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Inhibition of nitric oxide synthase and soluble guanylate cyclase induces cardiodepressive effects in normal rat hearts

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Abstract

Exogenous nitric oxide (NO) has been shown to modulate the contractile force of rat cardiac myocytes. We sought to determine whether endogenous NO-production in the isolated normal rat heart has an effect on myocardial contractility. Hearts of male Wistar rats were investigated using a constant flow perfused non-paced Langendorff preparation. Changes of contractile parameters such as left ventricular peak pressure, dP/dt_{max} and dP/dt_{min}, and of coronary perfusion pressure and heart rate were recorded after infusion of the NO-synthase inhibitors *N*ω-nitro-L-arginine (L-NOARG, 0.1 mM, 1.0 mM, $n = 6$), *N*ω-methyl-L-arginine (L-NMMA, 0.1 mM, 1.0 mM, $n = 9$) and methylene blue (2 μM, 20 μM, $n = 6$), the NO-donor sodium (*Z*)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolat (DEA/NO, 0.01 μM, 0.1 μM, $n = 12$), the specific inhibitor of soluble guanylate cyclase 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 0.1 μM, $n = 7$) and L-arginine (0.1 mM, 1.0 mM, $n = 6$). All NO-synthase inhibitors reduced the contractile function of the ventricular muscle before changes in coronary perfusion pressure were evident. The negative inotropic effect of L-NMMA was absent in the presence of an equimolar concentration of L-arginine. ODQ reduced contractile force and coronary perfusion pressure in parallel. By contrast, L-arginine and DEA/NO improved the contractile force of the left ventricle and DEA/NO decreased coronary perfusion pressure. Heart rate was reduced by L-NOARG (1 mM) and methylene blue (20 μM), while DEA/NO (0.1 μM) and L-arginine (1 mM) had a positive chronotropic effect. All these changes were significant ($P < 0.05$). These results suggest that endogenous NO-production exerts a positive effect on myocardial contraction that is mediated by activation of guanylate cyclase. In addition, NO might be involved in regulation of heart rate. © 1997 Elsevier Science B.V.

Keywords: N^{ω}-nitro-L-arginine; Nitric oxide (NO) donors; Heart muscle; Contractility; Guanylate cyclase, soluble; Heart rate; (Rat)

1. Introduction

Contrary to numerous investigations on the vasorelaxant effect of nitrovasodilators only a few reports described the influence of these drugs on myocardial contractility. It has been shown that cGMP, which is generated after nitric oxide-induced activation of soluble guanylate cyclase, inhibits Ca^{2+}-entry into isolated ventricular myocytes (Nawrath, 1977). This effect is most likely mediated by cGMP-dependent protein kinase (Méry et al., 1991). In accordance, the nitrovasodilator sodium nitroprusside reduces the contractile force of isolated cardiomyocytes (Brady et al., 1993) and a high concentration of NO itself (100 μM) depresses contractions of isolated rabbit papillary muscle (Ishibashi et al., 1993).

On the other hand there is evidence for a positive effect of nitrovasodilators on myocardial contraction. Early investigations have shown that the organic nitrate glyceryl trinitrate, which is bioactivated to NO (Feelisch and Noack, 1987), improves the contraction of left ventricular heart muscle in the dog in vivo (Raff et al., 1970) and of isolated human myocardium and the guinea pig heart in vitro (Strauer, 1971; Korth, 1975). In the cat papillary muscle the nitrovasodilator sodium nitroprusside elicits a similar effect (Diamond et al., 1977). Another investigation reported on an increase of isoprenaline stimulated Ca^{2+} entry into isolated ventricular cardiomyocytes initiated by cGMP (Ono and Trautwein, 1991). It was also demonstrated that the endocardium has a positive influence on myocardial contraction (Brutsaert et al., 1988; Smith et al., 1991). Recently, we showed that NO alters the contractile force of isolated cardiomyocytes in a biphasic manner. Increasing the concentration of NO switches a positive and

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probably cAMP-dependent effect on contraction of rat cardiomyocytes to a negative one, which is most likely mediated by activation of cGMP-dependent protein kinase (Kojda et al., 1996). A biphasic effect of NO was also observed in isolated cat papillary muscles (Mohan et al., 1996).

Some recent studies provided evidence for an endogenous NO-production in the myocardium by demonstrating expression of endothelial and inducible nitric oxide synthase in cardiomyocytes and cardiac tissues from rats (Balligand et al., 1994, 1995; Kitakaze et al., 1995; Luss et al., 1995). Expression of inducible nitric oxide synthase by cytokines is associated with an intense production of NO and reduces the contractile force of the myocardium (Finkel et al., 1992; Balligand et al., 1994), while constitutive expression of endothelial nitric oxide synthase contributes to NO-dependent parasympathetic signalling in rat cardiomyocytes (Balligand et al., 1995). However, endogenous NO production in other cell types such as vascular endothelial cells (Palmer et al., 1988) might also influence cardiac functions. In the present study we sought to determine if the activity of constitutive NO-synthase and soluble guanylate cyclase alters myocardial contractile force in the isolated rat myocardium by using specific inhibitors of these enzymes. In addition, we comparably investigated inotropic effects of NO-donor released NO at a concentration, which most likely occurs in the rat coronary circulation under constant flow Langendorff conditions.

