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Role of lipocortin-1 in the anti-hyperalgesic actions of dexamethasone

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1 The effect of dexamethasone, lipocorton-1_{2-26} and an antiserum to lipocortin-1_{2-26} (LCPS1) upon the hyperalgesic activities in rats of carrageenin, bradykinin, tumour necrosis factor α (TNFα), interleukin-1[2], interleukin-6 (IL-6), interleukin-8 (IL-8), prostaglandin Eβ (PGE_2) and dopamine were investigated in a model of mechanical hyperalgesia.

2 Hyperalgesic responses to intraplantar (i.pl.) injections of carrageenin (100 μg), bradykinin (500 ng), TNFα (2.5 pg), IL-1β (0.5 pg), and IL-6 (1.0 ng), but not responses to IL-8 (0.1 ng), PGE_2 (100 ng) and dopamine (10 μg), were inhibited by pretreatment with dexamethasone (0.5 mg kg^{-1}, subcutaneously, s.c., or 0.04–5.0 μg/paw).

3 Inhibition of hyperalgesic responses to injections (i.pl.) of bradykinin (500 ng) and IL-1β (0.5 pg) by dexamethasone (0.5 mg kg^{-1}, s.c.) was reversed by LCPS1 (0.5 ml kg^{-1}, injected s.c., 24 h and 1 h before hyperalgesic substances) and hyperalgesic responses to injections (i.pl.) of bradykinin (500 ng), TNFα (2.5 pg) and IL-1β (0.5 pg), but not responses to PGE_2 (100 ng), were inhibited by pretreatment with lipocortin-1_{2-26} (100 μg/paw). Also, lipocortin-1_{2-26} (30 and 100 μg ml^{-1} and dexamethasone (10 μg ml^{-1}) inhibited TNFα release by cells of the J774 (murine macrophage-like) cell-line stimulated with LPS (3 μg ml^{-1}), and LCPS1 partially reversed the inhibition by dexamethasone. These data are consistent with an important role for endogenous lipocortin-1_{2-26} in mediating the anti-hyperalgesic effect of dexamethasone, with inhibiton of TNFα production by lipocortin-1_{2-26} contributing, in part, to this role.

4 Although arachidonic acid by itself was not hyperalgesic, the hyperalgesic response to IL-1β (0.25 pg, i.pl.) was potentiated by arachidonic acid (50 μg) and the potentiated response was inhibited by dexamethasone (50 μg, i.pl.) and lipocortin-1_{2-26} (100 μg, i.pl.). Also, lipocortin-1_{2-26} (30 and 100 μg ml^{-1}) inhibited/abolished PGE_2 release by J774 cells stimulated with LPS (3 μg ml^{-1}). These data suggest that, in inflammatory hyperalgesia, inhibition of the induction of cyclo-oxygenase 2 (COX-2), rather than phospholipase A_2, by dexamethasone and lipocortin-1_{2-26} accounts for the anti-hyperalgesic effects of these agents.

5 The above data support the notion that induction of lipocortin by dexamethasone plays a major role in the inhibition by dexamethasone of inflammatory hyperalgesia evoked by carrageenin, bradykinin and the cytokines TNFα, IL-1β and IL-6, and provides additional evidence that the biological activity of lipocortin resides within the peptide lipocortin-1_{2-26}. Further, the data suggest that inhibition of lipocortin-1_{2-26} of eicosanoid production by COX-2 also contributes to the anti-hyperalgesic effect of lipocortin-1.

Keywords: Inflammatory hyperalgesia; annexin 1; dexamethasone; bradykinin, tumour necrosis factor; interleukin-1; interleukin-6; interleukin-8; prostaglandins

Introduction

In a rat paw pressure test, carrageenin-evoked hyperalgesia resulted from the combined effect of the release of cyclo-oxygenase products and sympathomimetic amines (Nakamura & Ferreira, 1987). In this model, carrageenin caused hyperalgesia by releasing bradykinin, which stimulated the release of tumour necrosis factor α (TNFα). The TNFα induced interleukin-1β (IL-1β) and IL-6, which stimulated the production of cyclo-oxygenase products, and IL-8, which stimulated production of sympathomimetics (Cunha *et al*., 1992, Ferreira *et al*., 1993). These data are broadly consistent with data obtained in another model of inflammatory hyperalgesia: inflammatory hyperalgesia caused by Freund's complete adjuvant, to which TNFα and IL-1β contributed (Garabedian *et al*., 1995; Woolf *et al*., 1996; 1997).

