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Positive inotropic effect of exogenous and endogenous NO in hypertrophic rat hearts

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1 Recent evidence suggests that nitric oxide (NO) modulates the contractile force of isolated cardiomyocytes in a biphasic manner. We sought to examine whether myocardial hypertrophy induced by long-term hypertension changes the effects of NO on myocardial contractility.

2 We used constant flow perfused non-paced Langendorff preparations of hearts of 3 months old Wistar rats (WIS, $n=23$) and of stroke-prone spontaneously hypertensive rats (SHR) at the age of 10 months (SHR10, $n=16$) and 15 months (SHR15, $n=8$). Changes of left ventricular peak pressure (LVP), $+dP/dt_{max}$, $-dP/dt_{max}$, coronary perfusion pressure (CPP) and heart rate (HR) were recorded after infusion of noradrenaline (NA, 0.1 μmol l^{-1}), glyceryl trinitrate (GTN, 1–100 μmol l^{-1}), S-nitroso-N-acetyl-D,L-penicillamine (SNAP, 1–10 μmol l^{-1}) and N^{ω}-nitro-L-arginine (L-NOARG, 0.1–1 mmol l^{-1}).

3 Long-term hypertension induced myocardial hypertrophy and an abnormal response to NA. The relative heart weight (in mg kg^{-1}) increased from 2.95 ± 0.04 (WIS) to 6.67 ± 0.34 (SHR15), while the increase in $+dP/dt_{max}$ induced by NA was absent in SHR15. Hearts of SHR10 showed an intermediate response.

4 Both SNAP and GTN significantly increased LVP, $+dP/dt_{max}$ and $-dP/dt_{max}$ in hearts of WIS and of SHR. In WIS but not in SHR10, SNAP also increased HR. In SHR10 the lowest concentration of SNAP (1 μmol l^{-1}) showed no effect on contractility but a significantly diminished reduction of CPP suggesting inactivation of extracellularly released NO in the coronary circulation of SHR.

5 L-NOARG significantly reduced contractility in hearts of WIS and of SHR to a similar extent. At a concentration of 1 mmol l^{-1} L-NOARG also reduced HR.

6 These results suggests that positive inotropic effects of exogenous and endogenous NO are not changed in hypertension induced myocardial hypertrophy.

Keywords: Stroke-prone spontaneously hypertensive rats; SHR; N^{ω}-nitro-L-arginine; nitric oxide donors; glyceryl trinitrate; heart muscle; contractility; heart failure

Introduction

Pharmacological effects of organic nitrates such as glyceryl trinitrate (GTN) are initiated by enzymatic release of nitric oxide (NO) from the nitrate ester groups (Ahlner *et al*., 1991). In a recent investigation we demonstrated that this bioactivation also occurs in rat cardiac myocytes (Kojda *et al*., 1996). In these cells GTN and low concentrations of NO improved the contractile response to electrical field stimulation by a adenosine 3′:5′ -cyclic monophosphate (cyclicAMP)-dependent mechanism. Further investigations on newly developed organic nitrates showed that this positive inotropic activity also occurs in isolated hearts of normal Wistar rats (Kojda *et al*., 1995). There is also evidence for a positive inotropic action of NO produced by the endocardium (Brutsaert *et al*., 1988; Mohan *et al*., 1996).

In contrast, high concentrations of NO depress the contractility of rat cardiac myocytes (Brady *et al*., 1992; Kojda *et al*., 1996). The underlying mechanism most likely involves activation of guanosine 3′:5′-cyclic monophosphate (cyclic GMP)-dependent protein kinase resulting in diminished transmembrane Ca^{2+} current and/or a decreased myofilament response to Ca^{2+} (Hartzell & Fischmeister, 1986; Ono & Trautwein, 1991; Shah *et al*., 1994). It has been speculated that this effect of NO might contribute to the contractile dysfunction of the ventricular muscle in heart failure. Expression of inducible NO-synthase, which produces large amounts of NO, occurs in rat cardiac myocytes (Balligand *et al*., 1994) and in the failing human myocardium (De Belder *et al*., 1995; Haywood *et al*., 1996). On the other hand, induction of inducible NO-synthase is not consistently found in experimental and clinical heart failure (Decking *et al*., 1995; Thoenes *et al*., 1996). In this study, we investigated the inotropic action of the spontaneous NO-donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP), the organic nitrate GTN and of endogenous NO-production in both, normal and severely hypertrophic and dysfunctional hearts from rats.

Methods

This study was performed in isolated hearts of 24 normal male Wistar rats (WIS, 3 months, body weight 313 ± 4 g) and of male stroke-prone spontaneously hypertensive rats at an age of 10 months ($n=16$, SHR10, body weight 325 ± 4 g) and 15 months ($n=8$, SHR15, 318 ± 14 g), respectively. The wet heart weights of WIS, SHR10 and SHR15 were 0.93 ± 0.02 g, 1.65 ± 0.04 g and 2.21 ± 0.14 g, respectively. Previous investigations showed that SHR15 have altered baseline haemodynamic and humoral characteristics (Stasch *et al*., 1987; 1995). Some of these previous results are shown in Table 1.

The hearts were rapidly excised and perfused by the technique of Langendorff at a constant pressure of 110 cm H_2O with an oxygenated (95% O_2, 5% CO_2) modified Krebs-Henseleit-buffer (KH-buffer, pH 7.4, 37°C) of the following composition (in mmol l^{-1}): Na^+ 143.07, K^+ 5.87, Ca^{2+} 1.60, Mg^{2+} 1.18, Cl^- 125.96, HCO_3^- 25.00, $H_2PO_4^-$ 1.18, SO_4^{2-} 1.18 and glucose 5.05. A manometer connected to a small

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Table 1 Baseline cardiovascular and humoral characteristics of WIS and SHR15

Parameter	*WIS*	*SHR15*
Systolic blood pressure (mmHg)	145±3	214±7
Left ventricular end diastolic pressure (mmHg)	7.0±6.0	24.3±0.7
Plasma renin activity (ng $ml^{-1} h^{-1}$)	3.3±0.4	15.3±2.6
Plasma-levels of ANP (pg ml^{-1})	88±23	470±38

Data from Stasch *et al*., 1987; 1995.

balloon filled with 50% ethanol was inserted into the left ventricle via the mitral valve to measure heart rate (HR), left ventricular peak pressure (LVP), $+dP/dt_{max}$ (refers to the maximal increase of left ventricular pressure per time unit during ventricular contraction) and $-dP/dt_{max}$ (refers to the maximal decrease of left ventricular pressure per time unit during ventricular relaxation). A tip manometer placed near the aortic valve measured coronary perfusion pressure (CPP). The manometers were connected to a computer (imc, Meßsysteme GmbH, Berlin, Germany) to provide on-line recording. The end-diastolic pressure imposed by the balloon in each group of hearts was set to 8 mmHg. To accomplish this we used a special device connected to the balloon (Ballon-Kit, Hugo Sachs Elektronik, March-Hugstetten, Germany).

