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Specificity and inhibition of glucocorticoid-induced macrocortin secretion from rat peritoneal macrophages

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1 The secretion of the phospholipase A_2-inhibitor macrocortin and the binding of dexamethasone were studied in suspensions of rat peritoneal macrophages.

2 Corticosteroid-induced macrocortin secretion was specific for glucocorticoids and did not occur in response to glucocorticoid antagonists or other steroids or in response to non-steroid macrophage activators (formyl-methionyl-leucyl-phenylalanine f-MLP), the calcium ionophore A23187, phorbol myristate acetate (PMA) and lipopolysaccharide-*E.-coli*(LPS)).

3 The apparent potency of competition by secretory glucocorticoids for dexamethasone binding to the macrophage parallelled their ability to induce secretion, implying that these binding sites represent the receptors by which macrocortin secretion is initiated.

4 Agents which interfere with microtubule assembly (colchicine, vinblastine and trimethylcolchicinic acid) and prostacyclin and dibutyryl cyclic AMP inhibit macrocortin secretion.

5 Inhibition studies of glucocorticoid-induced macrocortin secretion also suggest dependence upon metabolic energy, a source of Ca^{2+} and proteolysis and glycosylation prior to secretion.

Introduction

Glucocorticoids are indirectly able to inhibit phospholipase A_2 and thereby prevent the biosynthesis of a number of pro-inflammatory mediators, such as the prostaglandins, thromboxanes and leukotrienes (Nijkamp, Flower, Moncada & Vane, 1976; Tam, Hong & Levine, 1977; Blackwell, Flower, Nijkamp & Vane, 1978; Flower & Blackwell, 1979; Di Rosa & Persico, 1979). This action depends upon steroid interaction with specific receptors, and the modulation of RNA and protein synthesis within the target cells (Danon & Assouline, 1978; Flower & Blackwell, 1979; Russo-Marie, Paing & Duval, 1979; Di Rosa & Persico, 1979; Carnuccio, Di Rosa & Persico, 1980; Hirata Schiffman, Venkatasubramanian, Salomon & Axelrod, 1980).

We and others (Flower & Blackwell, 1979; Di Rosa & Persico, 1979; Carnuccio *et al.* 1980; Hirata *et al.*, 1980; Hirata 1981) have demonstrated that the anti-phospholipase effect is exerted by a 'second messenger' synthesized and secreted by the cells in response to glucocorticoid stimulation. We have isolated this material from rat peritoneal macrophages and shown it to be a protein with a molecular weight of 15,000 daltons and have subsequently named the material macrocortin (Blackwell, Carnuccio, Di Rosa, Flower, Pasente & Parsico, 1980). A protein with similar properties and a molecular weight of 40,000 daltons has also been isolated from glucocorticoid-treated rabbit neutrophils (Hirata *et al.*, 1980; 1981). Furthermore, we have also shown that macrocortin exists as a pre-formed store within the rat peritoneal macrophage and its secretion is induced by glucocorticoids (Blackwell *et al.*, 1980; Carnuccio, Di Rosa, Flower & Pinto, 1981).

This paper concerns investigations into the mechanism of glucocorticoid-induced macrocortin secretion from rat peritoneal macrophages.

Methods

Preparation of membrane phospholipase A_2

Cells from the basophilic leukaemia line RBL-1 were maintained in Dulbecco's M.E.M. (containg 10% heat-inactivated foetal calf serum) in an incubator until required. For preparation of the enzyme approximately 1×10^8 cells were washed free of medium with Tris HCl buffer (pH 7,5, 100 mM) and then resuspended in 20 ml of the same buffer. The cells were lysed by repeated ($3 \times$) freeze-thawing and the preparation was then centrifuged at 10,000 *g*

for 20 min at 4°C in a Beckman LS150 Ultracentrifuge. The resultant supernatant was decanted and centrifuged at 100,000 *g* for 60 min at 4°C and the microsomal pellet thus obtained was resuspended in the Tris HCl buffer to a protein concentration of 10 mg/ml (as measured by the biuret reaction, Gornal, Bardawill & David, 1949).

The enzyme preparation was stored at − 70°C for 3 months with no appreciable loss of activity.

Isolation of rat peritoneal macrophages

Peritoneal macrophages from adult male Wistar rats (200–250g) were harvested according to methods previously described (Blackwell *et al.*, 1980) and resuspended in modified Krebs solution (2 g/litre glucose and 100 μg/ml bovine serum albumin) to a cell concentration of 1×10^7 cells/ml.

