Acute Effects of Nitric Oxide and Cyclic GMP on Human Myocardial Contractility

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ABSTRACT

Evidence that the activity of nitric oxide synthase and the generation of nitric oxide (NO) within the myocardium are enhanced in several cardiovascular disorders is increasing. Findings whether NO exerts a direct effect on cardiac contractility are contradictory. Therefore, the direct effect of the NO donor sodium nitroprusside (SNP) on isometric force of contraction of human atrial and ventricular myocardium was investigated, and the question was addressed whether the effects of NO on cardiac contractility are mediated via cGMP. Experiments were performed on isolated electrically driven (1Hz, 37°C) human right atrial trabecula and left ventricular papillary muscle preparations from nonfailing and terminally failing hearts. SNP led to a concentration-dependent decrease of force of contraction (FOC) with a maximum effect at 100 μmol/l. In atrial trabecula, SNP (100 μmol/l) caused an acute decrease in basal FOC as well as in FOC after application of isoprenaline or IBMX by 12.5 ± 5% (P < .05), 16.6 ± 3.7% (P < .05) and 18.3 ± 4.2% (P < .05), respectively. The negative inotropic effects could be attenuated by the guanylyl cyclase inhibitor methylene blue. In papillary muscle preparations, NO release caused a maximum decrease in basal and in isoprenaline-enhanced FOC of 11.0 ± 1.9% (P < .05) and 23.6 ± 1.5% (P < .05), respectively. In the presence of isoprenaline, the reduction of FOC was less pronounced in failing than in nonfailing papillary muscles. 8-bromo-cGMP caused a 38.2 ± 5.2% decrease in atrial trabecula contractility. Both SNP and 8-bromo-cGMP caused a shortening of the contractile twitch with a premature onset of relaxation. As determined by radioimmunoassay, exposure of atrial trabecula to SNP (100 μmol/l) led to a 6-fold increase in myocardial cGMP concentrations, which could be attenuated by methylene blue. In conclusion, NO exerts a negative inotropic effect on human atrial and ventricular myocardium which seems to be mediated via generation of cGMP. The release of NO within the myocardium in a variety of cardiovascular disorders might explain decreases in cardiac contractility. The control of NO release could be an important target for future therapeutic interventions in these pathological conditions.

Nitric oxide is an ubiquitous molecule which plays an important role in intercellular as well as autacoid signal transmission. It is generated from the L-arginine-citrulline pathway by the enzyme NOS. There are at least three distinct isoforms of this molecule. Two isoforms are constitutive (cNOS) and generate NO in a Ca++-calmodulin-dependent manner. The other isoform is inducible (iNOS) and generates large amounts of NO independent from Ca++ (for review, see Moncada et al., 1991; Schmidt, 1994). The latter isoform is expressed after stimulation with different cytokines in a variety of cells such as macrophages, vascular smooth muscle cells, endothelial cells and cardiac myocytes (for review, see Dinerman et al., 1993). The most important second messenger transmitting the effects of NO is cGMP, NO being a powerful activator of guanylyl cyclase (Rapoport and Murad, 1983; Förstermann et al., 1986). One important function of the potent vasodilator NO is the endothelial-dependent regulation of the vascular tone (Furchgott and Zawadzki, 1980; Rapoport and Murad, 1983; Förstermann et al., 1986; for review, see Dinerman et al., 1993). Other roles are the impairment of platelet function (Radomski et al., 1987) and inhibition of leukocyte adhesion to the endothelium (Kubes et al., 1991) and its involvement in neurotransmission (for review, see Dinerman et al., 1993).

The effects of NO on myocardial contractility are a matter of controversy. Recent studies have shown that in vitro NO, either released from endocardial endothelium or from exogenous NO donors such as SNP, leads to an abbreviation of the contraction cycle of the isometric twitch with a hastening of

