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Suppression by Dexamethasone of Inducible Nitric Oxide Synthase Protein Expression *In Vivo*
A Possible Role for Lipocortin 1

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ABSTRACT. Western blot and densitometric analysis of organ homogenates from lipopolysaccharide (LPS)-treated rats (1–10 mg kg^{-1}, i.p.) exhibited a strong induction of inducible nitric oxide synthase (iNOS) expression seen at all the doses tested (1, 3, and 10 mg kg^{-1}, $n = 3$). In particular, 3 hr after challenge of rats with LPS, iNOS was detectable in the liver, kidney, aorta, spleen and lung. Dexamethasone (DEX) (0.1–1 mg kg^{-1}; −1 hr) dose-dependently reduced iNOS expression in lung homogenates after exposure to LPS (1 mg kg^{-1}; $P < 0.05$). A partial reversal of DEX-induced suppression of iNOS expression in lung homogenates 3 hr after challenge with LPS was observed in rats which received a specific anti-lipocortin 1 sheep serum (LCS3; 1 mL kg^{-1} 24 hr prior to the steroid), with an inhibition of 35 ± 8%, as compared to animals passively immunised with normal sheep serum where dexamethasone exhibited an inhibition of 60 ± 7% ($n = 4$). Peritoneal macrophages collected from rats treated with LPS (1 mg kg^{-1}; 3 hr) and cultured for 16 hr, released significant amounts of nitrite (51 ± 1 μM) into the cell supernatants; this was reduced (−70 ± 6%) after pre-treatment with dexamethasone (0.3 mg kg^{-1}) and this effect was neutralised if animals were passively immunised with LCS3 ($P < 0.01$; $n = 4$). Thus lipocortin 1 mediates, at least in part, the inhibitory action exerted by dexamethasone on both iNOS protein expression in lung and iNOS activity (as measured by nitrite release) in primary peritoneal cells of rats. BIOCHEM PHARMACOL **55**;3:279–285, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. annexin 1; glucocorticoid; macrophages; LPS; endotoxaemia

Circulatory shock is often caused by the release of endotoxin (or bacterial lipopolysaccharide; LPS)† from Gram-negative organisms into the blood stream. The accompanying vasodilatation is associated with the induction of inducible nitric oxide synthase (iNOS) which leads to the generation of large amounts of nitric oxide and hence vascular smooth muscle relaxation [1, 2]. LPS and other bacterial toxins induce iNOS protein and activity in a number of cells and tissues *in vitro* as well as *in vivo* [3–5]. The induction of iNOS by LPS and cytokines, both *in vivo* and *in vitro*, can be reduced by treatment of animals or cells with glucocorticoids such as dexamethasone (DEX). This suggests that one of the protective effects of glucocorticoids in LPS-induced endotoxaemia is through the suppression of iNOS expression [6, 7].

Glucocorticoids exert most of their biological actions through interaction with a glucocorticoid receptor and subsequent modulation of gene expression. Glucocorticoid binding activates the receptor complex which translocates to the nucleus and interacts with glucocorticoid response elements in the promoter region of susceptible genes, leading to either an induction (such as for lipocortin 1– also called annexin 1) or suppression (such as for cyclo-oxygenase 2) of gene expression [8]. Several effects of glucocorticoids are mediated by lipocortin 1, a protein which is found in increasing amounts, often on the plasma membrane of cells, after exposure to these hormones [9–11]. Human recombinant lipocortin 1 has anti-inflammatory properties in a number of models reducing eicosanoid release, cytokine fever, paw oedema, neutrophil migration and cell growth and differentiation [12–16] and these effects mimic those of glucocorticoids in the same models. Neutralising antibodies or antisense oligonucleotides to lipocortin 1 prevent some of the effects of DEX *in vitro* [17, 16] while neutralising antibodies reduce the anti-inflammatory effects of glucocorticoids *in vivo* [15, 18].

