Endogenous Vascular Hydrogen Peroxide Regulates Arteriolar Tension In Vivo

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Background—Although many studies suggested direct vasomotor effects of hydrogen peroxide (H$_2$O$_2$) in vitro, little is known about the vasomotor effects of H$_2$O$_2$ in vivo.

Methods and Results—We have generated mice overexpressing human catalase driven by the Tie-2 promoter to specifically target this transgene to the vascular tissue. Vessels of these mice (cat$^+$) expressed significantly higher levels of catalase mRNA, protein, and activity. The overexpression was selective for vascular tissue, as evidenced by immunohistochemistry in specimens of aorta, heart, lung, and kidney. Quantification of reactive oxygen species by fluorescence signals in cat$^+$ versus catalase-negative (cat$^-$) mice showed a strong decrease in aortic endothelium and left ventricular myocardium but not in leukocytes. Awake male cat$^+$ at 3 to 4 months of age had a significantly lower systolic blood pressure (sBP, 102.7±2.2 mm Hg, n=10) compared with their transgene-negative littermates (cat$, 115.6±2.5$ mm Hg, P=0.0211) and C57BL/6 mice (118.4±3.06 mm Hg, n=6). Treatment with the catalase inhibitor aminotriazole increased sBP of cat$^+$ to 117.3±4.3 mm Hg (P=0.0345), while having no effect in cat$^-$ (118.4±2.4 mm Hg, n=4, P>0.05). In contrast, treatment with the NO-synthase inhibitor nitro-L-arginine methyl ester (100 mg · kg BW$^{-1}$ · d$^{-1}$) increased sBP in cat$^+$ and C57BL/6 to a similar extent. Likewise, phosphorylation of vasodilator-stimulated phosphorylase in skeletal muscle, left ventricular myocardium, and lung was identical in cat$^+$ and cat$. Endothelium- and NO-dependent aortic vasodilations were unchanged in cat$^+$ and exogenous H$_2$O$_2$ (10 μmol/L)–induced vasoconstriction.

Conclusions—These data suggest that endogenous H$_2$O$_2$ may act as a vasoconstrictor in resistance vessels and contribute to the regulation of blood pressure. (Circulation. 2005;112:2487-2495.)

Key Words: hydrogen peroxide ■ blood pressure ■ catalase, vascular ■ aminotriazole ■ contractility

Hydrogen peroxide is a nonradical reactive oxygen species that is produced in many different cell types in the human body, including vascular endothelial and smooth muscle cells.$^1$ A large number of reports during the past 20 years have suggested that hydrogen peroxide may be an important mediator in the vasculature, eg, a regulator of vasomotor tone.$^2$–$^4$ However, in vitro studies have yielded conflicting results on the effect of hydrogen peroxide on vasomotor tone,$^5$–$^6$ suggesting that the vasomotor response to hydrogen peroxide depends on experimental conditions, such as the type of vessel studied, the species studied, and the concentration range used. For example, endothelial hydrogen peroxide has been shown to relax mouse and human mesenteric arteries,$^7$–$^8$ but not human radial arteries.$^9$

The steady-state concentration of hydrogen peroxide in human plasma, blood cells, and vascular cells is unknown but is most likely in the lower micromolar range or less.$^1$ In contrast, the vast majority of studies investigated the vasomotor effects of exogenous hydrogen peroxide at much larger concentrations (up to 10 mmol/L), and both vasodilator and vasoconstrictor effects were reported.$^3$–$^5$, $^10$ Vasodilator effects of hydrogen peroxide may be mediated by activation of K$^+$ channels,$^7$ activation of endothelial NO production,$^{11}$–$^{12}$ decreased myosin phosphorylation,$^{13}$ and inhibition of myosin ATPase,$^{14}$ whereas vasoconstriction is attributed to an increase of intracellular Ca$^{2+}$,$^{15}$ to the generation of arachidonic acid metabolites with vasoconstrictor activity,$^{16}$ and to direct Ca$^{2+}$–independent tonic effects on the smooth muscle contractile apparatus.$^{17}$–$^{18}$

