

## Serotonin produces monoamine oxidase-dependent oxidative stress in human heart valves

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Submitted 26 June 2009; accepted in final form 5 August 2009

**Peña-Silva RA, Miller JD, Chu Y, Heistad DD.** Serotonin produces monoamine oxidase-dependent oxidative stress in human heart valves. *Am J Physiol Heart Circ Physiol* 297: H1354–H1360, 2009. First published August 7, 2009; doi:10.1152/ajpheart.00570.2009.—Heart valve disease and pulmonary hypertension, in patients with carcinoid tumors and people who used the fenfluramine-phentermine combination for weight control, have been associated with high levels of serotonin in blood. The mechanism by which serotonin induces valvular changes is not well understood. We recently reported that increased oxidative stress is associated with valvular changes in aortic valve stenosis in humans and mice. In this study, we tested the hypothesis that serotonin induces oxidative stress in human heart valves, and examined mechanisms by which serotonin may increase reactive oxygen species. Superoxide ( $O_2^{\cdot-}$ ) was measured in heart valves from explanted human hearts that were not used for transplantation.  $O_2^{\cdot-}$  levels (lucigenin-enhanced chemoluminescence) were increased in homogenates of cardiac valves and blood vessels after incubation with serotonin. A nonspecific inhibitor of flavin-oxidases (diphenyliodonium), or inhibitors of monoamine oxidase [MAO (tranylcypromine and clorgyline)], prevented the serotonin-induced increase in  $O_2^{\cdot-}$ . Dopamine, another MAO substrate that is increased in patients with carcinoid syndrome, also increased  $O_2^{\cdot-}$  levels in heart valves, and this effect was attenuated by clorgyline. Apocynin [an inhibitor of NAD(P)H oxidase] did not prevent increases in  $O_2^{\cdot-}$  during serotonin treatment. Addition of serotonin to recombinant human MAO-A generated  $O_2^{\cdot-}$ , and this effect was prevented by an MAO inhibitor. In conclusion, we have identified a novel mechanism whereby MAO-A can contribute to increased oxidative stress in human heart valves and pulmonary artery exposed to serotonin and dopamine.

serotonin; reactive oxygen species; carcinoid syndrome; superoxide; valvulopathy

CARCINOID VALVE DISEASE is present in ~50–60% of patients with the carcinoid syndrome (2, 39, 54). Several circulating hormones including serotonin (5-hydroxytryptamine) and dopamine are released by carcinoid tumors (2, 18, 23, 44). Serotonin is released by carcinoid tumors, and its metabolism is associated with development and progression of myxomatous changes in heart valves and pulmonary hypertension (12, 38, 47). Similar findings were described in patients taking fenfluramine-phentermine for weight control (10, 16, 48, 52), pergolide for Parkinson's disease (60, 61), and other drugs such as ergot derivatives (48). Interestingly, fenfluramine also increases circulating serotonin levels (49). The combination fenfluramine-phentermine was removed from the market in

1997, and pergolide was removed in 2007, after Food and Drug Administration advisories about increased risk of valvular disease. However, mechanisms whereby serotonin elicits myxomatous valvular diseases have remained poorly understood.

Several studies in animals suggest a role for serotonin in carcinoid heart disease and drug-induced valve disease. Tryptophan hydroxylase I, the limiting enzyme in serotonin synthesis, is increased in canine myxomatous valve disease (14). In rats exposed to long-term administration of serotonin, increased cell proliferation and thickening of the heart valves, that resembles the changes reported in patients with carcinoid heart disease, are observed (15, 20). In addition, mice with the serotonin transporter gene knocked out also manifest valvular dysfunction, hyperplasia, and fibrosis of the valve leaflets (34).

In several cell types, including valvular interstitial cells (21, 43) and vascular smooth muscle cells (SMC) (9, 28–30), serotonin increases cell proliferation. There is evidence for activation and nuclear translocation of mitogen-activated protein kinases, transforming growth factor- $\beta$ 1, and other proliferative pathways by serotonin (28–30).

Reactive oxygen species (ROS), especially superoxide ( $O_2^{\cdot-}$ ), appear to participate in serotonin-induced mitogenesis (19, 26, 28–30). Antioxidants can prevent the mitogenic effects of serotonin (19, 26, 28–30). Nicotine adenine dinucleotide phosphate oxidase [NAD(P)H oxidase] may be a significant source of  $O_2^{\cdot-}$  after stimulation of SMC with serotonin, because inhibitors of NAD(P)H oxidase such as diphenyliodonium (DPI) inhibit proliferative signals in response to serotonin in pulmonary artery SMC and rat mesangial cells (19, 29, 30). Furthermore, recent evidence suggests that ROS may contribute to development of structural changes in stenotic aortic valves in humans and mice (36, 37). However, it is not known if serotonin increases oxidative stress in heart valves. Thus it is possible that increased oxidative stress in heart valves exposed to high concentrations of serotonin might increase proliferation of valve interstitial cells and contribute to the development of myxomatous valve disease.