The hearts were beating spontaneously throughout the experimental procedure. All experiments were done in constant volume Langendorff perfusion. The pulmonary artery was cannulated to measure coronary flow. The constant flow rate was adapted to the coronary flow measured under constant pressure Langendorff conditions after an equilibration period of 30 min. Variations of the constant flow between the hearts of the same group and between the different groups of rats are listed in Table 2. A constant proportion of 10% of the KH-buffer flow rate was applied with a double perfusor pump by use of a 50 ml syringe connected to a catheter placed in the aorta near the aortic valve. The other syringe in the pump was used to infuse the drugs diluted in KH-buffer and drug application was performed by switching from the KH-buffer containing syringe to the syringe containing the drug. This system ensured that application of the drugs was not associated with variations in coronary flow.

After equilibration, noradrenaline (NA), different concentrations of GTN, SNAP and N^{ω}-nitro-L-arginine (L-NOARG) were infused. Each drug application was followd by a 15 min wash-out period with buffer. In preliminary experiments with constant flow perfused WIS-hearts ($n=6$) NA showed half-maximal effective concentrations (pD_2-values, 95% confidence intervals in parentheses) of 6.56 (7.21 to 5.91) for the increase of HR, 6.66 (6.85 to 6.47) for the increase of LVP, 6.54 (6.79 to 6.30) for the increase of $+dP/dt_{max}$ and 6.59 (6.76 to 6.42) for the increase of $-dP/dt_{max}$. Based on these results we used a repeated infusion of NA (final concentration 0.1 μmol l^{-1}) to study the response of the hearts to adrenergic stimulation. After the second NA-infusion the experiments were performed according to the following experimental protocols. In 14 hearts of WIS GTN (100 μmol l^{-1}) and then L-NOARG (0.1 and 1.0 mmol l^{-1}) were infused; in 9 hearts of WIS SNAP (1 and 10 μmol l^{-1}) and GTN (1 and 10 μmol l^{-1}) were infused; in 8 hearts of SHR10 SNAP (1 and 10 μmol l^{-1}) and then GTN (1 and 10 μmol l^{-1}) were infused; in another 8 hearts of SHR10 L-NOARG 0.1 and 1 mmol l^{-1} were infused and in 8 hearts of SHR15 GTN (100 μmol l^{-1}) and then L-NOARG (0.1 and 1 mmol l^{-1}) were infused. In 7 hearts 0.005% (v/v) dimethylsulphoxide (640 μM, as vehicle for SNAP), 1.1 mg ml^{-1} glucose monohydrate (5.6 mmol l^{-1}, as vehicle for GTN) and hydrocloric acid (0.1 mmol l^{-1}, as vehicle for L-NOARG) were infused. The infusions of these vechicles did not change any measured parameter of cardiac function (data not shown).

Substances and solutions

SNAP was synthesized according to Field *et al*. (1978) as described previously (Kojda *et al*., 1994). NO-release from SNAP was measured in KH-buffer by use of a polarographic method (ISO-NO-electrode, WPI, Berlin, Germany). The maximal concentration of NO measured at a concentration of 10 μmol l^{-1} SNAP in the KH-buffer at 37°C in the presence of 150 mmHg of oxygen was 492.3±4.3 nmol l^{-1} ($n=3$).

All chemicals, except GTN, were obtained from Sigma, Deisenhofen, Germany, or Merck, Darmstadt, Germany in analytical grade. One millilitre of the stock solution of GTN contained 1 mg of GTN (4.404 mmol l^{-1}) and 49 mg of glucose monohydrate. The solution was purchased from Pohl-Boskamp GmbH & Co. Hohenlockstedt, Germany. Stock solutions of NA (hydrochloride salt, 10 mmol l^{-1}) and L-NOARG (100 mmol l^{-1}) were prepared in distilled water or hydrochloric acid (10 mmol l^{-1}, pH 2), respectively. The stock solution of SNAP (200 mmol l^{-1}) was prepared in dimethylsulphoxide. All stock solutions were freshly prepared each day, protected from daylight, kept on ice until use and diluted with KH-buffer as required. All concentrations indicated in the text, figures and tables are expressed as final concentrations in the perfusion buffer.

Statistics

All data were analysed by means of a computer programme (SAS PC Software 6.04, PROC ANOVA) and are expressed as mean values and s.e.mean of experiments from n different animals. Significant differences were evaluated by use of either paired or unpaired two-tailed Student's t test (Graph Pad Prism, also used to create the graphs) and a P value below 0.05 was considered significant.

Results

Long-term hypertension in SHR10 and SHR15 was associated with myocardial hypertrophy. The severity of hypertrophy was related to the age of hypertensive rats (Figure 1). The heart wet weight was significantly stronger increased in SHR15 as compared to SHR10, although the body weight was similar in all groups.

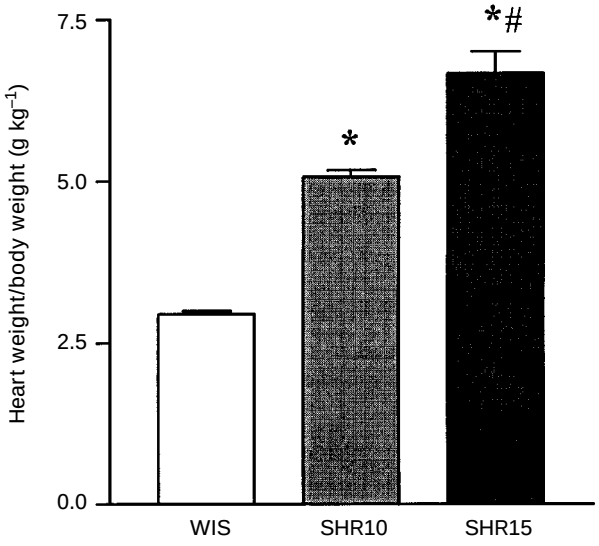

Figure 1 Myocardial hypertrophy in stroke-prone spontaneously hypertensive rats at the age of 10 months (SHR10, $n=16$) and of 15 months (SHR15, $n=8$) as compared to 3 months old Wistar rats (WIS). Plotted are the mean values (±s.e.mean) of the heart wet weight/body weight ratio. *$P<0.05$ vs WIS, unpaired t test; #$P<0.05$ vs SHR10, unpaired t test.

Myocardial hypertrophy in SHR was associated with changes in baseline parameters. The hearts of both groups of SHR showed a decrease in coronary flow and HR. Only in SHR10 CPP, $+dP/dt_{max}$, $-dP/dt_{max}$ and LVP were significantly increased (Table 2). These results indicate that myocardial hypertrophy in SHR is associated with an increased coronary resistance. They also show that only in SHR10 ventricular enlargement is associated with an increased contractility.

Original recordings of changes of LVP induced by the drugs are shown in Figure 2. Infusion of 0.1 μmol l^{-1} NA significantly increased HR, LVP, $+dP/dt_{max}$ and $-dP/dt_{max}$ in WIS-hearts (Figure 3). The effects on contractility were greatly reduced in SHR10 and almost absent in SHR15 (Figure 3). In contrast, hearts of SHR15 showed a significantly stronger increase in HR as compared to WIS and SHR10. These results demonstrate an abnormal response of SHR-hearts to adrenergic stimulation.

