Potentiation of Nitric Oxide-Mediated Vascular Relaxation by SC52608, a Superoxide Dismutase Mimic (43848)

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Abstract. **Nitric oxide (NO) produced by the vascular endothelium is an endogenous contributor to the regulation of vascular relaxation and the maintenance of blood pressure. The effective half-life of NO and the relaxation of aortic rings by NO is enhanced by a reduction in the concentration of superoxide radicals with superoxide dismutase (SOD). In the current study, SC52608, a newly synthesized SOD mimic with a manganese core, was tested for its ability to potentiate the activity of NO both *in vitro* and *in vivo*. SC52608 relaxation of rat aortic segments was endothelium dependent as well as concentration dependent. The maximum relaxation following KCl contraction was 44% with 300 μ*M* SC52608. Cyclic GMP concentrations in the segments were increased 1.6- and 3.2-fold with 5 and 300 μ*M* SC52608, respectively. N-monomethyl-l-arginine pretreatment of aortic rings abolished the relaxation and cyclic GMP accumulation mediated by SC52608. In a smooth muscle cell reporter system of nitric oxide synthase activity, SC52608 potentiated the increase in cyclic GMP elicited by NO in a concentration-dependent manner with a maximum increase of 5.2-fold at 100 μ*M*. Injection of SC52608 into conscious, restrained rats resulted in a dose-dependent decrease of blood pressure. Therefore, the data suggest that SC52608 potentiates the actions of nitric oxide on vascular tone, cyclic GMP, and blood pressure by enhancing the half-life of NO through a mechanism that mimics the action of SOD.**

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Systemic vascular tone is carefully regulated by a balance between contraction and relaxation factors. A major endogenous contributor to the relaxation of blood vessels is nitric oxide (NO) released from vascular endothelial cells (1, 2). Released NO has been shown to stimulate cytosolic guanylate cyclase in vascular smooth muscle cells with the resulting increase in smooth muscle cell cyclic GMP (cGMP) mediating relaxation (2). The contribution of NO to vascular tone has been further demonstrated by selective inhibition of nitric oxide synthase with N-nitro-l-arginine (NNA) resulting in a significant increase in vascular tone accompanied by decreased levels of smooth muscle cell cGMP (2).

The biological half-life of NO is very short (seconds) and results in the conversion of NO to inactive nitrite and nitrate. Superoxide radicals can inactivate NO, and hence, the effective biological half-life of NO and the associated vascular relaxation are enhanced by the addition of superoxide dismutase (SOD) which destroys superoxide radicals (3, 4). Enzymes catalyzing the dismutation of superoxide contain manganese, copper/zinc, or iron in their active sites (5). Manganese, as well as manganese-containing preparations have been reported to mimic SOD enzymes as determined by electron spin resonance (6) and cytochrome *c* competition (7) assays. In contrast, Weiss *et al.* (8) demonstrated by direct kinetic analysis that manganese and manganese-desferal complexes had no SOD activity. They proposed that manganese may interact stoichiometrically with superoxide (8). Regardless of the interrelationship between manganese and superox-

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ide, manganese has been shown to potentiate the vascular relaxation effect of endogenous NO by a mechanism consistent with enhancement of the biological half-life of NO (9).

SC52608 (Fig. 1) was synthesized as a Mn(II) complex of a macrocyclic pentaamine (1,4,7,10,13-pentaazacyclopentadecane, [Mn([15]aneN$_5$)Cl]Cl) which demonstrated SOD activity as shown by stopped-flow kinetic analysis (10). Thus, SC52608 may extend the biological half-life of NO by acting as an SOD mimic. We proposed to evaluate the interrelationship of SC52608 with NO in the regulation of vascular smooth muscle tone. Therefore, the present study was designed to examine the ability of SC52608 to potentiate the relaxation effect of endogenous NO in aortic rings, to increase intracellular cGMP concentration in a vascular smooth muscle cell reporter system of nitric oxide activity, and to decrease mean arterial pressure in the rat.

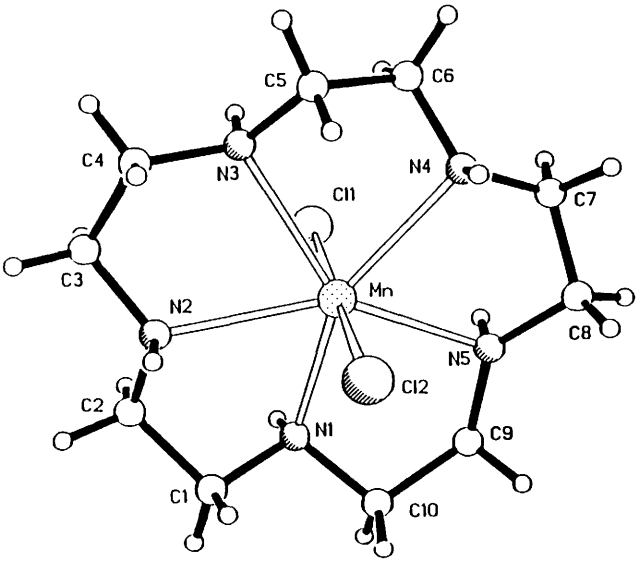

Figure 1. Structure of SC52608.