ABBREVIATIONS: FOC, force of contraction; NO, nitric oxide; NOS, nitric oxide synthase; cNOS, constitutive nitric oxide synthase; iNOS, inducible nitric oxide synthase; MB, methylene blue; cGMP, cyclic 3’-5’-guanosine monophosphate; SNP, sodium nitroprusside; IBMX, isobutylmethylxanthine; R-PIA, R-N^2-phenylisopropyladenosine; KCN, potassium cyanide; L-NMMA, N^2-monomethyl-L-arginine.
myocardial relaxation and a slight reduction in peak developed tension. These observations have been made in ejecting heart experiments (Grocott-Mason et al., 1994), in isolated papillary muscle preparations (Brutsaert et al., 1988; Smith et al., 1991) and in isolated cardiomyocytes (Brady et al., 1993) from different species. Hare et al. (1995), performing experiments on canine hearts in vivo, suggested that NO mediates vagal inhibition of the inotropic response to beta adrenergic stimulation. Only recently, Paulus et al. (1994) demonstrated that intracoronary infusion of NO in humans reduced left ventricular pressure development, hastened left ventricular relaxation and improved left ventricular diastolic distensibility. On the other hand, early studies demonstrated positive inotropic effects of glyceryl trinitrate in feline and human papillary muscles (Strauer, 1973) and of SNP in feline atria (Diamond et al., 1977). Nawrath et al. (1995) and Weyrich et al. (1994) reported no effects of NO donors on basal contractility of atrial myocardial preparations from different species including man. In the latter study, a negative inotropic effect of NO on isolated rat papillary muscle strips could only be observed in the presence of high concentrations of isoprenaline and of a very high concentration of NO (500 nmol/l) (Weyrich et al., 1994). Of special interest is a recent report by Mohan et al. (1996), in which a concentration-dependent biphasic effect of NO and cGMP on isolated cat papillary muscle was demonstrated. Low concentrations of NO and cGMP exerted a positive inotropic effect, and high concentrations exerted a negative inotropic effect.

The question whether NO is a cardiodepressant substance or not is of great pathophysiological and clinical importance because evidence that NO might be involved in a great number of pathological conditions is increasing. One condition is myocardial inflammation and septic cardiomyopathy. Finkel et al. (1992) were the first to demonstrate that cytokines impaired contractility of isolated hamster papillary muscle and that the effect of the cytokines could be inhibited by the NOS-inhibitor l-NMMA. Brady et al. (1992) demonstrated that endotoxin treatment of guinea pigs led to a decrease in isolated myocyte contractility of the animals. Similarly, Balligand et al. (1993) demonstrated that incubation of isolated ventricular myocytes from adult rat hearts in medium conditioned by endotoxin-activated rat alveolar macrophages led to a reduction of the inotropic myocyte response to beta adrenergic stimulation. In both studies, the negative inotropic effects could be antagonized by the NOS inhibitor l-NMMA or N\textsuperscript{G}-nitro-l-arginine methyl ester, which indicated that the effects are mediated by NO. In a guinea pig model of cardiac transplantation, it was demonstrated that iNOS mRNA, protein and enzyme activity are induced in cardiac allograft rejection (Yang et al., 1994). The importance of NO generation for inflammatory myocardial dysfunction is underlined by findings that there are an enhanced expression of iNOS and increased cGMP levels in septic human hearts (Thoenes et al., 1996), that iNOS mRNA is increased in human heart failure because of dilative cardiomyopathy, ischemic heart disease or valvular heart disease (Haywood et al., 1996) and that there is a relation between iNOS mRNA expression and contractile dysfunction in human cardiac allografts (Lewis et al., 1996). Furthermore, in endomyocardial biopsies obtained from patients with congestive cardiomyopathy and previous myocarditis an increased activity of iNOS could be demonstrated (de Belder et al., 1993).

The aim of this study was to examine the inotropic effects of exogenous NO and of its possible second messenger cGMP on human myocardium, because so far functional data concerning the effects of NO on the human heart are sparse and contradictory. The functional effects of the guanylyl cyclase inhibitor methylene blue and myocardial cGMP levels after exposure of the myocardium to NO donors were determined to elucidate whether the inotropic effects of NO are mediated via the guanylyl cyclase-cGMP pathway.

**Methods**

**Human myocardial tissue.** Experiments were performed on right atrial myocardium obtained during open heart surgery and on papillary muscles from terminally failing (New York Heart Association class IV) and nonfailing hearts obtained during cardiac transplantation. In the latter case, organ donor history and two-dimensional echocardiography revealed no evidence of heart disease. General anesthesia was performed with flunitrazepam, fentanyl and pancuronium bromide with isoflurane. Cardiac surgery was performed while on cardiopulmonary bypass. Only noninfarcted tissue was used and scars were carefully trimmed away. Tissue pieces were suspended in ice-cold cardioplegic solution (modified Brevitschneider solution containing (in mmol/l): NaCl, 15; KCl, 10; MgCl\(_2\), 4; histidine HCl, 180; tryptophane, 2; mannitol, 30; and potassium dihydrogen oxoglutarate; l and were delivered immediately from the operation room to the laboratory.