Assay of macrocortin using inhibition of phospholipase A_2

The method used was essentially that of the double-isotope assay for phospholipase A_2 as described by Blackwell *et al.*(1978) which uses a specifically labelled substrate and a labelled internal standard. Reaction mixtures comprised the following reagents: 0.2 ml Tris HCl buffer 100 mM pH 7.4, 0.1 ml 50 mM $CaCl_2$, 0.02 ml 0.1% Triton X-100, 0.1 ml enzyme preparation, 0.2 ml inhibitor or buffer and 0.1 ml labelled substrate (18.3 nmol 2-[1-^{14}C] oleoyl-phosphatidylcholine and 18.1 pmol[^{3}H]-oleic acid). Samples were incubated for 10 min at 37°C in a shaking water bath, after which 2.5 ml *n*-hexane was added and the radioactive fatty acids were selectively extracted by vortex mixing for 30 s. The two phases were separated by centrifugation and 1 ml aliquots of the upper (hexane) phase were removed, taken to dryness and the radioactivity measured by liquid scintillation counting techniques.

The $^{14}C/^3H$ ratio was estimated and the ratio of the two isotopes in an aliquot of the aqueous solution was also determined. This gave the value for the 'total hydrolysis' and the actual hydrolysis was calculated from the following formula:

$$\text{Units oleic acid liberated} = \frac{\text{d/min } ^{14}\text{C in sample} \times \text{d/min } ^3\text{H in control}}{\text{d/min } ^3\text{H in sample} \times \text{d/min } ^{14}\text{C in control}} \times S$$

where S is the number of units of oleate present in the phosphatide (i.e. nmol, μg etc), and the control tube contains all reagents except the enzyme preparation.

The membrane preparation contained no detectable PLA_1, PLC or PLD activity and the rate of phospholipid hydrolysis was linear for the duration of the incubation period, liberating 0.9 nmol substrate mg^{-1} min^{-1}. The enzymatic activity was stimulated by Ca^{2+} concentrations up to 10 mM but increasing concentrations of the ion produced no further stimulation. Supramaximal $CaCl_2$ concentrations (50 mM) were routinely used for the macrocortin assay.

Whole cell [^{3}H]-dexamethasone-binding assay

Triplicate 2 ml samples of rat peritoneal macrophages in Krebs solution (1×10^7 cells/ml) were incubated with 10 mM [^{3}H]-dexamethasone in the presence of the following unlabelled steroids: dexamethasone, triamcinolone, hydrocortisone, cortexolone, progestrone and oestradiol or the appropriate vehicle. The cell preparations were then incubated at 37°C for 30 min with gentle agitation after which they were decanted onto cellulose acetate microfilters supported on a multiwell Millipore filtration apparatus and the supernatants were removed under vacuum. The cells were washed with 5 × 10 ml aliquots of ice-cold Krebs solution and the filters were transferred to counting vials and solubilized with 1 ml of NCS tissue solubilizer. The radioactivity associated with each sample was estimated by liquid scintillation counting. Non-specific binding was taken to be the radioactivity associated with cells incubated in the presence of 10 μM unlabelled dexamethasone (usually less than 5% of the total binding), and results were calculated as d/min bound per 10^7 cells and expressed as a percentage of the binding in controls incubated with 10 nM [^{3}H]-dexamethasone alone.

Estimation of protein synthesis in isolated peritoneal macrophages

Radiolabelled[^{14}C]-lysine (0.1 μCi, 282 pmol) was added to duplicate 2 ml aliquots of the cell suspension which were then incubated in a shaking water bath at 37°C. After 2 h the cells were homogenized in the buffer and the proteins were precipitated with 5 vol of ice-cold 1 M perchloric acid. The precipitate was sedimented using a bench centrifuge and the supernatant discarded. The pellet was then washed twice with 5 vol of 0.5 M perchloric acid and then with 5 vol ethanol/ether (1:1) and the final pellet was solubilized with 5 N NaOH. The radioactivity in aliquots of the digest was measured by liquid scintillation counting after neutralization with acetic acid and the protein content was estimated by the biuret reaction (Gornall *et al.*, 1949). The results were calculated as pmol lysine incorporated per mg protein and expressed as percentage of control incorporation.