It has been known for many years that glucocorticoid drugs such as dexamethasone inhibit both the early and late changes that contribute to the inflammatory process. Lipocortin-1, a glucocorticoid-inducible protein of 37 kDa, has been identified as a potential endogenous mediator of the anti-inflammatory actions of glucocorticoids (Flower & Rothwell, 1994). Recombinant human lipocortin-1 (346 amino acids) and an N-terminal polypeptide, lipocortin-1_{1-188}, mimic a variety of anti-inflammatory effects of glucocorticoids (Relton *et al*., 1991). More recently, an N-terminal peptide comprising just 25 amino acids, lipocortin-1_{2-26}, has been shown to mimic a variety of the anti-inflammatory effects of lipocortin-1 (Perretti *et al*., 1993). The peptide was about 200 times less active than lipocortin-1 on a molar basis although both molecules gave maximum inhibitions of IL-1-induced leukocyte migration (Perretti *et al*., 1993). Further, in mice, immunoneutralization of endogenous lipocortin-1 with antiserum to lipocortin-1_{2-26} (LCPS1) exacerbated the acute inflammatory response to zymosan (Perretti *et al*., 1996).

Given the anti-inflammatory effect of lipocortin-1_{2-26} and the pro-inflammatory effect of anti-lipocortin-1_{2-26}, we have investigated the effects of these two agents and the glucocorticoid dexamethasone on the cascade of endogenous mediators that contribute to carrageenin-evoked inflamma-

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tory hyperalgesia measured in a rat paw pressure test. In addition we have investigated the effects of lipocortin-1_{2-26}, anti-lipocortin-1_{2-26} (LCPS1) and dexamethasone on the production of TNFα and prostaglandin E_2 (PGE_2) by cells of the J774 (murine macrophage-like) cell-line stimulated with the pro-inflammatory agent lipopolysaccharide (LPS, 3 μg ml^{-1}).

Methods

Animals

Male Wistar rats, 130–180g, were housed in temperature controlled-rooms (22–25°C) with water and food *ad libitum* until use.

Nociceptive test

A constant pressure of 20 mmHg was applied to the hind paws of rats and discontinued when they presented a typical freezing reaction (reaction time). This reaction was characterized by a reduction in escape movements: animals usually made several attempts to escape from the position imposed by the experimental situation. These were followed by alterations in respiratory frequency with the onset of a typical shivering reaction. The intensity of hyperalgesia was quantified as the variation in reaction time (delta reaction time) obtained by subtracting values measured 3 h after administration of hyperalgesic substances from (control) reaction times measured before injection at zero time (Ferreira *et al*., 1978). Reaction times were typically 32–34 s (with s.e.means of 0.5–1.0 s) before injection and 2–4 s at 3 h after supramaximal stimulation with hyperalgesic agents.

Experimental protocol

In vivo *measurements* Hyperalgesia was measured 3 h after injection of PGE_2 (100 ng), carrageenin (100 μg), bradykinin (500 ng), TNFα (2.5 pg), IL-1β (0.25 or 0.5 pg), IL-6 (1.0 ng), IL-8 (0.1 ng) and dopamine (10 μg), each injected in 100 μl, into the hind paws (intraplantar, i.pl.) of rats. Dexamethasone (0.5 mg in 0.5 ml kg^{-1}, subcutaneously, s.c., or 0.04–5.0 μg in 50 or 100 μl, i.pl.), lipocortin-1_{2-26} (100μg in 50 or 100 μl, i.pl.) or phosphate buffered saline (50 or 100 μl) was injected, into the (same) hind paws, 10 min before the hyperalgesic substances. LCPS1 (0.5 ml kg^{-1}) or non-immune serum (0.5 ml kg^{-1}) was injected, s.c., 24 h and 1 h before hyperalgesic substances.