**Isolated cardiac muscle strip preparation and measurement of FOC.** Immediately after excision, atria and papillary muscles were placed in ice-cold pre-aerated Tyrode’s solution. The experiments were performed on isolated electrically driven muscle preparations. Muscle strips of uniform size with muscle fibers running approximately parallel to the length of the strips were dissected under microscopic control with scissors in aerated modified Tyrode’s solution (composition below). Connective tissue was carefully trimmed away. The muscles were suspended in an organ bath (75 ml) maintained at 37°C and containing a modified Tyrode’s solution of the following composition (in mmol/l): NaCl, 119.8; KCl, 5.4; MgCl\(_2\), 1.05; CaCl\(_2\), 1.8; NaHCO\(_3\), 22.6; Na\(_2\)HPO\(_4\), 0.42; glucose, 5.0; ascorbic acid, 0.28; EDTA, 0.05. The bathing solution was continuously aerated with 95% O\(_2\) and 5% CO\(_2\). The muscles were stimulated by two platinum electrodes using field stimulation from a Grass S88 (Grass, Quincy, MA) stimulator (frequency 1 Hz; impulse duration 5 ms; intensity 10—20% greater than threshold). Each muscle was stretched to the length at which FOC was maximal. The developed tension was measured isometrically with an inductive force transducer (W. Fleck, Mainz, Germany) attached to a Gould recorder (Gould, Cleveland, OH). Preparations were allowed to equilibrate for at least 90 min, with the bathing solution being changed once after about 45 min.

**Determination of myocardial cGMP content.** For determination of myocardial cGMP content, frozen myocardial tissue was pulverized and solubilized in 6% (vol/vol) trichloroacetic acid containing 10 μmol/l HCl yielding at a final concentration of 100 mg tissue in 1000 μl solution. After centrifugation at 2000 g over 15 min, the supernatant was washed with water-saturated diethylether in order to remove trichloroacetic acid. Afterwards, the cGMP containing solution (composition below) was used and scars were carefully trimmed away. The muscles were suspended in an organ bath (75 ml) maintained at 37°C and containing a modified Tyrode’s solution of the following composition (in mmol/l): NaCl, 119.8; KCl, 5.4; MgCl\(_2\), 1.05; CaCl\(_2\), 1.8; NaHCO\(_3\), 22.6; Na\(_2\)HPO\(_4\), 0.42; glucose, 5.0; ascorbic acid, 0.28; EDTA, 0.05. The bathing solution was continuously aerated with 95% O\(_2\) and 5% CO\(_2\). The muscles were stimulated by two platinum electrodes using field stimulation from a Grass S88 (Grass, Quincy, MA) stimulator (frequency 1 Hz; impulse duration 5 ms; intensity 10—20% greater than threshold). Each muscle was stretched to the length at which FOC was maximal. The developed tension was measured isometrically with an inductive force transducer (W. Fleck, Mainz, Germany) attached to a Gould recorder (Gould, Cleveland, OH). Preparations were allowed to equilibrate for at least 90 min, with the bathing solution being changed once after about 45 min.

**SNP was obtained from Schwarz Pharma, Monheim, Germany; KCN and carbachol were from Merck, Darmstadt, Germany; methylene blue, isoprenaline, IBMX and R-PIA were from Sigma, Deisenhofen, Germany. All chemicals used were of analytical grade or the best grade commercially available. All compounds were dissolved in deionized and twice-distilled water.
Statistics. The data shown are mean ± S.E.M. Statistical significance was analyzed by the Student's t test according to Wallenstein et al. (1989). P < .05 was considered as significant.

Results

Effect of SNP on isometric FOC of atrial trabecula.
In isolated electrically driven atrial trabecula, SNP led to a significant reduction of the basal FOC. The exemplary original recording in figure 1 shows the decrease in the isometric contraction amplitude 240 sec after application of SNP (100 μmol/l) into the organ bath. KCN, which, besides NO, is also released from SNP, had no lasting effect on the FOC of atrial preparations. The bar graph in figure 2 gives mean values of individual experiments with six different hearts for the effect of 100 μmol/l SNP on atrial trabecula FOC, which indicates that the mean reduction of basal FOC caused by 100 μmol/l SNP was 12.5 ± 1.7% (P < .05). Once established, the effect of SNP was not reversible by washing out the substance from the organ bath.

The acute negative inotropic effect of SNP was slightly, but not significantly more pronounced after isometric FOC had been increased by isoprenaline (0.03 μmol/l) (fig. 3A, upper tracing) or by the phosphodiesterase inhibitor IBMX (0.03 μmol/l). In the presence of isoprenaline, the decrease in contraction amplitude caused by SNP was 16.6 ± 3.7% (P < .05, isoprenaline vs. isoprenaline + SNP, fig. 3B), in the presence of IBMX 18.3 ± 4.2% (P < .05, IBMX vs. IBMX + SNP, not shown).

