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Exogenous nitric oxide reduces oxygen consumption of isolated ventricular myocytes less than other forms of guanylate cyclase stimulation

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Abstract

We tested the hypothesis that increasing cyclic GMP with nitric oxide (NO) would reduce cardiac myocyte metabolism less than other forms of guanylate cyclase stimulation. The steady state O_2 consumption (VO_2) of a suspension of ventricular myocytes in 2.0 mM Ca^{2+} isolated from hearts of New Zealand white rabbits was measured in a glass chamber using Clark-type oxygen electrode. The cellular cyclic GMP levels, determined by radioimmunoassay, were increased by (1) adding 3-morpholinosydnonimine (SIN-1, 10^{-8}–10^{-5} M) and nitroprusside (10^{-8}–10^{-5} M), NO donors-soluble guanylate cyclase stimulators; (2) carbon monoxide (CO, 1.5×10^{-8}–1.5×10^{-6} M), soluble guanylate cyclase stimulator and (3) guanylin (10^{-8}–10^{-5} M), particulate guanylate cyclase stimulator. The baseline myocyte cyclic GMP level was 86 ± 13 fmol/10^5 myocytes with a corresponding VO_2 of 268 ± 21 nl O_2/min per 10^5 myocytes. An inverse relationship between cellular cyclic GMP levels and VO_2 existed in these myocytes. The regression equations for the four treatments were: $VO_2 = -0.45\times[\text{cyclic GMP}] + 294.4$, $r = 0.94$ for SIN-1; $VO_2 = -1.46\times[\text{cyclic GMP}] + 444.7$, $r = 0.96$ for CO; $VO_2 = -1.25\times[\text{cyclic GMP}] + 389.1$, $r = 0.84$ for guanylin and $VO_2 = -0.55\times[\text{cyclic GMP}] + 322.8$, $r = 0.79$ for nitroprusside. The regression lines of the two NO donors were parallel. A similar result was also evident for the regressions of CO and guanylin. However, the slopes of both the SIN-1 and nitroprusside regression line were significantly less steep than that of either the CO or guanylin lines. Therefore, VO_2 is reduced less for a similar increase in cyclic GMP with NO donors compared to direct stimulation with CO or guanylin. These results suggest that NO has metabolic effects on myocytes in addition to its stimulatory effects on cellular cyclic GMP. © 1998 Elsevier Science B.V.

Keywords: cGMP; Cardiac myocyte; Nitric oxide (NO); Guanylate cyclase stimulation; Oxygen consumption; (Rabbit)

1. Introduction

Cyclic GMP has negative metabolic and functional effects on cardiac myocytes (Lohmann et al., 1991; Murad, 1994). Others also reported a direct negative inotropic effect on the heart (Brady et al., 1993; Shah et al., 1995). In the rabbit heart, increases in cyclic GMP reduce myocardial O_2 consumption (Weiss et al., 1994). Cyclic GMP has also been reported to antagonize the positive effects of cyclic AMP (Lohmann et al., 1991; Mery et al., 1993). However, not all studies on cardiac myocytes report negative metabolic or functional effects of increases in cyclic GMP (Weyrich et al., 1994; Mohan et al., 1995; Kojda et al., 1996). Weyrich et al. (1994) demonstrated that increasing cyclic GMP using nitric oxide (NO) did not exert a major regulatory effect on cardiac function in unstimulated cardiac myocytes. A recent study (Kojda et al., 1996) in rat cardiac myocytes suggested that a moderate increase in the basal level of cyclic GMP was associated with an improved contractile response, while marked elevations in cyclic GMP decreased contractility. The reasons for these variable results have not been fully studied.

