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Topical glucocorticoids inhibit neurogenic inflammation: involvement of lipocortin 1

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Abstract

Topical glucocorticoid treatment (betamethasone-17-valerate (0.018 mg/cm^2, 3 h pretreatment) significantly inhibited neurogenic oedema formation induced by electrical antidromic stimulation (2 Hz, 15 V, 0.1 ms for 5 min) of the rat saphenous nerve; a response mediated by neuropeptides released from activated capsaicin-sensitive sensory C-fibres. Oedema formation was estimated by measurement of extravasation of i.v. injected ^{125}I-albumin into skin. The inhibitory effect of the topical glucocorticoid was reversed by passive immunisation of rats with polyclonal antibody to the glucocorticoid-inducible anti-inflammatory protein lipocortin 1 (1 ml/kg, s.c., 24 h pretreatment) whilst a non-immune serum was without effect. Similarly the glucocorticoid receptor antagonist RU38486 (20 mg/kg, 2 and 20 h pretreatment) abrogated the response indicating specific binding to glucocorticoid receptors. Topical glucocorticoid treatment also inhibited the oedema produced by intradermal substance P (0.1 nmol) in the dorsal skin of rats. Topical glucocorticoid inhibited neurogenic oedema formation partly through a mechanism dependent upon lipocortin 1. This inhibition may be partly due to a post-junctional effect upon substance P activity/binding however a pre-junctional component cannot be excluded.

Keywords: Lipocortin 1; Neurogenic inflammation; Edema; Topical glucocorticoid

1. Introduction

The topical steroids are the first-line treatment for many types of inflammatory skin diseases, although their mechanism of action has remained rather speculative. Studies from our group have suggested that one possible action may be at the level of the sensory C-fibre nerve-ending to alter the activity of pro-inflammatory neuropeptides released from these fibres following stimulation.

Over the past few years there has been a growing interest in the possible involvement of sensory nerves in the pathogenesis of several inflammatory diseases. Specifically relevant to this study is the potential involvement of over-activation of these fibres in skin diseases, including psoriasis (Bernstein et al., 1986; Anand et al., 1991). Antidromic stimulation of these sensory afferent fibres induces the release of neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP) (for review see Holzer, 1988). Both of these neuropeptides are pro-inflammatory in several species with substance P causing increases in both microvascular permeability and vasodilatation, and CGRP potentiating the effect of substance P through its vasodilator action (Gamse and Saria, 1985; Brain and Williams, 1985). More recently it has also been suggested that these two neuropeptides may affect the cellular component of an inflammatory response as both possess chemotactic activity for polymorphonuclear cells (Perretti et al., 1993; Ahluwalia and Perretti, 1994).

Topical steroid treatment inhibits changes in skin

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blood flow of the rat induced by mild local heating of the skin (Ahluwalia and Flower, 1993a), a response also inhibited by depletion of neuropeptides from sensory fibres with capsaicin (Ahluwalia and Flower, 1993b). These results suggest that the steroids could act at the level of the sensory fibres to alter the vasodilatation response.

Glucocorticoid treatment of rat skin is also associated with increases in the glucocorticoid-inducible protein lipocortin 1 (Ahluwalia et al., 1994). Lipocortin 1 is a member of a family of proteins possessing a highly conserved repeated 70 amino acid motif which confers calcium and phospholipid binding properties. This protein possesses potent anti-inflammatory properties in vivo (Cirino et al., 1989; Perretti and Flower, 1993) and treatment of animals with anti-lipocortin 1-neutralising antiserum reverses the anti-inflammatory effects of steroid treatment implicating lipocortin 1 in this action in several models (for review see Perretti, 1994).

In light of these studies we have now investigated whether topical glucocorticoid treatment inhibits neurogenic inflammation, and whether lipocortin 1 is involved in this glucocorticoid effect. To do this we have used antidromic stimulation of the rat saphenous nerve which results in increases in skin blood flow of the hind paw and oedema formation (Lembeck and Holzer, 1979), the major mediators of which are CGRP and substance P, respectively (Escott and Brain, 1993; Garret et al., 1991). This model provides an ideal system to explore the effects of topical glucocorticoids on neurogenic inflammation of the skin.