2. Methods

2.1. Animal preparation

Isolated hearts of 37 normal male Wistar rats at an age of 3–4 months and an average body weight of 311 ± 6 g were investigated. The average wet weight of the hearts was 921 ± 20 mg. 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) Krebs–Henseleit-buffer (pH 7.4, 37°C) of the following composition (in mM): 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. Measurement of heart rate, left ventricular peak pressure, dP/dt_{max} and dP/dt_{min} was performed with a manometer connected to a small balloon filled with 50% ethanol that was inserted into the left ventricle via the mitral valve. A tip manometer placed near the aortic valve measured coronary perfusion pressure. The manometers were connected to a computer (imc, Meßsysteme, Berlin) to provide on-line recording. The oxygen content of the buffer was continuously measured using an electrode (Radiometer, Willich).

Throughout the experimental procedure the hearts were beating spontaneously and all experiments were done at 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. The average rate of constant coronary flow was 10.4 ± 0.4 ml/min/g ($n = 37$). A constant proportion of 10% of this flow rate was applied with a double perfusor pump using 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 Krebs–Henseleit-buffer. Drug application was performed by switching from the Krebs–Henseleit-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.

2.2. Experimental protocol

After equilibration, different concentrations of noradrenaline, sodium (*Z*)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolat (DEA/NO), L-arginine, *N*ω-nitro-L-arginine (L-NOARG), *N*ω-methyl-L-arginine (L-NMMA), 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and methylene blue were applied. A 15 min washout period with buffer followed each drug application. Preliminary experiments using constant flow perfused hearts of Wistar rats showed that 0.1 μM noradrenaline is below the half-maximal effective concentration of this drug (data not shown). Based on these results we used a repeated infusion of 0.1 μM noradrenaline to investigate the response of all hearts to adrenergic stimulation. After the second noradrenaline infusion the experiments were performed according to following experimental protocols.

In 12 hearts DEA/NO (0.01 and 0.1 μM) was infused. In 6 of these hearts this was followed by infusion of 2 μM methylene blue and in the other 6 hearts by infusion of 20 μM methylene blue. In 9 hearts 0.01 mM, 0.1 mM and 1 mM L-NMMA were infused. In 6 hearts 0.1 mM and 1.0 mM L-arginine and then 0.1 and 1.0 mM L-NOARG were infused. In 3 hearts a combination of L-arginine and L-NMMA (each at 0.1 mM and 1.0 mM) and then 0.1 mM and 1.0 mM L-arginine alone were infused. In 7 hearts 0.001% (volume/volume) dimethylsulfoxide (127 μM) and then 0.1 μM ODQ in 0.001% (volume/volume) dimethylsulfoxide were infused.

The delay time from the onset of the drug-infusion to exposure of the hearts was 30 s. With some exceptions the effects of the drugs were maximal within 2 min after exposure of the hearts. The effects were measured at that time and are related to the directly preceding equilibration values. In case of noradrenaline we measured the effects of the second infusion. ODQ needs a longer incubation time for its pharmacological actions (Garthwaite et al., 1995; Moro et al., 1996; Schrammel et al., 1996). Maximal effects occurred after 30 min and were measured at that time. With the exception of ODQ, methylene blue and L-NOARG, which were always applied at the end of an

experiment, the sequence of drug infusions and of the corresponding concentrations were randomized. In the 22 hearts subjected to methylene blue ($n = 12$), ODQ ($n = 7$) and a combination of L-NMMA and L-arginine ($n = 3$) the total duration of the experiment at constant flow perfusion exceed 2 h but not 2.5 h.

2.3. Measurement of NO-release

NO-release from DEA/NO (Keefer et al., 1996) was determined in Krebs–Henseleit-buffer using a polarographic method (ISO-NO-electrode, WPI, Berlin). The maximal concentration of NO measured at a concentration of 10 μM DEA/NO in the Krebs–Henseleit-buffer at 37°C in the presence of 150 mm Hg of oxygen was 3384 ± 24 nM ($n = 3$) and occurred after 90 s.

2.4. Substances and solutions

DEA/NO was a gift from Professor Dr. L. Keefer, National Cancer Institute, Frederick, MD and ODQ was obtained from Alexis Chemicals, Grünberg. All other chemicals were obtained from Sigma, Deisenhofen, or Merck, Darmstadt in analytical grade.

Stock solutions of noradrenaline (10 mM), L-NMMA (100 mM) and methylene blue (10 mM) were prepared in distilled water. The stock solution of L-NOARG (100 mM) was prepared in 0.01 M hydrochloric acid (pH 2). The stock solution of DEA/NO was prepared in 0.01 M sodium hydroxide (pH 12). The stock solution of ODQ (10 mM) was prepared in dimethylsulfoxide. All stock solutions were freshly prepared each day, protected from daylight, kept on ice and immediately before use diluted with Krebs–Henseleit-buffer as required. All concentrations indicated in the text, figures and tables are expressed as final concentrations in the perfusion buffer.

