The ischemia/reperfusion (I/R) syndrome occurring during revascularization results in the progressive loss of cardiac myocytes, ultimately leading to heart failure.1

A wealth of reports indicate a pivotal role for apoptosis in cardiac myocyte death after I/R.2–4 Indeed, cardiomyocyte apoptosis was found during the early phase of reperfusion after myocardial ischemia in animal models5,6 and after cardiac transplantation in humans.7,8 In addition, prevention of apoptosis strongly reduced postischemic cardiac damage.

Abundant evidence suggests that reactive oxygen species (ROS) play an important role in the development and progression of postischemic myocardial damage, leading to apoptosis.9,10

The induction of oxidative stress is related to an activation of several enzymatic systems including monoamine oxidases (MAOs).11–13 MAOs catalyze oxidative deamination of several monoamines (eg, serotonin [5-hydroxytryptamine[5-HT]], noradrenaline, dopamine), resulting in significant ROS production.14 Based on their substrate preference and inhibitor specificity, 2 functional isoenzymes, MAO-A and MAO-B, have been identified.15 In the heart, MAO-A is a predominant enzyme involved in the deamination of endogenous or exogenous amines.16 Recently, we have shown that oxidative stress induced by MAO-A is responsible for receptor-independent, serotonin-mediated apoptosis during postischemic myocardial injury.17

Presently the link between MAO-produced ROS and apoptosis is not well defined. Promising candidates for mediating I/R-induced apoptosis are members of the sphingolipid family. Among these lipids, ceramide and sphingosine 1-phosphate (S1P) are potent messenger molecules playing opposite roles in cell fate.18 Whereas ceramide accumulation has been associated with cell growth arrest and apoptotic cell death, its downstream metabolite, S1P, is a growth promoter.
and survival factor (reviewed previously). The dynamic balance between ceramide and S1P depends in part on the activity of sphingosine kinase-1 (SphK1), an enzyme playing an important role in cell survival. In heart, S1P has been shown to be involved in cardioprotection. Indeed, preincubation with S1P prevents the mortality induced by hypoxia in neonatal rat ventricular myocytes. In addition, SphK1 is upregulated during ischemic preconditioning and linked to enhanced myocardial survival. In contrast, ceramide signaling has been reported to be required for I/R-induced death of myocardial cells. Importantly, hypoxia/reoxygenation-induced ceramide generation was shown to rely on ROS production.

Herein we report the involvement of the ceramide/S1P balance and SphK1 activity in MAO-A- and ROS-mediated apoptosis of H9c2 cardiomyoblasts, as well as in vivo during I/R-induced cardiac damage in MAO-A knockout (KO) and wild-type (WT) mice. Our results support the notion that SphK1 inhibition could be a central event in I/R cardiac injury.

Materials and Methods

Cell Lines

The embryonic rat heart–derived myogenic cell line H9c2 was obtained from the American Type Culture Collection. These cells were cultured in DMEM, supplemented with 10% FCS and 4 mmol/L glutamine. Cells were grown at 37°C in an atmosphere of 5% CO2 and used below the 35th passage.

Materials

Culture medium, serum, and antibiotics were obtained from Invitrogen. Escherichia coli diacylglycerol kinase and n-octylglucoside were from Calbiochem. Alkaline phosphatase, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and serotonin were from Sigma. Culture medium, serum, and antibiotics were obtained from Invitrogen. Escherichia coli diacylglycerol kinase and n-octylglucoside were from Calbiochem. Alkaline phosphatase, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and serotonin were from Sigma.