Recent studies in mouse and rat mesenteric arteries and mouse aorta showed vasoconstrictor effects at up to 10 μmol/L of hydrogen peroxide, whereas higher concentrations induced sustained and almost irreversible vasodilatation.$^{16}$–$^{19}$
These data suggest a biphasic action of hydrogen peroxide on the vasomotor tone of conductance and small mesenteric arteries, in which lower micromolar concentrations induce predominantly vasoconstrictor effects. Although there are no data on the effects of hydrogen peroxide on the vascular tone of small skeletal muscle resistance arteries, it seems possible that the vasomotor effects of endogenously produced hydrogen peroxide contribute to the regulation of blood pressure. In hypertension, the plasma concentration of hydrogen peroxide is increased and is positively correlated to plasma renin activity and systolic blood pressure (sBP) but negatively correlated to cardiac contractility and renal function. To investigate the effects of endogenous vascular hydrogen peroxide on small skeletal resistance vessels in vivo, we studied a transgenic mouse with a vascular-specific overexpression of human catalase.

**Methods**

**Generation of Transgenic Mice**

We generated a transgenic construct in which human catalase (hCat) was inserted between murine Tie-2 Promotor (2.1 kb) and a 10-kb Tie-2 intron fragment, designated as Tie-2 enhancer, and this construct was used to target catalase gene expression to the vasculature as described previously. Founder mice showing approximately 100-fold higher catalase expression were crossed 10 times to C57BL/6 mice to generate pure C57BL/6 background (catn). Mice were used at 12 to 16 weeks of age. Transgenic-negative littermates (cat) served as controls. In addition, C57BL/6 mice were used as nontransgenic controls. In some experiments, mice carrying a vascular-specific overexpression of wild-type endothelial nitric oxide synthase (eNOS) driven by the murine Tie-2 promotor were used. Vascular overexpression of eNOS (eNOScatn) was evident by a 1.66 ± 0.13-fold eNOS protein content in the aorta (n = 5, P < 0.05) compared with transgene negative littermates (eNOScat).

**Ethical Statement**

Permission for this study was provided by the regional government (AZ: 23.05-230-3-94/00 and 23.05-230-3-65/99), and the experiments were performed according to the guidelines for the use of experimental animals as given by the Deutsches Tierschutzgesetz and to the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health.

**Preparation of 100g Supernatants**

To prepare protein homogenates, mouse thoracic aortas were flash-frozen in liquid nitrogen, homogenized, solubilized in lysis buffer (50 mmol/L Tris-HCl, 1 mmol/L DTT, 1 μmol/L proteinase inhibitors), and centrifuged 10 minutes at 100g. Supernatants were stored at −70°C until used for Western blots.

**Western Blotting**

Western blot analysis was performed in a blinded manner, as described previously, using commercially available monoclonal antibodies directed against eNOS (Transduction Laboratories), rabbit antisemur M4 against vasodilator-stimulated protein (VASP, Alexis), monoclonal anti-phospho-Ser239 antibody 16C2 (Vasop-harm), and a polyclonal antibody for catalase (Calbiochem) as well. Blots were developed by use of enhanced chemiluminescence (Roche) and exposed to x-ray film. The autoradiographs were analyzed by densitometry (Geldoc, Bio-Rad). Total protein levels were determined by the Bradford method.

**Immunohistochemistry**

Different organs of the mice (heart, aorta, lung, kidney) were removed, immediately fixed in Bouin’s fluid, and embedded in paraffin wax. Sections 8 μm thick were stained for catalase (anti-body diluted 1:100, anti-human, from rabbit; Calbiochem). After incubation for 60 minutes at room temperature, the reaction was detected by an ABC system (Vector Elite Kit) using biotinylated second antibodies and PO-conjugated streptavidin. Before the reaction, all sections were digested for 30 minutes with 0.1% trypsin.