2.5. Statistics

All data were analyzed by a standard computer program (SAS PC Software 6.04, PROC ANOVA) and are expressed as mean values and standard error of the mean (S.E.M.). Significant differences were evaluated using either unpaired or paired two-tailed students *t*-test (Graph Pad Prism, also used to create the graphs) and a *P*-value below 0.05 was considered as significant.

3. Results

The baseline values of heart rate and of the parameters of contractile force were not altered during the experiments indicating a stable preparation (Table 1). All drugs elicited significant changes of the parameters of contractile force, while coronary perfusion pressure and heart rate were not consistently altered. The hearts showed a normal response to noradrenaline as indicated by a significant increase in heart rate, left ventricular peak pressure, dP/dt_{max} and dP/dt_{min} (Table 1). Original recordings of changes of left ventricular peak pressure induced by application of the drugs are shown in Fig. 1.

3.1. Effects on myocardial contractile force

To investigate inotropic effects of exogenous NO, we subjected 12 hearts to 0.01 and 0.1 μM of the NO-donor DEA/NO. This drug, which spontaneously releases NO as measured polarographically (see Section 2), induced a significant increase of left ventricular peak pressure, dP/dt_{max} and dP/dt_{min}, indicating a positive inotropic action (Table 2). Infusion of 1 mM L-arginine, the physiological precursor of endogenous NO-production, also in-

Table 1
Effect of the duration of the experiment and of 0.1 μM noradrenaline on myocardial function of the rat hearts at constant flow perfusion

Condition	Time (h)	CPP (mm Hg)	dP/dt_{max} (mm Hg s^{-1})	dP/dt_{min} (mm Hg s^{-1})	LVP (mm Hg)	HR (beats min^{-1})
Equilibration	0.5	75.9 ± 5.8	$+1793 \pm 83$	-1113 ± 60	53.5 ± 2.5	257 ± 6
Noradrenaline	1.0	82.8 ± 7.1	$+2485 \pm 139$ *	-1662 ± 107 *	72.2 ± 4.0 *	285 ± 5 *
Equilibration	1.5	87.2 ± 7.2	$+1787 \pm 108$	-1065 ± 71	53.7 ± 3.3	255 ± 4
Equilibration	2.0	98.1 ± 6.9 *	$+1731 \pm 93$	-1028 ± 64	56.4 ± 3.3	243 ± 6

Values for coronary perfusion pressure (CPP), dP/dt_{max}, dP/dt_{min}, left ventricular peak pressure (LVP) and heart rate (HR) were measured at the indicated time points as described in Section 2. The effects of the second noradrenaline infusion are presented. All values are expressed as mean and standard error of the mean (S.E.M.) of 37 individual hearts. Significant differences to the values obtained at the first equilibration period are indicated (* $P < 0.05$, paired *t*-test).

Table 2
Changes of cardiac contractile parameters, coronary vasomotor tone and heart rate induced by a NO-donor

Drug	CPP (%)	dP/dt_{max} (%)	dP/dt_{min} (%)	LVP (%)	HR (%)
DEA/NO (0.01 μM)	-3.3 ± 1.4 *	7.6 ± 2.3 *	10.2 ± 3.1 *	8.2 ± 3.2 *	1.4 ± 0.6 *
DEA/NO (0.1 μM)	-10.3 ± 3.2 *	11.5 ± 2.7 *	16.0 ± 3.3 *	11.0 ± 2.6 *	3.4 ± 1.2 *

Values are expressed as mean percentage and standard error of the mean (S.E.M.) of changes of the parameters related to the baseline value before drug infusion as observed in 12 hearts (* $P < 0.05$ vs. baseline, paired *t*-test).