In vitro *measurements* Cells of the J774 murine macrophage-like cell line were allowed to adhere to 96-well plastic tissue culture plates (5×10^5 cells/well) for 1 h at 37°C, in an atmosphere of air containing 5% CO_2. The monolayers were then washed three times with phosphate buffered saline (PBS), pH 7.4, and incubated at 37°C with the following stimuli: (a) RPMI medium (200 μl/well), (b) LPS (3 μg ml^{-1}), (c) lipocortin-1_{2-26} (100 μg ml^{-1}), (d) lipopolysaccharide (LPS, 3 μg ml^{-1}) plus lipocortin-1_{2-26} (30 and 100 μg ml^{-1}), (e) LPS (3 μg ml^{-1}) plus dexamethasone (10 μg ml^{-1}) and (f) LPS (3 μg ml^{-1}) plus LCPS1 (100 μl ml^{-1}) or non-immune serum (100 μl ml^{-1}). After overnight incubation, the concentrations of TNFα and PGE_2 in the supernatants were measured by ELISA and radioimmunoassay, respectively, with methods similar to those described previously (Salmon, 1978; Cunha *et al*., 1993).

Results are presented as means with s.e.means of groups of 5 animals for the *in vivo* measurements and of triplicate wells for the *in vitro* assays. Three independent *in vitro* assays were performed. Differences between responses (delta reaction times, TNF concentrations and PGE_2 concentrations) were evaluated by ANOVA, followed by Bonferroni *t* test.

Drugs

Recombinant human IL-1β, IL-6, IL-8 (72 amino acids) and TNFα were NIBSC preparations coded 86/680, 88/514, 89/520 and 87/650. The specific activities of these materials are IL-1β: 100 000 international units (iu)/1 μg/ampoule, IL-6: 100,000 iu/1 μg/ampoule, IL-8: 1 000 iu/1 μg/ampoule, TNFα: 40,000 iu/1 μg/ampoule. Recombinant murine TNFα was the NIBSC preparation coded 88/532 (200 000 u/1 μg/ampoule). PGE_2 was a gift from the Upjohn Co. (U.S.A.). Carrageenin was a gift from the FMC Corporation (Philadelphia, U.S.A.). Bradykinin, dopamine and arachidonic acid were purchased from Sigma (St. Louis, U.S.A.). Purified bacterial endotoxin from *E. coli* 055:B5 (referred to here as lipopolysaccharide, LPS) was purchased from Difco Laboratories Ltd (West Molesey, Surrey, U.K.). Lipocortin-1_{2-26} was generously synthesized by Dr M. Toda (ONO Pharmaceutical Co., Osaka, Japan). Peptide preparations were more than 95% pure as analysed by high performance liquid chromatography (h.p.l.c.). Amino acid composition and Mr were confirmed by mass spectrometry. LCPS1 is a sheep polyclonal antiserum raised against the synthetic peptide lipocortin-1_{2-26}, and previously has been shown to neutralize the actions of dexamethasone and lipocortin-1 (Perretti *et al*., 1996).

The endotoxin (LPS) content of the above materials, as measured in the Limulus Amoebocyte Lysate test, was of the order of 0.25 iu μg^{-1}, which is equivalent to a little over 10^{-15} g of endotoxin in a hyperalgesic dose of IL-1β (0.5 pg), for example. The threshold dose of LPS for induction of hyperalgesia in the above model is likely to be approx. 100 ng, i.e. 10^{-7} g (based upon published data: Ferreira *et al*., 1993). Therefore, the doses of the hyperalgesic agents used contained amounts of endotoxin up to eight logs less than the estimated threshold hyperalgesic dose for endotoxin.)

Results

Effect of dexamethasone on the hyperalgesic responses to PGE_2, carrageenin, bradykinin, TNFα, IL-1β, IL-6, IL-8 and dopamine

Injection of PGE_2 (100 ng), carrageenin (100 μg), bradykinin (500 μng), TNFα (2.5 pg), IL-1β (0.5 pg), IL-6 (1.0 ng), IL-8 (0.1 ng) and dopamine (10 μg), each into one hind paw evoked hyperalgesia, measured 3 h after injection (Figure 1).