**Smooth Muscle Layer Thickness**

Aortic rings (1 cm long) of catcat and catn were fixed in buffered 4% formalin and embedded ethanol-free in the water-soluble plastic resin Technovit 7100 (Heraeus-Kulzer) to prevent shrinkage and lipid extraction. Sections 4 μm thick were stained with 1% toluidine blue, and intima-media thickness was measured by use of the image analyzing system CUE-3 ( Olympus Ltd, version 4.5, 1993). The measurements were routinely performed in a double-blind experimental approach by a technical assistant who had no knowledge of the specific scientific background of the study.

**Detection of Reactive Oxygen Species**

The hydrogen peroxide level was monitored with 5- and 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate bis(acetomethyl)ester (DCDHF-DA, Molecular Probes), which can be oxidized to the fluorescent compound 2′,7′-dichlorofluorescein. The intimal layer of longitudinally cut mouse aortic rings was incubated with 5 μmol/L DCDHF-DA for 30 minutes at 37°C and rinsed 3 times with PBS, and fluorescence was visualized on a Leica TCS SP2 confocal microscope (excitation, 488 nm; emission, 525 nm). Slices of left ventricle of catcat and catn mice were pretreated for 1 hour at 37°C with vehicle or with the catalase inhibitor aminotriazole (1 mmol/L), followed by incubation with 5 μmol/L DCDHF-DA, and quantification of fluorescence intensity. In addition, the basal level of oxidative stress was measured in leukocytes by means of dihydroethidine fluorescence intensity as analyzed by fluorescence-activated cell sort (FACS).

**Measurement of Blood Pressure and Heart Rate**

SBP and heart rate were measured in awake male catcat (n = 8), catn (n = 8), and C57Bl/6 mice (n = 6) at 3 to 4 months of age using an automated tail-cuff system (Visitech Systems) as described previously. In some experiments, catcat and catn mice (n = 4 each) were treated with aminotriazole (666 mg · kg−1 · d−1, dissolved in drinking water) for 14 days. In another subset of experiments, catcat (n = 6) and C57Bl/6 (n = 5) mice were treated for 28 days with N-nitro-l-arginine (100 mg · kg BW−1 · d−1), and blood pressure and heart rate were recorded before, during, and after the treatment period.

**Organ Bath Experiments**

Preparation of thoracic ring segments was performed in HEPES-containing Krebs-Henseleit buffer, and the organ bath experiments were performed in the same buffer lacking HEPES. After a 60-minute equilibration period, aortic rings were repeatedly subjected to 80 mmol/L KCl. The vasoconstriction that developed during the last of 3 KCl applications was taken as the maximal receptor-independent vasoconstriction. The function of the endothelium was examined by cumulative addition of acetylcholine (1 mmol/L–10 μmol/L) after submaximal precontraction with phenylephrine. In catcat (n = 5) and catn (n = 4) mice, endothelium-dependent vasodilation to the NO donor DEA-NO (0.1 mmol/L–10 μmol/L) was followed by a cumulative application of phenylephrine (1 mmol/L–10 μmol/L). Thereafter, the aortic rings were incubated for 30 minutes with aminotriazole (1 mmol/L) or vehicle, and a concentration-response curve for phenylephrine (1 mmol/L–10 μmol/L) was performed. Reaction to increasing concentrations of hydrogen peroxide (10 mmol/L–100 mmol/L) was examined in endothelium-intact or denuded aortic segments of catcat and catn/C57BL/6 mice after precontraction with phenylephrine (0.1 μmol/L).

**Substances and Solutions**

All chemicals were obtained from Merck or from Sigma in analytical grade. Stock solutions were prepared daily, diluted with Krebs-buffer as required, kept on ice, and protected from daylight until use. All
concentrations indicated in the text and figures are expressed as final bath concentrations.

Statistics
All data were analyzed by standard computer programs (GraphPad Prism PC Software, Version 3.0, ANOVA) and are expressed as mean values and SEM. Significant differences were evaluated by use of either the Newman-Keuls multiple comparison test after 1-way ANOVA, 2-way ANOVA, or Student t test. A probability value of less than 0.05 was considered significant.

