Inhibition of guanylate cyclase stimulation by NO and bovine arterial relaxation to peroxynitrite and H2O2

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Iesaki, Takafumi, Sachin A. Gupte, Pawel M. Kaminski, and Michael S. Wolin. Inhibition of guanylate cyclase stimulation by NO and bovine arterial relaxation to peroxynitrite and H2O2. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H978–H985, 1999.—The inhibitor of soluble guanylate cyclase (sGC) stimulation by nitric oxide (NO), 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), was examined for its effects on the prolonged relaxation of endothelium-removed bovine coronary (BCA) and pulmonary (BPA) arteries to peroxynitrite (ONOO−) and on H2O2-elicited relaxation and sGC stimulation. Our previous studies suggest that ONOO− causes a prolonged relaxation of BPA by regenerating NO and that a 2-min exposure of BCA or BPA to 50 nM NO causes an ONOO−-elicited relaxation. The relaxation of K+ -precontracted BCA to 50 nM NO or 100 µM ONOO− was essentially eliminated by 10 µM ODQ. ODQ also eliminated relaxation to 0.1 nM-10 µM of NO donor S-nitroso-N-acetyl-penicillamine (SNAP), but it did not alter relaxation to 1–300 µM H2O2. Similar responses were also observed in BPA. ODQ did not increase lucigenin-detectable superoxide production in BCA, and it did not alter luminol-detectable endogenous ONOO− formation observed during a 2-min exposure of BCA to 50 nM NO. In addition, ODQ did not affect tissue release of NO after 2 min exposure of BCA to 50 nM NO. The activity of sGC in BPA homogenate that is stimulated by endogenous H2O2, was not altered by ODQ, whereas sGC activity in the presence of 10 µM SNAP (fungal catalase) was reduced by ODQ. Thus relaxation of K+ -precontracted BCA and BPA to ONOO− appears to be completely mediated by NO stimulation of sGC, whereas the actions of ODQ suggest that NO is not involved in H2O2-elicited relaxation and sGC stimulation. This study did not detect evidence for the participation of additional mechanisms potentially activated by ONOO− in the responses studied.

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The probe 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) is a relatively new inhibitor of the stimulation of sGC, which appears to function by converting the NO-Fe2+ heme of sGC to its Fe3+ oxidation state (24). When the heme of sGC is in its Fe3+ form, it does not seem to support activation of cGMP production as a result of it not readily binding NO (8). In contrast to this apparently selective action of ODQ, other agents that antagonize H2O2-elicited vascular relaxation and sGC stimulation appear to function through additional mechanisms, including the formation of superoxide anion (4, 31), which inhibits catalase (14), and by the Fe2+ of this heme group (8, 33). The purified form of sGC has also been demonstrated to be activated by additional physiological mediators, including H2O2 (5) and carbon monoxide (CO; see Ref. 8). However, only limited information is available on the role of these other stimuli of sGC in the vascular relaxant responses to vasodilators, which potentially function through mechanisms other than NO. Multiple additional redox-related agents have been suggested to alter the activity of sGC in tissues (29). Peroxynitrite (ONOO−) is another agent that has been shown to both stimulate the activity of sGC and promote vascular relaxation through a thiol-dependent mechanism, which may involve the metabolic generation of NO (1, 15, 17, 28, 34).

Our previous studies have suggested that H2O2 produces vascular relaxation and sGC stimulation as a result of its metabolism by catalase, and we have provided evidence that the compound I species of catalase mediates the stimulation of sGC (2, 3, 5). It has been shown that vascular relaxation responses to H2O2 can be associated with increases in tissue levels of cGMP (2, 3). The relaxation of bovine pulmonary arteries (BPA) to H2O2 is attenuated by agents that antagonize the stimulation of sGC, including methylene blue (2), LY-83583 (4, 20), superoxide anion (4), and hemoglobin (32), and it is also inhibited by agents that modulate the metabolism of peroxide by catalase (3, 19–21). One aspect of the cGMP-mediated vascular relaxation to H2O2 that has not been given adequate consideration is the possible role of NO in this response, perhaps originating from a co-oxidation reaction catalyzed by compound I of catalase. Although our previous studies (2, 34) have ruled out a role for hydroxyl radical in the vascular relaxant responses to H2O2 and ONOO−, both of these relaxing agents are reactive oxidant species that could potentially stimulate sGC or activate additional vasodilator mechanisms by an oxidative mechanism that does not involve NO. For example, it has been observed in cat cerebral arterioles that H2O2 and ONOO− elicit a vasodilator response through the activation of ATP-dependent K+ channels, by a mechanism not involving the stimulation of sGC (30).

