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Nitric oxide synthase inhibition impairs myocardial efficiency and ventriculo-arterial matching in acute ischemic heart failure

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Abstract

Background and aims: The effect of nitric oxide (NO) manipulation in acute heart failure has not been sufficiently investigated. Therefore, we assessed the impact of NO-synthase (NOS) inhibition on left ventricular (LV) function and energetics as well as overall hemodynamics, in a porcine model of acute ischemic LV failure. *Methods*: Acute heart failure was induced by left coronary artery microembolization in fourteen anesthetized pigs. LV pressure–volume relationships and mechanical work (PVA) were assessed 30 min after stable heart failure, using pressure-conductance catheters. Myocardial oxygen consumption (MVO_2) was determined from coronary flow and coronary arteriovenous oxygen difference. Microembolization led to a significant decrease in cardiac output, arterial pressure and LV systolic and diastolic performance. Animals were then randomized to a control group ($n=7$) or to receive 15 mg/kg N^{ω}-Nitro-L-arginine-metyl ester ($n=7$), an inhibitor of NO synthase (NOS). *Results*: Measurements 15 min later revealed that NOS inhibited animals had significantly reduced cardiac output (1.53 ± 0.45 vs. 2.13 ± 0.49 l/min, $P=0.003$) and stroke work (1054 ± 461 vs. 1296 ± 348 mmHg ml, $P=0.03$), and also displayed a significant increase in the slope of the MVO_2–PVA relationship (2.57 ± 0.53 vs. 1.92 ± 0.15, $P=0.008$), i.e. an inefficient chemomechanical coupling. NOS inhibition did not alter contractility, diastolic function or arterial pressure, but afterload was significantly increased compared to controls (arterial elastance 6.03 ± 1.48 vs. 2.74 ± 0.34 mmHg/ml, $P=0.009$). *Conclusion*: Inhibition of NOS in experimental acute heart failure increased afterload without altering left ventricular systolic and diastolic function. Consequently, cardiac output was reduced. Furthermore, mechanoenergetic efficiency was severely impaired. NOS inhibition in acute heart failure and cardiogenic shock warrants further investigations.

Keywords: Heart failure; Nitric oxide; Energy metabolism; Ventricular function; Hemodynamics

1. Introduction

Cardiogenic shock is the leading cause of death in patients hospitalized for acute coronary syndromes. In the course of a myocardial infarction, approximately 7% develop shock, with mortality rates as high as 60–80% [1–3] In recent years, thrombolysis, angioplasty or surgical revascularization has improved survival [3,4]. However, despite aggressive treatment, outcome remains poor with unacceptably high mortality [5]. Further research is clearly needed to enlighten the cellular and hemodynamic mechanisms of cardiogenic shock and to develop new treatment strategies.

Because nitric oxide (NO) is thought to impair contractility [6], increased myocardial production of NO has been proposed as a contributor to the progression of chronic cardiac failure [7]. Chronically failing hearts display increased expression of nitric oxide synthase (NOS) [8] and cardiac NO production is augmented in chronic heart failure [9]. Importantly, myocardial NO production is also increased during acute ischemia [10] and coronary NO levels increase substantially during acute cardiac decompensation [11]. Therefore, a deleterious role of endogenous NO in acute ischemic left ventricular failure and cardiogenic shock could be envisioned.

In a recent study, Cotter and associates reported a remarkable effect of NOS-inhibition in human cardiogenic shock [12]. Ten out of 11 patients in severe shock after myocardial infarction could be weaned from mechanical

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Table 1
Dose–response study using N^{ω}-Nitro-L-arginine-metyl ester (L-NAME)

	After Heart Failure	L-NAME 0.5 mg/kg	L-NAME 1 mg/kg	L-NAME 2.5 mg/kg	L-NAME 5 mg/kg	L-NAME 10 mg/kg	L-NAME 15 mg/kg	L-NAME 20 mg/kg
HR	129 ± 14	130 ± 18	129 ± 13	131 ± 23	126 ± 12	119 ± 19	108 ± 24	112 ± 22
MAP (mmHg)	65 ± 10	62 ± 14	67 ± 19	66 ± 29	68 ± 24	68 ± 26	72 ± 28	73 ± 28
CO (l/min)	2.56 ± 0.42	2.67 ± 0.51	2.34 ± 0.53	2.45 ± 0.60	1.84 ± 0.18*	1.70 ± 0.20*	1.57 ± 0.22*	1.75 ± 0.17*
SV (ml)	19.9 ± 1.0	20.5 ± 3.2	18.1 ± 4.6	18.8 ± 6.5	14.6 ± 4.4*	14.3 ± 5.2*	15.3 ± 4.4*	15.9 ± 5.2*
SW (mmHg ml)	1202 ± 135	1180 ± 142	1224 ± 499	1106 ± 678	915 ± 502*	868 ± 584*	1035 ± 515*	995 ± 571*
SVR(dynes s/cm^5)	2055 ± 436	1862 ± 489	2290 ± 563	2693 ± 1291	2957 ± 1009*	3163 ± 917*	3665 ± 1126*	3321 ± 1250*
Ea (mmHg/ml)	3.84 ± 0.73	3.67 ± 0.86	3.92 ± 1.03	4.22 ± 1.56	5.53 ± 0.72*	5.49 ± 0.27*	5.61 ± 0.85*	5.35 ± 1.01*
PRSW (mmHg)	34.8 ± 9.5	33.3 ± 11.2	28.2 ± 8.6	29.9 ± 12.2	29.3 ± 5.6	25.2 ± 5.6	28.5 ± 11.3	22.6 ± 10.5
dp/dt_{max} (mmHg/s)	1040 ± 169	1013 ± 254	899 ± 177	953 ± 205	941 ± 301	896 ± 383	1001 ± 385	963 ± 423
Tau (ms)	30.5 ± 3.4	33.6 ± 4.9	30.2 ± 4.2	32.4 ± 5.8	31.8 ± 4.2	35.7 ± 6.6	33.3 ± 6.1	33.3 ± 6.1
dp/dt_{min} (mmHg/s)	1055 ± 120	1011 ± 213	1103 ± 192	1057 ± 302	1028 ± 209	941 ± 317	1048 ± 257	1042 ± 259

