Physiological Concentrations of Nitric Oxide Do Not Elicit an Acute Negative Inotropic Effect in Unstimulated Cardiac Muscle

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Abstract We examined the effect of several nitric oxide (NO) donors, authentic NO gas, and L-arginine in isolated cat and rat papillary muscles. We did not observe significant inotropic effects in response to any NO donor (ie, SPM-5185, C87-3754, and S-nitroso-N-acetylpenicillamine [SNAP]) from 1 mmol/L to 100 mmol/L. Similarly, authentic NO, at concentrations far in excess of those that maximally dilate the coronary vasculature (ie, 500 nmol/L), also failed to exert a detectable inotropic effect in these preparations. However, in the presence of 5 μmol/L norepinephrine, 500 nmol/L NO exerted a 12±3% decrease in isolated rat papillary muscle contractility (P<.05). Addition of L-arginine up to 25 mmol/L exerted no inotropic effects in isolated rat papillary muscles. However, at 50 mmol/L, L-arginine decreased contractile force by 21±4% (P<.01). On further examination, the negative inotropic effect of 50 mmol/L L-arginine appeared to be nonspecific, since the inactive stereoisomer, D-arginine, at 50 mmol/L exerted the same effect. Further studies in isolated adult rat cardiac myocytes elicited similar results, in that 50 mmol/L of L- and D-arginine equally decreased contraction amplitude and the underlying cytosolic calcium transient. Moreover, 500 mmol/L of the NO donor SPM-5185 only modestly decreased contraction amplitude or intracellular calcium in isolated rat cardiac myocytes. These results indicate that administration of physiological concentrations of exogenous NO does not acutely depress the inotropic state of the rat or cat heart to a physiologically significant extent. Only in the presence of high concentrations of norepinephrine did NO exert a statistically significant negative inotropic effect, and this effect was a modest one. These data demonstrate that physiological levels of NO do not exert a major regulatory effect on cardiac contractility. (Circ Res. 1994;75:692-700.)

Key Words • isolated cardiac myocytes • L-arginine • nitric oxide donor • cardiac contractility • papillary muscles

Nitric oxide synthase (NOS) exists in several isoforms, all of which use L-arginine as the substrate to produce nitric oxide (NO).1,2 Endothelium-derived NO is synthesized by a constitutive NOS and is critical for the preservation of vascular homeostasis.1,3 The constitutive NO synthase is associated with low levels of NO production, which are continuously generated (ie, basal production).4 Despite these modest levels of NO production, NO released via the constitutive pathway is sufficient to maintain vascular tone and inhibit neutrophil and platelet adhesion to the vascular endothelium under control conditions.5,6 These physiological concentrations apparently are between 0.1 and 1 nmol/L in the coronary circulation.7 Similarly, therapeutic concentrations (ie, ~500 nmol/L) of agents that release physiological concentrations of NO (ie, NO donors) also inhibit neutrophil and platelet adhesion to endothelial cells and preserve the ischemic/reperfused myocardium.8,9 Although low concentrations of NO clearly have cardioprotective effects in ischemic reperfused hearts, it is not known whether these concentrations exert any direct effect on cardiac contractility.

Another form of NOS, known as inducible NOS, can be activated by cytokines and endotoxin. Its expression peaks after 6 to 12 hours in various cell types (eg, macrophages and vascular smooth muscle).11-13 Although direct measurements of endogenous NO are still not available because of its short half-life in vivo, indirect measurements indicate that NO is produced at significantly higher levels via the inducible NOS pathway.4 These high concentrations of NO are thought to depress cardiac contractility during the late stages of septic shock.14 N⁰-Nitro-L-arginine methyl ester (L-NAME) and N⁰-monomethyl-L-arginine (L-NMMA), two NOS inhibitors, do not directly alter myocyte contractility, indicating that cardiac myocytes do not synthesize appreciable amounts of constitutive NO in their normal state.11,14-17 However, Finkel et al18 reported that tumor necrosis factor-α (TNF-α) depressed contractility of isolated papillary muscles within 3 to 5 minutes, an effect that could be reversed by L-NMMA. Since L-arginine reversed the effects of L-NMMA, the authors speculated that the acute negative inotropic effects of TNF-α were elicited by a constitutive NOS. However, these studies do not conclusively prove whether NO released via the constitutive NOS pathway is sufficient to acutely depress cardiac contractility. More recently, Brady et al19 bypassed the NO biosynthetic pathway by
directly adding sodium nitroprusside, an NO donor, to isolated cardiac myocytes. The authors demonstrated that myocyte contraction amplitude was decreased by ≈20% (ie, from 5.8% to 4.7% of resting cell length) in the presence of 10 μmol/L sodium nitroprusside. However, no one has compared physiological amounts of NO in these diverse preparations and compared them with effects on vascular smooth muscle.