In this study, we tested the hypothesis that serotonin increases ROS in human heart valves and explored mechanisms for production of ROS by serotonin in heart valves. We found that high concentrations of serotonin increase  $O_2^{\cdot-}$  in heart valves and blood vessels. Because amines, including serotonin and dopamine, are metabolized by monoamine oxidase (MAO) (4, 42, 55), we incubated the valves with an MAO inhibitor to prolong and augment the effects of serotonin. Surprisingly, the MAO inhibitor greatly reduced the serotonin-induced increase in  $O_2^{\cdot-}$  in cardiac valves and blood vessels. We also found that MAO-dependent metabolism of dopamine increased  $O_2^{\cdot-}$  lev-

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els. We used pharmacological interventions and recombinant human protein to demonstrate that MAO-A-mediated degradation of serotonin can be a critical contributor to  $O_2^{\cdot-}$  production in heart.

## METHODS

Normal human cardiac valves (pulmonary, tricuspid, and mitral) and proximal segments of pulmonary artery and aorta were obtained from donor hearts that were not suitable for transplantation. Hearts were obtained through the Iowa Donor Network and the National Disease Research Interchange (Philadelphia, PA) <12 h after organ harvesting and maintained in cold University of Wisconsin solution as described previously (36). Because clinical information was not obtained from the donor patients (except for age and sex), the University of Iowa Institutional Review Board indicated that informed consent was not required from each patient. Tissue was homogenized in a cocktail of protease inhibitors in PBS and stored at  $-80^\circ\text{C}$  until analysis.

**$O_2^{\cdot-}$  measurement.** Homogenized heart valve and vascular tissue were used to examine the levels of  $O_2^{\cdot-}$  using lucigenin-enhanced chemoluminescence. Tissue homogenates have been used in the past to study the activity of MAO in several animal tissues, including brain, heart, and liver (1, 5, 17, 22). Homogenates from pulmonary artery were used as a positive control because it is known that serotonin increases  $O_2^{\cdot-}$  in this tissue (28–30). Protein was quantified in each sample. Tissue homogenate containing 250  $\mu\text{g}$  of protein was placed in a cuvette containing 5  $\mu\text{M}$  lucigenin in PBS to obtain a total volume of 500  $\mu\text{l}$ . Samples were then incubated in the presence of serotonin at  $37^\circ\text{C}$  in a mixture of 95%  $O_2$  and 5%  $CO_2$  for 4 h. Oxygen concentration was calibrated with an oxygen analyzer (Beckman OM11). These conditions have been used for examination of responses to serotonin in rat aorta (40) and evaluation of NAD(P)H oxidase activity in mouse blood vessels (7, 50). Luminescence in each vial was read in an FB12 luminometer (Berthold, Germany).

Enzyme inhibitors were added to the samples 30 min before the addition of serotonin and maintained during the incubation period. The following drugs were used: 10  $\mu\text{M}$  DPI (a nonspecific flavin-containing enzyme inhibitor), 10  $\mu\text{M}$   $N^G$ -nitro-L-arginine methyl ester [L-NAME, an inhibitor of all three isoforms of the nitric oxide synthase (NOS)], 10  $\mu\text{M}$  allopurinol (a xanthine oxidase inhibitor), 100  $\mu\text{M}$  apocynin [an NAD(P)H oxidase inhibitor], 10  $\mu\text{M}$  indomethacin (a cyclooxygenase inhibitor), 200 U/ml catalase (36), 1–10  $\mu\text{M}$  tranylcypromine (an MAO-A/B inhibitor), and 1  $\mu\text{M}$  clorgyline (an MAO-A inhibitor) (56). All reagents were obtained from Sigma.

In addition, some samples were incubated with 1 mM dopamine dissolved in PBS. Clorgyline (1  $\mu\text{M}$ ) was added 30 min before dopamine to inhibit MAO. Luminescence in each vial was read in a luminometer after 4 h of incubation.