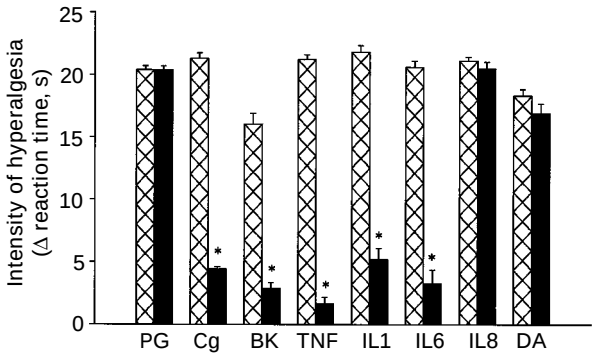

Figure 1 Effect of systemic administration of dexamethasone on the hyperalgesic responses to PGE_2, carrageenin, bradykinin, TNFα, IL-1β, IL-6, IL-8, and dopamine. Responses were measured 3 h after injection (in 100 μl, i.pl.) of PGE_2 (PG, 100 ng), carrageenin (Cg, 100 μg), bradykinin (BK, 500 ng), TNFα (TNF, 2.5 pg), IL-1β (IL-1, 0.5 pg), IL-6 (1.0 ng), IL-8 (0.1 ng), and dopamine (DA, 10 μg). Dexamethasone (0.5 mg kg^{-1}, s.c., solid columns) or saline (0.5 ml kg^{-1}, s.c., cross-hatched columns) was given 1 h before hyperalgesic substances. Means ± s.e.means in groups of 5 rats are shown; $^*P < 0.001$.

Dexamethasone (0.5 mg kg^{-1}, s.c.), injected 1 h before these hyperalgesic agents, inhibited ($P<0.001$) responses to carrageenin, bradykinin, TNFα, IL-1β and IL-6 but not responses to PGE_2, IL-8 and dopamine (Figure 1). Similarly, injection of dexamethasone (0.04–5.0 μg/paw), 1 h before the hyperalgesic agents, inhibited $P<0.001$), in a dose-dependent manner, responses to carrageenin, bradykinin, TNFα, IL-1β and IL-6 but not responses to PGE_2, IL-8 and dopamine (Figure 2).

Abolition by an anti-lipocortin-1_{2-26} antiserum of the anti-hyperalgesic effect of dexamethasone

Inhibition by dexamethasone (0.5 mg kg^{-1}, s.c.) of bradykinin (500 ng/paw) evoked and IL-1β (0.5 pg/paw) evoked hyperalgesia (measured 3 h later) was abolished by treatment with LCPS1 (0.5 ml kg^{-1}, s.c., 24 h and 1 h before the dexamethasone, Figure 3).

