Background—Serotonin (5-hydroxytryptamine [5-HT]), released by activated platelets during cardiac ischemia, is metabolized by the mitochondrial enzyme monoamine oxidase A (MAO-A). Because hydrogen peroxide is one of the byproducts of 5-HT degradation by MAO-A, we investigated the potential role of reactive oxygen species generated by MAOs in 5-HT–dependent cardiomyocyte death and post–ischemia-reperfusion cardiac damage.

Methods and Results—Treatment of isolated adult rat cardiomyocytes with 5-HT induced intracellular oxidative stress and cell apoptosis. The apoptotic cascade triggered by 5-HT involves release of cytochrome c, upregulation of proapoptotic Bax protein, and downregulation of antiapoptotic Bcl-2 protein. These effects were prevented by inhibition of amine transporter or MAO, antioxidants, or iron chelation. In contrast, cardiomyocyte apoptosis was only slightly affected by the 5-HT2B receptor antagonist SB 206553. In vivo, inhibition of MAO-A largely reduced myocardial ultrastructural damage induced by 30 minutes of ischemia followed by 60 minutes of reperfusion in the rat heart. Cardioprotective effects of MAO inhibitors were associated with the prevention of postischemic oxidative stress, neutrophil accumulation, and mitochondrial-dependent cell death and were not reverted by SB 206553. Administration of MAO-A inhibitors during ischemia was still effective in preventing cardiac damage.

Conclusions—Our results supply the first direct evidence that oxidative stress induced by MAO is responsible for receptor-independent apoptotic effects of 5-HT in cardiomyocytes and postischemic myocardial injury. These findings provide new insight into the mechanisms of 5-HT action in the heart and may constitute the basis for novel therapies. (Circulation. 2005;112:3297-3305.)

Key Words: apoptosis ■ ischemia ■ enzymes ■ myocytes ■ serotonin

S

erotonin (5-HT) is a biogenic amine produced in the
central nervous system by cells originating in the raphe
nuclei of the brainstem. In periphery, serotonin is produced
predominantly by intestinal enterochromafin cells and stored
in platelets. Serotonin affects a wide variety of physiological
functions through the interaction with specific G-coupled
membrane receptors. In the heart, 5-HT has been involved in
regulation of normal cardiac development1 and in different
diseases, including arrhythmia,2 ventricular hypertrophy,3
and cardiac valvular insufficiency associated with carcinoid
tumors.4 During the last years, several studies showed that
serotonin accumulates in heart during ischemia-reperfusion
(I/R) and contributes to the progression of myocardial injury
and dysfunction.5-7 The deleterious effects of serotonin have
been related to indirect mechanisms involving coronary
vasoconstriction8 and reactive oxygen species (ROS)–depen-
dent stimulation of cardiac sympathetic afferents.5-9 At present, the
possibility that serotonin acts directly on cardiac cells to induce
apoptosis and necrosis has not been investigated. However, this
possibility is suggested by our recent studies showing that the
serotonin-degrading mitochondrial enzyme monoamine oxidase A
(MAO-A) is an important source of hydrogen peroxide (H2O2) in
the heart.10 Monoamine oxidases are outer mitochondrial mem-
brane enzymes that, based on genetic criteria,11 substrate specificity,
and inhibition by synthetic compounds,12 have been subdivided into
2 major forms, A and B. The degradation of biogenic amines
(serotonin and catecholamines) has been considered the major
physiological function of these enzymes. However, we have re-
cently shown that in kidney H2O2 produced by MAOs during
dopamine degradation mediates cell apoptosis.13 Although heart
contains large amount of MAO-A,14 the role of this enzyme in

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regulation of cardiac functions is still not clearly defined. Some studies indicated that cardiac MAO-A regulates noradrenaline concentrations. Our demonstration that MAO-A is also an important source of H₂O₂ in the heart suggests that this enzyme may contribute to ROS-dependent cardiomyocyte apoptosis. To verify this hypothesis, we investigated the potential role of ROS generated by MAO-A during 5-HT degradation in cardiomyocyte death and the role of MAO in post-I/R cardiac damage.