Hydrogen peroxide; nitric oxide; redox signalling
alteration of the metabolism of peroxide by catalase (3, 19, 21). In the present study, we examined the actions of the ODQ probe on components of the hypothesized mechanisms of relaxation of endothelium-removed bovine coronary arteries (BCA) and BPA elicited by H$_2$O$_2$ and ONOO$^-$ to better establish the processes involved.

**MATERIALS AND METHODS**

Materials. The following reagents were used for the studies. ODQ, ONOO$^-$, the thromboxane A$_2$-receptor-agonist U-46619, and cGMP enzyme immunossay kits were from Cayman Chemicals (Ann Arbor, MI). NO gas (200 parts per million (ppm) NO, balance N$_2$) was from Matheson Gas Products (East Rutherford, NJ). Bis-N-methylacylindiamine nitrate (lucigenin), 5-amino-2,3-dihydro-1,4-phtalalindinedione (luminol), 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron), diethyldithiocarbamic acid (DETCA), HEPES, GTP, 3-isobutyl-1-methylxanthine (SNAP), phosphocreatine, creatine phosphokinase, catalase from Aspergillus niger (6,660 U/mg protein), collagenase (type IV), soybean trypsin inhibitor (type 1-S), elastase (type VI), EDTA, MOPS, lactic acid, and methylene blue were from Sigma Chemicals (St. Louis, MO). S-nitroso-N-acetyl-penicillamine (SNAP) was synthesized by methods previously published (13). Lactate solution (1 M) was prepared by dissolving lactic acid in distilled water followed by adjustment of pH with NaOH (1 M) to 7.4. Other chemicals were analyzed reagent grade and were obtained from Baker Chemical (Phillipsburg, NJ).

Measurement of changes in force in BCA and BPA. Bovine hearts and lungs were obtained from a slaughterhouse and were maintained in ice-cold oxygenated PBS solution while being transported to our laboratory. Isolated endothelium-removed coronary and pulmonary arterial rings were prepared by adaptation of previously described methods (2, 20). Briefly, the left anterior descending coronary artery and the second branches of the main lobar pulmonary artery were isolated and cleaned from surrounding tissue. The endothelium was mechanically removed by gentle rubbing. Arterial rings (~4 mm in diameter and length) were mounted on wire hooks attached to force displacement transducers (model FT-03; Grass Instruments, Quincy, MA) for measurement of changes in isometric force on a polygraph (model 7; Grass Instruments). The rings were incubated in individually thermostated (37°C) 10-ml baths (Metro Scientific Farmingdale, NY) for 2 h at an optimal passive tension of 5 g in Krebs bicarbonate buffer (pH 7.4) containing the following (in mM): 118 NaCl, 4.7 KCl, 1.5 CaCl$_2$, 25 NaHCO$_3$, 1.2 KH$_2$PO$_4$, and 5.6 glucose, gassed with 21% O$_2$-5% CO$_2$ - Balance N$_2$). At the end of the 2-h incubation, the vessels were contracted with Krebs bicarbonate buffer containing 100 mM l-arginine. In a continuously gassed (21% O$_2$-5% CO$_2$, balance N$_2$) 1-cm$^2$ spectrophotometer cuvette mounted in a thermostated (37°C) cell holder on the surface of a Lucte light guide (with a shutter cover) directed into a cooled photomultiplier tube (model 9235B; Thorn EMI). An amplifier-discriminator (model C604; Thorn EMI) and photon counter (model C660; Thorn EMI) were employed to quantitate chemiluminescence. The counts were integrated for 5-s periods by the photon counter, and an analog signal of the integrated counts was continuously recorded on a polygraph recorder (Grass model 7) together with changes in force. The chemiluminescent data were expressed as counts per 5 s per gram tissue. Although multiple reactive radicals can promote luminol chemiluminescence, our previous work (6, 7) provides evidence that the increase that is observed during exposure to NO gas originates from the vascular preparation.

