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The ineffectiveness of the NO-cyclic GMP signaling pathway in the atrial myocardium

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1 This study was performed to determine whether nitric oxide (NO) has direct effects on force of contraction (Fc) in atrial myocardium from rats, rabbits, guinea-pigs, frogs, and man.

2 Glyceryl trinitrate, isosorbide dinitrate, 3-morpholino-sydnonimine hydrochloride (SIN-1), and S-nitroso-N-acetylpenicillamine (SNAP) did not signficantly reduce Fc in the various preparations investigated, either given alone or after stimulation of α- or β-adrenoceptors.

3 SNAP did not change the time course of contractions in rat, guinea-pig and human preparations.

4 8-Bromo-guanosine-3':5'-cyclic monophosphate (8-Br-cyclic GMP) produced a negative inotropic effect in rat, guinea-pig and human atrial preparations and shortened time to peak tension and relaxation time in human preparations.

5 High K^+ (85 mmol l^{-1})-induced contracture in rat heart muscle was reduced by 8-Br-cyclic GMP but not by SIN-1.

6 N-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase, failed to influence muscarinic effects on Fc or frequency from rat and guinea-pig hearts.

7 We conclude that NO, under the experimental conditions described here, has no direct effects on the heart, although cyclic GMP may be involved in the regulation of myocardial contraction.

Keywords: Heart; nitric oxide; cyclic GMP; nitrovasodilators

Introduction

Nitric oxide (NO) synthesized from L-arginine is regarded as an ubiquitous chemical messenger involved in the signal transduction in a variety of organ systems including vascular and neuronal tissues leading to activation of soluble guanylate cyclase and subsequent increases in guanosine 3':5'-cyclic monophosphate (cyclic GMP) levels (for review see Dinerman *et al.*, 1993). The role of the NO-signaling pathway in the modulation of cardiac function is, however, not well understood.

Nitrovasodilators are supposed to mediate their effects on smooth muscle via NO (Rapoport *et al.*, 1983). Their ability to influence myocardial contractility has been discussed controversially. Positive inotropic effects of glyceryl trinitrate have been described in feline and human papillary muscles (Strauer, 1973) and of sodium nitroprusside in feline atria (Diamond *et al.*, 1977). In contrast, negative inotropic actions of sodium nitroprusside in canine atria (Endoh & Yamashita, 1981) or in ferret papillary muscles (Smith *et al.*, 1991) and in the perfused heart of the ferret (Fort & Lewis, 1991) have been demonstrated. Rodger & Shahid (1984) have shown that sodium nitroprusside was without inotropic effect, either positive or negative, in rabbit right ventricular papillary muscles. In anaesthetized dogs, a transient positive inotropic effect of glyceryl trinitrate was observed which has been related to sympathetic reflex activity induced by the fall in arterial blood pressure (Ross & Jorgensen, 1968). Moreover, a negative intropic effect of cytokines on the heart which is thought to be mediated through a myocardial inducible NO synthase has been demonstrated (Finkel *et al.*, 1992).

To further elucidate the role of NO in the heart, we studied the effects of nitrovasodilators on force of contraction (Fc) in a variety of isolated atrial heart muscle preparations from rats, rabbits, guinea-pigs, frogs, and man. In addition, to determine whether the L-arginine-NO pathway is involved in the cholinergic regulation of the heart, we studied the effects of the NO synthase inhibitor N-monomethyl-L-arginine (L-NMMA) on the response of left and right atrial preparations from rats and guinea-pigs to acetylcholine or carbachol.