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Most studies use NO to increase cyclic GMP levels. Many of the actions of NO are mediated by activation of soluble guanylate cyclase leading to an increase in intracellular cyclic GMP levels (Schmidt et al., 1993; Murad, 1994). NO, either produced by endogenous NO synthase or by exogenous NO donors, has been shown to exert negative inotropic, chronotropic and metabolic effects on cardiac myocytes, on isolated hearts and on intact hearts in situ in several species including humans (Grocott-Mason et al., 1994; Murad, 1994; Paulus et al., 1994; Weiss et al., 1994; Hare et al., 1995). However, both Weyrich et al. (1994) and Crystal and Gurevicius (1996) reported that NO had no acute inotropic effects in isolated rat and cat papillary muscles and in the in situ dog heart. In addition, contrary positive effects of NO on myocardial function and metabolism have also been demonstrated (Mohan et al., 1995; Kojda et al., 1996). These discrepancies could be caused by methodological differences, including those related to the in vitro preparation of the myocardium used, the dose of NO studied and the animal species studied. However, possible additional mechanisms of action for NO besides its stimulatory effect on soluble guanylate cyclase might also exist. Several additional mechanisms have been suggested (Schluter et al., 1994; Gross et al., 1996; Reid, 1996; Xie et al., 1996). No one has compared the effect of increasing cyclic GMP through the guanylate cyclase stimulating effect of NO on myocyte oxygen consumption with other agents that stimulate guanylate cyclase through different mechanisms.

We hypothesized that increasing the intracellular level of cyclic GMP would decrease cardiac myocyte oxygen consumption (VO_2) to a smaller extent when NO was used to stimulate guanylate cyclase compared to other agents that also stimulate myocyte guanylate cyclase. To test this hypothesis, steady state VO_2 and corresponding cyclic GMP levels were measured in cardiac myocyte preparations isolated from the hearts of New Zealand white rabbits. The effects of NO on myocyte VO_2 and cyclic GMP were compared with other agents that also stimulate guanylate cyclase at various dosages. This is the first study to quantitatively compare the relationship between myocyte VO_2 and cyclic GMP increased by various guanylate cyclase stimulating agents.

2. Materials and methods

New Zealand white rabbits ($n = 11$), weighing 2–3 kg, were used in this study. Cardiac ventricular myocytes were prepared as described previously (Gong et al., 1993), with the following modifications. The rabbits were anesthetized (35 mg/kg sodium pentobarbital) and then heparinized (10 units/g body weight given i.v. via ear vein) and the heart was rapidly removed after an overdose of pentobarbital (60 mg/kg). Retrograde aortic perfusion of the heart was immediately begun at 70 mm Hg constant pressure with HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethane-sulfonic acid, $pK = 7.5$) buffered minimal essential medium (MEM). This solution contains (in mM): 117 NaCl, 5.7 KCl, 11 $NaHCO_3$, 1.5 NaH_2PO_4, 1.7 $MgCl_2$, 21.1 HEPES, 11.7 glucose, amino acids and vitamins. We added 2 mM L-glutamine, 10 mM taurine and the pH was adjusted to 7.2 with NaOH. This low-Ca^{2+} MEM solution had an osmolarity of 296 mOsm and the free Ca^{2+} activity was 2–5 μM. After 5 min of perfusion with low-Ca^{2+} MEM, the heart was perfused at 50 mmHg with a 60 ml volume of low-Ca^{2+} MEM supplemented with 0.1% collagenase (Worthington type II). All perfusion solutions were maintained at 37°C and equilibrated with a water–saturated gas mixture (85% O_2/10% N_2/5% CO_2). After 25 min of collagenase perfusion with recirculation, the heart was removed from the perfusion apparatus and cut into 8–10 pieces in MEM containing 1.0 mM $CaCl_2$ and 0.5% bovine serum albumin (Fraction V, Sigma). This Ca^{2+}-MEM was supplemented with 0.1% collagenase. The tissue suspension was gently swirled in 50 ml centrifuge tubes at 37°C by a wrist action shaker (Multi-Mixer, Lab-Line Inst., Melrose Park, IL) at 2 cycles/s for 5 min. A slurry containing isolated heart cells was decanted from the tissue suspension. The isolated cells were washed three times with the aid of low-speed centrifugation ($34 \times g$) to complete removal of collagenase and some subcellular debris and resuspended in low Ca^{2+}-MEM. Incubation of the remaining tissue with collagenase was repeated at least two more times. The combined, washed cells were then maintained at room temperature. The viability of the myocytes in MEM suspension was about 70–80%. Yields were typically $10–14 \times 10^8$ rod-shaped cells per heart. Cells previously isolated in this fashion have been shown to have an intact cell coat as well as a functional sarcolemma and normal permeability barriers to extracellular ions, ADP and succinate (Wittenberg and Robinson, 1981).

