Inhibition of hepatic gluconeogenesis by nitric oxide: a comparison with endotoxic shock

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Isolated hepatocytes incubated in the presence of the NO donors *S*-nitroso-*N*-acetylpenicillamine (SNAP) and 3-morpholinosydnonimine (SIN-1) displayed a time- and dose-dependent inhibition of glucose synthesis from lactate plus pyruvate as the substrate which correlated with NO production, but not nitrite production. Neither the parent compound of SNAP, *N*-acetyl-DL-penicillamine (NAP), nor nitrite or nitrate had any significant effect on glucose output, indicating that the inhibition was due to the generation of NO within the incubation medium. The concentrations of NO required for this effect (< 800 nM) are within the range reported to occur in intact tissues and *in vivo*. The magnitude of the inhibitory effect of SNAP (~ 50 %) was

comparable with that of endotoxin treatment of the rat with lactate plus pyruvate as the substrate. When the effect of SNAP on glucose synthesis and lactate plus pyruvate synthesis from a number of different substrates was examined, this showed a pattern comparable with that observed after endotoxin treatment of the rat, suggesting that NO may be the inhibitory mediator of the effects of bacterial endotoxin on hepatic gluconeogenesis. The NO donor had no effect on the flux through 6-phosphofructo-1-kinase, supporting the concept that the primary site of inhibition of gluconeogenesis by both NO and endotoxin resides at the level of phosphoenolpyruvate formation.

INTRODUCTION

Gram-negative bacterial infection or treatment of animals with bacterial endotoxin is characterized by profound alterations in glucose homeostasis, typically an initial transient hyperglycaemia followed by a prolonged and frequently fatal hypoglycaemic phase [1–9]. During the initial phase there is a pronounced mobilization of hepatic glycogen [10–12] and increased flux through 6-phosphofructo-1-kinase [8,13], whereas in the latter phase hepatic glucose synthesis from lactate, pyruvate and a number of other substrates is decreased [1–9]. We have suggested that the inhibition of gluconeogenesis results primarily from a lowered substrate flux through phosphoenolpyruvate carboxykinase, with the increase in 6-phosphofructo-1-kinase activity being of secondary importance [8,9]. Evidence suggests that the effects of endotoxin on carbohydrate metabolism are not the result of a direct effect of the lipopolysaccharide at the level of the hepatic parenchymal cell [1,5], but the result of an interaction of the bacterial endotoxin with the Kupffer cells in the liver [10–13]. The hyperglycaemic phase has been attributed to the release of prostaglandins by the Kupffer cells [10–12], although the rapid stimulation of 6-phosphofructo-1-kinase has been proposed to be independent of any changes in either prostaglandins or cytokines [13].

The mechanism underlying the inhibition of gluconeogenesis remains to be established [13]; however, recent studies into the mechanisms of hepatocellular dysfunction during sepsis suggest that the longer-term responses involve the release of cytokines from the Kupffer cells which interact with the hepatocytes to induce NO synthase with concomitant NO production [14–17]. The time of induction of the Ca²⁺-independent NO synthase within the liver parenchymal cells both *in vivo* and *in vitro* correlates with the inhibition of glucose synthesis [16,18] and is

antagonized by co-administration to the intact animal of dexamethasone or cortisol [19], hormones known to be able to alleviate the effects of the endotoxin on glucose metabolism. The aim of this study was to investigate whether NO inhibits glucose synthesis in isolated hepatocytes by the use of artificial NO donors and whether the effects are comparable with those of bacterial endotoxin on hepatic carbohydrate metabolism.

EXPERIMENTAL

Materials

N-Acetyl-DL-penicillamine (NAP) and enzymes were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Collagenase (type IV) was obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A. *S*-Nitroso-*N*-acetylpenicillamine (SNAP) and *S*-nitroso-glutathione (SNOG) were given by the Wellcome Research Laboratories, Beckenham, Kent. 4-Morpholinosydnonimine (SIN-1) was from Cassella A.G., Frankfurt, Germany. All other chemicals were of AnalaR grade or similar from BDH Chemicals, Poole, Dorset, U.K., or Sigma.

