Effect of dexamethasone on polypeptides synthesised in polymorphonuclear leucocytes

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Received 5 November 1984; revised version received 7 January 1985

The effect of a glucocorticoid on protein synthesis in human polymorphonuclear leucocytes (PMNLs) was investigated by two-dimensional gel electrophoresis. In the peripheral blood PMNLs of healthy laboratory personnel, the rate of incorporation of L-[^{35}S]methionine into a least nine polypeptides was consistently influenced by dexamethasone in a dose-dependent manner, being increased in the case of seven polypeptides and decreased in the remainder.

Polymorphonuclear leucocyte *Protein synthesis* *Two-dimensional gel electrophoresis* *Glucocorticoid inflammation* *Rheumatoid arthritis*

1. INTRODUCTION

Glucocorticoids exert an anti-inflammatory effect, both clinically in humans and also in a variety of animal models of inflammation such as the carrageenin-induced pouch in rats [1]. One of the cell types involved in acute inflammation is the phagocytic PMNL which migrates from the circulation to the site of inflammation. Chemotactic activity for PMNLs increases at this site and is attributable in the early stages of inflammation to leukotriene B_4 [2], a lipoxygenase metabolite of arachidonic acid.

PMNLs possess receptors for glucocorticoids [3] and gene transcription is presumed to be affected in them in a manner similar to that in the classic glucocorticoid target cells such as the hepatocytes and thymocytes. Amongst the biological effects of glucocorticoids upon PMNLs are a receptor-mediated suppression of phospholipase A_2-dependent release of arachidonic acid [4], a reduction in chemotactic activity released into the medium [5], and an inhibition of plasminogen activator production [6]. Induction of synthesis of a protein inhibitor of phospholipase A_2, termed macrocortin or lipomodulin [7], has been identified in several cell types, including rabbit PMNLs, in response to incubation with glucocorticoids. However, it is not yet established whether this accounts fully for all the effects of glucocorticoid seen in PMNLs in vivo and in vitro, nor whether glucocorticoids influence the synthesis of other proteins by PMNLs. As a first step towards resolving these questions, we have pulse-labelled human peripheral blood PMNLs with L-[^{35}S]methionine in the presence and absence of dexamethasone, a synthetic glucocorticoid, and subjected the cell homogenates, prepared in the presence of a protease inhibitor, to two-dimensional gel electrophoresis. The synthesis of a number of polypeptides was found to be induced or repressed dose-dependently in response to dexamethasone.

Abbreviations: PMNLs, polymorphonuclear leucocytes; PAGE, polyacrylamide-gel electrophoresis; IEF, isoelectric focusing; NEPHGE, non-equilibrium pH gradient gel electrophoresis

2. MATERIALS AND METHODS

2.1. *Materials*

Dextran T500 and Percoll were obtained from

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

Pharmacia, Dulbecco's Ca and Mg free phosphate-buffered saline (PBS) and RPMI 1640 medium from Gibco, L-[^{35}S]methionine (high specific activity) from Amersham, and Protosol from New England Nuclear.

2.2. *Preparation of PMNLs*

Venous blood from healthy volunteers was treated with EDTA and diluted with 0.5 vol PBS and the red cells allowed to sediment in the presence of 1.5% dextran at 37°C. In all further procedures, the cells were kept cold. The cells in the supernatant were separated on a 55%/67.8% Percoll in PBS step gradient and PMNLs were collected from the concentration boundary. PMNLs were washed twice in PBS, residual red cells being lysed by a 30 s hypotonic (0.2% PBS) incubation on ice, washed twice more in PBS and once briefly in RPMI 1640. All solutions were sterile prior to use.

2.3. *Incubation of PMNLs and preparation of material for PAGE*

Aliquots of 2×10^6 cells were incubated for 4.5 h in 200 μl RPMI 1640 medium lacking methionine but supplemented with 0.1% (w/v) bovine serum albumin and 64 units of gentamycin sulphate in an atmosphere of 5% CO_2/95% air at 37°C either in the presence of vehicle (0.005% (v/v) ethanol) or in dexamethasone, concentration as indicated in figure legends. L-[^{35}S]Methionine, 62.5 μCi, was included during the final hour. Cells were then collected and washed twice in cold PBS containing 5 mM unlabelled methionine, and solubilised in 80 μl lysis buffer [8] containing 1 mM phenylmethylsulphonyl fluoride and 2% ampholines (1.6% pH 5–8, 0.4% pH 3.5–10; LKB). The samples were freeze-thawed 5 times prior to loading onto the gels, to shear DNA.

