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Antiflammin-2, a nonapeptide of lipocortin-1, inhibits leukocyte chemotaxis but not arachidonic acid mobilization

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Abstract

We have studied the effects of antiflammin-2, a fragment corresponding to amino acids 246–254 of lipocortin-1 (HDMNKVLDL), on arachidonate mobilization and metabolism and we also determined the effect of antiflammin-2 on the chemotaxis of phagocytes. Our results demonstrated that antiflammin-2 was not able to diminish significantly [^{3}H]arachidonic acid mobilization stimulated by 4β-phorbol-12-myristate 13-acetate or calcium ionophore A23187 in murine 3T6 fibroblasts or resident peritoneal macrophages. Further, antiflammin-2 had no effect on arachidonate metabolism. In contrast, a glucocorticoid such as dexamethasone reduced significantly [^{3}H]arachidonic acid release and arachidonate metabolism induced in both cells. This study confirms the inhibitory effect of antiflammin on leukocyte migration and suggests that it acts partly through the inhibition of leukocyte binding to endothelial cells.

Keywords: Antiflammin; Arachidonic acid; Phospholipase A_2; Chemotaxis; Inflammation; Anti-inflammatory drug

1. Introduction

Glucocorticosteroids are highly effective in modulating chronic inflammatory and immune responses, although their mode of action remains uncertain (Barnes and Adcock, 1993). However, we now know that this anti-inflammatory activity of glucocorticoid hormones is the result of several modulating effects exerted on cells and mediators involved in the induction of the inflammatory process (Schleimer et al., 1989). Thus, several studies indicate that the anti-inflammatory action of glucocorticoids is mediated by the induction of regulatory proteins. Some of these proteins, called lipocortins (Di Rosa et al., 1984) or uteroglobins (Miele et al., 1987) have been characterized and shown to have an inhibitory effect on phospholipase A_2 (E.C. 3.1.1.4.), thereby preventing arachidonic acid mobilization and the subsequent biosynthesis of pro-inflammatory mediators such as eicosanoids (Davidson and Dennis, 1989; Mukherjee et al., 1992; Leyton et al., 1994).

The first successful attempt to find active lipocortin- or uteroglobin-derived peptides came with the identification of a region of homology between both proteins (Miele et al., 1988). The corresponding nonapeptides have been termed antiflammins. Antiflammin-1 (MQMKKVLDS) is equivalent to the 9-amino acid C-terminal portion of α-helix 3 in uteroglobin, whereas antiflammin-2 (HDMNKVLDL) corresponds to the 246–254 sequence of lipocortin-1. Initially, both antiflammins were reported to inhibit, in a concentration-dependent manner, phospholipase A_2 activity in vitro and to reduce the oedema response to carrageenin when they were injected locally into the rat paw in vivo (Miele et al., 1988). However, these effects of antiflammins have been questioned by several authors (Van Binsbergen et al., 1989; Marki et al., 1990; Hope et al., 1991). Irrespective of this, several independent investigators have confirmed the anti-inflammatory nature of antiflammins. Thus, antiflammins inhibited the synthesis of platelet-activating factor from human neutrophils (Camussi et al., 1990), platelet aggregation induced by ADP (Vostal et al., 1989) or by collagen (Lloret and Moreno, 1992), ocular inflammation in rats (Chan et al., 1991), carrageenan rat paw oedema (Lloret and Moreno, 1994) and murine ear oedema (Cabrè et al., 1992; Lloret and Moreno, 1992, 1995). In these latter studies, we demonstrated, using different experimental models of in-