Dose increments were followed by 15 min of stabilization and assessment of general hemodynamics and left ventricular mechanical function. MAP, mean arterial pressure; CO, cardiac output; SV, stroke volume; SW, stroke work; SVR, systemic vascular resistance; Ea, total arterial elastance; PRSW, preload Recruitable stroke work; dP/dt_{max} and dP/dt_{min}, maximal and minimal first derivative of pressure alteration; Tau, time constant of relaxation. *$P<0.05$ compared to measurements after heart failure induction. Four animals were investigated in the 'low-dose' range (0.5–2.5 mg/kg) and 6 animals were investigated in the 'high dose' range (5–20 mg/kg).

ventilation and intra-aortic balloon pumping after NOS-inhibition. Seven of the patients were alive at 1-month follow up. The authors did not investigate the mechanisms behind these remarkable results, but suggested that increased contractility could play a role. Other possible mechanisms could be improved diastolic function or myocardial efficiency, or secondary effects due to alterations in vascular resistance.

In order to differentiate these possibilities, the present study was designed to investigate the effect of NOS inhibition in an animal model of acute ischemic heart failure. Specifically, the impact of NOS-inhibition on left ventricular function and ventriculo-arterial interplay were assessed. Since NO is known to affect ventricular energetics [13,14], we also assessed LV oxygen consumption and mechanical work during NOS-inhibition. We hypothesized that NOS-inhibition would improve left ventricular contractility at the prize of increased myocardial oxygen demands.

2. Methods

The experimental protocol was approved by the local steering committee of the Norwegian Animal Experiments Authority. All animals received care in compliance with the European Convention on Animal Care, and the investigation conformed with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Dose–response study

To assure that we achieved adequate NOS inhibition, we performed a dose–response study with the NOS inhibitor N^{ω}-Nitro-L-arginine-metyl-ester (L-NAME) before the protocol outlined below was started. After surgery, instrumentation and heart failure induction (see below), the dose–response study was performed by a 2-min bolus infusion of L-NAME in increasing doses up to 20 mg/kg. Each dose-step was followed by 15 min of stabilization and subsequent assessment of general hemodynamics and both systolic- and diastolic properties as outlined below. Four pigs were studied after infusion of low dose L-NAME (0.5–2.0 mg/kg) and 6 pigs (including the 4 in the low-dose group) were studied after high dose infusion (5–20 mg/kg). The dose–response data are given in Table 1. Using the lower doses (0.5–2.5 mg/kg), there were no significant alterations in any values from baseline measurements. In the high dose range (5–20 mg/kg), cardiac output, stroke volume, systemic vascular resistance and arterial elastance were altered significantly at all doses compared to both baseline and measurements in the low dose range, but without differences between the different doses. From the rather large standard deviation in measurements in the low dose range, we deduced that there was a large inter-animal variation in the dose needed to achieve NOS inhibition. Therefore, to be certain that adequate NOS inhibition was achieved in all animals, we chose a dose from the high dose range. Furthermore, at 15 mg/kg there was a tendency, although not significant in this study, towards an increased LV contractility. Therefore, we chose the dose of 15 mg/kg in the main study. This is also in the NOS inhibitor bolus dose range used in several other studies, and similar doses have been shown to inhibit NO synthase [15,16].

2.2. Surgery and instrumentation

Fourteen domestic castrated male pigs (30 ± 2 kg) were used in the main study. The animals were fasted

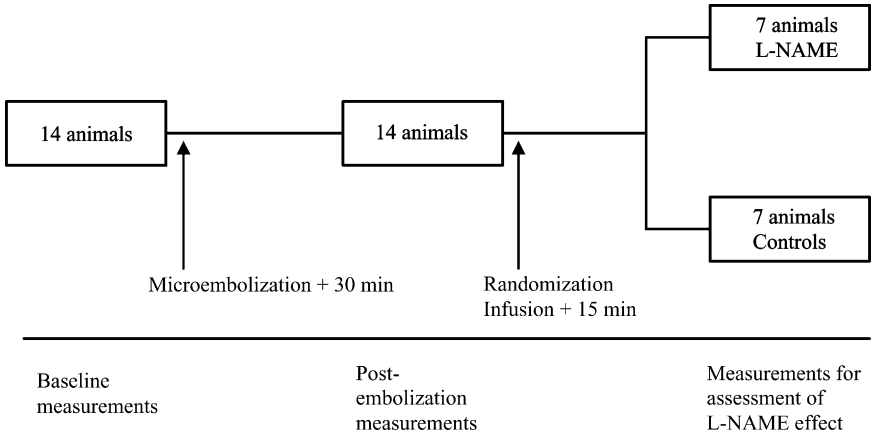

Fig. 1. Outline of the experimental protocol. After baseline assessment of hemodynamics and left ventricular systolic and diastolic function, animals underwent left coronary artery microembolization. After 30 min of stabilization, new measurements confirmed the presence of heart failure. Animals were then randomized to receive infusion of either L-NAME or NaCl. Fifteen minutes after infusions, all measurements were repeated.