Preparation of Mitochondria and Western Blot Analysis of Cytochrome c

Mitochondrial preparations were performed as previously described. In brief, cell samples were collected by centrifugation at 600g for 5 minutes at 4°C. The cell pellets were washed once with ice-cold PBS and resuspended with 5 volumes of ice-cold buffer A (20 mmol/L HEPES-KOH pH 7.5, 0.1% BSA, 1 mmol/L sodium EDTA, 1 mmol/L DTT, 0.1 mmol/L phenyl methanesulfonyl fluoride, 20 μg/mL leupeptin, 10 μg/mL aprotinin, and 10 μg/mL pepstatin A) containing 250 mmol/L sucrose. After swelling on ice for 5 minutes, the cells were homogenized with 15 to 20 strokes of a no. 22 Kontes Dounce homogenizer with the B pestle, and the homogenates were centrifuged at 750g for 5 minutes at 4°C. The supernatants were then pelleted at 10 000g for 15 minutes at 4°C. Resulting pellets containing mitochondria were resuspended in cold buffer A. The supernatants were further cleared at 20 000g for 30 minutes at 4°C. For Western blot analysis, anti–cytochrome c monoclonal antibody (PharMingen) and anti–cytochrome oxidase subunit II monoclonal antibody (Molecular Probes) were used as primary antibodies. Proteins were visualized by ECL (Pierce) using anti-mouse or anti-rabbit horseradish peroxidase–conjugated IgG (Bio-Rad).

Western Blot Analysis of SphK1 Expression

Western blotting was performed as previously described. Rabbit anti-SphK1 (gift from Dr Stuart Pitson, Institute of Medical and Veterinary Science, Adelaide, Australia) and mouse α-tubulin antibody (Sigma) were used as primary antibodies.

SphK1 Assay and Mass Measurements of Sphingolipids

SphK1 activity, ceramide and S1P levels were measured as previously described in detail.

RNA Interference Experiments

Transient interference was achieved by double-stranded SphK1-specific small interfering RNA (siRNA) or scrambled siRNA. SiRNA transfection was performed according to the manufacturer’s instructions (Invitrogen). Briefly, 75 pmol/L siRNA were complexed with 1:125 final dilution of OligofectAMINE (Invitrogen) reagent and applied to 1:10 cells in a final volume of 250 μL of Opti-MEM (Invitrogen) without FCS or antibiotics. After incubation for 4 hour at 37°C under 5% CO2, 150 μL of DMEM with 30% FCS was added 20 hours later medium was changed to 1 mL DMEM with 10% FCS.

Preparation of Rat Ventricular Cardiomyocytes

Enriched cultures of neonatal (day 1 to 3) cardiomyocytes wereprepared from hearts of Sprague-Dawley rats by a previously described enzymatic method. After treatment with collagenase type II (Sigma), and subsequent digestion with trypsin and deoxyribonuclease, cells were resuspended in complete medium (50% [vol/vol] DMEM, 50% [vol/vol] Ham’s F-12 medium, 1% nonessential amino acids, 2 mmol/L L-glutamine, penicillin [100 U/mL], streptomycin [100 μg/mL], streptomycin [100 μg/mL]) containing 10% FCS and incubated for 40 minutes at 37°C. Cells allowing selective attachment of nonmyocytes. Cardiomyocyte-enriched suspensions were plated in complete medium containing 10% FCS. Each dish was incubated with complete serum-free medium (50% [vol/vol] DMEM, 50% [vol/vol] Ham’s F-12 medium, 1% BSA)) for 16 hours before experiment.

Animal Studies

We used 3-month-old males of the Tg8 strain of MAO-A KO mice and their WT C3H/HeOuJ controls. An insertional deletion in the Mao-a locus occurred following DNA injection into a 1-cell embryo of the C3H/HeOuJ inbred strain. The X-linked MAO-A mutation was maintained on the C3H/HeOuJ background by repeated backcrossing. In the current study, all subjects were generated from homozygous breeding pairs to avoid direct competition between mutant and WT pups, which is known to be detrimental to MAO-

Hydrogen Peroxide Production

Levels of intracellular hydrogen peroxide (H$_2$O$_2$) were determined spectrofluorometrically by the oxidation of the specific probe 2′,7′-dichlorodihydrofluorescein diacetate (H$_2$DCFHDA) (Molecular Probes) to 2′,7′-dichlorofluorescein (DCF), as previously reported.
A–deficient pups (at risk of a 30% deficit in body weight). At the time of weaning, males were housed individually to prevent stress associated with the frequent fighting initiated by MAO-A KO mice. Mice were maintained on a 14 hours light/10 hours dark cycle, in a pathogen-free facility, and had free access to food and water. They were handled in accordance with the principles and procedures outlined in Council Directive 86/609/EEC (homozygous breeding is consistent with the commitment to reduce excess production of animals). Tg8 breeders were obtained from the French CNRS animal repository (CDTA, Orléans, France) and C3H/HeOuL breeders were purchased from Charles River Laboratory.