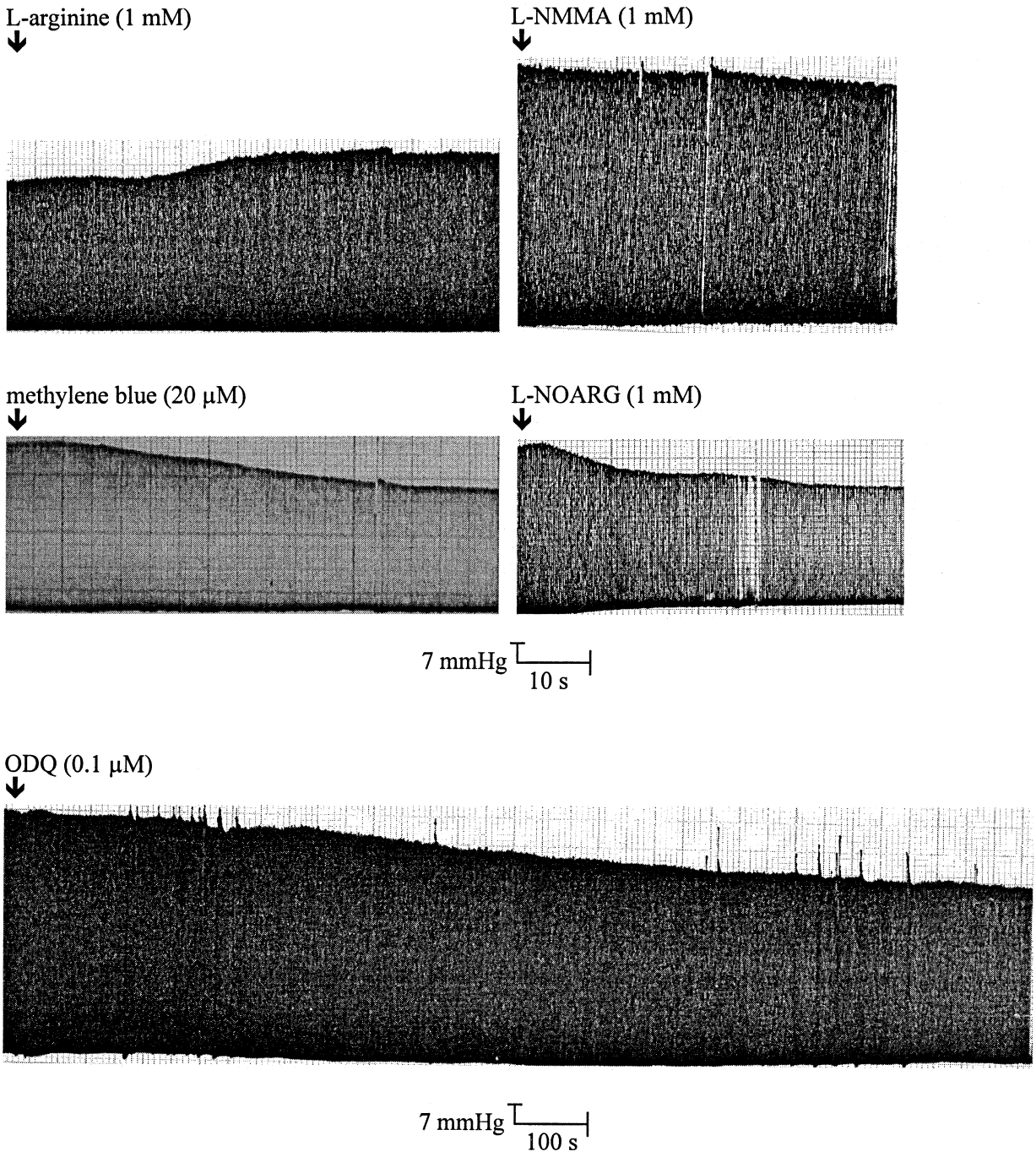

Fig. 1. Original recordings showing the effect of L-arginine, L-NMMA, methylene blue, L-NOARG and ODQ on left ventricular pressure of isolated hearts from Wistar rats. The time point of subjection of the hearts with the drugs are indicated by an arrow.

creased left ventricular peak pressure, dP/dt_{max} and dP/dt_{min}, while a lower concentration (0.1 mM) had no effect (Fig. 2). These results show that both, application of exogenous NO and stimulation of endogenous NO production by L-arginine increase the contractile force of the isolated rat myocardium.

Pharmacological inhibition of endogenous NO-production by infusion of the NO-synthase inhibitors L-NMMA and L-NOARG had the opposite effect. Both drugs concentration-dependently decreased left ventricular peak pressure, dP/dt_{max} and dP/dt_{min}, indicating a negative inotropic action (Figs. 3 and 4). At a concentration of 1 mM, the depressant effect of L-NOARG on dP/dt_{max} and dP/dt_{min}, was significantly stronger than that of L-NMMA ($P < 0.05$, unpaired t-test). The negative inotropic effect of L-NMMA was blunted by coinfusion with an equimolar concentration of L-arginine. At a concentration of 1 mM of both drugs the percentual changes of left ventricular peak

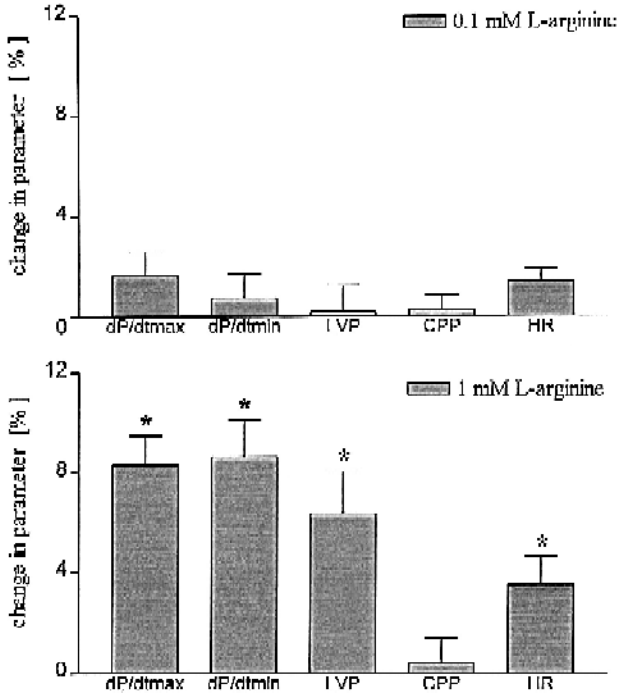

Fig. 2. Effect of infusion of 0.1 mM (upper panel) and of 1 mM (lower panel) L-arginine on contractile parameters (LVP = left ventricular peak pressure), coronary perfusion pressure (CPP) and heart rate (HR) of spontaneously beating constant volume perfused Langendorff-hearts of normal Wistar rats ($n = 9$). Given are the mean values ($\pm$S.E.M.) of percentual changes related to the basal values before application of L-arginine (* $P < 0.05$ vs. baseline, paired t-test).