Estimation of RNA synthesis in isolated peritoneal macrophages

The procedure was identical to that outlined in the previous section except that the incorporation of [^{3}H]-uridine (0.1 μCi, 33 pmol) was measured.

Effects of drugs on macrocortin secretion from rat peritoneal macrophages

Duplicate 2 ml aliquots of the cell suspension were incubated at 37°C with various concentrations of the drugs under test which were dissolved in saline, Tris buffer, or 0.1% EtOH in saline, together with the appropriate vehicle controls. After 30 min pre-incubation, dexamethasone (2.5 μM) or hydrocortisone (20 μM final conc.) was added whilst controls received phosphate buffer alone. The samples were then incubated for a further 2 h at 37°C in a shaking water bath. At the end of the incubation period the cells were removed by centrifugation, the supernatants decanted into stoppered glass tubes and placed in a 70°C water bath for 5 min to inactivate proteolytic enzymes and endogenous phospholipase A_2. Aliquots (0.2 ml) were then assayed for macrocortin activity against the membrane phospholipase A_2 preparation. Inhibitory activity in each drug sample was compared with steroid controls and the results expressed as a percentage of that inhibition produced by the steroid. The amount of inhibitory activity detected in 0.2 ml medium (from cells incubated in the presence of glucocorticoid and assayed as described) was variable, ranging between 47.6%–94.6% with a mean inhibition of 67.4 ± 2.7%. In some experiments the medium from glucocorticoid-treated cells was purified and found to contain protein which co-chromatographed in several column systems with macrocortin obtained from corticosteroid-treated perfused guinea-pig lungs (Blackwell, Carnuccio, Di Rosa, Flower, Langham, Parente, Persico, Russell-Smith & Stone, 1982).

Stimulation of macrocortin secretion from rat peritoneal macrophages

The experimental procedures for these studies were identical to those outlined in the previous section except that drugs were examined for their ability to induce macrocortin secretion in the absence of added corticosteroids.

Materials

The following chemicals were purchased from the Sigma Chemical Co: actinomycin D, aldosterone-21-hemisuccinate, colchiceine, colchicine, cycloheximide, cytochalasin B, dibutyrylcyclic AMP, 2-deoxyglucose, dexamethasone, 2,4-dinitrophenol (2,4-DNP), hydrocortisone-21-acetate, lipopolysaccharide-*E. coli* (LPS), lumicolchicine (α and β), formyl-methionyl-leucyl-phenylalanine (f-MLP) oestradiol 17β, papaverine, phorbol myristate acetate (PMA), progesterone, puromycin, testosterone-17-hemisuccinate, N-α-P-tosyl, L-lysyl-chloromethyl-ketone (TLCK), trimethylcolchicinic acid (TMCA), triamcinolone acetonide, theophylline and vinblastine.

Tunicamycin and the calcium ionophore A23187 were obtained from Calbiochem; EDTA and EGTA from BDH; dexamethasone phosphate (DECADRON) from Merck, Sharpe and Dohme; and cortexolone from Aldrich Chemical Company. The radiochemicals [^{3}H]-dexamethasone (45 Ci/mmol), [^{3}H]-oleic acid (8 Ci/mmol),[^{14}C]-lysine monohydrochloride (354 mCi/mmol), [^{3}H]-uridine (3 Ci/mmol) were obtained from Amersham International and 2-[1-^{14}C] oleoyl-phosphatidylcholine (19 Ci/mmol) was synthesized according to methods previously described (Blackwell *et al.*, 1978). RBL-1 cells were a gift from Dr Priscilla, Piper, Royal College of Surgeons, England.

Results

Binding of corticosteroids to whole cell preparations

Competition for the binding of [^{3}H]-dexamethasone to whole cell preparations of rat peritoneal macrophages was specific for glucocorticoids or known glucocorticoid antagonists (Table 1). Non-labelled glucocorticoids (dexamethasone, triamcinolone and hydrocortisone) competed from the binding with the labelled ligand in the order of potency dex-

Table 1 Competition by various steroids for binding of [^{3}H]-dexamethasone to glucocorticoids receptors on rat peritoneal macrophages

Competing steroid	*Concentration* (μM)	[^{3}H]-*dexamethasone binding* % Control*
Dexamethasone	0.1	27.4 ± 3.2
	1	6.8 ± 2.2
Triamcinolone	0.1	37.2 ± 1.9
Hydrocortisone	1	42.4 ± 4.7
Cortexolone	1	61.6 ± 2.9
Progesterone	1	51.7 ± 3.7
Oestradiol 17β	1	99.0 ± 4.1

*The results show the mean±s.e.mean for three separate experiments.