2.1. Myocyte oxygen consumption measurement

Steady state VO_2 was recorded continuously with a two-channel Oximeter (University of Pennsylvania, Philadelphia, PA) fitted in a customized glass recording chamber. All experiments described below were performed under normoxic condition. The recording started with a PO_2 at 115 mmHg and ended at no less than 25 mmHg. Anaerobic metabolism occurs only at PO_2 levels below 5–10 mmHg (Rose et al., 1987). Gradients in PO_2 were not likely in the chamber, since the cell suspension was well stirred.

The chamber was of glass construction and contained a small Teflon coated stirring bar to maintain the cells in suspension by slow rotation. The cuvette was mounted on a magnetic stirrer. A ground glass stopper was used to eliminate the gas phase. This stopper also provided access to the assay medium via a central hole (1.3 mm internal diameter) for addition of agents during the experiment.

The volume of the recording chamber is 1.5 ml. Myocytes were added to the chamber and their number determined. The average number of rod-shaped myocytes added to the chamber in these studies was $5.4 \times 10^4 \pm 7.4 \times 10^3$. The total volume of all drugs added to the chamber were less than 100 μl so no significant dilution of the myocyte suspension occurred.

The VO_2 was measured polarographically with a Clark-type electrode. The electrode was calibrated by putting the electrode into a solution saturated with two known concentrations of oxygen. When calibrated, a 95% response could be obtained within 3–4 s. The rate of fall in oxygen tension within the chamber was used to determine VO_2 of the myocytes over time. Oxygen consumption was expressed as nl of O_2/min per 100 000 myocytes. Oxygen determinations were obtained in the same MEM solution used to resuspend the cells. The sample was stirred at a rate sufficient to keep the cells suspended and yet not so rapidly as to compromise the viability of the cells. Inspection of cellular morphometry, made at random, indicated that at the completion of the experiment there was little loss of viability even after several consecutive cycles of deoxygenation–reoxygenation. We found at least 70% of the cells were quiescent rod shaped cells at the completion of the experiment.

2.2. Protocol

The following protocol was used for the VO_2 recording. Myocytes were suspended in the chamber with 2.0 mM Ca^{2+}-MEM at an appropriate myocyte concentration and the cells were stabilized for 10–15 min. A 5 min recording was obtained as baseline. (1) For the NO donor groups, two separate experiments using two NO donors were conducted. In the first experiment, 3-morpholino-sydnonimine (SIN-1, Sigma, St. Louis, MO, $n = 11$) at 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M was added to the chamber and the corresponding VO_2 was recorded consecutively for 5 min at each dose. For the second experiment, nitroprusside (Sigma, St. Louis, MO, $n = 5$) following the same protocol as in SIN-1 was used. (2) For the carbon monoxide (CO) group ($n = 11$), CO saturated MEM solution was added to the chamber causing final CO concentrations of 1.5×10^{-8}, 10^{-7} and 10^{-6} M and the corresponding VO_2 were recorded consecutively for 5 min at each dose. The calculation of the concentration of CO was based on the solubility of CO at 37°C and the extent of dilution in the recording chamber. For the guanylin (RBI, Natick, MA, $n = 11$) group, guanylin at doses of 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M was added to the chamber and the corresponding VO_2 was recorded consecutively for 5 min at each dose. At the end of each run, 2,4-dinitrophenol (Sigma, St. Louis, MO), was added (5×10^{-5} M) to the chamber. This always at least doubled the baseline VO_2 values.

The same experimental manipulations were done on a portion of the myocytes incubated in Elermyer flask following the same treatment regimen (timing, dosage, temperature, stirring, etc.) except that air was not eliminated from the flask. These myocytes were frozen in liquid nitrogen upon completion of each respective drug treatment in less than 15 s for later cyclic GMP determination.

2.3. Cyclic GMP measurement

In order to determine cyclic GMP levels, frozen samples were warmed to 0°C and homogenized in ethanol using a Brinkmann Polytron in an ice bath. The homogenate was centrifuged at $30\,000 \times g$ for 15 min in a Sorvall RC-5B centrifuge. The supernate was recovered. The pellet was resuspended in 1 ml of 2:1 ethanol–water and centrifuged as before. The combined supernatants were evaporated to dryness in a 60°C bath under a stream of nitrogen gas. The final residue was dissolved in 1.5 ml of assay buffer (0.05 M sodium acetate, pH 5.8, containing sodium azide). Cyclic GMP levels were determined by radioimmunoassay using a scintillation proximity assay (Amersham). This assay measures the competitive binding of ^{125}I-labeled cyclic GMP to a cyclic GMP specific antibody. All assays were performed in duplicate. After construction of a standard curve, cyclic GMP levels were determined directly from the counts in picomoles divided by the number of myocytes per tube times 100 000.