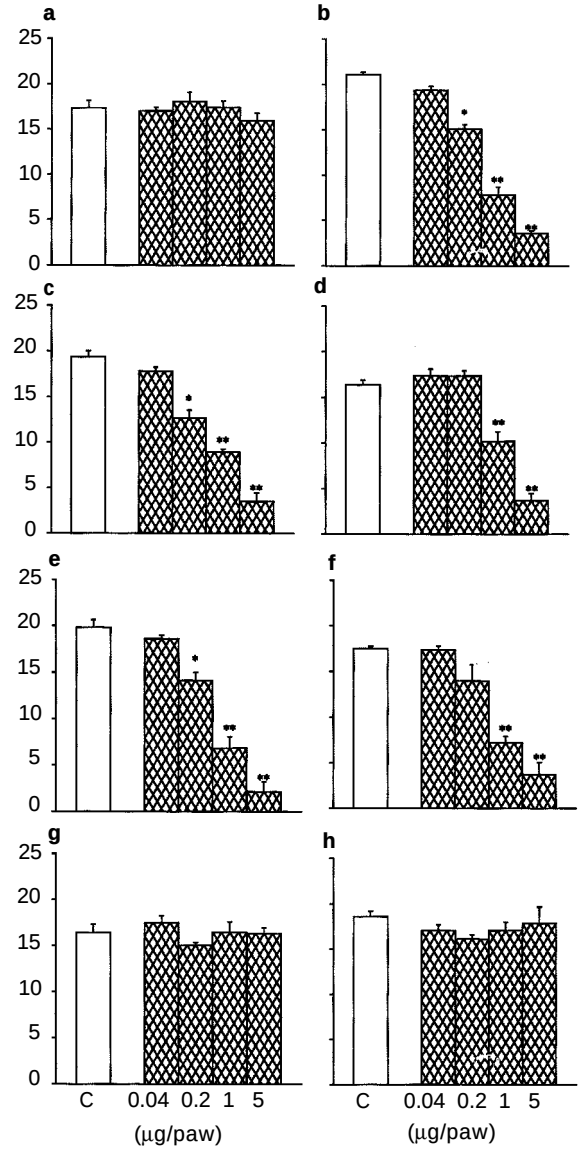

Figure 2 Effect of local administration of dexamethasone on the hyperalgesic responses to (a) PGE_2, (b) carrageenin, (c) bradykinin, (d) TNFα, (e) IL-1β, (f) IL-6, (g) IL-8 and (h) dopamine. Responses were measured 3 h after injection (in 100 μl, i.p.) of PGE_2 (100 ng), carrageenin (100 μg), bradykinin (500 ng), TNFα (2.5 pg), IL-1β (0.5 pg), IL-6 (1.0 ng), IL-8 (0.1 ng) and dopamine (10 μg). Dexamethasone (0.04–5.0 μg in 100 μl, i.p., hatched columns) or saline (control, C, 100 μl, open columns) was given 1 h before hyperalgesic substances. Means ± s.e.means in groups of 5 rats are shown; $^*P<0.05$, $^{**}P<0.001$.

Effect of lipocortin-1_{2-26} on the hyperalgesic responses to PGE_2, bradykinin, TNFα and IL-1β

Injection of PGE_2 (100 ng), bradykinin (500 ng), TNFα (2.5 pg) and IL-1β, each into one hind paw, evoked hyperalgesia, measured 3 h after injection (Figure 4). Lipocortin-1_{2-26} (100 μg, i.pl.), injected 1 h before these hyperalgesic agents, markedly inhibited ($P<0.001$) responses to bradykinin, TNFα and IL-1β, but not the response to PGE_2 (Figure 4).

Reversal by dexamethasone and lipocortin-1_{2-26} of arachidonic acid-induced potentiation of the hyperalgesic effect of IL-1β

Injection of IL-1β (0.25 pg) into one hind paw evoked hyperalgesia, measured 3 h after injection and this response was markedly potentiated ($P<0.0001$) by arachidonic acid (50 μg, i.pl.), given 10 min before the IL-1β (Figure 5). The potentiated response to arachidonic acid + IL-1β was markedly attentuated ($P<0.0001$) by dexamethasone (5 μg) and lipocortin-1_{2-26} (100 μg), each given i.pl. (in 50 μl), 40 min before the IL-1β (Figure 5).

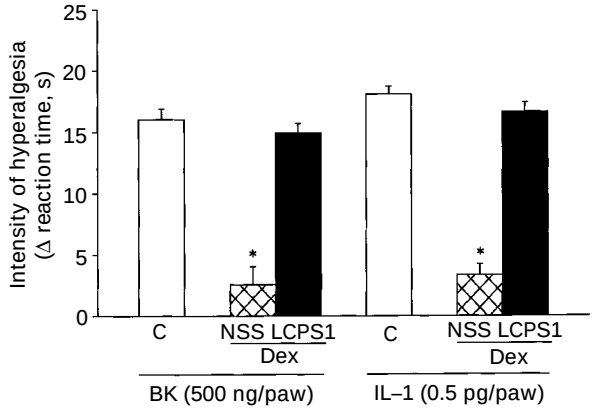

Figure 3 Abolition by LCPS1 of the anti-hyperalgesic effect of dexamethasone. Responses were measured 3 h after injection (in 100 μl, i.pl.) of bradykinin (BK, 500 ng) and IL-1β (IL-1, 0.5 pg). Dexamethasone (Dex, 0.5 mg kg^{-1}, s.c.), saline (0.5 ml kg^{-1}, s.c.), or no pretreatment (control, C) was given 1 h before hyperalgesic substances. LCPS1 (0.5 ml kg^{-1}, solid columns) or non-immune serum (NSS, 0.5 ml kg^{-1}, cross-hatched columns) was injected, s.c., 24 h and 1 h before dexamethasone. Means ± s.e.means in groups of 5 rats are shown; $^*P<0.001$.