In view of these uncertainties and unresolved questions, we examined the inotropic effects of authentic NO, newly developed organic NO donors that release NO without the need for metabolic transformation by smooth muscle cells, and the precursor of endogenous NO synthesis (ie, L-arginine) on cardiac contractility in rat and cat myocardium in preparations ranging from isolated cardiac myocytes to isolated papillary muscles and compared their effects in vascular smooth muscle preparations isolated from the same animal.

Materials and Methods

Isolated Cat Coronary Artery Ring Studies

Adult male cats (2.5 to 4.0 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV). After a midsternal thoracotomy, the heart was quickly removed and placed into Krebs-Henseleit (K-H) solution consisting of (mmol/L) NaCl 118, KCl 4.75, CaCl2 1.25, MgSO4·7H2O 1.19, NaHCO3 12.5, and glucose 10.0. Both left anterior descending coronary artery and left circumflex coronary artery segments (internal diameter, 0.3 to 0.5 mm) were dissected, cleaned of fat and connective tissue, and carefully cut into rings 2 to 3 mm in length. The rings were then mounted on stainless-steel hooks, suspended in 7-mL tissue baths, and subsequently connected to Grass FT-03 force displacement transducers to record changes in force by using a Grass model 7 oscillographic recorder (Grass Instrument Co.). The baths were filled with 7 mL K-H solution and aerated at 37°C with a gas mixture of 95% O2/5% CO2. Coronary rings were initially stretched to give a preload of 0.5 g of force and equilibrated for 90 minutes. During this period, the K-H solution in the tissue baths was replaced every 20 minutes. After equilibration, the rings were then precontracted by 100 mmol/L U-46619 (9,11-epoxymethanoprostaglandin H2), a thromboxane A2 mimetic, to generate ≈0.5 g of developed force. Once a stable contraction was obtained, either 5-nitroso-N-acetylpenicillamine (SNAP), a nitrosothiol NO donor, SPM-5185 [N-nitratopivaloyl-3-(N3-acetylalanyl)-cysteine ethyl ester], a cysteine-containing NO donor, or C87-3754 [3-[1-(2,6-dimethylpiperi
dino)-sydnonimine-hydrochloride-hydrate], a sydnonimine NO donor, was separately added to the bath cumulatively (SNAP, 1, 2.5, 5, 10, 25, 50, and 250 mmol/L; SPM-5185 and C87-3754, 1, 2.5, 5, 10, and 50 μmol/L). Relaxation responses were expressed as a percentage of the U-46619-induced contraction.

Authentic NO was bubbled for 1 hour from a gas cylinder containing 1% NO/99% N2 into O2-purged distilled water or K-H solution in a specially designed airtight chamber. Just before addition, the NO solution was withdrawn from the bubbling chamber into an airtight glass syringe. The solution was then added to the 7-mL vascular ring bath at volumes of 0.1 to 0.3 mL; measured NO concentrations in the baths were 160 to 500 mmol/L, respectively, with these NO additions. Vasorelaxation responses of coronary artery rings to NO solution were assessed as described above.

Isolated Cat Papillary Muscles

Adult male cats (2.5 to 4.0 kg) were anesthetized with sodium pentobarbital, and their hearts were quickly excised. Papillary muscles <1.5 mm in diameter were dissected from the right ventricle in warmed oxygenated K-H solution, and a 0.8 silk suture was tied to each end. The preparations were suspended individually in the tissue chambers and subsequently connected to Grass FT-03 force displacement transducers to record changes in force with a Grass model 7 oscillographic recorder. Each muscle was stretched to the length at which contractile force was maximal (preload, 1.0 to 2.0 g). The preparations were electrically paced at 1 Hz with pulses of 5-millisecond duration at a voltage ≈50% greater than threshold. All preparations were allowed to equilibrate in drug-free K-H solution for 40 to 60 minutes until complete mechanical stabilization had been achieved. All NO-donating compounds were freshly dissolved in prewarmed and preaerated K-H solution and cumulatively added to the bath chambers. Fresh NO in K-H solution (0.1 to 0.6 mL) was added to a 7-mL tissue bath cumulatively. Measured NO concentrations in the bath were 160 to 960 mmol/L. After each observation, muscles were washed several times and allowed to recover for 10 to 20 minutes until their mechanical function completely returned to control values.