Results
Characterization of Catalase Overexpression
Injection of the catalase-Tie-2-promotor construct resulted in a marked overexpression of catalase, as evidenced by Southern blot in the myocardium (not shown), competitive reverse transcription–polymerase chain reaction (31.7±4.2 6 pg mRNA/μg total RNA in cat++ versus 4.5±0.6 pg mRNA/μg total RNA in cat−, n=3, P<0.01), Western blot (2.2±0.3-fold, P<0.05), and activity (43.2±11.5×10−3 s−1/mg protein of cat++ versus 14.2±2.0×10−3 s−1/mg protein in cat−, n=4, P<0.05) in the aorta, the lung, and the heart. Immunohistochemistry performed in cross sections of the aorta, the heart, the lung, and the kidney of cat++ showed that the overexpression occurred specifically in blood vessels (Figure 1). Examination of the steady-state content of hydrogen peroxide in endothelial cells by confocal microscopy using longitudinally cut aortic segments pretreated with DCDHF-DA revealed a significant reduction of the fluorescence signal in cat++ (1973±244) compared with cat− (3808±664, n=8, P=0.0021). Likewise, DCDHF-DA–induced fluorescence was much smaller in slices of left ventricular myocardium of cat++ compared with cat−, whereas this difference disappeared in the presence of aminotriazole, which increased the fluorescence signal to the same degree in mice of both strains (Figure 2). In striking contrast, oxidative stress in leukocytes as measured by dihydroethidine fluorescence using FACS analysis was not different in cat− (43±6 U, n=10) and cat++ (44±28 U, n=9) but increased strikingly in cat++ treated with aminotriazole (107±5, n=5). These data indicate that cat++ mice specifically overexpress catalytically active catalase protein in vascular cells.

Smooth Muscle Layer Thickness
To evaluate a possible effect of catalase overexpression on the media thickness of the vasculature, we measured the smooth muscle layer thickness of different arteries. There was no difference between cat− and cat++ (n=3, each) in the aorta (45.18±2.37 versus 45.25±1.06 μm, P=0.978) and in arteries of the heart (5.0±0.23 versus 5.12±0.08 μm, P=0.642) and the lung (9.33±0.44 versus 8.91±0.24 μm, P=0.455). These data suggest that catalase overexpression has no effect on vascular media thickness.

Blood Pressure and Heart Rate
sBP was significantly lower in cat++ mice (102.7±2.2 mm Hg, n=10) compared with their transgene-negative littermates (cat−, 115.6±2.5 mm Hg, n=8) or C57BL/6 mice (118.4±3.06 mm Hg, n=6, Figure 3A). Catalase overexpression had no effect on heart rate (data not shown). The decrease of sBP in cat++ was identical to that in eNOS++ mice (105.3±2.6 mm Hg, n=6, Figure 3B). These data indicate that overexpression of catalase causes the same degree of sBP reduction as overexpression of eNOS.

Aminotriazole Treatment
Treatment of cat++ (n=4) and cat− (n=4) with the catalase inhibitor aminotriazole significantly raised sBP in cat++ mice, whereas it had little effect on the sBP of cat− (Figure 3C). There was no change of heart rate caused by inhibition of catalase (data not shown). The recovery of sBP by aminotriazole treatment suggests that the reduction of blood pressure in cat++ mice is caused by overexpression of catalase and a subsequent decrease of the vascular hydrogen peroxide steady-state concentration.

Aortic eNOS Expression
To evaluate a possible effect of catalase overexpression on vascular eNOS expression, we compared aortic eNOS protein levels in cat− and cat++. As shown in Figure 4, overexpression...
Figure 2. Effect of catalase inhibitor aminotriazole on endogenous hydrogen peroxide content in the slices of left ventricle of cat++ and cat*. A, Endothelial hydrogen peroxide levels after treatment with vehicle or catalase inhibitor aminotriazole (1 mmol/L) as measured by use of dichlorofluorescein fluorescence. B represents the mean dichlorofluorescein fluorescence intensity in arbitrary units (P < 0.05 vs cat++, n = 4, 1-way ANOVA).

of catalase had no effect on aortic eNOS protein content. In contrast, Western blot analysis in eNOS++ mice demonstrated a significantly elevated aortic eNOS protein level of 166 ± 13.7% (n = 5, P < 0.05, Figure 4). These findings indicate that the reduction of sBP in cat++ mice is not caused by an increase of aortic eNOS protein expression.