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flammation, that both peptides have potent anti-inflammatory actions, in the absence of any effect on several purified phospholipases A_2, and it was suggested that antiflammins could interfere with any step in the process of phospholipase A_2 activation in whole cell systems. There is, however, an alternative mechanism to explain the anti-inflammatory effect of antiflammins, which may involve an antichemotactic effect on cells implicated in the development of the inflammatory response such as phagocytes. Thus, at the site of infection, glucocorticoids prevent the ingress of polymorphonuclear cells and impair the competence of those cells present (Tsurufuji et al., 1984). There are many ways in which glucocorticoids could control the egress of neutrophils from the vascular system to the inflammatory focus. There could be decreased synthesis of the chemotactic factor, decreased expression of adhesion molecules on the vascular wall, some action on the neutrophil itself. Furthermore, this effect of glucocorticoids on leukocyte migration is mediated through the production of the protein lipocortin-1 by neutrophils (Flower and Rothwell, 1994). We must consider that several authors have described this antichemotactic effect of lipocortins and uteroglobins (Vasanthakumar et al., 1988; Perretti and Flower, 1993).

The purpose of this paper was to study the effect of antiflammins on the mobilization and metabolism of arachidonic acid in mouse resident peritoneal macrophages and mouse fibroblasts 3T6, and to determine the effect of antiflammin-2 on chemotaxis of phagocytes.

2. Materials and methods

2.1. Materials

[5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid (180–240 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA, USA). Dexamethasone, calcium ionophore A23187, 4β-phorbol-12-myristate 13-acetate (PMA), *N*-formyl-Met-Leu-Phe (FMLP) and sialidase (*V. cholerae*, type III) were purchased from Sigma (St. Louis, MO, USA). Antiflammin-2 (molecular weight 1084) was supplied by Bachem Feinchemikalien (Bubendorf, Switzerland). Purity was always > 95% as determined by high pression liquid chromatography; amino acid composition and molecular mass were confirmed by mass spectrometry (data from the manufacturer). Antiflammin-2 was stored as lyophilized powder at 4°C and dissolved in either phosphate buffer or cell culture medium prior to use. PMA and dexamethasone were kept as stock solutions in dimethylsulfoxide (DMSO) and samples from each experiment were freshly diluted in culture medium. The final concentration of DMSO never exceeded 0.1%. Ionophore A23187 was stored as an ethanol solution. Ethanol was evaporated and A23187 was resuspended in medium when needed. All other reagents were of analytical grade.

2.2. Cell cultures

Mouse peritoneal macrophages were collected from male CD-1 mice (Charles River, France) (20–25 g), which had been killed by carbon dioxide asphyxiation, and the peritoneal cavity was lavaged with Hank's balanced salt solution containing 1% bovine serum albumin, 20 units of heparin/ml, 100 units of penicillin/ml and 100 μg/ml of streptomycin. Lavage fluids were pooled and centrifuged at 400 × *g* for 10 min at 4°C to pellet cells. Macrophages were resuspended in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with the antibiotics and 10% heat-inactivated foetal calf serum (Gibco BRL), counted and plated in plastic 12-well cluster dishes (Costar, Cambridge, MA, USA). Macrophages were allowed to adhere for 2 h at 37°C in an atmosphere of CO_2/air (1:19) and 100% humidity. The non-adherent cells were removed by washing the cell layer with phosphate-buffered saline (PBS). The adherent cells, which we had previously demonstrated by morphological criteria to be > 95% macrophages, were maintained in medium RPMI 1640 supplemented with 10% foetal calf serum and antibiotics.

Stock cultures of murine Swiss 3T6 fibroblasts were obtained from Dr N. Suesa (Lab. Menarini, Barcelona, Spain) and were routinely maintained in RPMI 1640 supplemented with foetal calf serum (10%) and antibiotics in a subconfluent state with subculture every 3–4 days at 37°C in 5% CO_2-95% air. For experimental use, subconfluent cultures were removed by treatment with trypsin/EDTA and seeded into 12-well plates at a density of 10^3 cells/well. Cells were allowed to replicate to 10^4 cells/well prior to each experiment.