To test the specificity of the MAO inhibitors, we examined effects of the inhibitors on NAD(P)H oxidase, which is another potential source of  $O_2^{\cdot-}$  that has been associated with serotonin-induced oxidative stress, in pulmonary artery SMC (29, 30). For the evaluation of NAD(P)H oxidase-mediated  $O_2^{\cdot-}$  production, homogenates were preincubated for 30 min in the presence of DPI, tranylcypromine, clorgyline, or vehicle (PBS). Samples were exposed to 100  $\mu\text{M}$  NAD(P)H, and luminescence in each cuvette was measured.

**$O_2^{\cdot-}$  generation by recombinant MAO-A.** Recombinant human MAO-A (Sigma) was incubated with serotonin for 4 h in 5  $\mu\text{M}$  lucigenin in PBS at  $37^\circ\text{C}$  in a mixture of gases of 95%  $O_2$  and 5%  $CO_2$ . MAO-A was preincubated in the presence of tranylcypromine (10  $\mu\text{M}$ ) or PBS (vehicle) for 30 min before the addition of 100  $\mu\text{M}$  serotonin or PBS (control).  $O_2^{\cdot-}$  generation was detected by lucigenin-enhanced chemoluminescence in a luminometer.

**Electron paramagnetic resonance.** Recombinant human purified 100  $\mu\text{g}/\text{ml}$  MAO-A (Sigma) was added to a solution containing 0.1 mM serotonin or vehicle (PBS), 100 mM phosphate buffer, 250  $\mu\text{M}$  of the iron-chelating agent diethylenetriaminepentaacetic acid (DETAPAC), 1% albumin, and 50 mM 5,5-dimethylpyrroline-1-oxide (DMPO) as a spin trap. Electron paramagnetic resonance (EPR) was performed with a Bruker EMX spectrometer. Data were collected during 30 min at room temperature and ambient air. EPR parameters were 3510.3 G center field; 80 G scan width; 9.854 GHz microwave frequency; 20 mW power;  $2 \times 10^5$  receiver gain; modulation frequency of 100 kHz; modulation amplitude of 1.0 G; with the conversion time and time constant both being 40.96 ms with five scans for each 1,024-point spectrum.

**Quantitative real-time RT-PCR.** Heart valve and vascular tissues were homogenized in 0.5 ml of TRIzol (Invitrogen) and stored at  $-80^\circ\text{C}$  until collection was complete. RNA extraction, quantification, and RT were performed as described previously (8). RT (1  $\mu\text{l}$ ) was used for TaqMan real-time PCR to obtain the cycle threshold ( $C_t$ ) for MAO-A and  $\beta$ -actin. TaqMan Primers/probes were obtained from Applied Biosystems. Expression levels of MAO-A were determined relative to that of  $\beta$ -actin (which was constant among samples), using the  $\Delta\Delta C_t$  method as described previously (8, 36).

**Statistics.** Results are expressed as means  $\pm$  SE. Statistical significance was determined by one-way ANOVA and post hoc analysis with the Tukey test using the statistical program SAS (SAS Institute, Cary, NC) and VassarStats calculator (Vassar College, Poughkeepsie, NY). A significant difference was considered as  $P < 0.05$ .

## RESULTS

Incubation of homogenates of human heart valves (tricuspid and pulmonary) and pulmonary artery with 100  $\mu\text{M}$  serotonin significantly increased levels of  $O_2^{\cdot-}$  (Fig. 1). Serotonin also increased  $O_2^{\cdot-}$  levels in the mitral valve and proximal aorta

