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In vivo quantification of endotoxin-induced nitric oxide production in pigs from $Na^{15}NO_3$-infusion

Borislav Šantak,[1] Peter Radermacher, Thomas Iber, Jens Adler, Ulrich Wachter, Damian Vassilev, Michael Georgieff & Josef Vogt

Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Universitätsklinik für Anästhesiologie, Klinikum der Universität, Ulm, Germany

1 In this investigation the NO production rate is quantified in the pig during normotensive endotoxin-induced shock with increased cardiac output and during subsequent treatment with the NO synthase inhibitor N^{ω}-monomethy-L-arginine (L-NMMA). NO production rate was derived from the plasma isotope-enrichment of ^{15}N-labelled nitrate ($^{15}NO_3^-$).

2 Three groups of animals (control, $n=5$; endotoxin, $n=6$; endotoxin + L-NMMA, $n=6$) were anaesthetized and instrumented for the measurement of systemic and pulmonary haemodynamics. Each animal received a primed-continuous infusion of stable, non-radioactively labelled $Na^{15}NO_3$ (bolus 30 mg, infusion rate 2.1 mg h^{-1}). Arterial blood samples were taken 5, 10, 15, 30, 60 and 90 min later and every 90 minutes until the end of the experiment.

3 Continuous i.v. infusion of endotoxin was incrementally adjusted until mean pulmonary artery pressure (PAP) reached 50 mmHg and subsequently titrated to keep mean PAP ≈ 35 mmHg. Hydroxyethylstarch was administered as required to maintain mean arterial pressure (MAP) > 60 mmHg. Six hours after the start of the endotoxin continuous i.v. L-NMMA (1 mg kg^{-1} h^{-1}) was administered to the endotoxin + L-NMMA group. Haemodynamic data were measured before as well as 9 h after the start of the endotoxin.

4 After conversion of NO_3^- to nitro-trimethoxybenzene and gas chromatography-mass spectrometry analysis the total NO_3^- pool, basal NO_3^- production rate and the increase per unit time in NO_3^- production rate were calculated from the time-course of the $^{15}NO_3^-$ plasma isotope-enrichment. A two compartment model was assumed for the NO_3^- kinetics, one being an active pool in which newly generated NO_3^- appears and from which it is eliminated, the other being an inactive volume of distribution in which only passive exchange takes place with the active compartment.

5 Although MAP did not change during endotoxin infusion alone, cardiac output (CO) increased by 42 ± 40% ($P<0.05$ versus baseline) by the end of the experiment due to a significant ($P<0.05$ versus baseline) fall in systemic vascular resistance (SVR) to 65 ± 25% of the baseline value. L-NMMA given with endotoxin did not change MAP, and both CO and SVR were maintained close to the pre-shock levels.

6 Baseline plasma NO_3^- concentrations were 43 ± 13 and 40 ± 10 μmol l^{-1} in the control and endotoxin animals, respectively, and did not differ at the end of the experiment (39 ± 8 and 44 ± 15 μmol l^{-1}, respectively). The mean NO_3^- pool and basal NO_3^- production rate were 1155 ± 294 μmol and 140 ± 32 μmol h^{-1}, respectively, without any intergroup difference. Endotoxin significantly increased NO_3^- production rate (23 ± 10 μmol h^{-2}, $P<0.05$ versus control (6 ± 7 μmol h^{-2}) and endotoxin + L-NMMA groups). L-NMMA given with endotoxin (-1 ± 2 μmol h^{-2}, $P<0.05$ versus control and endotoxin groups) had no effect.

7 Analysis of the time course of the $^{15}NO_3^-$ plasma isotope enrichment during primed-continuous infusion of $Na^{15}NO_3$ allowed us to quantify the endotoxin-induced increase in NO_3^- production rate independently of total NO_3^- plasma concentrations. Low-dose L-NMMA blunted the increase in NO_3^- production rate while maintaining basal NO_3^- formation.