pressure, dP/dt_{max} and dP/dt_{min} were $-5.6 \pm 4.3\%$, $-0.6 \pm 0.2\%$ and $1.3 \pm 5.1\%$ ($n = 3$), respectively. None of these changes were significant. Methylene blue (20 μM), which is an inhibitor of NO-synthase and soluble guanylate cyclase, significantly decreased left ventricular peak pressure, dP/dt_{max} and dP/dt_{min}, while a 10 times lower concentration (2 μM) had no effect (Fig. 5). ODQ, which is a specific inhibitor of soluble guanylate cyclase, elicited similar effects (Fig. 6). These results suggest that basal endogenous NO-production is involved in the regulation of myocardial contractility of the rat heart. Its supportive action on contractility is most likely mediated by cGMP.

3.2. Effects on coronary perfusion

The basal coronary perfusion pressure progressively increased over the time of the experiment. A significant rise in basal values was observed after 2 h of constant volume perfusion with oxygenated Krebs–Henseleit-buffer (Table 1) indicating increased vascular resistance in the coronary circulation at that time point. Infusion of NO released by DEA/NO induced a significant decrease of coronary perfusion pressure (Table 2), while inhibition of soluble guanylate cyclase by ODQ had the opposite effect (Fig. 6). By contrast, none of the NO-synthase inhibitors showed an effect on coronary perfusion pressure (Figs. 3–5) at the timepoint of measurement (1.5–2 min, see also Fig. 2). The effect of a longer perfusion period with NO-synthase inhibitors was investigated using L-NMMA. After 5 min of infusion with 100 μM L-NMMA there was a significant increase in coronary perfusion pressure of $17.2 \pm 6.2\%$ as related to the baseline value before drug infusion ($n = 7$, $P < 0.05$, paired t-test). These results suggest that exogenous NO and the activity of NO-synthase and/or soluble guanylate cyclase dilate coronary resistance vessels during constant flow perfusion conditions.

3.3. Effects on heart rate

Both, application of exogenous NO released by DEA/NO and stimulation of endogenous NO-production by L-arginine slightly increased heart rate (Table 2, Fig. 2). In accordance, pharmacological inhibition of NO-synthase elicited a reduction of heart rate. High concentrations of the NO-synthase inhibitors L-NOARG (1 mM) and methylene blue (20 μM) showed a pronounced negative chronotropic effect (Figs. 4 and 5). By contrast, even the highest concentration of L-NMMA did not change heart rate (Fig. 3). A similar result was observed after infusion

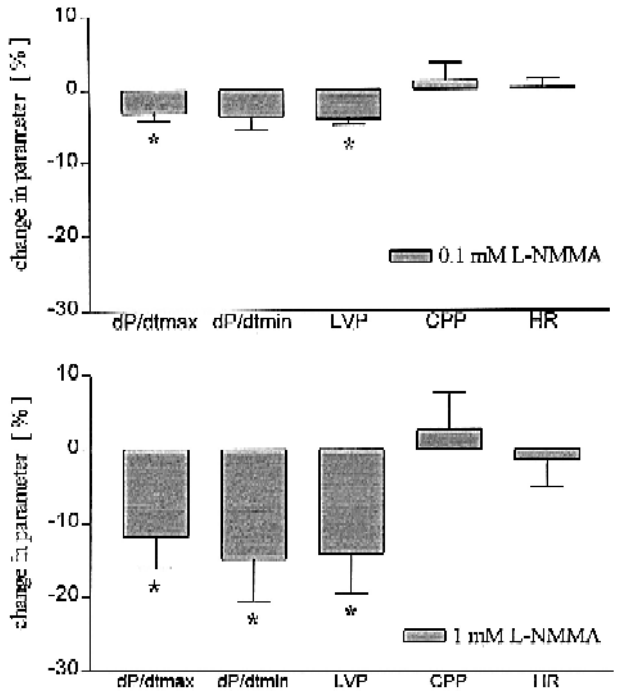

Fig. 3. Effect of infusion of 0.1 mM (upper panel) and of 1 mM (lower panel) *N*ω-methyl-L-arginine (L-NMMA), an inhibitor of NO-synthase, on contractile parameters (LVP = left ventricular peak pressure), coronary perfusion pressure (CPP) and heart rate (HR) of spontaneously beating constant volume perfused Langendorff-hearts of normal Wistar rats ($n = 10$). Given are the mean values ($\pm$S.E.M.) of percentual changes related to the basal values before application of L-NMMA (* $P < 0.05$ vs. baseline, paired t-test).