overnight and premedicated with intramuscular ketamine (20 mg/kg, Warner Lambert Nordic, Sweden) and atropine (1 mg, Nycomed Pharma, Norway). Anesthesia was induced by intravenous pentobarbital sodium (10 mg/kg, Nycomed Pharma) and fentanyl (0.01 mg/kg, Pharmlink, Sweden) and maintained with continuous infusion of pentobarbital sodium (4.0 mg kg^{-1} h^{-1}), fentanyl (0.02 mg kg^{-1} h^{-1}) and midazolam (0.3 mg kg^{-1} h^{-1}, Alpharma, Norway). Animals were tracheostomized, intubated and ventilated with 60% oxygen. The internal jugular veins were catheterized for infusions and measurement of central venous pressure (CVP). Mean arterial pressure (MAP) was measured in the descending thoracic aorta. After sternothomy, the left hemiazygos vein was ligated and transit time flow probes (CardioMed CM-4000, Medi-Stim AS, Norway) were placed on the three main coronary arteries and on the pulmonary trunk for determination of coronary flow and cardiac output (CO). A balloon (Sorin Biomedical, CA) was introduced in the inferior caval vein for preload reduction. A combined pressure-conductance catheter (7 Fr., 12 electrodes, Sentron, the Netherlands) was inserted into the left ventricular cavity via the left carotid artery. Myocardial venous blood was drawn from a catheter placed in the great cardiac vein. Finally, a catheter was inserted into the pulmonary trunk for measurement of pulmonary artery pressure (MPAP). After surgery, the animals received 150 mg amiodarone (Cordarone, Sanofi Winthrop, Sweden) to prevent ventricular arrhythmias. Furthermore, 20 mg/kg hexamethonium chloride (Hexamethonium, Sigma, St. Louis, MO) was administered as a bolus to avoid autonomous reflex influences on hemodynamics during measurements and interventions. Finally, 2500 IU heparin was given intravenously. Animals were stabilized for 30 min before baseline measurements.

2.3. Experimental protocol

The experimental protocol is outlined in Fig. 1. To assess contractility, pressure–volume data were recorded during transient (12–15 s) preload reduction. The slope of the stroke-work-end-diastolic volume relationship (PRSW, Preload Recruitable Stroke Work) was used as the main index of contractility [17]. Pressure–volume recordings with concomitant MVO_2 determination were performed at 6 different steady state preloads in order to assess the PVA–MVO_2 relationship [18]. After these baseline measurements, heart failure was induced by repeated injections of 5-mg boluses of 50 μm polystyrene microspheres (NEM-005, NEN Lifescience Products, Boston, MA) in the left coronary main stem until CO was reduced by approximately 30% and MAP was under 70 mmHg. Measurements 30 min after the last microembolization confirmed the presence of stable ischemic LV failure. Animals were then randomized to receive either a 2-min intravenous infusion of 15 mg/kg of the NOS inhibitor L-NAME (Alexis biochemicals) in 0.9% NaCl ($n=7$), or vehicle only (NaCl) (controls, $n=7$). Hemodynamic and energetic measurements were repeated 15 min after pharmacological infusion. At the end of the experiment, animals were sacrificed by intracardiac injection of KCl and infusion of high-dose pentobarbital.

2.4. Analyses

The conductance catheter technique has been described previously [19]. Pressure- and conductance signals were processed using a conductance conditioner (Leycom, Sigma 5DF, Cardiodynamics, the Netherlands) and displayed on a computer using the software Conduct PC, CPC V3.15 (Leycom). The slope factor α was calculated from the ratio

between conductance- and transit time derived cardiac outputs. Parallel conductance was estimated by the saline dilution technique [19].

Pressure–volume area (PVA) represents total mechanical work [18]. PVA was calculated as:

$$PVA = SW + [ESP \times (ESV - V_0)/2] - [EDP \times (ESV - V_0)/2]$$

where SW is Stroke Work calculated from integrated pressure–volume data; ESP and ESV are end-systolic pressure and volume, respectively. V_0 is the extrapolated x-intercept of quadratic fitted end-systolic pressure–volume relationships, and EDP is end-diastolic pressure. Left ventricular coronary blood flow (LVCBF) was estimated:

$$LVCBF = CBF/W \times LVW$$

Where CBF is total coronary blood flow, W is total- and LVW is left ventricular myocardial weight. Myocardial oxygen consumption (MVO_2) was calculated:

$$MVO_2 = (LVCBF \times avdO_2 \times Hb \times 1.39)/HR \times 20.2$$

where $avdO_2$ is difference between aortic and myocardial venous oxygen saturation, Hb is hemoglobin in g/ml, 1.39 is a constant (in ml O_2/g Hb) and HR is heart rate. The factor 20.2 J/ml O_2 was used to convert MVO_2 to units equivalent to mechanical energy units. Arterial elastance (Ea) was calculated as end-systolic pressure divided by stroke volume.

2.5. Statistics

Linear, curvilinear and exponential relationships were estimated by least squares fit regression using a spreadsheet (Excel 2000, Microsoft), and all data were further analyzed in a statistical software package (SPSS 9.0, SPSS inc., Chicago, IL). After having excluded that large deviation from normal distribution of the data existed, differences between groups at baseline were evaluated by a two-sample *t*-test. In order to assess the impact of microembolization, *t*-test for paired data was used. Effect of NOS-inhibition was evaluated by 2-way ANOVA (time and group) for data before and after infusion in the L-NAME and control groups. In the dose–response study, the response was analyzed by repeated measurements of variance with Bonferroni correction for multiple comparisons. All values are given as mean ± S.D. Significance is reported at the 5% level.