Infusion of 1 μmol l^{-1} SNAP significantly increased all parameters of contractile force in WIS-hearts (Figure 4). A similar response was observed after infusion of 10 μmol l^{-1} SNAP, but the increase of LVP was significantly lower. At this concentration of SNAP HR was significantly increased as compared to the baseline value. In contrast, 1 μmol l^{-1} SNAP did not elicit any significant effect on contractile force in hearts of SHR10 except a small increase of $+dP/dt_{max}$ (Figure 4). A higher concentration of SNAP (10 μmol l^{-1}) induced a significant increase of $+dP/dt_{max}$ and LVP that was not different from the effect of 10 μmol l^{-1} l^{-1} SNAP in WIS. In SHR10-hearts SNAP did not influence HR. These results indicate that NO is capable of increasing contractility in isolated hearts of WIS. This effect is diminished in SHR10-hearts and absent in SHR15-hearts.

All concentrations of GTN (1–100 μmol l^{-1}) increased myocardial contractility in WIS and in SHR but had no effect on HR (Figure 5, Table 3). In hearts of WIS these effects of GTN were similar at all concentrations. In contrast, the hearts of SHR10 responded significantly less to 1 μmol l^{-1} GTN (Table 3), while the hearts of SHR15 showed a significantly

Table 2 Baseline parameters of myocardial function after the equilibration period

Species	n	*Coronary flow* (ml min^{-1} g^{-1})	*CPP* (mmHg)	$+dP/dt_{max}$ (mmHg s^{-1})	$-dP/dt_{max}$ (mmHg s^{-1})	*LVP* (mmHg)	*HR* (beats min^{-1})
WIS	23	10.2±0.3	77±8	2235±171	1408±131	63±4	277±13
SHR10	16	5.5±0.2*	98±10*	3124±208*	2008±171*	120±9*	160±8*
SHR15	8	3.9±0.3*#	92±11	2181±435#	1454±258#	82±15#	154±12*

Values were measured before infusion of the drugs and are expressed as mean±s.e.mean. *$P<0.05$ vs WIS, unpaired t test; #$P<0.05$ vs SHR10, unpaired t test.

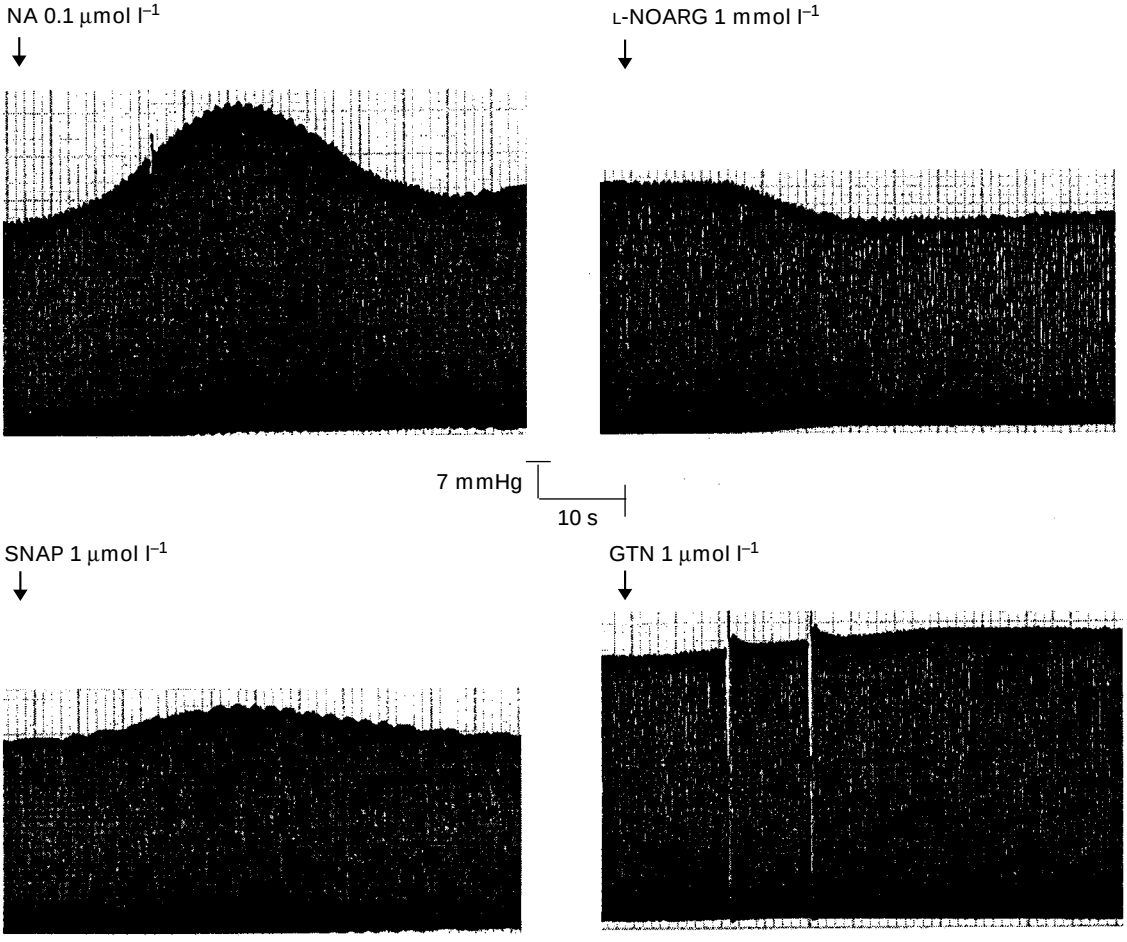

Figure 2 Original recordings showing the effect of NA, GTN, SNAP and L-NOARG on left ventricular pressure of isolated hearts from Wistar rats. The timepoints the hearts were subjected to the drugs are indicated by an arrow.

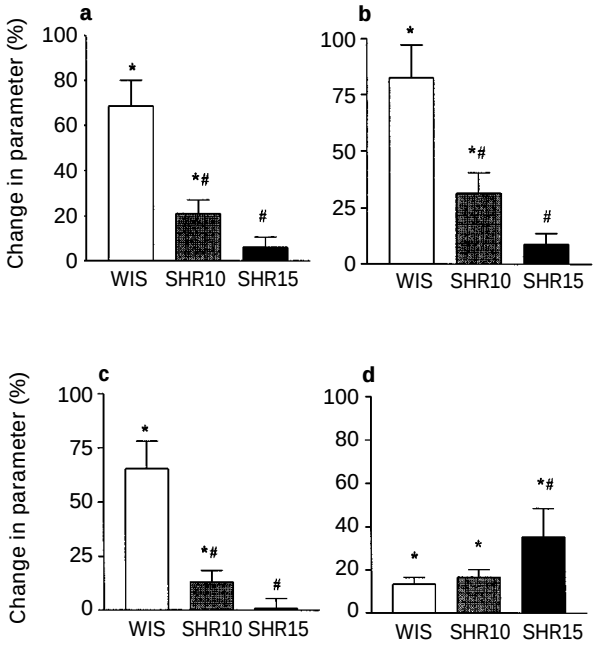

Figure 3 Effect of an infusion of 0.1 μmol l^{-1} noradrenaline on (a) $+dP/dt_{max}$, (b) $-dP/dt_{max}$, (c) left ventricular peak pressure (LVP) and (d) heart rate of isolated hearts from stroke-prone spontaneously hypertensive rats at an age of 10 months (SHR10, $n=16$) and of 15 months (SHR15, $n=8$) as compared to normal Wistar rats (3 months, WIS, $n=23$). Given are the mean values and s.e.mean of percentage changes related to the basal values before drug application $^*P<0.05$ vs baseline, paired t test; $\#P<0.05$ WIS vs SHR10, unpaired t test.