2.3. Incorporation and release of [^{3}H]arachidonic acid and its metabolites

After a 20 h incubation of macrophages or a replication period for fibroblasts, the medium was removed and replaced with 0.5 ml of RPMI containing 0.1% fatty acid free bovine serum albumin and 0.1 μCi of [^{3}H]arachidonic acid, and the samples were incubated for another 6 h at 37°C. Cells were then washed three times in medium containing 0.5% bovine serum albumin to remove unincorporated [^{3}H]arachidonic acid. The macrophages and fibroblasts used in these studies incorporated 48 ± 4% and 56 ± 3%, respectively, of the total [^{3}H]arachidonic acid. Then, the labelled cells were incubated with various agents for the times indicated. After this period, the medium was removed for analysis of radioactivity release. At the end of each experiment, the cell monolayer was overlaid with 1% Triton X-100, and the cells were scraped off the dishes. Finally, the radioactivity present in the medium and in the cell fraction was measured by scintillation counting, using a Packard Tri-Carb 1500 counter. The amount of [^{3}H]arachidonic acid released into the medium as a result

of the specified treatment was determined and expressed as a percentage of cell-incorporated [^{3}H]arachidonic acid, which was determined in solubilized cells. Background release from untreated cells (about 9 ± 3% of [^{3}H] incorporated in macrophages, and 12 ± 2% incorporated in fibroblasts) has been subtracted from all data.

To determine the amount of [^{3}H]arachidonic acid metabolized, 0.5 ml of cell medium was extracted with ethyl acetate (5 ml) acidified with 1% formic acid. The organic phase was evaporated under a nitrogen stream, resuspended in 100 μl chloroform, and resolved in thin layer chromatography plates with diethyl ether:hexane:acetic acid (60:40:1, v/v/v). Thin layer chromatography plates were exposed to Rhodamine B (0.2%) and activated at 100°C. [^{3}H]Arachidonic acid and [^{3}H]arachidonic acid metabolites will fluoresce under U.V. (370 nm). Finally, the radioactive spots were quantified by scraping them off and scintillation counting them.

2.4. Mononuclear chemotaxis assay in vitro

Fifty milliliters of heparinized peripheral blood were obtained from healthy human volunteers and mixed with 8 ml of 6% dextran. Mononuclear cells were further separated from white blood cells on Ficoll gradients. After centrifugation at 1200 × *g* for 20 min at 20°C, mononuclear cells were removed from the interface, washed twice in Hank's modified medium and kept at 4°C until use. The purity of the mononuclear cells was > 95% as assessed by May-Grünwald-Giemsa staining.

Chemotaxis assays were carried out in Boyden chambers according to Venge (1979). 10^6 blood peripheral mononuclear cells in 0.5 ml of RPMI with BSA were placed in the upper chamber, which was separated from the bottom one by a Millipore filter of 3 μm pore size and 150 μm thickness. The bottom chamber contained RPMI-BSA with or without FMLP (10 nM). After a 60-min incubation at 37°C, the upper chamber was emptied and washed with PBS containing 2 mM EDTA to remove the cells that had not entered into the filters. To quantify chemotaxis, the filters were removed, washed in PBS, and stained with 0.2% crystal violet in 10% ethanol. The filters were thoroughly washed in water and the dye was eluted with 33% acetic acid to measure the absorbance at 540 nm.

2.5. FMLP-induced neutropenia

We used the method established by Perretti et al. (1993). Mice received an intravenous injection of vehicle (PBS), sialidase or antiflammin-2 30 min before FMLP (10 ng i.v.) administration. We used the sialidase concentration chosen by Harris et al. (1995). Total leukocyte numbers in heparinized mouse blood samples were measured with a Coulter-Counter, whereas differential counting was performed in a Neubauer haemocytometer following staining with Turk's solution (1 part blood:10 parts Turk's). The total number of PMN for each mouse was then calculated.

2.6. Statistical analysis

For the cell culture experiments, data are expressed as the means ± standard error of the mean (S.E.M.). All experiments in vitro were performed at least three times to ensure consistency of the observations. Significance of differences between data points and control was determined using a 2-tailed Student's *t*-test with $P < 0.05$ confidence limits.