Isolated Rat Papillary Muscles and Rat Aortic Rings

Male Wistar rats (250 to 300 g) were anesthetized with sodium pentobarbital (50 mg/kg), and their hearts were quickly excised. Papillary muscles <1 mm in diameter were dissected from the left ventricle in oxygenated K-H solution, and their mechanical response to authentic NO, NO donors, arginine, and aspartic acid was studied as described above for cat papillary muscles. The pH of the 50-mmol/L L-arginine and t-arginine solutions was found to be 9.74±0.15 and 9.66±0.13, respectively. In some experiments, NO gas was added to papillary muscles in the presence of 10 nmol/L to 5 μmol/L norepinephrine.

Rat aortic rings were isolated from the same rats as those in which the papillary muscles were obtained. The aorta was trimmed of fat and connective tissue and cut into rings of 2 to 3 mm. The rings were mounted in 7-mL tissue baths as described above for the cat coronary arteries. After equilibration for 90 minutes in K-H solution, the aortic rings were precontracted with 5 μmol/L norepinephrine bitartrate (Sterling-Winthrop). After 10 to 15 minutes, developed force stabilized, and NO gas could be added to the bath under control conditions or under conditions of enhanced constrictor tone in response to 5 μmol/L norepinephrine.

Measurement of NO Concentrations

NO was measured using an ISO-N0 meter obtained from World Precision Instruments, Inc. This NO meter was connected to a polarographic NO electrode described by Shibuki and Okada. The current flow across this electrode is proportional to the NO concentration at the surface of the membrane on the electrode. Calibration and amperometric measurements were made according to the methods of Tsukahara et al and recorded on a strip-chart recorder (BD 40, Kipp and Zonen). All values reported were means of at least six measurements reported as nanomolar NO concentrations.

Isolated Rat Cardiac Myocytes

Ca2+-tolerant quiescent cardiomyocytes were isolated from the hearts of adult male Sprague-Dawley rats (350 to 400 g) by retrograde aortic collagenase perfusion as described previously. The cells were finally resuspended at a concentration of 5 mg cell protein per milliliter in incubation buffer composed of (mmol/L) NaCl 121, HepES 10, NaHCO3 5, KCl 4.7, KH2PO4 1.2, MgSO4 1.2, CaCl2 1.25, and glucose 10, along with 1.8% bovine serum albumin at pH 7.4. The cardiomyocytes were loaded with fura 2 acetoxymethyl ester (2 to 4 μmol/L) by incubation at room temperature for 25 minutes with gentle shaking.

After loading with fura 2, the cardiomyocytes were washed and placed in an open flow chamber mounted on the heated
stage of a Zeiss inverted epifluorescence microscope. The stage (X20 glycerin immersion objective, Nikon Inc) and chamber were thermostatically regulated at 37°C. The chamber was equipped with platinum electrodes to allow electrical field stimulation of the myocytes within the field of view. A stimulation of 0.25 Hz was used throughout all experiments. Fluorescence was collected alternately at excitation wavelengths of 340 and 380 nm (10-nm bandwidth filters) to excite the Ca2+-bound and Ca2+-free forms of this ratiometric dye, respectively. The emission wavelength was 510 nm (120-nm bandwidth filter). The cells were perfused continuously at a flow rate of 7 mL/min with modified incubation buffer containing 0.01% bovine serum albumin and 2 mmol/L CaCl2. To ensure that a steady state was achieved, [Ca2+]i, and the contractile parameters were obtained under control conditions, at 10-minute intervals after preincubation with L-arginine, d-arginine, SPM-5185, and SPM-5267 and after washout. The imaging system, techniques for obtaining digital images of cellular fluorescence at high time resolution, and calculation of [Ca2+]i have been described in detail previously.21 Cell lengths were determined at each time point during the contractions from a combination of the 340- and 380-nm fluorescence images by using an edge detection program that compensated for any time-dependent changes in cellular fluorescence. Since absolute length varied significantly from cell to cell, contraction was expressed as the maximum percent change from the resting cell length or as shortening expressed as percentage of resting cell length. Cardiomyocytes were studied under control conditions and conditions of adrenergic stimulation with 1 μmol/L norepinephrine.