Materials and Methods

Materials. Male Sprague-Dawley rats (300–350 g) purchased from Charles River (Wilmington, MA) were used in these studies. All chemicals and reagents were of the highest purity available from Sigma Chemical Co. (St. Louis, MO). Zaprinast (MB22948) and SC52608 were synthesized by Monsanto Co. (St. Louis, MO).

Synthesis and Structure of SC52608. The details for the synthesis of SC52608 were outlined by Riley and Weiss (9). Briefly, SC52608 was produced by reacting the free base 1,4,7,10,13-pentaazacyclopentadecane, [15]aneN$_5$, with anhydrous $MnCl_2$ in MeOH under dry N_2. The resultant white complex was recrystallized from EtOH and shown by single-crystal x-ray diffraction to have the seven-coordinate structure shown in Figure 1. The crystal data were collected on a Four-Circle Nicolet (Siemens, Heidelberg, Germany) Autodiffractometer using full (0.90°-wide) ω scans and graphite-monochromated MoKα radiation. The structure was solved using direct methods incorporating the Siemens SHELXTL-Plus software package as modified at Crystallics Co., Lincoln, Nebraska. Further details of the crystal structure, other physical properties, and characterization of the SOD activity of SC52608 were detailed by Riley and Weiss (10).

Rat Aortic Rings. Thoracic aortas were removed from rats following anesthesia (10 mg/kg xylaxine plus 50 mg/kg ketamine, intramuscular injection) and exsanguination. The connective tissue was carefully trimmed to avoid damage to the endothelium. Rings were cut into 3-mm lengths and placed into tissue baths as described (11). Where indicated, the endothelial cells were removed by gentle rubbing of the ring preparation. Aortic rings were maintained at 37°C in Krebs-bicarbonate buffer with the following composition (in m*M*): NaCl (130); $NaHCO_3$ (25); KCl (5); NaH_2PO_4 (1.2); $MgSO_4$ (1.2); $CaCl_2$ (2.5); D-glucose (11.1); and ascorbic acid (0.1), pH 7.2 bubbled with 5% CO_2/95% O_2. The rings were preloaded with 1 g of tension and equilibrated for 30 min with two buffer changes.

After stabilization of the baseline, the rings were contracted with 30 m*M* KCl (final concentration) and the presence of functional endothelium was determined by relaxation with acetylcholine (1 μ*M*). Under the conditions used in these experiments, this concentration of acetylcholine produced maximum relaxation in the ring segments. Contraction responses to acetylcholine were detected at concentrations in excess of 10 μ*M*. Rings without endothelium were relaxed with sodium nitroprusside (0.1 μ*M*) after a lack of relaxation response to acetylcholine was noted. After 10 min of acetylcholine or nitroprusside treatment, the tissues were washed for 30 min with two buffer changes. The tissues were recontracted with KCl and washed several times until repetitive contractions were similar in magnitude. Following KCl contraction, cumulative concentrations of SC52608 were added and the responses were determined for 10 min before the addition of the next concentration. Isometric tension was recorded and relaxation was determined as the percentage of maximum tone developed to the contracting agent. At the end of each experiment, the tissue was washed and its functional integrity was confirmed by contraction with KCl followed by relaxation with acetylcholine or nitroprusside.

Determination of cGMP in Aortic Rings. Aortic rings were prepared as described above and equilibrated in Krebs-bicarbonate buffer (30 min). Tissues were blotted dry, weighed, and placed into individual microcentrifuge tubes with fresh buffer. KCl (30 m*M*)

and zaprinast (Type V, specific cGMP phosphodiesterase inhibitor; 100 μ*M*) were added for 10 min prior to SC52608 addition. Following preincubation, rings were treated with SC52608 (5 or 300 μ*M*) for 90 sec and the reaction was stopped by placing the tissue into ice-cold 0.1 *N* HCl. The cGMP formed was extracted by homogenizing the tissue with a Polytron homogenizer (Setting 8 for 30 sec). The tissue extract was centrifuged and the cGMP content was determined by radioimmunoassay (12). In those studies utilizing N-monomethyl-l-arginine (NMA, nitric oxide synthase inhibitor), this compound was added at the same time as the KCl and zaprinast.