Methods

Cell Culture

Calcium-tolerant adult rat ventricular myocytes were obtained from hearts of male Sprague-Dawley rats (250 to 275 g) as previously described. Briefly, hearts were perfused retrogradely with Ca²⁺/H₁1001-free Krebs-Henseleit bicarbonate buffer and dissociated in the same buffer containing 0.02 mg/mL trypsin and 0.02 mg/mL deoxyribonuclease. The cells were filtered and sedimented through 60>H₁9262 g/mL BSA (Sigma-Aldrich) to separate ventricular myocytes from nonmyocytes as described by Ellingsen et al. The cell pellet was resuspended and plated in ACCT medium consisting of DMEM containing 2 mg/mL BSA, 2 mmol/L-carnitine, 5 mmol/L creatine, 5 mmol/L taurine, 100 IU/mL penicillin, and 100 μg/mL streptomycin.

Evaluation of Apoptosis and Necrosis

In vitro, morphological changes in the nucleus were detected as described by Meilhac et al. Necrosis and apoptosis were evaluated concomitantly on intact cultured cells after fluorescent staining using vital fluorescent dyes SYTO-13 (0.6 mol/L), a permeant DNA intercalating green probe, and propidium iodide (15 mol/L), a nonpermeant intercalating orange probe (Molecular Probes), and counted with an inverted fluorescence microscope (Fluovert FU, Leitz). Normal nuclei exhibited loose chromatin colored green by SYTO-13; apoptotic nuclei exhibited condensed green chromatin; necrotic cells exhibited orange nuclei with loose chromatin.

In vivo, apoptosis was evaluated with the DeadEnd Fluorometric TUNEL system according to the manufacturer’s instructions (Promega). Briefly, the deparaffinized heart sections were incubated in a 20 g/mL proteinase K solution to permeabilize the tissues, rinsed, and fixed in 4% paraformaldehyde. The sections were then incubated with terminal deoxynucleotidyl transferase (25 U/L) and fluorescein-12-dUTP for 1 hour at 37°C. After rinsing in 1 PBS, the slides were immersed in propidium iodide solution (1 g/L) for 15 minutes.

<table>
<thead>
<tr>
<th>TABLE 1. Area at Risk and Infarct Size</th>
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<tbody>
<tr>
<td>LV WT, g</td>
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<tr>
<td>I/R</td>
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<tr>
<td>P+I/R</td>
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<tr>
<td>C+I/R</td>
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<td>P+SB+I/R</td>
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<td>C+SB+I/R</td>
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LV indicates left ventricular; WT, weight; P, paragyline; C, clorgyline; and SB, SB 206553. Data are mean±SEM. *P<0.001 vs I/R.

<table>
<thead>
<tr>
<th>TABLE 2. Scoring Method of Myocyte and Endothelial Injury</th>
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<tr>
<td>Injury</td>
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<td>Myocyte</td>
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Western Blot Analysis

The proteins levels of Bax, Bcl-2, and procaspase-3 were assessed by immunoblotting on cells and heart lysates. The blot was stripped completely of antibodies before reprobing with a polyclonal anti-actin antibody used as a standard. The quantity of proteins loaded from cardiac myocyte extracts was normalized with Ponceau red.

Caspase-3 Activity

Caspase-3 activity was assessed with EnzChek Caspase-3 Assay Kit #1 (Molecular Probes) according to the manufacturer’s instructions. Briefly, cells were harvested in 1× cell lysis buffer. After centrifugation (5000 rpm for 5 minutes), the supernatant was transferred to a microplate, and 2× substrate working solution (10 mmol/L Z-DEVD-AMC substrate) was added. The microplate was incubated in dark at room temperature for 30 minutes. Substrate cleavage was monitored fluorometrically with a SpectraMax Gemini spectrophotometer (Molecular Devices) with excitation and emission wavelengths of 350 and 450 nm.