Table 2 Ability of steroids and other agents to induce macrocortin secretion from rat peritoneal macrophages

Compound	*Concentration* (μM)	*% macrocortin secretion induced by 2.5 μM dexamethasone**
Hydrocortisone	20	96.4 ± 7.3
Triamcinolone	5	89.3 ± 7.3
Cortexolone	50	0
Progesterone	50	0
Oestradiol 17β	50	0
Testosterone	50	0
Aldosterone	60	0
f-MLP	0.1-1	0
A-23187	1,10	0
PMA	100	0
Cytochalasin B	50	0
LPS	2 μg/10^7 cells	0

*The results show the mean ± s.e. mean for five separate experiments. For abbreviation, see 'Materials'.

amethasone > triamcinolone > hydrocortisone. The glucocorticoid antagonists cortexolone and progesterone also competed with [^{3}H]-dexamethasone for the specific binding sites but oestradiol 17β did not (Table 1).

Stimulation of macrocortin secretion

Macrocortin secretion is apparently only brought about by agents which are glucocorticoid receptor agonists, as aldosterone, cortexolone, oestradiol-17β, progesterone and testosterone failed to induce secretion (Table 2) whereas hydrocortisone and triamcinolone were effective. In addition, the secretion of stored macrocortin was not mobilized by non-glucocorticoid macrophage stimulators such as f-MLP, A23187, PMA, cytochalasin B or LPS (Table 1). However, in two experiments phagocytosis of zymosan or heat killed (*B. pertussis*) caused an elevation of intracellular macrocortin levels possibly by an overall increase in cellular metabolism following the phagocytic stimulation.

Table 3 Effects of inhibitors of protein and RNA synthesis on glucocorticoid-induced macrocortin secretion from rat peritoneal macrophages

Compound	IC_{50}* (μM)	IC_{100}* (μM)
Actinomycin D	0.32 ± 0.03	1.04 ± 0.08
Cycloheximide	1.81 ± 0.07	5.58 ± 0.14
Puromycin	7.41 ± 0.14	24.71 ± 2.17

*The results show the mean ± s.e. mean for seven separate experiments.

Table 4 RNA and protein synthesis by rat peritoneal macrophages: effects of modulators of macrocortin secretion

Drug	*Concentration* (μM)	*RNA synthesis* (% control)*	*Protein synthesis* (% control)*
Puromycin	10	98.3 ± 4.7	46.5 ± 3.4
Colchicine	50	97.4 ± 5.3	99.7 ± 4.3
Actinomycin D	0.8	27.4 ± 6.1	97.2 ± 2.8
Dexamethasone	2	98.2 ± 4.7	95.2 ± 4.9

*The results are the mean ± s.e. mean of three separate experiments.

Inhibition of glucocorticoid-induced macrocortin secretion

Compounds such as actinomycin D which inhibit RNA synthesis and cycloheximide or puromycin which inhibit protein synthesis, prevent the glucocorticoid-induced inhibition of phospholipase A_2 and prostaglandin biosynthesis in renal papillary tissue, perfused lungs and isolated leucocytes (Danon & Assouline, 1978, Flower & Blackwell, 1979; Carnuccio *et al.*, 1980). These effects were confirmed in the present study and the IC_{50} values for inhibition by these compounds of macrocortin secretion are shown in Table 3, and their effects upon total RNA and protein synthesis are shown in Table 4.

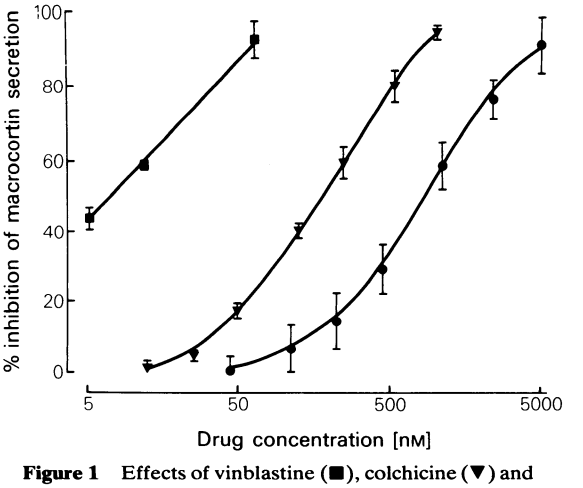

Figure 1 Effects of vinblastine (■), colchicine (▼) and trimethylcolchicinic acid (●) on glucocorticoid-induced macrocortin secretion from rat peritoneal macrophages (each point is the mean of three separate experiments; s.e.means shown by vertical lines).

