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Hydrocortisone inhibits prostaglandin production but not arachidonic acid release from cultured macrophages

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We have investigated the action of hydrocortisosone on arachidonic acid mobilisation in cultures of mouse peritoneal macrophages, mouse L_{929} cells and the mouse macrophage-like cell line RAW_{264}. Hydrocortisone inhibits both arachidonic acid release and prostaglandin production by L_{929} cells. However, prostaglandin production by macrophages or RAW_{264} cells is inhibited with a concomitant stimulation rather than inhibition of arachidonic acid release. These data suggest that hydrocortisone acts at the level of phospholipase activity in fibroblasts but at a later stage of prostanoid production in macrophages.

Glucocorticoid steroid *Macrophage* *Prostaglandin*

1. INTRODUCTION

The anti-inflammatory action of glucocorticoid steroids has been attributed to their ability to inhibit prostaglandin and leukotriene synthesis [1]. These inflammatory mediators are derived from arachidonic acid, which is stored in lipid body and membrane phospholipids [2] and released by phospholipases A_2 and C [3]. Evidence has been presented that steroids exert their inhibitory action at the level of phospholipase activity, firstly because exogenous arachidonic acid can overcome steroid inhibition of prostanoid synthesis, and secondly because arachidonic acid release from fibroblasts is inhibited by steroids to the same extent as prostanoid production [4]. We show here, however, that prostaglandin E_2 (PGE_2) production by resting mouse peritoneal macrophages is inhibited by hydrocortisone, with a concomitant stimulation rather than reduction in arachidonic acid release. Because macrophages are the major source of eicosanoid products amongst leucocytes [5], our observations suggest that a locus of steroid inhibition other than phospholipase activity may play an important role in the anti-inflammatory actions of glucocorticoid hormones.

Arachidonic acid metabolism was studied in BALB/c resting peritoneal macrophages after 24 h in culture [6] and in the macrophage-like virally transformed RAW_{264} [7] cell line as well as the mouse L_{929} fibroblast line [8]. By pre-labelling cells with [^{14}C]arachidonic acid, the effect of hydrocortisone on both total arachidonic acid derived metabolite release, and on individual eicosanoid products, identified by thin-layer chromatography (TLC), could be investigated [9] (fig.7, [6]). In addition, PGE_2 production was measured by radioimmunoassay [10] using a variety of different stimuli to induce arachidonic acid mobilisation in the presence and absence of hydrocortisone.

2. MATERIALS AND METHODS

Macrophages from female BALB/c mice, killed by CO_2 inhalation, were prepared by peritoneal lavage with RPMI 1640, containing 10% foetal calf serum (FCS) (Flow) and penicillin (100 units/ml) and streptomycin (100 μg/ml). Cells were plated into 24 well plates (Linbro) at a density of 2.5×10^5 macrophages per well in a 5% CO_2 humidified incubator at 37°C. After 90 min, non-adherent cells were removed by washing in the

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same medium. Experiments were carried out 24 h later. L_{929} cells were grown in the same medium at an initial density of about 10^6 cells per well. Both macrophages and L_{929} cells were labelled for 2 h with [^{14}C]arachidonic acid (Amersham; 60 mCi/mM, 0.2 μCi/ml, 0.5 ml per well). After 5 washes with serum-free RPMI, test stimuli [6] were added to duplicate or triplicate cultures (0.5 ml/well). RAW_{264} cells were treated exactly as were the L_{929} cells. Arachidonic acid release was induced with 10% FCS, 0.2% sheep red blood cell (SRBC) sensitised with 100 μg/ml IgG2b Sp_2 monoclonal antibody (Seralab), zymosan (Sigma), calcium ionophore A23187 (Sigma) or ATP (Sigma) dissolved in RPMI 1640 containing antibiotics. Hydrocortisone hemisuccinate (Sigma) was freshly prepared and used at a final concentration of 2.5 μg/ml. At various times, supernatants were sampled, microfuged (1 min, 5000 × g) and counted (25 μl) in PCS scintillant (Amersham).