Hydrogen Peroxide Production

H₂O₂ production was determined by the oxidation of 2',7'-dichlorodihydrofluorescein (DCFH) (Molecular Probes) to a fluorescent 2',7'-dichlorofluorescein (DCF). DCFH was added to a final concentration of 20 μmol/L to a fluorescent 2× fluorescence microscope (Fluovert FU, Leitz) were used to quantify fluorescence intensity with SigmaScan Pro 5 software.

Measurement of the GSH/GSSG Ratio

The ratio between reduced (GSH) and oxidized (GSSG) glutathione was determined with the Bioxytech GSH/GSSG-412 kit (OxisResearch) adapted for cells. Briefly, cells were washed with 1× PBS and harvested into GSH assay buffer. After homogenization, they were centrifuged at 10 000 g for 15 minutes. Then, 5% metaphosphoric acid was added to the supernatant. After centrifugation at 10 000 g for 2 minutes, the supernatant was divided into 2 samples, 1 for GSH and 1 for GSSG measures. The assays were performed at 412-nm OD during 3 minutes with a SpectraMax 340 pc spectrophotometer (Molecular Devices).

Animals

The experimental protocol was designed in compliance with the recommendations of the European Economic Community (86/600/CEE) for the care and use of laboratory animals and was approved by the animal care committee of the University of Florence (Italy). Male Wistar albino rats weighing 200 to 250 g (Harlan, Milan, Italy) were anesthetized with intraperitoneal injection of sodium thiopenthal (Pentothal, Abbott; 50 mg/kg). A cannula was inserted into the trachea, and the animals were ventilated with air with a Palmer pump (U. Basile). All rats underwent thoracotomy at the fifth left intercostal space, the pericardium was opened, and a loose 5-0 braided silk suture was placed around the left anterior descending coronary artery, together with a small silicon ring to permit an easy removal of the ligature. Ischemia (30 minutes) was induced by tightening the threads of the coronary suture. Reperfusion (60 minutes) was obtained by reopening the chest and cutting the ligature around the coronary artery. In all animals, survival time was recorded.

Experimental Protocol

The protocol included 8 groups of rats treated as follows: Group 1 (n = 10), sham-operated rats in which no tightening of the coronary sutures was performed; group 2 (n = 10), rats pretreated with saline and subjected to 30 minutes of ischemia followed by 60 minutes of reperfusion; group 3 (n = 10), rats pretreated with the MAO inhibitor pargyline (10 mg/kg) 30 minutes before ischemia and subjected to 30 minutes of ischemia followed by 60 minutes of reperfusion; group 4 (n = 10), rats treated under the same conditions as group 3 but with the MAO-A inhibitor clorgyline (5 mg/kg); group 5 (n = 6), rats pretreated for 3 days with the 5-HT uptake blocker imipramine, or 1 mol/L 5-HT₁ receptor antagonist SB 206553 before addition of 5-HT (10 μmol/L) for 24 hours. B, Quantification of cardiomyocytes stained with SYTO-13 and propidium iodide. Percentage of apoptotic and necrotic cells presented in A. Data result from counting 3 fields of ~100 cells each per dish. Values are mean±SEM of the percentage of morphologically apoptotic and necrotic cells from three separate experiments. C, Caspase-3 activity was measured fluorometrically with 20 μmol/L Ac-DEVD-AMC as described in Methods. Data are mean±SEM from 3 independent experiments. ***P<0.001 vs control, ###P<0.001 vs 10 μmol/L 5-HT alone.