2. Materials and methods

2.1. Oedema measurement following saphenous nerve stimulation

Male Wistar rats (250–330 g) were anaesthetised using sodium pentobarbitone (45 mg/kg, i.p.), both paws were shaved, a depilatory cream applied to remove any of the remaining hair and the animals left for a minimum of 1 h before carrying out any further procedure. After this time, the saphenous nerve in both paws were carefully dissected free and ligated to prevent any central reflexes following stimulation, following the method described by Escott and Brain (1993) with minor modifications. The nerve of the left leg only, the right serving as a sham control, was placed on bipolar platinum electrodes. At 15 min after this procedure 2 μCi/kg of ^{125}I-labelled human serum albumin in Evan's blue dye (1%) was injected into the tail vein and the nerve stimulated for 5 min (2 Hz, 1 ms pulse duration at 15 V). This level of stimulation led to submaximal oedema formation as assessed in preliminary experiments. After nerve stimulation blood was collected by intracardiac puncture and the animal killed with anaesthetic overdose. The skin from the left paw in which Evans blue dye extravasation could be observed was removed and weighed. An equivalent area of skin was taken from the sham paw and weighed. The radioactivity found in both skin samples and plasma was measured using a γ-counter.

To investigate the effect of topical glucocorticoid treatment upon this response the skin of the hind paw was treated with betamethasone-17-valerate (0.018 mg/cm^2 dissolved in acetone) pipetted evenly over the skin of the paw (approximately 5 cm^2 area) 3 h prior to use, a regimen which is known to be anti-inflammatory and which results in a concomitant induction of lipocortin 1 (Ahluwalia et al., 1994). Control animals were treated with the acetone vehicle only. To investigate the involvement of lipocortin 1 in the effect of steroid treatment, one group of animals was treated with a specific anti-lipocortin 1 sheep antiserum (Croxtall and Flower, 1992) (1 ml/kg, s.c. 24 h prior to use), whilst the other control group were treated with a non-immune sheep serum only. To determine whether the inhibitory effect of the topical steroid was due to specific binding to glucocorticoid receptors some animals were treated with the glucocorticoid receptor antagonist RU38486. This drug (20 mg/kg) was administered orally both 2 and 20 h prior to topical steroid treatment.

2.2. Oedema measurement following exogenous mediator administration

Male Wistar rats were anaesthetised as described above, the dorsal skin shaved and a depilatory cream applied. The formation of local oedema in the skin of rats was measured using the technique described by Williams (1979). Briefly, 2 μCi/kg of ^{125}I-labelled human serum albumin in Evans blue dye (1%, 1 ml/kg) was injected i.v. and then various agents were injected i.d. (100 μl volume into each site) into the back of the rat in duplicate, these comprised: substance P (0.1 nmol) alone or in the presence of CGRP (10 pmol), CGRP (10 pmol) alone, compound 48/80 (1 μg), 5-hydroxytryptamine (5-HT) (1 nmol), PAF (10 nmol) and saline. After 30 min blood samples were taken by intracardiac puncture, the rat killed by anaesthetic overdose and the skin removed. The skin sites were punched out, as visualised by the blue areas) with a 17 mm diameter hole punch and radioactivity in both these sites and the plasma was assessed using a γ-counter.

To investigate the effect of steroid treatment upon oedema formation animals were pretreated with betamethasone-17-valerate 3 h prior to administration of i.d. injections. Control animals were treated with vehicle at the same time.

2.3. Data and statistical analysis

Plasma extravasation into the skin is expressed as μl of plasma, where the radioactivity in 1 μl of plasma from the same animal was measured and used to calculate the equivalent μl of plasma in each of the skin sites. In the case of the electrical stimulation experiments the oedema is expressed per 100 mg of tissue and is a net value with the response of the unstimulated contralateral paw being subtracted from that of the stimulated paw. All values are expressed as means ± S.E.M. Statistical differences between treatments were determined using Student's *t*-test for unpaired data. Values were considered to be statistically different when $P < 0.05$.

2.4. Drugs

Betamethasone-17-valerate, substance P, 5-HT creatinine sulphate, PAF, Evans blue and normal sheep serum were all purchased from Sigma Chemical Company, Poole, UK. α-CGRP (human) was purchased from Bachem, UK and ^{125}I-human serum albumin from Amersham International, UK. RU38486 (mifepristone) was a generous gift of Dr R. Deraedt of Roussel-Uclaf, Romainville, France. Betamethasone-17-valerate was dissolved in acetone on the day of use. Stock solutions of PAF were prepared in absolute ethanol, 5-HT and CGRP were made up in sterile water, whilst substance P was dissolved in 0.1 N acetic acid. All stock solutions were kept at −20°C. All dilutions were made in sterile saline (0.9% NaCl) on day of use. Evans blue was dissolved in sterile saline. A suspension of RU38486 was made in sterile water on day of use.