Materials

Bovine serum albumin, aspartic acid, L-arginine, d-arginine, and L-NMMA were obtained from Sigma Chemical Co. L-NAME was obtained from Calbiochem-Novabiochem Co. U-46619 and SNAP were obtained from Biomol Laboratories. SM-5185 and SPM-5267 were obtained from Schwarz Pharma. C87-3754 was obtained from Cassella AG. Human recombinant superoxide dismutase (hSOD) was obtained from Grünenthal GmbH. Fura 2 acetoxymethyl ester was obtained from Molecular Probes Inc.

Statistical Analysis

All values in the text and figures are presented as mean±SEM of n independent experiments. Data were subjected to ANOVA followed by the Bonferroni correction for post hoc t test comparison. For experiments using single cardiomyocytes, each cell acted as its own control, and the drug treatments were analyzed by a paired t test. Values of P<.05 were considered to be statistically significant.

Results

Vasodilator and Inotropic Effects of NO Donors and Authentic NO in Cat Papillary Muscles

To confirm that SNAP, SPM-5185, and C87-3754 released NO and were physiologically active, these NO donors were tested in isolated cat coronary artery rings. Fig 1 (left panel) illustrates the concentration-response curves to SNAP, SPM-5185, and C87-3754 in cat coronary artery rings. All three NO donors produced a concentration-dependent relaxation to U-46619–contracted coronary artery rings, indicating that the NO donors were active under the conditions of our experiments. SNAP, SPM-5185, and C87-3754 fully relaxed coronary artery rings at 50 nmol/L, 5 μmol/L, and 10 μmol/L, respectively. However, none of these NO donors exerted any detectable inotropic effect on cat ventricular papillary muscles from 1 nmol/L up to 100 μmol/L. This highest concentration is up to 1000 times

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**Fig. 1.** Cumulative concentration-response curves to nitric oxide donors (S-nitroso-N-acetylpenicillamine [SNAP], SPM-5185, and C87-3754) in cat coronary artery rings contracted with 100 nmol/L of U-46619 (left) and cat right ventricular papillary muscles (right). Results are expressed as mean±SEM of 9 to 12 coronary artery rings and 8 to 10 papillary muscles. **P<.01 vs control value.
higher than the maximal vasodilator concentrations (Fig 1, right panel) of these NO donors. In contrast, addition of 10 to 100 μmol/L sodium pentobarbital to the same preparations induced a negative inotropic effect of 75% to 80% (Fig 1, right panel), indicating that the papillary muscles were indeed responsive to negative inotropic agents.

To eliminate the possibility that the lack of inotropic effect in response to NO donors was due to their failure to release NO in a cardiac preparation, we studied the effect of authentic NO gas in both coronary artery rings and cardiac papillary muscles. Fig 2 represents typical recordings of responses of a cat coronary artery ring and a cat papillary muscle to NO. Addition of 0.2 mL of an NO solution corresponding to 320 nmol/L NO induced a full vasorelaxation in an isolated cat coronary artery ring. However, addition of a saturated NO solution up to 0.6 mL (ie, 960 nmol/mL) had no detectable inotropic effect. Moreover, it is well known that addition of hSOD may prolong the half-life of NO and thus enhance its biological effect. However, addition of 150 μg/mL hSOD alone or hSOD in the presence of NO simultaneously failed to exert any significant inotropic effect in cat papillary muscles. In seven papillary muscles, developed force was 98±3% of control for 150 μg/mL hSOD alone and 97±2% of control for hSOD plus 960 nmol/L NO solution. These values were not significantly different from each other or from 100% (ie, basal contractility of these muscles).

**Inotropic Effects of NO and L-Arginine in Rat Papillary Muscles**

To determine whether this lack of inotropic effect of NO solution or NO donors on papillary muscles was due to a peculiarity of cat cardiac muscle, we observed their inotropic effect in isolated rat papillary muscles. Fig 3 summarizes the results obtained from 9 to 11 rat papillary muscles studied. Addition of SNAP (1 to 250 nmol/L), SPM-5185, and C87-3754 (1 to 50 μmol/L) or addition of 0.1 to 0.6 mL NO solution failed to exert any detectable inotropic effect. However, addition of 10 to 100 μmol/L sodium pentobarbital to the same preparations induced a prominent negative inotropic effect. Cardiac contractile force decreased 81±4% at 100 μmol/L pentobarbital. These results indicate that the lack of the inotropic responses to NO is not specific for cat cardiac muscle.