In experiments in which NO gas was used, a 200 ppm NO gas mixture was delivered in a manner that produced an ~50 nM steady-state buffer concentration of NO (6, 7), based on measurements made with an NO electrode (World Precision Instruments, Sarasota, FL). The method of exposure to NO employed was also designed not to decrease the P$_O_2$ in the tissue bath, as confirmed by monitoring its P$_O_2$ content with an O$_2$ electrode (Yellow Springs Instruments, Yellow Springs, OH). Relaxation was expressed by percent change of steady-state level of contraction.

Chemiluminescence measurement of superoxide production. Endothelium-removed BCA rings were prepared as indicated above for organ bath studies. The arterial rings were placed in plastic scintillation miniwells containing 250 µM lucigenin and other additions in a final volume of 1 ml of air-equilibrated Krebs solution buffered with 10 mM HEPES-NaOH (pH 7.4). As previously described (20), the chemiluminescence elicited by superoxide anion in the presence of lucigenin was measured in a liquid scintillation counter (Mark V; TaManufact, Elko Grove Village, IL) with a single active photomultiplier tube positioned in out-of-coincidence mode. All manipulations were performed in the darkroom with minimum lighting. The temperature was initially 37°C but subsequently was equilibrated with the ambient temperature. After 5 min of dark adaptation, vials containing all components, with the exception of arterial rings (blanks), were counted one time for 0.1 min over the next 10 min. This procedure was repeated two more times after placement of ~50 mg of endothelium-removed arterial rings in each vial. Blanks were then subtracted from the average of the relatively constant levels of chemiluminescence produced under each condition by the arteries to obtain the data reported as counts per minute per gram tissue.

Chemiluminescence measurement of endogenous ONOO$^-$ formation. Experiments in which arteries were simultaneously studied for changes in force and chemiluminescence measurement of ONOO$^-$ on exposure to 200 ppm NO gas for 2 min were conducted employing the force measurement methods described above in a single photon-counting apparatus constructed in a light-tight box similar to that previously described (6, 7). In these experiments, arteries were incubated in Krebs bicarbonate buffer containing 100 mM luminol in a continuously gassed (21% O$_2$-5% CO$_2$, balance N$_2$) 1-cm$^2$ spectrophotometer cuvette mounted in a thermostated (37°C) cell holder on the surface of a Lucte light guide (with a shutter cover) directed into a cooled photomultiplier tube (model 9235B; Thorn EMI). An amplifier-discriminator (model C604; Thorn EMI) and photon counter (model C660; Thorn EMI) were employed to quantitate chemiluminescence. The counts were integrated for 5-s periods by the photon counter, and an analog signal of the integrated counts was continuously recorded on a polygraph recorder (Grass model 7) together with changes in force. The chemiluminescent data were expressed as counts per 5 s per gram tissue. Although multiple reactive radicals can promote luminol chemiluminescence, our previous work (6, 7) provides evidence that the increase that is observed during exposure to NO gas originates from ONOO$^-\textit{formation}$ in the vascular preparation.