3. Results

3.1. Endogenous neuropeptide activity

Electrical stimulation of the left saphenous nerve resulted in plasma extravasation in the left paw, with no oedema formation in the contralateral control paw. Treatment with topical glucocorticoid inhibited oedema formation significantly reducing the response by approximately 55% ($n = 9$, $P < 0.01$, see Fig. 1). Pretreatment of animals with non-immune sheep serum had no effect upon the activity of the glucocorticoid which inhibited oedema formation to a similar extent as in untreated animals, i.e. 65% inhibition ($n = 6$, $P < 0.05$, see Fig. 1). However, in animals treated with antiserum to lipocortin 1 the anti-inflammatory activity of the steroid was partially reversed with only a 29% inhibition ($n = 6$) of the response which was not significantly different from the vehicle control (Fig. 1). The glucocorticoid receptor antagonist RU38486 reversed the

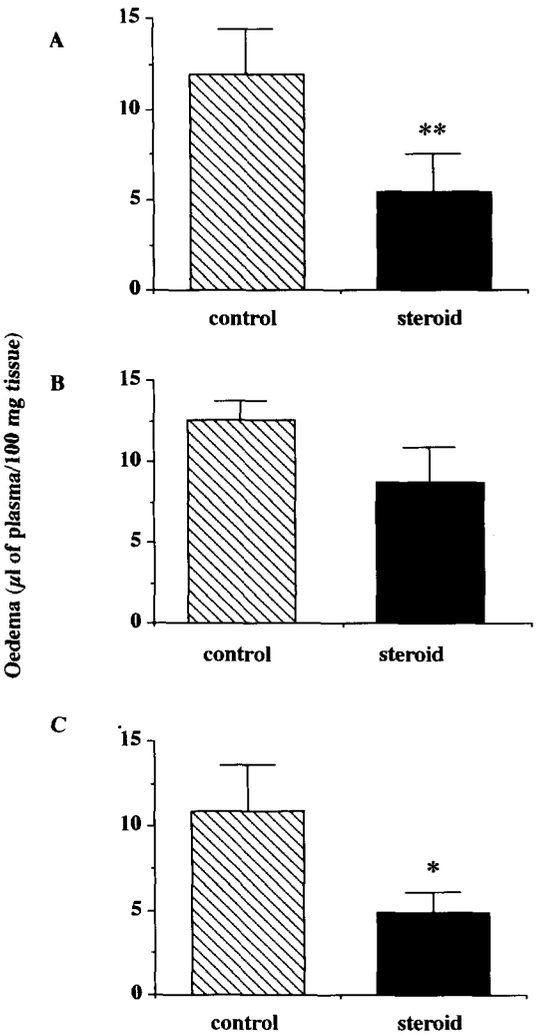

Fig. 1. Pretreatment with a specific neutralising anti-serum to the glucocorticoid-inducible protein lipocortin 1 (1 ml/kg, 24 h pretreatment s.c.) reverses the anti-inflammatory effect of topical betamethasone-17-valerate (0.09 mg in acetone) upon oedema induced by electrical stimulation (2 Hz, 0.1 ms duration, 15 V for 5 min) of the rat saphenous nerve. Each graph shows oedema formation in vehicle-treated animals shown by the hatched bars and the glucocorticoid-treated paws shown by the solid bars and each value is a mean ± S.E.M. (represented by the vertical bars). Panel A shows responses in animals with no systemic treatment ($n = 7$ for control and $n = 6$ for steroid-treated), B shows the responses in rats pretreated with a sheep antibody to lipocortin 1 ($n = 4$ for controls and $n = 6$ for steroid-treated) and panel C the responses in animals pretreated with control non-immune sheep serum ($n = 5$ for controls and $n = 6$ for steroid-treated. Statistical significance is shown as $^{*}P < 0.05$ and $^{**}P < 0.01$.

effect of the topical glucocorticoid treatment thus blocking its inhibitory action upon oedema formation ($n = 5$, Fig. 2). Pretreatment of animals with RU38486 alone had no effect upon oedema formation in paws treated with the vehicle giving a response of 14.5 ± 2.2 μl/100 mg tissue ($n = 6$), which was not significantly different from untreated animals (14.5 ± 2.3 μl/100 mg tissue, $n = 6$).