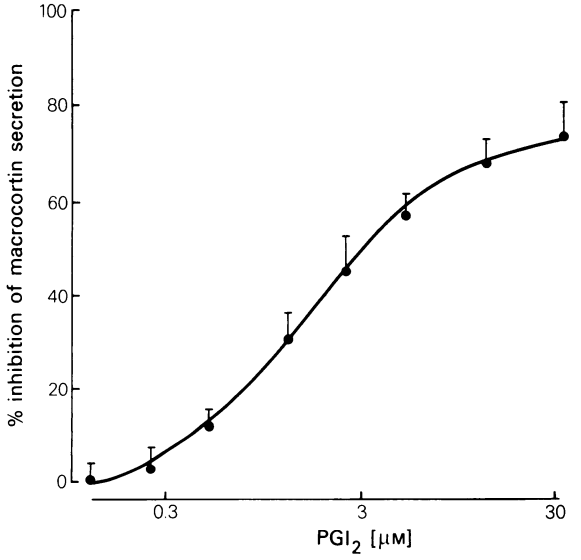

Figure 2 Inhibition of glucocorticoid-induced macrocortin secretion by PGI_2 (prostacyclin) (each point is the mean of three individual experiments; s.e.mean shown by vertical lines).

Inhibitors of microtubule assembly were also tested for their ability to inhibit glucocorticoid-induced macrocortin secretion. Agents such as vinblastine (IC_{50} 8.3 nM) colchicine (IC_{50} 170 nM) and TMCA (IC_{50} 920 nM) were potent inhibitors of macrocortin secretion (Figure 1). The photoisomeric products of colchicine (α- and β-lumicolchicine), as well as colchiceine were without effect even at concentrations in excess of 5 μM, but at these concentrations the agents had toxic effects upon macrophage viability as measured by dye exclusion tests.

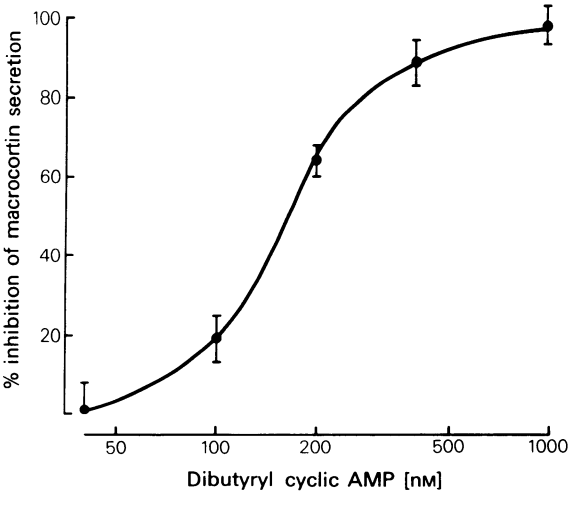

Figure 3 Inhibition of glucocorticoid-induced macrocortin secretion from rat peritoneal macrophages by dibutyrylcyclic AMP (each point is the mean from at least three separate experiments; s.e.mean shown by vertical lines).

Table 5 Effects of metabolic inhibitors, chelating agents, protease and glycosylation inhibitors on glucocorticoid-induced macrocortin secretion from rat peritoneal macrophages

Compound	*IC_{50}*	*IC_{100}*
2-Deoxyglucose	1.2 mM	3.9 mM
2,4-Dinitrophenol	26 μM	51 μM
EDTA	—	5 mM
EGTA	—	5 mM
TLCK	192 μM	1.2 mM
Tunicamycin	2.6 μg/ml	>20 μg/ml

Incubation of peritoneal macrophages with prostacyclin (PGI_2) produced a dose-dependent inhibition of glucocorticoid-induced macrocortin secretion (Figure 2). A maximal inhibition of 75% was obtained with concentrations of 26 μM. Since PGI_2 is, at least in platelets, the most potent stimulator of adenylate cyclase yet discovered (Tateson, Moncada & Vane, 1977; Gorman, Bunting & Miller, 1977) it is possible that the effects of PGI_2 on macrocortin secretion might also be brought about by the elevation of intracellular levels of cyclic AMP, which in turn might control the secretory process. (Indeed, intracellularcyclic AMP levels increased by over 300% when macrophages were incubated in the presence of 26μ M PGI_2).

