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Characterization and partial purification of 'renocortins': two polypeptides formed in renal cells causing the anti-phospholipase-like action of glucocorticoids

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1 Anti-inflammatory steroids reduce prostaglandin E_2 (PGE_2) synthesis in rat renomedullary interstitial cells in culture by inhibiting the release of arachidonic acid from membranous phospholipid stores, exhibiting antiphospholipase-like properties.

2 After treatment of the cells with dexamethasone 10^{-6} M, these cells release a protein in the supernatant.

3 This supernatant is able to inhibit PGE_2 secretion in untreated cells and to inhibit phospholipase A_2 activity in an *in vitro* system.

4 Using chromatofocusing separation, we showed that two distinct proteins exist with isoelectric points of 5.8 and 8.3.

5 Using gel permeation separation, we showed that two proteins exist with apparent molecular weights of 15,000 and 30,000 daltons.

6 We conclude that, in renal cells in culture, anti-inflammatory steroids induce the synthesis and the release of two polypeptides which we have named 'Renocortins' (induced by corticoids in renal cells) causing the antiphospholipase-like action of glucocorticoids.

7 Our results are in good agreement with others, but as renal cells are not directly involved in the inflammatory process, we suggest that this steroid-induced phenomenon is not solely involved in the inflammatory reaction but is of more general physiological relevance.

Introduction

Anti-inflammatory steroids reduce prostaglandin synthesis in intact cells (Tashjian, Voelkel, McDonough & Levine, 1975; Newcombe, Fahey & Ishikawa, 1977; Parente, Ammendola, Persico & DiRosa, 1978) and in isolated organs (Kantrowitz, Robinson, McGuire & Levine, 1975; Bonilla & Dupont, 1976; Floman & Zor, 1976) by inhibiting the release of the polyunsaturated fatty acid precursors (Gryglewski, Panczenko, Korbut, Grodzinska & Ocetkiewicz, 1975; Hong & Levine, 1976; Nijkamp, Flower, Moncada & Vane, 1976; Blackwell, Flower, Nijkamp & Vane, 1978) for both cyclo-oxygenase and lipoxygenase pathways. This inhibitory action of steroids is related to the glucocorticoid function of the anti-inflammatory steroids and consequently requires glucocorticoid receptor occupancy (Russo-Marie, Paing & Duval, 1979) and RNA and protein synthesis (Danon & Assouline, 1978; Flower & Blackwell, 1979 Russo-Marie *et al.*, 1979; Tsurufuji, Sugio & Takesama, 1979). This inhibitory action is abolished by addition of exogenous arachidonic acid (Russo-Marie & Duval, 1982) demonstrating that this protein inhibits the phospholipase-induced release of arachidonic acid. The synthesis of such proteins under anti-inflammatory steroid treatment has been demonstrated in rat macrophages, 'macrocortin' (Carnuccio, DiRosa & Persico, 1980; Blackwell, Carnuccio, DiRosa, Flower, Parente & Persico, 1980), in rabbit neutrophils 'lipomodulin' (Hirata, Schiffmann, Venkatasubramanian, Salomon & Axelrod, 1980). Both macrophages and neutrophils are directly involved in the inflammatory process. The present paper shows that rat renomedullary interstitial cells in culture when treated with the anti-inflammatory steroid, dexamethasone, synthesize and release two distinct proteins which we have

named 'renocortins' (induced by corticoids in renal cells). These proteins cause the antiphospholipase-like action of glucocorticoids. These results suggest that the steroid-induced phenomenon is not solely involved in the inflammatory process but is of a more general physiological relevance.