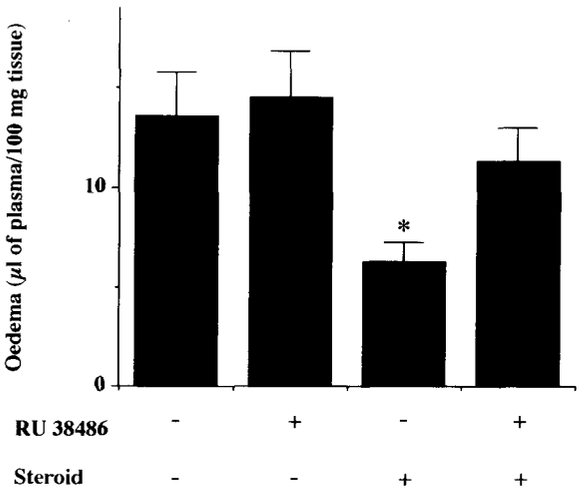

Fig. 2. Blockade of glucocorticoid receptors using the antagonist RU38486 partially inhibits the anti-inflammatory effect of betamethasone-17-valerate (0.09 mg in acetone) upon oedema induced in the rat paw following electrical stimulation of the saphenous nerve. Animals were pretreated with RU38486 (20 mg/kg) 2 and 20 h o.p. prior to experimentation. Values shown are means ± S.E.M. (represented by the vertical bars) of $n = 6$. Statistical significance is shown as * $P < 0.05$.

3.2. Exogenous mediator activity

Topical steroid treatment inhibited the oedema responses to intradermal PAF and 5-HT (Fig. 3) confirming our previous findings. This treatment also inhibited the responses to both substance P alone and in combination with CGRP ($P < 0.05$). The steroid had no effect, however, upon the response to CGRP alone or compound 48/80 (Fig. 3). Intradermal application of saline was unaffected by topical glucocorticoid treatment with values of 6.1 ± 1.3 μl ($n = 6$) of oedema in drug-treated animals and 9.5 ± 1.8 μl ($n = 6$) in vehicle-treated controls (not significantly different).

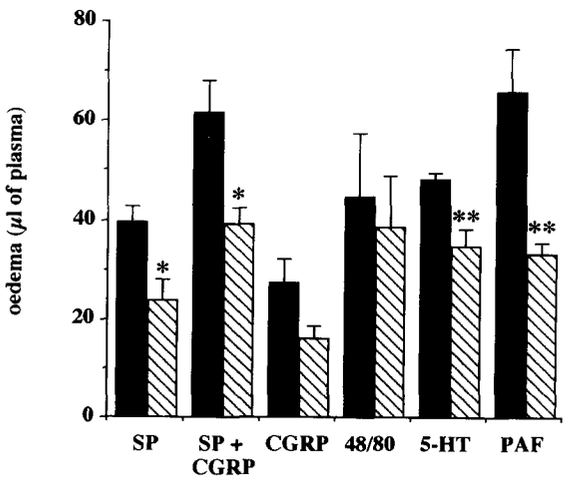

Fig. 3. Effect of topical betamethasone-17-valerate upon oedema formation in rat skin in response to a variety of putative mediators of inflammation. The solid bars represent the responses to each mediator in vehicle-treated animals and the hatched bars the responses in glucocorticoid-treated rats. The stimuli consist of substance P (substance P; 0.1 nmol) in the absence and presence calcitonin gene-related peptide (CGRP; 10 pmol), CGRP alone, compound 48/80 (48/80; 1 μg), 5-hydroxytryptamine (5-HT; 1 nmol) and platelet activating factor (PAF; 10 nmol). Saline caused an oedema response of 6.1 ± 1.3 μl ($n = 6$) in drug-treated animals and 9.5 ± 1.8 μl ($n = 6$) in vehicle-treated controls. Values shown are means ± S.E.M. (represented by the vertical bars) of $n = 6$. Statistical significance is shown as * $P < 0.05$ and ** $P < 0.01$.