NO Bioavailability
To evaluate whether overexpression of catalase changes the bioavailability of endogenous NO, C57BL/6 and cat++ mice were treated with the NO synthase inhibitor Nω-nitro-l-arginine methyl ester (L-NAME) for 6 weeks. As expected, L-NAME elevated blood pressure significantly in C57BL/6 mice, from 118 ± 3 to 146 ± 5.6 mm Hg (n = 6, P < 0.001, Figure 5A). This 23.3 ± 4.8% increase of sBP observed in C57BL/6 mice was almost identical to the 20.3 ± 2.8% increase of sBP in cat++ (Figure 5B), in which an elevation from 103 ± 2.3 to 125 ± 4.5 mm Hg occurred (n = 6, P < 0.001). In both mouse strains, termination of L-NAME treatment induced a complete restoration of sBP to pretreatment values. We have also measured VASP phosphorylation to further substantiate that the NO/cGMP system is not involved. There was no change in the total VASP protein content in cat++ in the lung (120 ± 12%, n = 4), the skeletal muscle (92 ± 10%, n = 4), and the heart muscle (109 ± 19%, n = 3), tissues known to contain mostly resistance vessels. Likewise, phosphorylated VASP protein was also identical in cat+++ and cat++, as indicated by the relative Western blot signal intensity in cat++ in the lung (117 ± 36%, n = 8), the skeletal muscle (126 ± 20%, n = 4), and the heart (95 ± 29%, n = 4).

Studies of Isolated Aortic Segments
Aortic responses to increasing concentrations of acetylcholine (Figure 6A) were similar in cat+++ and cat++, confirming that the bioavailability of endothelial NO is not altered by a reduction of vascular hydrogen peroxide levels. Likewise, the efficiency of the NO/cGMP pathway remained in cat+++, as evidenced by aortic responses to the spontaneous NO donor DEA-NO (Figure 6B). Thus, vascular overexpression of catalase had no effect on endothelium-dependent and-independent NO-mediated relaxation of conductance vessels.

Subjection of aortic rings to a single dose of 80 mmol/L KCl induced a strong vasoconstriction in cat+++ (9.65 ± 0.34 mN, n = 4). Aortic rings of cat++ showed a significantly lower maximal response to 80 mmol/L KCl of 8.05 ± 0.46 mN (n = 4, P = 0.0322), although there was no change of the media thickness in this vessel type (see above). These data suggest that overexpression of catalase decreases the contractile response of vascular smooth muscle cells caused by depolarization. In contrast, concentration-dependent vasoconstriction to phenylephrine was similar in cat+++ and cat++ (Figure 7). Neither the half-maximal effective concentration given as pD2 values in −log mol/L for phenylephrine in cat++ (6.46 ± 0.2, n = 4) and cat+++ (6.45 ± 0.2, n = 4) nor maximal vasoconstrictions to phenylephrine in cat++ (5.49 ± 0.54 mN, n = 4) and cat+++ (5.48 ± 0.54 mN, n = 4) were different. However, although inhibition of catalase with aminotriazole had only little effect on phenylephrine constrictions in cat+++, it significantly increased phenylephrine constrictions in cat+++ (P = 0.023, n = 4, Figure 7), suggesting a contribution of endogenous hydrogen peroxide to adrenergic vasoconstriction in conductance vessels.

To investigate vasoconstrictor effects of exogenous hydrogen peroxide, denuded aortic rings of cat+++ and cat+++ were subjected to increasing concentrations of hydrogen peroxide. Small vasoconstrictor effects were observed up to 50 μmol/L hydrogen peroxide in cat+++ only, whereas higher concentrations induced strong and irreversible vasodilator effects in cat+++ and cat++ (Figure 8). After washout of vasodilator concentrations of hydrogen peroxide, aortic rings did not respond to KCl or phenylephrine any longer (data not shown).