Keywords: Endotoxin; septic shock; nitric oxide production rate; nitrate production rate; stable isotope infusion; $^{15}NO_3^-$ isotope enrichment; N^{ω}-monomethy-L-arginine

Introduction

There is experimental evidence that endotoxin-induced overproduction of nitric oxide (NO) may be the 'final mediator' (Booke *et al.*, 1996) of systemic vasodilatation associated with septic shock (Rees, 1995). Therefore the effects of treatment with inhibitors of NO synthase is currently under investigation (Kilbourn *et al.*, 1990; Thiemermann *et al.*, 1995). Since NO has both protective and deleterious properties (Wright *et al.*, 1992) assessing the NO production rate may be important: In fact, while early inhibition of NO synthesis caused intestinal vascular leakage and increased mortality (Laszlo *et al.*, 1994) inhibition several hours after induction of NO overproduction attenuated vascular injury (Laszlo *et al.*, 1994) and improved survival (Nava *et al.*, 1992).

Several methods are available to estimate the release of NO (Archer, 1993; Marzinzig *et al.*, 1997). Among these the determination of plasma concentrations of the stable NO metabolites, i.e. nitrite (NO_2^-) and nitrate (NO_3^-), are the most commonly used methods in man and in intact animals. However, plasma NO_3^-, does not reliably reflect NO production: haemodynamic alterations following administration of endotoxin caused little or no effect on arterial (Preiser *et al.*, 1994;

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[1] Author for correspondence at: Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Universitätsklinik für Anästhesiologie, Klinikum der Universität, Parkstrasse 11, D-89073 Ulm (Donau), F.R.G.

Klemm *et al.*, 1995) or mixed-venous (Hussain *et al.*, 1997) NO_3^- concentrations. Hence, simple measures of plasma NO_3^-, may lead to substantial errors of estimated NO formation, in particular in disease states where renal function and/or extracellular volume are altered (Zeballos *et al.*, 1995).

The present study was performed to quantify the change in NO production rate in the pig during a normotensive endotoxin-induced shock with increased cardiac output as well as during subsequent NO synthase inhibition with N^{ω}-monomethy-L-arginine (L-NMMA). NO production rate was derived from the plasma isotope-enrichment of stable, non-radioactively ^{15}N-labelled nitrate ($^{15}NO_3^-$) during primed-continuous infusion of $Na^{15}NO_3$. We hypothesized that in the shock state the time-dependent decay curve of the plasma isotope-enrichment would be shifted downwards due to 'dilution' of the continuously infused $^{15}NO_3^-$ by the increased endogenous NO_3^- formation.

Methods

Animal preparation

The study protocol was approved by the University Animal Care Committee as well as the federal authorities for animal research of the Regierungspräsidium Tübingen (Baden-Württemberg, F.R.G.). Seventeen domestic pigs (40 ± 2 kg) were fasted for 24 h with water *ad libitum*. The animals were anaesthetized with intramuscular atropine (2.5 mg; Atropinsulfat, Braun, Melsungen, F.R.G.) and azaperone (100 mg; Stresnil, Janssen, Neuss, F.R.G.) followed by cannulation of an ear vein and i.v. administration of sodium pentobarbitone (10 mg kg^{-1}; Nembutal, Sanofi Wintrop, Munich) and ketamine (2.5 mg kg^{-1}; Ketavet, Parke-Davis, Berlin). The pigs were orally intubated, and their lungs were mechanically ventilated (FiO_2 0.4; PEEP 3 cmH_2O; Servo 900B, Siemens, Erlangen, F.R.G.) with a tidal volume of 15 ml kg^{-1} at a respiratory rate of 8–10 breaths min^{-1} adjusted to maintain Pa_{CO_2} between 35–40 mmHg. During the surgical preparation the inspired gas mixture consisted of N_2O and O_2, during the observation period of air and O_2. Anaesthesia was maintained by continuous administration of pentobarbitone (300 mg h^{-1}, i.v.), and depth of anaesthesia was controlled by continuous EEG monitoring (Neurotrac, Interspec Inc., Cronshohocken, PA). The spectral edge frequency was always below 15 Hz, the median power frequency was 5–10 Hz. In previous experiments this pentobarbitone infusion rate allowed full anaesthesia to be maintained without any additional muscle relaxation (Stein *et al.*, 1990). Buprenorphine (0.3 mg; Temgesic, Boehringer, Mannheim, F.R.G.) was added (i.v.) every 4 h and prior to any surgical or noxious stimuli in order to prevent a rise in heart rate and arterial pressure due to inadequate anaesthesia. Muscle paralysis was obtained with alcuronium (20 mg h^{-1}; Alloferin, Hoffmann-La Roche AG, Basel, Switzerland). The right and left jugular veins as well as the right and left femoral arteries were surgically exposed. A central venous catheter for drug, isotope and fluid infusion was inserted into the superior V. cava, a balloon-tipped thermodilution pulmonary artery catheter (93A 754 7F, Baxter Healthcare, Irvine, CA) was placed for the measurement of central venous, pulmonary artery (PAP) and pulmonary artery occluded pressure (Medex MX 80 pressure transducers, Medex Inc., Hillard, OH). Cardiac output (CO) was determined by thermodilution (66S Monitor, Hewlett Packard, Palo Alto, CA), the data present being the mean of 4–5 injections of 10 ml ice-cold saline randomly spread over the respiratory cycle. Systemic vascular resistance (SVR) was calculated by use of the standard formula. Arterial catheters were placed in the femoral artery for continuous blood pressure recording and blood sampling. Ringer lactate solution (10 ml kg^{-1} h^{-1}) was infused i.v. as a maintenance fluid. Ascites formation was assessed by measuring the fluid loss via an abdominal drainage tube.