3. Results

3.1. Effects of microembolization on hemodynamic and energetic parameters

Induction of acute LV failure led to a significant decline in LV performance parameters, CO and MAP, confirming the presence of acute heart failure with both systolic and diastolic components. Details are given in Table 2. Systemic vascular resistance (SVR) and arterial elastance (Ea) were not altered by microembolization. Stroke work was reduced to a greater degree than MVO_2, leading to deterioration of mechanical efficiency (SW/MVO_2), another characteristic of left ventricular failure [20].

Table 2
Effects of microembolization

	Before microembolization	After microembolization
CO (l/min)	2.79 ± 0.55	2.01 ± 0.53*
SW (mmHg ml)	2406 ± 353	1295 ± 409*
MAP (mmHg)	89 ± 13	66 ± 11*
HR (beats/min)	94 ± 14	90 ± 14
SV (ml)	29.5 ± 6.2	22.3 ± 5.5*
PRSW (mmHg)	60.3 ± 9.1	25.7 ± 6.9*
dP/dt_{max} (mmHg/s)	1407 ± 186	966 ± 145*
dP/dt_{min} (mmHg/s)	1493 ± 192	825 ± 205*
EDP (mmHg)	11.3 ± 5.8	22.0 ± 5.5*
Tau (ms)	32.5 ± 5.4	43.4 ± 9.3*
Slope EDPVR	0.051 ± 0.031	0.074 ± 0.041*
SVR (dynes s/cm^5)	2677 ± 818	2862 ± 1004
SW/MVO_2	0.26 ± 0.07	0.19 ± 0.04*

Parameters before and 30 min after microembolization. CO, cardiac output. SW, stroke work. MAP, mean arterial pressure. HR, heart rate. SV, stroke volume. PRSW, Preload Recruitable Stroke Work. dP/dt_{max} and dP/dt_{min}, maximal and minimal first derivative of pressure alteration; EDP, end-diastolic pressure; Tau, time constant of relaxation; EDPVR, end-diastolic pressure–volume relationship; SVR, systemic vascular resistance; SW/MVO_2 relationship between stroke work and myocardial oxygen consumption (mechanical efficiency). *$P<0.01$ for impact of microembolization. $N=14$ (all animals, both groups).

3.2. Effects of NOS-inhibition

Hemodynamic variables for NOS inhibited animals and controls are given in Table 3 and Fig. 2. Compared to controls, NOS-inhibited animals had an increased afterload and decreased CO. However, there were no significant differences in indices of left ventricular systolic- and diastolic function between NOS-inhibited animals and controls (Table 3). NOS-inhibition did not affect left ventricular myocardial oxygen consumption or mechanical efficiency (SW/MVO_2, 0.21 ± 0.03 in controls vs. 0.13 ± 0.03 in L-NAME group, $P=0.07$), and we found no difference between the two groups in y-axis intercept of the $PVA–MVO_2$ relationship (unloaded MVO_2). However, as shown in Fig. 3, animals in the L-NAME group had a significantly increased slope of the $PVA–MVO_2$ relationship.

4. Discussion

This is the first experimental study to examine the impact of NOS-inhibition on LV mechanics and energetics

Table 3
Differences between NOS-inhibited animals and controls

	Controls	L-NAME	*P*-values (between groups)
HR (beats/min)	88 ± 17	96 ± 13	0.83
MAP (mmHg)	57.9 ± 9.6	75.7 ± 27.5	0.28
MPAP (mmHg)	19.7 ± 1.7	27.6 ± 2.2	0.00006
SW (mmHg ml)	1296 ± 348	1054 ± 461	0.027
MVO_2 (J/beat/100 g)	0.86 ± 0.22	1.05 ± 0.39	0.93
Unloaded MVO_2 (J/beat/100 g)	0.30 ± 0.15	0.42 ± 0.26	0.54
Slope PVA–MVO_2	1.92 ± 0.15	2.57 ± 0.53	0.009
PRSW (mmHg)	27.2 ± 10.1	30.7 ± 5.0	0.76
dP/dt_{max} (mmHg/s)	957 ± 197	1006 ± 205	0.66
Tau (ms)	41.9 ± 10.9	45.4 ± 10.6	0.071
dp/dt_{min} (mmHg/s)	753 ± 250	964 ± 325	0.42

HR, heart rate; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; MVO_2, myocardial oxygen consumption; Unloaded MVO_2, oxygen consumption for non-mechanical processes; Slope PVA–MVO_2 relationship, inverse value of slope indicates myofibrillar efficiency; Unloaded MVO_2 indicate oxygen consumption for non-contractile processes such as basal metabolism and E–C coupling; PRSW, preload recruitable stroke work; dP/dt_{max} and dP/dt_{min}, maximal and minimal first derivative of pressure alteration; Tau, time constant of relaxation.

in acute ischemic heart failure. The main findings in our study were:

1. NOS-inhibition induced a profound increase in peripheral arterial resistance and arterial elastance, with a concomitant further reduction of cardiac output in already failing hearts (Fig. 2).
2. The mechanoenergetic relationship revealed decreased myofibrillar efficiency in NOS inhibited animals (Fig. 3).

