Nitric oxide controls cardiac substrate utilization in the conscious dog

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Abstract

Objectives: The aim of this study was to determine whether the acute inhibition of nitric oxide (NO) synthase causes changes in cardiac substrate utilization which can be reversed by a NO donor. Methods: NO synthase was blocked by giving 30 mg/kg of nitro-l-arginine (NLA) i.v. to 15 chronically instrumented dogs. Hemodynamics and blood samples from aorta and coronary sinus were taken at control and at 1 and 2 h after NLA. In five dogs, 0.4 mg/kg of the NO donor 3754 was given i.v. 1 h after NLA. In six dogs, angiotensin II was infused over 2 h (20–40 ng/kg/min) to mimic the hemodynamic effects of NLA. Results: Two h after NLA: mean arterial pressure was 153±4 mmHg; MVO₂ increased by 38%; cardiac uptake of lactate and glucose increased, respectively, from 20.0±5.0 to 41.0±9.3 μmol/min and from 1.1±0.7 to 6.8±1.5 mg/min (all P<0.05 vs. control). Cardiac uptake of free fatty acids decreased by 43% after 1 h (P<0.05) and returned to control values at 2 h. Cardiac respiratory quotient increased from 0.76±0.03 to 1.05±0.07, indicating a shift to carbohydrate oxidation. All these changes were reversed by the NO donor. In the dogs receiving angiotensin II infusion, MVO₂ increased by 28% and lactate uptake doubled (both P<0.05), but no other metabolic changes were observed. Conclusions: The acute inhibition of NO synthase by NLA causes a switch from fatty acids to lactate and glucose utilization by the heart which can be reversed by a NO donor, suggesting an important regulatory action of NO on cardiac metabolism. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Energy metabolism; Hemodynamics; Nitric oxide; Oxygen consumption

1. Introduction

Over the last 10 years, several studies in vitro and in vivo have demonstrated or suggested that one of the functions of nitric oxide (NO) consists of the regulation of important metabolic pathways. NO modulates oxygen consumption by inhibiting the complexes I, II and IV of the mitochondrial electron transport chain [1] and the aconitase, although the effect of NO on this enzyme is still controversial [2]. Some studies indicate that NO facilitates glucose utilization, likely by potentiating the action of insulin [3] or stimulates total substrate oxidation, by activating a cGMP-dependent protein kinase [4]. Others suggest an opposite role, since NO inhibits GAPDH, a key enzyme of the glycolytic pathway [5]. Finally, a recent study from our laboratory has described an association between the fall in cardiac NO production and a switch in myocardial substrate uptake and oxidation during decompensation of pacing-induced heart failure [6]. In end stage failure, the heart shifts from prevalent free fatty acids (FFA) to carbohydrate utilization. A similar switch in uptake occurs also in normal animals after competitive blockade of NO synthase (NOS) by nitro-l-arginine (NLA) [6,7]. Another recent study has shown that NO inhibits myocardial glucose uptake, via cGMP, in vitro [8], supporting our previous findings in vivo.

On the basis of these studies, we have proposed a modulatory action of NO, tonically released by microvascular endothelium, on myocardial oxygen consumption [7–9] and metabolic pathways. In fact, a much higher concentration of constitutive NOS is present in endothelial cells compared to cardiomyocytes [10]. Hypothetically, an
impaired endothelial production of nitric oxide, as may occur in atherosclerosis, hypertension, cardiac ischemia and heart failure [6,11–14], could affect myocardial metabolism, and therefore cardiac performance. Chronic heart failure and cardiac ischemia are associated with endothelial dysfunction and are also characterized by a defective oxidation of FFA and intermediates of FFA metabolism such as acyl-CoA and acyl-carnitine accumulate in ischemic, postischemic and failing myocardium [15–17]. These intermediates can alter the biophysical equilibrium in myocytes and contribute to the development of cardiac arrhythmias [15]. Moreover, since the early 60s it has been shown that glucose rather than FFA oxidation improves recovery from acute myocardial infarction [18]. If NO really plays a key role in the regulation of myocardial substrate utilization, it follows that nitrovasodilators, NO-releasing agents traditionally employed in the treatment of heart failure and myocardial ischemia, may be beneficial by directly modulating cardiac oxygen consumption and substrate utilization. The aims of the present investigation were to determine if: (1) acute inhibition of NOS can result in altered substrate utilization by the heart and (2) this can be reversed by acute administration of a NO donor. The efficacy of the NO donor would confirm that cardiac metabolic changes observed after inhibition of NOS are effectively due to the lack of NO and could provide new insights into the therapeutic action of nitrovasodilators in the treatment of chronic heart failure and cardiac ischemia.