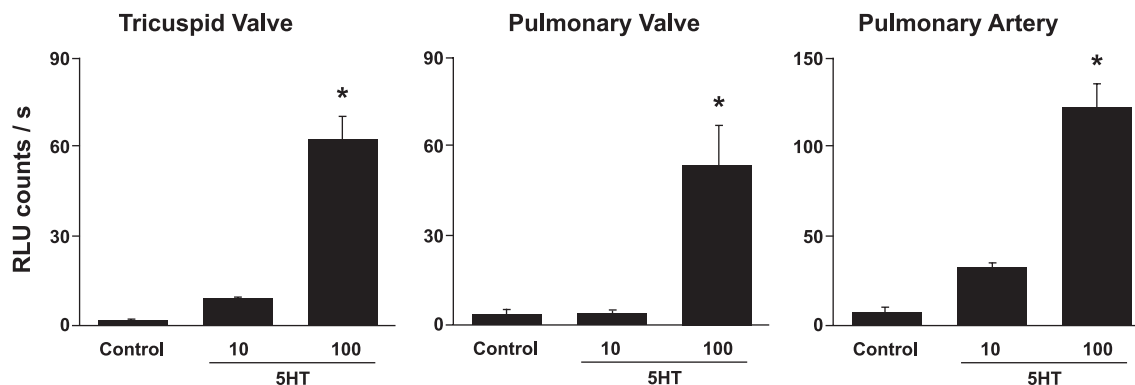


Fig. 1. Superoxide levels are increased in human tricuspid and pulmonary valves, and pulmonary artery, after incubation with serotonin (5-HT, 10  $\mu\text{M}$  and 100  $\mu\text{M}$ ) compared with control tissue [incubated with vehicle (PBS)]. \* $P < 0.05$  vs. control;  $n = 8$ –10 valves and pulmonary arteries.

[Supplemental Fig. 1 (Supplemental material for this article can be found on the *American Journal of Physiology: Heart and Circulatory Physiology* website)].

DPI (a nonspecific inhibitor of flavin oxidases) prevented the serotonin-induced increase in  $O_2^{\cdot-}$  in heart valves and pulmonary artery. Apocynin [an inhibitor of NAD(P)H oxidase] did not attenuate the increase in  $O_2^{\cdot-}$  in response to serotonin in any of the tissues (Fig. 2). A cyclooxygenase inhibitor (indomethacin) did not prevent the increase in  $O_2^{\cdot-}$  after serotonin treatment (Supplemental Fig. 2). Similarly, inhibitors of NOS (L-NAME), xanthine oxidase (allopurinol), did not attenuate the serotonin-induced increase in  $O_2^{\cdot-}$  in a subset of samples (data not shown).

We found that MAO-A is expressed in human tricuspid and pulmonary valves, and in pulmonary artery (Fig. 3). Incubation of homogenates of tricuspid and pulmonary valves, or pulmonary artery, with tranlycypromine (a nonselective MAO-A/B inhibitor) or clorgyline (an MAO-A inhibitor) abolished the increase in  $O_2^{\cdot-}$  in response to serotonin (Fig. 4). Tranlycypromine and clorgyline also prevented the increase in  $O_2^{\cdot-}$  after treatment with serotonin in mitral valve and aorta (Supplemental Fig. 1).

Addition of exogenous NADPH to homogenates of tricuspid or pulmonary valves or pulmonary artery increased  $O_2^{\cdot-}$  (Supplemental Fig. 3). Preincubation with DPI [NAD(P)H oxidase and flavin oxidases inhibitor] attenuated the increase in  $O_2^{\cdot-}$ . MAO inhibitors (tranlycypromine and clorgyline) did not attenuate the increase in  $O_2^{\cdot-}$  after addition of NADPH to homogenates of cardiac valves or pulmonary artery.

Incubation of recombinant human MAO-A with serotonin produced a significant increase in  $O_2^{\cdot-}$  levels, which was attenuated by tranlycypromine (Fig. 5). Recombinant human MAO-A was also studied with EPR. A DMPO-OH signal, suggestive of the presence of  $O_2^{\cdot-}$  in the sample, was obtained when serotonin and MAO-A were added together (Fig. 6).

Incubation of pulmonary valve homogenates with another MAO-A substrate, dopamine, increased  $O_2^{\cdot-}$  significantly (Fig. 7). The dopamine-induced increase in  $O_2^{\cdot-}$  was significantly attenuated by clorgyline.

## DISCUSSION

Elevated levels of serotonin are associated with development of myxomatous valve disease and pulmonary artery hypertension by mechanisms that are currently not well understood (2,

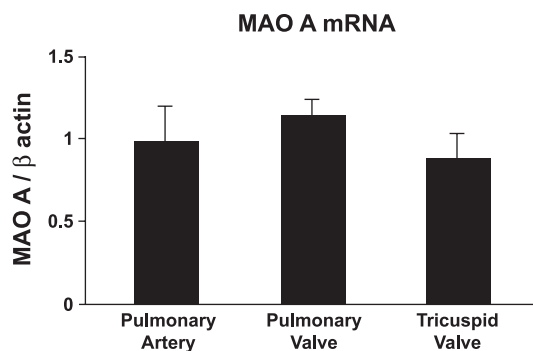


Fig. 3. Transcripts for monoamine oxidase (MAO)-A (real-time PCR) are expressed in pulmonary artery, and in pulmonary and tricuspid valves. There were no significant differences between tissues ( $n = 11$  valves and pulmonary arteries).