2.4. Statistics

Results are expressed as mean ± S.E.M. A repeated measure analysis of variance (ANOVA) was used to compare variables measured in the experimental and control conditions. Duncan's multiple range test was used to compare differences post hoc. This analysis was also used to determine differences between groups and treatments for myocyte VO_2 and cyclic GMP levels. A least squares regression analysis was used to compare the relationship between VO_2 and cyclic GMP between the treatment groups. In all cases, $P < 0.05$ was accepted as significant.

3. Results

The mean value of basal VO_2 was 268 ± 21 nl O_2/min per 10^5 rod shaped myocytes. The corresponding cellular cyclic GMP level was 86 ± 13 fmol/10^5 myocytes. The effects of SIN-1, CO, NP and guanylin on myocyte VO_2 in the various experiments performed are summarized in Fig. 1. Adding either SIN-1, CO, nitroprusside, or guanylin to the myocytes suspension medium resulted in a dose-dependent decrease in the O_2 consumption of the myocytes. The two NO donors, SIN-1 and nitroprusside, at their highest doses (10^{-5} M) lowered the VO_2 to 180 ± 13 and 210 ± 17 nl O_2/min per 10^5 myocytes, respectively. This represented about a 32% reduction of the VO_2 in the NO donor group over control. When non-NO donors, CO and

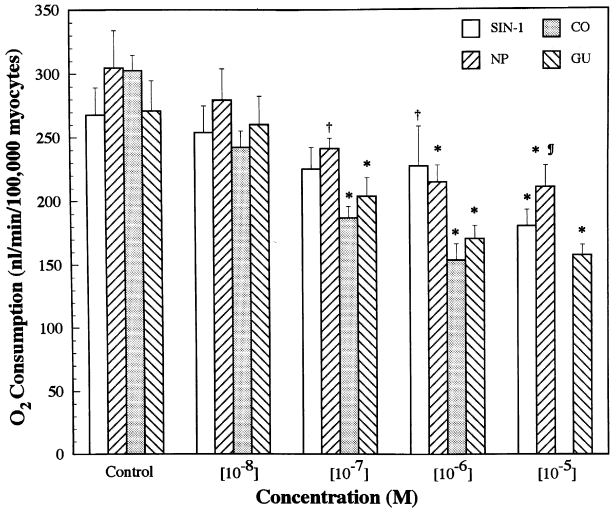

Fig. 1. The effects of 3-morpholino-sydnonimine, nitroprusside (NP), carbon monoxide and guanylin on the O_2 consumption of isolated myocytes from rabbit hearts. The number of animals in which myocyte O_2 consumption was determined were: $n=11$, control; $n=11$, 10^{-8} to 10^{-5} SIN-1; $n=11$, 1.5×10^{-8} to 1.5×10^{-6} CO; $n=5$, 10^{-8} to 10^{-5} nitroprusside. * Significantly different from control. †Significantly different from carbon monoxide. ¶Significantly different from guanylin.

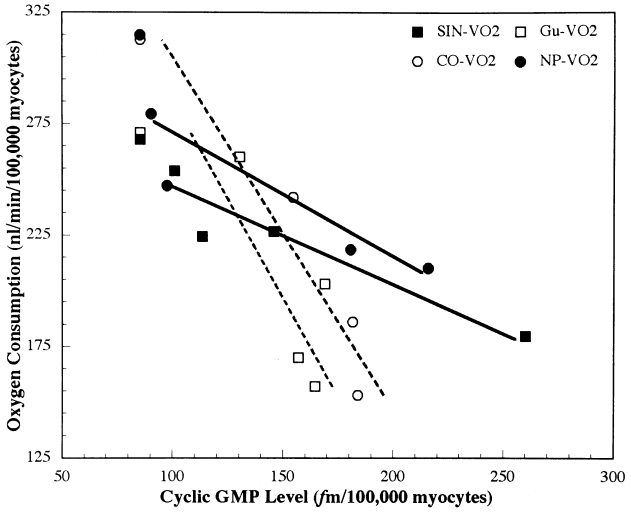

Fig. 3. The relationship between myocyte O_2 consumption and the level of cyclic GMP in myocytes treated with of 3-morpholino-sydnonimine (dashed), nitroprusside (dashed), carbon monoxide (solid) and guanylin (solid). The standard errors of the values are presented in Figs. 1 and 2. The slope of the lines for the NO donor groups were significantly less steep, than those groups in which guanylate cyclase was stimulated by other means.