Nitric Oxide Synthase. RAW 264.7 macrophages were grown in Dulbecco's Modified Eagle Medium (Gibso, Gaithersburg, MD) supplemented with 10% fetal bovine serum at 37°C, in a humidified atmosphere of 5% CO_2/95% air. NOS activity was induced in these cells by treatment with 10 μg/ml lipopolysaccharide (LPS) and 10% fetal bovine serum in MEM containing Earle's salts (without phenol red) and 2 m*M* L-glutamine. Crude extracts from LPS-treated cells were obtained by suspending the cells (30–100 × 10^6/ml) in 50 m*M* Tris, pH 7.6, 0.5 m*M* dithiothreitol and lysing them with three 10 s bursts of a Polytron in the presence of the following protease inhibitors: 0.1 mg/ml phenylmethylsulfonyl fluoride, 0.01 mg/ml soybean trypsin inhibitor, 0.01 mg/ml antipain, 0.01 mg/ml leupeptin, and 0.01 mg/ml aprotonin. The lysate was then centrifuged at 100,000*g* for 1 hr at 4°C, after which glycerol was added to a final concentration of 10% to both the supernatant and pellet. The crude extract of nitric oxide synthase was stored at −80°C with a final protein concentration of 4.7 mg/ml.

Nitric Oxide Synthase Activity Measured by Citrulline Production. Nitric oxide synthase catalyzes the conversion of L-arginine to NO and L-citrulline. This reaction was monitored as previously reported with minor modifications (13, 14). Enzyme was incubated at 37°C for 15 min in a reaction volume of 100 μl with the following components added together to start the reaction: 50 m*M* Tris (pH 7.6), 1 mg/ml bovine serum albumin, 1 m*M* dithiothreitol, 2 m*M* $CaCl_2$, 10 μ*M* flavin adenine dinucleotide, 10 μ*M* tetrahydrobiopterin, 30 μ*M* L-arginine containing L-[2,3-^{3}H] arginine resulting in a specific activity of 150–200 cpm/pmol, and 1 m*M* NADPH. The reaction was terminated by the addition of 400 μl of cold stop buffer consisting of 10 m*M* EGTA, 100 m*M* Hepes (pH 5.5), and 1 m*M* citrulline. The reaction mixture was applied to a Dowex 50W X-8 column (1 ml, Na-form, 100–200 mesh) preequilibrated with stop buffer. L-[2,3-^{3}H] citrulline was eluted with two 0.75-ml washes of water and radioactivity determined by liquid scintillation counting.

Nitric Oxide Detection by Cellular cGMP Reporter System. This assay was a modification of that described by Murad and colleagues (15). Guanylate cyclase laden rat vascular smooth muscle cells were grown to confluence on a 48-well tissue culture plate. The cells were washed twice with Earle's salts containing amino acids. A nitric oxide generating system (250 μl total volume) consisting of induced nitric oxide synthase (47 μg, see above), L-arginine (1 m*M*), and 3-isobutyl-1-methylxanthine (cyclic nucleotide phosphodiesterase inhibitor, 1 m*M*) was then added to each well on ice. Finally, NADPH (1 m*M*) was added and the plate was transferred immediately to a 37°C water bath to initiate the reaction. After 5 min, the assay was stopped by cooling to 4°C, the cGMP formed was extracted and subsequently quantified by radioimmunoassay (12).

***In Vivo* Studies.** Mean arterial pressure and heart rate were determined during SC52608 bolus injection or infusion using a standard protocol (16). In brief, polyethylene catheters (PE50) were inserted into the femoral artery and vein of anesthetized rats. The animals were placed into individual restraining cages and allowed to regain consciousness. Infusion of 0.9% sodium chloride via the femoral vein (0.07 ml/kg/min) was begun and continued throughout the study period. Observations were started following an equilibration period of 90 min after the start of infusion. Blood pressure was measured continuously for the next 150 min via the femoral artery with a pressure transducer (type 041-500-503; Cobe, Lakewood, CO) connected to a Grass model 7E polygraph. Mean arterial pressure and heart rates were recorded at 3-min intervals by a computerized data acquisition system. SC52608 was administered either as a bolus or infused via the femoral vein in a sodium chloride (0.9±) vehicle.

Protein Determination. Protein concentrations were determined according to Bradford (17) using bovine serum albumin as standard.

Data Evaluation. Data are presented as the average ± SEM. Significance was determined by Student's *t* test for unpaired data with significance indicated as $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. The details of group comparisons are described in the figure legends.

Results

SC52608 relaxation of rat aortic ring segments was first studied for concentration and endothelium dependence. Endothelium intact aortic rings contracted with KCl (30 m*M*) developed 1.64 ± 0.20 g (n = 9) of tension. The presence of endothelium was confirmed by relaxation (52% ± 3.6%) to acetylcholine (1 μ*M*). Removal of the endothelium resulted in rings which contracted with 2-fold greater tension (3.54 ± 0.26 g, n =

4) and demonstrated no relaxation response to acetylcholine (1 μM). These preparations still responded to sodium nitroprusside (0.1 μM) with 93% ± 1.0% relaxation. Relaxation of aortic rings to SC52608 was both endothelium and concentration dependent (Fig. 2 and 3). A maximal relaxation of 44% was obtained with 300 μM SC52608 in intact ring segments. Endothelium denuded ring segments demonstrated a slight relaxation response to SC52608 at the highest concentration tested (300 μM, Fig. 2 and 3).