Methods

Preparations

Sprague-Dawley rats (200-500 g) of either sex were anaesthetized with ether, male guinea-pigs (300-800 g) and rabbits (1000-1200 g) were stunned with a blow on the head and bled from the carotid arteries. Female frogs (30-50 g) were kept in ice-water for 15 min and then decapitated. The hearts were quickly removed and immersed into warmed and oxygenated Tyrode solution. Whole hearts were pinned down on Sylgard to cut off left or right atria which were supplied at two ends with silk ligatures. Human atrial heart muscle preparations were obtained from patients undergoing open heart surgery. The patients ($n=8$; 3 female, 5 male) ranged in age from 35 to 80 years and suffered from coronary heart disease or aortic/mitral valve dysfunctions without or with signs of heart failure. Most patients had been treated with β-adrenoceptor blockers and/or diuretics, calcium antagonists, cardiac glycosides before surgery. General anaesthesia was performed with narcotic combinations, skeletal muscle relaxants and analgesics. At surgery, approximately 1 cm^2 of atrial myocardium was removed from the right atrial appendage as a part of the cannulation procedure for cardiopulmonary bypass. Immediately after excision, the tissue was immersed in cool (4°C) preoxgenated Tyrode solution. The time between excision and the beginning of laboratory processes was 10 min. The atrial heart muscle samples were transferred to a dissection chamber

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containing oxygenated warm Tyrode solution, pinned down on Sylgard, and cut into 4–6 strips. Both ends were ligated with fine silk sutures.

Experimental procedure

All preparations were mounted next to two platinum electrodes built in a muscle holder. They were then placed vertically in 5 ml organ baths and connected via stainless steel wires to an inductive force-displacement transducer whose output was fed to a Hellige carrier frequency preamplifier (resting tension 1.0 g). Left atrial heart muscle preparations were electrically driven by square wave pulses (Grass S4; 1 ms duration; voltage 20% above threshold) at 0.5 Hz (rat), 1.0 Hz (man, rabbit) or 3.0 Hz (guinea-pig). The cardiac preparations from frogs were stimulated electrically (Grass S4; 3–4 ms duration; voltage 20% above threshold) at a frequency of 0.5 Hz. The various frequencies were chosen to warrant stable contractions during the experimental procedure, although changes in the driving rate did not change qualitatively the response to the drugs which were investigated in this study. This possibility was seriously considered, since L-NMMA has been described to reverse the force-frequency-relationship in isolated hamster papillary muscles from negative to positive (Finkel *et al.*, 1995). Twitch responses were recorded under isometric conditions at the apex of the preload active tension curve. Force of contraction (Fc) was monitored in left atrial preparations, frequency in right atrial preparations. K^+-induced contractures were produced in rat left atria.

The Tyrode solution, containing (mmol l^{-1}): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.6, EDTA 0.05, was equilibrated with 95% O_2 and 5% CO_2 at 37°C (pH 7.4). The studies on frog heart preparations were performed at room temperature. The high potassium (85 mmol l^{-1}) solution for inducing contractures in rat left atria was prepared by replacing the appropriate amount of NaCl of the Tyrode solution with KCl. All light-sensitive substances were protected from degradation by using dark-room illumination during the experimental procedures.

The preparations were allowed to equilibrate for at least 45–60 min. In the case of K^+-induced contractures, electrical stimulation was stopped 10 s before the solution change.

Chemicals

The following drugs were used (abbreviations and sources in parentheses): 3-morpholino-sydnonimine hydrochloride (SIN-1) (Cassella, Frankfurt/Main, F.R.G.); S-nitroso-N-acetylpenicillamine (SNAP) (Calbiochem, Bad Soden/F.R.G.); zaprinast (M&B 22948) (Rhône-Poulenc Rorer, Daggenham, Great Britain); atropine hydrochloride, 8-bromo-guanosine-3':5'-cyclic monophosphate (8-Br-cyclic GMP), substance P, (Serva, Heidelberg/F.R.G.); atenolol, isosorbide dinitrate, N-monomethyl-L-arginine (L-NMMA), phenylephrine (PE) (Sigma, Munich/F.R.G.); isoprenaline sulphate (Boehringer, Ingelheim/F.R.G.). Acetylcholine (ACh), carbamoylcholine hydrochloride (carbachol), glyceryl trinitrate and all other chemicals were purchased from Merck, Darmstadt (F.R.G.). The concentration-dependent spontaneous release of NO from both SIN-1 and SNAP in aqueous solution was detected qualitatively in the gas phase of sealed vials, filled with drug-containing Tyrode solution, by use of NO chemiluminescence analyzer (Sievers 270 B, Fa. Gerstel; Mülheim/F.R.G.). All drug solutions were freshly prepared before the experiments. Stock solutions of SNAP and isosorbide dinitrate were prepared in dimethylsulphoxide (DMSO), all other drugs were dissolved in water and further diluted in Tyrode solution. Glyceryl trinitrate was purchased in solution (1% dissolved in 96% ethyl alcohol). The effects of solvents in their final concentrations in the test solution were taken into account by control experiments using the solvents without drugs. The effects were either negligible (DMSO) or, where relevant (ethyl alcohol), described in the Results section.