3. Results

To evaluate the action of antiflammin-2 on arachidonate mobilization, we labelled macrophages isolated from mouse peritoneal cavity and murine 3T6 fibroblasts with [^{3}H]arachidonic acid. The release of label into the cellular supernatant was considered to reflect phospholipase A_2 activity. The supernatant, PMA- or A23187-stimulated peritoneal macrophages contained 15 ± 1.5% or 23 ± 1.1% of total [^{3}H]arachidonic acid incorporated into cells, respectively. Fig. 1 shows that neither dexamethasone (1 μM) nor antiflammin-2 (100 μM) inhibited [^{3}H]arachidonic acid release induced by either agonist during the first 6 h of incubation. However, after prolonged incubation with dexamethasone (24 h), a partial inhibition (50%) of the ionophore-induced arachidonate release became evident. In contrast, dexamethasone was much more efficient in inhibiting PMA-induced arachidonic acid release at this time. Thus, treatment with dexamethasone for 24 h resulted in 90–95% inhibition of PMA induced release of arachidonate (Fig. 1).

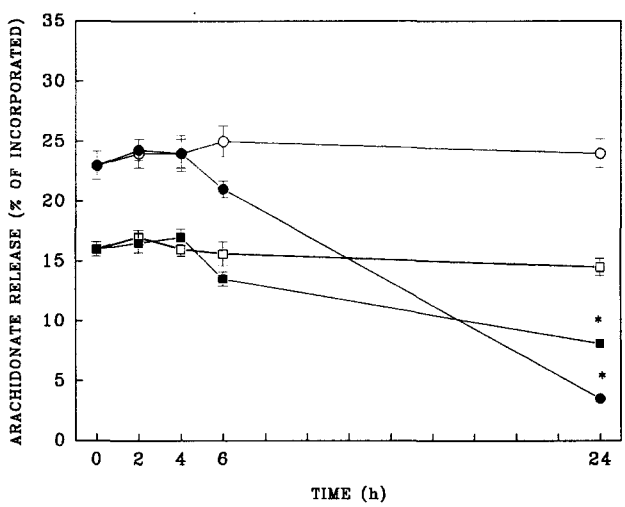

Fig. 1. Time-course of the effect of dexamethasone (filled symbols) and antiflammin-2 (hollow symbols) on [^{3}H]arachidonic acid release elicited by murine resident peritoneal macrophages. Cell activation was induced by PMA (1 μM, circles) and calcium ionophore A23187 (1 μM, squares) for 2 h at 37°C. Dexamethasone (1 μM) and antiflammin-2 (100 μM) were incubated with macrophages prior to cell activation at the indicated times. Bars represent the means ± S.E.M. of 5–6 determinations. * Significantly different from the non-treated cells ($P < 0.05$).

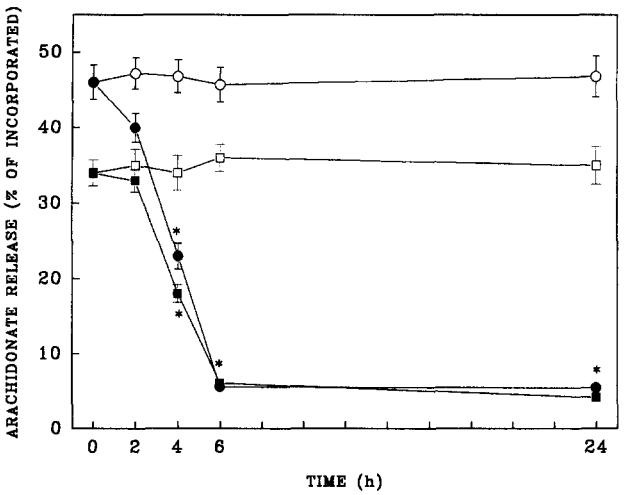

Fig. 2. Time-course of the effect of dexamethasone (filled symbols) and antiflammin-2 (open symbols) on [^{3}H]arachidonic acid release elicited by murine 3T6 fibroblasts. Cell activation was induced by PMA (1 μM, circles) and calcium ionophore A23187 (1 μM, squares) for 2 h at 37°C. Dexamethasone (1 μM) and antiflammin-2 (100 μM) were incubated with fibroblasts prior to cell activation at the indicated times. Bars represent the means ± S.E.M. of 5–6 determinations. * Significantly different from the non-treated cells ($P < 0.05$).

