The myeloperoxidase-derived oxidant hypochlorous acid (HOCl) is thought to contribute to endothelial dysfunction, but the mechanisms underlying this inhibitory effect are unknown. The present study tested the hypothesis that HOCl and L-arginine (L-Arg) react to form novel compounds that adversely affect endothelial function by inhibiting nitric oxide (NO) formation. Using spectrophotometric techniques, we found that HOCl and L-Arg react rapidly ($k = 7.1 \times 10^6 \text{M}^{-1} \text{s}^{-1}$) to form two major products that were identified by mass spectrometry as monochlorinated and dichlorinated adducts of L-Arg. Pretreatment of bovine aortic endothelial cells with the chlorinated L-Arg metabolites (Cl-L-Arg) inhibited the A23187-induced formation of the NO metabolite nitrate ($\text{NO}_3^-$) and nitrite ($\text{NO}_2^-$) in a concentration-dependent manner. Preincubation of rat aortic ring segments with Cl-L-Arg resulted in concentration-dependent inhibition of acetylcholine-induced relaxation. In contrast, blood vessels relaxed normally to the endothelium-independent vasodilator sodium nitroprusside.

In vivo administration of Cl-L-Arg to anesthetized rats increased carotid artery vascular resistance. A greater than 10-fold excess of L-Arg was required to reverse the inhibitory effects of Cl-L-Arg in vivo and in vitro. Reaction of HOCl with L-arginine (d-Arg) did not result in the formation of inhibitory products. These results suggest that HOCl reacts with L-Arg to form chlorinated products that act as nitric-oxide synthase inhibitors.

Reactive inflammatory mediators formed in a variety of acute and chronic diseases compromise vascular function. Adhesion and infiltration of leukocytes in the vessel wall are critical components of tissue injury induced by these agents, resulting in increased vascular permeability, elevated activities of oxidative enzymes, and endothelial dysfunction (1–6).

Under inflammatory conditions, neutrophil activation initiates the assembly of cellular components of an NADPH oxidase that generates the oxidants superoxide ($\text{O}_2^-$) and hydrogen peroxide ($\text{H}_2\text{O}_2$). The heme protein myeloperoxidase (MPO) is an additional component of neutrophils that is released from intracellular granules into phagocytic vacuoles as well as the extracellular space (7). Myeloperoxidase catalyzes the oxidation of chloride by $\text{H}_2\text{O}_2$, resulting in the formation of the chlorinating and oxidizing species hypochlorous acid (HOCl) (8, 9). Hypochlorous acid reacts avidly with a variety of cellular substrates, including thiols, nucleotides, unsaturated fatty acids, and amines (10–12). It is estimated that nearly 50% of the $\text{H}_2\text{O}_2$ produced by an activated neutrophil is converted to HOCl by MPO and that, under inflammatory conditions, HOCl may reach concentrations to effectively damage host tissues (11).

Myeloperoxidase and HOCl are emerging as critical pathogenic mediators of vascular injury associated with sepsis, atherosclerosis, and ischemia-reperfusion injury (1–3, 13). Hypochlorous acid contributes to structural and functional alterations of the vasculature under pathologic conditions. In this respect, HOCl facilitates the degradation of extracellular matrix proteins by inhibiting TIMP-1 and enhancing the activities of matrix metalloproteinases (14, 15). It also reduces $\alpha$-antiprotease activity, leading to an increase in elastase concentration and tissue degradation at sites of inflammation (15–17). The MPO/HOCl system also promotes atherogenesis via modification of the apolipoprotein moeity of very low density lipoprotein, thus promoting cholesterol ester accumulation in macrophages (18).