2.4. *Electrophoresis and fluorography*

Either IEF for 16.5 h at 400 V followed by 1 h at 800 V, or NEPHGE for 4.5 h at 400 V was used as the first dimension of two-dimensional gel electrophoresis as in [8,9]. Tube gels (12.5 cm × 2 mm diam.) were then equilibrated with SDS-containing sample buffer for 2 × 20 min prior to applying to 15% (w/v) polyacrylamide slab gels. Electrophoresis in the second dimension was for 17 h at 55 V, and matched pairs of dexamethasone or vehicle treated samples were run concurrently in the same tank. Gels were prepared for fluorography as in [10] and exposed to Fuji RX medical X-ray film at −70° for 7–14 days.

2.5. *Gel solubilisation and assay for radioactivity*

Labelled polypeptide spots of interest were excised from the dried gel by aligning with the X-ray film. They were rehydrated, incubated with 0.2 ml Protosol overnight at 37°C and assayed for radioactivity with non-aqueous scintillant.

3. RESULTS

Circulating PMNLs have a low protein synthetic activity and are not thought to require protein synthesis for two of their major activities, viz, chemotaxis and phagocytosis. Furthermore, the cells have a short half-life in vivo and in vitro. PMNLs from 5 healthy volunteers were incubated for 4.5 h over which time their viability remained higher than 92% as shown by the nigrosine exclusion test. Pulse-labelling with L-[^{35}S]methionine was between 3.5 and 4.5 h. Cells incubated in vehicle incorporated 1–2.5 × 10^6 cpm per 10^7 cells per h in these conditions and cells incubated with 1 μM dexamethasone incorporated methionine at 70–90% of this rate. The fluorograms of two-dimensional polyacrylamide-gels using IEF in the first dimension show polypeptides of isoelectric point between 5.0 and 7.5 into which labelled methionine has been incorporated in a 1-h pulse. The two-dimensional (IEF) PAGE pattern of polypeptides from cells of one of 5 healthy volunteers is shown in fig.1, incubated with vehicle (A) or dexamethasone (B). Although the overall profiles from the 5 volunteers possess dissimilarities, the rate of methionine incorporation into the polypeptides marked 1–6 in fig.1B is increased in all 5 individuals, and into polypeptides R1, R2 (fig.1A) is decreased in each case. No other changes due to dexamethasone have been observed in any of the volunteers. An increase in rate of methionine incorporation with dexamethasone incubation into one other more basic polypeptide was detected using NEPHGE in the first dimension (polypeptide 7, fig.1C,D). Also marked in fig.1D are the positions of polypeptides induced by dexamethasone which correspond to polypeptides 4 and 6 on fig.1B. The M_r values of polypeptides 1–7 were as