guanylin, were used at their highest dosages (1.5×10^{-6} M and 10^{-5} M, respectively), the myocyte VO_2 was decreased to 153 ± 13 and 157 ± 29 nl O_2/min per 10^5 myocytes. This corresponded to a maximum VO_2 reduction of 45% in the non-NO donor groups. The difference in the reduction between NO donor groups and non-NO groups was significant ($P<0.01$).

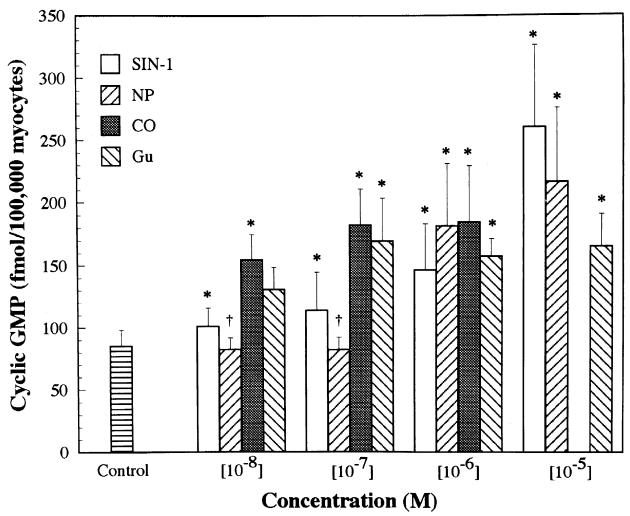

Fig. 2. The effects of 3-morpholino-sydnonimine, nitroprusside, carbon monoxide and guanylin on the level of cyclic GMP in isolated myocytes from rabbit hearts. The numbers of animals in which myocyte cyclic GMP was determined were: $n=11$, control; $n=6$, 10^{-8} to 10^{-5} SIN-1; $n=11$, 1.5×10^{-8} to 1.5×10^{-6} CO; $n=5$, 10^{-8} to 10^{-5} nitroprusside. * Significantly different from control. †Significantly different from carbon monoxide.

When dinitrophenol was added to the chamber, O_2 consumption increased. In one group, baseline O_2 consumption was 268 ± 20 and this was decreased by SIN-1. This value increased to 464 ± 39 with the addition of dinitrophenol. Baseline O_2 consumption was 312 ± 12 and this was decreased by CO in another group. This value then increased to 561 ± 28 with the addition of dinitrophenol. In the guanylin group, baseline O_2 consumption was 271 ± 24 and this was increased to 492 ± 63 by dinitrophenol. In the nitroprusside group, baseline O_2 consumption was 315 ± 25 and this was increased to 568 ± 47 by dinitrophenol.

The corresponding levels of myocyte intracellular cyclic GMP are shown in Fig. 2. The NO donors, as well as CO and guanylin, significantly increased the myocyte intracellular cyclic GMP levels in a dose dependent fashion. SIN-1 and nitroprusside, at 10^{-5} M, caused a maximal increase of intracellular cyclic GMP to 260 ± 66 and 216 ± 60 fmol/10^5 myocytes, respectively. The level of cyclic GMP was increased less with either CO or guanylin. The maximally increased cyclic GMP levels were 184 ± 46 and 165 ± 26 fmol/10^5 myocytes, respectively.

Fig. 3 shows the regression plots of myocyte cyclic GMP versus O_2 consumption. This compares the effects of intracellular cyclic GMP levels, elevated by three different mechanisms, on the myocyte VO_2. An inverse relationship between cellular cyclic GMP level and VO_2 was found in all myocyte groups. The regression equations for the four treatments were: $VO_2=-0.45\times[\text{cyclic GMP}]+294.4$, $r=0.94$ for SIN-1; $VO_2=-1.46\times[\text{cyclic GMP}]+444.7$, $r=0.96$ for CO; $VO_2=-1.25\times[\text{cyclic GMP}]+$

389.1, $r = 0.84$ for guanylin and $VO_2 = -0.55 \times$ [cyclic GMP] + 322.8, $r = 0.79$ for nitroprusside. The regression lines of the two NO donors were parallel to each other. The regression lines of CO and guanylin were also parallel to each other. However, the slopes of both the SIN-1 and nitroprusside regression lines were significantly less steep than that of either the CO or guanylin lines. At any given level of increased intracellular cyclic GMP caused by the NO donors, the VO_2 of the myocytes was higher than the same level of cyclic GMP caused by either CO or guanylin.