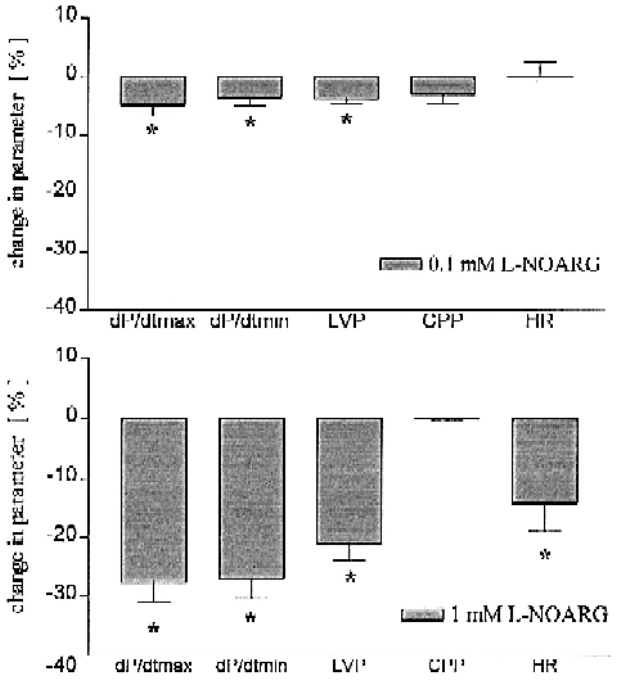

Fig. 4. Effect of infusion of 0.1 mM (upper panel) and of 1 mM (lower panel) *N*ω-nitro-L-arginine (L-NOARG), an inhibitor of NO-synthase, on contractile parameters (LVP = left ventricular peak pressure), coronary perfusion pressure (CPP) and heart rate (HR) of spontaneously beating constant volume perfused Langendorff-hearts of normal Wistar rats ($n = 6$). Given are the mean values (± S.E.M.) of percentual changes related to the basal values before application of L-NOARG (* $P < 0.05$ vs. baseline, paired *t*-test).

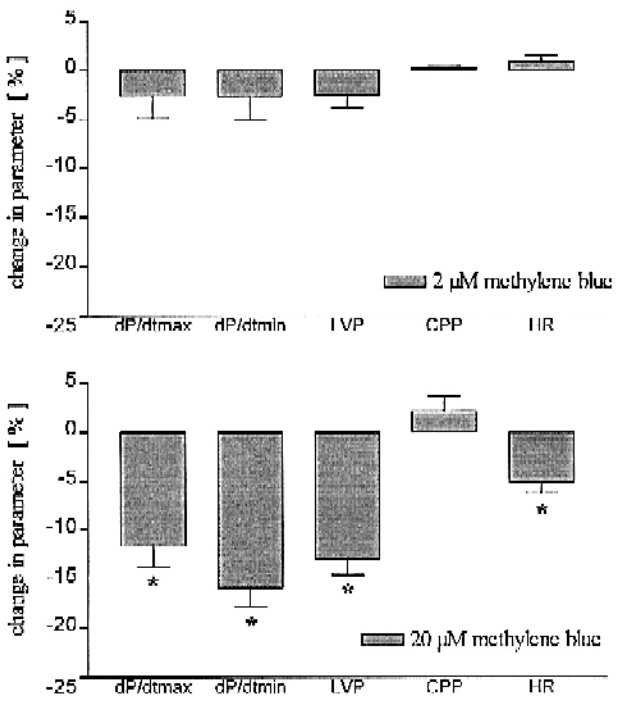

Fig. 5. Effect of infusion of 2 μM (upper panel) and of 20 μM (lower panel) methylene blue, an inhibitor of NO-synthase and soluble guanylate cyclase, on contractile parameters (LVP = left ventricular peak pressure), coronary perfusion pressure (CPP) and heart rate (HR) of spontaneously beating constant volume perfused Langendorff-hearts of normal Wistar rats ($n = 6$). Given are the mean values (± S.E.M.) of percentual changes related to the basal values before application of L-arginine (* $P < 0.05$ vs. baseline, paired *t*-test).

of ODQ (Fig. 6). These results indicate that endogenous and exogenous NO might increase the spontaneous activity of the sinus node in the isolated rat heart, an effect of NO that is probably not mediated by cGMP.

4. Discussion

The aim of the present study was to investigate the effects of endogenous NO-production on myocardial function of the normal heart. The main finding is that pharmacological inhibition of NO-synthase and soluble guanylate cyclase depressed myocardial contractile function. Furthermore, inhibition of NO-synthase, but not of soluble guanylate cyclase, was associated with a reduction in heart rate. These results suggest that endogenous NO-production contributes to the maintenance of myocardial contractility and heart rate.

4.1. Effects on myocardial contractile force

The negative inotropic effect of L-NMMA (Fig. 3), L-NOARG (Fig. 4), and methylene blue (Fig. 5) is consistent with previous reports demonstrating a similar effect of L-NMMA in the isolated rat heart stimulated with isoprenaline (Klabunde et al., 1992) and in the dog heart in vivo (Klabunde et al., 1991; Lechevalier et al., 1994). Presumably, these inotropic effects are induced by inhibition of NO-synthase, which is the main pharmacological action of these drugs (Martin et al., 1985; Palmer et al., 1988; Mülsch and Busse, 1990). Accordingly, inotropic effects of L-NMMA are absent in the presence of equimolar concen-