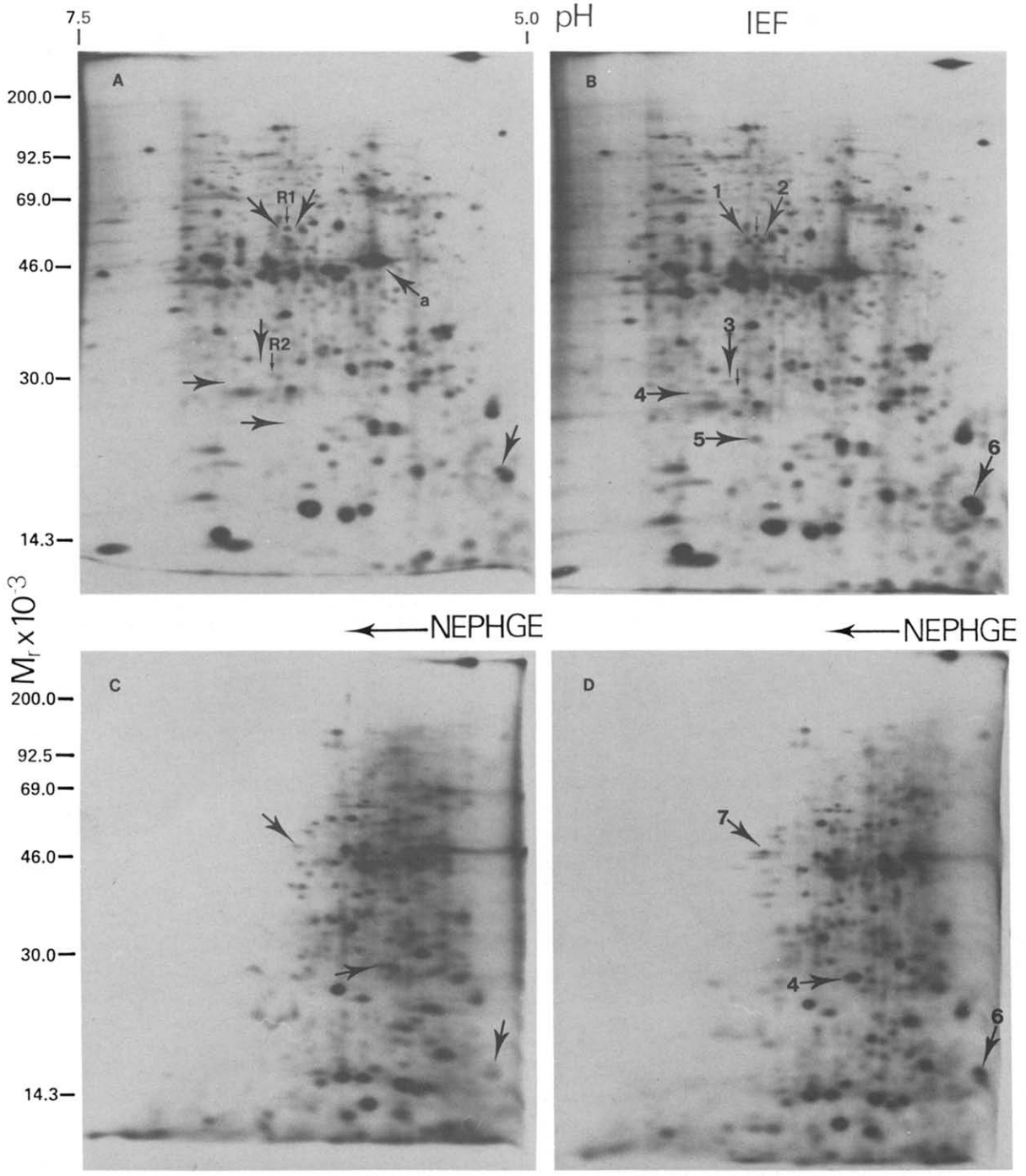

Fig. 1. Fluorograms of two-dimensional polyacrylamide-gels of pulse-labelled PMNL proteins from cells incubated in the presence of vehicle (A,C) or 1 μM dexamethasone (B,D). Approximately equal amounts of label from vehicle-treated and dexamethasone-treated samples were applied to matched pairs of gels. (A,B): IEF in the first dimension; (C,D): NEPHGE in the first dimension. Also shown are the positions of M_r marker proteins run in the second dimension alongside first dimension gels. Dexamethasone-affected polypeptides are indicated by numbered arrows and the position of actin (a) is shown, identified by 2-dimensional electrophoresis of muscle actin and M_r marker proteins run concurrently with a 2-dimensional gel of a labelled PMNL lysate.

follows: 1,2 = 55 000; 3 = 29 000; 4 = 27 000; 5 = 22 000; 6 = 16 000; 7 = 49 000. The M_r values of R1 and R2 were 55 000 and 28 000, respectively.

The effect of dexamethasone concentration on the induction of these changes in polypeptide synthesis was investigated by incubating cells from one volunteer in either vehicle or 1 nM, 10 nM, 100 nM, 1 μM or 10 μM dexamethasone. Pulse labelling was as before and each sample was subjected to two-dimensional PAGE with IEF or NEPHGE in the first dimension. The degree of effect due to dexamethasone was observed to increase with dexamethasone dose over the range 0–1 μM at 3.5–4.5 h. The two most highly labelled spots induced by dexamethasone (polypeptide 4 in fig.1D, polypeptide 6 in fig.1B) are shown in fig.2A. They were excised from each gel and assayed for radioactivity along with 5 unaffected polypeptides, which acted as reference points between gels. Small variations in sample loading, loss in equilibration and second dimension gel penetration were accounted for by comparing radioactivity in the 5 unaffected polypeptides in each gel with reference to unaffected polypeptides in vehicle-treated samples, and correcting the radioactivity in induced polypeptides accordingly.

The dose-effect curve for the 16-kDa polypeptide (fig.2B) shows a 4.7-fold stimulation in the rate of synthesis of the polypeptide with 1 μM dexamethasone over the rate in hormone-free cells. Half-maximal response occurs around 10 nM dexamethasone, a concentration sufficient to saturate half the number of putative dexamethasone receptors in human PMNLs [3]. Incorporation of methionine into the 27-kDa polypeptide also increases with dexamethasone at the time point studied, with a maximum 3-fold stimulation in synthesis at 1 μM, but it is less affected by lower dexamethasone concentrations (10 nM and less). This may be because induction of this polypeptide does not commence until the second or third hour of incubation even using receptor-saturating levels of dexamethasone (results to be presented elsewhere). The full effect of dexamethasone on the 27-kDa polypeptide at lower concentrations may therefore not be seen until after 4.5 h. By contrast, an increased rate of methionine incorporation into the 16-kDa polypeptide is seen as an early event in response to dexamethasone, it being 70% maximal within the first hour.