Determination of Area at Risk and Infarct Size

After 60 minutes of reperfusion, in some animals of each group, the left anterior descending coronary artery was reoccluded, and 1 mL of 1% Evans blue dye was injected into the carotid artery. Right ventricular and atrial tissues were removed, and heart was sliced...
transversely (12 to 13 slices) and immersed in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 20 minutes. The left ventricular sections were fixed in 10% formalin for 24 hours, weighed, and visualized under an Olympus microscope equipped with a closed-circuit television camera (Sony). In each photograph, the area at risk (unstained by Evans blue dye) and the infarcted area (unstained by TTC) were outlined and measured by planimetry. The mass of tissue in each region was calculated using the areas measured in each slice and the wet weight of each slice. Infarct size was expressed as a percentage of the area at risk (Table 1).

**Morphology**

Cardiac tissues were fixed in cold 4% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4, for 3 hours at room temperature and were postfixed in 1% osmium tetroxide in 0.1 mol/L phosphate buffer, pH 7.4, for 1 hour at 4°C. Semithin sections (2 μm) were cut, stained with toluidine blue sodium tetraborate, and studied by light microscopy. Electron microscopic examination was carried out on ultrathin sections stained with uranyl acetate and alkaline bismuth subnitrate and viewed under a Jeol 1010 electron microscope at 80 kV. In each fragment, 2 series of 6 to 8 ultrathin sections cut at 2 different levels were examined and photographed (Table 2). Myocyte and microvascular endothelium injury was quantified from electron micrographs (magnifications ranging from ×3000 to ×200000).

**Evaluation of Myeloperoxidase Activity**

Myeloperoxidase (MPO) activity, a marker for neutrophil accumulation, was evaluated according to the method of Bradley et al. 21 Frozen samples of left ventricular tissue (100 mg) were homogenized in 5 mmol/L potassium phosphate buffer and then centrifuged at 30 000g for 30 minutes at 4°C before extraction. The resulting pellets were used to measure MPO activity after suspension in 50 mmol/L phosphate buffer containing 0.5% hexadecyltrimethyl ammonium bromide. The pellets were centrifuged at 40 000g for 15 minutes; 0.2 mL supernatant specimen was added to 0.8 mL 50 mmol/L phosphate buffer (pH 6.0) containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.0005% H2O2. Absorbance was measured spectrophotometrically at 460-nm wavelength for 2 minutes. MPO activity, normalized to the protein content of the supernatant, was expressed as milliunit per milligram of protein.

**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. 22 The cardiac tissues were placed in 50 mmol/L Tris-HCl (pH 7.4) containing 180 mmol/L KCl and 10 mmol/L EDTA in a total volume of 2 mL and homogenized. Briefly, 0.02 mol/L HCl and 0.4% thiobarbituric acid were added to the homogenates and heated to 95°C for 20 minutes. After the addition of 2 mL of 1-butanol, the mixture was centrifuged at 2000 rpm for 10 minutes at 4°C. Absorbance of the upper layer was read spectrophotometrically at 548-nm wavelength. Results are expressed as nanomole of MDA per milligram of protein.

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**Figure 2.** Effect of 5-HT on Bax/Bcl-2 expression and cytochrome c release in cardiomyocytes. Cardiac myocytes were pretreated for 30 minutes with or without 1 μmol/L pargyline, 50 μmol/L deferoxamine, 1 μmol/L pyruvate, or 25 μmol/L imipramine before the addition of 5-HT (10 μmol/L) for 16 hours. The expression of Bax (A) and Bcl-2 (B) proteins and the intracellular distribution of cytochrome c (C) were determined by Western blot in cell lysates (left). Ponceau red was used to confirm equal loading of the extracts. Right, Quantification of Western blot data. Results are mean±SEM from 3 independent experiments. **P<0.01, ***P<0.001 vs control.
Statistical Analysis

Data are given as mean ± SEM. The ANOVA procedure, followed by Student-Newman-Keuls multiple comparison test or Student’s t test, was used for statistical comparison as appropriate (GraphPad Software). A value of P < 0.05 was considered statistically significant.