Methods

Cell culture and cell line

Monolayer tissue cultures of rat renomedullary interstitial cells were obtained by the technique previously described (Russo-Marie, *et al.*, 1979). Briefly, renal medullae from two rats were removed, homogenized and injected subcutaneously into different places in the abdominal wall of a syngenic rat. Four days later, the yellow vascularized nodules formed were removed aseptically, trypsinized and cultured. Cultures were maintained at 37°C in a 5% CO_2 in air atmosphere and achieved confluence in 21 to 25 days. After confluence, cells were subcultured and the cell line was maintained to the 45th passage. Under similar culture conditions, these cultures were shown by Muirhead, Germain, Leach, Pitcock, Stephenson, Brooks, Brosius, Daniels & Hinman (1972), to retain the characteristics of medullary interstitial cells. Cultures were grown in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS) (GIBCO-Biocult, Glasgow, Scotland).

Treatment of cells with dexamethasone

Cells were grown for 24 h after seeding in 10 ml of fresh culture medium plus 10% FCS in T75 cm flasks (Falcon). The medium was then removed for assay of basal prostaglandin E_2 (PGE_2) content and replaced by 10 ml of new medium. Dexamethasone 10^{-6} M was added to some flasks. After an additional 24 h incubation period, medium from control and dexamethasone-treated flasks were collected. An aliquot of all supernatants was assayed for new PGE_2 content in order to determine the initial inhibition produced by dexamethasone.

Dialysis of supernatants

Supernatants (40 ml) from control and dexamethasone-treated cells were collected, dialysed twice against ammonium acetate buffer 100 mM (5 l) pH 7.4, and a third time against ammonium acetate buffer 10 mM (5 l), pH 7.4. [^{3}H]-dexamethasone (NEN, Paris) (about 10^5 ct/min) was added before dialysis in order to check the disappearance of dexamethasone from the supernatants. The dialysates were then lyophilized. After lyophilization, the dry residues were diluted in culture medium and tested either for their ability to inhibit PGE_2 secretion in untreated cells or to inhibit phospholipase A_2 activity.

In some experiments, the dissolved residue (i.e. after dialysis and lyophilization) was heated 5 min at 70°C before testing; and in other experiments, the dissolved residue was treated by trypsin (Sigma chemical corp. St Louis, Mo) for 1 h at 37°C. After a 1 h incubation period, trypsin activity was stopped by the addition of 10 mg/ml of soybean trypsin inhibitor (Sigma Chemical Corp.). Supernatants were then tested for their ability to inhibit PGE_2 secretion.

Biological activity of supernatants of control and dexamethasone-treated flasks as measured by the inhibition of prostaglandin E_2 secretion and phospholipase A_2 activity

Inhibition of prostaglandin E_2 secretion Cells were grown for 24 h after seeding in 24 multiwell Linbro plates (Linbro, Scotland) with 1 ml of medium + 10% FCS. Medium was discarded, and replaced by 0.5 ml of the samples for testing. After a 40 min incubation period, supernatants were recovered and PGE_2 secretion measured in all samples.

The sample was extracted with 2 vol. acetone to precipitate proteins; 2 vol. hexane were added, the organic phase containing neutral lipids discarded and the aqueous phase containing prostaglandins acidified to pH 4 with citric acid (70%). This phase was then extracted with 2 vol. chloroform. The organic phase was recovered, evaporated under nitrogen and resuspended in 1 ml of $NaCl/PO_4$ buffer, 100 mM, pH 7.4, containing 0.1% sodium azide and 0.1% gelatin; 100 µl aliquots of the eluates were then incubated with 100 µl of anti PGE_2 antiserum (Pasteur Institute, Paris), and 100 µl of [^{3}H]-PGE_2 (NEN, Paris), (containing approximately 2,000 ct/min). Standard curve was run in parallel using non-labelled PGE_2 (Upjohn company, Kalamazoo, Michigan). The whole reaction was carried out with the $NaCl/PO_4$ buffer described above. After 20 min incubation at room temperature and 4 h incubation at 4°C, each sample was mixed with 1 ml of dextran-coated charcoal (2.5 g of Norit A, 0.25 g of dextran T70 in 1 l of $NaCl/PO_4$ buffer) and allowed to stand for 10 min at 4°C. The samples were then centrifuged and supernatant fluids counted by liquid scintillation spectrometry.