Evaluation of results

Results are shown as original records or expressed as mean ± standard error of mean (s.e.mean). Peak levels of phasic contractions (Fc) or tonic tension were evaluated and are given as % of control values. The time course of myocardial contractions was described by the determination of time to peak tension (TTP) and relaxation time (RT), evaluated, for accuracy, slightly above diastolic tension at 10% of peak tension. Concentration-response curves were constructed by fitting a sigmoid curve using GraphPad Inplot Software. Student's paired two-tailed t test was used to determine the significance of differences between means. Statistically significant differences are marked by one or two asterisks corresponding to $P<0.05$ or $P<0.01$, respectively.

Results

Figure 1 shows the effects of three nitrovasodilators, i.e. glyceryl trinitrate, SIN-1, and isosorbide dinitrate, in cumulatively increasing concentrations on force of contraction in rat left atria. Fc was barely affected apart from a slight reduction which reflects run down of isolated preparations, also seen without the addition of drugs, following the same time course of experimentation. Similar results as shown in Figure 1 were also obtained with the rabbit left atria (not shown).

Another nitrovasodilator, SNAP, was equally ineffective in rat left atria at 100 μmol l^{-1}, given alone or after stimulation of α- or β-adrenoceptors with phenylephrine (in the presence of atenolol 10 μmol l^{-1}) or isoprenaline, respectively (Figure 2). SNAP also failed to influence TTP or RT under these conditions (Table 1). In contrast, 8-Br-cyclic GMP (100 μmol l^{-1} reduced Fc by about 50% (Figure 2). Since nitrovasodilators are thought to act via an increase in cyclic GMP levels, inhibition by zaprinast of the cyclic GMP-specific phosphodiesterase is expected to potentiate the response to these substances. In the presence of zaprinast 30 μmol l^{-1} Fc was slightly reduced by about 5%. But also under these conditions, glyceryl trinitrate and SIN-1 remained ineffective in rat left atria (not shown). We have also considered the possibility that endogenously released NO might affect cardiac function. This was studied by the application of substance P, based on the fact that local cardiac effects of substance P have been described (Chiao & Caldwell, 1995) and the effects of substance P in other tissues are mediated, at least partially, by NO (Jin *et al.*, 1993; Ziche *et al.*, 1993). Fc in isolated left atria remained, however, virtually unaffected after the application of substance P 100 μmol l^{-1} (in the presence of atropine 1 μmol l^{-1} and atenolol 10 μmol l^{-1} 97.3% of control values; $n=3$).

To test whether NO donors are effective in the heart during tonic tension, contractures were induced in rat left atria by

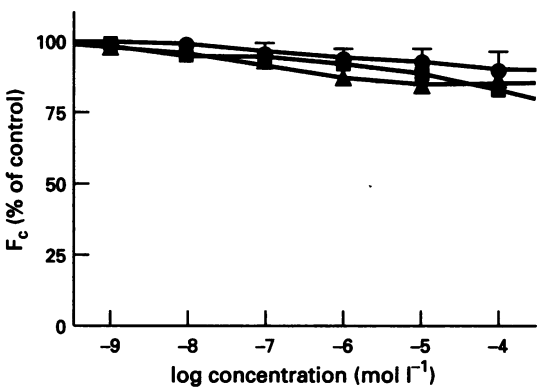

Figure 1 Effects of nitrovasodilators on force of contraction (Fc) in rat left atria. Cumulative concentration-response-relationships of (●) isosorbide dinitrate; (■) glyceryl trinitrate and (▲) 3-morpholino-sydnonimine (SIN-1). Symbols represent means ± s.e.mean, $n=3$ for each drug.

high extracellular K^+ (85 mmol l^{-1}). SIN-1 10 μmol l^{-1} did not change the level of high K^+-induced contracture, whereas 8-Br-cyclic GMP 100 μmol l^{-1} significantly relaxed the preparations (Figure 3).