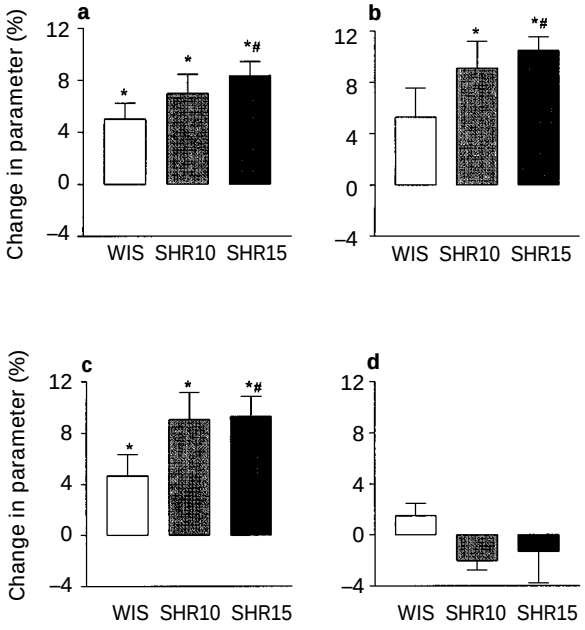

Figure 5 Effect of an infusion of 100 μmol l^{-1} GTN on (a) $+dP/dt_{max}$, (b) $-dP/dt_{max}$, (c) left ventricular peak pressure (LVP) and (d) heart rate of isolated hearts from stroke-prone spontaneously hypertensive rats at an age of 10 months (SHR10, $n=8$) and of 15 months (SHR15, $n=8$) as compared to normal Wistar rats (3 months, WIS, $n=14$). Given are the mean values and s.e.mean of percentage changes related to the basal values before drug application $^*P<0.05$ vs baseline, paired t test; $\#P<0.05$ WIS vs SHR10, unpaired t test. The effects of lower concentrations of GTN are shown in Table 4.

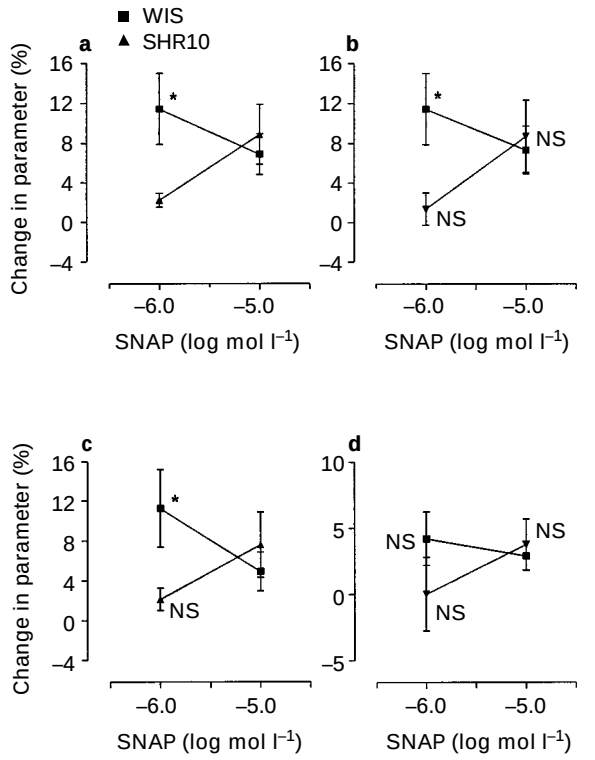

Figure 4 Effect of an infusion of 1 μmol l^{-1} and 10 μmol l^{-1} SNAP on (a) $+dP/dt_{max}$, (b) $-dP/dt_{max}$, (c) left ventricular peak pressure (LVP) and (d) heart rate of isolated hearts from stroke-prone spontaneously hypertensive rats at an age of 10 months (SHR10, $n=8$) as compared to 3 months old normal Wistar rats (WIS, $n=9$). Given are the mean values and s.e.mean (vertical lines) of percentage changes related to the basal values before drug application. All changes were significantly different from baseline except those indicated by NS; $^*P<0.05$ WIS vs SHR10, unpaired t test.

greater positive inotropic response to 100 μmol l^{-1} GTN (Figure 5). These results indicate that GTN – even at a concentration as low as 1 μmol l^{-1} – increases contractility in hearts of WIS and SHR.

Inhibition of endogenous NO-production by infusion of the NO-synthase inhibitor L-NOARG (100 μmol l^{-1}) caused a significant decrease in contractility in all groups (Figure 6). HR was not altered. A higher concentration of L-NOARG (1 mmol l^{-1}) induced stronger effects on contractility and a significant reduction of HR (Table 4). These results indicate a role for endogenous NO-production in regulating heart function.

Some of the drugs induced significant changes of CPP (Table 5) indicating an effect on coronary resistance vessel tone. A strong reduction of CPP, i.e. decrease in coronary resistance, was observed in hearts of WIS after infusion of SNAP. This effect was significantly diminished in SHR10. GTN (10–100 μmol l^{-1}) induced a lower reduction of CPP. At concentrations of 1 μmol l^{-1} and of 10 μmol l^{-1} this effect was smaller in SHR10. None of the drugs altered CPP in SHR15. These results demonstrated that SNAP induces a stronger dilatation in coronary resistance vessels than GTN and that the vasodilator activity of both drugs is attenuated in hearts of SHR10.

Discussion

The aim of the present study was to compare the effects of NO-donors and NO-synthase inhibitors on myocardial function in normal and hypertrophic isolated rat hearts. The main finding is that the organic nitrate GTN, the spontaneous NO-donor SNAP and endogenously produced NO induce a positive inotropic effect in both normal hearts of WIS and hypertrophic hearts of SHR, which exhibit a markedly impaired inotropic and an increased chronotropic response to NA.

Table 3 Changes in parameters of contractile force and heart rate induced by different concentrations of glyceryl trinitrate

Drug	*Species*	n	$+dP/dt_{max}$ (%)	$-dP/dt_{max}$ (%)	*LVP* (%)	*HR* (%)
GTN 1 μmol l^{-1}	WIS	9	6.33±2.6*	9.23±3.4*	5.34±2.7	0.17±1.6
	SHR10	8	2.19±0.7*#	2.27±0.9*#	1.19±1.2#	4.88±1.7*#
GTN 10 μmol l^{-1}	WIS	9	3.18±0.9*	6.22±1.9*	4.90±2.3	−0.64±1.4
	SHR10	8	2.93±0.9*	3.91±1.1*	2.55±1.0*	−0.15±0.9

Values are expressed as mean percentage (±s.e.mean) change of the parameter related to the baseline value before drug infusion. *$P<0.05$ vs baseline, paired t test; #$P<0.05$ WIS vs SHR10, unpaired t test.