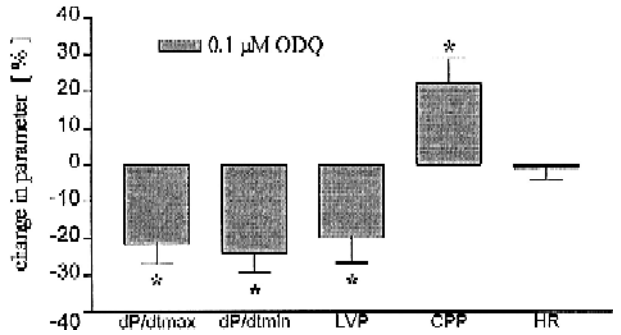

Fig. 6. Effect of infusion of 0.1 μM 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a specific inhibitor of soluble guanylate cyclase, on contractile parameters (LVP = left ventricular peak pressure), coronary perfusion pressure (CPP) and heart rate (HR) of spontaneously beating constant volume perfused Langendorff-hearts of normal Wistar rats ($n = 7$). Given are the mean values (± S.E.M.) of percentual changes related to the basal values before application of ODQ (* $P < 0.05$ vs. baseline, paired *t*-test).

trations of L-arginine (see Section 3), the physiological precursor of endogenous NO-production (Palmer et al., 1988). The NO-synthase inhibitor methylene blue has been shown to inhibit also soluble guanylate cyclase, but this effect is comparably small at the concentrations used in our study (Martin et al., 1985; Mayer et al., 1993). Inhibition of soluble guanylate cyclase by ODQ, which is a specific inhibitor of this enzyme (Garthwaite et al., 1995; Moro et al., 1996; Schrammel et al., 1996), also reduced the contractile force of isolated constant flow perfused rat hearts (Fig. 6) suggesting that inotropic effects of endogenous NO-production are mediated by cGMP.

Endogenous NO-production in the heart can take place not only in endothelial cells but also in cardiomyocytes. The occurrence of endothelial and of inducible NO-synthase has been demonstrated in isolated cardiomyocytes and cardiac tissues from rats (Balligand et al., 1994, 1995; Luss et al., 1995). Other data provided evidence for NO-synthase activity in the same cell type (Kitakaze et al., 1995). Agents known to release endogenous NO from endothelial cells such as bradykinin and acetylcholine induced an increase in cGMP-content of cardiomyocytes that was blunted by N^G-nitro-L-arginine-methylester. This NO-synthase inhibitor is considered to be a prodrug of L-NOARG (Pfeiffer et al., 1996). Presumably, endogenous NO-production in cardiomyocytes of normal rats is based on the activity of endothelial NO-synthase. A contribution of inducible NO-synthase is unlikely, because this enzyme is not detectable by western-blot analysis in normal rats (Wu et al., 1996). The negative inotropic activity of NO-synthase inhibitors shown here is therefore assumingly caused by inhibition of endothelial NO-synthase resulting in reduction of endogenous NO-production in the myocardium.

The effects on myocardial contractile force induced by inhibition of NO-synthase and of soluble guanylate cyclase suggest that endogenous NO acts as a positive inotropic agent in the normal rat heart. The concentration of endogenously produced NO in ventricular myocytes of the isolated rat heart is unknown. Measurements on the surface of endothelial cells have shown that stimulation of NO-production results in a maximal NO-concentration of approximately 500 nM (Malinski and Taha, 1992). In the isolated guinea pig heart the concentration of NO released luminally into the coronary circulation is approximately 2–20 nM (Kelm and Schrader, 1990). To investigate whether nanomolar concentrations of NO are capable to exert a positive inotropic effect, hearts from normal rats were subjected to 10 nM and 100 nM of DEA/NO. This drug is a spontaneous NO-donor (Morley et al., 1993; Keefer et al., 1996). Polarographic measurements demonstrated a NO-release from DEA/NO that resulted in a maximal concentration of NO of approximately 30% of the initial DEA/NO-concentration after 1.5 min (see Section 2). This finding is consistent with the results of other reports (Morley et al., 1993; Christodoulou et al., 1996) and suggests that the maximal NO-concentration in the coronary circulation after infusion of 10 and 100 nM of DEA/NO is approximately 3 and 30 nM, respectively. As shown in Table 2, infusion of 10 nM and 100 nM of DEA/NO significantly increased the contractile force of the isolated rat myocardium indicating that NO at a concentration in the lower nanomolar range is indeed capable to exert a positive inotropic action. This finding confirms the results of a previous study in isolated rat cardiomyocytes demonstrating an increase of the contractile force of isolated rat cardiomyocytes induced by 1 μM of DEA/NO (Kojda et al., 1996). DEA/NO has also been shown to exert a positive inotropic effect in the dog heart in-vivo (Preckel et al., 1997). Other investigators have found a positive inotropic action of NO in multicellular myocardial preparations of the cat (Brutsaert et al., 1988; Mohan et al., 1996). Summarizing these results it is likely that the positive inotropic effect of endogenous and exogenous NO reported here is the result of a direct inotropic action of NO rather than an indirect effect related to coronary vasodilation (see below). The mechanism of the direct effect of NO on cardiac muscle is presumably mediated by a cAMP dependent pathway as demonstrated in isolated rat cardiomyocytes (Kojda et al., 1996).