Nitrate isotope-enrichment measurements

As soon as steady-state conditions for blood flow and vascular pressures had been obtained after the stabilization phase, stable, non-radioactively labelled $Na^{15}NO_3$ was given as an i.v. bolus (30 mg) followed by continuous infusion (2.1 mg h^{-1}). Arterial blood samples were drawn 5, 10, 15, 30, 60 and 90 min after the bolus and subsequently every 90 min until the end of the experiment. After centrifugation 250 μl of plasma were deproteinized with acetonitril, and the plasma NO_3^- was converted with 1,3,5-trimethoxybenzene to 1-nitro-2,4,6-trimethoxybenzene (Tesch *et al.*, 1976; Green *et al.*, 1982). In order to avoid interference with non-nitrate sources in plasma resulting in underestimation of $Na^{15}NO_3$ isotope enrichment (Rhodes *et al.*, 1995), the Tesch nitrate assay was modified using silver sulfate instead of hydrogen peroxide for removal of chloride ions. After gas chromatography separation, the $^{15}NO_3^-$ plasma isotope-enrichment defined as the ratio $[^{15}NO_3^-]/[^{14}NO_3^-]$ was assessed by mass spectrometry in the chemical ionization, selected ion-monitoring mode by comparing the abundance of unlabelled (mass 214) and labelled NO_3^- (mass 215) with reference to standard curves derived from solutions with known $[^{15}NO_3^-]/[^{14}NO_3^-]$ ratios. In four animals of the control and five of the endotoxin group, plasma NO_3^- levels were measured by using an ion exchange liquid chromatography system equipped with a conductivity detector (Everett *et al.*, 1995).

Mathematical procedure

The basal NO_3^- production rate as well as the change of NO_3^- production rate per time were calculated from the time course of the $^{15}NO_3^-$ plasma isotope-enrichment based on the conjecture that (1) NO is the main source of NO_3^- in the plasma (Nathorst Westfeld *et al.*, 1995; Rhodes *et al.*, 1995), and (2) NO is rapidly converted into nitrite (Ignarro *et al.*, 1993; Rhodes *et al.*, 1995) which is then completely oxidized to NO_3^- within a short time (95 % in <1 h) (Wennmalm *et al.*, 1992; Moshage *et al.*, 1995). A two compartment model was assumed for the NO_3^- kinetics, one compartment being an active pool where newly generated NO_3^- appears and from which it is eliminated, the other being an inactive volume of distribution where only passive exchange takes place with the active compartment (Figure 1). It was assumed that (1) there is a constant baseline NO_3^- production rate prior to the induction of sepsis, (2) the induction of sepsis results in a linear increase in NO_3^- production rate per unit time, and (3) for any