Results

Serotonin-Dependent Cardiac Cell Death

Treatment of cardiac myocytes with 5-HT for 24 hours induced a significant increase in SYTO-13 cell labeling (35% versus control; P < 0.001) and caspase-3 activity (2.5-fold versus control; P < 0.01) with a maximal effect at 10 μmol/L. Figure 1B and 1C). Higher 5-HT concentrations (50 to 100 μmol/L) resulted in both apoptotic and necrotic cell death (Figure 1B). The apoptotic effect of 5-HT was associated with mitochondrial dysfunction. Indeed, as shown in Figure 2, cardiomyocytes incubated with 5-HT displayed a significant increase in the expression of the proapoptotic protein Bax. The increase in Bax was concomitant with a decrease in the expression of the antiapoptotic protein Bcl-2. Analysis of cytochrome c distribution showed that the alteration of Bax and Bcl-2 expression was followed by release of cytochrome c from mitochondria and its accumulation in the cytosol (Figure 2). Cardiomyocyte apoptosis by 5-HT was concomitant with mitochondrial dysfunction. Indeed, as shown in Figure 2, cardiomyocytes incubated with 5-HT displayed a significant increase in the expression of the proapoptotic protein Bax. The increase in Bax was concomitant with a decrease in the expression of the antiapoptotic protein Bcl-2. Analysis of cytochrome c distribution showed that the alteration of Bax and Bcl-2 expression was followed by release of cytochrome c from mitochondria and its accumulation in the cytosol (Figure 2). Cardiomyocyte apoptosis by 5-HT was concomitant with intracellular oxidative stress and H2O2 generation (Figure 3). Indeed, we showed that 5-HT induced a decrease in the intracellular GSH/GSSG ratio (Figure 3A). In addition, 5-HT (10 to 100 μmol/L) also induced dependent intracellular H2O2 formation (Figure 3B), as measured with the fluorescent probe DCF.

Serotonin effects on SYTO-13 labeling, caspase-3 activity, Bax increase, Bcl-2 decrease, cytochrome c redistribution, and oxidative stress production were fully prevented by cardiomyocyte treatment with the MAO inhibitor pargyline, the amine transporter inhibitor imipramine, the iron chelator deferoxamine, or the antioxidant pyruvate. In contrast, the increase in 5-HT–dependent cardiomyocyte apoptosis was unaffected by cell pretreatment with the 5-HT2B receptor antagonist SB 206553. Taken together, these data show that 5-HT induces cardiomyocyte apoptosis by a mechanism involving H2O2 generation by MAO-A.

Involvement of MAOs in the Postreperfusion Cardiac Damage

To determine the relevance of H2O2 production by MAOs in vivo, we investigated the effects of MAO inhibition in cardiac damage after IR. Figure 4 shows the effect of MAO inhibition on myocardial infarct size of rats subjected to 30 minutes of myocardial ischemia and 60 minutes of reperfusion with or without pretreatment with MAO inhibitors (pargyline and clorgyline). The area at risk is not modified by the different treatments (Table 1). Infarct size is significantly reduced in animals treated with pargyline or clorgyline compared with saline-treated ischemic rats. Pretreatment with the 5-HT2B receptor antagonist SB 206553 for 3 days did not reverse the effect of the MAO inhibitors (Figure 4 and Table 1), indicating that the cardioprotective properties of pargyline and clorgyline were not due to 5-HT receptor activation. On the other hand, MAO inhibitors also induced cardioprotection when given 20 minutes after the onset of ischemia (infarct size, 57.3 ± 5.9% in the...
I/R animals and 26.2 ± 3.6% in the pargyline-treated I/R animals, *P* < 0.01; 31.7 ± 4.3% in clorgyline-treated I/R animals, *P* < 0.01).