The animals were anesthetized with an intraperitoneal injection of ketamine HCl 35 mg/kg and xylazine 5 mg/kg. A polyethylene tube size 90 was carefully inserted into the trachea, taped in place to prevent dislodgment, and connected via a loose junction to a small ventilator (model 683; Harvard Apparatus), set at a tidal volume and at a rate of 60 strokes per minute. Ligation of the left coronary artery was performed as previously described.17 After 30 minutes of ischemia, the ligature was released and the heart reperfused for 180 minutes. The protocol included different groups: mice subjected to 30 minutes of ischemia followed by 180 minutes of reperfusion and sham-operated animals, for both WT and MAO-A KO mice.

**Determination of Area at Risk and Infarct Size**

At the end of the infarction protocol, the ligature around the coronary artery was repositioned and Evans blue dye (1.5%, 0.1 mL) was injected into the left ventricular cavity to measure the myocardial ischemic area at risk. The animals were euthanized immediately, and the heart was removed and then cut from apex to base into 4 to 5 transverse slices of equal thickness. The slices were then incubated in 1% triphenyltetrazolium chloride solution in isotonic pH 7.4 phosphate buffer at 37°C for 20 minutes. The slices were subsequently fixed in 10% formalin solution for 6 hours to assess myocardial tissue viability and determine myocardial infarct size. Red-stained viable tissue was easily distinguished from the infarcted pale/unstained necrotic tissue. The areas of infarcted tissue, the risk zone, and the whole left ventricle were determined by computer morphometry. The area for each region was averaged from slices. Infarct size was expressed as a percentage of the ischemic risk area.

**Determination of Malondialdehyde Production**

Malondialdehyde (MDA), an end product of lipid peroxidation, was determined by measurement of the chromogen generated from the reaction of MDA with 2-thiobarbituric acid, as described previously.17 Results are expressed as nanomole of MDA per milligram of protein.

**Evaluation of Posts ischemic Apoptosis**

DNA fragmentation was visualized in situ on formalin-fixed and paraffin-embedded sections by the terminal transferase-mediated dUTP nick-end labeling (TUNEL) procedure with the apoptosis detection kit of Promega (Madison, Wis). Briefly, deparaffinized sections were incubated in a 20 µg/mL proteinase K solution to permeabilize the tissues, then rinsed. The sections were incubated with 1 µL of terminal deoxynucleotidyl transferase (25 U/µL) and fluorescein-12-dUTP in equilibration buffer (25 mmol/L Tris-HCl [pH 6.6], 200 mmol/L potassium cacodylate [pH 6.6], 2.5 mmol/L cobalt chloride, 0.25 mg/mL BSA, 0.2 mmol/L DTT) for 1 hour at 37°C. Then, after rinsing in saline sodium citrate and PBS, the slides were immersed in 40 mL of PI solution (1 µg/µL) for 15 minutes. The positive control was treated by DNase I (1 µg/µL) before being processed with the TUNEL procedure.

**Caspase-3 Activity**

The activity of caspase-3 was determined by using the fluorescence substrate Ac-DEVD-AMC (Bachem). The frozen kidney cortices were homogenized with 10 mmol/L HEPES (pH 7.4) containing 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 42 mmol/L KCl, 5 mmol/L MgCl2, 1 mmol/L DTT, 1 mmol/L phenyl methanesulfonyl fluoride, 2 µg/mL leupeptin, and 1 µg/mL pepstatin A. The homogenate was then centrifuged at 10,000g for 10 minutes. Supernatant (containing 250 µg of total protein) was incubated with 40 µmol/L of Ac-DEVD-AMC for 60 minutes at 37°C. Fluorescent AMC (7-amino-4-methyl-coumarin) product formation was quantified at an excitation wavelength of 380 nm and emission of 460 nm using a fluorometer.26

**Troponin Measurement**

 Plasma troponin I was measured by a quantitative rapid assay using the Cardiac Reader (Roche Diagnostics).

