CyPA in the cellular response to oxidative stress.

Understanding the mechanisms by which ROS modulate cell function is important for cardiovascular disease, because ROS are increased in ischemia-reperfusion, hypertension, and atherosclerosis. It has become clear that atherogenesis demonstrates cellular and molecular responses that resemble an inflammatory disease. \(^{29}\) Monocytes, polymorphonuclear leukocytes, and T lymphocytes are markedly increased in regions of human atheroma. \(^{30,31}\) ROS produced by white blood cells in the vessel wall in vivo is unknown; at sites of inflammation, production of ROS is enhanced, and \(H_2O_2\)
levels have been estimated at 0.1 to 1.0 mmol/L, which is greater than values used in this study. Because antioxidants such as vitamin E and probucol have had significant beneficial effects on coronary events and restenosis after angioplasty, it will be important to clarify the mechanisms by which ROS contribute to the initiation and progression of cardiovascular disease.

In the present study, we tested several approaches to generate ROS, including 200 μmol/L H₂O₂ and 1 μmol/L LY83583, 50 μmol/L menadione, and 100 μmol/L xanthine plus 1 U/mL xanthine oxidase. Concentrations used in the present study are similar to those in a large number of studies in the literature. Although this level of ROS is rarely achieved in normal physiological conditions, such levels may be present transiently in pathophysiological conditions, such as inflammation and vessel injury. In addition, we showed that CyPA was induced and secreted in cells transfected with the O₂⁻-generating enzyme mox-1. The level of oxidative stress in mox-1-transfected cells is quite low, indicating the physiological relevance of the present findings.

The mechanisms that regulate CyPA secretion and expression are unknown. Our findings that antioxidants (except SOD) inhibited late phase ERK1/2 activation and CyPA secretion in response to LY83583, as well as our results with mox1-transfected cells, support the hypothesis that increased ROS stimulate expression and secretion of CyPA. Indeed, induction of cyclophilins together with other stress proteins has been shown in endothelial cells treated with exogenous oxidants. Our data suggest that hydrogen peroxide is the predominant ROS responsible for CyPA induction and ERK activation, because hydrogen peroxide leads to CyPA induction and catalase inhibits secretion of CyPA and late ERK activation, whereas SOD stimulates these events. Furthermore, there also seems to be a relationship in vivo among inflammation, ROS, and cyclophilin release, as shown by the high CyPA levels in serum from patients with human immunodeficiency virus type-1, rheumatoid arthritis, and sepsis. Because these diseases are usually accompanied with ROS generation by neutrophils, lymphocytes, and vessel wall
cells, it is possible that ROS may stimulate the CyPA secretion and expression in vivo. In the balloon-injured carotid model, we found dramatic increases in CyPA expressed in the neointima. The time course and location of CyPA expression within the balloon-injured artery is consistent with this hypothesis, because balloon injury is associated with increased ROS production.27,47

Although increasing evidence shows that cyclophilins are secreted, the present study is the first to show that a cyclophilin acts as a growth factor. We showed that CyPA activated ERK1/2, increased DNA synthesis, and protected cells against apoptosis, suggesting that CyPA shares common signal pathways with growth factors. We have also observed that CyPA increased intracellular calcium in VSMCs and BAPTA-AM (an intracellular calcium chelator) abrogated ERK1/2 activation in response to CyPA (data not shown). In addition to VSMC, we observed that CyPA stimulated ERK1/2 activation in mox1-transfected NIH3T3 cells as well as in endothelial cells and lymphocytes (data not shown). These results suggest a broad role for CyPA as an extracellular signal mediator.

Cyclophilins possess PPIase activity.48,49 However, the PPIase activity of these proteins is not involved in their immunosuppressive effects,50 and the biological functions of this enzymatic activity remain poorly understood. Our finding that CsA inhibits CyPA-induced ERK1/2 suggests a role for PPIase activity in mediating signal transduction. In preliminary experiments, we have observed that although a mutant CyPA (R55A) is 1000-fold less active as a PPIase, it failed to stimulate ERK1/2 activation (data not shown). Taken together, these results indicate that the PPIase activity of CyPA is required for ERK1/2 activation and suggest the existence of a plasma membrane receptor that may be modified or activated by PPIase activity. In support of this concept, host-derived CyPA was shown to be incorporated into human immunodeficiency virus type-1 virions, and this incorporation was essential for viral infectivity. Because infectivity was inhibited by CsA, the results suggested that an interaction with a cellular receptor for CyPA was important in infectivity.51 Thus, additional characterization of the nature of the CyPA receptor and the role of PPIase activity will provide insights into the physiological importance of these novel functions of CyPA.

Analysis of LY-CM showed that several factors were secreted from VSMC in response to oxidative stress.21 In this study, we proved that CyPA was a SOXF that played an important role in ROS-induced late phase ERK1/2 activation and cell growth. Immunodepletion of CyPA inhibited LY-CM–induced ERK1/2 activation by ~50% (Figure 2). hrCyPA-induced ERK1/2 activation was totally blocked by CsA (Figure 5A), but LY-CM–induced ERK1/2 activation was only inhibited by 50% with CsA (Figure 5C). hrCyPA was also ~50% as effective as LY-CM in preventing apoptosis (Figure 7). Taken together, these results suggest that other factors in LY-CM likely contribute to ROS-induced late phase ERK1/2 activation, DNA synthesis, and inhibition of apoptosis.

In summary, the present study demonstrates a novel role for CyPA as a secreted redox-sensitive mediator and VSMC growth factor. Understanding the mechanisms by which ROS stimulate CyPA secretion and CyPA stimulates cell growth and inhibits apoptosis should provide important insights into the cellular response to oxidative stress.

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References


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