To determine whether the effect of SNP was concentration-dependent and whether it was monophasic or biphasic as reported recently (Mohan et al., 1996), the cumulative effect of increasing concentrations of SNP on isometric FOC was measured. As indicated in figure 4, SNP exerted a concentration-dependent negative inotropic effect on basal as well as on isoprenaline- or IBMX-enhanced FOC. The effect was significant at a concentration of 0.1 μmol/l (basal and IBMX) and 10 μmol/l (isoprenaline) and reached its maximum at 100 μmol/l. No positive inotropic effect was observed.

Isoprenaline itself caused a concentration-dependent increase in atrial trabecula FOC with a maximum at 0.1 μmol/l. Donation of SNP (100 μmol/l) before isoprenaline slightly, although not significantly shifted the atrial contractile concentration-response curve to the right (EC₅₀ values: control, n = 7, 0.003 (0.001–0.007) μmol/l; SNP, n = 7, 0.014 (0.007–0.029) μmol/l, fig. 5).

Effect of methylene blue on NPN-modulated FOC. In the presence of the guanylyl cyclase inhibitor methylene blue (10 μmol/l), the negative inotropic effect of SNP on basal as well as on isoprenaline- or IBMX-increased FOC was significantly reduced (fig. 4). However, as depicted in figures 2 and 3, the negative inotropic effect of SNP could not be completely antagonized by the guanylyl cyclase inhibitor. Also, methylene blue did not completely abolish the right-shift of the isoprenaline concentration-response curve (fig. 5). Methylene blue itself had no significant inotropic effect.

Effect of carbachol and R-PIA on atrial FOC. To have a marker for the potency and efficacy of the negative inotropic effect of the NO donor SNP in comparison with other physiological mechanisms involved in the inhibitory regulation of FOC, its effects were compared with those of carbachol and the A₁ adenosine receptor agonist R-PIA. As expected, carbachol and R-PIA caused a significant concentration-dependent decrease in FOC of atrial trabecula (fig. 6) with a

Fig. 1. Original recording demonstrating the effect of SNP (100 μmol/l) and of KCN (100 μmol/l) on isolated human atrial trabecula in vitro. Addition of SNP led to a lasting decrease in basal isometric contraction amplitude, whereas no lasting negative inotropic effect was observed after addition of KCN.

Fig. 2. (A) Original recording demonstrating the effect of SNP (100 μmol/l) on isometric FOC of isolated human atrial trabecula in vitro. Addition of SNP led to a decrease in isometric FOC. A much smaller and retarded effect was observed when MB (10 μmol/l) had also been added to the organ bath. (B) Bar graph demonstrating mean values (± S.E.M.) for the effect of SNP (100 μmol/l) and SNP (100 μmol/l) plus MB (10 μmol/l) on isoprenaline-enhanced isometric FOC. Data are obtained from experiments with four atria. Basal FOC is taken as 100%.

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maximum reduction in FOC caused by carbachol of 64 ± 6% (P < .01) and by R-PIA of 76 ± 8% (P < .01). Obviously, carbachol and R-PIA were much more efficacious (P < .01) than SNP.

**Effect of SNP on FOC of human papillary muscle.** In isolated electrically driven human papillary muscle strip preparations, acute addition of SNP (100 μmol/l) exerted a similar negative inotropic effect on basal and on isoprenaline-increased FOC as on atrial trabecula. Results are summarized in figure 7. SNP led to a mean reduction of FOC of 11.0 ± 1.9% (P < .05) in muscle strips from nonfailing hearts and of 10.2 ± 1.7% (P < .05 SNP vs. control) in muscle strips from failing hearts. In the nonfailing myocardium, the negative inotropic effect of SNP was significantly (P < .05, control + SNP vs. isoprenaline + SNP) more pronounced in the presence of isoprenaline in an mean maximum reduction in FOC of 23.6 ± 1.5% (P < .05 SNP vs. control). In contrast, in the failing myocardium, the negative inotropic effect of SNP was only slightly, but not significantly more pronounced in the presence of isoprenaline (−12.8 ± 1.2%, P < .05, isopren-
Figure 8 shows that isoprenaline led to a concentration-dependent increase in FOC in nonfailing and in failing myocardium, which, as expected, was significantly less pronounced in the latter (NF: 256 ± 64% vs. NYHA IV: 152 ± 4%, P < .001). SNP (100 μmol/l) led to a right-shift of the concentration-response curves, which could be antagonized by methylene blue.