Radiolabelled products were also analysed by TLC. Total supernatants were extracted and analysed after 2–18 h stimulation [9], using Whatman LK5D silica plates in a solvent system of ethyl acetate:2,2,4-trimethylpentane:acetic acid:water (110:50:20:100, v/v) and then autoradiographed at −70°C using Kodak X-Omat film for 1–14 days. Identity of labelled products was decided by comparison with authentic prostaglandin and arachidonic acid markers. Quantitation of products was carried out by carefully cutting out strips corresponding to identified bands after autoradiography, and counting them in PCS scintillant.

Radioimmunoassays of PGE_2 [10] were carried out on unlabelled macrophages or mouse L_{929} cells plated at similar densities to those used above, into quadruplicate wells of 48 well plates (Costar), after 2–18 h stimulation.

3. RESULTS AND DISCUSSION

In neither primary macrophage cultures nor RAW_{264} cells could we detect a steroid-induced inhibition of total ^{14}C metabolite release (fig.1) or a reduction in ^{14}C-labelled arachidonic acid release, quantified after TLC. Nonetheless, PGE_2 production by primary macrophages, measured by radioimmunoassay or after TLC, and PGD_2 production by RAW_{264} cells measured after TLC, were inhibited (30–65%) by hydrocortisone (table 1) (PGD_2 is the major prostanoid released by stimulated RAW_{264} cells). In contrast, both arachidonic acid release and PGE_2 production by mouse L_{929} cells were inhibited by hydrocortisone (fig.1, table 1) in agreement with the original observations on mouse MC5-5 cells [4].

Because total ^{14}C counts released from macrophages or RAW_{264} cells are unaffected, or even stimulated by hydrocortisone treatment, whilst prostaglandin levels are reduced, arachidonic acid must either be diverted to other eicosanoid products, or be present at higher levels in the supernatants of steroid-treated cells. Higher levels of [^{14}C]arachidonic acid, quantitated after TLC, are indeed present in steroid-treated macrophage and RAW_{264} supernatants, concomitant with the reduction in prostaglandin levels [e.g., with hydrocortisone (2.5 μg/ml) after 18 h incubation with 10% FCS; macrophage PGE_2 production down 29%, arachidonic acid release up 57%]. This implies that the locus of hydrocortisone inhibition lies after the phospholipase-catalysed release of arachidonic acid, and before or during its conversion by cyclooxygenase or lipoxygenases. Such a side of inhibition is compatible with the ability of exogenous arachidonic acid to stimulate PGE_2 production in steroid-treated cells, particularly as it is still possible to observe an inhibitory effect of steroid on exogenous arachidonic acid conversion ([4], fig.3, unpublished).

Mast cell arachidonic acid release is also refractory to steroid inhibition, except when induced by IgE-mediated stimulation [11]. We have demonstrated a lowering of Fc γ 2b receptor expression on steroid-treated macrophages [6] which results in reduced prostanoid production when cells are stimulated with IgG_{2b} immune complexes. An analogous mechanism could explain the selective steroid inhibition of IgE-mediated arachidonic acid release in the mast cell. Although a steroid-induced inhibition of arachidonic acid release has been detected in polymorphonuclear cells [14] (PMNs) others have failed to detect any inhibition of prostaglandin synthesis [12], whilst the stimulation of lipoxygenase products by *N*-formyl-Met-Leu-Phe has been shown to be independent of phospholipase activity in these cells [13]. The variable effects of glucocorticoid steroids on prostaglandin synthesis and arachidonic acid release in various cell types are summarised in table 2. These