4.1. Effect of NOS-inhibition on LV mechanical properties

Experimental studies of myocardial contractile effects of NO have yielded apparent contradictory results [6,21] This can be explained by a biphasic, dose dependent effect of NO on the myocardium. In a study using isolated cat papillary muscles, Mohan and colleagues found that administration of low concentrations of NO donors induced a positive inotropic effect, but during administration of higher doses, a negative inotropic effect was seen [22]. In the chronically failing heart, increased NOS expression have been associated with negative effects on cardiac contractility [23,24]. Whether NOS expression in the myocardium is a result of heart failure or being related to its cause, is controversial [25,26]. Furthermore, some investigations have failed to demonstrate a positive inotropic effect of NOS inhibition in cardiac failure [21]. In the present study, NOS inhibition did not alter the maximum first derivative of pressure rise (dP/dt_{max}) or PRSW. We conclude that very early in acute ischemic heart failure, the concentration of NO present in the myocardium is not in the range where contractility is significantly affected, and that endogenous NO plays no or little role in the contractile failure during acute ischemia.

When considering the time aspect of shock development, the emergence of different isoforms of NOS could possibly explain a dual response on contractility. A cardiodepressive effect of NO could be associated with the expression of inducible NOS (iNOS) occurring later in the development of heart failure [27]. However, the constitutive form of NOS, eNOS, has been demonstrated to mediate positive inotropic effects in certain circumstances [15]. This opens for differentiated actions of NO depending on the isoenzyme source, with iNOS derived NO being a contributor to cardiac decompensation. A significant iNOS activity in our study is unlikely, since Thielman et al. found no evidence for iNOS expression 8 hours after microembolization in dogs [28]. In the phase of heart failure investigated by us, iNOS activity was probably not present, and if a heart failure of NO is mediated thorough iNOS, this could explain the unaltered LV function after NOS inhibition found in our study.

Intracoronary infusion of a NO donor has been shown to hasten relaxation and improve LV distensibility in healthy humans [29]. Furthermore, in a dog model of pacing induced heart failure, a rise in LV end-diastolic pressure was associated with a decline in cardiac NO production [30]. In our study, NOS inhibition did not alter relaxation, diastolic stiffness or end-diastolic volume. Consequently, endogenous NO does not seem to play a role in the impairment of diastolic function in ischemia induced heart failure. However, it should be noted that assessment of both systolic and diastolic properties could have been confounded by the L-NAME induced load alterations.

While contractility and diastolic function were unchanged in NOS-inhibited animals, L-NAME led to a significant increase in SVR and Ea. The increased SVR was not accompanied by a significant incline in arterial pressure, indicating that the failing LV did not manage to handle the increased afterload. Therefore, we conclude that the reduced stroke volume, stroke work and cardiac output in the L-NAME group are exclusively due to the ventriculo-arterial mismatch caused by the peripheral vasoconstrictory effects of NOS-inhibition.

4.2. Effects of NOS-inhibition on cardiovascular efficiency

Microembolization significantly reduced left ventricular contractility while arterial elastance (Ea) was unchanged. The resulting mismatch in ventriculo-arterial coupling is known to impair mechanical efficiency (reduced ratio of SW/MVO_2) [31]. Accordingly, we found a highly significant reduction of mechanical efficiency after microembolization.

A key observation in our study was that NOS-inhibition led to a significantly increased Ea, while contractility was unchanged. This implies further worsening of ventriculo-arterial coupling, and one could expect an additional impair-