2. Methods

2.1. Surgical procedure and instrumentation

Male dogs (n=21) weighing 25–27 kg were sedated with acepromazine maleate (1 mg/kg, i.m.), anesthetized with sodium pentobarbital (25 mg/kg, i.v.) and ventilated with room air. A thoracotomy was performed in the left fifth intercostal space. Catheters (Tygon) were placed in the descending thoracic aorta and left atrial appendage for pressure measurements. A third catheter was inserted in the coronary sinus by perforating its wall. The length of the inserted segment was such that the tip of the catheter led blood away from the right atrium. During surgery, a blood sample was taken from the catheter and immediately measured on a pH/blood gas analyzer. If the PO$_2$ was >25 mmHg, the catheter was repositioned and finally tied into place. A solid-state pressure gauge (P6.5, Konigsberg) was inserted in the left ventricle (LV) through the apex. A Doppler flow probe (Parks Electronics) was placed around the left circumflex coronary artery. A human, screw-type, unipolar myocardial pacing lead was placed on the LV. Wires and catheters were run subcutaneously to the intrascapular region, the chest was closed in layers and the pneumothorax was reduced. Antibiotics were given after surgery and the dogs were allowed to fully recover. After 10 days, dogs were trained to lie quietly on the laboratory table. The protocols were approved by the Institutional Animal Care and Use Committee of the New York Medical College and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Hemodynamic recordings

The aortic catheter was attached to a P23ID strain-gauge transducer for measurement of aortic pressure. LV pressure was measured using the solid-state pressure gauge. The first derivative of LV pressure, LV dP/dt, was obtained using an operational amplifier (National Semiconductor LM 324), and triangular wave signals with known slope were substituted for the pressure signals to calibrate the differentiator directly. Coronary blood flow was measured with a pulsed Doppler flowmeter (Model 100, Triton). This technique has been used in our previous studies [6,7,19]. All signals were recorded on a 14-channel tape recorder (Bell and Howell 3700B) and played back on an eight-channel direct-writing oscillograph (Gould RS 3800). Mean values of aortic pressure and coronary flow were obtained by filtering the respective signals at 2 Hz. Heart rate was measured using a cardiotachometer (model 9857B, Beckman) from the LV pressure pulse interval. For some data analysis, the tape was played back and the analog signals stored in computer memory through an analog–digital interface (National Instruments), at a sampling rate of 250 Hz.

Mean coronary resistance was obtained by dividing mean aortic pressure by mean coronary flow (mmHg·min/ml).

2.3. Cardiac metabolites and insulin

Blood samples from aorta and coronary sinus were collected into plastic syringes treated either with heparin or EDTA and immediately stored on ice. Special care was taken to withdraw blood slowly from the coronary sinus catheter to avoid potential contamination of the sample with right atrial blood. Blood gases were measured in a blood gas analyzer (Corning, model 170). PO$_2$ was multiplied by 0.003 to obtain the concentration of oxygen dissolved in plasma [20] and added to oxygen concentration measured by an hemoglobin analyzer (CO-Oximeter, Instruments Laboratory) to obtain total oxygen content in whole blood (v/v). Hematocrits were measured after centrifugation. Lactate was measured from whole blood using a lactate analyzer (YSI 1500 Sport, Yellow Springs Instrument). Glucose and FFA concentrations were determined in plasma after centrifugation of the blood samples at 1000 g for 15 min at 0°C. Glucose was measured using a glucose analyzer (Beckman Glucose 2, Beckman). FFA analysis was performed on plasma from...
EDTA treated samples using a colorimetric assay (NEFA C kit from Wako). The arterial—coronary sinus concentration difference (A—CS) of oxygen, lactate, glucose and FFA contents was multiplied by mean coronary blood flow, assumed as double of mean flow measured in the left circumflex coronary artery [21] to calculate cardiac consumption. We have described these methods previously [6,7].

In six dogs, synthetic human angiotensin II (Sigma) was infused over 2 h at the dose of 20–40 ng/kg/min. The infusion rate was adjusted to obtain the same values of mean arterial pressure measured in dogs receiving NLA and also the heart was paced at the same rate of 132±1 beats/min. Hemodynamic changes were recorded during the 2 h of infusion and blood samples were taken at 1 and 2 h.

2.6. Statistical analysis

Data are presented as mean±standard error of the mean (SEM). Statistical analysis was performed employing commercially available software (Sigma Stat 2.0). Changes in hemodynamics and metabolism over time were tested by one-way analysis of variance followed by Dunnett’s test. For all the statistical analysis, significance was accepted at P<0.05.