[^{3}H]Arachidonic acid mobilization was more manifest when prelabelled subconfluent 3T6 fibroblasts were incubated with PMA (1 μM) or ionophore A23187 (1 μM), release being 46 ± 2.6% and 34 ± 1.9% of the [^{3}H]arachidonic acid incorporated, respectively (Fig. 2). It is important to note that we observed a significant inhibition by dexamethasone (1 μM) of [^{3}H]arachidonic acid release in 3T6 fibroblasts stimulated with PMA (83%) or A23187 (85%). This effect of dexamethasone was dose-dependent (data not shown). This inhibition developed progressively with time, being limited at 2 h and approximately half-maximal at 4 h. However, our results also demonstrate that antiflammin-2 was not able to diminish significantly stimulated [^{3}H]arachidonic acid mobilization in 3T6 fibroblasts under our experimental conditions (Fig. 2).

Table 1
Effect of dexamethasone and antiflammin-2 on [^{3}H]arachidonate metabolism by murine 3T6 fibroblasts stimulated with 4β-phorbol-12-myristate 13-acetate (PMA)

	Percentage [^{3}H]arachidonic acid metabolized
Control	21.5 ± 2.1
PMA	82.3 ± 3.6
PMA + dexamethasone	38.5 ± 3.1 [a]
PMA + antiflammin-2	83.3 ± 2.6

Preconfluent 3T6 fibroblasts (2000 cells/cm^2) were labelled with [^{3}H]arachidonic acid. Dexamethasone (1 μM) and antiflammin-2 (100 μM) were incubated with fibroblasts 2 h prior to cell stimulation with PMA (1 μM) for 2 h at 37°C. Then, the medium was removed and [^{3}H]arachidonic acid and [^{3}H]arachidonate metabolites were measured by thin-layer chromatography and liquid scintillation spectroscopy. Measurements were performed in triplicate and expressed as the means ± S.E.M. of three experiments.
[a] $P < 0.05$, significantly different from non-treated values.

Table 2
Effect of dexamethasone and antiflammin-2 on mononuclear cell chemotaxis

Treatment	A_{540}	% control
PBS	0.23 ± 0.1	
FMLP	1.95 ± 0.2	100
FMLP + antiflammin-2 (1 μM)	1.18 ± 0.3 [a]	55
FMLP + antiflammin-2 (10 μM)	0.83 ± 0.1 [a]	35
FMLP + antiflammin-2 (100 μM)	0.64 ± 0.1 [a]	24

Blood peripheral mononuclear cell chemotaxis was measured in Boyden chambers as described in Materials and methods. Chemotaxis was activated with FMLP (10 nM). Antiflammin was preincubated with cells for 10 min before chemotaxis was stimulated. Values are the means ± S.E.M. of three experiments performed in triplicate. PBS, phosphate-buffered saline; FMLP, *N*-formyl-Met-Leu-Phe.
[a] $P < 0.05$, significantly different from non-treated cells.

Table 1 compares the ability of dexamethasone and antiflammin-2 to reduce the generation of [^{3}H]arachidonic acid metabolites by PMA-stimulated 3T6 fibroblasts when we added exogenous [^{3}H]arachidonic acid to cultured cells. This effect was manifest when fibroblasts were treated with dexamethasone (1 μM). Thus, the glucocorticoid inhibited the metabolism of [^{3}H]arachidonic acid induced by PMA (71%) and ionophore A23187 (60%). However, antiflammin-2 had no effect on arachidonate metabolism.