Figure 4 (a) shows the cumulative concentration-response relationships for ACh on Fc in rat left atria under control conditions and in the presence of an inhibitor of NO synthase. The ACh-evoked negative inotropic response was not changed in the presence of L-NMMA 100 μmol l^{-1}. L-NMMA itself did not produce significant changes of Fc.

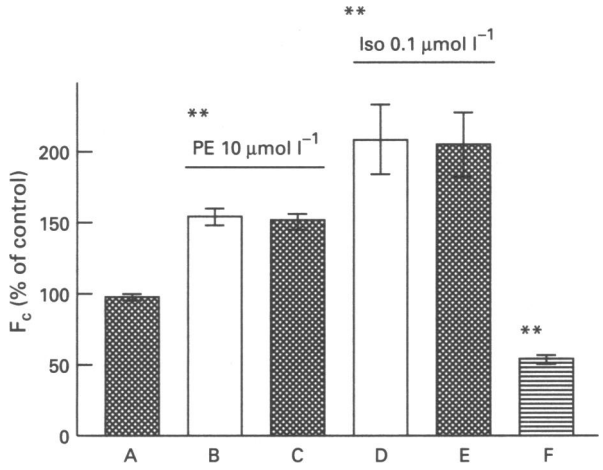

Figure 2 Influence of S-nitroso-N-acetylpenicillamine (SNAP) and 8-Br-cyclic GMP on force of contraction (Fc) in rat left atria. Effects of SNAP 100 μmol l^{-1} in untreated preparations (A) and during stimulation of α-adrenoceptors with phenylephrine 10 μmol l^{-1}, PE (C) or β-adrenoceptors with isoprenaline 0.1 μmol l^{-1}, Iso (E). Inotropic effects of PE (B) and Iso (D) *per se*. (F), inotropic effect of 8-Br-cyclic GMP 100 μmol l^{-1}. Columns represent means ± s.e.mean, $n = 4-13$. The asterisks denote statistically significant differences of values in the presence of PE, Iso, and 8-Br-cyclic GMP against the respective control values.

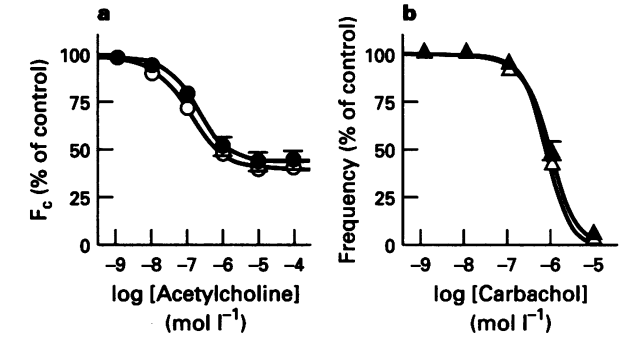

Figure 4 Influence of L-NMMA on the ACh-induced inotropic effect in rat left atria and on the chronotropic effect of carbachol in spontaneously beating rat right atria. (a) (○) ACh; (●) ACh in the presence of L-NMMA 100 μmol l^{-1} ($n = 7$, paired data). (b) (△). Carbachol; (▲) carbachol in the presence of L-NMMA 100 μmol l^{-1} ($n = 3$, unpaired data). Symbols represent means ± s.e.mean.

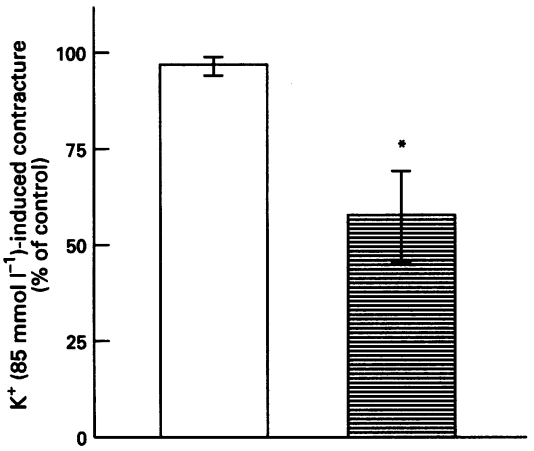

Figure 3 Effects of 3-morpholino-sydnonimine hydrochloride (SIN-1) 10 μmol l^{-1} (open column) and 8-Br-cyclic GMP 100 μmol l^{-1} (striated column) on K^+ (85 mmol l^{-1})-induced contracture in rat left atria. Columns represent means ± s.e.mean, $n = 4$, paired data. The asterisk denotes statistical significance (effect vs. control).