Preparation and incubation of hepatocytes

Male Sprague–Dawley rats (180–220 g) were used for all experiments. In experiments to investigate the effect of endotoxin treatment, the animals were injected with endotoxin (4 mg of trichloroacetic acid-extracted lipopolysaccharide from *Salmonella typhimurium*/kg body wt.) 18 h before the preparation of the hepatocytes as described in [7]. The concentration of endotoxin used in this study was carefully chosen to control the fall in plasma glucose concentration, and thus the variability in the degree of severity of the treatment, while still retaining a persistent inhibition of gluconeogenesis and elevated plasma lactate and

Abbreviations used: NAP, *N*-acetyl-DL-penicillamine; SNAP, *S*-nitroso-*N*-acetylpenicillamine; SIN-1, 3-morpholinosydnonimine; SNOG, *S*-nitroso-glutathione.

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urea values. Plasma urea and plasma lactate levels were significantly raised in the endotoxin-treated animals, the concentrations being 4.22 ± 0.13 (8) and 12.81 ± 1.34 (7) mM ($P < 0.001$) for the urea and 0.95 ± 0.06 (8) and 3.62 ± 0.55 (8) mM ($P < 0.01$) for the lactate levels for control and endotoxin-treated animals respectively. Plasma glucose levels were 4.3 ± 0.2 (11) and 4.0 ± 0.3 (10) mM. All animals were starved overnight and the hepatocytes prepared by collagenase digestion as described previously [7]. The cells were resuspended in Krebs–Ringer buffer [20] containing 0.5% (w/v) defatted BSA (final cell concentration 20 mg wet wt./ml) and diluted with an equal volume of Krebs–Ringer buffer containing the appropriate additions. The resulting cell suspension was incubated in 125 ml plastic Erlenmeyer flasks at 37 °C under an atmosphere of O_2/CO_2 (19:1) for the times shown. SNAP, NAP, SNOG and SIN-1 were dissolved in buffer immediately before addition of the cells. NO production was measured continuously in incubations by using an ISO-NO meter and sensor from World Precision Instruments Inc. (Stevenage, Herts., U.K.). Nitrite production was measured at the times indicated by removing 0.1 ml of cells and immediately adding to 0.1 ml of Griess reagent, both to stabilize the NO donors and to develop the coloured reagent [21]. The samples were centrifuged at 11 600 *g* for 30 s and the A_{540} of the supernatant was rapidly measured on an Anthos 2001 e.l.i.s.a. plate reader. Plasma nitrite levels were measured with the Griess reagent immediately after separation of the plasma. Cellular ATP levels and plasma urea concentrations were measured as described previously [7]. 6-Phosphofructo-1-kinase flux was measured by the release of 3H_2O from D-[3- 3H]glucose as in [7]. D-Glucose was measured fluorimetrically by using hexokinase [22] or by the glucose oxidase method as in [7]. NO synthase activity was measured in intact livers obtained from control and endotoxin-treated rats by the haemoglobin assay as described in [18]. Plasma and cellular lactate concentrations were measured fluorimetrically in neutralized/deproteinized extracts by using lactate dehydrogenase [23].

Results are expressed as means $\pm$ S.E.M. with the numbers of different cell preparations given in parentheses. Statistical analyses of results was carried out with a pooled *t* test for experiments comparing the effects of endotoxin treatment, or a paired *t* test when comparing the effects of NO donors.

RESULTS AND DISCUSSION

Effect of NO donors on gluconeogenesis, nitrite and NO production

Figure 1(a) shows the effect on glucose synthesis from 18 mM lactate plus 2 mM pyruvate of the time of incubation of hepatocytes with the NO donor SNAP (600 μ M). Inclusion of SNAP in the incubation medium resulted in a rapid inhibition of gluconeogenesis, the rate over the first 10 min being inhibited by 33%. This inhibition was maintained over the entire incubation period, and indeed became more pronounced at later time points. To ensure that the inhibition of glucose output was not due to a breakdown product of SNAP other than NO, the cells were also incubated with the parent compound of the NO donor, NAP, or the breakdown products of NO, nitrite and nitrate. Inclusion of NAP in the incubation medium at 600 μ M had no significant effect on glucose output at any time point measured, the rates between 20 and 40 min being 26.4 ± 2.6 , 18.8 ± 1.6 and 23.0 ± 1.7 nmol/20 min per mg wet wt. of cells ($n = 4$) for control cells and cells incubated in the presence of SNAP and NAP respectively. Similarly, incubation of hepatocytes in the presence