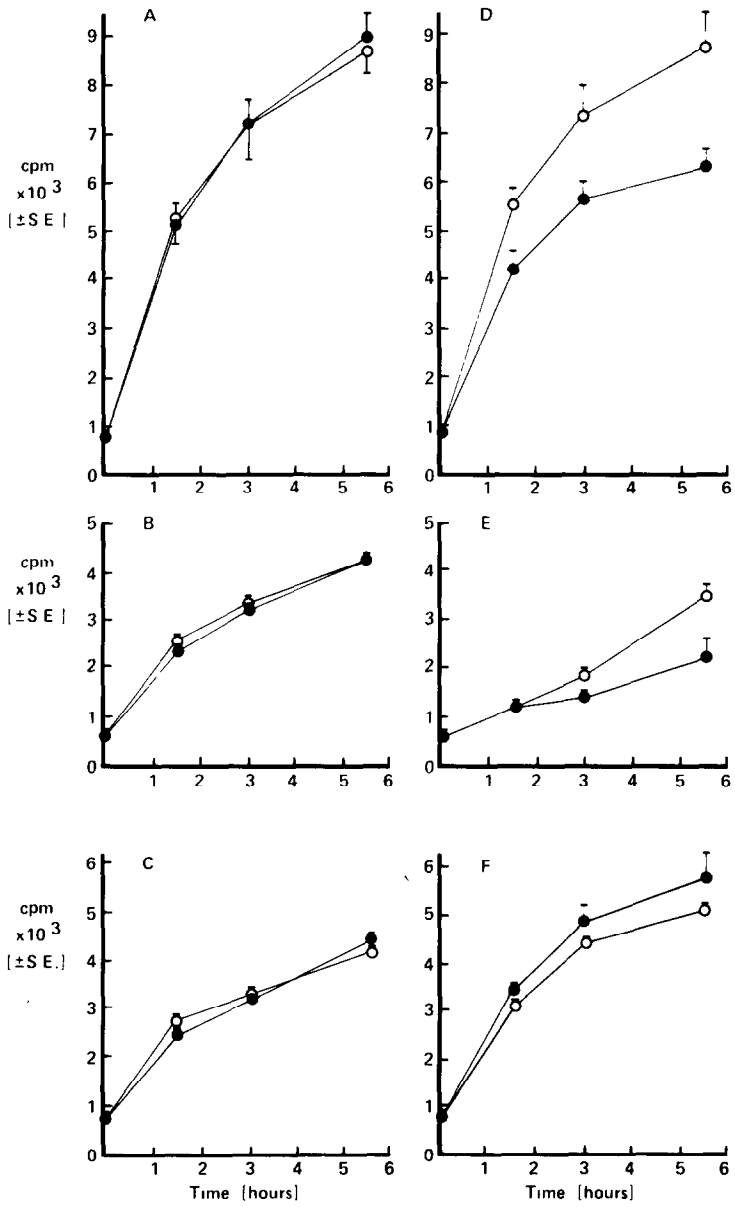

Fig.1. Time course of [^{14}C]arachidonic acid metabolite release from mouse peritoneal macrophages (A–C,F) and mouse L_{929} cells (D,F). Arachidonic acid release was stimulated by 10% FCS (A,D), 0.2% (SRBC)–IgG_{2b} immune complex [6] (B,E), zymosan (100 μg/ml) (C), or calcium ionophore A23187 (F) at 2.5 μg/ml. Similar results were obtained after short (2 h) or long (18 h) labelling times, and macrophages and RAW_{264} cells showed an identical lack of inhibition by hydrocortisone to all stimuli tested. (○) Control, (●) with 2.5 μg/ml hydrocortisone.

observations suggest that anti-inflammatory steroid action is cell-type dependent. We have used unstimulated mouse peritoneal macrophages after 24 h in culture for our experiments, and we cannot exclude the possibility that macrophages manipulated in a different way, for example, thioglycolate induced, may behave differently. Nonetheless, the use of defined populations of

Table 1

Effect of hydrocortisone on macrophage and L_{929} cell PGE_2 production and arachidonic acid metabolite release

Stimulus	PGE_2 (ng/ml)	+HC	% inhibition	% change [^{14}C]arachidonic acid metabolite release
RPMI 1640 (control)	1.9	0.8	58	+1.7
Zymosan (100 μg/ml)	51.2	35.4	32	+3.7
FCS (10%)	107.3	52.8	51	+1.8
0.2% IgG_{2b} immune complex	31	13.8	55	0
ATP (1 mM)	6.4	2.3	65	+8
A23187 (5 μg/ml)	3.7	3.5	6	+13
10% FCS on mouse L_{929} cells	9.8	6.5	33	−38
10% FCS on RAW_{264} cells – PGD_2 production (TLC)	–	–	42	+6

Table 2

Hydrocortisone effects on different cell types

Cells	Arachidonic acid release	Prostaglandin synthesis
Fibroblasts [4]	↓	↓
Mast cells [11]	−/↓	↓
Macrophages	↑	↓
PMN [12–14]	↓	−

↓, inhibition; −, no effect; ↑, stimulation

macrophages and the RAW_{264} line provide a useful system for the analysis of this novel locus of anti-inflammatory steroid action.

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