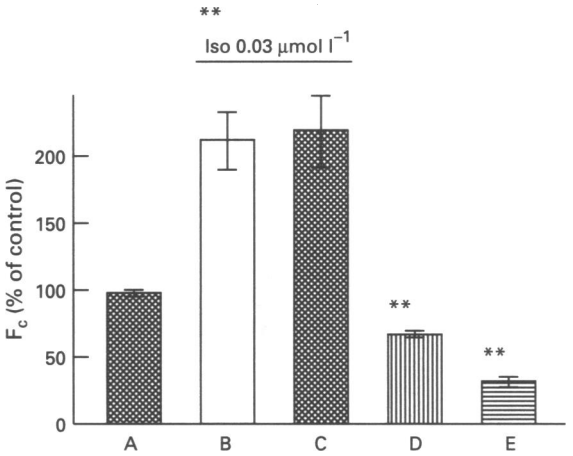

Figure 5 Influence of S-nitroso-N-acetylpenicillamine (SNAP) and 8-Br-cyclic GMP on force of contraction (Fc) in guinea-pig left atria. Effects of SNAP 100 μmol l^{-1} in untreated preparations (A) and during stimulation of β-adrenoceptors with isoprenaline 0.03 μmol l^{-1}, Iso (C). (B) Inotropic effects of Iso *per se*. Inotropic effects of 8-Br-cyclic GMP 100 μmol l^{-1} (D) and 1 mmol l^{-1} (E). Each column represents means ± s.e.mean, $n = 4-7$. The asterisks denote statistically significant differences of values in the presence of Iso, and 8-Br-cyclic GMP against the respective control values.

Table 1 Influence of S-nitroso-N-acetylpenicillamine (SNAP) on time to peak tension (TTP) and relaxation time (RT) in rat atrial heart muscle preparations

			Phenylephrine (10 μmol l^{-1})		*Isoprenaline* (0.1 μmol l^{-1})	
	Control	*SNAP* (100 μmol l^{-1})	*Control*	*SNAP* (100 μmol l^{-1})	*Control*	*SNAP* (100 μmol l^{-1})
TTP (ms)	25 ± 1	25 ± 1	23 ± 1	23 ± 1	22 ± 1	22 ± 1
RT (ms)	35 ± 1	35 ± 1	34 ± 2	33 ± 1	29 ± 1	29 ± 1

Values are means ± s.e.mean, $n = 3$.

For studying the effect of a muscarinic agonist on the frequency of spontaneously beating rat right atria, carbachol was used instead of ACh (b). Thus, varying results because of higher activities of ACh esterases in the sinoatrial region were avoided. The concentration-dependent negative chronotropic effect of carbachol was not changed by L-NMMA 100 μmol l^{-1}. Similar results were obtained in left and right atrial preparations from guinea-pigs (not shown).

Since the rat, in its cardiac pharmacology, may be a somewhat atypical species, we have also investigated the effects of SNAP and, for comparison, of 8-Br-cyclic GMP on Fc in isolated atrial preparations from guinea-pig left atria, either given alone or in the presence of isoprenaline 0.03 μmol l^{-1} (Figure 5). Time to peak tension and relaxation time were also not influenced by SNAP (Table 2). A concentration-dependent negative inotropic effect of 8-Br-cyclic GMP was also observed in this preparation (Figure 5).

In our search, across species, for a functional NO-cyclic GMP pathway in the heart, isolated human preparations were also included. In isolated strips from the right atrium, SIN-1 100 μmol l^{-1} produced a slight positive inotropic effect, whereas SNAP 100 μmol l^{-1} was ineffective; in contrast, 8-Br-cyclic GMP 100 μmol l^{-1} produced a strong negative inotropic effect (Figure 6). Time to peak tension and relaxation time were significantly reduced by 8-Br-cyclic GMP but not by SIN-1 or SNAP (Figure 6e and Table 3).