11, 38, 47). Because recent findings from our laboratory suggest a role for oxidative stress in the pathogenesis of aortic valve disease (36, 37), we hypothesized that oxidative stress may be found in heart valves exposed to high concentrations of serotonin. In the present study, we report two major findings. First, high concentrations of serotonin or dopamine increase  $O_2^{\cdot-}$  radicals in human heart valves and in vascular tissue. Second, MAO-A is a novel source of  $O_2^{\cdot-}$  in human heart valves. The data support a model in which increased  $O_2^{\cdot-}$ , derived from metabolism of amines by MAO-A, may contribute to the pathogenesis of valve disease.

About two-thirds of patients with carcinoid syndrome, especially those who tend to have the highest concentrations of serotonin in plasma (47), and high serotonin metabolism (38), develop carcinoid heart disease. Carcinoid heart disease in humans and in animal models is characterized by cellular proliferation, fibro-myxoid changes, and thickening of heart valves (2, 11, 15, 20, 34, 38, 47, 54). These changes are more frequent in the tricuspid and pulmonary valves than in the left side valves (2, 38, 39), presumably because lungs are a major site of metabolism for serotonin and other amines (24, 41), and the aortic and mitral valves therefore are exposed to lower concentrations of serotonin. In the present study, we examined human cardiac valves, and proximal segments of pulmonary artery and aorta. We focused especially on the tricuspid and pulmonary valves, and the pulmonary artery, because they are more commonly involved in carcinoid heart disease.

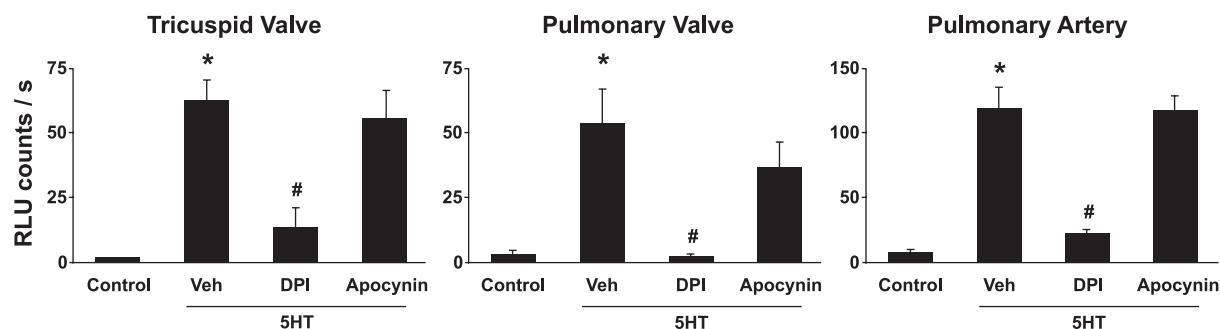


Fig. 2. 5-HT (100  $\mu$ M)-induced increase in superoxide in tricuspid and pulmonary valves, and pulmonary artery, is attenuated by diphenyliodonium (DPI, 10  $\mu$ M), an inhibitor of several flavin-containing oxidases. The increase in superoxide was not attenuated significantly by apocynin (100  $\mu$ M), an inhibitor of NAD(P)H oxidase. Vehicle (Veh) was PBS at pH 7.4.  $P < 0.05$  vs. control (\*) and vs. 5-HT only-Veh (#);  $n = 4$  for tricuspid valve,  $n = 6$  for pulmonary valve, and  $n = 7$  for pulmonary artery. Analysis used controls shown in Fig. 1.