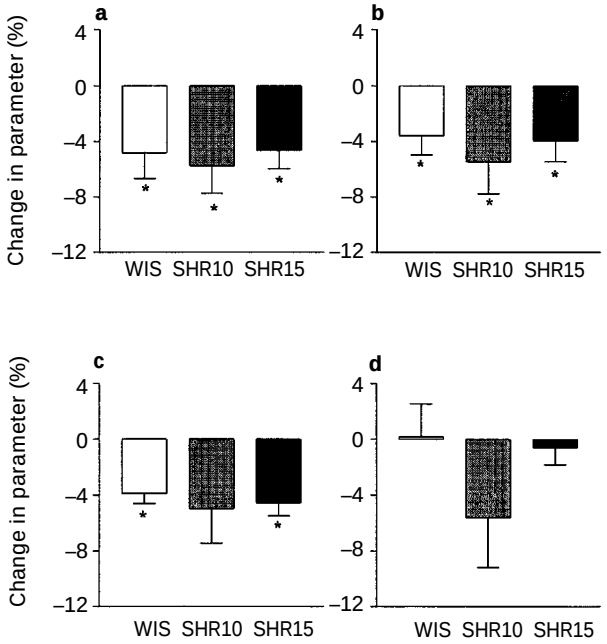

Figure 6 Effect of an infusion of 0.1 μmol l^{-1} L-NOARG on (a) $+dP/dt_{max}$, (b) $-dP/dt_{max}$, (c) left ventricular peak pressure (LVP) and (d) heart rate of isolated hearts from stroke-prone spontaneously hypertensive rats at an age of 10 months (SHR10, $n=8$) and of 15 months (SHR15, $n=8$) as compared to normal Wistar rats (3 months, WIS, $n=14$). Given are the mean values and s.e.mean of percentage changes related to the basal values before drug application *$P<0.05$ vs baseline, paired t test. The effects of a higher concentration of L-NOARG are shown in Table 5.

Table 4 Changes in cardiac contractile parameters and heart rate induced by 1 mmol l^{-1} L-NOARG

Species	n	$+dP/dt_{max}$ (%)	$-dP/dt_{max}$ (%)	*LVP* (%)	*HR* (%)
WIS	14	−29.4±7.8	−28.1±7.2	−25.6±6.3	−16.1±5.1
SHR10	8	−25.4±4.2	−20.5±4.6	−18.2±4.5	−18.1±4.2
SHR15	8	−20.6±3.2	−16.5±4.5	−16.1±4.7	−15.4±2.9

Values are expressed as mean percentage (±s.e.mean) change of the parameter related to the baseline value before drug infusion. All changes were significantly different from baseline (paired t test, $P<0.05$) but there were no significant differences between the species.

The positive inotropic effect of SNAP (Figure 4) and GTN (Figure 5, Table 3) in the rat isolated normal heart is consistent with previous findings showing similar effects of NO-donors in ventricular tissue or intact hearts of dogs (Raff *et al*., 1970; Preckel *et al*., 1997), cats (Diamond *et al*., 1977; Mohan *et al*., 1996), guinea-pigs (Korth, 1975), rats (Kojda *et al*., 1995; 1996; Kamelgard *et al*., 1995) and man (Strauer, 1971). Presumably, this effect of SNAP and GTN is caused by NO, as is the well-known vasodilator activity of these drugs. The release of NO from SNAP occurs spontaneously, while NO-release from GTN requires enzymatic degradation as demonstrated in vascular tissue *in vitro* and *in vivo* (Kowaluk & Fung, 1990; Noack & Feelisch, 1991; Feelisch & Kelm, 1991; Salvemini *et al*., 1992; Mülsch *et al*., 1995). The release of NO from GTN also occurs in the coronary circulation of Langendorff-hearts (Schrör *et al*., 1991).

In a previous investigation we have shown that bioactivation of GTN occurs in isolated cardiac myocytes from normal rats (Kojda *et al*., 1996). GTN and other organic nitrates such as isosorbide mononitrate and pentaerythritol tetranitrate increase cyclic GMP levels and contractile force in these cells. A similar effect was observed with low concentrations of different spontaneous NO-donors including SNAP. It is suggested, therefore, that the positive inotropic activity of GTN and SNAP observed in the present study was caused by a direct action of NO on ventricular myocytes and not caused by indirect mechanisms, such as coronary vasodilatation as discussed below. The mechanism of the direct effect of NO on cardiac muscle most likely includes a cyclic AMP-dependent pathway, as demonstrated in rat isolated cardiomyocytes (Kojda *et al*., 1996). The positive inotropic effect of NO might be counterregulated by a concurrently occurring attenuation of myocardial oxygen consumption (Xie *et al*., 1996).

The positive inotropic effect was present at concentrations of SNAP that were shown to release nanomolar concentrations of NO (see Methods), which are also produced endogenously (Malinski & Taha, 1992). Endogenous NO-production by endothelial nitric oxide synthase in the heart can take place not only in endothelial cells but also in cardiomyocytes (Balligand *et al*., 1995). A contribution of inducible NO-synthase is unlikely in WIS but conceivable in SHR. Inducible NO-synthase was shown to be expressed in SHR, while in WIS the enzyme was not detectable by Western-blot analysis (Wu *et al*., 1996). In the present study we observed a negative inotropic effect after infusion of the NO-synthase inhibitor L-NOARG at a concentration of 100 μmol l^{-1} (Figure 6). A 10 fold higher concentration of L-NOARG induced not only a much stronger negative inotropic activity but also a negative chronotropic effect (Table 4). It has been shown previously that inhibition of endogenous NO-synthase in rat isolated hearts promotes NA-release from this tissue, as measured by detection of NA in the coronary effluent (Schwarz *et al*., 1995). This action of NO-synthase inhibitors might have counteracted the results presented here. However, our results are consistent with previous findings demonstrating a negative inotropic effect of the NO-synthase inhibitor N^G-methyl-L-arginine (L-NMMA) in the rat isolated heart stimulated with isoprenaline (Klabunde *et al*., 1992) and in the dog heart *in vivo* (Klabunde *et al*., 1991; Lechevalier *et al*., 1994). In addition, it was shown that disruption of the endothelial NO-synthase gene causes bradycardia in the mouse, which is aggravated by oral treatment with the NO-synthase inhibitor N^G-nitro-L-arginine-methylester (Shesely *et al*., 1996; Kojda *et al*., 1997). Interestingly, the spontaneous NO-donor SNAP significantly increased the heart rate of WIS-hearts (Figure 4). Taken together, these results suggest that endogenous NO-production in the normal