4. Discussion

In the present study, NO donor and non-NO donor guanylate cyclase stimulators were used to assess the possible effects of NO on myocyte metabolism in addition to its effect on the stimulation of soluble guanylate cyclase. The major finding of this study was that the increase in cellular cyclic GMP caused by NO donors reduced myocyte VO_2 significantly less than an increase to the same level of cyclic GMP caused by non-NO donors in isolated rabbit ventricular myocytes. This suggests that NO has additional effects on myocyte VO_2 beside its effects on the level of cyclic GMP. Another finding was that guanylin, a peptide isolated from intestine, can strongly stimulate particulate guanylate cyclase in cardiac myocytes to increase the production of myocyte cellular cyclic GMP.

The use of isolated ventricular myocytes in this study eliminated concerns arising from the use of heart tissues containing heterogeneous cell types, which could act as confounding sources of both cyclic GMP and the VO_2 measured. The viability of the myocytes was confirmed at the end of each experiment by both re-checking the percentages of rod-shaped myocytes and their morphology (Wittenberg and Robinson, 1981). The O_2 consumption valves we found were similar to previous reports in isolated myocytes (Wittenberg and Robinson, 1981; Rose et al., 1987; Rumsey et al., 1990; Gong et al., 1997). We also demonstrated that the myocytes were capable of increasing their VO_2 at the end of each treatment series by adding dinitrophenol to the cell suspensions and this at least doubled the VO_2. Our measured baseline cyclic GMP levels were also similar to previous reports (Kojda et al., 1996; Gong et al., 1997; Lew et al., 1997). Measuring errors with regard to VO_2 and cyclic GMP determinations due to damaged cells have to be considered. Our cell preparation contains about 15% rounded cells. Most of them may still metabolize to an unknown extent. However, their relatively small contribution would only lead to a shift in the absolute values of the VO_2 and cyclic GMP levels without impairing the conclusions drawn from the experiments.

SIN-1 and nitroprusside spontaneously decompose in solution to release NO and are useful and widely accepted tools for studying the effects of various concentrations of NO both in vitro and in vivo (Feelisch, 1991). NO, through stimulation of soluble guanylate cyclase, can significantly increase the production of cyclic GMP in the myocytes. These increases in cyclic GMP decrease the metabolism and function of myocytes (Lohmann et al., 1991; Mery et al., 1993; Murad, 1994; Shah et al., 1995). In this study, this increase in myocyte cyclic GMP was about 3-fold with SIN-1 and 2.5-fold with nitroprusside compared to control conditions. CO can also stimulate soluble guanylate cyclase by a similar mechanism to that of NO (Farrugia et al., 1993; Rattan and Chakder, 1993; Kharitonov et al., 1995). It can increase cellular cyclic GMP in a concentration-dependent fashion (up to 300 μM). This effect is independent of its effects on myoglobin and/or its inhibitory effects on cell respiratory chain (Utz and Ullrich, 1991; Sarti et al., 1992; Rattan and Chakder, 1993). In this study, adding micromolar concentration of CO to the myocyte suspension more than doubled the myocyte cyclic GMP level over that at baseline. Guanylin, a peptide found in various mammalian tissues (Schulz et al., 1992; Forte and Currie, 1995), has been demonstrated to increase cellular cyclic GMP production by stimulating the cellular particulate guanylate cyclase activity (Forte and Currie, 1995). The effect of guanylin on cardiac ventricular myocyte cyclic GMP has not been reported previously to the best of our knowledge. In this study, we report for the first time that myocyte cellular cyclic GMP levels were significantly increased by adding guanylin to the myocyte suspension (Fig. 2). Our data imply that there is a common cyclic GMP pool in cardiac myocytes, since stimulation of both particulate and soluble guanylate cyclase produces similar decrements in metabolism.