The induction of iNOS in response to LPS and cytokines in several cell types and tissues is suppressed by DEX [19–21]. A link between the effect of glucocorticoids on iNOS and the glucocorticoid-inducible protein, lipocortin 1, has been recently shown [11]. In this study the involvement of lipocortin 1 in the protection exerted by DEX upon LPS-induced circulatory shock was proposed on the basis of the effects achieved with a neutralising anti-lipocortin 1 sheep serum, termed LCS3, which reversed the effect of

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† *Abbreviations:* LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthase; DEX, dexamethasone; DMEM, Dulbecco's Modified Eagle's medium; LCS3, anti-lipocortin 1 sheep serum; NSS, non-immune sheep serum.

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DEX on iNOS activity *in vivo*, as measured with the citrulline assay, and *in vitro*, as estimated by the release of the stable breakdown product of nitric oxide, nitrite ions [11]. However, this paper did not address two important points. First, the study focused on iNOS activity *in vivo* and did not investigate whether the involvement of endogenous lipocortin 1 in the effect of DEX was also evident at the level of protein expression. Second, the cellular investigation was based on experiments performed with a murine cell line (J.774 cells) rather than with rat primary cells.

Utilising a highly selective antibody raised against rat iNOS [22] the present investigation has considered the distribution of iNOS protein in several tissues, after treatment of rats with a range of doses of LPS, to establish the dose required for maximal iNOS expression. Similarly, the dose of DEX necessary to suppress iNOS expression *in vivo* was also established before investigating the role of lipocortin 1 in the effect of the steroid. Using the well characterised neutralising serum LCS3 [15, 23, 24], the role of lipocortin 1 in DEX-induced suppression of iNOS protein expression *in vivo* has now been studied. The potential modulatory effect of LCS3 on nitrite release from peritoneal macrophages *ex vivo* was also studied to ascertain whether data obtained using the macrophage cell line J.774 were also obtained with primary cells such as rat peritoneal macrophages.

MATERIALS AND METHODS

Animal Treatment

Male Wistar rats (250–350 g body weight) were purchased from Tuck and kept on a standard chow pellet diet and tap water *ad lib*. LPS (*Escherichia coli* serotype 0.127:B8; 1–10 mg kg^{-1}) was administered via the i.p. route. To investigate the effects of DEX on iNOS expression *in vivo*, animals were treated with different doses of the steroid (0.1–1 mg kg^{-1} given sub-cutaneously, s.c.) 1 hr prior to LPS administration. The possible mediator role of endogenous lipocortin 1 in the DEX suppression on iNOS expression was studied using a specific polyclonal sheep anti-human lipocortin 1 antiserum (LCS3) which was administered according to a protocol reported to be effective in mice [15] and rats [24]. In addition, LCS3 recognizes rat lipocortin 1 by Western blot analysis [23, 11]. Rats were pre-treated with control non-immune sheep serum (NSS) or with the specific neutralising anti-human recombinant lipocortin 1 sheep serum (LCS3, 1 mL kg^{-1} s.c. in both cases) 23 hr prior to administration of vehicle or DEX (0.3 mg kg^{-1} s.c.). Given in this way, LCS3 reaches a peak of anti-lipocortin 1 titre in the serum 24 hr after the s.c. injection, with a half-life of 5–7 days [15]. LPS (1 mL kg^{-1} i.p.) was injected 1 hr after DEX and peritoneal cells and organs collected as described below.