We next examined the effect of antiflammin-2 on human blood peripheral mononuclear cell chemotaxis induced in vitro by FMLP. As we can see in Table 2, antiflammin-2 was highly inhibitory for mononuclear cell chemotaxis, and this effect was dose-dependent.

Recently, Perretti et al. (1993) reported that intravenous injection of FMLP to mice caused a dose-dependent fall in the number of circulating neutrophils in a time-dependent way. At the chosen dose of 10 ng given intravenously, FMLP reduced PMN numbers by 58% at 2 min under our experimental conditions (Table 3). FMLP-induced neu-

Table 3
Effect of dexamethasone and antiflammin-2 on FMLP-induced neutropenia

Treatment	PMN (× 10^6)	% control
PBS	1.51 ± 0.20	100
FMLP	0.63 ± 0.03	42
FMLP + sialidase	1.21 ± 0.15 [a]	80
FMLP + dexamethasone	1.46 ± 0.26 [a]	97
FMLP + antiflammin-2	1.19 ± 0.24 [a]	79

Mice received intravenously 100 μl of PBS (phosphate-buffered saline), antiflammin-2 (100 μg per mouse) or sialidase (0.01 mU per mouse) 30 min before i.v. challenge with 10 ng FMLP (*N*-formyl-Met-Leu-Phe). Dexamethasone (1 mg/kg) was administered orally 3 h before FMLP injection. In all cases blood was collected 2 min after FMLP administration. Values are the means ± S.E.M. for 5–6 mice.
[a] $P < 0.05$, significantly different from non-treated animals.

tropenia was significantly attenuated when mice were pretreated with sialidase. Thus, the dose of sialidase that modified the number of circulating neutrophil leukocytes in the absence of any toxic effect (0.01 mU) reduced FMLP neutrophil migration by approximately 50%. A similar effect was obtained with both pharmacological pretreatments: with dexamethasone (1 mg/kg, p.o.) and antiflammin-2 (100 μg i.v.), which significantly reduced the chemotactic effect induced by FMLP in vivo on PMN (Table 3).

4. Discussion

The exposure of phagocytes to PMA or calcium ionophore A23187 in vitro enhanced the release of arachidonic acid and arachidonate metabolism, suggesting that the enzyme(s) responsible for arachidonic acid release from the *sn*-acyl position of membrane phospholipids is activated. A major target candidate for this activation is the high-molecular-mass (85 kDa) intracellular phospholipase A_2, which specifically cleaves arachidonic acid from the *sn*-position of phospholipids (Mayer and Marshall, 1993). Translocation of intracellular phospholipase A_2 from the cytosol to the membrane after changes in intracellular calcium concentrations (Clark et al., 1991; Lloret et al., 1995) and protein phosphorylation (Lin et al., 1993; Lloret and Moreno, 1996) could be involved in intracellular phospholipase A_2 activation.

Lipocortins have been shown to be members of a family of proteins induced by glucocorticoids, which can bind to anionic phospholipids and actin filaments in a calcium-dependent manner and which inhibit phospholipase A_2 activity only under certain conditions by sequestration of its substrate rather than by interactions with the enzyme (Davidson and Dennis, 1989). This effect of lipocortin-1 has been related to a powerful anti-inflammatory activity in acute inflammation (Cirino et al., 1990). Uteroglobin is a progesterone-induced protein inhibitor of phospholipase A_2 (Randall et al., 1991) with anti-inflammatory actions (Miele et al., 1994). On the basis of computer analysis, Miele et al. (1988) identified a region of local sequence similarity between uteroglobin and lipocortin-1 and designed several synthetic nonapeptides named antiflammins because they retained the anti-phospholipase A_2 activity and the anti-inflammatory actions of both proteins. However, our previous results did not appear to indicate a direct interaction between antiflammins and phospholipase A_2 or the enzymes involved in arachidonate metabolism. Results presented here confirm that antiflammin-2, a nonapeptide derived from the sequence of lipocortin-1, did not have a significant effect on arachidonic acid mobilization or arachidonate metabolism in either of the two type cells, murine resident peritoneal macrophages or murine 3T6 fibroblasts, which respond to glucocorticoid treatment.