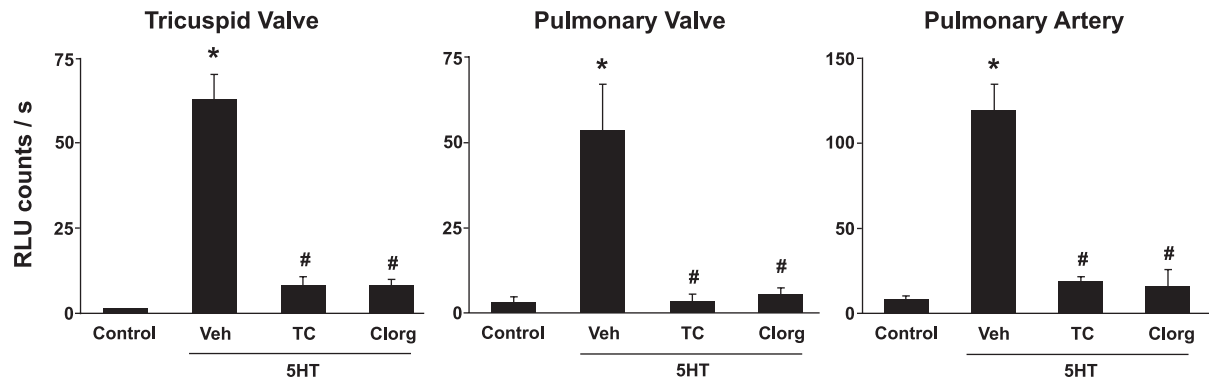


Fig. 4. Inhibition of MAO with tranlylcypromine (TC, 10  $\mu$ M) significantly attenuated the increase in superoxide in tricuspid and pulmonary valves, and pulmonary artery homogenates incubated with 100  $\mu$ M 5-HT. A specific inhibitor of MAO-A [1  $\mu$ M clorgyline (Clorg)] also attenuated increases in superoxide. Vehicle was PBS at pH 7.4.  $P < 0.05$  vs. control (\*) and vs. 5-HT only-Veh (#);  $n = 4$  for tricuspid valve,  $n = 6$  for pulmonary valve, and  $n = 7$  for pulmonary artery. Analysis used controls shown in Fig. 1.

The data indicate that serotonin increases  $O_2^{\cdot-}$  in heart valves and blood vessels. Data from cultured pulmonary artery SMC suggest that  $O_2^{\cdot-}$  may participate in serotonin-induced mitogenesis in multiple pathways including: 1) activation and translocation of mitogen-activated protein kinases (28), and the phosphatidylinositol 3-kinase pathway (29); 2) activation of cell cycle proteins (53); and 3) transactivation of other mitogenic receptors such as the platelet-derived growth factor receptor (30). Antioxidants attenuate the mitogenic effects of serotonin in pulmonary artery SMC (28–30). Similarly, dexfenfluramine-induced proliferation of SMC requires ROS and is attenuated by antioxidants (27). Therefore, we speculate that serotonin or fenfluramine-induced oxidative stress may also play a critical role in proliferation of valve interstitial cells and valvular thickening in vivo.

A key finding in this study is that serotonin-mediated increases in  $O_2^{\cdot-}$  are not primarily dependent on activation of NAD(P)H oxidase. Some studies in which DPI [an NAD(P)H oxidase inhibitor] was used concluded that NAD(P)H oxidase is the primary source of  $O_2^{\cdot-}$  in serotonin or dexfenfluramine-induced oxidative stress in cultured pulmonary artery SMC (27, 29, 30). DPI, however, inhibits multiple flavin oxidases and is not specific for NAD(P)H oxidase (45). Apocynin [a somewhat more specific inhibitor of NAD(P)H oxidase] did not attenuate the increase in  $O_2^{\cdot-}$  mediated by serotonin.

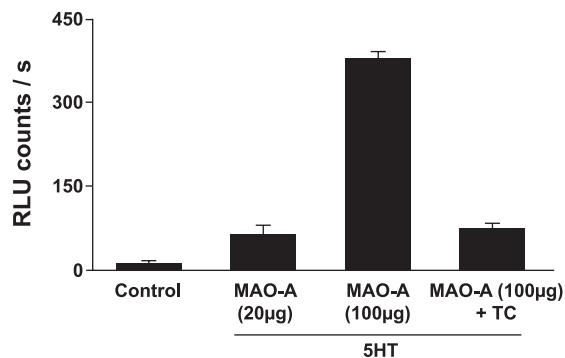


Fig. 5. MAO-A-derived superoxide. Superoxide is generated by human recombinant purified MAO-A incubated with 1 mM 5-HT or PBS as vehicle (control). The increase in superoxide was markedly attenuated by coincubation with an inhibitor of MAO (10  $\mu$ M tranlylcypromine). Results were obtained from 2 independent experiments, with 2 samples of MAO in each.