of either 600 μ M nitrite or 600 μ M nitrate also had no effect on glucose output, the rates of glucose synthesis being 17.7 ± 1.3 , 17.8 ± 1.7 , and 17.8 ± 1.4 ($n = 4$) nmol/20 min per mg wet wt. respectively for control cells and cells incubated in the presence of nitrite and nitrate respectively. To confirm that the inhibition was due to NO release, the experiment was repeated with SIN-1, as the NO-generating agent. SIN-1 produced a comparable degree of inhibition over the first 20 min of the incubation (glucose output being lowered by 39% over the first 10 min); however, the effect of SIN-1 was transient and the rate returned to that of the control between 20 and 40 min, indicating that the effect of the NO donor was reversible. In preliminary studies we have also demonstrated that 600 μ M SNOG is capable of inhibiting glucose output by parenchymal cells, the rates of glucose production being 23.2 ± 3.5 and 12.5 ± 1.4 ($n = 4$, $P < 0.05$) nmol/20 min per mg wet wt. for control and SNOG-treated cells respectively. This demonstrates that three different NO donors are all capable of inhibiting glucose synthesis and strengthens the argument for NO being the active mediator.

Figure 1(b) shows the correlation between glucose output and both nitrite formation and also NO production, the former frequently being used as an indicator of NO production. There was no detectable formation of nitrite or NO in the control cell incubations. Although both SNAP and SIN-1 increased nitrite formation, there was no correlation with the extent of the inhibition of gluconeogenesis by the two NO donors. Addition of

Figure 1 Effect of time of incubation of isolated hepatocytes with 600 μ M SNAP or 600 μ M SIN-1 on gluconeogenesis, nitrite and NO formation

Hepatocytes were incubated with vehicle (▲), 600 μ M SNAP (■) or 600 μ M SIN-1 (●) for the appropriate times and (a) glucose output from 18 mM lactate plus 2 mM pyruvate was determined, or (b) nitrite (black symbols) or NO (white symbols) was measured as described in the Experimental section. The results shown are means $\pm$ S.E.M. for 4 different cell preparations, except for the NO measurements, which show a representative experiment from one of four incubations: * $P < 0.05$ for differences between values obtained from control cells and cells incubated in the presence of the NO donors.

SNAP produced a relatively small but immediate rise in nitrite formation; however, there was only a slight further increase over the next 1 h of incubation. In contrast, SIN-1 produced a relatively constant rate of nitrite formation over 40 min, with a lowering of the rate only being apparent between 40 and 60 min. In contrast with the results obtained with nitrite, measurement of NO directly produced results correlating well with the inhibition of glucose output by the cells. Both SNAP and SIN-1 produced a sharp rise in NO concentration within the first few minutes after addition to the cells, reaching a peak at 4 min, followed by a subsequent decline over the next 1 h. The maximal concentration of NO produced by SNAP was 3.2-fold higher than that measured in the presence of SIN-1, and an elevated but constantly decreasing level was maintained throughout the incubation period. In contrast, NO production by SIN-1 was not detectable after 30 min, consistent with the lack of effect of SIN-1 on glucose output after this time. This was despite the continued increased formation of nitrite under the same conditions and suggests that the NO produced by SIN-1 is rapidly converted into peroxynitrite by interaction with the superoxide ions also produced during SIN-1 degradation, such that the steady-state concentration of NO was considerably less than that in incubations with SNAP [24].

Studies measuring the effectiveness of NO donors on the activation of guanylate cyclase have shown a similar distinction between the efficacy of SNAP and SIN-1 in producing NO [25]. The small amounts of nitrite produced by SNAP degradation compared with that of SIN-1 in the above experiments may reflect different proportions of nitrite and nitrate formed by the two NO donors under the conditions used. Attempts to measure nitrate by conversion into nitrite, followed by subsequent assay by the Griess reagent, were precluded by our inability to prevent further degradation of the NO donors during the conversion procedure; similarly, attempts to measure the two metabolites by h.p.l.c. were prevented by overlapping of the substrate, SNAP and nitrite peaks.