Numerous studies show that endothelial dysfunction is associated with neutrophil adhesion and/or the elaboration of neutrophil-derived products in models of inflammation and ischemia-reperfusion injury (19–23). Increased tissue concentrations of MPO and HOCl production are associated with reduced NO bioactivity, but their cellular targets and mechanisms of action are unknown (24–26). Previous studies show that HOCl converts $\alpha$-amino acids to $N$-chloroamines (27, 28). Herein, data are presented showing that HOCl reacts with the amino acid L-Arg to form novel chlorinated metabolites. These compounds have a potent inhibitory effect on endothelium-dependent relaxation in vivo and in vitro and display similar pharmacological properties as traditional nitric-oxide synthase inhibitors.
obtained from Sigma. N\-acetyl-L-Arg, A23187, Ach, SNP, phenylephrine (PE), taurine, hypochlorous acid, and sodium phosphate were obtained from Calbiochem. Diaion\® CR11 beads were from Supelco (Bellefonte, PA). Water was deionized with a MilliQ system and had a conductance greater than 18 megohms. Hypochlorous acid concentration was determined by monitoring the absorbance of hypochlorite at 292 nm (ε = 350 M\(^{-1}\) cm\(^{-1}\)) in 0.1 N NaOH using a Beckman diode array spectrophotometer model DU 7000.

**Experimental Procedures**

**Materials**—L-Arg, L-Arg, N\-acetyl-L-Arg, A23187, Ach, SNP, phenylephrine (PE), taurine, hypochlorous acid, and sodium phosphate were obtained from Sigma. N\-Nitro-L-arginine methyl ester (L-NAME) and nitrile assay kits were from Calbiochem. Diaion\® CR11 beads were from Supelco (Bellefonte, PA). Water was deionized with a MilliQ system and had a conductance greater than 18 megohms. Hypochlorous acid concentration was determined by monitoring the absorbance of hypochlorite at 292 nm (ε = 350 M\(^{-1}\) cm\(^{-1}\)) in 0.1 N NaOH using a Beckman diode array spectrophotometer model DU 7000.

**Animals**—Ten-week-old male Harlan Sprague-Dawley rats were obtained from Harlan Breeding Laboratories (Indianapolis, IN). All rats were maintained at constant humidity (60 ± 5\%), temperature (24 ± 1\°C), and light cycle (6 a.m. to 6 p.m.) and were fed a standard rat pellet diet (Ralston Purina diet) ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and were consistent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85–23, revised 1985).

**Cell Culture**—Bovine aortic endothelial cells (BAEcs) were isolated from aortas obtained from a local abattoir. BAEcs were maintained in phenol red-free Medium 199 (M199) containing 5% fetal bovine serum, 5 mM sodium pyruvate, and 1 mM L-glutamine. Cells were maintained in a 37 °C incubator with 5% CO\(_2\). Me\(_2\)SO (1.3% v/v) was split and passed into the electrospray ionization interface of the mass spectrometer.

**Stopped-flow Spectrophotometry**—The apparent rate constant of the reactions of hypochlorite with L-Arg and taurine were determined at pH 7.4 in 100 mM sodium phosphate buffer. Reaction traces were obtained on an Applied Photophysics SKMV18 stopped-flow spectrophotometer (Leatherhead, United Kingdom). First order exponential fits were performed with the Applied Photophysics software using a Levenburg-Marquardt nonlinear fitting routine. Stoichiometry of the reaction of L-Arg (in 100 mM sodium phosphate, pH 7.4) with hypochlorite was determined on a HP 8453 UV-visible spectrophotometer. Consecutive additions of limiting L-Arg were added to express hypochlorite in a stirred 3-ml cuvette. Decay of hypochlorite was followed at 315 nm with an extinction coefficient determined to be 155 M\(^{-1}\) cm\(^{-1}\).

**Mass Spectrometry**—Electrospray ionization mass spectrometry (MS) was used to characterize the reaction products of HOCl and L-Arg. Samples of L-Arg or L-Arg mixed with an equimolar concentration of HOCl were flow-injected into the electrospray interface of a PE Sciex (Concorde, Ontario, Canada) API III triple quadrupole mass spectrometer operating in the positive ion mode. Parent ion/daughter ion pairs were analyzed by MS-MS to identify fragments of the principal products formed. We will refer to HOCl + L-Arg reaction sample as Cl-L-Arg, recognizing that it is a mixture of mono- and dichlorinated species.