Finally, we investigated the effects of NO donors in the frog heart which has been described to be strongly influenced by 8-Br-cyclic GMP (Singh & Flitney, 1981) and sodium nitroprusside (Flitney *et al.*, 1980). Figure 7 demonstrates the concentration-dependent effects of glyceryl trinitrate and SIN-1 in frog atrial heart preparations. The negative inotropic effects of glyceryl trinitrate (30 and 100 μmol l^{-1}) were significantly more pronounced than the effects elicited by the solvent ethyl alcohol alone (at the highest concentration of ethyl alcohol). SIN-1 did not significantly alter Fc at both concentrations used.

Discussion

The effects of nitrovasodilators on the heart have been discussed controversially (McDonald *et al.*, 1994; Schulz & Triggle, 1994). Under clinical conditions, cardiac performance

Table 2 Influence of S-nitroso-N-acetylpenicillamine (SNAP) on time to peak tension (TTP) and relaxation time (RT) in guinea-pig atrial heart muscle preparations

			Isoprenaline (0.1 μmol l^{-1})	
	Control	*SNAP* (100 μmol l^{-1})	*Control*	*SNAP* (100 μmol l^{-1})
TTP (ms)	32±1	33±1	30±1	29±1
RT (ms)	54±2	53±1	42±1	42±1

Values are means±s.e.mean, $n=4$.