Head space NO measurements were determined employing an NO chemiluminescence analyzer (model 20B; Sievers Instruments, Boulder, CO), using a slightly modified version of the methods used in our previous studies on ONOO$^-\textit{formation}$ (6, 7). To quantitate the amount of NO in the head space gas, the following protocols were followed. Fernbach flasks (6 ml) containing Krebs bicarbonate buffer (2 ml final volume) in the presence or absence of ~300 mg of endothelium-removed BCA were exposed to 50 nM NO for 2 min under conditions similar to the organ bath studies.
Immediately after this treatment, the Fernbach flasks were sealed and then deoxygenated with 95% N₂-5% CO₂ for 5 min to remove the NO derived directly from the 2-min exposure period. Next, a 5-min incubation that allows NO to accumulate in the head space, a single 0.5-ml aliquot was taken of the head space gas from each sealed Fernbach flask to quantify the amount of NO produced from each experimental condition by use of a Sievers NO analyzer. The amount of NO formed was quantified, after subtraction of an injection artifact blank, using NO standards and recovery of authentic NO from the Fernbach flask containing 2 ml of buffer. NO release was expressed as picomoles per gram tissue. Our previous work has provided evidence that the NO that is detected under these conditions appears to result from ONOO⁻ formation and a thiol-dependent mechanism of trapping and regenerating NO (6, 7).

Determination of sGC activity in bovine arterial homogenate. Bovine arterial homogenate was prepared by a previously described method (23). Briefly, after isolation of major lobar pulmonary arteries and removal of endothelium, the medial layer of the artery was finely minced with a commercial meat grinder and then was digested with a collagenase (91 mg/ml) solution containing soybean trypsin inhibitor (0.25 mg/ml) and elastase (0.125 mg/ml) in 20 mM MOPS-KOH buffer (pH 7.4) containing 250 mM sucrose (1 g tissue/2 ml buffer) at 37°C for 15 min. After the addition of glutathione (GSH) to a final concentration of 2 mM, the tissue was subsequently homogenized at 0–5°C in an Eberbach homogenizer at maximum speed with five 20-s treatments. The material retained on a stainless steel sieve was rehomogenized in 50% of the original volume of MOPS-sucrose buffer, the pooled vessel homogenate was filtered through four layers of cheesecloth, and the homogenate was reconcentrated to approximate tissue enzyme levels in the assay of sGC activity. The homogenate (15 ml) was reconcentrated eightfold by centrifugal filter having a pore size of 5,000 Da by centrifugation at 3,000 rpm over a period of 10–12 h at 4°C. It was subsequently diluted twofold into the sGC assay. Guanylate cyclase activity in the arterial homogenate was determined by measuring the formation of cGMP by an enzyme immunonassay using an adaptation of previously described sGC assay methods (2). Briefly, the reaction mixture (0.2 ml final volume) contained 20 mM MOPS-KOH (pH 7.4), 0.1 mM GTP, 2 mM MgCl₂, 0.3 mM of the phosphodiesterase inhibitor IBMX, a GTP-regenerating system consisting of 10 mM phosphocreatine and 150 U/ml creatine phosphokinase, 0.1 ml of concentrated homogenate, and test agents, as indicated. Assays of sGC were initiated by the addition of arterial protein. Incubations were conducted for 10 min at 37°C, they were terminated by addition of 0.1 ml of preheated 12 mM EDTA, and this was followed by boiling the assay mixtures for 10–15 min. Each tube was centrifuged at 15,000 rpm, and the supernatant, which was subsequently diluted fivefold, was used to estimate cGMP by enzyme immunonassay.

Statistical analysis. Results are expressed as means ± SE, with n equal to the number of animals employed or determinations made in separate preparations of pooled homogenates derived from several animals. Comparisons between groups were made with an ANOVA and a Student’s t-test with a Bonferroni correction for multiple comparisons. P < 0.05 was used to determine statistical significance.

RESULTS

Effects of ODQ on relaxation of BCA and BPA to the NO donor SNAP. As shown in Fig. 1, 10 µM ODQ markedly inhibited SNAP-induced relaxation of 30 mM K⁺-precontracted BCA (A) and BPA (B) over the entire range of concentrations examined. A small relaxation (14.3 ± 5.4%) was observed in the presence of ODQ in BCA, but not in BPA, only at the highest concentration of SNAP (10 µM) examined.