Cells were then rinsed with Hanks balanced salt solution and dissolved in 1 ml of NaOH 1 N. The cellular protein content was measured according by the method of Lowry, Rosebrough, Farr & Randall (1951). The secretion of PGE_2 was expressed in pg of PGE_2 per µg of cellular protein. Results were expressed as percentage inhibition of PGE_2 production in

dexamethasone-treated cells as compared to control cells.

Inhibition of phospholipase A_2 activity In order to observe a phospholipase A_2 inhibiting effect, it was necessary to adapt both phospholipid and phospholipase A_2 concentrations so that phospholipids (the substrate) would not be in excess in relation to the enzyme phospholipase A_2. These experimental conditions were required because of the high level of phospholipids in foetal calf serum (15 μg per ml of culture medium containing 10% foetal calf serum).

Preliminary experiments had shown that hydrolysis of phosphatidyl choline was linear up to 35–40% as long as phospholipase A_2 concentration was modified according to the substrate concentration. Consequently, in our assays, phospholipase A_2 concentration was always chosen so that the hydrolysis of the substrate did not exceed 35%.

Two different substrates were used: phosphatidyl choline-1-α-1-palmitoyl-2 arachidonoyl [arachidonoyl-1-^{14}C] (54 mCi/mmol, NEN, Paris) and phosphatidyl choline 1-α-[dipalmitoyl-^{14}C] (95 mCi/mmol, NEN, Paris). The incubation was carried out in Tris base buffer, 50 mM, pH 8.0, containing 5 mM $CaCl_2$, 0.025 μCi of the labelled phosphatidyl choline, and various amounts of culture supernatants and of phospholipase A_2 from porcine pancreas (900 u/mg protein, Sigma Chemical Corp.) in a final volume of 0.5 ml. After a 5 min preincubation period at 37°C, the reaction was started by addition of phospholipase A_2. After an additional 5 min incubation at 37°C, the reaction was stopped by adding 2 ml chloroform/methanol (2:1). The reaction tubes were mixed vigorously. The lower phase containing lipids was evaporated to dryness and lipids were separated on silica gel plates (Merck, Darmstadt, Germany), in the developing system, chloroform/methanol/water (65:25:4). Radioactive spots were visualized by autoradiography, scraped and counted by liquid scintillation spectrometry.

The percentage of phospholipid hydrolysis was compared in control and dexamethasone-treated supernatants and results expressed as percentage inhibition found in dexamethasone-treated supernatants versus controls.

Chromatofocusing experiments

Supernatants from 3 control T75 flasks (30 ml) and 3 dexamethasone-treated T75 flasks (30 ml) were pooled and layered onto a PB 94 chromatofocusing column (9 × 1.6 cm) (Pharmacia, France). This technique segregates the proteins according to their ionic properties. Proteins were eluted with two polybuffer exchange systems which produce linear pH gradient, one allowing a 6–9 linear pH gradient and the other one allowing a 7–4 linear pH gradient. In all experiments [^{3}H]-dexamethasone was added in order to detect the zone where dexamethasone eluted. The effluent was collected in 15 ml fractions. The radioactivity, the optical density and the pH were recorded. In order to determine the percentage inhibition of PGE_2 secretion, 1 ml of each fraction was layered in triplicate on untreated cells in 24 multiwell plates and PGE_2 secretion was measured as described above; results were expressed as percentage inhibition exhibited by fractions derived from dexamethasone treated cells versus control cells.