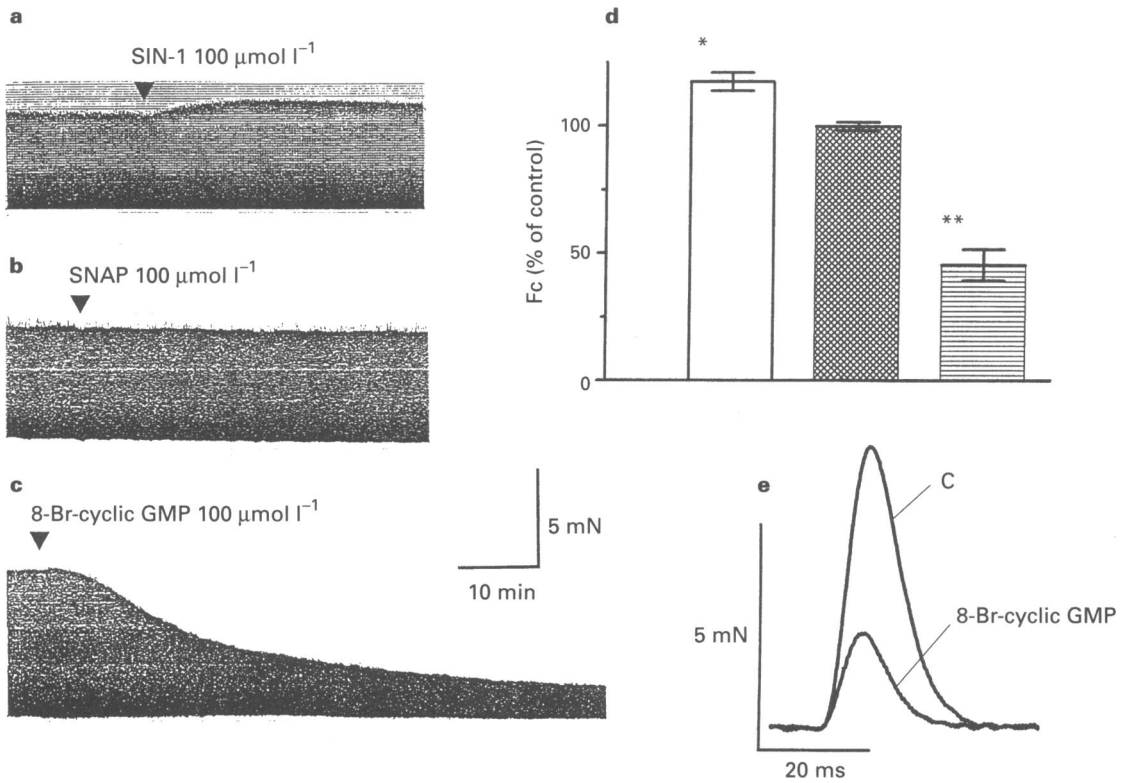

Figure 6 Effects of 3-morpholino-sydnonimine (SIN-1) 100 μmol l^{-1}, S-nitroso-N-acetylpenicillamine (SNAP) 100 μmol l^{-1} and 8-Br-cyclic GMP 100 μmol l^{-1} on Fc and time course of contraction in human atrial heart muscle preparations. (a, b, c, e). Original records. (d) Columns represent means±s.e.mean for SIN-1 (open), SNAP (cross-hatched) and 8-Br-cyclic GMP (horizontally hatched), $n=5-8$. The asterisks denote statistically significant differences of values in the presence of SIN-1, and 8-Br-cyclic GMP against the respective control values.

Table 3 Influence of S-nitroso-N-acetylpenicillamine (SNAP) and 8-Br-cyclic GMP on time to peak tension (TTP) and relaxation time (RT) in human atrial heart muscle preparations

	Isoprenaline (0.1 μmol l^{-1})					
	Control	*SNAP* (100 μmol l^{-1})	*Control*	*SNAP* (100 μmol l^{-1})	*Control*	*8-Br-cyclic GMP* (100 μmol l^{-1})
TTP (ms)	87±3	81±2	80±8	82±7	86±2	77±3*
RT (ms)	165±5	169±4	117±48	116±6	191±13	165±10*

Values are means±s.e.mean, $n=3-7$. *$P<0.05$, significantly different from control.

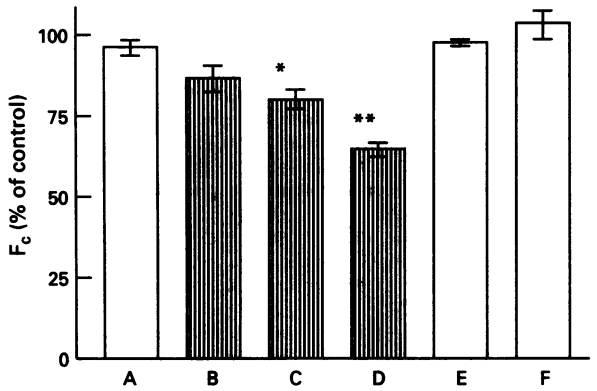

Figure 7 Influence of glyceryl trinitrate, ethyl alcohol, and 3-morpholino-sydnonimine (SIN-1) on Fc in cardiac preparations from frogs. Negative inotropic effect of glyceryl trinitrate 10, 30 and 100 μmol l^{-1} (B, C and D, respectively) ($n=3$, paired data) and influence of ethyl alcohol 0.22% (A) which corresponds to the solvent content reached at glyceryl trinitrate 100 μmol l^{-1} (unpaired data, same sample size). SIN-1 10 μmol l^{-1} (E) and 100 μmol l^{-1} (F) did not significantly change Fc ($n=6$, paired data). Columns represent means±s.e.mean. Asterisks denote statistically significant differences of values in the presence of glyceryltrinitrate against the respective control values.

is not impaired by the use of vasodilators but rather improved (Ross & Jorgensen, 1968; Monrad *et al.*, 1986).

The increasing interest in the effects of NO in various organ systems gave rise to numerous recent investigations on the modulatory role of NO also in heart muscle. Several reports have suggested that the direct effects of nitrovasodilators on the heart are moderate to none (Strauer, 1973; Diamond *et al.*, 1977; Lincoln & Keely, 1980; Laustiola *et al.*, 1983; Rodger & Shahid, 1984; Yanagisawa *et al.*, 1988; Ishibashi *et al.*, 1993; Kennedy *et al.*, 1994). Other evaluations revealed modulatory effects on the heart function (Endoh & Yamashita, 1981; Fort & Lewis, 1991; Smith *et al.*, 1991; Shah & Lewis, 1993). A recent study showed that physiological concentrations of nitric oxide do not elicit an acute negative inotropic effect in unstimulated cardiac muscle (Weyrich *et al.*, 1994). Organic nitrovasodilators are possibly not metabolized by mammalian ventricular muscle (Brady *et al.*, 1993). This may reduce an inotropic response to nitrovasodilators in single cardiomyocytes, but only to a minor extent in multicellular heart muscle preparations where coronary microvessels are also present. This eventual shortcoming is anyway prevented by the use of either SIN-1 or SNAP which spontaneously release NO, by different mechanisms, in aqueous solutions (Kröncke *et al.*, 1993).