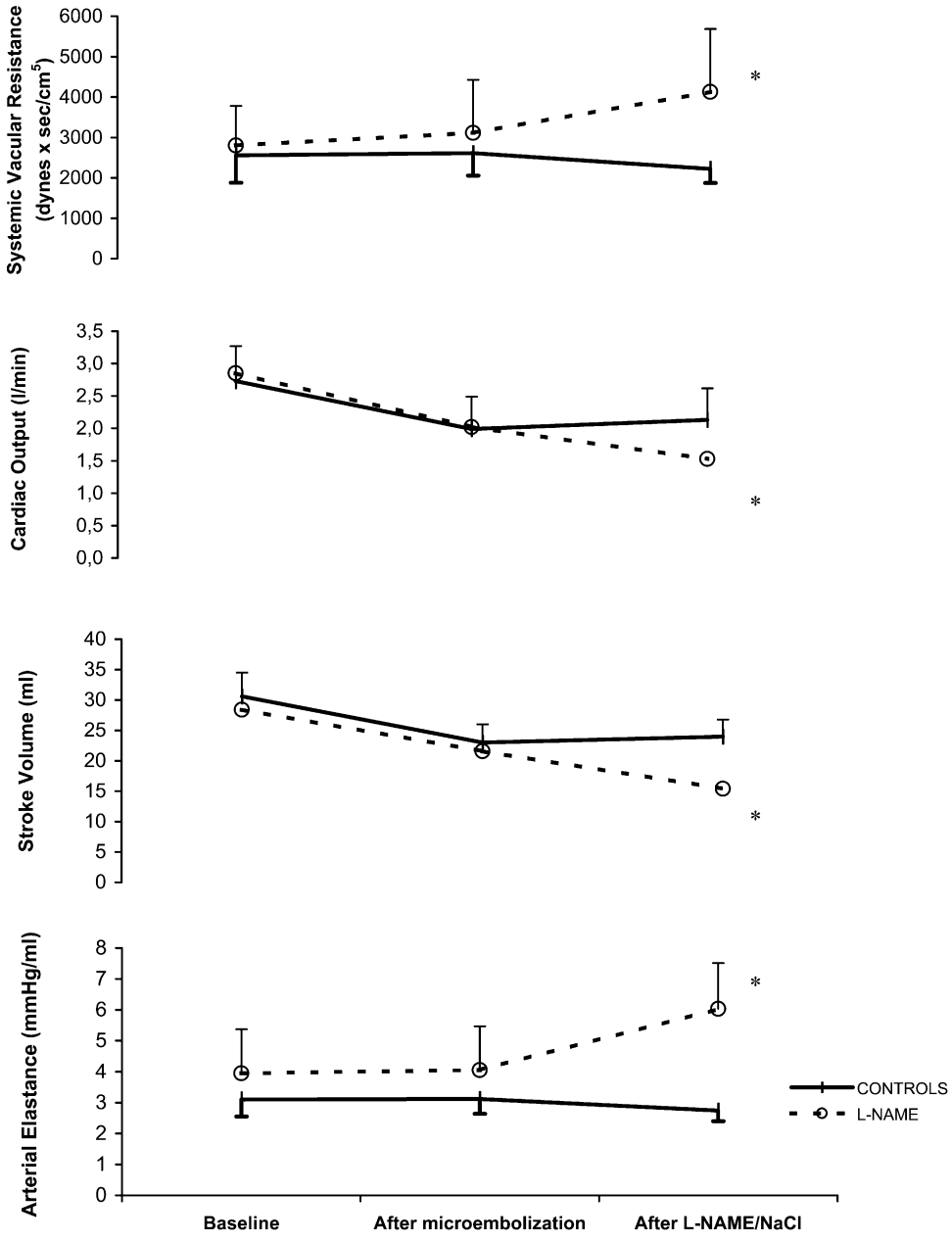

Fig. 2. Hemodynamics in L-NAME and control groups. Average and S.D. are shown for Controls and L-NAME groups. NOS-inhibited animals displayed significantly increased systemic vascular resistance and total arterial elastance compared to controls. Stroke volume and Cardiac Output was significantly reduced in L-NAME animals. *$P<0.01$ compared to controls (two-way ANOVA).

ment of mechanical efficiency. We found a tendency towards further deterioration of mechanical efficiency (SW/MVO_2) in NOS-inhibited animals, but this was not statistically significant ($P=0.07$).

While SW/MVO_2 depicts all over cardiovascular efficiency, the intrinsic myocardial efficiency is studied using the PVA–MVO_2 relationship. The *y*-axis intercept of this relationship (unloaded MVO_2) represents oxygen consumption for non-contractile processes [18]. The preserved unloaded MVO_2 indicates that endogenous NO plays no role in regulation of energy expenditure for basal metabolic processes or E–C coupling in acute ischemic heart failure. This is in contrast to observations in dogs with normal cardiac function where NOS-inhibition increases MVO_2, probably related to increased contractility and basal metabolism [13].

The PVA–MVO_2 relationship describes efficiency in processes converting oxygen to mechanical work, i.e. oxygen-to-ATP conversion and ATP consumption in myofibrillar contraction [18]. The slope of this relationship was significantly increased in NOS-inhibited animals. Several in vitro studies indicate that NO inhibits complexes in the

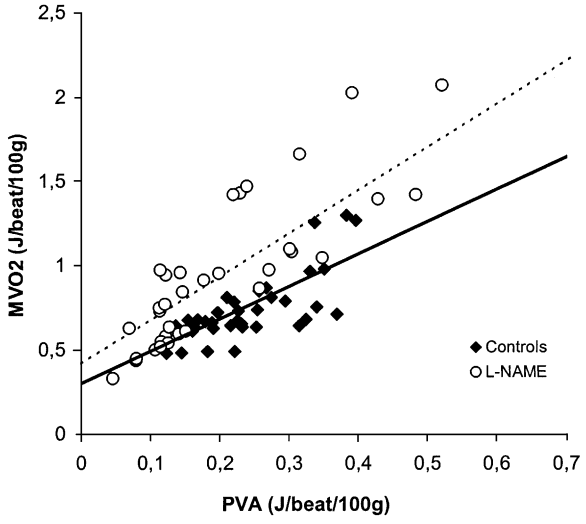

Fig. 3. MVO_2–PVA data for all animals in Control and L-NAME groups. Lines depict average values for the MVO_2–PVA relationship in controls (—) and L-NAME (–) groups. NOS inhibited animals had significantly increased slope of the relationship ($P=0.008$), indicating decreased mechanoenergetic efficiency in this group (two-way ANOVA).

mitochondrial respiratory chain [32–34] Consequently, one could envision that NOS-inhibition increased oxygen utilization and impaired efficiency through attenuating the mitochondrial oxygen-to-ATP generation efficiency. However, since ATP is the energy source for basal metabolic processes and E-C-coupling, as well as for contractile processes, inefficiency in oxygen-to-ATP conversion should also be reflected by an increase in unloaded MVO_2. This was not found. Therefore, the most likely explanation for our findings is that endogenous NO preserved efficiency in cross bridge cycling after microembolization, and consequently that NOS-inhibition impaired this efficiency. Previous studies have shown an impact of NOS inhibition on myocardial oxygen consumption in heart failure [35,36]. Furthermore, in an in-vivo study in pigs, Heusch et al. also found indirect evidence for an effect of NO on myofibrillar efficiency [15].

4.3. Limitations of the study

Our results do not support non-selective NOS inhibition alone as a strategy to improve myocardial function in this early phase of acute cardiogenic shock.

One reason for the apparent inconsistency between the present investigation and the study by Cotter et.al., could be the time aspect. We investigated NOS-inhibition within the first few hours of acute heart failure, while Cotter and associates included patients with cardiogenic shock that persisted for more than 24 h. Several studies have indicated that vasodilation is a common denominator in the late phases of several kinds of shock [37]. Increased peripheral production of nitric oxide has been shown to play an important role in this uncontrolled and detrimental vasodilation. It is therefore possible that the vasoconstriction of NOS-inhibition could be beneficial at a later time point in cardiogenic shock.