Effects of ODQ on lucigenin-detectable superoxide production in BCA. The data in Fig. 2 summarize the effects of ODQ on lucigenin-detectable superoxide anion in endothelium-removed BCA. The lucigenin-derived chemiluminescence under control conditions, including 0.1% DMSO (a vehicle for ODQ), was not altered by the presence of 10 µM ODQ. When vessels were pretreated with an inhibitor of Cu,Zn-superoxide dismutase [SOD; 10 mM DETCA incubation for 30 min followed by washout (4)], there was a significant increase in chemiluminescence to 234% of the control level. ODQ did not increase the chemiluminescence, even under the conditions in which Cu,Zn-SOD was
inhibited by the DETCA pretreatment. Tiron (10 mM), a scavenger of intracellular superoxide, markedly decreased \((P, 0.05)\) the chemiluminescence in vessels pretreated with DETCA by 89%, confirming the detection of superoxide anion by lucigenin.

Effects of ODQ on NO- or ONOO\(^{-}\)-induced relaxation of BCA and BPA. Data in Fig. 3 show the relaxation responses of 30 mM K\(^{+}\)-precontracted BCA or BPA to either 50 nM NO or 100 µM ONOO\(^{-}\) in the absence or presence of 10 µM ODQ. Both NO and ONOO\(^{-}\) caused almost full relaxation of BCA (A), and these responses were essentially eliminated by 10 µM ODQ (96% inhibition of relaxation for NO and 99% inhibition for ONOO\(^{-}\)). Similar responses were observed in BPA (B). Relaxations to NO and ONOO\(^{-}\) were also markedly attenuated by 10 µM ODQ (87% inhibition of relaxation for NO and 99% inhibition for ONOO\(^{-}\)).

Effects of ODQ on luminol-detectable ONOO\(^{-}\) formation in BCA. Luminol-detectable tissue chemiluminescence under basal conditions (gassed with 21% O\(_2\)-5% CO\(_2\), balance N\(_2\)) was 135.6 ± 11.0 counts·5 s\(^{-1}\)·g\(^{-1}\), and exposure to 30 mM K\(^{+}\) did not alter the tissue-derived chemiluminescence signal (140.8 ± 21.0 counts·5 s\(^{-1}\)·g\(^{-1}\)), indicating that changes in chemiluminescence are not dependent on the contractile state of the tissue, as reported previously (6, 7). Exposure of BCA to 50 nM NO for 2 min increased the chemiluminescence, consistent with detection of an increase in endogenous levels of ONOO\(^{-}\). Figure 4 shows the increase in chemiluminescence after subtraction of level of chemiluminescence observed during contraction with 30 mM K\(^{+}\) for each condition. ODQ did not alter the increase in levels of luminol chemiluminesence caused by NO. In these experiments, all changes in force were similar to those observed in the organ bath studies described in Fig. 3A (data not shown).
5-min deoxygenation period removed all detectable NO from the NO-pretreated Krebs buffer, as reported previously (6, 7). ODQ did not alter the amount of NO trapped and subsequently released after exposure of BCA to NO and deoxygenation.

Effects of ODQ on NO and H₂O₂ stimulation of guanylate cyclase activity in BPA homogenate. The data in Fig. 6 summarize sGC activity in BPA homogenates. The presence of a fungal catalase significantly reduced the basal activity of sGC (control) by 53%, suggesting that the basal activity of sGC was stimulated by endogenously produced H₂O₂ under this experimental condition. ODQ did not significantly inhibit this H₂O₂-stimulated basal activity of sGC. In the presence of fungal catalase, sGC activity was markedly increased by 10 µM SNAP (to 583% of the activity in the presence of fungal catalase), and this SNAP-stimulated sGC activity was markedly attenuated by 10 µM ODQ.