**Statistical Analysis**

The statistical significance of differences between the mean values was evaluated by unpaired Student’s t test. All statistical tests were 2-sided and the level of significance was set at P<0.05. Calculations were performed using Instat (GraphPad Software).

**Results**

**Serotonin Induces H9c2 Cells and Neonatal Cardiomyocytes Apoptosis, Which Is Mediated by SphK1 Inhibition and Upregulation of the Ceramide/S1P Ratio**

The experiments were performed in H9c2 cells, which predominantly express the MAO-A isoenzyme (data not shown). H9c2 treated with increasing serotonin concentration displayed a dose-dependent decrease in cell viability, associated with 40% increase in H2O2 content (Figure 1B). Syto 13/PI staining demonstrated that serotonin-induced cell death could be attributed to apoptosis (Figure 1C). Western blot analysis revealed that H9c2 apoptosis was concomitant to cytochrome c release from mitochondria (Figure 1D). All of these effects were prevented by both irreversible MAO inhibitor pargyline and N-acetyl-L-cysteine, an H2O2 scavenger (Figure 1A through 1D).

To determine the potential involvement of sphingolipid metabolism in cardiomyoblast apoptosis, we assessed H9c2 cells treated with serotonin for 16 hours for sphingolipid content and SphK1 activity. H9c2 apoptosis was associated with ~2-fold increase in intracellular ceramide (Figure 2A) and a significant decrease of intracellular S1P levels (Figure 2B). Similar to the onset of apoptosis, these effects were blocked by both pargyline and NAC. Thus, in H9c2 cells, serotonin treatment drastically modified the balance between ceramide and S1P toward proapoptotic ceramide, which resulted in a 3-fold increase in intracellular ceramide/S1P ratio. Concomitantly with S1P decrease, serotonin treatment resulted in approximately 40% inhibition of SphK1 activity (Figure 2C) and significant decrease in SphK1 protein level (Figure 2D). Importantly, both serotonin-induced shift of sphingolipid balance and SphK1 inhibition were abrogated by inhibition of MAOs or prevention of H2O2 accumulation.

Additional studies were conducted with neonatal rat cardiomyocytes to validate the data obtained in H9c2 cells. Serotonin induced modification of the sphingolipid biostat status. Ceramide content was indeed increased (Figure 3A) and S1P decreased (Figure 3B) after serotonin treatment. SphK1 activity was also markedly diminished (Figure 3C). Of note, these changes were inhibited by pargyline and NAC (Figure 3A through 3C).
SphK1 Inhibition or Addition of Ceramide Mimics Serotonin-Induced Apoptosis in H9c2

To validate the role of sphingolipid balance and SphK1 activity with regard to cell viability, we treated H9c2 cells with permeant C2-ceramide, siRNA to SphK1, and a pharmacological inhibitor of SphK1 (SKI). All treatments led to decreases in cell viability (Figure 4A) that could be attributed to apoptosis (Figure 5A). Pharmacological inhibition of SphK1 (Figure 4B) by SKI resulted in drastic changes in the sphingolipid balance, giving rise to the proportional decrease in the intracellular S1P and concomitant increase in ceramide (Figure 4C).