4. Discussion

The mechanism of the anti-inflammatory action of the topical glucocorticoids is undoubtedly multifactorial, but recent evidence points to an effect at the level of sensory C-fibres. Steroid treatment suppressed local heat-induced increase in skin blood flow, a response dependent upon an intact capsaicin-sensitive C-fibre system (Ahluwalia and Flower, 1993a; Ahluwalia and Flower, 1993b) but did not reduce the response to capsaicin per se (Ahluwalia and Flower, 1993a). Glucocorticoid treatment in vitro had no effect upon capsaicin-induced vasodilator responses in the perfused rabbit ear but did alter the release of neuropeptide which occurred following capsaicin treatment (Ahluwalia and Flower, 1994). The data suggested two different modalities of neuropeptide release, one following acute capsaicin treatment and another tonic release of neuropeptide which is sensitive to glucocorticoid treatment. It was in light of these results that we chose antidromic stimulation of the rat saphenous nerve leading to neurogenic inflammation which used a physiological rather than a pharmacological stimulus.

Topical glucocorticoid treatment using betamethasone-17-valerate significantly attenuated oedema produced in the paw skin following antidromic electrical stimulation of the saphenous nerve. This inhibitory effect of this treatment was in part reversed by prior treatment of animals with a specific antiserum which neutralises lipocortin 1 in vivo in the rat (Perretti and Flower, 1993; Duncan et al., 1993) implying that the anti-inflammatory effects of the topical glucocorticoid in this system are, at least partly, mediated by this protein. To control for any effects of the serum alone animals were also immunised with a non-immune sheep serum. This had no effect either upon the absolute oedema in control animals or on the inhibitory activity of the glucocorticoid itself. Together these results suggest that the anti-inflammatory effect of the glucocorticoid in this particular model may, in part at least, be brought about by the glucocorticoid-inducible protein lipocortin 1 acting at the level of the sensory neurone. The oedema response to the electrical stimulation used in these experiments is known to be due to activation of sensory afferent C-fibres since systemic pretreat-

ment with capsaicin blocked the response (Jancso et al., 1967). Selective tachykinin NK_1 receptor antagonists (Garret et al., 1991; Moussaoui et al., 1993), also block the oedema response following antidromic stimulation suggesting that the response is in fact due to the release of substance P from nerve fibre endings. Thus it would seem reasonable to suggest that the glucocorticoid treatment is in some way interfering with sensory nerve-mediated responses whether it be pre-or post-junctional.

In the rat the same glucocorticoid treatment regimen as used here induces lipocortin 1 on the surface of cells which is maximal at 3 h (Ahluwalia et al., 1994). This induction mirrors the inhibitory activity seen here and provides further support for the proposal that lipocortin 1 may be involved in the glucocorticoid-induced inhibition of neurogenic inflammation. Moreover the ability of the antibody to reverse the effect of the glucocorticoid would suggest that the lipocortin 1 is either soluble or on the cell surface.

In other models the inhibitory activity of glucocorticoids at sensory C-fibres appears to be prejunctional (Ahluwalia et al., 1994). However, our present studies reported here demonstrate that glucocorticoids also inhibit the activity of substance P in a post-junctional manner. This does not exclude the possibility that there may also be a prejunctional effect of these agents. This effect of betamethasone-17-valerate upon substance P activity is in agreement with findings in the rat trachea showing that the oedema induced by substance P was blocked by prior treatment with dexamethasone: Piedimonte et al. (1990, 1991) suggested that this inhibitory effect was due to an upregulation of the enzymes which catabolise the neuropeptide, thereby restricting its duration of action. It is possible that the effect of betamethasone-17-valerate upon the response to substance P in our model is brought about in the same manner, however this is something which needs to be pursued further. Since it is well established that substance P may also degranulate mast cells (an event which apparently is not receptor-mediated (Devillier et al., 1985)), we also investigated the effect of topical glucocorticoid treatment upon the inflammatory response to the mast cell degranulator compound 48/80. We found no amelioration of the oedema response to this substance following topical betamethasone-17-valerate suggesting that the effect of the glucocorticoid upon the response to substance P was not due to an action at the level of the mast cell. We have also verified that the inhibitory action of topical glucocorticoid treatment in this model is receptor-mediated since the response was reversed following prior treatment with RU38486.

From these results it would appear that induction of lipocortin 1 on the cell surface following topical glucocorticoid treatment is associated with anti-inflammatory activity in this model of neurogenic inflammation. Our study does not exclude other contributing mechanisms of action such as an effect upon the processing of substance P or an effect upon NK_1 receptor numbers.

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