Effects of ODQ or methylene blue on H₂O₂- or lactate-induced relaxation. In experiments in which H₂O₂-induced relaxation was evaluated, 0.1 µM U-46619 was used as a contractile agent because contraction with KCl appears to reduce the relaxation to H₂O₂ (2). As shown in Fig. 7, 10 µM ODQ did not alter H₂O₂-induced relaxation of endothelium-removed BCA (A) or BPA (B) over the entire range of concentrations tested. In addition, lactate-induced relaxation of endothelium-removed BCA precontracted with 25 mM K⁺ was not altered by 10 µM ODQ, whereas relaxation to lactate was inhibited by 10 µM methylene blue (see Fig. 8). The presence of 10 µM methylene blue significantly decreased the relaxation to 5 and 10 mM lactate by 45 and 34%, respectively.

DISCUSSION

The virtually complete elimination of the observed prolonged relaxation to exogenous and endogenously

![Fig. 5. Effects of 10 µM ODQ on the release of NO after the formation of ONOO⁻ during a 2-min exposure of endothelium-removed bovine coronary arteries to 50 nM NO. Release of NO after a 5-min deoxygenation period and a 5-min period of NO accumulation is expressed as pmol/g tissue. Control, tissue control (0.1% DMSO); ODQ, in the presence of 10 µM ODQ (n = 9).](image)

![Fig. 6. Effects of 10 µM ODQ on soluble guanylate cyclase (sGC) activity in a reconcentrated pulmonary arterial homogenate that is activated by endogenously formed H₂O₂ (Control) or the NO donor (10 µM SNAP). Note that the presence of 1 µM fungal catalase (CAT) was used for removal of the stimulation of sGC by endogenously formed H₂O₂ during the study of activation by SNAP. The activity of sGC is expressed as cGMP production (pmol·min⁻¹·g tissue⁻¹). Control, basal activity; +CAT, in the presence 1 µM fungal catalase; +ODQ, in the presence of 10 µM ODQ; +CAT +SNAP, in the presence of 1 µM fungal catalase and 10 µM SNAP; +CAT +SNAP +ODQ, in the presence of 1 µM fungal catalase, 10 µM SNAP, and 10 µM ODQ (n = 10–12).](image)

![Fig. 7. Effect of 10 µM ODQ on the relaxation of endothelium-removed bovine coronary (A, n = 10) and pulmonary (B, n = 10) arteries precontracted with 0.1 µM U-46619 to increasing cumulative concentrations of H₂O₂.](image)
formed ONOO\textsuperscript{-} in BCA and BPA by ODQ indicates that the previously hypothesized mechanism involving NO generation and sGC stimulation is the primary process that mediates this response. This is a rather unexpected observation because ONOO\textsuperscript{-} is thought (27) to undergo multiple other chemical reactions with biological constituents that should alter the function of signaling processes in addition to our previously hypothesized mechanism involving the thiol-dependent regeneration of NO. In contrast, the actions of ODQ on H\textsubscript{2}O\textsubscript{2}-elicited relaxation of BCA and BPA and the stimulation of sGC activity by endogenously formed H\textsubscript{2}O\textsubscript{2} are consistent with an absence of a role for NO in these responses. This observation essentially eliminates the possible role of NO (and CO) formation from reactions catalyzed by the metabolism of peroxide by catalase (or other enzymes) in the relaxation to H\textsubscript{2}O\textsubscript{2} that is thought to be mediated through the stimulation of sGC. Moreover, the marked difference in the actions of ODQ on responses to exogenous and endogenously formed H\textsubscript{2}O\textsubscript{2} compared with ONOO\textsuperscript{-} further eliminates the possible role of a common mechanism in the relaxation responses examined in this study of intermediates with hydroxyl radical-like reactivity, which can be generated from these agents.