Organs and Peritoneal Cell Preparation

Animals were killed by CO_2 inhalation 3 hr after LPS treatment. Organs were removed and snap frozen in liquid nitrogen for later use. Peritoneal cells were collected and prepared as described by Perretti *et al.* [25]. Briefly, after sterile preparation of the abdominal wall, a small incision was made and 10 mL of sterile saline containing EDTA (3 mM) was injected into the abdominal cavity. The peritoneal fluid was removed by aspiration and collected into sterile tubes maintained on ice. Cells from 3 animals in each treatment regime were pooled. The cells were pelleted at 300 × *g* at 4° and then washed 3 times in EDTA-free sterile saline. Cells (~80% macrophages) were suspended in Dulbecco's modified Eagle's medium (DMEM) containing 20% foetal calf serum and placed in 24-well plates at a density of 2 million in 0.5 mL per well. After a 3 hr period, non-adherent cells were removed by gentle washing (3 times with warm DMEM) and adherent cells (~95% macrophages) incubated in DMEM supplemented with 5% foetal calf serum and 0.5% gentamycin sulphate for a further 24 hr (at 37° in 5% CO_2 atmosphere).

Detection of Nitrite Release in the Cell Supernatant

The supernatant of cultured macrophages was removed and assayed for nitrite accumulation by the Griess reaction as an indication of iNOS activity [26]. Briefly, an equal volume of Griess reagent (4% sulphanilamide and 0.2% naphtylethylenediamine dihydrochloride in 10% phosphoric acid) was added to an equal volume of sample and the colorimetric difference in optical density at 540 nm and 620 nm read immediately. This method detects free nitrite and not nitrate (the subsequent metabolite of nitrite). Nitrate measurement was not performed in this study because as the free nitrite levels released from peritoneal macrophages were already high it was felt that the back-conversion of nitrate to more nitrite was unlikely to give any extra information. The values thus obtained were compared with standards of sodium nitrite dissolved in DMEM and the concentration of nitrite released calculated and expressed as concentration (μM).

Western Blotting Analysis

Tissues were homogenised on ice in PBS containing EDTA (10 mM), Triton X-100 (1%), PMSF (1 mM), pepstatin A (0.05 mM) and leupeptin (0.2 mM) and centrifuged at 5,000 × *g* for 5 min (4°). Peritoneal cell pellets were resuspended and lysed in the EDTA/Triton buffer. The protein content of the supernatant or cell lysate was assessed using the Bradford assay [27]. Samples were boiled for 10 min in a 1:1 ratio (v:v) of gel loading buffer (20 mM Tris base, 2 mM EDTA, 10% mercaptoethanol, 2% SDS, 20% glycerol and 0.2% bromophenol blue; pH = 6.8). Equal quantities of the proteins present in the tissue supernatants or cell lysates were resolved by one dimensional SDS gel electrophoresis on a 7.5% SDS gel with molecular weight markers (SDS-7B; Sigma) and transferred by electrophoresis (400 mA, 80V, 60 min) to nylon membranes (PVDF; Millipore). The membranes were incu-

bated overnight with rabbit anti-murine iNOS antibody (1:2000 at 4°; Ref. 22). The western blots were incubated with sheep anti-rabbit IgG linked to horseradish peroxidase conjugate. Primary antibody conjugation was visualised with diaminobenzidine and hydrogen peroxide as the enzyme substrate.

Drugs

All drugs and reagents were purchased from Sigma (Poole, Dorset) except for DEX which was purchased as sodium phosphate salt from David Ball Laboratories (Warwick). Solutions for injection were prepared in sterile, endotoxin free distilled water.

Data Analysis and Statistics

All data are presented as mean ± SE mean of n independent experiments (performed with 3–4 rats per group) unless otherwise stated. Blots were quantified by densitometric analysis of each protein band compared to a background reading from each membrane using a Seescan image analysis system and the data is expressed in relative optical density (OD) units. Differences between the experimental groups were analysed using one way analysis of variance taking a P value < 0.05 as significant.

RESULTS

LPS Induction of iNOS Expression in Several Rat Organs

Administration of LPS (10 mg kg^{-1} i.p.) to rats induced, at the 3 hr time-point, a strong expression of a 135 kDa protein band (the molecular weight of iNOS) which was recognised by the specific anti-iNOS rabbit serum. This dose of LPS and time-point have previously been shown to produce a high degree of iNOS activity [7]. Figure 1A illustrates that this strongly immunoreactive protein band seen at 135 kDa (iNOS) was present in a number of rat tissues including the liver, lung, kidney, aorta and spleen after challenge with the endotoxin. No iNOS band was seen in any of the rats treated with vehicle alone (Fig. 1B). A dramatic iNOS protein induction was seen in the lung supporting a previous study showing a large induction of NOS activity in this organ [7]. The lung was therefore selected and used in all our subsequent studies as a reproducible and convenient tissue in which to monitor the potential effect of different treatments upon iNOS expression.