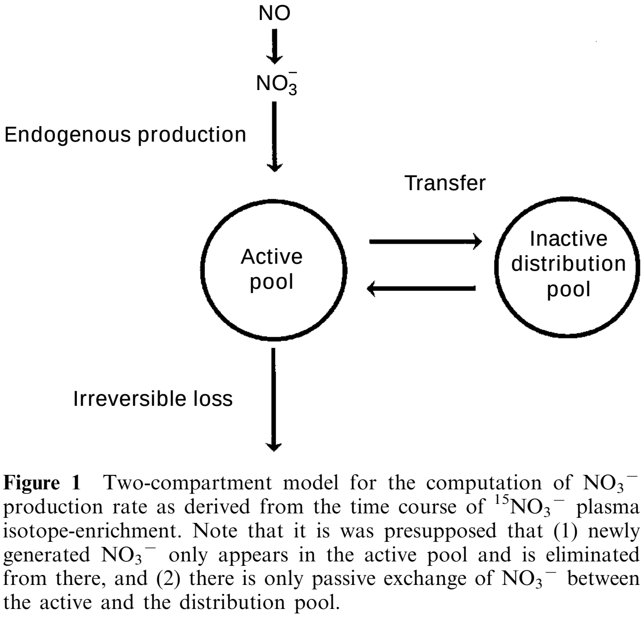

Figure 1 Two-compartment model for the computation of NO_3^- production rate as derived from the time course of $^{15}NO_3^-$ plasma isotope-enrichment. Note that it is was presupposed that (1) newly generated NO_3^- only appears in the active pool and is eliminated from there, and (2) there is only passive exchange of NO_3^- between the active and the distribution pool.

given time point the NO_3^- production rate equals the elimination rate, in other words, the NO_3^- pool size of the active compartment is constant. Under these conditions time-dependent changes in the isotope-enrichments R for the two compartments can be described according to the following equations:

$$\frac{dR_1}{dt} = \frac{1}{Q_1} \cdot (T(NO_3^-) \cdot (R_2 - R_1) - P(NO_3^-) \cdot R_1 + p(^{15}NO_3^-)) \qquad (1)$$

$$\frac{dR_2}{dt} = \frac{1}{Q_2} \cdot T(NO_3^-) \cdot (R_1 - R_2) \qquad (2)$$

where $T(NO_3^-)$ is the NO_3^- transfer rate between the two compartments, $P(NO_3^-)$ the time-dependent endogenous NO_3^- production rate, $p(^{15}NO_3^-)$ the infusion rate of the $^{15}NO_3^-$, R_2 and R_1 the $^{15}NO_3^-$ plasma isotope enrichments, and Q_1 and Q_2 the NO_3^- pools of the respective compartment. A plasma isotope decay curve was generated by numerical integration of equations (1) and (2) and fitted to the measured enrichment values with a non-linear regression based on the least squares-method. From these decay curves the total NO_3^- pool as well as the basal production rate and the endotoxin-induced per-time-increase in production rate were calculated. It was assumed that this linear increase of the NO_3^- production rate began about 4 h after the start of the endotoxin infusion and the total NO_3^- production rate at the end of the experiment was then computed as the sum of the baseline NO_3^--production rate plus the excess change of the NO_3^--production multiplied by the time interval of 5 h. Typical time courses of the $^{15}NO_3^-$ plasma isotope-enrichment are presented in Figure 2.

Protocol

The animals were randomly assigned to three groups: control ($n=5$), endotoxin ($n=6$) and endotoxin + L-NMMA ($n=6$). After the preparation the animals were allowed to recover from the surgical trauma for at least 2 h. Under steady-state haemodynamic conditions, i.e. when stable CO and constant vascular pressures had been achieved, the $Na^{15}NO_3$ infusion was started as described above. After another 90 min either an endotoxin (*E. coli* lipopolysaccharide B 0111:B4, DIFCO Laboratories, Detroit, MC; 20 mg l^{-1} in 5% saline) (1 ml h^{-1}), or saline infusion was started. The endotoxin infusion rate was incrementally increased until mean PAP reached 50 mmHg and then subsequently adjusted to result in moderate pulmonary hypertension with mean PAP ≈35 mmHg. Hydroxyethylstarch (HAES-steril 6%, Fresenius, Bad Homburg, F.R.G.) was administered as required to maintain mean arterial pressure (MAP) >60 mmHg. In the endotoxin + L-NMMA group, L-NMMA (546C88, 5 mg l^{-1}, Glaxo Wellcome, Beckenham, UK) was administered as a continuous infusion (1 mg kg^{-1} h^{-1}) 6 h after the start of the endotoxin infusion. Systemic and pulmonary haemodynamics were measured before as well as 9 h after the start of the endotoxin or saline infusion, respectively. After the second set of data had been obtained the animals were killed by KCl injection.

Statistical analysis

All haemodynamic and NO_3^- data are presented as mean ± s.d., graphic presentation of haemodynamics is mean ± s.e.mean. Because of substantial interindividual differences within the three experimental groups present before any experimental intervention the results for CO and SVR are presented as % of baseline levels. Differences between the groups were analysed by use of a rank-sign analysis for unpaired samples. Time-dependent differences within the groups were tested by using a rank-sign test for paired variables. A P value <0.05 was considered significant.