3. Results

3.1. Hemodynamics

Values of mean arterial pressure and blood flow in the left circumflex coronary artery at control and after NLA or NLA+NO donor (3754) are displayed in Fig. 1. NLA caused a sustained increase in pressure at 1 and 2 h and a significant increase in blood flow at 1 h. 3754 Caused a return to control values after 5 min and the effect persisted for 1 h. LV systolic pressure increased from 132±3 mmHg at control to 169±4 and 168±4 mmHg at 1 and 2 h after
3.2. Cardiac substrate uptake and insulin concentration

MVO₂ and uptake of FFA, glucose and lactate, in dogs receiving NLA alone or NLA + NO donor, are shown in Fig. 2. All changed significantly after NLA and the changes were reversed by the NO donor. FFA uptake decreased significantly 1 h after NLA, but returned to control values after 2 h. The action of the NO donor on cardiac metabolism occurred within 5 min after administration, as shown in Fig. 2. Changes in the concentration of cardiac substrates in arterial plasma are listed in Table 1. Arterial FFA decreased significantly from control 1 h after NLA and was still lower after 2 h. This decrease was rapidly reversed by the NO donor. Arterial plasma glucose and lactate concentrations did not change significantly. Fig. 3 shows the changes of insulin concentration in arterial plasma after NLA and NLA + NO donor. Insulin concentration decreased by 50% at 1 and 2 h after NLA and this change was partially reversed by the NO donor.

The changes in MVO₂ and uptake of FFA, glucose and lactate, together with the changes in arterial concentration of these substrates, during angiotensin infusion, are listed in Table 2. FFA and glucose uptake did not change whereas lactate uptake doubled.

3.3. Cardiac RQ

Changes in cardiac RQ in dogs receiving NLA alone or NLA + NO donor are shown in Fig. 4. RQ increased significantly from control at 1 and 2 h after NLA. The NO donor 3754 abolished these changes. RQ calculated from real production of CO₂ and MVO₂ were 0.90 ± 0.06 and 2.26 ± 0.11 mmHg·min⁻¹·ml⁻¹ at 1 and 2 h after NLA. Values of cardiac RQ pressure to values not statistically different from control. During infusion of angiotensin II are reported in Table 2. dP/dtmax decreased from 2709 ± 57 mmHg/s to 2220 ± 117 and 2261 ± 112 mmHg/s at 1 and 2 h after NLA, respectively (P < 0.05). One h after 3754, dP/dtmax was further decreased to 1800 ± 120 mmHg/s. This was most likely due to reduced preload by the NO donor. Mean coronary vascular resistance was 3.5 ± 0.2 mmHg·min⁻¹·ml⁻¹ and did not change significantly after NLA or NLA + NO donor (3754).

Angiotensin infusion caused the following increases in hemodynamic values: mean arterial pressure from 105 ± 5 mmHg at control to 151 ± 2 and 156 ± 5 mmHg at 1 and 2 h, respectively (P < 0.05); LVSP from 120 ± 6 mmHg at control to 169 ± 3 and 170 ± 4 mmHg at 1 and 2 h (P < 0.05). dP/dtmax from 2537 ± 78 mmHg/s at control to 2825 ± 68 and 2886 ± 112 mmHg/s at 1 and 2 h, respectively (P < 0.05); mean coronary flow in the left circumflex coronary from 34.0 ± 2.4 ml/min at control to 37.0 ± 3.3 and 39.0 ± 3.7 ml/min at 1 and 2 h, respectively (nonsignificant); mean coronary resistance increased from 3.1 ± 0.2 mmHg·min⁻¹·ml⁻¹ to 4.2 ± 0.3 and 4.2 ± 0.5 mmHg·min⁻¹·ml⁻¹, respectively (P < 0.05).

4. Discussion

The present study demonstrates that acute inhibition of NO production causes a reduction of myocardial utilization of FFA and an increase in carbohydrate (lactate + glucose) utilization. The metabolic changes can be rapidly reversed by a NO donor. In particular, the increase in lactate uptake is likely driven by the elevated ventricular afterload, since it occurs also in the control group receiving angiotensin II. On the other hand, the increase in glucose uptake and in cardiac RQ are present only when NOS is blocked and are not insulin-dependent. These findings strongly suggest that NO is involved in regulation of cardiac substrate utilization and reveal a potential novel pharmacological action of nitrovasodilators in conditions of reduced synthesis of NO. The effects of the NO donor on cardiac metabolism could be potentiated by the supersensitivity of the soluble...
guanylate cyclase to exogenous NO, which occurs after NOS blockade, as shown in vitro and in vivo [28]. In a previous study [6], we have described an association between the fall in cardiac NO production and the switch in substrate utilization in end stage heart failure. The involvement of NO in this metabolic alteration was suggested by similar changes in cardiac uptake observed after administration of NLA [6,7]. The present study provides a further basis to the previous results.