In a similar manner, endothelium-intact aortic rings contracted with prostaglandin ($PGF_{2\alpha}$, 2 μM) developed 0.84 ± 0.18 g (n = 8) of tension and relaxed in a concentration-dependent manner to SC52608 with a maximum of 88% ± 2% at 100 μM (Fig. 4). No further increase in relaxation was noted at concentrations up to 300 μM. The maximum relaxation response to SC52608 was 2-fold greater in $PGF_{2\alpha}$ contracted versus KCl contracted rings (88% vs 44%).

Blockade of the relaxation response to SC52608 in endothelium-intact aortic rings was accomplished by pretreatment (10 μM for 10 min) with NMA (Fig. 5). This concentration of NMA has been demonstrated to partially inhibit endogenous nitric oxide synthase as determined by an increase in aortic ring tension following NMA exposure (18). In the present study, 10 μM NMA completely inhibited relaxation responses to all concentrations of SC52608 examined.

Previous studies have demonstrated the inability of cyclooxygenase inhibitors to prevent endothelium-dependent relaxations and the inability of prostacyclin to relax rat aorta (19–21). Nevertheless, in order to test the possibility that the relaxation activity of SC52608 may be dependent upon the basal release of prostanoids, indomethacin (a cyclooxygenase inhibitor) was added to endothelium intact rings contracted with KCl. No change of tension (n = 4) was determined by the addition of indomethacin (10 μM, 30 min) to rings following a stable baseline contraction to 30 mM KCl (1.62 ± 0.12 g compared to 1.61 ± 0.13 g tension before versus after indomethacin, respectively). Indomethacin-treated rings relaxed with 5.6% ± 1.2%, 20% ± 0.64%, 30% ± 0.87%, 32% ± 0.48%, and 49% ± 1.8% of maximum at 0.05, 0.5, 5, 50, and 300 μM SC52608, respectively while control rings not treated with indomethacin demonstrated 4.4% ± 0.58%, 17% ± 2.9%, 32% ± 1.8%, 36% ± 2.6%, and 51% ± 1.5% relaxation with 0.05, 0.5, 5, 50, and 300 μM SC52608, respectively. Thus, pretreatment with indomethacin (a cyclooxygenase inhibitor) had no significant effect

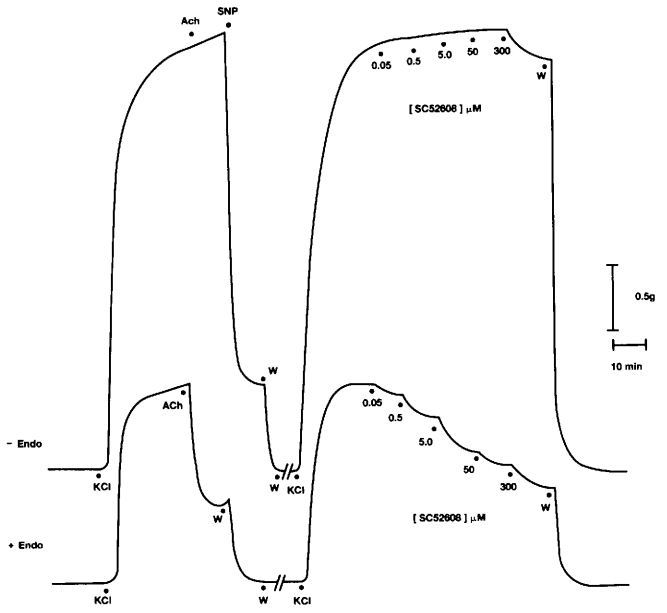

Figure 2. SC52608 relaxation of rat aortic rings. Representative physiograph tracings of rat aortic segments with (+Endo) or without (−Endo) endothelium. SC52608 was administered at the concentrations indicated. KCl-potassium chloride (30 mM); ACh-acetylcholine (1 μM); SNP-sodium nitroprusside (0.1 μM); W-wash.

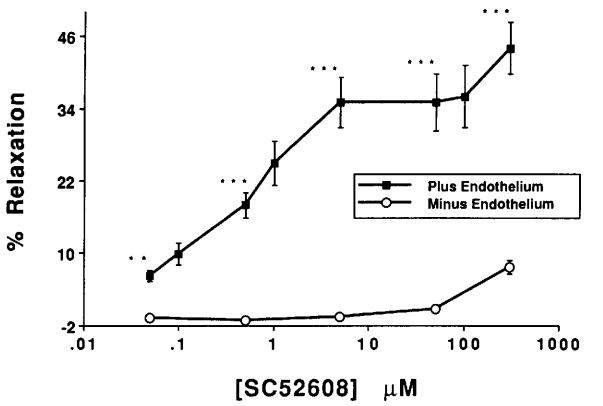

Figure 3. Effect of SC52608 on rat aortic rings contracted with KCl. The data were calculated as a percentage of the maximum contraction. Statistical significance was determined between rings with versus without endothelium. n = 6–9.