Gel permeation experiments

Supernatants from control and dexamethasone-treated cells were separated by gel permeation on an ultrogel AcA 54 column (IBF reagents, Villeneuve la Garenne, France); this column allows separation of the proteins according to their size. The range of permeation of the gel was between 5,000 and 70,000 daltons. The column (85 × 2.6 cm) was first equilibrated with ammonium acetate buffer 100 mM, pH 7.4, and then dextran blue was eluted in order to determine the void volume. The column was calibrated by measuring the elution volume (Ve) of known molecular weight proteins: bovine serum albumin, haemoglobin, ovalbumin, α-chymotrypsin and cytochrome C. Supernatants (40 ml) from 4 T75 control and 4 T75 dexamethasone-treated flasks were layered on the top of the column. [^{3}H]-dexamethasone was added in order to determine the low molecular weight fraction where it eluted. The elution buffer was ammonium acetate buffer 100 mM, pH 7.4; 4 ml fractions were collected. The radioactivity and the optical density of each fraction was recorded. Fractions were lyophilized, dissolved in medium containing 10% FCS and tested in triplicate on 24 multiwell plates. PGE_2 secretion was measured as described above and results expressed as in the preceding experiments.

Results

Effects of supernatants on prostaglandin E_2 secretion over a 40 min incubation period in untreated cells grown in 24 multiwell plates

Supernatants from control and dexamethasone-treated cells were dialysed as described in methods; addition of trace amounts of [^{3}H]-dexamethasone (10^5 ct/min) showed that over 99% of dexamethasone was eliminated by dialysis. The dialysates were lyophilized. The lyophilizates were tested for their action on prostaglandin secretion in untreated cells over a 40 min incubation period.

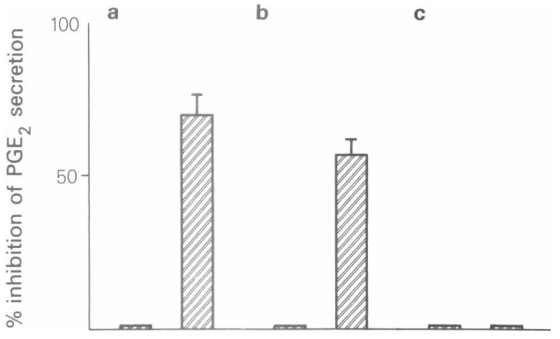

Figure 1 Effects of lyophilizates after dialysis of supernatants derived from control cells (left hatched columns) and cells treated with 10^{-6} M dexamethasone (right hatched columns) for 24 h on prostaglandin E_2 (PGE_2) secretion in untreated cells over a 40 min incubation period. (a) Inhibition of PGE_2 secretion by supernatants dialysed and lyophilised. (b) Inhibition of PGE_2 secretion by supernatants which have been heated 5 min at 70°C after dialysis and lyophilisation. (c) Inhibition of PGE_2 secretion by sypernatants which have been treated by trypsin for 1 h at 37°C after dialysis and lyophilization.

Lyophilizates derived from dexamethasone-treated cells showed an inhibitory effect on PGE_2 secretion when compared to those derived from control cells. Ten ml of medium derived from about 700 μg of cellular protein was dialysed, lyophilized and the residue dissolved in 3 ml of new medium: 0.5 ml of this medium was layered in triplicate on wells containing approximately 30 μg of cellular protein. Supernatants derived from dexamethasone-treated cells produced a 70 ± 6% (mean ± s.d.) inhibition of PGE_2 secretion when compared to supernatants derived from control cells. In summary, the PGE_2 secretion of 1 mg of untreated cells was inhibited by 70% by the product of 4 mg of cells incubated with 10^{-6} M dexamethasone for 24 h (Figure 1a). The same experiments were performed but the dissolved residue was heated for 5 min at 70°C; the inhibition was of 56 ± 5% (mean ± s.d.) (Figure 1b). The same experiments were performed but the dissolved residue was treated with trypsin and then with soybean trypsin inhibitor. Under these experimental conditions supernatants from dexamethasone treated cells were no longer inhibitory (Figure 1c); (control cells treated with 0.5 ml of fresh medium containing 1 mg/ml of trypsin and 10 mg/ml of soybean trypsin inhibitor had no effect on PGE_2 secretion). In addition, the inhibitory effect was lost after boiling the supernatants.