Figure 2 shows the effect of increasing concentrations of SNAP on glucose output and NO production. Glucose production was decreased over the entire concentration range used, although this was only significant above 100 μ M SNAP. NAP had no significant effect at any of the concentrations used up to 900 μ M (results not shown). The formation of NO showed a similar dose-response, suggesting an inverse ratio between NO production and gluconeogenesis. The fall in glucose output was not accompanied by a significant decrease in the cellular ATP content, the mean values being 2.95 ± 0.37 (8), 2.86 ± 0.52 (4), 2.84 ± 0.46 (4), 2.96 ± 0.28 (8), 2.62 ± 0.36 (8) and 2.90 ± 0.48 (4) nmol/mg wet wt. for cells treated with 0, 50, 100, 300, 600 and 900 μ M SNAP respectively at the 40 min time point, suggesting that the effect was not simply due to a decreased cell viability. Except for incubations containing less than 100 μ M SNAP, the inhibition of gluconeogenesis was maintained over a second hour of incubation. However, at the higher doses of SNAP there was a significant fall in ATP after 2 h, suggesting some cell damage following prolonged exposure to NO (results not shown).

Comparison of the effects of SNAP and endotoxin treatment of the rat on gluconeogenesis from different substrates

Studies into the mechanism of the inhibition of hepatic protein synthesis by endotoxin have clearly indicated the cytokine-induced formation of NO as the mediator, and this response can be mimicked by NO donors [14–17]. The concentrations of SNAP required to inhibit glucose synthesis were comparable

Figure 2 Effect of SNAP concentration on hepatic gluconeogenesis and NO concentrations

Hepatocytes were incubated with 18 mM lactate plus 2 mM pyruvate with the indicated concentrations of SNAP, and glucose output (■) was measured between 20 and 40 min. The peak NO production (●) was measured in a separate incubation as described in the Experimental section. The results shown are means $\pm$ S.E.M. for 4–8 different cell preparations for glucose output. The data for NO are taken from a representative experiment taken from one of four incubations. * $P < 0.05$ for differences between control cells and cells incubated in the presence of SNAP.

Table 1 Effect of endotoxin treatment of the rat and SNAP on gluconeogenesis in isolated hepatocytes

Hepatocytes were prepared from control and endotoxin-treated rats and incubated as described in the Experimental section. The control cells were incubated in the absence or presence of either 600 μ M NAP or 600 μ M SNAP. Glucose output from the different substrates was measured between 20 and 40 min. All substrates were added to a final concentration of 10 mM, except for lactate plus pyruvate, which were added at 10 mM and 1 mM respectively. Results are expressed as nmol of glucose produced/20 min per mg wet wt. of cells: * $P < 0.05$, ** $P < 0.01$ ($n = 6$) for the differences between control cells and cells prepared from the endotoxin-treated animal and cells incubated in the presence of NAP and SNAP.

Substrate	Control	Endotoxin	NAP	SNAP
Lactate + pyruvate	21.6 ± 1.8	$8.7 \pm 1.5^{**}$	20.7 ± 1.8	$12.6 \pm 1.1^*$
Alanine	11.8 ± 1.4	$4.3 \pm 0.8^*$	11.2 ± 1.7	$5.4 \pm 0.9^*$
Asparagine	8.9 ± 0.8	$3.3 \pm 0.6^{**}$	8.2 ± 0.6	$4.0 \pm 0.7^*$
Glycerol	18.3 ± 1.1	$8.1 \pm 1.6^{**}$	16.3 ± 1.3	$11.9 \pm 0.8^*$
Dihydroxyacetone	26.6 ± 1.6	$12.3 \pm 2.1^{**}$	26.9 ± 1.9	$22.2 \pm 1.5^*$
Fructose	79.7 ± 8.5	72.0 ± 8.2	83.8 ± 8.1	73.5 ± 6.7

with those previously reported to inhibit hepatic protein synthesis [17], and the decrease in glucose production by the NO donor is comparable in magnitude with the extent of inhibition of gluconeogenesis observed in livers from endotoxin-treated animals [1–9]. To examine the possibility that the inhibitory effect of endotoxin treatment of the rat might be due to the production of NO, the effect of the lipopolysaccharide on gluconeogenesis from a number of substrates feeding into the pathway at different sites was correlated with that of SNAP and NAP (Table 1). The addition of 600 μ M NAP to the incubation had no significant effect on glucose synthesis from any substrate. Endotoxin treatment resulted in an inhibition of glucose synthesis from substrates feeding into the pathway before phosphoenolpyruvate, consistent with the suggestion that the major effect of endotoxin treatment resides at the level of the conversion of oxaloacetate into phosphoenolpyruvate [7,9]. However, the endotoxin also diminished glucose synthesis from both dihydroxyacetone and glycerol, whereas that from fructose remained

Table 2 Effect of endotoxin treatment of the rat and SNAP on lactate plus pyruvate production in isolated hepatocytes

The experimental protocol was as described in Table 1. Lactate plus pyruvate formation was measured between 20 and 40 min. Results are expressed as nmol of lactate plus pyruvate formed/20 min per mg wet wt. of cells: * $P < 0.05$ ($n = 6$) for the differences between control cells and cells prepared from the endotoxin-treated animal and cells incubated in the presence of NAP and SNAP.