To clarify whether addition of the substrate for NOS enhances endogenous NO production and thus may exert inotropic activity, we studied the effect of L-arginine, the precursor of endogenous NO synthesis, on isolated rat papillary muscles. At concentrations of 1 to 10 mmol/L (pH 7.4), l-arginine did not exert any acute inotropic effects (contractile force, 97±4% and 98±3% of control at 1 and 10 mmol/L, respectively). Moreover, addition of 10 mmol/L l-arginine for periods of up to 30 minutes did not produce any detectable inotropic effects. However, addition of a very high concentration of l-arginine (50 mmol/L) induced a significant negative inotropic effect in rat papillary muscles. At 50 mmol/L
L-arginine, rat papillary muscle contractions decreased to 21±4% of the control value (Fig 4). Surprisingly, addition of D-arginine, the inactive stereoisomer of L-arginine that does not generate NO, also exerted the same negative inotropic effects as observed with L-arginine (Fig 4). Moreover, the L-arginine-induced negative inotropic effect could not be blocked by either L-NMMA at concentrations up to 25 mmol/L (Fig 4) or L-NAME, another NOS inhibitor, at concentrations up to 50 mmol/L. The contractile force at 50 mmol/L L-arginine decreased 21±4% and 23±3% in the presence and absence of 50 mmol/L L-NAME, respectively. These values are not significantly different from each other. Therefore, the inotropic effect of L-arginine does not appear to be related to NO generation, since L-NMMA or L-NAME could not block this effect and D-arginine exerted the same inotropic effect as L-arginine.

We examined the effect of NO gas on norepinephrine-stimulated aortic rings and papillary muscles isolated from the same rats. Norepinephrine (5 μmol/L) significantly increased contractile force in the papillary muscle and increased tone in the aortic rings. NO was added to the baths and was measured by using the NO-selective electrode described in "Materials and Methods." At 146 nmol/L NO, there was a significant vasorelaxation of the aortic rings but no significant decline in the cardiac muscle contractility. However, at 500 mmol/L, NO completely relaxed the aortic rings and exerted a 12% decrease in papillary muscle contractility (P<.05). Thus, NO can exert a significant inotropic effect in the presence of a β-adrenergic agonist. However, the effect is modest and is significant only at a high NO concentration. To more fully investigate the effects of NO on cardiac muscle under adrenergic stimulation, we studied rat papillary muscles in the presence of 10 nmol/L to 5 μmol/L norepinephrine. Fig 5 summarizes these results. Although 10 nmol/L norepinephrine did not significantly increase contractile force, 100 nmol/L, 1 μmol/L, and 5 μmol/L norepinephrine significantly increased contractile force (P<.05, P<.01, and P<.01). However, NO gas from 52 to 500 nmol/L had no effect on contractile force at any norepinephrine concentration, except that 500 nmol/L NO exerted a significant decrease in contractile force at 5 μmol/L norepinephrine. Thus, even under high adrenergic stimulation, physiological concentrations of NO do not depress contractility of rat papillary muscles. Only at very high concentrations of NO was even a modest negative inotropic effect observed in the presence of norepinephrine.

**Effect of SPM-5185 and L-Arginine on Isolated Rat Cardiac Myocytes**

Finally, we evaluated the effects of NO donors and precursors on electrically triggered Ca²⁺ transients and contraction in isolated rat ventricular myocytes. Fig 6, top panel, shows mean contraction responses of cardiomyocytes in the presence of L-arginine, D-arginine, SPM-5185, or SPM-5267 (an analogue of SPM-5185 that is not an NO donor) compared with their initial control myocytes. L-Arginine had little effect at 5 mmol/L but caused a 35% decrease in cardiomyocyte shortening after 10 minutes of exposure at a concentration of 50 mmol/L (P<.01, Fig 6, top panel). Similar responses in cell shortening were observed with 50 mmol/L D-arginine. In addition to the depression of peak contractility at 50 mmol/L, both L- and D-arginine decreased the rate of shortening and prolonged the time...
to peak contraction (Fig 6, top panel). In the presence of 500 nmol/L SPM-5185, there was only a 13% decrease in cell shortening ($P<.05$). This was the maximum response of these cardiomyocytes to the NO donor, since addition of 10 μmol/L SPM-5185 had no further effect. The NO-free control compound, SPM-5267, had no effect on cell shortening at either 500 nmol/L or 10 μmol/L. For each of the test agents that depressed cardiomyocyte contraction, the effects were reversed at least partially during 10 minutes of washout. The inhibition of cardiomyocyte shortening induced by SPM-5185 and L- and D-arginine was paralleled by
decreases in the amplitude of the Ca\(^{2+}\) transient. Fig 6, bottom panel, shows mean Ca\(^{2+}\) transients for each experimental group compared with their initial control transients. None of the test agents caused significant changes in basal Ca\(^{2+}\). In contrast to the altered kinetics of contraction, the depression of peak Ca\(^{2+}\) in the presence of 50 mmol/L L- or D-arginine was not associated with any shift in the time course of the Ca\(^{2+}\) transient. In cells treated with SPM-5185, the time courses of both the Ca\(^{2+}\) transient and contraction were essentially unchanged apart from the reduced amplitudes of these processes (Fig 6, bottom panel). In agreement with the lack of effect on contraction, SPM-5267 had no effect on the Ca\(^{2+}\) transient. For the group of cardiomyocytes treated with SPM-5185, the reduced amplitude of the Ca\(^{2+}\) transient was highly correlated with the depression of cell contraction with a slope of 0.828 (r=0.923, P<0.001). The effects of L- and D-arginine on Ca\(^{2+}\) and contraction were less well correlated (r=0.791 and 0.790, respectively), and the slopes of these relations were much less steep (0.445 and 0.571, respectively). Taken together with the differential effects of the two arginine isomers on the kinetics of Ca\(^{2+}\) and contraction, these data suggest that high levels of arginine may influence cardiomyocyte contraction through a second mechanism in addition to the inhibition of the Ca\(^{2+}\) transient.