Light microscopic analysis of postischemic semithin sections demonstrated attenuated myofibril hypercontraction and interstitial edema in pargyline-treated rats (Figure 5A). In addition, ultrastructural cell lesions induced by myocardial ischemia were detected by electron microscopy. Indeed, quantification of ultrastructural tissue damage (Table 2) revealed that myocyte injury was significantly increased in the I/R hearts (sham-operated, 0.3 ± 0.2; I/R, 2.8 ± 0.1; *P* < 0.001) and was largely prevented in pargyline-treated rats (I/R, 2.8 ± 0.1; pargyline + I/R, 1.3 ± 0.3; *P* < 0.01). Similarly, endothelial cell damage was significantly increased in the I/R hearts (sham-operated, 0.2 ± 0.1; ischemic-reperfused, 2.9 ± 0.1; *P* < 0.001) and was strongly reduced by pargyline treatment (I/R, 2.9 ± 0.1; pargyline + I/R, 1 ± 0.2; *P* < 0.001).

**Effect of MAO Inhibition on Lipid Peroxidation and Phagocyte Infiltration**

Oxidative stress and phagocyte infiltration have been proposed as key mediators triggering postreperfusion cardiac damage. As shown in Figure 6, MDA levels (Figure 6A) and MPO activity (Figure 6B), markers of lipid peroxidation and infiltration of ROS-generating inflammatory cells (ie, neutrophils and macrophages), respectively, were increased after 1 hour of reperfusion in ventricles from saline-treated rats compared with sham-operated animals. Treatment with the MAO inhibitor pargyline or the MAO-A inhibitor clorgyline resulted in a significant reduction in myocardial MDA levels and MPO activity compared with untreated I/R animals.

Cardiac Cell Apoptosis, Bax and Bcl-2 Expression, and Procaspase-3 Cleavage

Finally, to determine whether MAO-dependent ROS production may contribute to postreperfusion cardiomyocyte apoptosis, we examined the effects of pargyline on TUNEL staining, Bax/Bcl-2 balance, and procaspase-3 cleavage. As shown in Figure 7A and 7B, I/R induced a significant increase in the number of TUNEL-positive cells compared with sham-operated animals. This effect was strongly prevented by rat treatment with pargyline or clorgyline. I/R also increases the expression of the proapoptotic protein Bax, decreases the expression of the antiapoptotic protein Bcl-2, and induces procaspase-3 cleavage (Figure 7C and 7D). In rats treated with pargyline, the amounts of Bax, Bcl-2, and procaspase-3 were similar to those found in sham-operated animals.

**Discussion**

The major finding of this study is the demonstration that 5-HT behaves as a proapoptotic factor in cardiomyocytes and that its effect occurs independently of receptor stimulation. The novel mechanism of action of 5-HT that we describe requires 5-HT receptor–mediated effects on ROS production and antiapoptotic effects on cellular functions. The demonstration that 5-HT is able to regulate directly cardiomyocyte functions is relatively recent. As reported in a variety of cell types, 5-HT displays trophic and protective properties in cardiac cells. Via 5-HT<sub>3a</sub> receptors, 5-HT induces cardiac hypertrophy and prevents cardiomyocytes from apoptosis. The 5-HT<sub>3a</sub> receptor–mediated antiapoptotic effect of 5-HT involves inhibition
acid metabolism, and nonphagocyte NADPH oxidase, have been
5-HT in cardiac cells requires ROS production. Various sources of
5-HT may contribute in different ways to the initiation and
5-HT may have opposite effects on cardiomyocyte survival. It is
depending on the concentrations and the mechanisms of action,
those previously reported by Nebigil and coworkers3 suggest that,
c, upregulation of the proapoptotic Bax protein, and downregulation
drial proapoptotic pathway as shown by the release of cytochrome
ramine. The effect of 5-HT involves the activation of the mitochon-
drimal proapoptotic pathway as shown by the release of cytochrome
c, upregulation of the proapoptotic Bax protein, and downregulation