**Detection of Chlorinated L-Arginine Formed by Neutrophilic HL-60 Cells**—Differentiated HL-60 cells (4 x 10\(^5\) cells/ml) were suspended in 2 ml of PBS containing 1 mM CaCl\(_2\) and 1 mM L-Arg. Cells were then activated with PMA (10 \(\mu\)M) at 37 °C for 30 min. The cells were next centrifuged for 5 min (2,000 x g; 4 °C), and the supernatant was split and passed through a 0.45-\(\mu\)m cellulose filter. The UV absorbance spectrum of this material was then obtained. Trifluoroacetic acid (3\%) was added to the reaction mixture prior to monitoring liquid chromatography–mass spectrometry (LC-MS). Reaction mixtures were separated by high performance liquid chromatography using a 10 cm x 4.6 mm inner diameter, C-8 Aquapore reverse-phase column under isocratic conditions. The mobile phase (10 mM ammonium acetate) flow rate was 1.0 mls/min. Specific parent ion/daughter ion combinations were used in multiple reaction ion monitoring (MMR) to detect L-Arg (175/70) and Cl-L-Arg (209/70 and 243/146), respectively. Data were analyzed using commercially available software (MassSpec version 3.3, PE Sciex, Inc.).

**Measurement of NO Metabolites**—Nitric oxide production in BAECs was assessed by monitoring the formation of the metabolites nitrate (NO\(_2\)), nitrite (NO\(_3\)), and nitrate (NO\(_3\)). BAECs were pretreated with Cl-L-Arg (0–10 \(\mu\)M) in serum-free media for 1 h followed by washout. Cells were then exposed to the calcium ionophore A23187 (1 \(\mu\)M) for 2 h. Superoxide dismutase (200 units/ml) was added to the incubation medium to minimize cellular superoxide content. Aliquots of the media were sampled at the end of this period. Nitrate in samples was enzymatically reduced to NO\(_2\) by treatment with Escherichia coli–enriched nitrate reductase. Total NO\(_2\) was used as an index of NO production. Nitrite was detected using the fluorophore 2,3-diaminonaphthalene (Calbiochem). Under alkaline conditions, NO\(_2\) converts 2,3-diaminonaphthalene to the fluorescent compound 1(H)-naphthotriazole. Nitrite concentration was monitored by the spectrofluorometric excitation of 1(H)-naphthotriazole at 380 nm and emission at 450 nm. A standard curve was constructed for NaNO\(_2\) (0.1–1,000 \(\mu\)M).

**Vessel Reactivity Studies**—Isometric tension was measured in isolated aortic ring segments of Harlan Sprague-Dawley rats. Upon sacrifice, the aorta was excised and cleansed of fat and adhering tissue. The vessel was cut into individual ring segments (2–3 mm in width) and suspended from a force-displacement transducer in a tissue bath. Ring segments were bathed in a bicarbonate-buffered, Krebs-Henseleit solution of the following composition (mM): NaCl, 118; KCl, 4.6; NaHCO\(_3\), 27.2; KH\(_2\)PO\(_4\), 1.2; MgSO\(_4\), 1.2; CaCl\(_2\), 1.75; Na,EDTA, 0.03; and glucose, 11.1. A passive load of 2 g was applied to all ring segments and maintained at this level throughout the experiment. At the beginning of each experiment, indomethacin-treated ring segments were depolarized with KCl (70 mM) to determine the maximal contractile capacity of the vessel. Rings were then thoroughly washed and allowed to equilibrate.
In subsequent experiments, vessels were submaximally contracted (50% of KCl response) with PE (3 x 10^-3 to 8 x 10^-3 M). When tension development reached a plateau, Ach (10^-2 to 3 x 10^-2 M) was added cumulatively to the bath to evoke endothelium-dependent relaxation. Endothelium-independent relaxation was tested by cumulative addition of the NO donor SNP. In some experiments, ring segments were pretreated with Cl-L-Arg at concentrations of 0–10 μM for 1 h, followed by thorough rinsing. The concentration of Cl-L-Arg in these experiments is expressed in terms of molar equivalents of arginine. Parallel experiments were performed using D-Arg and the product of HOCl (Cl-D-Arg). In other experiments, rings were pretreated with a fixed amount of Cl-L-Arg (10 μM) and increasing concentrations of L-Arg (0–1,000 μM), prior to monitoring endothelium-dependent relaxation. Additional control experiments were performed to compare the relative sensitivity of ring segments to inhibition of Ach-mediated relaxation by L-NAME. Real time data were collected for all experiments and downloaded to an IBM PC for analysis using WorkBench PC for Windows (Strawberry Tree, Inc./DASYTECH version 3). Dose-response profiles for different experimental conditions were analyzed and tested for differences in relaxation parameters.