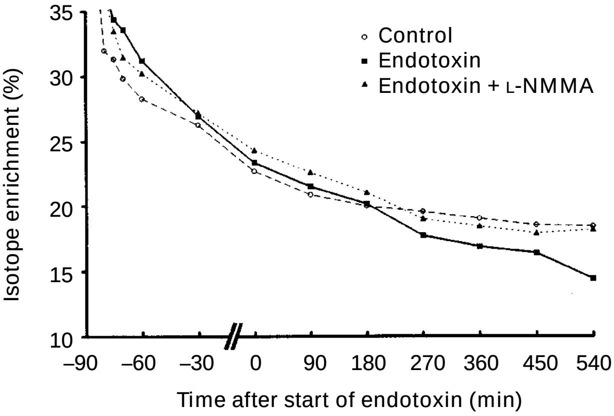

Figure 2 Typical time course of the $^{15}NO_3^-$ plasma isotope enrichment defined as the ratio $[^{15}NO_3^-]/[^{14}NO_3^-]$ in the control, the endotoxin + L-NMMA and the endotoxin group. Note that while the two former decay curves were virtually identical the latter deviated downwards 4 h after the start of the endotoxin infusion. This 'dilution' of the continuously infused $^{15}NO_3^-$ demonstrates an increased endogenous NO_3^- production rate.

Results

Figure 3a–c summarizes the haemodynamic data in the three experimental groups before endotoxin administration and at the end of the experiment. As intended by the protocol, volume infusion resulted in unchanged MAP in the three experimental groups (Figure 3a). Endotoxin administration resulted in a 42 ± 40% ($P<0.05$ versus baseline) increase in CO by the end of the experiment (Figure 3b) due to a fall ($P<0.05$ versus baseline) of SVR to 65 ± 25% of the pre-shock level (Figure 3c). Infusion of L-NMMA did not change MAP and maintained CO and SVR close to the values before endotoxin challenge (Figure 2). The ascites formation was higher in the endotoxin group than in both the endotoxin + L-NMMA and the control group (2540 ± 180 versus 2120 ± 210 and 260 ± 30 ml, respectively, $P<0.05$).

Baseline NO_3^- production rates were 153 ± 36, 138 ± 29 and 131 ± 33 μmol h^{-1} in the three groups, respectively, without any difference between the groups (Figure 4a). The mean basal NO_3^- production rate of the three experimental groups taken together was 140 ± 32 μmol h^{-1} corresponding to a range of 2.3–4.3 μmol kg^{-1} h^{-1}. The total NO_3^- pools were 1191 ± 182, 1200 ± 479 and 1087 ± 200 μmol, respectively, and there was again no intergroup difference (Figure 4b). Endotoxin caused a rise ($P<0.05$ versus control and endotoxin + L-NMMA group) in NO_3^- production rate of 23 ± 10 μmol h^{-1} when compared to the control animals (6 ± 7 μmol h^{-2}) (Figure 4c). Subsequent infusion of L-NMMA completely blunted this rise in NO_3^- production rate (−1 ± 2 mol h^{-2}), and the difference was statistically significant ($P<0.05$) when compared to the two other groups (Figure 2c). The excess NO_3^- production resulted in a higher total NO_3^- production rate at the end of the experiment, i.e. 9 h after induction of sepsis, in the endotoxin animals (245 ± 54 μmol h^{-1}) when compared to the control (181 ± 38 μmol h^{-1}) and the endotoxin + L-NMMA group (126 ± 25 μmol h^{-1}) ($P<0.05$ versus control and endoxotin + L-NMMA).

Baseline plasma NO_3^- concentrations were 43 ± 13 and 40 ± 10 μmol l^{-1} in the control and endotoxin animals, respectively, and did not differ at the end of the experiment (39 ± 8 and 44 ± 15 μmol l^{-1}, respectively). NO_3^- concentrations in the ascites fluid were the same as in the plasma.