Another reason for the apparent disagreement between these two studies could be the additional treatment given in the clinical study. Patients in the aforementioned study received maximal treatment with coronary revascularization, aspirin, heparin, loop diuretics, intra-aortic balloon pumping and beta-adrenergic stimulation. Importantly, when considering the effect of blocking NO in these patients, NOS-inhibition has been shown to increase contractility when given simultaneously with beta-adrenergic stimulation [38]. The heart failure model employed by us is also obviously different from the clinical entity of post-infarct cardiogenic shock. Microembolisation does not necessarily give the same kind of ventricular damage as occluded coronary arteries, especially if the myocardium is revascularized. However, several important studies have implied microembolisation as a very important mechanism behind acute coronary syndromes, particularly after revascularization [39,40]. We investigated the effects of NOS-inhibition after blocking the sympathetic activity with hexamethonium. Thus, the experiments were focused on the effect of NOS inhibition, avoiding the confounding effects of sympathetic activation during heart failure. However, most heart failure patients receive beta-blockers, rendering our sympathetic inhibited model comparable to a clinical setting. The use of amiodarone in our protocol could theoretically have influenced ventricular mechanical function as well as energetics. However, the drug was also used in control animals, so the findings reported were due to NOS-inhibition alone.

A final reason for the differences between the two studies could be the degree of NOS inhibition. The dose–response study performed by us indicated that 15 mg/kg L-NAME totally inhibited NOS, as no significant alteration were seen in hemodynamics after infusion of a higher dose. However, due to a possible biphasic effect of NO on the myocardium, partial NOS inhibition could be beneficial in cardiogenic shock. In the study by Cotter et.al., a significantly lower dose (1 mg/kg) of the NOS-inhibitor L-NMMA was used. In our dose–response study, no cardiac or hemodynamic effects were seen at low doses. NOS inhibition appeared to start at a dose between 2.5 and 5 mg/kg. However, there were large inter-animal variations in both SVR and CO at these doses, indicating that some animals were inhibited while others were not. To achieve a partial NOS inhibition we would need continuous evaluation of cardiac and systemic NO production, adjusting the dose according to the individuals' response in NO production. Unfortunately, we were not able to measure NOS activity in our study.

Our findings indicate that a high-dose NOS-inhibition alone early in acute ischemic left ventricular failure and cardiogenic shock does not increase contractility or improve

the energetic profile of the myocardium. Further investigations are warranted to elucidate the effects of NOS inhibition during acute cardiogenic shock.

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References

[1] Hands ME, Rutherford JD, Muller JE, Davies G, Stone PH, Parker C, et al. The in-hospital development of cardiogenic shock after myocardial infarction: incidence, predictors of occurrence, outcome and prognostic factors. The MILIS Study Group. J Am Coll Cardiol 1989;14(1):40–6.

[2] Goldberg RJ, Gore JM, Alpert JS, Osganian V, de Groot J, Bade J, et al. Cardiogenic shock after acute myocardial infarction. Incidence and mortality from a community-wide perspective, 1975 to 1988. N Engl J Med 1991;325(16):1117–22.

[3] Holmes DR Jr, Bates ER, Kleiman NS, Sadowski Z, Horgan JH, Morris DC, et al. Contemporary reperfusion therapy for cardiogenic shock: the GUSTO-I trial experience. The GUSTO-I Investigators. Global utilization of streptokinase and tissue plasminogen activator for occluded coronary arteries. J Am Coll Cardiol 1995;26(3):668–74.

[4] Hochman JS, Sleeper LA, Webb JG, Sanborn TA, White HD, Talley JD, et al. Early revascularization in acute myocardial infarction complicated by cardiogenic shock. SHOCK Investigators. Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock. N Engl J Med 1999;341(9):625–34.

[5] Goldberg RJ, Samad NA, Yarzebski J, Gurwitz J, Bigelow C, Gore JM. Temporal trends in cardiogenic shock complicating acute myocardial infarction. N Engl J Med 1999;340(15):1162–8.

[6] Flesch M, Kilter H, Cremers B, Lenz O, Sudkamp M, Kuhn-Regnier F, et al. Acute effects of nitric oxide and cyclic GMP on human myocardial contractility. J Pharmacol Exp Ther 1997;281(3):1340–9.

[7] Paulus WJ. The role of nitric oxide in the failing heart. Heart Fail Rev 2001;6(2):105–18.

[8] Drexler H, Kastner S, Strobel A, Studer R, Brodde OE, Hasenfuss G. Expression, activity and functional significance of inducible nitric oxide synthase in the failing human heart. J Am Coll Cardiol 1998;32(4):955–63.

[9] Node K, Kitakaze M, Yoshihara F, Sasaki T, Kuzuya T, Hori M. Increased cardiac levels of nitric oxide in patients with chronic heart failure. Am J Cardiol 2000;86(4):474–7.

[10] Node K, Kitakaze M, Sato H, Koretsune Y, Karita M, Kosaka H, et al. Increased release of nitric oxide in ischemic hearts after exercise in patients with effort angina. J Am Coll Cardiol 1998;32(1):63–8.

[11] O'Murchu B, Miller VM, Perrella MA, Burnett JC Jr. Increased production of nitric oxide in coronary arteries during congestive heart failure. J Clin Invest 1994;93(1):165–71.

[12] Cotter G, Kaluski E, Blatt A, Milovanov O, Moshkovitz Y, Zaidenstein R, et al. L-NMMA (a nitric oxide synthase inhibitor) is effective in the treatment of cardiogenic shock. Circulation 2000;101(12):1358–61.