Measurement of Vascular Resistance—Rats were anesthetized with ketamine (80 mg/kg)/xylazine (5 mg/kg). A catheter was inserted in the right femoral artery for measurement of blood pressure. An additional catheter was inserted in the left jugular vein for drug administration. A Doppler flow probe (Transonic Systems, Inc.) was inserted around the right common carotid artery. The study was divided into three periods: 1) control infusion of 0.9% NaCl; 2) sequential infusion of 1.5, 15, and 150 mmol/kg Cl-L-Arg for 10 min each; and 3) infusion of 1.5 mmol/kg L-Arg. Vascular resistance was calculated from blood pressure and blood flow measurements (r = BP/F) for each infusion.

Statistical Analysis—All results are expressed as the mean ± S.E. Dose-response profiles for different experimental conditions were analyzed and tested to determine differences in relaxation responses using
HOCI and L-Arginine-derived NOS Inhibitors

RESULTS

Characterization of the Reaction Products of HOCI and L-Arginine—Hypochlorous acid absorbs maximally at 292 nm, while L-Arg has an absorbance (A) maximum at 210 nm at pH 7.4 (Fig. 1). On mixing HOCI and L-Arg, the HOCI absorbance disappeared, and a new broad band was evident at 252 nm, consistent with the formation of a new product(s) (Fig. 1).

Using stopped-flow spectrometry, we found that HOCI rapidly reacted with L-Arg with an apparent second order rate of reaction of $k = 7.1 \times 10^3 \text{ M}^{-1} \text{s}^{-1}$ (Fig. 1). As a control, we measured the reaction rate of HOCI with taurine, which yielded a value of $1.6 \times 10^6 \text{ M}^{-1} \text{s}^{-1}$ in agreement with previous measurements (10).

To characterize the reaction products of HOCI and L-Arg, we employed positive ion electrospray ionization mass spectrometry. Representative mass spectra for L-Arg and Cl-L-Arg are shown in Fig. 2. Electrospray ionization of L-Arg gave a major peak with an $m/z$ of 175, corresponding to the molecular ion $+H^+$ under these positive ionization conditions (Fig. 2). In the HOCI-treated sample, L-Arg was almost completely consumed, and major new products were detected. Peaks were observed at $m/z$ 209/211 with a ratio indicating the presence of one chlorine. Peaks were also observed at 243/245 with a ratio consistent with the presence of two chlorines (Fig. 2). MS-MS analyses revealed that parent ion/daughter ion monitoring of $m/z$ 175 and 209/211 share a common fragment with an $m/z$ of 70. A pair of chlorinated species detected at $m/z$ 146/148 was identified as a daughter fragment of the dichlorinated product (data not shown).