The actions of the synthetic cyclic AMP analogue, dibutyryl cyclic AMP on glucocorticoid-induced macrocortin secretion were also examined (Figure 3). Incubation of macrophages with this compound produced a profound inhibition of the secretion with total inhibition occurring at doses of 1 μM with an IC_{26} of 180 nM.

The secretion of proteins from intact cells generally requires metabolic energy and thus is sensitive to the effects of inhibitors of intermediary metabolism. Therefore metabolic poisons such as 2,4-DNP and 2-deoxyglucose would be expected to inhibit glucocorticoid-induced macrocortin secretion and indeed this was the case (Table5). Similarly, the role of Ca^{2+} in many secretory processes is well defined, and in these studies the chelation of intracellular and extracellular Ca^{2+} by EDTA and EGTA inhibited macrocortin secretion (Table 5). Tosyl-lysyl chloromethyl ketone (a protease inhibitor and tunicamycin (a glycosylation inhibitor) also prevented glucocorticoid-induced macrocortin secretion (Table 5).

Discussion

Data from this study indicate that steroid-induced macrocortin secretion from rat isolated peritoneal macrophages is specific for glucocorticoids, as non-glucocorticoid steroids such as aldosterone, testosterone and oestradiol as well as non-specific macrophage activators including f-MLP, PMA, LPS and A23187 failed to induce the secretion of macrocortin. Thus, in order to induce macrocortin secretion, it is likely that the steroid must interact with specific receptors. The relative affinities of corticosteroids for the glucocorticoid receptor from the rat peritoneal macrophages (Table 1) are in good accord with the results obtained by Werb (1978) in an excellent study examining the specificity of glucocorticoid receptors from several macrophage sources. The glucocorticoid antagonists coretexolone (11-deoxycortisol) and progesterone also competed with [^{3}H]-dexamethasone for the specific binding sites. These agents can prevent the manifestation of a glucocorticoid response in many cell systems (for review see Raspe, 1971) and can inhibit the induction of macrocortin-like proteins in the isolated pefused lung (Flower & Blackwell, 1979).

Colchicine and vinblastine inhibit protein secretion from many cell sources, including amylase from the murine pancreas (Williams & Lee, 1976), fibrinogen from cultured rat hepatocytes (Feldmann, Maurice, Sapin & Benhamou, 1975), and antibodies from rat plasma cells (Antoine, Maurice, Feldmann & Avrameas, 1980). Likewise they prevented glucocoticoid-induced macrocortin secretion (Figure 1). These agents did not interfere with glucocorticoid binding to specific receptors (concentrations in excess of 1 mM did not influence [^{3}H]-dexamethasone binding to specific receptors) and the effects of colchicine were not due to inhibition of gross protein or RNA synthesis (Table 4). Although the inhibition of the synthesis of specific proteins cannot strictly be ruled out, other workers have reported that inhibition of protein and RNA synthesis occurs between concentrations of 10^{-4}–10^{-2}M colchicine and 10^{-6}–10^{-4}M vinblastine (Seybold, Bieger & Kern, 1975; Delahunty & Johnson, 1976). Thus it seems likely that colchicine and vinblastine prevent the secretion of macrocortin rather than its synthesis, perhaps by the prevention of tubulin polymerisation and inhibition of microtubular assembly which is essential for protein secretion (Le Marchand, Patzelt, Assimacopoulos-Jeannet, Loten & Jeanrenaud, 1974). The mode of action of TMCA is probably different as it does not compete with radiolabelled colchicine for tubulin binding sites (Mizel & Wilson, 1972). Therefore, if colchicine, vinblastine and TMCA all exert their inhibitory effects on glucorcorticoid-induced macrocortin secretion at the same site, then this site is unlikely to be at the level of tubulin polymerisation. Rather they might interfere with the fusion of Golgi-derived secretory vesicles with the plasma membrane, possibly in a manner analogous to the effects of these drugs on amylase secretion from rat parotid glands (Patzelt, Brown & Jeanrenaud, 1977). The precise mechanism of this latter inhibitory activity is unknown but might involve adenylate cyclase or changes in intracellular-cyclic AMP levels (Hagmann & Fishman, 1980).