SphK1 Overexpression Renders H9c2 Resistant to Both Serotonin and SKI

To confirm the critical role of SphK1 inhibition in serotonin-induced apoptosis, we stably transfected H9c2 cells with SphK1 (H9c2/SphK1) and empty vector (H9c2/Neo). The average SphK1 activity in H9c2/neo cells was 158±11 pmol/mg protein per minute (similar to SphK1 activity of H9c2 parental cells), whereas a 20-fold increase in SphK1 activity (3071±118 pmol/mg protein per minute) was observed in H9c2/SphK1 cells. The increase in basal SphK1 activity in H9c2/SphK1 cells resulted in an augmentation of basal intracellular S1P content (from 20.05±2.26 pmol/mg protein to 27.69±2.06 pmol/mg protein) and a concomitant decrease in basal levels of ceramide (from 2.58±0.14 pmol/µg protein to 2.05±0.07 pmol/µg protein). Unlike empty vector–transfected cells, H9c2/SphK1 cells exhibited a significant resistance to apoptosis induced by serotonin, C2-ceramide, or siRNA to SphK1 (Figure 5A and 5B). This increased resistance was associated with a smaller values of ceramide/S1P ratio after treatment with serotonin or SKI in H9c2/SphK1 cells in comparison with H9c2/Neo cells (Figure 5D and 5E). Indeed, a 3-fold increase in ceramide/S1P ratio was found with serotonin-treated H9c2/Neo cells, as compared with a 1.7-fold increase with SphK1-overexpressing cells (calculated from the relative amounts of ceramide and S1P shown in Figure 5D and 5E). In a similar fashion, SKI treatment led to a 6.5-fold increase of ceramide/S1P ratio (2.14:0.33) in H9c2/Neo cells versus a 2.4-fold increase...
(1.81:0.75) in only H9c2/SphK1 cells. These smaller values of ceramide/S1P ratio in H9c2/SphK1 cells were provisioned by sustained SphK1 activity in response to serotonin and SKI (91% and 70%, respectively) in contrast to 64% and 31%, respectively, in H9c2/Neo cells (Figure 5C).

H2O2 Treatment Leads to SphK1 Downregulation

After establishing that serotonin-induced SphK1 downregulation was inhibited by antioxidant, it was of interest to assess whether addition of exogenous H2O2 could mimic the effect of serotonin on SphK1 activity. As shown in Figure 6A, addition of H2O2 led to a very strong inhibition of SphK1 activity. This was further illustrated by decrease in S1P content (Figure 6A, inset). Of note, pretreatment with NAC could totally impede SphK1 inhibition and S1P level decline induced by addition of H2O2 (Figure 6A). We next investigated the role of SphK1 in regulating H2O2 intracellular levels and apoptosis after H2O2 treatment.
Although SphK1 overexpression did not lower the content of H$_2$O$_2$ (Figure 6B), it did block the loss of cell viability of H9c2 cells treated by H$_2$O$_2$ (Figure 6C). As anticipated, pretreatment with NAC could lower the intracellular content of H$_2$O$_2$ and cell death triggered by exogenously added H$_2$O$_2$ (Figure 6B and 6C). These findings support the notion that H$_2$O$_2$ is an important regulator of SphK1 activity.

Cardioprotection of MAO-A KO Mice Is Associated With a Decrease in Production of ROS and Ceramide and an Increase in SphK1 Activity

To determine the relationship between sphingolipid signaling and MAO-mediated I/R-induced injury, we used a strain of MAO-A–deficient mice (MAO-A KO) and compared them with the WT strain. Both groups were subjected to 30 minutes of ischemia, followed by 180 minutes reperfusion and were

Figure 4. SphK1 inhibition induces H9c2 apoptosis. Cells were treated for 24 hours with or without 10 μmol/L SKI or 10 μmol/L C$_2$-ceramide (C$_2$-Cer) or transfected with siRNA against SphK1 for comparison with scrambled siRNA and incubated for 72 hours. A, Cell viability was measured using the MTT assay. H9c2 cells were treated for 16 hours with SKI and then tested for ceramide and S1P levels (B) and SphK1 activity (C). Columns indicate mean of 3 independent experiments performed in triplicate; bars, SE. The 2-tailed probability values between the means are as follows: ***P<0.001; **P<0.01; ns, not significant.

Figure 5. SphK1 overexpression promotes H9c2 resistance to apoptosis. H9c2/Neo and H9c2/SphK1 were treated with 100 μmol/L serotonin (5-HT), 10 μmol/L SKI, or 10 μmol/L C$_2$-ceramide (C$_2$-Cer) for 24 hours or transfected with siRNA against SPHK1 for comparison with scrambled siRNA and incubated for 72 hours. A, Representative images of H9c2 cell nuclei stained with Syto13-PI. B, Cell viability was measured at 24 hours using the MTT assay. H9c2/Neo and H9c2/SphK1 cells were treated for 16 hours with serotonin or SKI and then tested for SphK1 activity (C), ceramide levels (D), and S1P levels (E). Columns indicate mean of 3 independent experiments performed in triplicate; bars, SE. The 2-tailed probability values between the means are as follows: ***P<0.001; **P<0.01; ns, not significant.
Figure 8B. On the contrary, the postreperfusion level of ceramide following ROS production (Figure 8B). Ceramide revealed a significant increase in the level of proapoptotic markers in MAO-A KO mice after 1 hour of reperfusion was significantly lower when compared with WT mice (Figure 8A).