**Effect of 8-bromo-cGMP on myocardial contractility.** To investigate whether the effects of NO are mediated by cGMP, the inotropic effect of the stable cGMP analog 8-bromo-cGMP was compared with those of SNP. As can be depicted from the original recording in figure 9A, 8-bromo-cGMP (100 μmol/l) caused a pronounced decrease in the contraction amplitude of the examined atrial trabecula, as did SNP. Interestingly, the maximum effect of 8-bromo-cGMP developed more slowly than the maximum effect of SNP. The negative inotropic effect of 8-bromo-cGMP was concentration-dependent, the maximum effect being at 100 μmol/l (fig. 9B). This concentration led to a mean decrease in isometric FOC by 38.2 ± 5.2%. Again, no positive inotropic effect was observed.

**Effect of SNP and 8-bromo-cGMP on the time course of contraction.** Effects of SNP and 8-bromo-cGMP on time-dependent parameters of the contractile twitch are summarized in table 1. SNP and cGMP both led to a minor shortening of the contractile twitch with a reduction of time to peak tension (P < .05) and premature onset of relaxation. The velocity of tension increase was not altered. Only 8-bromo-cGMP, but not SNP led to a decrease in half-maximal relaxation time.

**Regulation of myocardial cGMP content by SNP.** Also to investigate whether the effects of NO might be mediated by stimulation of guanylyl cyclase and generation of cGMP, the effect of SNP on myocardial cGMP concentrations was examined. Myocardial cGMP content was determined in the trabecula, which had been used previously for functional experiments and had been frozen under liquid nitrogen when the maximum inotropic effect of the examined substances had been reached. Exposure of human atrial trabecula to SNP (100 μmol/l) led to a 6-fold increase in myocardial cGMP content (P < .001). This increase could partly be antagonized by preincubation of the trabecula with methylene blue (10 μmol/l). Methylene blue itself had no significant effect on myocardial cGMP levels (fig. 10). As can be depicted from figure 10, lower panel, changes in myocardial cGMP levels inversely reflected changes in FOC. The fact that methylene blue did not completely attenuate the SNP-induced increase...
in myocardial cGMP levels is in accordance with the incomplete abolishment of the negative inotropic effect of SNP.

**Discussion**

The present study demonstrates that the NO donor SNP exerts a significant negative inotropic effect on human myocardium. SNP led to a concentration-dependent decrease in basal, isoprenaline- or IBMX-enhanced isometric FOC in isolated atrial trabecula. For the first time, it was demonstrated that this effect holds true also for left ventricular human myocardium. The effects of SNP were similar to those of the stable cGMP analog, 8-bromo-cGMP, which also had a sustained negative inotropic effect on human atrial trabecula. In accordance with previous observations (Brady et al., 1993), this effect developed more slowly than the effect of SNP, which might be because the second messenger penetrates the myocardial tissue more slowly than NO itself. Experiments were performed on isolated atrial trabecula or papillary muscle preparations. In contrast to experiments in vivo or with isolated hearts, changes in coronary blood flow and peripheral vascular resistance by NO or 8-bromo-cGMP were not modulators for the FOC in this experimental setting. Therefore, it can be concluded that NO and cGMP directly depress the cardiac FOC. The observations that the guanylyl cyclase inhibitor methylene blue attenuated the effect of SNP and that exposure of human atrial trabecula to SNP caused an increase in myocardial cGMP content suggest that the negative inotropic effect of SNP and thereby NO is mediated via activation of guanylyl cyclase and generation of cGMP.

Our observation that SNP decreases isometric force development in human myocardial preparations is in accordance to previous investigations in other species which also demonstrated a negative inotropic effect of NO and cGMP. Brady et al. (1993) observed in isolated guinea pig cardiac myocytes that length shortening during contraction was attenuated by NO released from the endothelium or by SNP and 8-bromo-cGMP by 20% and that the effect of SNP could be reversed by methylene blue. In contrast to our experimental setting, which does not allow differentiation between the direct effects of NO and 8-bromo-cGMP on cardiac myocytes and those effects which are mediated by other myocardial cell types, the experiments by Brady et al. (1993) demonstrated that both substances directly affect myocyte contractility. Similar effects were observed when the effect of NO and 8-bromo-cGMP on isolated ferret papillary muscle were determined (Smith et al., 1991; Shah et al., 1991). In isolated ejecting hearts of the ferret, the NO-releasing substance bradykinin caused a significant reduction in peak left ventricular pressure (Fort and Lewis, 1993). In humans, it could be demonstrated that intracoronary infusion of SNP suppressed left ventricular pressure development (Paulus et al., 1994), whereas intracoronary l-NMMA infusion enhanced the positive inotropic response to dobutamine (Hare et al., 1995).