Discussion

This study was performed to quantify the NO formation rate in the pig during endotoxin-induced shock, in which appropriate colloid infusion had allowed us to obtain normotension and increased cardiac output, as well as during subsequent NO inhibition with L-NMMA. NO production rate was derived from the time course of the plasma isotope-enrichment of stable, non-radioactively labelled $^{15}NO_3^-$ during primed-continuous infusion of $Na^{15}NO_3$. This approach allowed us to avoid the potential drawbacks of simple measures of NO_3^- blood levels and thereby to estimate NO formation independently of total plasma NO_3^- concentrations

Our method of estimating NO production is based on the assumptions that (1) NO is the only source of plasma NO_2^- and NO_3^- except for nutrition-induced variations, and that (2) NO is rapidly converted to NO_2^- and, subsequently, NO_3^-.

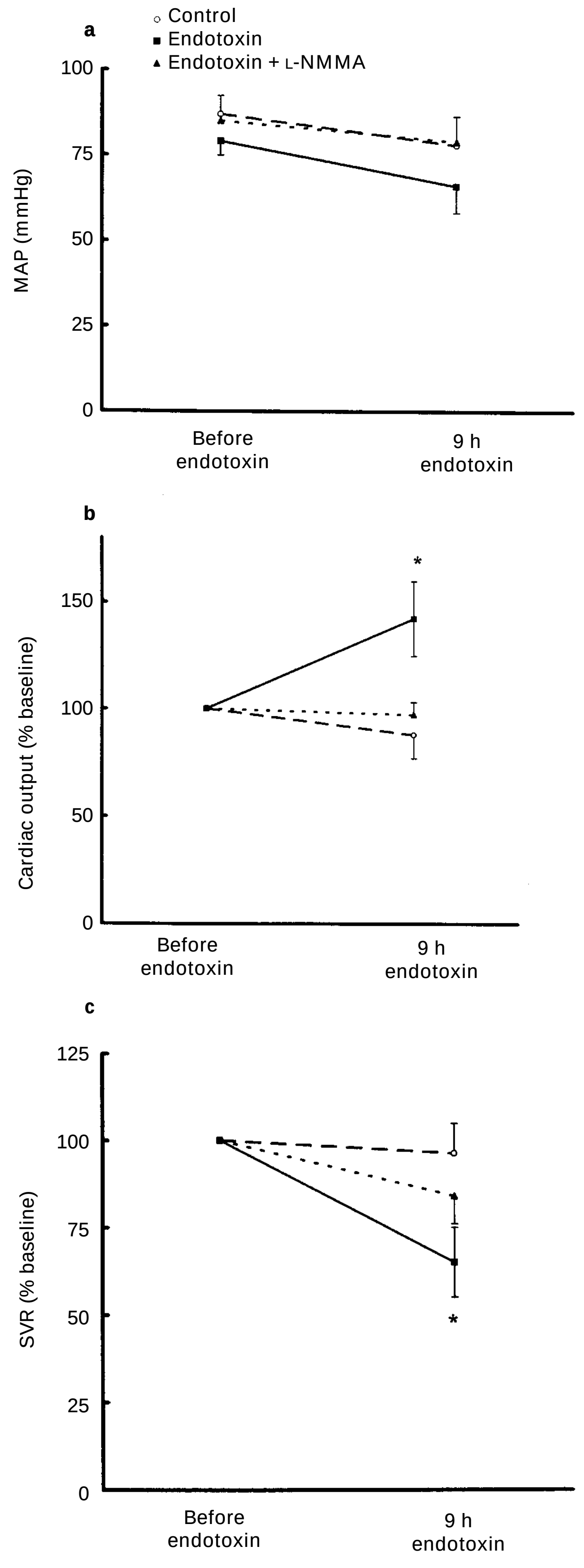

Figure 3 (a) Mean arterial pressure (MAP), (b) cardiac output (CO) and (c) systemic vascular resistance (SVR) before and at the end of the experiment in the control, ($n=5$), the endotoxin, ($n=6$), and the endotoxin + L-NMMA group, ($n=6$). Data are means and vertical lines show s.e.mean. * Represent significant ($P<0.05$) increase (CO) and decrease (SVR), respectively, versus baseline levels.