Table 5 Changes in coronary perfusion pressure induced by infusions of the drugs

		Changes in coronary perfusion pressure (% of baseline)					
Drug	*Concentration*	*WIS*	n	*SHR10*	n	*SHR15*	n
NA	0.1 μmol l^{-1}	2.89±1.1*	23	2.58±1.9	16	1.07±0.8	8
SNAP	1 μmol l^{-1}	−17.42±4.0*	9	−3.31±1.4*#	8	–	
	10 μmol l^{-1}	−14.33±4.7*	9	−6.63±2.5*#	8	–	
GTN	1 μmol l^{-1}	−2.39±1.4	9	0.34±1.5	8	–	
	10 μmol l^{-1}	−2.73±1.0*	9	0.46±0.9	8	–	
	100 μmol l^{-1}	−2.43±0.7*	14	−1.71±0.5*	8	0.40±2.2	8
L-NOARG	0.1 mmol l^{-1}	−2.95±1.6	14	0.87±0.9	8	0.40±1.1	8
	1.0 mmol l^{-1}	−0.32±0.9	14	−2.45±1.6	8	−1.40±0.9	8

Values are expressed as mean percentage (±s.e.mean) change of coronary perfusion pressure related to the baseline values before drug infusion. *$P<0.05$ vs baseline, paired t test; #$P<0.05$ WIS vs SHR10, unpaired t test.

heart is involved in the contractile function of the ventricular myocardium. The underlying mechanism is most likely identical to the mechanism of action shown to mediate the positive inotropic effect of exogenous NO (Kojda *et al*., 1996). Elucidation of the effect of endogenous NO-production on HR and the underlying mechanism require further investigation.

The positive inotropic effect of the NO-donors GTN and SNAP and of endogenous NO-production also occurs in isolated hearts of SHR. These hearts show an age-dependent development of myocardial hypertrophy (Figure 1) that is associated with an impaired inotropic and an increased chronotropic response to β-adrenoceptor stimulation by NA (Figure 3). We used NA to indicate functional differences in left ventricular responsiveness between WIS and SHR. Our results indicate that neither structural nor functional myocardial changes induced by long-term hypertension alter the positive inotropic response of isolated hearts from SHR to exogenous NO. In addition, endogenous NO-production is probably not involved in the decreased inotropic response of SHR-hearts to NA.

As shown in Figure 4, low concentrations of SNAP did not induce a positive inotropic effect in SHR10-hearts. This lack of effect might be the result of a partial inactivation of NO, which is extracellularly liberated by this NO-donor (Kowaluk & Fung, 1990; Noack & Feelisch, 1991). The significantly reduced vasodilator efficacy of SNAP in the coronary circulation of SHR10 supports this suggestion (Table 5). Inactivation of NO released by SNAP might involve a reaction with superoxide radicals that have been shown to be increased in SHR (Grunfeld *et al*., 1995).

Our findings may help elucidate the effects of endogenous NO-production in heart failure. It is well established that long-term hypertension in man is associated with myocardial hypertrophy, which strongly increases the risk of hypertension-induced heart failure (Schwartzkopf *et al*., 1993; Vogt *et al*., 1993). SHR15 have been shown to develop not only severe hypertension associated with myocardial hypertrophy, but also a high left ventricular end diastolic pressure, a marked activation of the renin angiotensin aldosterone system and an enhanced release of atrial natriuretic peptide (Stasch *et al*., 1987; 1995). A comparison between SHR15 and age matched Wistar rats is given in Table 1. These pathological changes also occur in hypertension-induced heart failure in man (Strauer, 1989). Furthermore, SHR15 show a decrease of the elevated blood pressure from 250 mmHg to 214 mmHg starting at the age of one year ('decapitated hypertension') (Stasch *et al*., 1987). This observation indicates the occurrence of a severely reduced cardiac output, which might be related to the inadequate response to β-adrenoceptor stimulation shown here. A reduction of ejection fraction to approximately 50% is already apparent in 6 months old SHR but at this age a 'decapitated hypertension' is absent (Motz *et al*., 1983). Taken together, these results suggest the presence of hypertension-induced heart failure in SHR15.

The changes in myocardial contractility described here were probably not caused by changes in CPP. GTN and SNAP are potent vasodilators and increase coronary flow in a constant pressure perfused isolated heart by decreasing vascular resistance in the coronary circulation. According to Gregg's phenomenon (Gregg, 1963), an increase in coronary perfusion associated with enhanced coronary artery transmural pressure results in augmented cardiac oxygen consumption and contractility. The underlying mechanism may be related to a distension of coronary vessels (garden-hose hypothesis) and a subsequent increase in cardiac sarcomere lengths (Arnold *et al*., 1968; Poche *et al*., 1971). In the present study we used a constant volume perfused preparation and observed a significant reduction of CPP by GTN and by SNAP (Table 5). It is unlikely that this vasodilator activity contributed to the positive inotropic action of GTN and SNAP. A reduction in CPP would rather produce a negative garden-hose effect associated with a decrease in myocardial contractility. In addition, there is evidence that a pharmacological alteration of coronary perfusion pressure at constant flow conditions in the range of 50 mmHg does not change myocardial oxygen consumption in the isolated heart (Zborowska-Sluis *et al*., 1977). A similar conclusion can be drawn from the results of the present study. The reduction of CPP induced by SNAP was significantly different in hearts of SHR10 and of WIS (Table 5), while the increase in contractility was similar (Figure 3). In accordance with these data, GTN induced comparable inotropic effects in hearts of WIS and of SHR being independent of changes in CPP.

Conceivably, GTN induced redistribution of coronary flow to endocardial regions; the so-called positive coronary steal phenomenon (Winbury *et al*., 1971), might have caused an increase in contractility. This phenomenon, i.e. a selective increase in endocardial oxygen tension, occurs in the ischaemic heart and is the result of the selective action of GTN on coronary conductance vessels, combined with the maximal dilatation of coronary resistance vessels in ischaemic regions mediated by autoregulation (Sellke *et al*., 1990; Ahlner *et al*., 1991; Harrison & Bates, 1993). Vasodilators such as dipyridamole (Winbury *et al*., 1971) and SNAP (Table 5) potently dilate coronary resistance vessels and would therefore redistribute coronary flow to non-ischaemic regions and produce a negative coronary steal phenomenon associated with a negative inotropic effect. In the present study both SNAP and GTN showed a positive inotropic effect. Thus, it is unlikely that redistribution of coronary flow induced by GTN contributed to its positive inotropic effect.

Limitations of the study

In this study we used 3–4 months old WIS as controls. This decision was based on a previous investigation showing that the relative heart weight of 15 months old WIS

(2.8 ± 0.2 g kg^{-1} body weight) was not different from that of 3–4 months old WIS used here (Stasch *et al*., 1987; 1995). These results indicate the absence of age-dependent myocardial hypertrophy in WIS. In addition, the 15 months old WIS had normal plasma renin activity, normal plasma ANP levels, normal left ventricular end diastolic pressure and a normal blood pressure apparently identical to 3–4 months old WIS. These results probably exclude the possibility of spontaneous heart failure in 15 months old WIS. However, we cannot fully exclude the possibility of some differences in functional responses to NA, GTN, SNAP or L-NOARG of hearts isolated from 15 months old WIS as compared to 3–4 months old WIS. On the other hand, we believe that such differences, if there are any, are extremely small.

In summary, our results provide evidence for a direct positive inotropic effect of NO in isolated hearts of aged SHR, which exhibit structural and functional changes occurring in hypertension-induced heart failure.