Discussion
The aim of this study was to determine the effect of endogenous vascular hydrogen peroxide on the vasomotor tone of resistance vessels in vivo. Our new finding is that a reduction of steady-state concentrations of vascular hydrogen peroxide induced by a vascular specific overexpression of human catalase resulted in a marked reduction of sBP in mice. This hypotension was completely reversed by treatment with the catalase inhibitor aminotriazole but was independent of vascular eNOS protein content, eNOS activity, and the
efficiency of the NO/cGMP pathway as evidenced by Western blot analysis, chronic treatment with the NOS inhibitor L-NAME, and organ bath experiments, respectively. These data suggest that endogenous hydrogen peroxide is a vasoconstrictor in resistance vessels in vivo. We assume that the vasoconstrictor effects of hydrogen peroxide in resistance vessels might contribute to the development of essential hypertension.

Our data obtained in transgenic mice overexpressing catalase strongly support the concept that reactive oxygen species, such as hydrogen peroxide, generated in the vascular wall can modulate arterial tone and are involved in the pathogenesis of such vascular diseases as hypertension. As predicted, catalase overexpression driven by the Tie-2 promotor construct was vascular-specific in our mouse model. This was evident in the aorta, the lung, the heart, and the kidney. Vascular specificity was further investigated by measuring dihydroethidine fluorescence in leukocytes by FACS analysis, and no difference was found between cat and cat++, suggesting an absence of catalase overexpression in nonvascular cells, as found in a variety of organs. Our cat++ mouse is the first transgenic animal model in which a mammalian gene was overexpressed, driven by the Tie-2 promotor. In the original publication describing the discovery of the Tie-2 promotor, Schläger et al suggested an endothelium-specific expression induced by this promotor. However, this was not directly proved by histology of cross sections of vascularized tissues, and our results rather suggest a vascular-specific instead of an endothelium-specific expression pattern induced by the Tie-2 promotor.

In our animal model, catalase overexpression was associated with a substantial reduction of blood pressure in resting awake mice, and this hypotension was completely reversible by the catalase inhibitor aminotriazole. Recently, Yang et al reported that catalase-overexpressing mice generated by means of an 80-kb P1 clone containing the entire human catalase gene show no change of hydrogen peroxide release from the aorta and have normal blood pressure. Thus, specific targeting of catalase overexpression to the vasculature appears to be important to unmask a vasotonic effect of endogenous hydrogen peroxide.

It has been shown that vascular hydrogen peroxide has many associations with the vascular NO/cGMP pathway. For example, hydrogen peroxide may either enhance endogenous NO generation by increasing the activity and expression of eNOS or impair endothelial production of NO in response to such mediators as the calcium ionophore A23187, bradykinin, and ADP. Hydrogen peroxide might also play a role in flow-mediated vasodilation, and catalase most likely consumes NO, which partially inhibits the reaction of the enzyme with hydrogen peroxide. We investigated whether hypotension in cat++ is associated with changes of the vascular eNOS content or the bioavailability of endogenous NO. Aortic eNOS protein was not changed, suggesting that the reduction of vascular hydrogen peroxide in cat++ had no impact on the regulation of endothelial eNOS expression.

The lack of influence on eNOS expression of a reduced vascular hydrogen peroxide content probably results from the complex regulation of vascular eNOS expression, leaving a
We were also unable to detect differences of the bioavailability of vascular NO in resistance and conductance vessels. Oral treatment with the NOS inhibitor L-NAME induced an identical rise of sBP in cat\(^n\) and cat\(^++\), and the approximately 15-mm Hg difference in sBP remained. Likewise, aortic endothelium-dependent vasodilation in cat\(^n\) was normal, and measurement of serine-239–VASP phosphorylation\(^{36}\) showed a similar degree of activation of the NO/cGMP pathway in tissues known to contain mostly resistance vessels. These data do not support the hypothesis that endogenous hydrogen peroxide impairs endogenous NO production and bioavailability in BP-regulating skeletal resistance vessels.