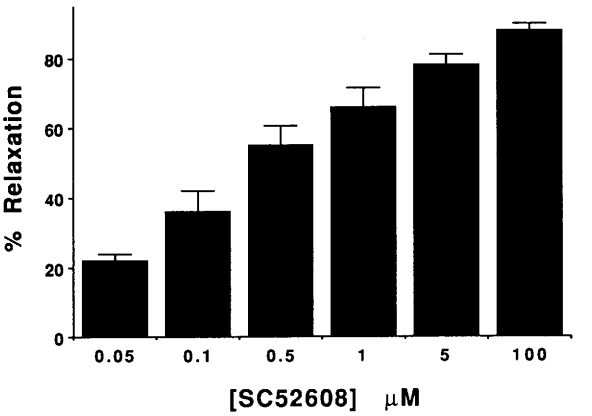

Figure 4. SC52608 mediated relaxation of rat aortic rings following $PGF_{2\alpha}$ contraction. Intact endothelium of the segments was demonstrated by relaxation with acetylcholine. At every concentration tested the data from treated versus untreated rings were statistically different with $P < 0.01$. n = 4.

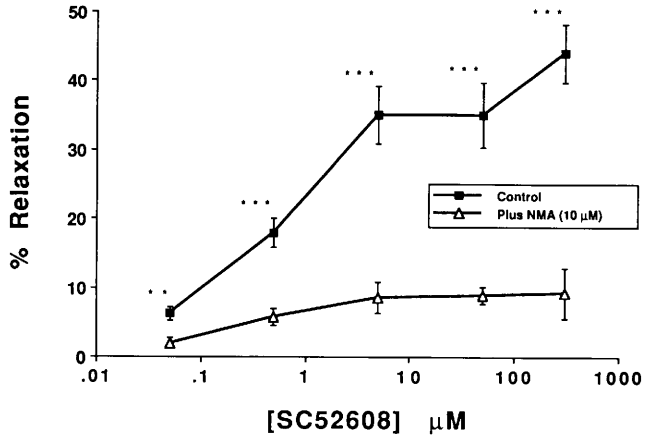

Figure 5. Blockade of SC52608 relaxation in rat aortic rings by NMA. The segments were endothelium intact and were preincubated with NMA for 10 min before contraction by KCl. The data from control versus NMA-treated segments were compared statistically. n = 4–10.

upon the relaxation response of aortic rings to SC52608.

Tissue concentrations of cGMP were increased 1.6- and 3.2-fold above the control (zaprinast treated) tissue in response to SC52608 at 5 and 300 μ*M*, respectively (Fig. 6). The accumulation of tissue cGMP in response to SC52608 (at either concentration) was completely blocked by concurrent treatment with NMA (10 μ*M*). In control experiments, aortic rings were treated with acetylcholine (1 μ*M*). The tissue relaxed as previously described (Fig. 2, see above) and the cGMP concentration increased 18-fold (3140 ± 1040 fmol/mg wet weight, n = 4). NMA treatment (500 μ*M*) reduced basal levels of cGMP by 62% (65.6 ± 6.6 fmol/mg wet weight, n = 4). Also, NMA pretreatment (500 μ*M*) attenuated acetylcholine relaxation by one half (30% ± 5.9%, n = 4) and decreased cGMP accumulation to 3.1-fold above the level with NMA alone (204 ± 38 fmol/mg wet weight, n = 4).

Smooth muscle cells in culture have been demonstrated to be useful as a reporter system of nitric oxide synthase activity (15, 18). SC52608 increased the cellular concentration of cGMP in this system above the level found with the addition of NOS alone (Fig. 7). This effect was concentration dependent and resulted in a 5.2-fold increase of intracellular cGMP concentration with the addition of 100 μ*M* SC52608.

In order to demonstrate the dependence of SC52608 activity upon the complexed manganese, the ring structure of the compound was synthesized without incorporation of the metal (SC52612). SC52612 did not induce relaxation in rat aortic rings and did not increase cGMP concentrations in the smooth cell reporter system (data not shown). As a control for these experiments, SC52608 was used following treatment with SC52612 (inactive compound) to substantiate functional integrity of the aortic ring or smooth muscle cell reporter system. In this paradigm, SC52608 (5 μ*M*) mediated a 26% ± 4.7% (n = 4) relaxation of aortic rings which was not statistically different from the response to SC52608 presented in Figure 3. Also, the smooth muscle cell reporter system treated with SC52608 (10 μ*M*) following exposure to SC52612 increased cGMP concentration to 480 ± 30 fmol/well which was not different from the response to SC52608 noted in Figure 7.