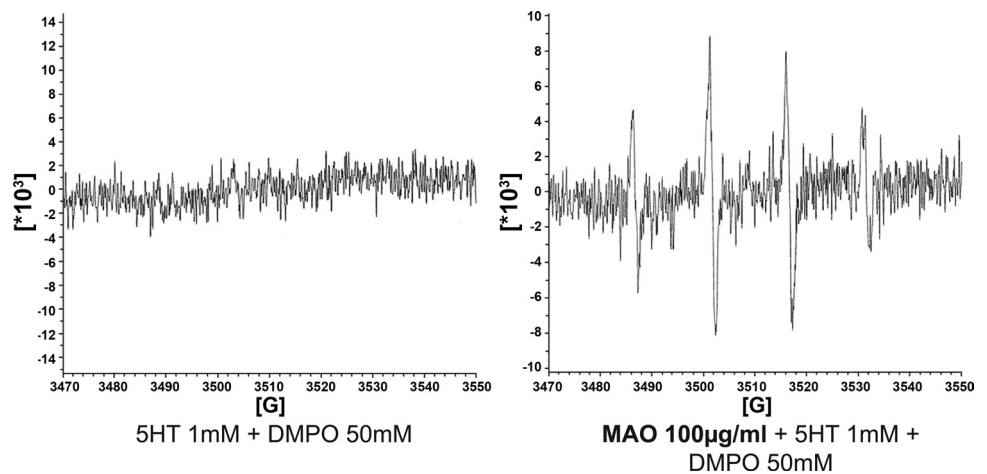
A major finding in this study is that the increase in  $O_2^{\cdot-}$  induced by serotonin was attenuated by inhibition of the flavin-containing enzyme MAO (46). Serotonin is metabolized avidly by MAO, which is localized in the outer mitochondrial membrane (4, 42, 55). MAO is present in two isoforms, MAO-A (which is expressed in a variety of tissues, including brain, liver, heart, kidney, and blood vessels and catalyzes the degradation of serotonin, dopamine, and norepinephrine) and MAO-B (which is expressed predominantly in the central nervous system and degrades dopamine and phenethylamine) (4, 55). In this study, we found that MAO-A was expressed in tricuspid and pulmonary valves, and in pulmonary artery. Furthermore, incubation with tranlylcypromine (an MAO-A/B inhibitor) or clorgyline (an MAO-A inhibitor) attenuated significantly the increase in  $O_2^{\cdot-}$  in tissue homogenates exposed to serotonin. Moreover, two different assays (lucigenin-enhanced chemoluminescence and EPR) also indicated that MAO-A is a source of  $O_2^{\cdot-}$  when recombinant human MAO-A is incubated with serotonin.

Although the chemistry of metabolism of serotonin and other amines by MAO is not clear (51), it appears that ROS are released as a byproduct during the reaction (58, 59). In another flavin oxidase, xanthine oxidase, the reaction with xanthine produces hydrogen peroxide or  $O_2^{\cdot-}$ . Generation of different ROS varies with electron flux through the flavin group (6, 32). We speculate that a similar mechanism may exist for MAO.

MAO-dependent oxidative stress is associated with multiple pathological processes, including proliferation of SMCs (9) and renal epithelial cells (57), cardiomyocyte hypertrophy (3), and renal ischemia-reperfusion injury (25). Oxidative stress produced by MAO-dependent degradation of amines is also increased in aged hearts in rats (31). Therefore, ROS generated from MAO-dependent degradation of serotonin may be important for the understanding of proliferation of valve interstitial cells in carcinoid heart disease and drug-induced valvulopathies.

We found that, in homogenates of cardiac valves, MAO (and not other oxidases), appears to be the primary source of  $O_2^{\cdot-}$  after stimulation with serotonin. Because we used two different MAO inhibitors (tranlylcypromine and clorgyline) to study the role of MAO in serotonin-induced oxidative stress, it was important to test the selectivity of these compounds. This is critical, because it is known that tranlylcypromine may inhibit

Fig. 6. Superoxide generation by MAO-A. *Left*: 1 mM 5-HT dissolved in a buffer solution [1% albumin, 50 mM 5,5-dimethylpyrroline-1-oxide (DMPO), 250 mM DETAPAC] was analyzed in the spectrometer. There is no change in the baseline recording during a 30-min period. *Right*: a signal indicative of DMPO-OH adducts (product of the DMPO + superoxide reaction) appears when human purified recombinant MAO-A is added to a buffer solution containing 1 mM 5-HT. Figure shows a representative trace.



eicosanoid metabolism (33). Therefore, we examined whether other common sources of  $O_2^{\cdot-}$  (cyclooxygenase, NOS, xanthine oxidase) were important sources of  $O_2^{\cdot-}$  in this preparation and assessed NAD(P)H oxidase activity in the presence of the MAO inhibitors. Inhibition of cyclooxygenase with indomethacin did not significantly attenuate the increase in  $O_2^{\cdot-}$ , after incubation with serotonin, in the tricuspid valve or pulmonary artery. Similarly, inhibitors of the three isoforms of NOS (with L-NAME) and xanthine oxidase (allopurinol) did not attenuate the serotonin-induced increase in  $O_2^{\cdot-}$  in a subset of samples of pulmonary artery. Importantly, we found that the MAO inhibitors, tranylcypromine and clorgyline, do not affect the NADPH-stimulated increase in  $O_2^{\cdot-}$  levels in homogenates of heart valve or pulmonary artery. Thus it is unlikely that the findings were the result of a nonspecific effect of the MAO inhibitors on other enzymes. In addition, NAD(P)H oxidase, NOS, xanthine oxidase, and cyclooxygenase do not appear to contribute importantly, in this preparation, to increases in  $O_2^{\cdot-}$  in heart valves or blood vessels exposed to high concentrations of serotonin.