LPS induction of iNOS in the rat lung did not exhibit significant dose-dependency within the dose range used (1–10 mg kg^{-1}). Figure 2A illustrates that bands of similar intensity were obtained after treatment with several different doses of LPS, the expression was just maximal at 1 mg kg^{-1} i.p. (1 mg kg^{-1}, 86 ± 5%; 3 mg kg^{-1}, 96 ± 20%; where 10 mg kg^{-1} gave 100% of maximal protein expression as calculated from relative optical density units; n = 3). The former dose was therefore selected and used in the following experiments. As stated above, lungs collected from rats treated with vehicle (sterile saline) showed no protein induction.

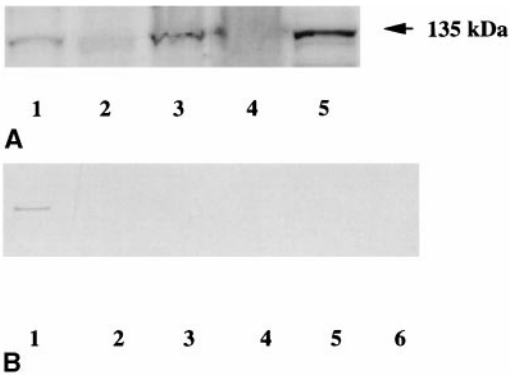

FIG. 1. Detection of iNOS protein in different rat organ homogenates. Organs were removed from rats 3 hr after treatment with LPS (10 mg kg^{-1}). Tissues were homogenised and equal quantities of protein were separated on a 7.5% SDS-acrylamide gel. After transfer to a nylon membrane the presence of iNOS protein was detected using a polyclonal antibody to murine iNOS at a dilution of 1:2000. The iNOS protein was shown as a protein band at 135 kDa in a number of organ homogenates including aorta (1), kidney (2), liver (3), spleen (4) and lung (5) (panel A). No protein bands which can react with anti-iNOS antibodies were seen in organ homogenates from vehicle treated animals (panel B: LPS-treated lung (1); vehicle treated aorta (2), kidney (3), liver (4), spleen (5) and lung (6)). Data are representative of n = 3–4 rats.

Effect of DEX on iNOS Induction in the Lung by LPS

Treatment of rats with increasing amounts of DEX (0.1–1 mg kg^{-1} s.c.) suppressed iNOS protein expression caused by LPS in a dose-dependent fashion (Fig. 2B). A comparable induction of iNOS protein was found after 1 mg kg^{-1} LPS in this set of experiments to that reported in Fig. 2A. A maximal inhibition of 42 ± 7% (as calculated from optical density units, $P < 0.01$, $n = 3$) was achieved with a dose of the steroid of 1 mg kg^{-1}, and a similar degree of inhibition (39 ± 3%, $n = 3$) was observed at 0.3 mg kg^{-1} (Fig. 2B). Only 22 ± 8% of inhibition was calculated with the DEX dose of 0.1 mg kg^{-1} ($n = 3$). The dose of 0.3 mg kg^{-1} DEX was therefore selected to evaluate the role played by endogenous lipocortin 1.