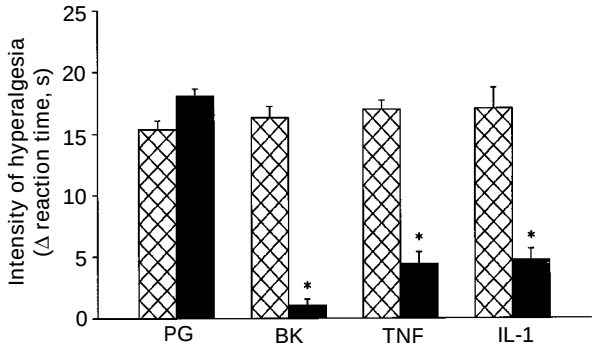

Figure 4 Effect of lipocortin-1_{2-26} on the hyperalgesic responses to PGE_2, bradykinin, TNFα and IL-1β. Responses were measured 3 h after injection (in 100 μl, i.pl.) of PGE_2 (PG, 100 ng), bradykinin (BK, 500 ng), TNFα (TNF, 2.5 pg) and IL-1β (IL-1, 0.5 pg). Lipocortin-1_{2-26} (100 μg in 100 μl, i.pl., solid columns) or saline (100 μl, i.pl., crosshatched columns) was injected 1 h before these hyperalgesic agents. Means ± s.e.means in groups of 5 rats are shown; $^*P<0.001$.

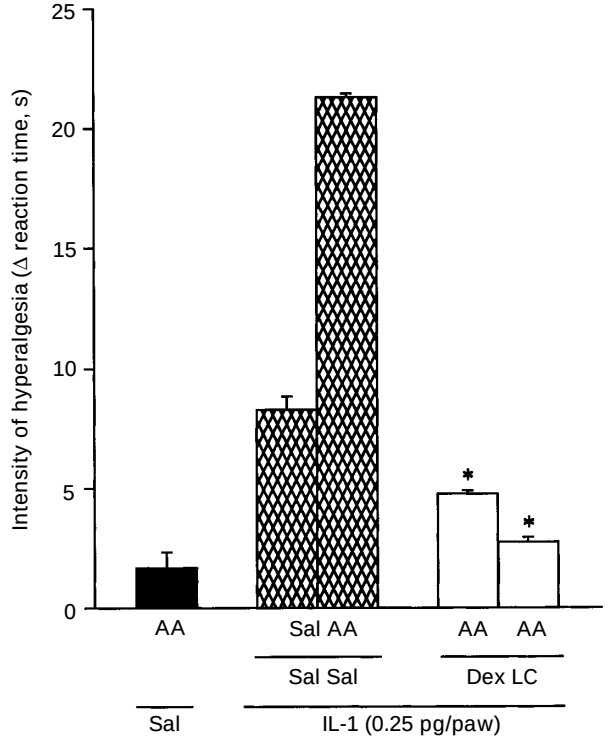

Figure 5 Reversal by dexamethasone and lipocortin-1_{2-26} of arachidonic acid-induced potentiation of the hyperalgesic effect of IL-1β. Animals were injected with saline (Sal, control, 50 μl, i.pl.), lipocortin-1_{2-26} (LC, 100 μg in 50 μl, i.pl.) or dexamethasone (Dex, 5 μg in 50 μl, i.pl.). Thirty minutes later, arachidonic acid (AA, 50 μg in 50 μl, i.pl.) or saline (Sal, 50 μl, i.pl.) was injected and, after a further 10 min, interleukin-1β (IL-1, 0.25 pg in 50 μl, i.pl.) or saline (50 μl, i.pl.) was injected. Means$\pm$s.e.means in groups of 5 rats are shown; $^*P<0.0001$ relative to rats injected with Sal + AA + IL-1.