drial proapoptotic pathway as shown by the release of cytochrome
c, upregulation of the proapoptotic Bax protein, and downregulation
of the mitochondrial apoptotic machinery via cross-talks between
the ERK and Akt pathways. Our results show for the first time that
5-HT may also promote cardiomyocyte apoptosis. This effect
occurs at concentrations (10 μmol/L) higher than those observed for
the antiapoptotic activity (1 μmol/L). In contrast to that observed for
the antiapoptotic properties of 5-HT, cell apoptosis by 5-HT does
not require receptor stimulation. Indeed, we showed that the
increase in cardiomyocyte apoptosis by 5-HT was unaffected by
cell treatment with the 5-HT1b receptor antagonist SB 206553 and
was completely prevented by the amine transporter inhibitor imip-
ramine. The effect of 5-HT involves the activation of the mitochon-
drial proapoptotic pathway as shown by the release of cytochrome
c, upregulation of the proapoptotic Bax protein, and downregulation
of the antiapoptotic Bcl-2 protein. Taken together, our results and
those previously reported by Nebigil and coworkers3 suggest that,
depending on the concentrations and the mechanisms of action,
5-HT may have opposite effects on cardiomyocyte survival. It is
conceivable that, in vivo, these dual concentration-dependent effects
of 5-HT may contribute in different ways to the initiation and
progression of ventricular dysfunction.

We showed that the receptor-independent apoptotic effects of
5-HT in cardiac cells requires ROS production. Various sources of
ROS, including mitochondrial electron-transport chain, arachidonic
acid metabolism, and nonphagocyte NADPH oxidase, have been
proposed to mediate cell apoptosis in the heart in response to
different agents.28 Concerning serotonin, we have identified the
mitochondrial enzyme MAO-A as the intracellular source of ROS
involved in cardiomyocyte apoptosis. During the oxidative deami-
nation of serotonin, this enzyme generates H2O2, which can be
inactivated by antioxidants or, in the presence of iron, converted to
hydroxyl radical by the Fenton reaction. At the concentrations
leading to cardiomyocyte apoptosis, we showed that serotonin
induces H2O2 generation and intracellular oxidative stress, which
were prevented by MAO inhibition, antioxidant, or iron chelation.
The localization of MAO-A within the outer mitochondrial mem-
brane and ROS production in mitochondrial environment may be
particularly relevant for triggering mitochondrial dysfunction
associated with apoptotic cell death. This possibility is supported by
previous findings showing that treatment of isolated mitochondria
from brain with the MAO substrate tyramine induced a decrease in
mitochondrial respiration concomitant with glutathion oxidation.29
According to these results, we showed that MAO-dependent ROS
generation induced an increase in the Bax/Bcl-2 ratio and mitochon-
drial cytochrome c release, leading to cardiac cell apoptosis. These
findings point out the critical role of ROS produced by MAO-A
during serotonin degradation in triggering the proapoptotic mito-
chondrial cascade.

Oxidative stress and mitochondrial dysfunction are considered
key mediators of cardiomyocyte apoptosis associated with post-I/R
cardiac damage.28 In addition, large amounts of serotonin are
released by activated platelets during vascular injury30 and I/R.3 It
is noteworthy that the 5-HT concentration inducing cardiomyocyte
apoptosis in vitro (10 μmol/L) is compatible with those potentially
observed in vivo. Indeed, it has been shown that, in cats5 and mice,1
platelets can release up to 27-μmol/L concentrations of 5-HT. In
addition, Fu and Longhurst5 have recently shown that large amounts
of 5-HT (5 μmol/L) can be released by activated platelets in cardiac
venous blood after I/R in cats. From these observations, we
investigated the potential role of MAO-A in oxidative stress and
cardiac damage in a rat model of cardiac I/R.