Increased cellular cyclic GMP can reduce cardiac myocyte metabolism and/or function (Lohmann et al., 1991; Murad, 1994). It has also been reported to antagonize the positive effects of catecholamines and cyclic AMP. When cyclic GMP levels were increased in this study, these increases were closely correlated with a decrease in myocyte O_2 consumption (Fig. 1). Nitric oxide donors, CO, and guanylin all significantly decreased myocyte O_2 consumption. However, at any given level of cyclic GMP, CO and guanylin decreased the VO_2 to a greater extent than NO although the later caused greater increases in the cyclic GMP level (Fig. 3). This could be related to additional effects of the NO donors or to other effects of CO and guanylin. Previously we had also demonstrated that zaprinast, a cyclic GMP phosphodiesterase inhibitor, also produced a greater decrease in myocyte VO_2 than nitroprusside for a similar increase in cyclic GMP (Gong et al., 1997). Thus, three independent means of changing cyclic GMP produce similar decrements in O_2 consumption which are different from those caused by NO donors. The need for a greater increase in cyclic GMP to produce a similar decrement in oxygen consumption suggests that there may be additional effects of NO on myocyte metabolism besides its action on the stimulation of guanylate cyclase.

Previous studies have indicated that at some doses NO can produce a positive inotropic effect (Mohan et al., 1995; Kojda et al., 1996). Others have also reported no functional effects of low doses of nitric oxide (Weyrich et al., 1994). These effects might be independent of the cyclic GMP stimulating effects of NO. These findings could be explained by the additional effects of NO suggested by the current study. Since our cells are non-beating but high Ca^{2+} challenged myocytes, the mechanisms responsible for the additional action of NO in the present study may be through cellular Ca^{2+} handling, such as modulation of the L-type Ca^{2+} current in cardiac myocytes. In this regard, there are reports that there can be stimulation of cardiac L-type Ca^{2+} channels (Ono and Trautwein, 1991; Kirstein et al., 1995), although most reports demonstrated reduced Ca^{2+} currents (Mery et al., 1993; Sperelakis et al., 1994). The inhibitory effects of NO on the L-type Ca^{2+} channel may act through cyclic GMP protein kinase pathway activation. Evidence exists that there are stimulatory effects of NO on the L-type Ca^{2+} channel due to cyclic GMP-induced inhibition of cyclic AMP-inhibited phosphodiesterase (Kirstein et al., 1995). There are also reports demonstrating that NO and/or cyclic GMP might also exert a direct stimulatory effect on L-type Ca^{2+} channels (Kirstein et al., 1995; Mery et al., 1993; Ono and Trautwein, 1991). It was possible in our study that the high concentrations of NO caused an increase in the conductance of Ca^{2+} channels besides increasing the cellular cyclic GMP levels, raising the intracellular Ca^{2+} concentration which in turn stimulates VO_2 and offsets the negative effect of cyclic GMP on VO_2. There are also other non-cyclic GMP mediated effects of exogenous NO related to inhibition of mitochondrial respiration and inhibition of creatine kinase (Gross et al., 1996; Xie et al., 1996). These are not likely explanations for a greater VO_2 with NO at a given level of cyclic GMP. There is also the possibility of free radical induced changes or *S*-nitrosylation of proteins and enzymes that could also explain the altered relationship between cyclic GMP and myocyte oxygen consumption using exogenous NO (Gross et al., 1996; Mohr et al., 1996). There may also be some compartmentalization of cyclic GMP production, although NO donors stimulate the soluble form of guanylate cyclase.

In summary, we used two NO donors, SIN-1 and NP, and two non-NO guanylate cyclase stimulators, CO and guanylin, to assess the effects of NO on myocytes metabolism in addition to its effect on the stimulation of soluble guanylate cyclase. Myocyte VO_2 was significantly decreased by all of these agents. This reduction parallels corresponding increases in myocyte cellular cyclic GMP levels. We found that with exogenous NO donors it required greater increases in cyclic GMP to reduce myocyte oxygen consumption compared to non-NO donor guanylate cyclase stimulators. This study provides evidence that there are additional effects of NO on O_2 consumption in ventricular cardiac myocytes besides its action on guanylate cyclase. This study may help to explain some of the controversial results of NO on myocardium. It is also demonstrated, for the first time, that guanylin, a peptide isolated from intestine, can strongly stimulate guanylate cyclase in cardiac myocytes to increase the production of cyclic GMP.

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