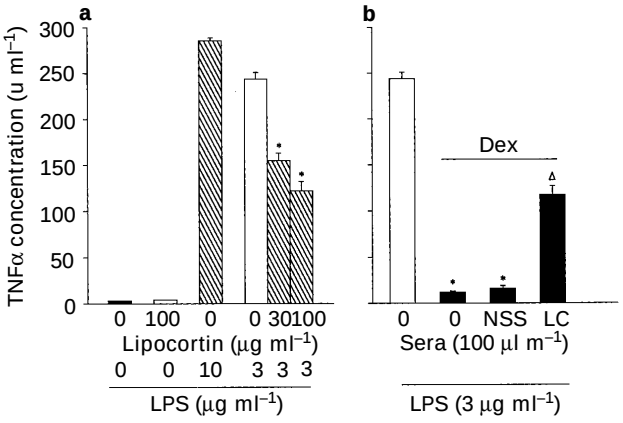

Figure 6 (a) Inhibition by lipocortin-1_{2-26} of TNFα production by LPS-stimulated cells of the murine macrophage-like cell-line J774. (b) Reversal by LCPS1 (LC) of the inhibition by dexamethasone of TNFα production by LPS-stimulated J774 cells. (a) J774 cells treated with RPMI medium (0) lipocortin-1_{2-26} (100 and 30 μg ml^{-1}) and stimulated, 30 min later, with RPMI medium (0) or LPS (10 or 3 μg ml^{-1}). (b) J774 cells treated with RPMI (0) or LCPS1 or sheep non-immune serum (NSS). Fifteen minutes later the cells were treated with dexamethasone (Dex, 10 μg ml^{-1}) and, after a further 30 min, stimulated with LPS (3 μg ml^{-1}). The concentration of TNFα was measured by ELISA. Means $\pm$s.e.means in groups of 5 rats are shown; $^*P<0.05$ relative to LPS-stimulated macrophages not treated with lipocortin-1_{2-26} or dexamethasone, $^{\Delta}P<0.05$ relative to macrophages treated with dexamethasone.

Effects of dexamethasone, lipocortin-1_{2-26} and anti-lipocortin-1_{2-26} serum on TNFα release by J774 cells stimulated with LPS

LPS at 3 μg ml^{-1} and 10 μg ml^{-1} stimulated J774 cells (5×10^{-5} cells/well) to produce 244 ± 7 and 286 ± 3 u ml^{-1} murine TNFα, respectively (Figure 6). Lipocortin-1_{2-26} at 30 μg ml^{-1} and 100 μg ml^{-1} reduced ($P<0.05$) the response to LPS (3 μg ml^{-1}) to 155 ± 8 u ml^{-1} TNFα and 122 ± 10 u ml^{-1} TNFα, respectively (Figure 6). The response to LPS (3 μg ml^{-1}) was abolished by dexamethasone (10 μg ml^{-1}) in the absence and presence of a control, non-immune serum (NSS, 100 μl ml^{-1}, Figure 6). The effect of dexamethasone was partially reversed (-52%, $P<0.05$) by LCPS1 (100 μl ml^{-1}), in the presence of which the response to LPS (3 μg ml^{-1}) + dexamethasone (10 μg ml^{-1}) was $117\pm$u ml^{-1} TNFα. LPS (3 μg ml^{-1}) also stimulated J774 cells (5×10^{-5} cells/well) to produce 5.80 ± 0.66 ng ml^{-1} PGE_2. Lipocortin-1_{2-26} at 30 μg ml^{-1} and 100 mg ml^{-1} reduced this response (to LPS) to 0.61 ± 0.34 ng ml^{-1} PGE_2 (-89%) and 0.05 ng ml^{-1} PGE_2 (-99%), respectively.

Discussion

Previously we showed that carrageenin evoked hyperalgesia by releasing bradykinin, which stimulated the release of TNFα. The TNFα then activated two, distinct pathways: one which involved induction of IL-1β, IL-6 and prostaglandins and another which involved induction of IL-8 and sympathomimetics (Cunha *et al*., 1992; Ferreira *et al*., 1993). These cytokines evoked hyperalgesia at doses which did not cause oedema. The finding of the present study, that dexamethasone inhibited hyperalgesic responses to carrageenin, bradykinin, TNFα, IL-1β, IL-1β/arachidonic acid and IL-6 but not hyperalgesic responses to IL-8, dopamine and PGE_2, is consistent with the existence of these two pathways, with dexamethasone inhibiting the (local) production of prostaglandins and other eicosanoids (Blackwell *et al*., 1980; Hirata *et al*., 1980), and also TNFα, IL-1β and IL-6 (Lew *et al*., 1988; Wage & Baake, 1988; Barton *et al*., 1991) to inhibit one pathway and inhibiting the (local) production of TNFα and IL-8 (Waage & Baake, 1988; Seitz *et al*., 1991) to inhibit the other pathway.