Effect of DEX on Ex Vivo *Peritoneal Cell Nitrite Release and iNOS Protein Expression*

Ex vivo culture of peritoneal macrophages isolated from rats treated with LPS (1 mg kg^{-1}) for 16 hr resulted in a substantial release of nitrite (≈50 μM) in the cell supernatant (Fig. 3A). This nitrite release was significantly attenuated by more than 50% when cells were harvested from rats treated with LPS + DEX (0.3 mg kg^{-1} s.c.) ($P < 0.01$, $n = 3$). Changes in nitrite release correlated with differences in cell activation, as indicated by the different

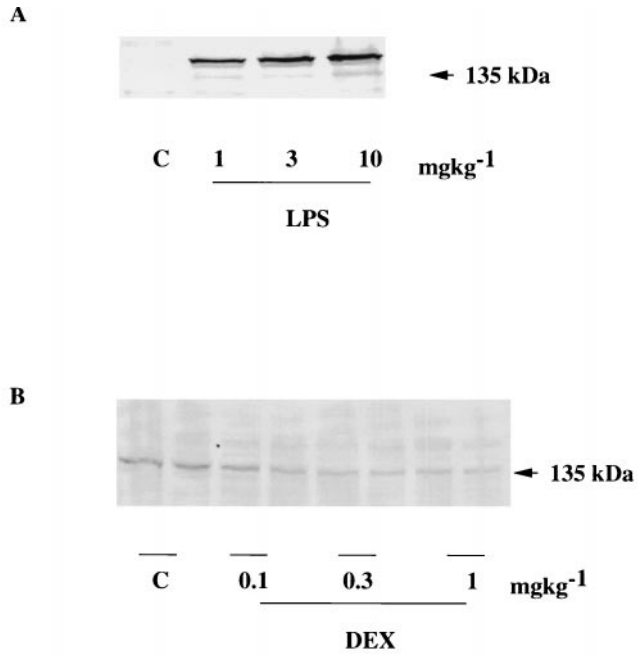

FIG. 2. Effects of increasing concentrations of LPS and DEX on lung iNOS expression. Panel A: lung homogenates taken from animals 3 hr after treatment with increasing doses of LPS (1–10 mg kg^{-1}). Panel B: lungs homogenates taken from rats treated with increasing doses of DEX (0.1–0.3 mg kg^{-1}) 1 hr prior to LPS (1 mg kg^{-1}). Samples were resolved on one dimensional SDS electrophoresis and probed for the presence of iNOS protein, which was observed as an immunoreactive protein band at 135 kDa. A large induction of iNOS protein was obtained at 1 mg kg^{-1} of LPS, and no significant changes were seen when the dose of LPS was increased (panel A; representative of n = 3 rats). DEX suppressed iNOS expression in response to LPS reaching a maximal reduction at 0.3 mg kg^{-1} (panel B; representative of n = 3 rats).

amount of iNOS protein expression present after cell collection, i.e. at the beginning of the overnight culture (data not shown). In the absence of iNOS expression, nitrite presence was not detectable in the supernatant of macrophages collected from rats which had not been treated with LPS (data not shown).

A Role for Endogenous Lipocortin 1 in the DEX Suppression of iNOS Expression

Passive immunisation of animals with NSS did not affect either the expression of iNOS in the lung in response to LPS or the suppression of LPS-induced iNOS expression by DEX (60 ± 7% inhibition, n = 4 experiments; $P < 0.05$ vs. animals treated with LPS alone). Administration of LCS3 alone reduced iNOS protein expression when compared to animals treated with NSS in response to LPS (LCS3 reduced iNOS expression by 32 ± 13%; $P < 0.05$). However, a much less pronounced suppression of iNOS induction was exerted by DEX in the rats passively immunised with LCS3, an effect that may be caused by transient increases in corticosterone following the injection of this antibody [25]. However, the inhibition observed with