Probes that have been used to inhibit the stimulation of sGC generally have been shown to possess distinct limitations for usage in the study of physiological systems due to the potential for multiple interactions. The ODQ probe has been shown rather convincingly to react with the NO-bound Fe\textsuperscript{2+} form of sGC by catalase (14). The data in the present study confirm that ODQ inhibits both NO-elicited relaxation and sGC stimulation, without causing alterations in lucigenin chemiluminescence-detectable superoxide anion levels or changes in the detection of NO by head space gas, the detection of ONOO\textsuperscript{-} by luminol chemiluminescence, or an apparent modification of the reaction between NO and superoxide anion. In contrast, ODQ did not alter the cGMP-associated relaxation to H\textsubscript{2}O\textsubscript{2} or sGC stimulation by endogenously formed H\textsubscript{2}O\textsubscript{2}. Although the absence of a detectable effect of ODQ on the responses to H\textsubscript{2}O\textsubscript{2} examined in the present study were somewhat unforeseen, these observations are consistent with the known actions of ODQ and hypothesized mechanism through which H\textsubscript{2}O\textsubscript{2} elicits cGMP-associated vascular relaxation and sGC stimulation. Thus ODQ appears to be a rather selective probe for the detection of processes involving NO-elicited stimulation of sGC under the conditions of the present study.

Our previous studies have provided evidence for a hypothesized multistep process (shown in Fig. 9) in the response of BCA and BPA to exogenous ONOO\textsuperscript{-} and endogenously formed ONOO\textsuperscript{-} involving a thiol-depen-

![Fig. 8: Effect of 10 µM ODQ or 10 µM methylene blue (MB) on the relaxation of endothelium-removed bovine coronary arteries precontracted with 25 mM KCl to increasing cumulative concentrations of lactate (n = 21 for control, n = 12 for ODQ, and n = 9 for MB).](http://ajpheart.physiology.org/)

![Fig. 9: Model showing the apparently selective site of inhibitory action of 10 µM ODQ on hypothesized mechanisms involving the stimulation of sGC through which BCA and BPA relax to exogenous or endogenously formed NO.](http://ajpheart.physiology.org/)
Plant regeneration of NO and activation of a relaxing mechanism mediated through the stimulation of sGC (6, 7, 34). Although the actual thiol-dependent mechanism involved in the regeneration of NO from ONOO\(^{-}\) in the intracellular environment of the blood vessel is not yet established, recent studies suggest that the activation of sGC from the reaction of NO with superoxide in the presence of GSH appears to occur through a mechanism that seems to involve the formation of S-nitroso-GSH and a copper-catalyzed release of NO from this thiolnitrite (16). In contrast, ONOO\(^{-}\) appears to nitrate the thiol of GSH to form an S-nitro-GSH species that spontaneously releases NO (1). The ODQ probe does not appear to interact with the multistep process involved in the ONOO\(^{-}\)-mediated thiol-dependent trapping and regeneration of NO. As discussed above, ODQ did not appear to alter the detected formation of ONOO\(^{-}\) from the interaction of endogenously formed superoxide with exogenous NO. It also did not seem to alter the amount of NO trapped and released as a consequence of endogenous ONOO\(^{-}\) formation. The near complete relaxant responses observed during the treatment of BCA or BPA to ~50 nM NO were essentially eliminated by ODQ, which is consistent with the potent inhibitory effect of ODQ on relaxation mediated through the stimulation of sGC. ODQ also antagonized the prolonged relaxation of BCA and BPA caused by exogenous ONOO\(^{-}\) or the interaction of exogenous NO with endogenously formed superoxide. The data in Fig. 5 demonstrate that ODQ did not alter the processes involved in the trapping and regeneration of NO as a result of an interaction of endogenous ONOO\(^{-}\) with GSH. Thus multiple steps in the hypothesized process (shown in Fig. 9) that contribute to the response of BCA and BPA to exogenous and endogenously formed ONOO\(^{-}\) involving a thiol-dependent regeneration of NO (6, 7, 34) appear not to be altered by the ODQ probe. Based on the evidence for a role of NO in the response to ONOO\(^{-}\), ODQ appears to attenuate the ONOO\(^{-}\)-mediated relaxation of BCA and BPA by selectively inhibiting the stimulation of sGC by NO.