One of the findings presented here is that dexamethasone needs 24 h to reduce [^{3}H]arachidonic acid mobilization stimulated by PMA or A23187 in peritoneal macrophages. These findings agree with the recent results reported by Gewert and Sundler (1995) and they could indicate the existence of a down-regulated phospholipase A_2 mechanism in resting mouse macrophages, as was suggested by these authors. It has recently been demonstrated that the rapid up-regulation of phospholipase A_2 in response to cell stimulation could be due to regulatory phosphorylation of the enzyme (Lin et al., 1992, 1993). The inhibitory action of dexamethasone on these events could explain the rapid impairment of [^{3}H]arachidonic acid mobilization induced by the corticosteroid in 3T6 fibroblasts stimulated with PMA or ionophore A23187. However, we should emphasize that antiflammin-2 had no effect on [^{3}H] AA release after short or long incubation times. However, one should keep in mind that lipocortin-1 (Maridonneau-Parini et al., 1989) or a lipocortin-1 fragment (Perretti et al., 1995) has been shown to interfere with receptor-mediated cell activation but not with stimuli such as PMA or A23187 which bypass this step.

Furthermore, antiflammin-2 had no effect on arachidonic acid metabolism when we supplied exogenous [^{3}H]arachidonic acid to cultured 3T6 fibroblasts stimulated with PMA. This is consistent with the earlier findings in vivo that arachidonic acid-induced murine ear oedema is not inhibited by the nonapeptide (Lloret and Moreno, 1992), and support the idea that antiflammins have no effect on enzymes involved in the arachidonate metabolism.

There is, however, an alternative mechanism to explain the anti-inflammatory effect of antiflammins, which may involve an antichemotactic action. We must consider that glucocorticoids are powerful inhibitors of cell trafficking in vivo. Various mechanisms have been proposed to account for their effectiveness, including the inhibition of adhesion molecule expression in endothelial cells (Cronstein et al., 1992). Furthermore, lipocortin-1 appears on the surface of neutrophils and monocytic cells after glucocorticoid injection (Goulding et al., 1990) and Perretti et al. (1993) speculate that it is through induction of these proteins that glucocorticoids act to inhibit white cell movement in inflammation.

We examined the activity of antiflammin-2 on monocyte and neutrophil migration. Antiflammin-2 reduced the chemotactic movement of monocytes induced by FMLP in vitro. These findings are consistent with the results of Camussi et al. (1990), who reported that antiflammin-2 inhibits neutrophil chemotaxis induced by C_{5a}.

The initial events in phagocyte migration in vivo are believed to be associated with the activation of adhesion molecules on the surface of phagocytes and endothelial cells. The rolling of leukocytes on the endothelium is a prerequisite for subsequent adhesion and it is mainly mediated by selectins (Lawrence and Springer, 1991; Lasky, 1992). Our data obtained in vitro did not allow us to determine whether antiflammin-2 interfered with leukocyte

trafficking. To obtain further information about this point, we measured the action of antiflammin-2 in a very simple model in vivo, FMLP-induced neutropenia. Sialidase, an enzyme widely used to remove carbohydrate moieties which are the counter-receptor for selectins (Rosen et al., 1989) induced a dose-dependent neutrophilia. Dexamethasone and antiflammin-2 also provided a significant protection in vivo against neutropenia caused by intravenous administration of FMLP. Similar data were recently obtained with an N-terminus peptide of human lipocortin-1 by Perretti et al. (1995) and by Harris et al. (1995), using platelet-activating factor-induced neutropenia.

Taken together, these observations suggest that the anti-inflammatory effects of antiflammins in vivo are not initially a result of an effect on arachidonic acid release or metabolism but may be due to their ability to suppress leukocyte trafficking to the inflammatory lesion. However, additional experiments should be performed to clarify whether antiflammins interfere with the expression or action of adhesion molecules.

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