In our in vitro studies, we showed that the 5-HT–degrading
enzyme MAO-A participates in the induction of the cardiomyocyte
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In our in vitro studies, we showed that the 5-HT–degrading
enzyme MAO-A participates in the induction of the cardiomyocyte
damage after I/R. Indeed, we demonstrated that rat pretreatment
with the irreversible MAO-A/B inhibitor pargyline or the selective
MAO-A inhibitor clorgyline significantly reduced infarct size. This
effect was not reversed by the 5-HT1b receptor antagonist SB
206553, indicating that the increase in cardiac 5-HT that may follow
MAO inhibition is not responsible for cardiac protection. These
findings are in agreement with previous results indicating that
stimulation of 5-HT receptors is involved in postreperfusion cardiac
damage rather than cardioprotection.6,31,32 Interestingly, MAO in-
hbitors may have clinical relevance for the prevention of postre-
perfusion cardiac injury by treating patients in the period occurring
between the ischemic event and reperfusion.

Electron microscopic study of postischemic rat hearts showed
that MAO inhibition prevented ultrastructural abnormalities, includ-
ing myofibril hypercontraction, interstitial edema, and mitochon-
drial swelling. The cardioprotective effects of MAO inhibition were
associated with a significant reduction of postreperfusion oxidative
stress. We found that MAO inhibition also prevented cardiac
accumulation of ROS-generating phagocytes. These results are in

Figure 6. Effect of MAO inhibition on myocardial postreperfusion
lipid peroxidation MDA (A) and MPO (B) activity. Sham indicates
sham-operated rats treated with saline; I/R, rats subjected to
ischemia followed by reperfusion; and P+I/R and C+I/R, rats
selected by the rule-based dissection algorithm. The natural
language model reads the text and produces a natural language
representation of the document. The model takes into account
the context and semantics of the text to generate a coherent and
accurate natural text representation.
agreement with previous reports suggesting that an initial burst of ROS at the onset of reperfusion may participate in delayed ROS generation by phagocytes. Prevention of postreperfusion cardiac damage and oxidative stress by MAO inhibition was associated with a decrease in caspase activation, upregulation of Bax, and downregulation of Bcl-2. Taken together, these results show that MAO-A is a major source of ROS-triggered myocardial apoptosis after reperfusion.

In conclusion, the identification of a new MAO-dependent mechanism involved in the 5-HT-triggered cardiomyocyte apoptosis opens new perspectives for the comprehension of the role of this biogenic amine in the heart. It is conceivable that the serotonin-MAO pathway may play a role not only in post-I/R syndrome but also in other heart diseases associated with cardiomyocyte apoptosis. Additional studies are necessary to verify this possibility and to define the potentiality of MAO-A as a pharmacological target for the prevention and therapy of cardiac diseases.

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**References**

In the heart, serotonin has been involved in the regulation of normal cardiac development and in different diseases, including arrhythmias, ventricular hypertrophy, and cardiac valvular insufficiency associated with carcinoid tumors. Recently, several studies showed that serotonin, released by activated platelets, accumulates in the heart during I/R and contributes to the progression of myocaridal injury and dysfunction. The availability of cardiac serotonin depends on the activity of the MAO-A. During serotonin degradation, MAO-A produces large amounts of H$_2$O$_2$. The major findings of this study are that serotonin behaves as a proapoptotic factor in cardiomyocytes and that its effect occurs independently of serotonin receptor stimulation. The novel mechanism of action of serotonin that we describe requires serotonin uptake into cardiac cells, its degradation by MAO-A, and H$_2$O$_2$ production. In addition, we demonstrate that H$_2$O$_2$ production by MAO-A plays a critical role in post-I/R apoptosis, leading to cardiac damage. The identification of a new MAO-dependent mechanism involved in the serotonin-triggered cardiomyocyte apoptosis offers new perspectives for the role of this biogenic amine in the heart. It is conceivable that the serotonin-MAO pathway may represent a potential pharmacological target for the prevention and therapy not only of cardiac I/R but also of other heart diseases associated with cardiomyocyte apoptosis.
Oxidative Stress by Monoamine Oxidase Mediates Receptor-Independent Cardiomyocyte Apoptosis by Serotonin and Postischemic Myocardial Injury

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