On the other hand, Nawrath et al. (1995) did not observe a negative inotropic effect of various NO donors, including SNP, in isolated atrial muscle strips from various species, including man. Similarly, Weyrich et al. (1994) reported that various NO donors failed to produce any inotropic effect on isolated papillary muscle preparations from cats and rats. Only in the presence of a high concentration of norepinephrine, an unphysiologically high concentration of NO exerted a significant decrease in FOC (Weyrich et al., 1994). The strength of this study was that release of NO into the organ bath was proved by determination of the NO concentration. Therefore, one cannot simply explain the difference between these and our findings with an inappropriate NO donor. This could have been concluded from the findings of Brady et al. (1993), who observed that organic nitrates are possibly not metabolized by mammalian ventricular muscle. Because Nawrath et al. (1995) also performed experiments on human myocardium, one can also not reduce contradictory findings to species differences. Possible explanations might be differences in the specific handling of the myocardium, e.g., tissue preparation in prewarmed Tyrode's solution might have caused damage to the endothelium, which has been shown to be of distinct relevance for the inotropic effect of NO (Mohan et al., 1996). Also, it has been suggested that effects of NO differ in atrial and ventricular myocardium as has been shown for acetylcholine, which possibly exerts its negative inotropic effect by endothelial release of NO (Endoh and Yashimita, 1981). According to our data, this does not seem to be the case for the effect of NO in humans.
TABLE 1
Effect of SNP (100 μmol/l) and 8-bromo-cGMP (100 μmol/l) on force of contraction and time-dependent parameters of contractile twitch of isolated electrically driven human atrial trabecula

<table>
<thead>
<tr>
<th>Force of Contraction</th>
<th>Time to Peak Tension</th>
<th>Half-maximal Relaxation Time</th>
</tr>
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<tbody>
<tr>
<td>Control (n = 9)</td>
<td>Δ% control</td>
<td>85.0 ± 5.5</td>
</tr>
<tr>
<td>SNP (n = 9)</td>
<td>−12.5 ± 1.7*</td>
<td>78.9 ± 5.9</td>
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<table>
<thead>
<tr>
<th>Force of Contraction</th>
<th>Time to Peak Tension</th>
<th>Half-maximal Relaxation Time</th>
</tr>
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<tr>
<td>Control (n = 6)</td>
<td>Δ% control</td>
<td>88.3 ± 2.5</td>
</tr>
<tr>
<td>8-bromo-cGMP (n = 6)</td>
<td>−38.2 ± 5.2**</td>
<td>83.3 ± 1.7</td>
</tr>
</tbody>
</table>

Data are given as mean ± S.E.M. * P < .05; ** P < .01.

**Human Atrial Trabeculae**

**Fig. 10.** (A) Bar graph demonstrating the effect of SNP (100 μmol/l), MB (10 μmol/l) and SNP in the presence of MB on the cGMP content of human atrial trabecula. Experiments were performed on trabecula from five individual hearts. Ordinate, cGMP concentration in femtomoles per milligram wet weight. Data are given as mean ± S.E.M. (B) Bar graph demonstrating the effect of SNP (100 μmol/l), MB (10 μmol/l) and SNP in the presence of MB on inotropic FOC of the very same human atrial trabecula as taken for cGMP determination after maximal FOC had developed. Ordinate, FOC (percent of basal FOC, basal FOC being 100%).

Of special interest is a recent study by Mohan et al. (1996) concerning the inotropic effect of different NO donors and 8-bromo-cGMP on isolated cat papillary muscles. This study suggests that NO and 8-bromo-cGMP exert a positive inotropic effect at low concentrations and a negative inotropic effect at high concentrations. Within the same concentration range and with a very similar experimental setup, a positive inotropic effect of SNP and 8-bromo-cGMP was not observed at either low or high concentrations in human myocardium.

One previously reported effect of NO donors and of 8-bromo-cGMP on myocardial contractile performance is a shortening of the contractile twitch with a hastening of myocardial relaxation. In the present study, SNP and 8-bromo-cGMP caused a slight shortening of time to peak tension with an earlier onset of relaxation. Maximum contraction velocities were not altered compared with controls. This observation is in accordance with the in vivo data in humans of Paulus et al. (1994), who observed a decrease in left ventricular electromechanical systole time because of an earlier onset of left ventricular isovolumic relaxation during intracoronary SNP infusion. In this study, SNP even caused a decrease in the absolute value for the velocity of pressure decrease. In the guinea pig isolated heart experiments of Grocott-Mason et al. (1994), left ventricular pressure decline was not only premature but also accelerated, at least in early diastole after SNP infusion. Fort and Lewis (1993) also described a significant reduction in the velocity of pressure decline during bradykinin infusion of isolated ejecting ferret hearts. However, this effect was independent of the absence or presence of a NOS inhibitor and thus possibly unrelated to NO. Taken together, there seem to be slight differences concerning changes in the time-dependent variables of the contractile cycle caused by NO donors, which might depend on the species chosen for the experiments and the experimental setting (in vivo, isolated ejecting heart, isolated atrial/papillary muscle experiments). However, the concurring result of all these studies is that NO, besides a decrease in peak force or pressure development, leads to an abbreviation of the contractile cycle.