The effects of NO are assumed to be mediated by activation of soluble guanylate cyclase and the intracellular accumulation of cyclic GMP (Moncada *et al.*, 1991). It is in line with a functional NO-cyclic GMP signaling pathway in the heart that cyclic GMP or derivatives can depress Fc (Nawrath 1976; 1977; Kohlhardt & Haap, 1978) and the calcium current (Hartzell & Fischmeister, 1986; Levi *et al.*, 1989; 1994; Wahler *et al.*, 1990; Méry *et al.*, 1991). Wahler and Dollinger (1995) showed that SIN-1 inhibits mammalian cardiac calcium current through activation of a cyclic GMP-dependent protein kinase. However, the situation regarding the effects of cyclic GMP on the heart also remains unsettled, since the electrophysiological results are inconsistent (Trautwein *et al.*, 1982; Wahler & Sperelakis, 1985; Thakkar *et al.*, 1988).

We have found that nitrovasodilators do virtually not affect myocardial contractility, either peak tension or time course of contraction, in concentrations which fully relax smooth muscle. This observation holds true for atrial heart muscle preparations isolated from rats, guinea-pigs, rabbits, and man, either unstimulated or stimulated by activation of α- or β-adrenoceptors. In frog heart preparations, glyceryl trinitrate can depress Fc to a greater extent than its solvent ethyl alcohol, however, SIN-1 exerted no negative inotropic effect. Therefore, the negative inotropic response to glyceryl trinitrate in frog heart which was not observed in mammalian preparations may be mediated by other mechanisms than activation of the NO signaling pathway. Tonic tension in rat left atria, induced by high external K^+ (85 mmol l^{-1}, was also unchanged by SIN-1 but significantly reduced by 8-Br-cyclic GMP.

The question remains unsettled why 8-Br-cyclic GMP depresses Fc and contracture, whereas organic nitrovasodilators which activate guanylate cyclase are virtually ineffective, even in the presence of zaprinast, an inhibitor of cyclic GMP phosphodiesterase. There are four possibilities to explain this discrepancy: First, guanylate cyclase may not be expressed in cardiomyocytes in sufficient amounts. Second, the guanylate cyclase-cyclic GMP system may be defective in atrial heart muscle. Third, cyclic GMP fails to stimulate the effector system to a striking amount as can 8-Br-cyclic GMP. Or fourth, the effects of 8-Br-cyclic GMP are rather unspecific and cannot be taken as effects of cyclic GMP itself. The latter possibility is not excluded but becomes less probable by the finding that inhibitors of cyclic GMP-dependent protein kinase antagonize the effects of 8-Br-cyclic GMP in rat left atria (Bäumner & Nawrath, 1995).

SIN-1, but not SNAP or glyceryl trinitrate or isosorbide dinitrate, exerted a slight positive inotropic effect in some preparations (statistically significant only in human preparations) which may be due to the release of endogenously stored catecholamines. We did, however, not investigate this effect in detail, since it was restricted to SIN-1.

The effects of cholinergic stimulation on the heart remained unaffected in the presence of L-NMMA indicating that NO generation also does not contribute to the effects of ACh or carbachol in the heart, in sharp contrast to the relaxing effects of ACh in smooth muscle which are clearly dependent on the generation of NO (Rees *et al.*, 1990).

Under the conditions described here, NO leaves myocardial contractility unchanged. It is, however, fair to assume that, in other or pathophysiological conditions, the NO-cyclic GMP signaling pathway may become operative also in the myocardium. This is suggested by the fact that both constitutive and inducible isoforms of NO synthase can be expressed in cardiac myocytes (Schulz *et al.*, 1995).

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