To determine the sites of chlorine addition to L-Arg, we employed spectrophotometric techniques. Progressive addition of HOCI (0–1 mM) to a fixed amount of L-Arg (1 mM) resulted in an increased absorbance at 252 nm ($A_{252}$) consistent with the formation of an $\alpha$-amino-monochloramine (Fig. 3; Ref. 29). HOCI also increased the absorbance at 207 nm, indicating chlorination of a guanidino nitrogen (29). In control experiments, we added HOCI to taurine, a simple amine that possesses an $\alpha$-amino group but lacks a guanidino nitrogen. At molar ratios up to 1:1, we found an increase in $A_{271}$ but no change in $A_{207}$ (not shown). Thus, an $\alpha$-amino-monochloramine derivative of taurine was the only product generated under these conditions. The lack of an $A_{271}$ peak in the taurine sample further supports our identification of the 207 peak in the L-Arg sample as an N-chloroguanidino derivative. Absorbance spectra for the HOCI + L-Arg reaction samples were corrected using molar extinction coefficients for chlorinated amines at 207 ($\epsilon = 3,000 \text{ M}^{-1} \text{cm}^{-1}$) and 252 ($\epsilon = 429 \text{ M}^{-1} \text{cm}^{-1}$) and are depicted in Fig. 3 (29). Collectively, these data are consistent with HOCI chlorinating the $\alpha$-amino group to form an $\alpha$-amino-monochloramine, followed by chlorination of the guanidino group to yield a dichlorinated product (29). The ratio of mono- to dichlorinated products formed under these conditions was 4:1, suggest-
Inhibition of Endothelial NO Production by Cl-Arg—We hypothesized that l-Arg chlorination products may share similar pharmacological properties with traditional NOS inhibitors. We therefore monitored the effect of Cl-Arg pretreatment on NO formation in A23187-stimulated BAECs. Over a 2-h treatment period, 1 μM A23187 stimulated a 44% increase in NO\textsubscript{3} and NO\textsubscript{2} above base line (Fig. 5). Prior exposure of BAECs to Cl-Arg, followed by washout, inhibited the A23187-mediated increase in NO\textsubscript{3} and NO\textsubscript{2} formation in a concentration-dependent manner, with 1 μM Cl-Arg blocking the peak increase in total NO\textsubscript{2} by 35% and 10 μM by 95% (Fig. 5).

In Vitro and in Vivo Responses to Cl-Arg—Since our results suggested that Cl-Arg inhibits NO formation by endothelial cells, we assessed the effects of this reaction product on endothelial-dependent relaxation in isolated blood vessels. Ring segments were incubated with Cl-Arg at concentrations between 0.1 and 10 μM. After a 1-h incubation period, the tissue bath was thoroughly rinsed, and residual effects of this treatment on vessel function were tested. Endothelium-dependent relaxation was monitored in precontracted ring segments by exposure to Ach. Endothelium-dependent relaxation in the absence of Cl-Arg treatment was normal (Fig. 6). Cl-Arg pre-treatment resulted in a concentration-dependent inhibition of Ach-induced relaxation with an ED\textsubscript{50} and maximum inhibitory effect (R\textsubscript{max}) occurring at 5 and 10 μM, respectively (Fig. 6). On a molar basis, Cl-Arg was equipotent to the commonly used NOS inhibitor L-NAME in inhibiting NO formation (not shown).

The inhibitory effect of Cl-Arg on endothelium-dependent relaxation persisted for time periods up to 2 h after washout. In contrast, endothelium-independent relaxation induced by the NO donor SNP was not altered by prior exposure to Cl-Arg, suggesting that the endothelium was the target of action of the chlorinated l-Arg metabolite (Fig. 6). The inhibitory effect of 10 μM Cl-Arg on endothelium-dependent relaxation could be reversed by concurrent incubation of ring segments with higher concentrations of l-Arg (Fig. 7). A 10-fold molar excess of l-Arg to Cl-Arg resulted in ~60% reduction in the inhibitory response to 10 μM Cl-Arg, while a 100-fold excess of l-Arg completely blocked the effects of Cl-Arg on NO bioactivity (Fig. 7).