Data concerning the effect of cGMP on the time course of the contractile cycle are very similar. This is important if one wants to strengthen the hypothesis that the effects of NO on cardiac contractility are mediated by cGMP. Shah et al. (1991) reported that in isolated ferret papillary muscle, 8-bromo-cGMP within a concentration range similar to that in our experiments reduced time to peak tension to a similar extent as peak developed tension. This led to an earlier onset of mechanical relaxation and a reduction of overall twitch duration. Similarly, a reduction in myocyte twitch amplitude and time to peak shortening by roughly 20% without changes in shortening velocity was observed in isolated adult rat ventricular myocytes after exposure to 8-bromo-cGMP (Shah et al., 1994). In this study, the effects of cGMP on time-dependent variables resembled very much those of SNP,
which suggested an identical negative inotropic mechanism. On the other hand, in accordance with earlier observations in ferret cardiac muscle (Shah et al., 1991), 8-bromo-cGMP led to a significant decrease in half-maximal relaxation time in human atrial trabecula in this study. This effect was not observed after exposure of atrial trabecula to SNP. One might speculate whether the effect of cGMP on relaxation time is concentration-dependent and whether the observed discrepancy between 8-bromo-cGMP and SNP is caused by the fact that the effect of 100 μmol/l SNP cannot be compared directly with the effect of 100 μmol/l 8-bromo-cGMP. These two substances do not depend on each other in a stoichiometric way; therefore, concentrations of both substances are difficult to compare. This might also explain why the negative inotropic effect of 8-bromo-cGMP was much more pronounced than the effect of an equimolar concentration of SNP. On the other hand, differences between cGMP and NO donors concerning their effects on the time course of the contractile twitch might also occur, because NO has other effects in addition to stimulation of guanylyl cyclase.

The results of myocardial cGMP quantification support the theory that NO effects on cardiac contractile performance are mediated via guanylyl cyclase activation and generation of cGMP. Not only was there a remarkable increase in myocardial cGMP concentrations after exposure to SNP, which proves that NO leads to an activation of the guanylyl cyclase in human myocardium, but also the effects of SNP on atrial and ventricular contractility and on myocardial cGMP concentrations could both be attenuated by the guanylyl cyclase inhibitor methylene blue. This finding is in agreement with observations in other studies (Brady et al., 1993; Mohan et al., 1996). Interestingly, the effect of SNP on both FOC and myocardial cGMP concentrations could not be completely antagonized by methylene blue. This might indicate that mechanisms other than activation of the guanylyl cyclase-cGMP pathway are involved as suggested previously (Stamler et al., 1993). These mechanisms include inhibition of enzyme activity by S-nitrosylation (as has been shown for cathepsin B, aldolase, γ-glutamylcysteinyl synthetase, alcohol and aldehyde dehydrogenases and glyceraldehyde-3-phosphate dehydrogenase) and by the binding of NO to Fe(II) or Fe(III) in heme groups (as shown for cytochrome P-450 or for NOS itself) (Stamler, 1994). Indeed, NO has led to increased superoxide anion (O₂⁻) and superperoxide (H₂O₂) production in rat heart mitochondria (Poderoso et al., 1996). Also, experiments with rat heart mitochondria preparations have demonstrated that NO interferes with mitochondrial electron transport either directly or indirectly via formation of peroxynitrite (Cassina and Radi, 1996). That another substance released from SNP is responsible for the remaining negative inotropic effect in the presence of methylene blue seems to be rather unlikely, because potassium cyanide, which is released from SNP together with NO and leads to an inhibition of oxidative phosphorylation of mitochondria, showed no lasting effect on atrial trabecula contractility.

There has been an ongoing discussion whether cGMP and cGMP-elevating substances affect basal contractility or only contractility in the presence of cAMP-elevating substances. Latter findings supported the theory that cGMP influenced cardiac contractility by opposing the effect of cAMP (Goldberg et al., 1975). In this study, the effect of SNP on atrial trabecula contractility was slightly, but not significantly more pronounced in the presence of isoprenaline and IBMX. Interestingly, in the ventricular myocardium, this difference between the negative inotropic effect of SNP in the absence or presence of cAMP-increasing substances was significant (P < .05) for papillary muscle preparations from nonfailing, but not from failing hearts. This observation might be from the fact that the generation of cAMP in response to isoprenaline is reduced because of the well-characterized desensitization of the adenylyl cyclase (Bristow et al., 1982; Böhm et al., 1990). Thus, the negative inotropic effect of NO, at least in ventricular myocardium, seems to be stronger in, but not dependent on the presence of elevated cAMP levels.