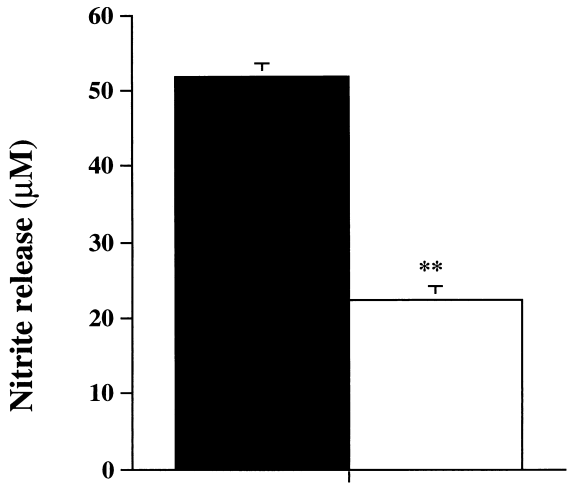

FIG. 3. The effect of LPS and DEX on *ex vivo* nitrite release in rat peritoneal cells. Peritoneal macrophages were collected from rats treated with either LPS (1 mg kg^{-1}; ■) or DEX (0.3 mg kg^{-1}) + LPS (□) and incubated for 16 hr. The cell supernatant was collected and tested for the presence of nitrite (an indicator of nitric oxide release) using the Griess reaction and calibrated with sodium nitrite standards dissolved in medium. Values are expressed in mean nitrite release (μM) ± SEM of triplicate observations from each rat (used 3–4 rats per group). **$P < 0.01$.

LCS3 + DEX was 35 ± 8% when compared to animals passively immunised with NSS and treated with LPS, but only 3 ± 7% inhibition was observed after steroid treatment of LCS3 immunised rats when compared to animals treated with LCS3 and LPS alone (see Fig. 4A).

A Role for Endogenous Lipocortin 1 in the DEX Suppression of Nitrite Release from Peritoneal Macrophages

Similar amounts of nitrite were measured in the supernatants of peritoneal macrophages collected from animals treated with the two sheep sera and LPS in the absence of DEX (Fig. 4B). However, treatment with the steroid greatly reduced the *ex vivo* production of nitrite induced by LPS in the NSS-group (71 ± 6% inhibition, n = 4, $P < 0.01$), whereas DEX failed to modify the release of nitrite in the animals which were treated with LCS3 (Fig. 4B).

DISCUSSION

Here we confirm that low doses of LPS can produce expression of iNOS protein in a number of tissues and that this induction is dose-dependently reduced by pre-treatment with DEX partially through a lipocortin 1-dependent mechanism. A high dose of LPS (10 mg kg^{-1}) induces a large amount of iNOS activity peaking at 3 hr, as measured by the conversion of arginine to citrulline, in a number of tissues [7]. This dose of LPS also causes a large haemodynamic response *in vivo* [7]. In accordance with this data, we find that this dose of endotoxin induces iNOS protein expression in a wide range of organs as estimated by Western blotting. A single study has previously reported