[13] Suto N, Mikuniya A, Okubo T, Hanada H, Shinozaki N, Okumura K. Nitric oxide modulates cardiac contractility and oxygen consumption without changing contractile efficiency. Am J Physiol 1998;275(1 Pt 2):H41–9.

[14] Shen W, Xu X, Ochoa M, Zhao G, Wolin MS, Hintze TH. Role of nitric oxide in the regulation of oxygen consumption in conscious dogs. Circ Res 1994;75(6):1086–95.

[15] Heusch G, Post H, Michel MC, Kelm M, Schulz R. Endogenous nitric oxide and myocardial adaptation to ischemia. Circ Res 2000;87(2):146–52.

[16] Datta P, Magder S. Hemodynamic response to norepinephrine with and without inhibition of nitric oxide synthase in porcine endotoxemia. Am J Respir Crit Care Med 1999;160(6):1987–93.

[17] Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, et al. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation 1985;71(5):994–1009.

[18] Suga H. Ventricular energetics. Physiol Rev 1990;70(2):247–77.

[19] Baan J, van der Velde ET, de Bruin HG, Smeenk GJ, Koops J, van Dijk AD, et al. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 1984;70(5):812–23.

[20] Nordhaug D, Steensrud T, Korvald C, Aghajani E, Myrmel T. Preserved myocardial energetics in acute ischemic heart failure-studies in an experimental pig model. Eur J Cardiothorac Surg 2002;22(1):135–42.

[21] Cotton JM, Kearney MT, MacCarthy PA, Grocott-Mason RM, McClean DR, Heymes C, et al. Effects of nitric oxide synthase inhibition on Basal function and the force-frequency relationship in the normal and failing human heart in vivo. Circulation 2001;104(19):2318–23.

[22] Mohan P, Brutsaert DL, Paulus WJ, Sys SU. Myocardial contractile response to nitric oxide and cGMP. Circulation 1996;93(6):1223–9.

[23] Heymes C, Vanderheyden M, Bronzwaer JG, Shah AM, Paulus WJ. Endomyocardial nitric oxide synthase and left ventricular preload reserve in dilated cardiomyopathy. Circulation 1999;99(23):3009–16.

[24] Hare JM, Givertz MM, Creager MA, Colucci WS. Increased sensitivity to nitric oxide synthase inhibition in patients with heart failure: potentiation of beta-adrenergic inotropic responsiveness. Circulation 1998;97(2):161–6.

[25] Fukuchi M, Hussain SN, Giaid A. Heterogeneous expression and activity of endothelial and inducible nitric oxide synthases in end-stage human heart failure: their relation to lesion site and beta-adrenergic receptor therapy. Circulation 1998;98(2):132–9.

[26] Drexler H. Nitric oxide synthases in the failing human heart: a doubled-edged sword? Circulation 1999;99(23):2972–5 (editorial; comment).

[27] Kojda G, Kottenberg K. Regulation of basal myocardial function by NO. Cardiovasc Res 1999;41(3):514–23.

[28] Thielmann M, Dorge H, Martin C, Belosjorow S, Schwanke U, van De SA, et al. Myocardial dysfunction with coronary microembolization: signal transduction through a sequence of nitric oxide, tumor necrosis factor- alpha, and sphingosine. Circ Res 2002;90:807–13.

[29] Paulus WJ, Vantrimpont PJ, Shah AM. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans. Assessment by bicoronary sodium nitroprusside infusion. Circulation 1994;89(5):2070–8.

[30] Recchia FA, McConnell PI, Bernstein RD, Vogel TR, Xu X, Hintze TH. Reduced nitric oxide production and altered myocardial metabolism during the decompensation of pacing-induced heart failure in the conscious dog. Circ Res 1998;83(10):969–79 (see comments).

[31] Burkhoff D, Sagawa K. Ventricular efficiency predicted by an analytical model. Am J Physiol 1986;250(6 pt 2):R1021–7.

[32] Abe K, Hayashi N, Terada H. Effect of endogenous nitric oxide on energy metabolism of rat heart mitochondria during ischemia and reperfusion. Free Radic Biol Med 1999;26(3–4):379–87.

[33] Xie YW, Shen W, Zhao G, Xu X, Wolin MS, Hintze TH. 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 1996;79(3):381–7.

[34] Riobo NA, Clementi E, Melani M, Boveris A, Cadenas E, Moncada S, et al. Nitric oxide inhibits mitochondrial NADH:ubiquinone

reductase activity through peroxynitrite formation. Biochem J 2001;359(Pt 1):139–45.
[35] Shinke T, Takaoka H, Takeuchi M, Hata K, Kawai H, Okubo H, et al. Nitric oxide spares myocardial oxygen consumption through attenuation of contractile response to beta-adrenergic stimulation in patients with idiopathic dilated cardiomyopathy. Circulation 2000; 101(16):1925–30.
[36] Chen Y, Traverse JH, Du R, Hou M, Bache RJ. Nitric oxide modulates myocardial oxygen consumption in the failing heart. Circulation 2002;106(2):273–9.
[37] Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. N Engl J Med 2001;345(8):588–95.
[38] Keaney JF Jr, Hare JM, Balligand JL, Loscalzo J, Smith TW, Colucci WS. Inhibition of nitric oxide synthase augments myocardial contractile responses to beta-adrenergic stimulation. Am J Physiol 1996;271(6 pt 2):H2646–52.
[39] Erbel R, Heusch G. Coronary microembolization–its role in acute coronary syndromes and interventions. Herz 1999;24(7):558–75.
[40] Erbel R, Heusch G. Spontaneous and iatrogenic microembolization. A new concept for the pathogenesis of coronary artery disease. Herz 1999;24(7):493–5 (editorial).