To date, the role of NO in the regulation of cardiac substrate utilization is almost unexplored. A recent study in isolated hearts has shown that cGMP, a second messenger of NO, inhibits cardiac glucose utilization and stimulates the synthesis of malonyl-CoA, an inhibitor of

**Table 1**
Cardiac substrate concentration in arterial plasma

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>NLA</th>
<th>NLA + NO donor (3754)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
<td>65</td>
</tr>
<tr>
<td>Arterial FFA (mequiv/l)</td>
<td>0.57±0.04</td>
<td>0.25±0.02*</td>
</tr>
<tr>
<td>Arterial lactate (mmol/l)</td>
<td>1.02±0.08</td>
<td>1.10±0.08</td>
</tr>
<tr>
<td>Arterial glucose (mg/dl)</td>
<td>90.0±2.0</td>
<td>84.0±1.3</td>
</tr>
</tbody>
</table>

*P<0.05 vs. control (time 0). Data are presented as mean±SEM.
long-chain FFA oxidation [8]. However, the heart was perfused with Krebs–Henseleit buffer containing glucose as the only substrate. Interestingly, our results indicate a similar inhibition by NO on glucose, but not on FFA, utilization. It is likely that the NO-mediated control on cardiac metabolism is in part different in vivo, where all the substrates are supplied and the oxygen delivery to the heart is optimal. Cardiac muscle utilizes primarily FFA in fasting state [23,24,29]. In dogs, we found a baseline cardiac RQ of 0.755, indicating that more than 80% of the oxidized substrates were FFA.

Factors other than NOS inhibition that could have caused profound alterations in cardiac metabolism in our experiments were the hemodynamic, hormonal, and neurohumoral changes. However, we can exclude that the marked elevation in blood pressure, after NLA, determined a switch in substrate utilization. During infusion of angiotensin II, MVO₂ increased by 25–28% and only lactate uptake increased twofold, whereas glucose and FFA uptake and RQ remained unchanged. These results indicate that the ventricular pressure development stimulates lactate uptake, yet the oxidation of FFA remains prevalent. Insulin stimulates cardiac glucose uptake and oxidation [17]. The observed switch in cardiac substrate consumption and the fall in FFA concentration could be indeed due to the release of insulin triggered in pancreatic beta-cells by the arginine analogue NLA [30]. Yet, our data indicate that insulin concentration fell by 50% after NLA, confirming previous reports of the inhibitory action of NOS blockade on the endocrine pancreas [31]. 3754 was able to raise insulin levels, although not completely. Another possible cause of altered cardiac metabolism occurring after NLA or NO donor could be the changes in neurovegetative activity. In our experiments, we did not measure sympathetic activity, but previous findings in conscious rabbits demonstrated that NOS blockade causes a reduction in sympathetic outflow [32] mainly due to the baroreflex response triggered by high blood pressure. However the baroreflex response was triggered also during angiotensin infusion, when only minimal changes in cardiac metabolism were found. Moreover, studies in vitro clearly indicate that NO/cGMP provides a direct control of glucose utilization [5,8]. Despite these considerations, we cannot exclude a role played by the neurovegetative changes. Finally, an important factor that strongly influences cardiac substrate utilization is metabolite concentration in arterial blood [17]. In particular, the human heart preferentially utilizes carbohydrates when arterial FFA concentration falls below the threshold of 0.3 mequiv/l [17]. The arterial plasma concentration of lactate and glucose did not change significantly after NLA, whereas FFA concentration fell below the threshold at 1 h after NLA, likely causing the reduction in uptake of FFA at that time point. Two h after

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MVO₂ (ml/min)</th>
<th>FFA uptake (μequiv/min)</th>
<th>Lactate uptake (μmol/min)</th>
<th>Glucose uptake (mg/min)</th>
<th>RQ</th>
<th>Arterial FFA (mequiv/l)</th>
<th>Arterial lactate (mmol/l)</th>
<th>Arterial glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>7.6±0.4</td>
<td>17.8±2.3</td>
<td>10.0±2.6</td>
<td>1.4±0.9</td>
<td>0.74±0.02</td>
<td>0.65±0.05</td>
<td>1.10±0.14</td>
<td>88.0±3.4</td>
</tr>
<tr>
<td>60</td>
<td>9.5±0.9*</td>
<td>16.6±1.8</td>
<td>19.8±2.6*</td>
<td>1.9±1.7</td>
<td>0.75±0.03</td>
<td>0.52±0.05*</td>
<td>1.20±0.11</td>
<td>90.0±1.9</td>
</tr>
<tr>
<td>120</td>
<td>9.7±1.0*</td>
<td>14.7±1.5</td>
<td>23.6±2.3*</td>
<td>1.9±1.5</td>
<td>0.79±0.03</td>
<td>0.49±0.06*</td>
<td>1.20±0.10</td>
<td>88.0±2.4</td>
</tr>
</tbody>
</table>