Previous experiments in this laboratory demonstrated the activity of manganese to potentiate NO relaxation and cGMP accumulation in aortic segments (9). In the present study, SC52608 relaxation activity in aortic rings has been shown to be dependent upon

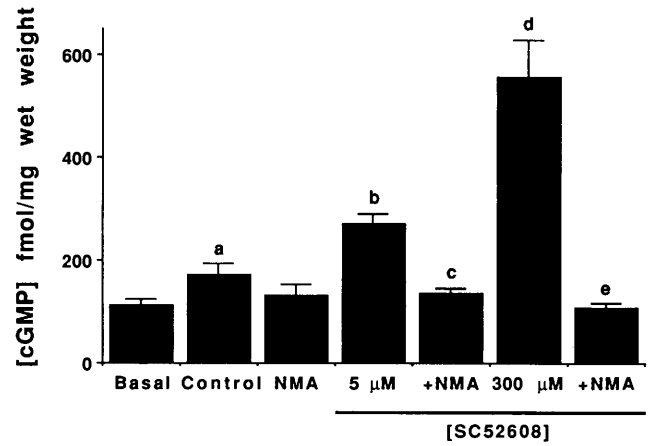

Figure 6. SC52608 potentiation of basal cGMP concentrations in rat aortic segments. All preparations except for the determination of basal levels were performed in the presence of zaprinast (100 μ*M*). Cyclic GMP concentrations were measured in rings after treatment with KCl alone (basal), KCl plus zaprinast (control), KCl plus zaprinast and NMA (NMA), SC52608 at the indicated concentrations, and SC52608 plus NMA (+NMA). (a) * vs basal, (b) * vs control, (c) * vs no NMA, (d) *** vs control, (3) *** no vs NMA. n = 4.

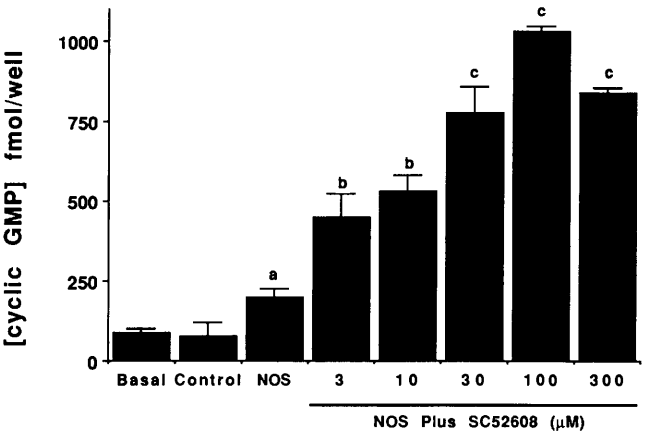

Figure 7. SC52608 potentiation of cGMP in a smooth muscle cell reporter system. Cyclic GMP concentrations were measured in rat aorta smooth muscle cells after no treatment (basal), SC52608 (300 μ*M*, control), nitric oxide synthase (NOS), and SC52608 at the indicated concentrations (in μ*M*) plus NOS. (a) ** vs basal, (b) ** vs NOS, (c) *** vs NOS. n = 4.

incorporated manganese (see above). However, there is a distinction between the relaxation of aortic segments by manganese or SC52608 (Fig. 8). Manganese chloride elicited a biphasic relaxation-contraction response relationship. At concentrations above 5 μM, manganese mediated contraction of the rings whereas SC52608 treatment resulted only in relaxation.

In conscious restrained rats, SC52608 delivered by bolus injection into femoral vein resulted in a dose-dependent decrease in mean arterial pressure (Fig. 9). A maximum decrease in pressure of 55 mm Hg was noted after injection of 30 mg/kg. The biological half-life of SC52608 *in vivo* was less than 5 min. Heart rate was not substantially altered by SC52608 except at the highest bolus dose (30 mg/kg) which caused a mild bradycardia.

Administration of SC52608 by intravenous infusion (1 mg/kg/min) resulted in a small decrease in arterial pressure similar to that noted during manganese treatment at the same concentration (Fig. 10). However, SC52608 had no apparent effect upon heart rate while manganese significantly decreased chronotropic activity.

Discussion

Previous studies of SOD have demonstrated the ability of this enzyme to prolong the biological half-life of NO and promote relaxation of vascular smooth muscle (3, 4). Manganese and manganese-containing preparations have been reported to mimic SOD enzymes as determined by electron spin resonance (6) and cytochrome *c* competition (7) assays. Gray and Carmichael (6) showed that the SOD activity of MnO_2-Desferal and MnO_2-Desferal-ascorbate complexes correlated with the manganese concentration present in the preparations suggesting that superoxide radical removal by these complexes is due to manganese. In contrast, manganese and manganese-desferal complexes showed no SOD activity in stopped-flow kinetic analysis (8). Weiss *et al.* (8) proposed that manganese and manganese-desferal complexes may interact stoichiometrically with superoxide.