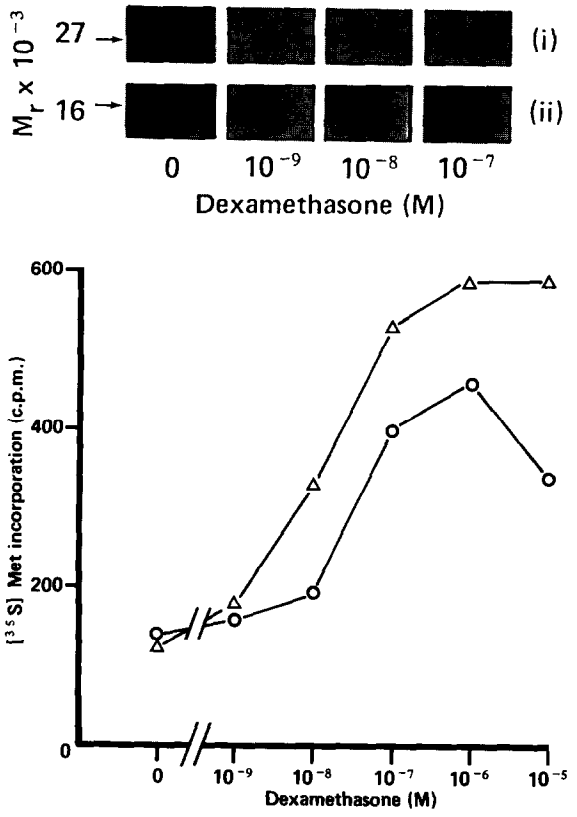

Fig.2. Effect of dexamethasone concentration on the incorporation of L-[^{35}S]methionine by PMNLs into two polypeptides. (A) Sections of fluorograms showing (i) 16-kDa polypeptide from IEF/2-dimensional PAGE and (ii) 27-kDa polypeptide from NEPHGE/2-dimensional PAGE, pulse-labelled in cells treated with 0,1 nM, 10 nM, or 100 nM dexamethasone. (B) The polypeptides were excised from the gels shown in (A), solubilised and assayed for radioactivity. Dexamethasone concentration-dependent incorporation of L-[^{35}S]methionine into 16-kDa polypeptide (△—△) and 27-kDa polypeptide (○—○) is shown after correction for small variations in overall amount of label between gels as assessed in 5 dexamethasone-unaffected polypeptides.

4. DISCUSSION

Dexamethasone consistently induced an increased rate of L-[^{35}S]methionine incorporation into at least 7 polypeptides and decreased the rate into at least two polypeptides in the PMNLs of all 5 healthy laboratory personnel investigated. Synthesis of polypeptides with M_r 16 000 and 27 000

was quantifiable and was found to be dexamethasone concentration-dependent over the range 0–1 μM. Such dependence was most pronounced between 0 and 100 nM dexamethasone, the concentration range over which the PMNL glucocorticoid receptors become saturated [3]. These results do not preclude the possibility of there being other dexamethasone-inducible polypeptides which, for instance, do not contain methionine, or are masked by adjacent, more prominently labelled polypeptides.

This study is based on methods used previously to detect glucocorticoid-induced proteins in other cell types such as hepatoma tissue-culture cells [11] and thymus cells [12]. In hepatoma cells, none of the class I induced polypeptides appear to correspond to any of the induced polypeptides shown here, whilst from thymus cells, only one induced polypeptide, that of M_r 22 000, appears to occupy a similar electrophoretic position to the polypeptide of corresponding molecular mass induced in PMNLs.

It is not yet known what roles these glucocorticoid-inducible polypeptides of PMNLs have, or even if all the polypeptides represent functionally separate activities, since some could prove to be sub-units of larger proteins. However, the induction of protein inhibitors of phospholipase A_2 by glucocorticoids has been observed in a number of cell types, including rabbit PMNLs [4] and rat peritoneal macrophages [13]. Inhibition of phospholipase A_2 leads to a reduction in arachidonic acid production and its pro-inflammatory metabolites, leukotrienes and prostaglandins [4]. Most of the anti-phospholipase activity has been found in 39–44 kDa fractions with some in a 16-kDa fraction [14]. It remains to be seen whether any of the polypeptides identified in the present study as dexamethasone-inducible are phospholipase inhibitory or what other roles they might have. We believe that further study of the effect of dexamethasone upon these polypeptides will shed light on the anti-inflammatory activity of glucocorticoids as seen in PMNLs. Furthermore, study of the induction of these polypeptides by dexamethasone in PMNLs of patients with rheumatoid arthritis will determine more precisely the mode of therapeutic action of glucocorticoids in chronic autoimmune inflammatory and allergic disorders.

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