MDA, a marker of lipid peroxidation, in ventricles of MAO-A KO and WT hearts subjected to I/R revealed that I/R-induced ROS generation was markedly reduced in the hearts of MAO-A KO animals (Figure 7B). TUNEL staining of heart tissues demonstrated that ceramide increase induced by serotonin in H9c2 cells relies on MAO-A–induced ROS generation. Indeed this increase could be completely abolished by both inhibition of MAO activity and antioxidant treatment.

More importantly, we link, for the first time, the production of proapoptotic ROS not only with the ceramide increase but also with the fall in the intracellular antiapoptotic S1P content caused by a decline of SphK1 protein and activity. So far, such SphK1 loss has been reported in only cancer cells undergoing apoptosis by a decline of SphK1 protein and activity. So far, such SphK1 loss has been reported in only cancer cells undergoing apoptosis in response to chemotherapeutic drugs.27,30,34 As proof of the strategic role for SphK1/S1P signaling, our studies showed that SphK1 overexpression markedly inhibited ROS-mediated, serotonin-induced cardiomyoblast apoptosis. Enforced SphK1 expression reduced ceramide elevation (triggered by serotonin) by driving ceramide metabolism toward the synthesis of S1P, known to oppose ceramide-induced proapoptotic effects.19 We further confirmed the specific function of SphK1 by showing first that siRNA against hSphK1 induced a strong loss of cell viability, thus suggesting that SphK1 activity was required for cell survival and that its attenuation was an important factor in cardiomyoblast cell death. Second, the pharmacological inhibition of SphK1 was able to induce H9c2 cell death, notably by tilting the ceramide/S1P biostat toward ceramide accumulation.

Importantly, in H9c2 cells, SphK1/S1P signaling appears to lie downstream of MAOs and ROS. First, both inhibitor of MAO activity and antioxidant successfully blocked serotonin-induced SphK1 inhibition. Second, we showed that independent ROS production by H2O2 led to a dramatic decrease in SphK1 activity and S1P levels in H9c2 cells, events that could be totally hampered by NAC antioxidant. This finding reveals the general ability of ROS increase to trigger SphK1 inhibition. It is noteworthy that SphK1 treatment could protect against H2O2-induced cell death in a manner similar to that of serotonin overexpression.

Finally, our studies show that in vitro findings could be translated in vivo by using a MAO-A KO mouse model that is protected from I/R-induced cardiac damage. The protection of MAO-A knockouts was related to significantly lower ROS generation following I/R, and, similar to our in vitro findings, SphK1 activity was much higher in hearts of MAO-A KO mice compared with WT mice (Figure 8C).

Discussion

Herein we report for the first time that inhibition of SphK1 activity and change of the ceramide/S1P sphingolipid balance are key events in MAO-mediated cardiomyocyte apoptosis.

Accumulation of ROS is an early and well-established step in apoptotic cascades induced by I/R.9,10 One of the sources of ROS production during I/R are MAOs, the enzymes responsible for the metabolic degradation of serotonin, noradrenaline, and dopamine. Serotonin-derived ROS has been shown to play an important role in I/R-associated events, such as myocardial dysfunction.11–13 We have recently reported that oxidative stress induced by MAO-A is accountable for receptor-independent, serotonin-mediated apoptosis during posts ischemic myocardial injury.17 I/R-induced apoptosis has also been associated with accumulation of the proapoptotic sphingolipid ceramide,23,24,33 an event shown to be ROS dependent.24 Currently we demonstrate that ceramide increase induced by serotonin in H9c2 cells was related to significantly lower ROS production by H2O2. Thus, the results showed that serotonin-derived ROS are capable of triggering SphK1 inhibition. It is noteworthy that SphK1 treatment could reveal the general ability of ROS increase to trigger SphK1 inhibition. It is noteworthy that SphK1 treatment could protect against H2O2-induced cell death in a manner similar to that of serotonin overexpression.