Substrate	Control	Endotoxin	NAP	SNAP
Glycerol	1.22 $\pm$ 2.53	5.11 $\pm$ 1.21	4.06 $\pm$ 0.99	2.90 $\pm$ 1.04
Dihydroxyacetone	33.1 $\pm$ 4.1	30.6 $\pm$ 9.8	35.0 $\pm$ 7.1	32.9 $\pm$ 6.1
Fructose	87.5 $\pm$ 9.7	47.2 $\pm$ 11.6*	94.8 $\pm$ 11.3	55.8 $\pm$ 10.0*

unchanged. The lack of effect of endotoxin treatment on glucose synthesis from fructose would support the conclusion that there is no major effect of sepsis on the degree of cycling at the level of glucose/glucose 6-phosphate [6]. Treatment of the cells with 600 μ M SNAP showed a similar profile to that of treatment with the lipopolysaccharide, although the degree of inhibition was slightly less, particularly with the substrates feeding into the pathway after phosphoenolpyruvate.

In contrast with previous studies [6,8], the work of Miller et al. [13] has suggested that the increase in PFK-1 flux following endotoxin treatment and the inhibition of gluconeogenesis can be dissociated, with the stimulation of PFK-1 flux being independent of both cytokine action and lowered glucose synthesis. To examine the possibility that NO may be responsible for the inhibition of glucose synthesis, but not the activation of PFK-1, we have measured PFK-1 flux in the presence of 600 μ M SNAP over the same time period used to demonstrate an inhibition of glucose synthesis. In contrast with the inhibition of gluconeogenesis, there was no effect of the NO donor on the de-tritiation of the [3 H]glucose; the rates for the control and SNAP-treated cells were 15.2 $\pm$ 1.9 and 14.2 $\pm$ 2.6 ($n = 4$) nmol of 3 H released/20 min per mg wet wt. of cells. This contrasts with the ability of endotoxin to increase PFK-1 flux [7–9,13]; however, it agrees with the concept that it is not the stimulation of PFK-1 which is primarily responsible for the inhibition of glucose synthesis in septic shock, but the NO-dependent inhibition of phosphoenolpyruvate formation [7,9,13].

Effect of SNAP and endotoxin treatment of the rat on lactate production from dihydroxyacetone, glycerol and fructose

Several studies have suggested that an increase in pyruvate kinase flux might account for the inhibition of glucose synthesis during sepsis [26,27]. However, no effect of treatment of the rat for 18 h with endotoxin has been reported on either total pyruvate kinase activity or the activity ratio [7,8]. Similarly, measurements of pyruvate kinase flux by the isotopic technique of Rognstad [28] have demonstrated either no change [6] or a decreased [7,9] rate of flux, with lactate plus pyruvate as the substrate. A number of studies have used the conversion of dihydroxyacetone into lactate as a measure of pyruvate kinase flux [29,30]; therefore we have compared the effect of both 600 μ M SNAP and endotoxin treatment of the rat as a further measure of this flux, which is independent of possible errors in the isotopic method. The results are shown in Table 2. With dihydroxyacetone as the substrate, no effect of endotoxin treatment of the rat, or treatment of the cells with either NAP or SNAP, was observed on lactate plus pyruvate formation. This is in contrast with the inhibition of glucose synthesis by both endotoxin and SNAP measured in the

same cell incubation (Table 1). The rate of pyruvate plus lactate formation was greatly depressed when dihydroxyacetone was replaced with glycerol as the substrate, as described previously [29,30], and again there was no effect of either endotoxin or SNAP. This is further confirmation that endotoxin has no effect on pyruvate kinase activity and that the depression of flux observed during gluconeogenesis from lactate plus pyruvate is the result of a decreased cytoplasmic concentration of phosphoenolpyruvate [7,9]. Similarly, SNAP had no effect on pyruvate kinase flux under these conditions, suggesting a common mechanism of action of SNAP and endotoxin treatment of the rat. When the conversion of fructose into lactate plus pyruvate was measured, this was significantly depressed by both SNAP and the endotoxin, further strengthening the correlation between the effects of NO and endotoxin. However, the mechanism responsible for this inhibition currently remains unclear.