To test for the selectivity of Cl-Arg in the inhibition of endothelium-dependent relaxation, we performed similar experiments using d-Arg, which is not a substrate for NO production. A stock solution of Cl-d-Arg was prepared by reacting 100 mM d-Arg and 100 mM HOCl. Ring segments were incubated with unmodified d-Arg or Cl-d-Arg at a concentration of 10 μM for 1 h. Tissue baths were then rinsed thoroughly. In contrast to Cl-Arg, incubation of rat aortic ring segments with Cl-d-Arg did not inhibit endothelium-dependent relaxation (Fig. 8).

Effects of Cl-Arg administration were tested in vivo by monitoring blood flow responses in anesthetized rats. Fig. 9 shows that intravenous administration of Cl-Arg results in a significant increase in resistance to carotid artery blood flow. Sequential infusion of increasing doses of Cl-Arg resulted in vasoconstriction, as reflected by a dose-dependent increase in carotid flow resistance (Fig. 9). The peak increase in vascular resistance occurred at 150 μmol/kg Cl-Arg. Subsequent infusion of a 10-fold molar excess of l-Arg (1.5 mmol/kg) resulted in a 67% decrease in carotid resistance.
Detection of Cl-L-Arg Generated by Activated HL60 Cells—In a final series of experiments, neutrophilic HL60 cells, bathed in PBS containing L-Arg, were stimulated with PMA to induce a respiratory burst. After 30 min, the PBS was collected for analysis. UV spectra revealed the formation of a product with similar absorbance properties as synthetic Cl-L-Arg (Fig. 10). In the absence of PMA, a reduced absorbance corresponding to L-Arg was observed at 210 nm. Nondifferentiated HL60 cells bathed in PBS containing L-Arg yielded a similar UV absorbance spectrum (not shown). Analysis by LC-MS indicated the presence of two major peaks in PBS of PMA-treated cells with discrete retention times. One of the products had a retention time that was identical to L-Arg (2.75 min). The other peak (retention time 5 2 min) was not detected in buffer samples in the absence of PMA (Fig. 10). The peaks were collected and directly injected into the mass spectrometer. The formation of parent/daughter ion pairs was assessed using MRM, which allows the detection of specific fragmentation products. MRM analysis revealed the presence of L-Arg and Nα-chloro-L-Arg (175/70 and 209/70, respectively). No parent/daughter ion pairs corresponding to Nα,Nε-dichloro-L-Arg (243/146) were observed. These results indicate that the primary chlorinated metabolite generated by neutrophilic HL60 cells is a monochloramine.

**DISCUSSION**

Results of the current studies show that HOCl reacts with L-Arg to form novel chlorinated products that inhibit endothelial NO synthesis. The rate at which this reaction proceeds is rapid and is of similar magnitude as that reported for HOCl with other amino acids (10). Results of mass spectrometry and spectrophotometric studies facilitated the structural analysis of these L-Arg metabolites. Our data suggest that the α-amino and guanidino nitrogens of L-Arg are sites of HOCl-dependent chlorination. Previous reports show that the HOCl-dependent formation of amino acid-based N-chloramines occurs via modification of the α-amino nitrogen (27). The reaction products of HOCl and L-Arg (at a 1:1 molar ratio) were the α-amino-monochloramine Nα-chloro-L-Arg and the dichlorinated product Nα,Nε-dichloro-L-Arg. The yield of Nα-chloro-L-Arg was 4-fold...
greater, suggesting that the monochloramine is the predominant species formed under these conditions. We also found evidence for the formation of an α-amino-dichloramine (N^α,N^α-dichloro-L-Arg) when HOCl was added in excess of L-Arg; however, the yield of this product was low.

Commercially available NOS inhibitors are widely used to study mechanisms of NO production and action in cells and tissues, and most inhibitors currently in use are structural variants of L-Arg. The mechanism of action of N^α-substituted analogues such as L-NNAME is to compete with L-Arg for binding to the active site of NOS. The reversibility of NOS inhibition varies among these compounds (30). One of the goals of our study was to explore the pharmacological properties of the chlorinated L-Arg metabolites. Specifically, we tested the hypothesis that chlorination of L-Arg interferes with NO synthesis. Results of cellular studies showed that prior exposure of BAECs to Cl-L-Arg inhibited the A23187-mediated formation of NO_3^- and NO_2^- in a concentration-dependent manner, suggesting that Cl-L-Arg acts as an inhibitor of NOS III.