The capactity of LCPS1 to reverse the inhibition by dexamethasone of the hyperalgesic responses to bradykinin and IL-1β, taken together with the finding that hyperalgesic responses to bradykinin, TNFα and IL-1β and IL-1β/arachidonic acid were inhibited by pretreatment with lipocortin-1_{2-26}, suggest that endogenous lipocortin-1 mediated the anti-hyperalgesic effect of dexamethasone and that this biological activity of lipocortin resided within the lipocortin-1_{2-26}. These suggestions accord with data from other models in which immunoneutralization of lipocortin-1 exacerbated acute inflammatory responses and neutralized the anti-inflammatory actions of dexamethasone (Perretti *et al*., 1996), and in which the biological activity of lipocortin was mimicked by the peptide lipocortin-1_{2-26}, albeit at higher doses than the parent protein (Peretti *et al*., 1993).

Dexamethasone abolished the production of TNFα by J774 cells stimulated with LPS whereas lipocortin-1_{2-26} was only partially effective (TNFα production was reduced by some 50%), suggesting that the inhibitory activity of dexamethasone on TNFα production was only partially mediated by lipocortin. This suggestion is supported by the finding that an antibody to lipocortin-1_{2-26} (LCPS1) only partially reversed the inhibitory effect of dexamethasone on TNFα release by J774 cells stimulated with LPS: TNFα production in the presence of dexamethasone was restored by about 50% by LCPS1.

Recently, glucocorticoids such as dexamethasone have been shown to have a biological activity additional to those described above: the capacity to stimulate production of IκB(α) which binds to and inhibits NF-κB, a transcription factor that plays a central role in the induction of a number of cytokine

genes, including TNFα, IL-1, IL-2, IL-3, IL-6, IL-8, interferonγ and granulocyte-macrophage colony-stimulating factor (Auphan *et al*., 1995; Scheinman *et al*., 1995). Several of these genes are also regulated by another transcription factor, AP-1 (Sterling *et al*., 1989; Park *et al*., 1993; Cockerill *et al*., 1993), which has a synergistic effect with NF-κB (Stein *et al*., 1993). The inhibition by glucocorticoids of the activities of both NF-κB and AP-1 provides additional mechanisms to explain the potent anti-inflammatory, anti-hyperalgesic and immunosuppressive effects of these drugs.

The finding that lipocortin-1_{2-26} abolished the inflammatory hyperalgesia mediated by cytokines and an antibody to this peptide abolished the anti-hyperalgesic effect of dexamethasone *in vivo* suggests that the anti-hyperalgesic effect of glucocorticoids in our nociceptive model is entirely dependent upon lipocortin release. Lipocortin-1_{2-26} may also have affected events other than the relese of pro-inflammatory cytokines, such as inhibition of the induction of the inducible cyclooxygenase enzyme (COX-2) responsible for the prostaglandin production that contributes to inflammatory hyperalgesia. Consistent with this possibility was the finding that arachidonic acid, by itself, was not hyperalgesic but potentiated the hyperalgesic effect of IL-1β, which is known to induce COX-2 (Geng *et al*., 1995). Further, both dexamethasone and lipocortin-1_{2-26} strongly inhibited this potentiation and lipocortin-1_{2-26} also abolished the production of PGE_2 by J774 cells stimulated with LPS. Although inhibition of phospholipase A_2 by dexamethasone and lipocortin-1_{2-26} is believed to underlie their anti-inflammatory effects (Flower *et al*., 1979; Hirata *et al*., 1982; Barnes, 1996), the above data suggest that, in inflammatory hyperalgesia, inhibition of the induction of COX-2 (rather than phospholipase A_2) by dexamethasone and lipocortin-1_{2-26} accounts for the anti-hyperalgesic effects of these agents. In addition, lipocortin/lipocortin-1_{2-26} may have a role in the effects of glucocorticoids on NF-κB and AP-1.

In a recent study, lipocortin-1_{1-188} did not inhibit LPS-stimulated release of TNFα and PGE_2 from human peripheral blood mononuclear cells (Sudlow *et al*., 1996). However, since the cells stimulated, the size and doses of the lipocortin fragment tested and the type and dose of the LPS used were all different in the earlier study, the reasons for this difference are not known. Clearly, further studies on the relative sensitivity to lipocortin, lipocortin-1_{1-188} and lipocortin-1_{2-26} of LPS-stimulated mononuclear cells and macrophages from different species are required.

The above data are consistent with an important role for endogenous lipocortin-1_{2-26} in mediating the anti-hyperalgesic effect of dexamethasone, with inhibition of the production of hyperalgesic cytokines and inhibition of the induction of COX-2 contributing to this role.

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