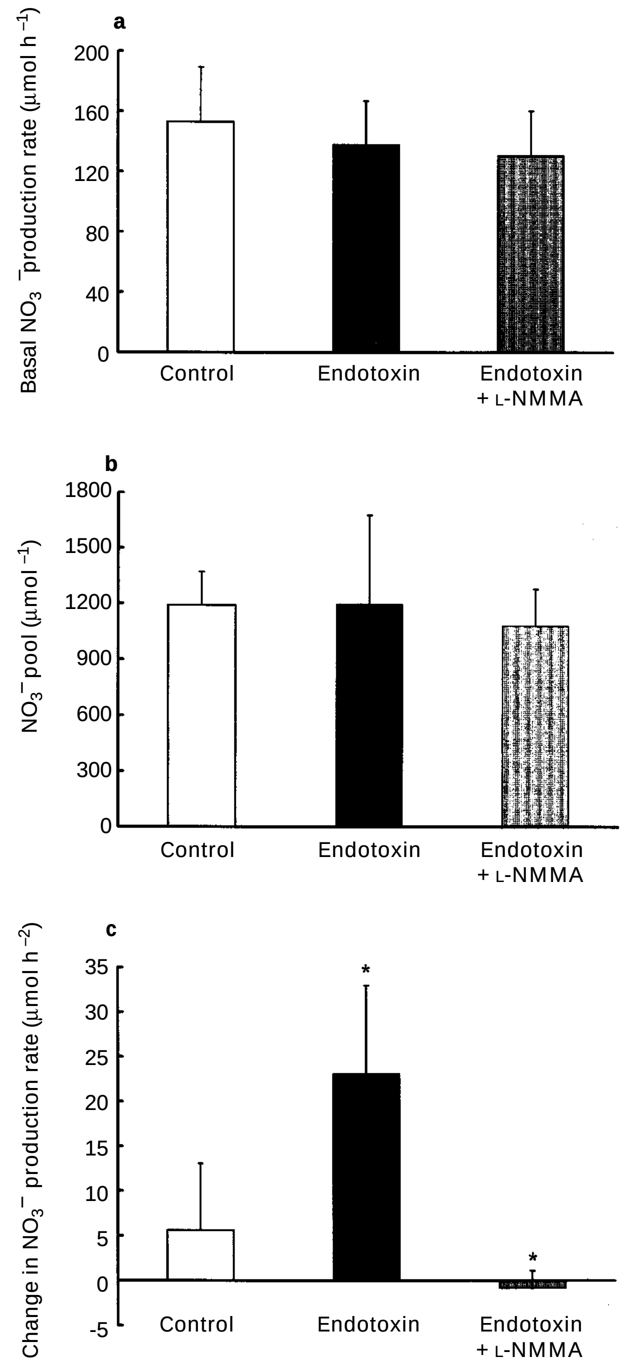

Figure 4 Baseline NO_3^- production rate (a), total NO_3^- pool (b) and per unit time-increase in NO_3^- production rate (c) in the control ($n=5$), the endotoxin ($n=6$), and the endotoxin + L-NMMA group ($n=6$). Data are mean ± s.d. * Represents significant ($P<0.05$) increase versus control and endotoxin + L–NMMA group.

The former has been confirmed by Rhodes *et al.* (1995) who showed that indeed 90% of the circulating NO_3^- are derived from the L-arginine : NO pathway. Nutrition-dependent variations in NO_3^- formation were excluded in our animals since they had received a standard low fibre diet for at least 48 h prior to the experiment. However, it could be argued that at least in the pig the kinetic of NO_3^- formation is not sensitive enough to indicate NO production (Klemm *et al.*, 1995) since NO_2^- is the predominant metabolite of NO (Ignarro *et al.*, 1993; Klemm *et al.*, 1995). In fact, plasma NO_2^- and NO_3^- concentrations do not always correlate (Klemm *et al.*, 1995; Moshage *et al.*, 1995; Marzinzig *et al.*, 1997), and in man NO_2^- levels may vary from 4–88 % of total plasma NO_2^- plus NO_3^- (Moshage *et al.*, 1995). However, it has to be noted that in the presence of oxygenated blood, NO_2^- is completely oxidized to NO_3^- within a short time (Ignarro *et al.*, 1993; Moshage *et al.*, 1995). Since we took blood samples every 90 minutes after the determination of the initial post-bolus isotope decay curve we think that the assumptions mentioned above were indeed valid for our approach.