Previous data obtained in endothelium-denuded mouse and rat mesenteric arteries have suggested that concentrations of exogenous hydrogen peroxide (0.1 to 10 \(\mu\)mol/L), which probably resemble in vivo conditions, induce either no response or a vasoconstriction.\(^{16,19}\) Such vasoconstrictor effects of hydrogen peroxide might be mediated by an increase of intracellular Ca\(^{2+}\),\(^{15}\) the generation of arachidonic acid metabolites with vasoconstrictor activity,\(^{16}\) and a direct Ca\(^{2+}\)-independent tonic effect on the smooth muscle contractile apparatus.\(^{17,18}\) These in vitro vasococontractile activities of exogenous hydrogen peroxide might also occur in vivo and might explain hypotension in cat\(^++\), which is dependent on catalase activity, as evidenced by the complete reversal after aminotriazole treatment. Inhibition of catalase activity by aminotriazole also unmasked a potentiation of adrenergic vasoconstriction by phenylephrine in aortic rings of cat\(^n\) that was attenuated in cat\(^++\), as expected. In addition, concentrations of hydrogen peroxide that most likely resemble in vivo conditions induced vasococontractor effects in aortic rings submaximally precontracted with phenylephrine. We suggest that the vasococontractor and/or calcium-sensitizing effect of hydrogen peroxide in vivo is already exploited at normal physiological concentrations that occur in the vascular wall. A further increase by treatment with aminotriazole does increase blood pressure slightly but not significantly. Likewise, aminotriazole did increase DCDHF-DA fluorescence of ventricular slices of cat\(^n\) slightly but not significantly, while having a much stronger effect in cat\(^++\).

Vasoconstriction to phenylephrine is mediated by \(\alpha_1/A_C\) receptors on vascular smooth muscle cells.\(^{37}\) Activation of these G-protein–coupled receptors results in stimulation of different intracellular enzymes that are involved in the signal transduction. The second messengers 1,4,5-inositol triphosphate (IP\(_3\)) and diacyl glycerol are generated by phospholipase C. Whereas IP\(_3\) releases calcium from the sarcoplasmic reticulum and thereby directly jvates vasoconstriction, diacyl glycerol activates protein kinase C and supports the intracellular calcium release.\(^{38}\) Hydrogen peroxide is known to interact with some proteins of this signal transduction pathway, such as phospholipase C, protein kinase C, and phosphoinositide 3-kinase, which might explain the molecular mechanisms underlying hydrogen peroxide–induced increases of vascular tone.\(^{39}\) It might be argued that aortic rings are not a suitable model to investigate the effects of hydrogen peroxide on resistance vessels. However, our data are consistent with the observed change of sBP, with the effects of aminotriazole in vivo and in vitro, and with a previous report showing that increases of blood pressure in response to vasoconstrictor agents such as norepinephrine and angiotensin II were less pronounced in mice with an unspecific overexpression of catalase.\(^{30}\) Thus, the involvement of endogenous hydrogen peroxide in adrenergic constriction of resistance vessels in vivo most likely contributes to hypotension in cat\(^++\).

We found that aortic rings of cat\(^++\) had a significantly lower maximal vasoconstrictor response to KCl. KCl induces a depolarization of the smooth muscle cell membrane and a subsequent influx of calcium through L-type calcium channels.\(^{40,41}\) It has been shown that the increase of the intracel-

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**Figure 4.** Quantitative evaluation of aortic eNOS expression in cat\(^n\) and cat\(^++\) and in eNOS\(^n\) and eNOS\(^++\). A and C, Representative blots for eNOS in cat\(^n\) and cat\(^++\) (A) and in eNOS\(^n\) and eNOS\(^++\) (C). The plots in B and D represent the mean values of eNOS protein expression in the aorta of cat\(^n\) and cat\(^++\) (B) and eNOS\(^n\) and eNOS\(^++\) (D) after densitometric analysis (n=5, each).
lular calcium concentration induced by KCl is not accompanied by a calcium release from the sarcoplasmic reticulum. This selective dependency of KCl-induced vasoconstrictions on the influx of extracellular calcium is widely used to initially evaluate the response of isolated vascular preparations to increased cytosolic calcium concentrations in smooth muscle cells. Hence, our data suggest that the vascular calcium contraction coupling might be less efficient in cat.