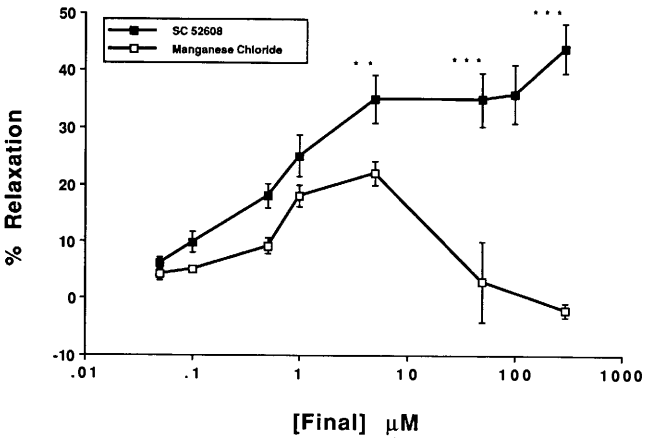

Figure 8. Comparison of manganese and SC52608 for relaxation of rat aortic rings. Segments were prepared as indicated in Figure 2. Significance was determined between SC52608 versus manganese treated rings. n = 4–9.

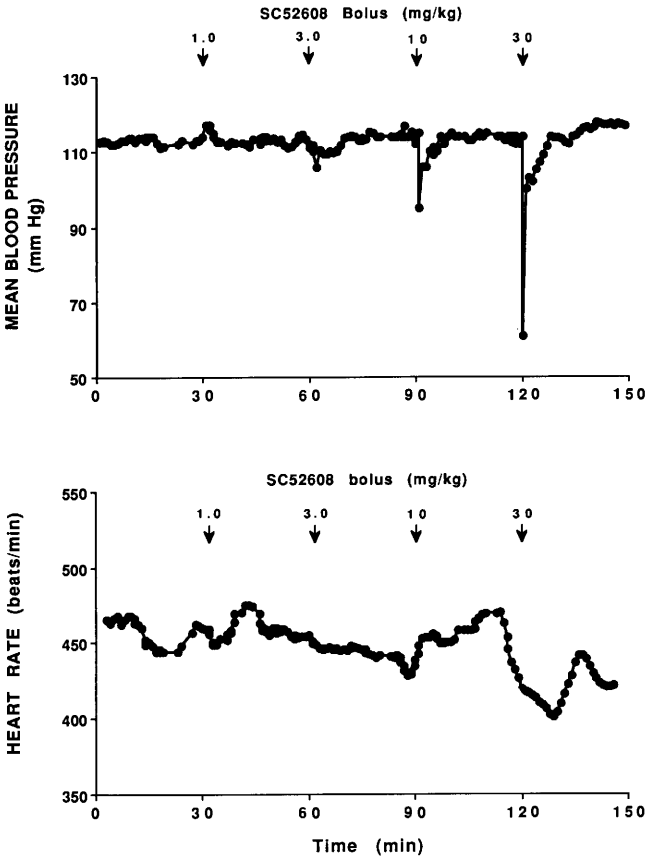

Figure 9. Effect of SC52608 injection upon blood pressure and heart rate in conscious normotensive rats. Experiments were run on four to six separate animals. Error bars are not shown for clarity. The standard error of the mean was ≤10% for each data point.

The activity of SC52608 as an SOD mimic was demonstrated in stopped-flow kinetic analysis (10). As an SOD mimic SC52608 may potentiate the biological half-life of NO. This mechanism for the vascular relaxation effect of SC52608 was supported in the aortic ring experiments by the endothelium dependence, the increase of cellular cGMP concentration, and the lack of relaxation activity for the mimic without incorporated metal (SC52612). Also, SC52608-mediated relaxation and cGMP accumulation in aortic rings was attenuated by the nitric oxide synthase inhibitor NMA. These data were consistent with SC52608 potentiation of endogenous NO released from vascular endothelial cells.

A comparison was made between relaxation of aortic rings mediated by SC52608 after contraction by KCl or $PGF_{2\alpha}$. SC52608 treatment resulted in 2-fold greater (88% vs 44%) relaxation in rings contracted with $PGF_{2\alpha}$ compared with those contracted with KCl. This increase in capacity to be relaxed by SC52608