In an additional series of isolated rat cardiomyocytes, we stimulated the cells with 1 mmol/L norepinephrine. Five rat cardiomyocytes shortened 2.8±0.4% of resting cell length under control conditions. In the presence of 1 mmol/L norepinephrine, these five myocytes shortened 15.2±0.6% (P<0.1). However, when 10 mmol/L SPM-5185 was added together with the 1 mmol/L norepinephrine, the degree of shortening was 16.1±0.6%, a value not significantly different from norepinephrine alone. Similarly, the intracellular Ca\(^{2+}\) transient amplitude increased from 151±10 nmol/L under resting conditions to 679±46 nmol/L on stimulation with 1 mmol/L norepinephrine (P<0.01). When 10 mmol/L SPM-5185 was added in the presence of norepinephrine, the Ca\(^{2+}\) transient was 704±45 nmol/L, a value not significantly different from the value obtained with norepinephrine alone. These data indicate that even under norepinephrine stimulation, high concentrations of an NO donor were ineffective in exerting a significant negative inotropic effect.

**Discussion**

NO is an important messenger that regulates physiological processes throughout the cardiovascular system. The goal of the present study was to determine the effect of physiological concentrations of NO, which would most likely be produced via the constitutive NOS pathway. We administered these therapeutic concentrations to isolated papillary muscles and to isolated individual cardiac myocytes in order to study myocardial contractility under different levels of organization. Very few studies exist of the inotropic effects of NO in vivo. Lefer et al.\(^{10}\) reported that SPM-5185 at 500 nmol/L attenuated myocardial necrosis in ischemic/reperfused dog hearts. The NO donor was infused directly into the coronary circulation, and no evidence of reduced cardiac contractility was observed. In fact, the NO donor was highly effective as a cardioprotective agent retarding myocardial necrosis. More recently, Hasebe et al.\(^{20}\) investigated the effect of endogenous NO on myocardial contractility in conscious dogs after post-ischemic cardiac stunning. These investigators found that inhibition of NO synthesis by L-NAME further depressed cardiac stunning (ie, exacerbated the decreased cardiac contractility). These results suggest that NO may be important for maintaining normal cardiac contractility. On the other hand, it is unlikely that vagal stimulation, which should release acetylcholine in the ventricul myocardium, could indirectly release NO as acetylcholine does on vascular endothelial cells. Therefore, we do not believe that a marked degree of cholinergic activation could decrease myocardial contractility via an NO mechanism.

Although few in vivo studies exist on the effect of NO release on cardiac contractility, a number of investigators have perfused NO donors or NOS inhibitors through the coronary circulation of isolated perfused hearts and subsequently measured cardiac function. Sodium nitroprusside at 10 μmol/L injected into the coronary circulation of isolated perfused ferret hearts decreased peak left ventricular pressure by 5% to 6%.\(^{20}\) However, infusion of the same concentration of sodium nitroprusside directly into the left ventricle had no effect on cardiac function. It is well known that vasodilating agents have significant indirect effects on cardiac contractility that are due to their coronary and systemic vasodilator effects, although it is possible that NO could diffuse directly from the capillary endothelial cells to cardiac myocytes. However, it is difficult to conclude that NO is a potent negative inotropic agent on the basis of the small decrease in peak left ventricular pressure observed in response to sodium nitroprusside infusion into the coronary circulation. In another study, Amrani et al.\(^{12}\) demonstrated that L-NMMA, an NOS inhibitor, significantly decreased dP/dt, an index of cardiac contractility, in the isolated working rat heart. The authors demonstrated, however, that this effect was largely attributed to decreases in coronary flow (ie, 39% of its basal values) since L-NMMA did not alter the contractile activity of isolated cardiac myocytes. Taken together, these results suggest that NO does not elicit a physiologically significant acute negative inotropic effect on the whole heart.