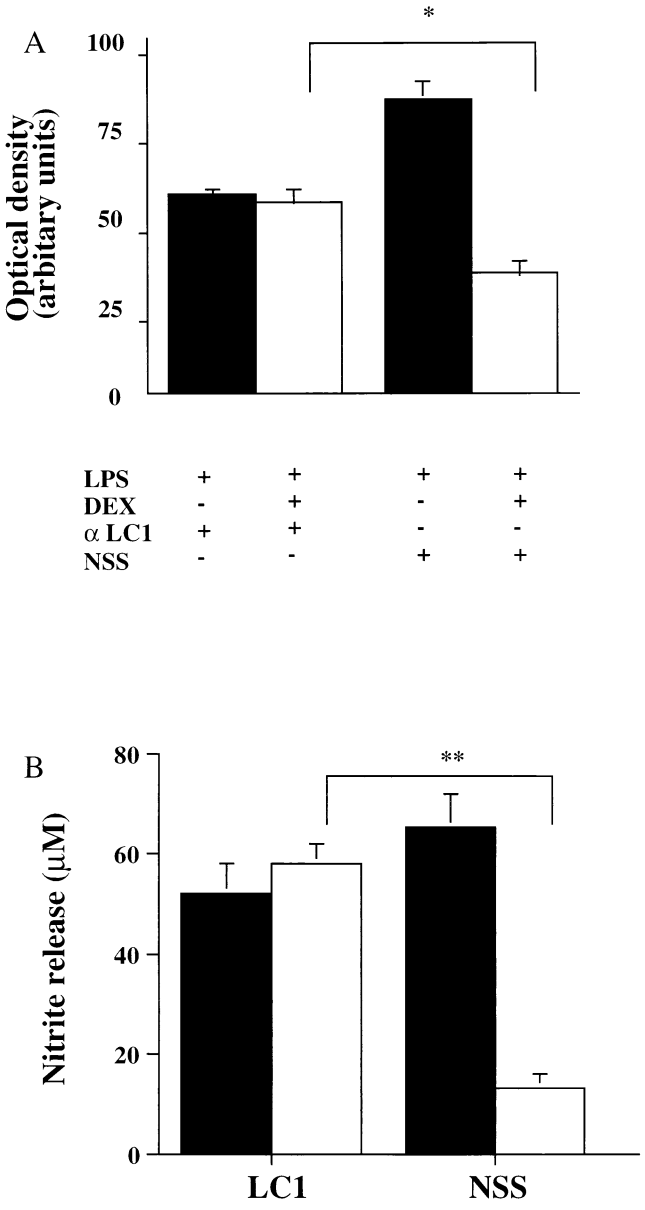

FIG. 4. The effect of endogenous lipocortin 1 on DEX suppression of iNOS induced by LPS in rat lung homogenates and peritoneal macrophages. Panel A: animals were passively immunised with either LCS3 (a sheep anti-lipocortin 1 antibody) or NSS (a control non-immune sheep serum) 24 hr before treating with DEX (0.3 mg kg^{-1}; $n = 4$ rats per group). After 1 hr rats were then treated with LPS (1 mg kg^{-1}) and the lungs collected 3 hr later. Organs were homogenised and the proteins resolved on one dimensional gel electrophoresis as described in Fig. 1 legend. A graphical representation of the optical densities recorded in arbitary units is shown with the readings taken and pooled from 4 different blots. Closed bars, LPS; open bars, DEX + LPS; *$P < 0.05$. Panel B: Following treatment of rats as in panel A, peritoneal macrophages were collected, cultured and the supernatant and tested for the presence of nitrite (an indicator of nitric oxide release) using the Griess reaction. Values are expressed in mean nitrite release (μM) ± SEM of triplicate observations ($n = 3$–4 rats per group). Closed bars, LPS; open bars, DEX + LPS; **$P < 0.01$.

induction of iNOS protein in rat tissues by LPS however, a very high dose of the endotoxin was used (50 mg kg^{-1}) [28]. This dose of LPS causes cardiovascular effects indistinguishable from those seen at a dose of 10 mg/kg and therefore the protein induction seen at 50 mg kg^{-1} is unlikely to reflect the haemodynamic changes caused by the toxin [28]. Indeed, we report here that iNOS protein induction is just maximal in the lung after treatment of the rat with as little as 1 mg kg^{-1} LPS. Studies with transgenic mice lacking the iNOS gene show that the haemodynamic response to LPS is not solely due to the production of nitric oxide [29, 30]. Other factor(s) produced in response to endotoxin, such as prostanoids, platelet-activating factor and cytokines, may be important in the overall pathology of endotoxaemia and could also synergise with the nitric oxide system *in vivo*. This may explain not only the LPS-induced haemodynamic changes seen in iNOS deficient mice, but may also explain why maximal iNOS expression is already reached at relatively low doses of LPS [31, 32]. Alternatively, it is possible that the high iNOS activity seen at LPS doses ≥10 mg kg^{-1} could be the result of the involvement of other pathways generating citrulline, or that activity is not necessarily directly proportional to the protein levels present in the tissue.