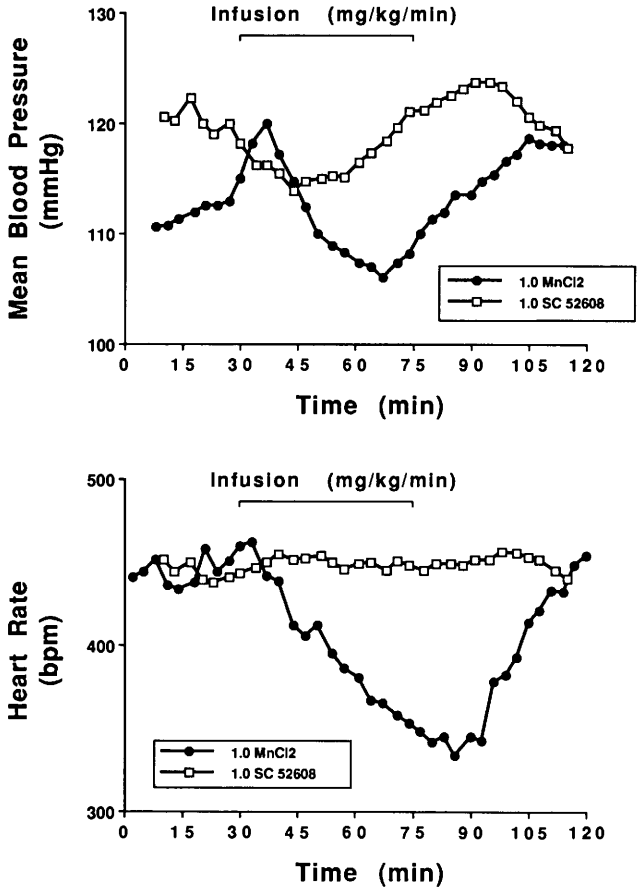

Figure 10. Comparison of SC52608 and manganese upon blood pressure and heart rate during infusion in conscious normotensive rats. Experiments were run on four to six separate animals. Error bars are not shown for clarity. The standard error of the mean was ≤10% for each data point.

was not due to increased tone in the rings by $PGF_{2\alpha}$ as the tissue contracted with prostaglandin developed less tone compared with those contracted with KCl (at the concentrations utilized). It appears that contractions of aortic rings induced by KCl undergo considerably smaller endothelium-dependent relaxations than do equivalent contractions induced by agents such as phenylephrine and $PGF_{2\alpha}$ which provide receptor-mediated contractions (28). The smaller relaxation responses in rings contracted by KCl may be a result of potassium-induced depolarization of the endothelial cells reducing the electrochemical gradient for calcium ion influx into these cells (29).

The smooth muscle cell reporter system for NO function represents a model of the intact aortic ring in which the cGMP response of the cells was measured directly without interference by other mediators released from endothelial cells. SC52608 potentiated the increase of cGMP mediated by addition of the NO generator in this model. This potentiation was observed at the same concentrations used in aortic rings and provided further evidence for the ability of SC52608 to protect NO.

Also in this study, SC52608 was tested for its ability to potentiate NO *in vivo*. Injection or infusion of SC52608 into conscious rats resulted in decreased blood pressure. Thus, SC52608 demonstrated the predicted *in vivo* activity of an SOD mimic to decrease basal tone by potentiation of NO.

In a prior report from this laboratory, manganese potentiated the biological effects of NO (9). There are several striking contrasts between the biological effects of manganese and SC52608. At concentrations above 5 μM, manganese caused a contraction of rat aortic rings, while SC52608 elicited relaxation at all concentrations tested. It should be noted that both SC52608 and manganese potentiated NO as measured by increased cellular concentrations of cGMP. Infusion of manganese into rats resulted in a decrease of blood pressure as well as a negative chronotropic effect. This work confirmed previous observations of the ability of manganese to suppress cardiac function (22, 23). In contrast, SC52608 decreased systemic blood pressure with no apparent effect upon cardiac activity. Thus, SC52608 potentiates NO in a manner similar to manganese without contractile activity at higher concentrations in aortic segments and without direct effects upon cardiac tissue. However, the actions of manganese on vascular smooth muscle are contractile at higher concentrations *in vitro* and negative chronotropic *in vivo* implying a dual mode of action.

SC52608 was synthesized as an effective scavenger of superoxide. Superoxide radical ion, a product of cellular respiration, activated leukocytes, and endothelial cells, has been demonstrated to be a mediator of ischemia reperfusion injury, inflammatory disease, and vascular disease (24–26). In many of the disease states noted above, SOD enzymes have been observed to provide beneficial effects. SC52608 has been studied extensively in an *in vitro* model of myocardial infarct and was shown to be an effective agent for reducing the superoxide-mediated injury resulting from reperfusion following ischemia (27). Thus, SC52608 may be an effective treatment in diseases which are mediated by an increase in superoxide production. The use of SC52608 to elucidate the role of superoxide in the disease states noted above will require further study.

In summary, this investigation demonstrated the effectiveness of SC52608, a newly synthesized manganese containing SOD mimic, to potentiate endogenous NO-mediated relaxation in rat aortic rings and exogenous NO generated by the addition of NOS into a smooth muscle cell reporter system. NMA blocked the SC52608 mediated decrease of vascular smooth muscle tone in a manner indicative of NMA attenuation of NOS activity. The data were consistent with the proposal that the mechanism of SC52608 mediated de-

crease of blood pressure was by acting as an SOD mimic. Thus, SC52608 potentiated NO resulting in enhancement of vascular smooth muscle cGMP concentration and decreased blood pressure.

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