To rule out the possibility that NO delivered via the coronary circulation may not reach cardiac myocytes at concentrations high enough to exert inotropic effects and to exclude indirect changes in cardiac contractility induced by NO due to its vasodilator properties,\(^{26}\) we studied the direct effect of NO donors and authentic NO on ventricular papillary muscle contractility. The NO donors studied (SNAP, SPM-5185, and C87-3754) all readily release NO in vitro without the need for cellular metabolism.\(^{27}\) This release of NO was confirmed, since these agents relaxed coronary artery rings completely (Fig 1). However, these NO donors had no detectable acute inotropic effects on either cat or rat papillary muscles. Similarly, authentic NO, at concentrations exceeding those which induced a maximal vasodilation, did not exert any detectable negative inotropic effects in isolated rat or cat papillary muscles. Our results clearly demonstrate that NO does not exert any significant acute negative inotropic effect in either cat or rat papillary muscles. These results, however, do not
The effects of SPM-5185 on cardiomyocyte contraction coincide with the study of Brady et al.,16 who found that similar concentrations of sodium nitroprusside or authentic NO also elicited a small reduction in myocyte shortening. Specifically, Brady et al found that 10 μmol/L sodium nitroprusside or authentic NO reduced the contractile amplitude of cardiac myocytes from 5.8% to 4.7% of resting length. At lower concentrations, these agents reduced the contractile amplitude by 4.3%, suggesting that this modest effect is the maximum effect possible. The results from our study and those of Brady et al demonstrate that NO does not have an appreciable effect on contractile amplitude when administered at therapeutic concentrations. However, in pathological conditions such as might occur in septic shock, where NO is produced via the inducible NOS pathway, excessive amounts of NO could contribute to decreased cardiac contractility if NO reached micromolar concentrations, a level not yet shown to circulate under any circumstances. At present, nanomolar concentrations have been reported in the coronary circulation in non–blood-perfused hearts.24 Even lower concentrations of NO would probably circulate in blood, since oxyhemoglobin rapidly inactivates NO. Alternatively, physiological concentrations of NO (ie, 1 to 2 nmol/L) may indirectly decrease the effect of β-adrenergic agonists on cardiac contractility after prolonged exposure to endotoxin (ie, 24 hours), since Balligand et al11 found that L-NMMA attenuated the positive inotropic response to isoproterenol in myocytes preincubated with medium derived from endotoxin-activated macrophages. However, Balligand and coworkers11,15 found that L-NMMA did not affect baseline contractile function of control myocytes, indicating that constitutive NO released by cardiac myocytes does not appear to alter basal cardiac contractile amplitude. However, Balligand et al11 reported that L-NMMA significantly increased the inotropic effect of isoproterenol in isolated adult cardiac myocytes. Our results showing that NO gas exerts a modest negative inotropic effect in the presence of norepinephrine are consistent with these findings.

In the present study, we found that L-arginine, the substrate for endogenous NO synthesis, does not significantly decrease myocyte contraction amplitude or intracellular Ca2+ fluxes at concentrations up to 10 mmol/L. In contrast, 50 mmol/L L-arginine depressed myocyte contraction amplitude and significantly decreased intracellular Ca2+. However, the same negative inotropic effect also occurred in response to d-arginine, indicating that the negative inotropic effect elicited by 50 mmol/L L-arginine is not related to NO generation. The nonspecific effect of high concentrations of L-arginine (ie, 50 to 100 mmol/L) on cardiac myocytes directly coincides with its nonspecific effect on isolated papillary muscles. The inability of L-arginine to elicit negative inotropic effects through NO generation is not surprising, since the availability of L-arginine in normal states is not rate limiting in the synthesis of NO.29 Moreover, other investigators have shown that NOS inhibitors, particularly L-NMMA and L-NAME, do not alter basal contractility in isolated cardiac myocytes.11,14-17 These results, in conjunction with previous studies, demonstrate that endogenous NO released via the constitutive pathway does not elicit significant acute negative inotropic effects.

coincide with Meulemans et al,28 who found that 10 μmol/L sodium nitroprusside induced early relaxation and increased peak twitch in a similar preparation of isolated cat papillary muscles. Although the apparent differences between their study and our present findings are not clear, the total reduction in peak tension reported by Meulemans et al was very small, ≈3% to 5%. It is unlikely that these small decreases in contractility would have an appreciable effect on cardiac output or cardiac contractility in vivo.