In mouse-tail arterioles, elevation in transmural pressure causes generation of reactive oxygen species, of which particularly hydrogen peroxide initiates myogenic constriction. It is interesting to speculate on a causal role of hydrogen peroxide in the development of essential hypertension. Experimental as well as clinical hypertension is associated with oxidative stress. Furthermore, previous studies indicate that in hypertension, the plasma concentration of hydrogen peroxide is increased and is positively correlated to plasma renin activity and sBP but negatively correlated to cardiac contractility and renal function. Still, it is not known whether vascular oxidative stress is a result of the disease or may be one underlying cause. Treatment options that have been shown to reduce vascular oxidative stress also reduce blood pressure. The direct demonstration of hypotension in mice carrying a vascular specific overexpression of catalase strongly argues for both direct vasoconstrictor effects of endogenous hydrogen peroxide on resistance vessels and a possible contribution of these effects to the development of hypertension.

One might consider limitations of our study on the basis of the fact that neither aminotriazole nor DCDHF-DA and dihydroethidine are entirely specific compounds. Unfortunately, there are no alternative chemical tools showing better specificity. Although DCDHF-DA detects primarily hydrogen peroxide, it is also oxidized by superoxide, although to a much lesser extent. Likewise, dihydroethidine detects primarily superoxide but is also known to be oxidized by hydrogen peroxide. The DCDHF-DA signal was much lower in the

Figure 7. Concentration-dependent constrictor response to increasing concentrations of phenylephrine in aortic rings of cat (A) and cat (B) after preincubation with vehicle (control) or aminotriazole (1 mmol/L, *P<0.05, n=4, ANOVA).
aortic endothelium and the left ventricular myocardium in cat’ compared with cat”, and this was associated with (1) a 6-fold increase of catalase mRNA expression, (2) a 2-fold increase of catalase protein expression, (3) a 3-fold increase of catalase activity in these tissues21, and (4) a 2-fold increase of the DCDHF-DA signal after treatment with aminotriazole, leaving little room to explain the oxidation of the fluorescent dyes by superoxide. In vitro data suggest that aminotriazole might inhibit not only catalase but also glutathione peroxidase, but the latter does not seem to occur in cardiovascular tissue of mammals in vivo.45 These data are consistent with our observation the aminotriazole strongly increased the blood pressure of cat’ but had only a small effect in cat”. However, we cannot completely rule out the possibility that aminotriazole slightly inhibited glutathione peroxidase in cardiovascular tissue of cat’ and cat”.

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CLINICAL PERSPECTIVE

The term vascular oxidative stress denotes an increase of a great variety of reactive oxygen species generated in the vascular wall. Although vascular oxidative stress is considered an important pathogenic factor in cardiovascular diseases, there is some evidence for physiological or even beneficial effects. A large number of reports during the past 20 years have suggested that the oxidant hydrogen peroxide may act as a regulator of vasomotor tone, but these in vitro studies have yielded conflicting results. The aim of our in vivo study was to evaluate possible physiological effects of the “hair bleach” hydrogen peroxide in the vasculature. By generation of a transgenic mouse with a vascular-specific overexpression of catalase, we established an animal model with a strongly reduced steady-state concentration of vascular hydrogen peroxide. In these animals, we found a profound hypotension that was completely reversed by treatment with the catalase inhibitor amiloratizole. These observations suggest a tonic effect of hydrogen peroxide on vascular smooth muscle and might be interpreted as a dual vascular role of hydrogen peroxide, again confirming Paracelsus’ law: “The dose determines a poison.” We cannot tell whether this newly discovered physiological effect of hydrogen peroxide occurs in humans, but this appears to be not entirely unlikely. We hope that our data might encourage clinical researchers to evaluate whether this tonic effect of hydrogen peroxide might contribute to the development of essential hypertension as previously suggested by the results of small clinical trials.
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