It has been suggested that the deleterious effects of both endotoxic and haemorrhagic shock at the level of the liver result from an inhibition of the mitochondrial respiratory chain [31–34], and this is also known to occur as a result of interaction of NO with aconitase and complexes I and II [35]. Evidence that this may underlie the inhibition of gluconeogenesis by endotoxin may be derived from the studies of Holtzman et al. [36], which indicate that endotoxin treatment increases the lactate/pyruvate ratio in liver. Similar conclusions can be drawn from the experiments in Table 2, where the lactate/pyruvate ratio is increased with all three substrates, the ratios being 5.0, 0.2 and 2.2 for control and 14.4, 2.2 and 3.0 for endotoxin treatment, with glycerol, dihydroxyacetone and fructose respectively. This confirms the previous observation of Holtzman et al. [36]. Similarly, mild inhibition of the respiratory chain is known to result in a similar profile of inhibition of gluconeogenesis with different substrates in the absence of significant effects on total cellular ATP concentrations, with the exception that glucose synthesis from fructose was also inhibited [30]. Although this may explain in part the action of NO and endotoxin, several factors suggest the involvement of at least one other site. Respiratory-chain inhibition is thought both to inhibit the pyruvate carboxylase reaction and simultaneously to stimulate the pyruvate kinase reaction, resulting in decreased flux through to glucose [30]. Although we have been able to demonstrate a decreased flux through pyruvate carboxylase [9], this was not coupled with an increase in pyruvate kinase flux ([6,7,9]; Table 2), but a decreased flux through phosphoenolpyruvate carboxykinase [7,9]. With respect to the latter, we have shown that endotoxin treatment of the rat results in a 40% decrease in the cytosolic content of GTP in subsequently isolated hepatocytes, the absolute values being 0.27 $\pm$ 0.03 ($n = 6$) and 0.17 $\pm$ 0.01 ($n = 5$) nmol/mg wet wt. ($P < 0.01$) for control and endotoxin-treated cells respectively. The changes in GTP correlate with the time of induction of NO synthase and the formation of NO within the liver [16,18] and with the inhibition of glucose synthesis (results not shown). It is established that the induction of NO synthase in the liver by endotoxin and cytokines results in a NO-dependent stimulation of the soluble guanylate cyclase, with the resultant cyclic GMP being released into the extracellular medium [37]. Therefore it is conceivable that this could deplete cellular GTP and that this could prove a unifying link between endotoxin, NO, and the inhibition of both gluconeogenesis and protein synthesis.

Conclusions

The concentrations of NO shown to be required to inhibit gluconeogenesis (200–800 nM) are well within the range reported

to occur in physiological settings, i.e. 400–500 nM from collagen-stimulated platelets in whole blood [38] or bradykinin- or ionomycin-stimulated vascular endothelial cells [39], 1300 nM in bradykinin-stimulated rabbit aorta [40] and 2700 nM in rat brain *in vivo* during cerebral ischaemia, and are therefore likely to be of physiological relevance [41]. Although further work is required to prove conclusively that NO is the mediator of the inhibitory effect of endotoxin on carbohydrate metabolism in septic shock, we have measured hepatic NO synthase activity under our conditions of treatment and have shown it to be elevated from 0.29 ± 0.01 to 4.36 ± 0.99 ($n = 3$; $P < 0.001$) nmol/min per g wet wt. of tissue after endotoxin administration. This is comparable with previous measurements [14–17] and indicates that the livers have a greatly increased capacity to synthesize intracellular NO in this model of sepsis. In addition, measurements of plasma nitrite concentrations show that there was a significant increase in systemic NO formation in the endotoxic rat, the plasma nitrite concentrations increasing from 3.1 ± 0.6 (5) to 12.3 ± 0.7 (3) μM ($P < 0.001$). Therefore the data presented here suggest that the inhibition of gluconeogenesis in endotoxaemia and sepsis could be caused by NO synthesized in the liver by the Ca^{2+} -independent NO synthase which is induced by endotoxin and cytokines.

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