We used the stable-isotope approach to estimate the NO_3^- kinetics in order to be independent of total plasma NO_3^- concentrations and thereby to avoid the drawbacks of simple measures of NO_3^- blood levels. In fact, the determination of the plasma NO_3^- concentrations alone would have been meaningless, since there were neither differences betwen the levels before starting the endotoxin infusion and at the end of the experiment nor between the control and endotoxin groups. These findings are in accordance with previous results by other authors in endotoxic pigs (Dimmeler *et al.*, 1995; Klemm *et al.*, 1995); haemodynamic alterations similar to those in our animals were observed without any change in plasma NO_3^- and NO_3^- levels. It is noteworthy that the plasma NO_3^- concentrations remained unchanged in the endotoxic animals although the NO_3^- production rate had doubled by the end of the experiment. This apparent contradiction is probably due to a substantial change in the total volume of distribution for NO_3^-: in the endotoxin animals *post-mortem* inspection revealed severe widespread interstitial edema, and the ascites formation had been approximately ten fold higher. Assuming that NO_3^-, being a small anion, distributes into the total body water (Wennmalm *et al.*, 1993) a considerable amount was probably lost into a 'third space' (ascites, interstitial space) without substantial NO_3^- backflow, and therefore plasma concentrations did not change.This reasoning is underscored by the observation that the volume of distribution for NO_3^- of 27.5 l as calculated from the plasma concentrations and the NO_3^- pool at the beginning of the experiment is nearly exactly that of the total body water content of 28.8 l as predicted from age and body weight (Setiabudi *et al.*, 1975).

The basal NO_3^- production rate in our experiments was three to five times higher than that of healthy human volunteers as derived from total urinary NO_3^- excretion (Green *et al.*, 1981; Tsikas *et al.*, 1994) or from conversion of ^{15}N-labelled arginine to [^{15}N]citrulline or $^{15}NO_3^-$ (Castillo *et al.*, 1995, 1996). However, it closely approximates to the total, i.e. urinary plus faecal, NO_3^- excretion in pigs described by Eggum *et al.* (1982) accounting for 2.5–3.4 μmol kg^{-1} h^{-1} depending on the fibre and microflora content of the diet. Thus although determined by using completely different methodological approaches there was a good agreement between the findings of Eggum *et al.* and our results for basal NO_3^- production rate in pigs .

We found a linear increase in NO_3^- production rate per unit time in both the endotoxin and the control animals. In the endotoxin group this increase was about four times as great as in the control group resulting in a nearly doubled NO_3^- production rate by the end of the experiment. This considerable increase in NO_3^- production rate confirms other studies in various species that endotoxin administered i.v. either as a bolus (Thiemermann *et al.*, 1995) or as a continuous infusion (Gardiner *et al.*, 1995) caused a several fold increase in NO_2^- or NO_3^- formation. We can only speculate as to why there also was a linear increase in NO_3^- production rate in the control animals. It is possible, however, that the surgical trauma associated with the placement of vascular catheters and the ascites drain may have caused this effect: in postoperative patients without sepsis or septic shock, serum NO_2^- and NO_3^- levels had been twice as high as in healthy controls (Evans *et al.*, 1993).

Surprisingly, infusing L-NMMA did not induce a decrease in the NO_3^- production rate per unit time, or in other words, basal NO_3^- formation was maintained throughout the experiment. This finding is of particular interest since L-NMMA, which is a non-selective inhibitor of both the inducible and the endothelial isoform (Gross *et al.*, 1990), has been shown to cause not only restoration of haemodynamics but also excessive vasoconstriction with organ ischaemia and damage (Wright *et al.*, 1992; Thiemermann, 1994). Our results suggest that at least in a well volume-infused shock model NO synthase inhibition with low doses of L-NMMA, such as in the present study (1 mg kg^{-1} h^{-1}), may restore systemic haemodynamics due to inhibition of excess NO formation without affecting the basal endothelial NO production (Nava *et al.*, 1992).

In summary, we have quantified the NO_3^- production rate in the pig during an endotoxin-induced shock as well as during subsequent NO synthase inhibition with L-NMMA. NO_3^- production rate was derived from the time course of $^{15}NO_3^-$ plasma isotope-enrichment during primed-continuous infusion of $Na^{15}NO_3$. This approach enabled us to be independent of total plasma NO_3^- concentrations and thereby to avoid the drawbacks of simple measures of plasma NO_3^- levels. With MAP kept constant by appropriate colloid infusion endotoxaemia resulted in increased CO due to decreased SVR. L-NMMA given together with endotoxin did not alter MAP, but maintained CO and SVR close to pre-shock levels. Endotoxin caused a linear increase in NO_3^- production rate per unit time which had nearly doubled at the end of the experiment. L-NMMA completely blunted this excess NO_3^- formation without any further effect on total NO_3^- production rate.

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