PGI_2 dose-dependently inhibited glucocorticoid-induced macrocortin secretion (Figure 2). That this was due to an elevation of intracellular cyclic AMP was suggested as dibutyryl cyclic AMP had a similar effect (Figure 3) although, theoretically dibutyryl cyclic AMP could also act as an inhibitor of the phosphodiesterase responsible for the breakdown of the endogenous cyclic AMP. However, in two further experiments (data not shown), incubation of the macrophages with theophylline and/or papaverine (1 mM), two inhibitors of cyclic AMP phosphodiesterase, did not directly inhibit glucocorticoid-induced macrocortin secretion but potentiated the effects of PGI_2. Cyclic nucleotides regulate a wide variety of cell functions including protein secretion (for review see Rasmussen & Goodman, 1977), and inhibition of glucocorticoid-induced macrocortin secretion could occur at a nuclear (Jungmann & Russell, 1977) and/or microtubular (Jameson & Caplow, 1981) level or may conceivably cause the phosphorylation of macrocortin itself. This may lead to its inactivation. Lipomodulin, derived from rabbit neutrophils is inactivated in a cell-free phosphorylation system using beef heart protein kinase (Hirata, 1981), although it is not known whether this effect occurs intracellularly.

The results obtained with chelating agents and metabolic inhibitors were not unexpected. For example, the concentrations needed to achieve complete inhibition of the secretory process (2-deoxyglucose 3.9 mM and 2,4-DNP 51 μM) are similar to those required to inhibit 48/80-induced histamine release from rat isolated mast cells (Diamant & Uvnas, 1961) and albumin secretion from rat isolated hepatocytes (Schreiber, Edwards & Schreiber, 1977). Moreover, these concentrations are approximately one order of magnitude less than those required to inhibit total protein synthesis (Schreiber *et al.*, 1977).

TLCK is a protease inhibitor that can also inhibit protein secretion from isolated hepatocytes (Edwards, Nagashima, Dryburgh, Wykes & Schreiber, 1979). The of action of TLCK in these experiments is uncertain as proteolysis could occur at several steps of the macrocortin-secretory pathway. One site could be cleavage of the pre-segment of the newly synthesized protein, but this is unlikely since macrocortin

exists as a preformed store within the cell. Another site could be removal of the pro-segment from a precursor of macrocortin. Some evidence indicates that macrocortin might be a fragment of a larger protein, possibly lipomodulin (Hirata *et al.*, 1981; Blackwell *et al.*, 1982), although several proteins from lysed macrophages and glucocorticoid-treated rabbit neutrophils which possess antiphospholipase activity and having a molecular weight in excess of 200,000 daltons have also been demonstrated (R.J. Flower personal communication, 1981; Hirata *et al.*, 1981). The actual proteolysis may take place during the Ca^{2+}-dependent formation of secretory vesicles from the Golgi apparatus (Judah & Quinn, 1978) or, during formation of secretory vesicles and their subsequent fusion with larger membranes. Glycosylation of proteins is often a pre-requisite for secretion and tunicamycin has been shown to inhibit protein secretion (Edwards *et al.*,1978) by preventing the formation of the essential acetyl glucosamine-lipid intermediates (Bettinger & Young, 1975). Although glycosylation usually occurs at the ribosome after translation (Hanover & Lennarz, 1981), tunicamycin can also inhibit glycosylation of proteins stored in the Golgi-complex (Jamieson, 1977). This compound also inhibits glucocorticoid-induced macrocortin secretion which is tentatively suggestive of a glycosylation process in this event.

In conclusion, it has been demonstrated that macrocortin secretion from rat isolated peritoneal macrophages can be modulated by a number of pharmacological agents, although the ability to stimulate secretion is, as yet, unique to glucocorticoids. This phenomenon is curious in that glucocorticoids are usually potent inhibitors of macrophage secretory processes, (Gery & Waksman, 1972; Golde & Cline, 1972; Werb, 1978) and to our knowledge, the literature cites only one example of a glucocorticoid-induced factor from macrophages, namely polymorph migration stimulator (PMS) first described by Stevenson (1974).

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