Using an in vitro bioassay system, preincubation of rat aortic ring segments with Cl-L-Arg inhibited Ach-induced relaxation in a concentration-dependent manner. The relative potency (ED_{50}) of Cl-L-Arg for the inhibition of Ach-induced relaxation was ~5 μM, which is similar to that reported for the commonly used NOS III inhibitors L-NMMA and L-NNAME (31). In contrast, the vasodilator response to SNP was not affected, revealing that vascular smooth muscle is not a target of Cl-L-Arg action, since the machinery required for vessel relaxation was fully intact. Rather, the inhibitory effect of Cl-L-Arg was mediated at the level of the vascular endothelium. In vivo studies showed that infusion of Cl-L-Arg in anesthetized rats resulted in a concentration-dependent reduction of basal flow, consistent with the vascular response to infusion of NOS inhibitors (30).

The inhibitory effect of chlorinated metabolites was specifically related to the modification of L-Arg, since incubation of ring segments with the reaction product of HOCl and d-Arg did not blunt endothelium-dependent relaxation. The production of NO from arginine is stereospecific for the L-isofromm, since d-Arg does not bind to NOS, and the inhibitory effect of Cl-L-Arg on endothelium-dependent relaxation appears to display stereospecificity as well (32). Furthermore, higher concentrations of L-Arg competed with the inhibitory effect of Cl-L-Arg. A 10-fold molar excess of L-Arg (100 μM) reduced the inhibitory effect of 10 μM Cl-L-Arg by ~60%, and a 100-fold excess completely blocked the response to Cl-L-Arg. The mechanism underlying the protective effect of L-Arg under these conditions is not clear and may be more complicated than a simple competitive interaction between Cl-L-Arg and endogenous L-Arg. At higher concentrations, L-Arg may effectively displace Cl-L-Arg binding to NOS III inside the endothelial cell.

Neutrophil adhesion contributes to the impairment of endothelial cell function in models of inflammation and ischemia-reperfusion injury, with neutrophil binding inhibiting both basal and stimulated release of NO (4). While some data suggest that increased vascular MPO content and HOCI formation contribute to endothelial dysfunction, few studies have directly assessed the effect of these molecules on NO bioactivity (26). A previous report showed that infusion of 2 μM HOCI into the guinea pig coronary circulation, followed by washout, resulted in a 50% reduction in basal flow (26). Additionally, the coronary flow response to the endothelium-dependent vasodilators Ach, bradykinin, and adenosine was completely blocked by prior exposure to HOCI (26). The mechanism by which HOCI inhibited vascular relaxation was not biochemical.

Other studies suggested that the biochemical modification of L-Arg results in the formation of an inactive substrate for NO production and that reduced NO bioactivity contributes to the pathogenesis of inflammatory cardiovascular disease (33). In this respect, it was shown that methylation of L-Arg is enhanced in endothelial cells in response to hypercholesterolemia, with the formation of dimethyl arginine, an inactive substrate for NO production (34, 35). In the current study, we present data showing that HOCI reacts with L-Arg, resulting in the formation of chlorinated metabolites. These products have a potent inhibitory effect on endothelium-dependent vasorelaxation in vivo and in vitro. A physiological role for Cl-L-Arg is supported by our observation that neutrophilic HL60 cells generate N^α-chloro-l-Arg, a component of synthetic Cl-L-Arg. We hypothesize that the MPO-derived oxidant HOCI decreases NO bioactivity by biochemical modification of L-Arg substrate and that this pathway may represent a significant pathogenic mechanism underlying endothelial dysfunction in diverse cardiovascular inflammatory diseases.

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