The inhibitory effects of agents that antagonize NO-elicted vascular relaxation and sGC activation, including methylene blue, LY-83583, superoxide anion, and hemoglobin, on similar responses to H\(_2\)O\(_2\) (2, 4, 20, 32) suggest consideration of a role for NO in the actions of peroxide. Because catalase has the ability to metabolize substances with markedly different chemical structures, such as azide (18) and cyanamide (23), to intermediates that produce NO, the possibility of a role for a co-oxidation reaction that generates NO needs to be evaluated as a potential explanation for the previously hypothesized role of peroxide metabolism by catalase in H\(_2\)O\(_2\)-elicted arterial relaxation and sGC activation. For example, although the most obvious interpretation for the observation that H\(_2\)O\(_2\) promotes an endothelium-dependent NO-mediated relaxation of rabbit aorta (10) is that it is stimulating NO synthase activity in the endothelium, it is also possible that this response could result from a peroxide-dependent reaction such as the regeneration of NO from its decomposition product nitrite (26). The lack of an effect of the ODQ probe on H\(_2\)O\(_2\)-elicted sGC activation and BCA and BPA relaxation is consistent with the absence of a role for NO in these responses. Observations that the relaxation of BPA to lactate is inhibited by methylene blue (23) and that it appears to be mediated through the generation of H\(_2\)O\(_2\) (19, 22, 23) suggested consideration of a role for NO in this response. The absence of an effect of ODQ on the relaxation to lactate indicates that NO is not a participant in the processes through which lactate causes relaxation. Because, as shown in the model in Fig. 9, lactate is thought to mediate relaxation as a result of its metabolism through the lactate dehydrogenase reaction increasing cytosolic NADH and superoxide anion-derived H\(_2\)O\(_2\) production originating from NADH oxidase, it seems that the ODQ probe does not significantly alter the function of any of the processes involved in both the formation of H\(_2\)O\(_2\) and its mechanism of activating sGC. Thus NO does not appear to participate in the cGMP-associated mechanism of relaxation to H\(_2\)O\(_2\), and the ODQ probe does not seem to inhibit sGC stimulation by H\(_2\)O\(_2\) or the multiple processes associated with the endogenous formation of H\(_2\)O\(_2\) and relaxation mediated by cGMP.

In summary, the results of the present study are consistent with the metabolic conversion of ONOO\(^{-}\) to NO and stimulation of sGC as the primary processes participating in the prolonged relaxation of BCA and BPA to ONOO\(^{-}\). Based on the evidence that the ODQ probe seems to function by selectively antagonizing the stimulation of sGC by NO through a mechanism that does not involve the generation of superoxide anion or an interaction with multiple additional processes shown in Fig. 9 that are of potential importance in vascular redox signalling, it is now possible to conclude that the relaxation responses of endothelium-removed BCA and BPA to H\(_2\)O\(_2\) are not mediated by NO. In addition, the actions of ODQ on responses to ONOO\(^{-}\) and H\(_2\)O\(_2\) do not support a role for a common mechanism involving intermediates that can form from these agents with hydroxyl radical-like reactivity. These observations in BCA and BPA are markedly different from the effects of H\(_2\)O\(_2\) and ONOO\(^{-}\) on cat cerebral arterioles, where a vasodilator response has been demonstrated to be mediated through the activation of ATP-dependent K\(^{+}\) channels, by a mechanism not involving the stimulation of sGC (30). Thus H\(_2\)O\(_2\) and ONOO\(^{-}\) appear to activate several different signaling mechanisms in vascular tissue, which may be of importance under conditions such as ischemia-reperfusion, inflammation, and vascular diseases that are associated with oxidant stress and alterations in the metabolism of NO.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-31089 and HL-43023. T. Iseki was supported by a Research Fellowship from the Uehara Memorial Foundation.

Part of the information herein was presented at the Experimental Biology '98 Meeting in San Francisco, CA (FASEB J. 12: A80, 1998).

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Received 9 December 1998; accepted in final form 31 March 1999.
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