To assess whether endogenous NO release exerts similar effects on cardiac contractility when compared with exogenous NO administration, we administered L-arginine, the precursor of NO synthesis, to rat papillary muscles. We found that concentrations of L-arginine up to 10 mmol/L had no significant effect on papillary muscle contractility. However, at 50 mmol/L, L-arginine exerted a significant negative inotropic effect similar to that reported by Finkel et al,18 who also found that 50 to 100 mmol/L L-arginine exerted a negative inotropic effect in hamster papillary muscle. However, on further examination, we found that the negative inotropic effect elicited by these extremely high concentrations of L-arginine was not related to NO generation, since d-arginine, the inactive stereoisomer of L-arginine, also induced a similar negative inotropic effect in rat papillary muscles. Moreover, the negative inotropic effect elicited by high concentrations of L-arginine could not be reversed by L-NMMA or L-NAME, two NOS inhibitors. Since Finkel et al10 did not use d-arginine, it is not clear whether their results with high concentrations (100 mmol/L) of L-arginine can be attributed to endogenous NO release or nonspecific actions of high concentrations of L-arginine. In this regard, we have demonstrated that high concentrations of aspartic acid, another amino acid, also elicited significant acute negative inotropic effects on rat papillary muscle clearly unrelated to NO synthesis. Differences between the results found in the present study and those found by Finkel et al18 could be due to differences in experimental design or species. Finkel et al also found that TNF-α exerted a negative inotropic effect within 2 to 3 minutes in hamster papillary muscles, an effect that was reversed by L-NMMA. However, we failed to observe any detectable acute negative inotropic effect in response to authentic NO or TNF-α alone or in combination.

To address the possibility that this lack of a negative inotropic effect was a result of the inability of NO to diffuse through all layers of cardiac tissue, we studied the effect of the NO donor SPM-5185 on contractile responses and intracellular Ca2+ in cardiac myocytes. In this isolated cell preparation, SPM-5185 caused a small depression of cell shortening. The maximal response to the NO donor represented a 13% depression of peak shortening (a reduction from 9.2% to 8.0% of initial cell length). Furthermore, the data obtained with fura 2 demonstrate that the reduced contractility of isolated cardiomyocytes can be explained by inhibition of the amplitude of the Ca2+ transient, with no change in basal (diastolic) Ca2+. The extent of these changes with maximal doses of SPM-5185 are very small compared with the effects of other inotropic agents in this preparation. For example, β-adrenergic stimulation increased the amplitude of contraction and the Ca2+ transient by about twofold.21,23
In summary, our results indicate that physiologically relevant concentrations of NO do not induce physiologically significant negative inotropic effects acutely in two different mammalian species in which we investigated the inotropic effect of NO on cardiac muscles at two different levels of organization (ie, isolated cardiac myocytes and isolated ventricular papillary muscles). The only condition in which a significant, albeit small, negative inotropic effect occurred is under the influence of high concentrations of norepinephrine. These results provide substantial evidence that low levels of NO, which inhibit neutrophil and platelet adhesion as well as maintain basal vascular tone, do not exert a major effect on cardiac contractility. If anything, abolition of endogenous NO reduces myocardial contractility in the conscious dog. Moreover, a recent study indicates that NO does not exert a significant negative chronotropic effect in isolated rat atrial preparations.

Acknowledgments

This study was supported in part by research grant GM-45434 from the Institute of General Medical Science of the National Institutes of Health (NIH), NIH postdoctoral fellowship HL-08507 (Dr Weyrich), a Deutsche Forschungsgemeinschaft postdoctoral fellowship (Dr Buerek), and a Japan Heart Foundation postdoctoral fellowship (Dr Murohara). The authors thank Robert Craig for expert technical assistance and Dr Dominique C. Renard-Rooney for assistance during the isolated myocytes studies.

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A S Weyrich, X L Ma, M Buerke, T Murohara, V E Armstead, A M Lefer, J M Nicolas, A P Thomas, D J Lefer and J Vinten-Johansen

doi: 10.1161/01.RES.75.4.692

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/75/4/692

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