Finally, incubation of pulmonary valve with dopamine (which, like serotonin, is a substrate for MAO) also increased  $O_2^{\cdot-}$  levels, and the response was inhibited by clorgyline. This is of interest because about one-third of patients with carcinoid syndrome also have high concentrations of dopamine in blood and urine (18, 23). The role of dopamine in myxomatous valve

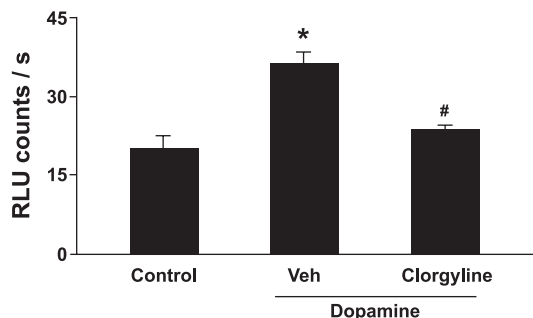


Fig. 7. Dopamine-induced increase in superoxide in homogenates of human pulmonary valve ( $n = 3$ ). Dopamine (1 mM) significantly increased superoxide levels in pulmonary valve compared with control tissue (incubated with PBS). The increase in superoxide produced by dopamine was attenuated by an MAO-A inhibitor (1  $\mu$ M clorgyline).  $P < 0.05$  vs. control (\*) and vs. dopamine treatment (#).

disease is not clear. Pergolide, a dopamine agonist, however, has been associated with valvulopathies in humans (60, 61).

We acknowledge important limitations in the present study. First, we used high concentrations of serotonin. Second, it is not possible to study the role of membrane signaling in homogenates. Although the intracellular concentration of serotonin in vascular tissue is not known, it appears to be higher than circulating levels in other tissues (35). It seems reasonable to speculate that intracellular serotonin and/or dopamine concentrations in patients with carcinoid syndrome may be even higher than blood levels because of active uptake of the amines. Two mechanisms are responsible for serotonin uptake: a high-affinity low-capacity uptake through the serotonin transporter and a low-affinity high-capacity uptake by the norepinephrine transporter (13). There are no data about mechanisms of serotonin uptake, or expression and function of these transporters, in human heart valves. Some authors speculate that, in circumstances where reduced expression or knockout of the serotonin transporter in animals has been associated with valve disease, increased circulating serotonin activates serotonin 2B receptors, leading to increased valve cell proliferation and valve disease (15, 34). We did not address the potentially important role of activation of serotonin receptors or transporter in serotonin-induced oxidative stress. We speculate, however, that serotonin uptake through the serotonin or norepinephrine transporter may be increased in conditions with high serotonin concentrations, leading to increased availability of serotonin for MAO. Increased metabolism of amines is associated with progression of carcinoid heart disease (38), and may be an important source of ROS in heart valves.

In summary, these findings identify a novel pathway whereby serotonin increases oxidative stress in heart valves through an MAO-A-dependent mechanism. MAO-dependent generation of ROS may be important for the understanding of mitogenic actions of serotonin in carcinoid valve disease, drug-induced valvulopathies, and pulmonary artery hypertension. A deeper understanding of MAO-A-dependent oxidative stress may also facilitate the avoidance of unwanted side effects from pharmaceutical drugs under development, as well as serious cardiovascular side effects from recreational drugs such as 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) that may alter the normal concentration of serotonin in human blood (49, 62).

## ACKNOWLEDGMENTS

We thank Dr. Gary Buettner and the spectroscopy facility at the University of Iowa for assistance with the EPR experiments.

## GRANTS

This work was supported by National Institutes of Health Grants HL-62984 and NS-24621 (D. D. Heistad.) and HL-092235 (J. D. Miller), support from the faculty development program from the Fulbright Commission and the Universidad de los Andes (R. Peña-Silva), a fellowship from the American Heart Association (0815525G) to R. Peña-Silva, and funds from a Carver Trust Research Program of Excellence at the University of Iowa (D. D. Heistad).

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