* P<0.05 vs. control (time 0); n=6. Data are presented as mean±SEM.
NLA, FFA uptake returned to control levels and RQ reached 1, indicating prevalent carbohydrate oxidation. Therefore, the changes in FFA utilization after NLA can be divided in two components: uptake and oxidation. Uptake returned to normal values after 2 h, when the arterial FFA concentration exceeded the threshold of 0.3 mequiv/l. The impairment of fat oxidation was not NFA concentration-dependent and was complete 2 h after NLA. To our knowledge, this is the first study describing a control by NO on cardiac FFA oxidation in vivo. On the basis of the present data, it is not possible to define the mechanism of control, nor the fate of the nonoxidized FFA, which were probably accumulated as FFA and/or as metabolic intermediates or were incorporated in the myocardial pool of triacylglycerols. However, the regulatory role of NO was confirmed by the action of 3754, which rapidly reversed these metabolic alterations.

Some interesting analogies can be proposed between myocardial metabolic changes due to competitive inhibition of NOS and metabolic abnormalities associated with cardiac diseases. For example, impaired release of NO from endothelial cells has been found in chronic heart failure [14,33], which is also characterized by a switch from FFA to carbohydrate utilization in the heart [6,34,35]. A clinical investigation on failing hearts documented an accumulation of long chain acyl-carnitine, an intermediate of FFA metabolism, in cardiac tissue [16]. Accumulation of FFA, acyl-CoA and acyl-carnitine also represents a characteristic event in ischemic and posts ischemic myocardium [15], when endothelial NOS activity is blunted [13], but does not occur after ischemic preconditioning [36], when endothelial NO production is typically enhanced [37]. Although the role of NO in posts ischemic myocardium remains controversial [38], the impairment of FFA oxidation observed during the postreperfusion period, but not after preconditioning, can be at least partially explained by the present data. On the other hand, prevalent glucose utilization could be advantageous, since less oxygen is required to obtain the same amount of ATP from carbohydrates [39]. The oxygen saving due to switch in substrate from palmitate to carbohydrate, in isolated hearts, is about 13% [40] and the increase in cardiac RQ that we found in our study suggests that, after NLA, MVO would theoretically be higher if the switch to carbohydrate utilization did not occur.

Several limitations of this study should be addressed. The first limitation regards the measured substrate. We did not measure pyruvate, acetate, amino acids and glycerol consumption, yet they represent a small percentage of total cardiac substrate [41]. The heart was studied as a black box: with the adopted methodology it is possible to determine the uptake but not the real fate of metabolites inside the cell. The biochemical mechanisms underlying the observed metabolic control of NO remain to be determined. We did not determine a dose–response of cardiac metabolism to NLA, so we cannot exclude that doses of NLA lower than 30 mg/kg could determine hemodynamic changes with no metabolic effects or vice versa. Finally, the calculation of RQ was based on CO2 content in whole blood, which was calculated using measures of pH, pCO2, hematocrit, total and reduced hemoglobin. However, partial values of RQ, which included only the CO2 directly measured in plasma by the blood gas analyzer, still increased significantly after NLA, indicating that the calculation of total CO2 did not generate misleading artefacts (data not shown).

In summary, this study suggests a control by NO on cardiac substrate utilization. We hypothesize that endogenous NO released in the heart functions as a paracrine factor that promotes FFA oxidation and inhibits carbohydrates oxidation. The main source of NO is likely the microvascular endothelium, which contains a much higher concentration of NOS compared to cardiomyocytes [10,42], although an important role of NOS activity in cardiomyocytes [10,43], cannot be excluded. The loss of NO, due to competitive inhibition of NOS, causes a switch from prevalent FFA to carbohydrate oxidation. This metabolic alteration is insulin-independent and can be rapidly reversed by exogenous NO, suggesting a novel pharmacological action of nitrovasodilators as regulators of myocardial metabolism in conditions of reduced availability of endogenous NO.

Acknowledgements

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