The precise mechanism by which NO and cGMP could affect cardiac contractility has not been completely understood so far (for review, see Lohmann et al., 1991). There is evidence from experiments with myocytes of different species that cGMP decreases Ica (Nawrath, 1977; Levi et al., 1989; Méry et al., 1991). In contrast to the functional data obtained in this study, in these electrophysiological studies, cGMP inhibited only cAMP-stimulated, but not basal Ica (Lohmann et al., 1977). This decrease in Ica might be caused by a stimulation of cAMP hydrolysis by cGMP (Endoh, 1979; Flitney and Singh, 1981; Walter, 1984). Evidence for the latter hypothesis comes from the observation that IBMX largely reverses the inhibitory effect of cGMP on cAMP-stimulated Ica (Hartzell and Fischmeister, 1986). Again, this is in contrast to our finding that in the presence of IBMX the effect of SNP on atrial contractility was even more pronounced. Another mechanism leading to a decrease in Ica independent from cAMP has to be taken into account, e.g., modulation of L-type Ca²⁺ -channel activity by cGMP-dependent protein kinase (Levi et al., 1989; Thakkar et al., 1988; Méry et al., 1991). In contrast to these considerations, Kirstein et al. (1995) demonstrated that the NO donor SIN-1 within a concentration range of 1 pmol/l to 10 nmol/l had a stimulatory effect on Ica in isolated human atrial myocytes, which was found to be caused by cGMP-induced inhibition of cGMP-inhibited phosphodiesterase. Ica was suppressed only at higher concentrations. In the present study, the lowest SNP concentration studied was 10 nmol/l. A negative inotropic effect started at a concentration of 100 nmol/l and higher. Thus, it might be possible that the stimulatory effect of NO/cGMP on Ica might have been missed in our experiments. A third mechanism explaining the negative inotropic effect of cGMP has been demonstrated only recently by Shah et al. (1994). They observed that 8-bromo-cGMP reduces the myofilament response to Ca²⁺ in intact adult ventricular myocytes. Most likely, this effect is mediated via cGMP-dependent protein kinase. This latter mechanism could explain why, despite an increase in Ica and low NO concentrations, there is no increase in FOC.

The physiological relevance of the negative inotropic effect of NO has repeatedly been a matter of controversy. Obviously, even at high SNP concentrations (100 μmol/l), the reduction in contractile force was significantly smaller than the decrease in contractility caused by other negative inotropic substances such as carbachol or R-PIA. However, an acute reduction in peak force development by roughly 10 to 20% under basal conditions and in the presence of cAMP-elevating positive inotropic substances is not negligible. Also, the results of the present study indicate that a significant negative inotropic effect occurs already at lower concentra-
tions of SNP, which indicates that physiological concentrations of NO also decrease cardiac contractility. So far, in vivo data on NO concentrations within the coronary system or within the heart under different pathological conditions are missing. However, there are data from healthy volunteers (Gilmer et al., 1996) and from women with preeclampsia (Lyell et al., 1995) on serum nitrate or nitrite concentrations, respectively, which both reflect serum NO levels. Both studies indicate that under physiological as well as pathological conditions, NO serum concentrations are within a concentration range similar to the negative inotropic SNP concentrations used in this study. Recent findings of an induction of NO synthase and an increase in NOS activity in congestive heart failure (Stoehr et al., 1994) are generated within the myocardium. Control of NO release, e.g., by inhibitors of NO synthase, might be an important therapeutic target in cardiovascular disorders such as septic cardiomyopathy, congestive heart failure after myocarditis or myocardial perfusion caused by decreased NO release.

In summary, the results in this study show that NO via stimulation of guanylyl cyclase and generation of cGMP exerts a negative inotropic effect on human atrial and ventricular myocardium. This inotropic effect of NO might be of functional relevance for the control of cardiac contractility by the endothelium. The negative inotropic potency of NO might be especially important in pathological conditions, when NO synthase activity is increased and high concentrations of NO are generated within the myocardium. Control of NO release, e.g., by inhibitors of NO synthase, might be an important therapeutic target in cardiovascular disorders such as septic cardiomyopathy, congestive heart failure after myocarditis or cardiac allograft rejection.

References


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