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References

AHLNER, J., ANDERSSON, R.G.G., TORFGÅRD, K. & AXELSSON, K.L. (1991). Organic nitrate esters: Clinical use and mechanisms of actions. *Pharmacol. Rev*., **43,** 351–423.

ARNOLD, G., KOSCHE, F., MIESSNER, A., NEITZERT, A. & LOCHNER, W. (1968). The importance of the perfusion pressure in the coronary arteries for the contractility and the oxygen consumption of the heart. *Pflügers Arch*., **299,** 339–356.

BALLIGAND, J.-L., KOBZIK, L., HAN, X., KAYE, D.M., BELHASSEN, L., O'HARA, D.S., KELLY, R.A., SMITH, T.W. & MICHEL, T. (1995). Nitric oxide-dependent parasympathetic signalling is due to activation of constitutive endothelial (type III) nitric oxide synthase in cardiac myocytes. *J. Bio. Chem*., **270,** 14582–14586.

BALLIGAND, J.-L., UNGUREANU-LONGROIS, D., SIMMONS, W.W., PIMENTAL, D., MALINSKI, T.A., KAPTURCZAK, M., TAHA, Z., LOWENSTEIN, C.J., DAVIDOFF, A.J., KELLY, R.A., SMITH, T.W. & MICHEL, T. (1994). Cytokine-inducible nitric oxide synthase (iNOS) expression in cardiac myocytes. Characterization and regulation of iNOS expression and detection of iNOS activity in single cardiac myocytes *in vitro*. *J. Biol. Chem*., **269,** 27580–27588.

BRADY, A.J.B., POOLE-WILSON, P.A., HARDING, S.E. & WARREN, J.B. (1992). Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. *Am. J. Physiol. Heart Circ. Physiol*., **263,** H1963–H1966.

BRUTSAERT, D.L., MEULEMANS, A.L., SIPIDO, K.R. & SYS, S.U. (1988). Effects of damaging endocardial surface on the mechanical performance of isolated cardiac muscle. *Circ. Res*., **62,** 358–366.

DE BELDER, A.J., RADOMSKI, M.W., WHY, H.J., RICHARDSON, P.J. & MARTIN, J.F. (1995). Myocardial calcium-independent nitric oxide synthase activity is present in dilated cardiomyopathy, myocarditis, and postpartum cardiomyopathy but not in ischaemic or valvar heart disease. *Br. Heart J*., **74,** 426–430.

DECKING, U.K.M., FLESCHE, C.W., GÖDECKE, A. & SCHRADER, J. (1995). Endotoxin-induced contractile dysfunction in guinea pig hearts is not mediated by nitric oxide. *Am. J. Physiol. Heart Circ. Physiol*., **268,** H2460–H2465.

DIAMOND, J., TEN EICK, R.E. & TRAPANI, A.J. (1977). Are increases in cyclic GMP levels responsible for the negative inotropic effects of acetylcholine in the heart? *Biochem. Biophys. Res. Commun*., **79,** 912–918.

FEELISCH, M. & KELM, M. (1991). Biotransformation of organic nitrates to nitric oxide by vascular smooth muscle and endothelial cells. *Biochem. Biophys. Res. Commun*., **180,** 286–293.

FIELD, L., DILTS, R.V., RAVICHANDRAN, R., LENHERT, G. & CARNAHAN, G.E. (1978). An unusual stable thionitrite from N-acetyl-D,L-penicillamine; x-ray crystal and molecular structure of 2-(acetylamino)-2-carboxy-1,1-dimethyl thionitrite. *JCS Chem. Commun*., **1157,** 249–250.

GREGG, D.E. (1963). Effect of coronary perfusion pressure or coronary flow on oxygen usage of the myocardium. *Circ. Res*., **13,** 497–500.

GRUNFELD, S., HAMILTON, C.A., MESAROS, S., MCCLAIN, S.W., DOMINICZAK, A.F., BOHR, D.F. & MALINSKI, T. (1995). Role of superoxide in the depressed nitric oxide production by the endothelium of genetically hypertensive rats. *Hypertension*, **26,** 854–857.

HARRISON, D.G. & BATES, J.N. (1993). The nitrovasodilators: New ideas about old drugs. *Circulation*, **87,** 1461–1467.

HARTZELL, H.C. & FISCHMEISTER, R. (1986). Opposite effects of cAMP and cGMP on Ca^{2+}-current in single heart cells. *Nature*, **323,** 273–275.

HAYWOOD, G.A., TSAO, P.S., VON DER LEYEN, H.E., MANN, M.J., KELLING, P.J., TRINDADE, P.T., LEWIS, N.P., BYRNE, C.D., RICKENBACHER, P.R., BISHOPRIC, N.H., COOKE, J.P., MCKENNA, W.J. & FOWLER, M.B. (1996). Expression of inducible nitric oxide synthase in human heart failure. *Circulation*, **93,** 1087–1094.

KAMELGARD, J., CRESTANELLO, J.A., FULLERTON, D. & WHITMAN, G.J. (1995). Nitric oxide as a positive inotropic agent in isolated rat hearts. *J. Surg. Res*., **59,** 482–484.

KLABUNDE, R.E., KIMBER, N.D., KUK, J.E., HELGREN, M.C. & FÖRSTERMANN, U. (1992). N^G-methyl-L-arginine decreases contractility, cGMP and cAMP in isoproterenol-stimulated rat hearts *in vitro*. *Eur. J. Pharmacol*., **223,** 1–7.

KLABUNDE, R.E., RITGER, R.C. & HELGREN, M.C. (1991). Cardiovascular actions of inhibitors of endothelium-derived relaxing factor (nitric oxide) formation/release in anesthetized dogs. *Eur. J. Pharmacol*., **199,** 51–59.

KOJDA, G., BECK, J.K., MEYER, W. & NOACK, E. (1994). Nitrovasodilator-induced relaxation and tolerance development in porcine vena cordis magna: Dependence on intact endothelium. *Br. J. Pharmacol*., **112,** 533–540.

KOJDA, G., BRIXIUS, K., KOTTENBERG, K., NIX, P., SCHLÜTER, K.D., PIPER, H.M. & NOACK, E. (1995). The new NO donor SPM3672 increases cGMP and improves contraction in rat cardiomyocytes and isolated heart. *Eur. J. Pharmacol*., **284,** 315–319.

KOJDA, G., KOTTENBERG, K., NIX, P., SCHLÜTER, K.D., PIPER, H.M. & NOACK, E. (1996). Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. *Circ. Res*., **78,** 91–101.

KOJDA, G., LAURSEN, J.B., RAMASAMY, S., KENT, J.D., SHESELY, E.G., SMITHIES, O. & HARRISON, D.G. (1997). Disruption of the eNOS-gen causes hypersensitivity of mouse aorta to vasoconstrictors and glyceryl trinitrate. *Naunyn-Schmiedeberg's Arch Pharmacol*., **355,** R41.

KORTH, M. (1975). Influence of glyceryl trinitrate on force of contraction and action potential of guinea-pig myocardium. *Naunyn Schmiedeberg's Arch. Pharmacol*., **287,** 329–347.