We showed a dose-dependent reduction in iNOS expression after administration of DEX (0.1–1 mg kg^{-1}) and this was in agreement with previous functional studies [31]. It is worth noting that a similar inhibitory action of DEX on iNOS expression occurs in response to other bacterial toxins such as lipoteichoic acid [5]. However, a high dose of DEX (10 mg kg^{-1}) was used in this latter study. In the present investigation it was possible to inhibit LPS induction of iNOS protein with doses of DEX as low as 0.3 mg kg^{-1}, suggesting that some of the protective effects exerted by the steroid in experimental endotoxaemia are effected *via* inhibition of iNOS expression. We believe that particular attention should be paid to the doses of endotoxin and DEX used, since the steroid failed to affect iNOS expression when induction was brought about by large amounts of endotoxin [28]. Under these conditions it is possible that many other direct or indirect pathways which may not be steroid sensitive could be involved.

A consistent nitrite release from peritoneal macrophages *ex vivo* was measured when the animals were treated with LPS. This measurement, widely used as a marker of iNOS induction, is related to the expression of the enzyme protein: peritoneal cells taken from animals lacking the iNOS gene showed no iNOS protein band and released only low levels of nitrite when cultured *in vitro* in the presence of interferon-γ [30]. Nitrite release was suppressed in animals treated with DEX prior to LPS challenge, this correlated to the extent of iNOS protein detected in peritoneal cells. These responses of primary cells taken from LPS-treated animals, with or without DEX pre-treatment, are similar to those seen *in vitro* [19, 33]. Therefore this assessment of *ex vivo* nitrite release provides a good method of monitoring iNOS activity after treatment of animals.

Glucocorticoids cause the expression of lipocortin 1 in many cells and tissues including rat thymus, human bronchoalveolar lavage fluid and rat peritoneal macrophages [9, 10]. In a recent study we have shown that endogenous lipocortin 1 mediates DEX suppression of iNOS expression in J774.2 cells *in vitro* and the protective effects of the steroid on the cardiovascular system after LPS administration *in vivo* [11]. In agreement with our previous data which monitored iNOS activity in the lung [11], here we show that passive immunisation of rats with anti-lipocortin 1 antibodies partially prevents the DEX suppression of iNOS expression in lung homogenates. In addition a complete reversal of DEX-induced inhibition of *ex vivo* nitrite release from peritoneal macrophages was observed in cells taken from rats treated with anti-lipocortin 1 antisera. This activity data was partially paralleled by changes in iNOS protein expression although in this case the reversal by DEX was incomplete. There may be many reasons for this partial discrepancy between iNOS activity and expression in macrophages; for example *in vivo*, LPS is likely to activate several pathways which will lead to iNOS induction, only some of which may be lipocortin 1 sensitive. It is also possible that the titre of anti-lipocortin 1 antibodies used in this study may be sufficient to neutralise some, but not all, of the DEX-induced lipocortin 1, thus allowing some residual effect of the steroid.

In the absence of DEX treatment, administration of anti-lipocortin 1 antibodies alone somewhat reduced the expression of iNOS in response to LPS alone in rat lung, an effect not seen when J774.2 cells are treated similarly *in vitro* [11]. This suggests that lipocortin 1 may play a role in the *in vivo* response to endotoxaemia possibly affecting iNOS expression indirectly, although the site of action remains, at the moment, unknown. However, we cannot exclude the possibility that this action on iNOS is the result of a non specific effect of LCS3 secondary, for instance, to an increase of basal corticosterone [24]. Against this hypothesis is the fact that treatment of rats with LCS3 did not affect the LPS-induced nitrite release from cultured peritoneal macrophages *ex vivo*. Further studies will be required to clarify this point.

In summary this study demonstrates that treatment of rats with LPS induces iNOS protein expression in a number of tissues and causes the release of nitrite from peritoneal cells incubated *ex vivo*. DEX prevented LPS-induced iNOS expression and activity *in vivo* by a mechanism partially involving endogenous lipocortin 1. This modulation of iNOS protein expression *in vivo* strengthens the concept that glucocorticoids can modulate gene expression indirectly through production of this mediator.

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