4.2. Effects on the coronary perfusion

It has been shown that endogenous NO-production in the coronary circulation contributes to the regulation of coronary resistance (Kelm and Schrader, 1990). In accordance, inhibitors of NO-synthase such as L-NMMA and L-NOARG reduce coronary flow or increase coronary perfusion pressure in constant pressure or constant flow heart-preparations, respectively (Bouma et al., 1992; Beresewicz et al., 1995; Amrani et al., 1995; Pabla and Curtis, 1995). In these studies the effects of NO-synthase inhibitors were measured after an incubation time of 10–80 min. An increase of coronary perfusion pressure was observed in the present study after infusion of the NO-synthase inhibitor L-NMMA for 5 min (see Section 3). In contrast, none of the NO-synthase inhibitors altered coronary perfusion pressure after an infusion period of 2 min (Figs. 3–5), the timepoint of measurement of the inotropic and chronotropic effects. It is unlikely therefore that the negative inotropic effects of L-NMMA, L-NOARG and methylene blue shown here were caused by constriction of coronary resistance vessels initiating ischemia in the myocardium. The lack of effect on coronary perfusion pressure rather indicates that the negative inotropic effect of the NO-synthase inhibitors is the result of reduced endogenous NO-production in cardiomyocytes and not of inhibition of NO-production in coronary microvascular endothelial cells.

Infusion of ODQ and of DEA/NO induced changes of coronary perfusion pressure (Fig. 6, Table 2) that occurred in parallel to the inotropic effects and might have con-

tributed to the alterations of contractile force of the isolated hearts. It has been shown earlier that an increase in coronary perfusion associated with enhanced coronary artery transmural pressure results in augmented cardiac oxygen consumption and contractility (Gregg, 1963). This effect is probably related to a distension of coronary vessels (garden-hose hypothesis) which increases cardiac sarcomere lengths (Arnold et al., 1968; Poche et al., 1971). On the other hand, it was reported that pharmacological alteration of coronary perfusion pressure in a constant flow perfused heart does not change myocardial oxygen consumption (Zborowska-Sluis et al., 1977).

In the present study we observed a substantial increase of coronary perfusion pressure after infusion of 0.1 μM ODQ (Fig. 6) indicating a contractile effect of this drug on coronary resistance vessels. These results demonstrate that the activity of soluble guanylate cyclase, which is most likely stimulated by endogenous NO-production, induces a dilation of coronary resistance vessels in constant flow perfused rat hearts. According to the garden-hose phenomenon one would expect that this effect results in an increased contractile force of the myocardium. It is possible therefore that the contractile effect of ODQ on coronary resistance vessels might have counteracted its negative influence on the contractile force of the myocardium. In contrast, DEA/NO induced a substantial decrease of coronary perfusion pressure, an observation that confirms earlier studies demonstrating a potent vasodilator effect of NO in coronary microvessels (Sellke et al., 1990; Kelm and Schrader, 1990). Again, this effect might have altered the inotropic response. In case of DEA/NO one would assume that the decrease of coronary perfusion pressure diminished its positive inotropic effect. Taken together, it cannot be excluded that changes of coronary perfusion pressure induced by ODQ and DEA/NO altered the inotropic effect of these drugs. In contrast, it is unlikely that the effects on coronary resistance are the primary cause of the observed changes in myocardial contractile force.

4.3. Effects on heart rate

The results of the present study suggest that exogenous and endogenous NO alters heart rate. Stimulation of endogenous NO-production by L-arginine and application of the NO-donor DEA/NO significantly increased heart rate, while inhibition of endogenous NO-production by L-NOARG and methylene blue had the opposite effect. These results are consistent with a previous report demonstrating a negative chronotropic effect of the NO-synthase inhibitor N^G-nitro-L-arginine-methylester in the isolated rat heart (Pabla and Curtis, 1995). In addition, disruption of the eNOS 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). The mechanism of chronotropic effects of endogenous and exogenous NO is unknown. An activation of soluble guanylate cyclase with subsequent production of cGMP is questionable, because ODQ, a specific inhibitor of this enzyme, showed no effect on heart rate (Fig. 6). Elucidation of the mechanism of action by which NO increases heart rate requires further investigations.

4.4. Limitations of the study

The use of isolated organ preparations such as the Langendorff-preparation has several limitations regarding the situation in-vivo. It is known that perfusion with saline buffers induces interstitial edema due to the relatively lower oncotic pressure of these buffers. Over the long term this results in an increase in coronary perfusion pressure, which may influence the investigation. The influence of coronary perfusion pressure on the results of the present investigation has already been discussed (see above). It is also known that the endocardium might have an influence on myocardial performance (Brutsaert et al., 1988). Thus, the damage of the endocardial surface induced by placing the intraventricular balloon might also have influenced the effects of the drugs on cardiac function obtained in the present study. Finally, it is known that isolated hearts do not operate with the same contractile force than the heart in-vivo. For these reasons, it is difficult to transfer the results of this study to the situation in-vivo.

In summary, our results provide evidence for positive inotropic and positive chronotropic effects of endogenous NO-production in the normal rat heart. These effects are most likely not related to NO induced alterations of coronary perfusion.

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