KOWALUK, E.A. & FUNG, H.-L. (1990). Spontaneous liberation of nitric oxide cannot account for *in vitro* vascular relaxation by S-nitrosothiols. *J. Pharmacol. Exp. Ther*., **255,** 1256–1264.

LECHEVALIER, T., DOURSOUT, M.-F., CHELLY, J.E. & KILBOURN, R.G. (1994). Cardiovascular effects of N^G-methyl-L-arginine in chronically instrumented conscious dogs. *J. Appl. Physiol*., **77,** 471–475.

MALINSKI, T. & TAHA, Z. (1992). Nitric oxide release from a single cell measured *in situ* by a porphyrinic-based microsensor. *Nature*, **358,** 676–678.

MOHAN, P., BRUTSAERT, D.L., PAULUS, W.J. & SYS, S.U. (1996). Myocardial contractile response to nitric oxide and cGMP. *Circulation*, **93,** 1223–1229.

MOTZ, W., PLOEGER, M., RINGSWANDL, G., GOELDEL, N., GARTHOFF, B., KAZDA, S. & STRAUER, B.E. (1983). Influence of nifedipine on ventricular function and myocardial hypertrophy in spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol*., **5,** 55–61.

MÜLSCH, A., MORDVINTCEV, P., BASSENGE, E., JUNG, F., CLEMENT, B. & BUSSE, R. (1995). In vivo spin trapping of glyceryl trinitrate-derived nitric oxide in rabbit blood vessels and organs. *Circulation*, **92,** 1876–1882.

NOACK, E. & FEELISCH, M. (1991). Molecular mechanism of nitrovasodilator bioactivation. In *Endothelial Mechanism of Vasomotor Control*. ed. Drexler, H., Zeiher, A.M., Bassenge, E. & Just, H., pp. 37–50. Darmstadt: Steinkopff Verlag.

ONO, K. & TRAUTWEIN, W. (1991). Potentiation by cGMP of β-adrenergic effect on Ca^{2+} current in guinea-pig ventricular cells. *J. Physiol.*, **443,** 387–404.

POCHE, R., ARNOLD, G. & GAHLEN, D. (1971). The influence of coronary perfusion pressure on metabolism and ultrastructure of the myocardium of the arrested aerobically perfused isolated guinea-pig heart. *Virchows Arch.*, **B8,** 252–266.

PRECKEL, B., KOJDA, G., SCHLACK, W., EBEL, D., KOTTENBERG, K., NOACK, E. & THÄMER, V. (1997). Inotropic effects of glyceryl trinitrate and spontaneous NO-donors in the dog heart. *Circulation*, (in press).

RAFF, W.K., DRECHSEL, U., SCHOLTHOLT, J. & LOCHNER, W. (1970). Herzwirkung des Nitroglycerins. *Pflügers Arch.*, **317,** 336–343.

SALVEMINI, D., MOLLACE, V., PISTELLI, A., ANGGARD, E. & VANE, J. (1992). Metabolism of glyceryl trinitrate to nitric oxide by endothelial cells and smooth muscle cells and its induction by *Escherichia coli* lipopolysaccharide. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 982–986.

SCHRÖR, K., WODITSCH, I. & FÖRSTER, S. (1991). Generation of nitric oxide from organic nitrovasodilators during passage through the coronary vascular bed and its role in coronary vasodilation and nitrate tolerance. *Blood Vessels*, **28,** 62–66.

SCHWARTZKOPF, B., MOTZ, W., VOGT, M. & STRAUER, B.E. (1993). Heart failure on the basis of hypertension. *Circulation*, **87,** IV66–IV72.

SCHWARZ, P., DIEM, R., DUN, N.J. & FÖRSTERMANN, U. (1995). Endogenous and exogenous nitric oxide inhibits norepinephrine release from rat heart sympathetic nerves. *Circ. Res.*, **77,** 841–848.

SELLKE, F.W., TOMANEK, R.J. & HARRISON, D.G. (1990). Influence of vessel size on the sensitivity of porcine microvessels to nitroglycerin. *Am. J. Physiol.*, **258,** 365–369.

SHAH, A.M., SPURGEON, H.A., SOLLOTT, S.J., TALO, A. & LAKATTA, E.G. (1994). 8-Bromo-cGMP reduces the myofilament response to Ca^{2+} in intact cardiac myocytes. *Circ. Res.*, **74,** 970–978.

SHESELY, E.G., MAEDA, N., KIM, H.S., DESAI, K.M., KREGE, J.H., LAUBACH, V.E., SHERMAN, P.A., SESSA, W.C. & SMITHIES, O. (1996). Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A.*, **93,** 13176–13181.

STASCH, J.P., KAZDA, S., HIRTH, C. & MORICH, F. (1987). Role of nisoldipine on blood pressure, cardiac hypertrophy, and atrial natriuretic peptides in spontaneously hypertensive rats. *Hypertension*, **10,** 303–307.

STASCH, J.P., KNORR, A., WEGNER, M. & HIRTH-DIETRICH, C. (1995). Prolonged inhibition of neutral endopeptidase 24.11 by sinorphan in stroke-prone spontaneously hypertensive rats. *Hypertens. Res.*, **18,** 137–143.

STRAUER, B.E. (1971). Evidence for a positive inotropic effect of nitroglycerol on isolated human ventricular myocardium. *Pharmacol. Res. Commun.*, **3,** 377–383.

STRAUER, B.E. (1989). Contractile, haemodynamic and neurohormonal abnormalities in heart failure. *Eur. Heart J.*, **10,** C2–C8.

THOENES, M., FÖRSTERMANN, U., TRACEY, W.R., BLEESE, N.M., NÜSSLER, A.K., SCHOLZ, H. & STEIN, B. (1996). Expression of inducible nitric oxide synthase in failing and non-failing human heart. *J. Mol. Cell. Cardiol.*, **28,** 165–169.

VOGT, M., MOTZ, W., SCHWARTZKOPF, B. & STRAUER, B.E. (1993). Pathophysiology and clinical aspects of hypertensive hypertrophy. *Eur. Heart J.*, **14** (suppl D), 2–7.

WINBURY, M.M., HOWE, B.B. & WEISS, H.R. (1971). Effect of nitroglycerin and dipyridamole on epicardial and endocardial oxygen tension-further evidence for redistribution of myocardial blood flow. *J. Pharmacol. Exp. Ther.*, **176,** 184–199.

WU, C.C., HONG, H.J., CHOU, T.Z., DING, Y.A. & YEN, M.H. (1996). Evidence for inducible nitric oxide synthase in spontaneously hypertensive rats. *Biochem. Biophys. Res. Commun.*, **228,** 459–466.

XIE, Y.W., SHEN, W.Q., ZHAO, G., XU, X.B., WOLIN, M.S. & HINTZE, T.H. (1996). Role of endothelium-derived nitric oxide in the modulation of canine myocardial mitochondrial respiration in vitro-Implications for the development of heart failure. *Circ. Res.*, **79,** 381–387.

ZBOROWSKA-SLUIS, D.T., MILDENBERGER, R.R. & KLASSEN, G.A. (1977). The role of coronary flow and pressure as determinants of myocardial oxygen consumption in the presence or absence of vasomotor tone. *Can. J. Physiol. Pharmacol.*, **55,** 471–477.

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