Finally, our studies show that in vitro findings could be translated in vivo by using a MAO-A KO mouse model that is protected from I/R-induced cardiac damage. The protection of MAO-A knockouts was related to significantly lower ROS generation following I/R, and, similar to our in vitro findings,
inhibition of MAO-mediated ROS resulted in abrogation of I/R-induced ceramide increase and SphK1 inhibition. Our results suggest that endogenous MAO-A–mediated ROS production is necessary for tipping the ceramide/S1P balance toward proapoptotic ceramide. Our results are supported by data from Jin et al, who showed that SphK1 activity was critical for ischemic preconditioning-induced cardioprotection, which could be abrogated by SphK1 inhibition.22

In conclusion, we propose a novel pathway linking MAO-A–generated ROS production and ceramide/S1P balance biostat in cardiac cells. We demonstrate, for the first time, that generation of ROS may result in SphK1 inhibition and, thus, in modification of sphingolipid biostat, which is a critical event in apoptosis progression. We propose that SphK1 might play an important role in post-I/R syndrome and, thus, present a potential as a target for anti-I/R therapies.

Sources of Funding
Supported by the Institut National de la Sante et de la Recherche Medicale (INSERM). D.P. was supported by a postdoctoral fellowship from Association Etudes et Recherches Urologiques. O.K. was supported by a postdoctoral fellowship from the Fondation pour la Recher-

**Sources of Funding**

Supported by the Institut National de la Santé et de la Recherche Médicale (INSERM). D.P. was supported by a postdoctoral fellowship from Association Etudes et Recherches Urologiques. O.K. was supported by a postdoctoral fellowship from the Fondation pour la Recher-

**Figure 7.** Postreperfusion myocardial injury in WT and MAO-A KO mice. WT and MAO-A KO mice were subjected to 30 minutes of ischemia, followed by 180 minutes of reperfusion. A, Representative images of hematoxylin and eosin–stained heart sections (×40). B, TUNEL staining of paraffin-embedded heart sections. Bright green dots correspond to TUNEL-positive nuclei (×40). C, Quantification of infarct size expressed as percentage of area at risk (left) and troponin I levels (right). D, I/R-induced apoptosis was evaluated by TUNEL staining (left) and caspase-3 activity (right) of heart sections. Troponin I levels in sham-treated animals were undetectable. Columns indicates means from 6 to 8 treated animals; bars, SE. The 2-tailed probability values between the means are as follows: ***P<0.001, **P<0.01, *P<0.05.

**Figure 8.** I/R-induced stress and changes in sphingolipid metabolism in WT and MAO-A KO mice. Mice were subjected to 30 minutes of ischemia, followed by 180 minutes of reperfusion. A, Myocardial MDA endproduct of lipid peroxidation in WT and MAO-A KO mice. B and C, Postreperfusion levels of ceramide (B) and SphK1 (C) in WT and MAO-A KO mice. Columns indicate means from 6 to 8 treated animals; bars, SE. The 2-tailed probability values between the means are as follows: ***P<0.001, **P<0.01, *P<0.05.
che Médicale (FRM). A.D. is a recipient of the Ministère de l’Enseignement Supérieur et de la Recherche.

Disclosures
None.

References
Oxidative Stress–Dependent Sphingosine Kinase-1 Inhibition Mediates Monoamine Oxidase A–Associated Cardiac Cell Apoptosis
Dimitri Pchejetski, Oxana Kunduzova, Audrey Dayon, Denis Calise, Marie-Hélène Seguelas, Nathalie Leducq, Isabelle Seif, Angelo Parini and Olivier Cuvillier

Circ Res. 2007;100:41-49; originally published online December 7, 2006; doi: 10.1161/01.RES.0000253900.66640.34
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/100/1/41

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/