Effects of supernatants on prostaglandin E_2 secretion and phospholipase A_2 activity

The same supernatants were tested in parallel for their action on PGE_2 secretion over untreated cells and on porcine phospholipase A_2. Supernatants were tested at various dilutions.

For testing their effect on PGE_2 secretion, the supernatants were effectively diluted (no other parameters were modified in the assay). The results are shown in Table 1. A linear inhibition can be observed; the initial dilution (1:6) corresponds to the dilution used in the other series of experiments described in Figure 1.

For testing their effect on porcine phospholipase A_2, because of the necessity to adjust the ratio, phospholipid/phospholipase A_2, both parameters were diluted in parallel, in which case, dilutions of the supernatants were in relation to phospholipase A_2.

The results are shown in Table 2. In these conditions, a constant inhibition of phospholipase A_2 was observed on both substrates. These results are in favour of a molecular 1:1 ratio between the inhibitor and phospholipase A_2, which reinforce the results

Table 1 Effects of supernatants on prostaglandin E_2 (PGE_2) secretion in untreated cells

Supernatant dilution	*Control supernatants* (pg/μg protein)	*Dexamethasone supernatants* (pg/μg protein)	*Inhibition* (%)	n
1/6	204.0 ± 34.6	70.8 ± 9.7	65	3
1/30	80.9 ± 13.3	60.9 ± 2.9	25	3
1/60	92.0 ± 4.2	75.2 ± 8.2	18	3

Supernatants from control and dexamethasone-treated cells were tested for their action on prostaglandin secretion in untreated cells over a 40 min incubation period. Ten ml of medium derived from about 700 μg of cellular protein (= 1 T75 flask) was dialyzed, lyophilized and dissolved in 3 ml of new medium: 0.5 ml of this medium was layered in triplicate in wells containing approximately 30 μg of cellular protein (dilution 1:6). The other dilutions are always referred to this first one.

The secretion of PGE_2 is expressed in pg of PGE_2 secreted per μg of cellular protein (mean ± s.d.).

Results are expressed as the percentage inhibition found in dexamethasone-treated supernatants versus control supernatants.

Table 2 Effects of supernatants on porcine pancreatic phospholipase A_2 activity

Supernatant dilution	*PLA_2* (units)	*Control supernatants* (% hydrolysis)	*Dexamethasone supernatants* (% hydrolysis)	*Inhibition* (%)	n	*Substrate*
1/6	3.0	33.3±0.7	27.6±4.6	17.0	3	Dipalmitoyl-PC
1/12	1.5	35.1±1.6	27.1±5.0	22.6	3	Dipalmitoyl-PC
1/24	.75	30.5±4.8	23.0±1.8	24.6	3	Dipalmitoyl-PC
1/48	.37	28.8±6.9	21.7±2.9	24.7	3	Dipalmitoyl-PC
1/6	3.0	23.6±1.1	20.3±0.7	14.0	2	1-Palmitoyl-2-arachidonoyl-PC
1/12	1.5	28.7±2.4	21.0±1.4	26.8	2	1-Palmitoyl-2-arachidonoyl-PC
1/48	.37	16.7±0.1	14.2±0.1	15.0	2	1-Palmitoyl-2-arachidonoyl-PC

Supernatants from control and dexamethasone-treated cells were tested for their action on porcine pancreatic phospholipase A_2 activity. The same procedure as in Table 1 was followed. The first dilution was chosen so that the supernatant of 1 T75 flask (about 700 μg of cellular protein) was diluted 1:6 in the assay. The other dilutions are referred to this first one.

Porcine pancreatic phospholipase A_2 units were always chosen according to the supernatant dilutions so that the percentage of hydrolysis never exceeded 35–40%. Values are mean ± s.d.

In the first set of experiments, the substrate used was phosphatidyl-choline 1-α-[dipalmitoyl-1-^{14}C]. In the second set of experiments, the substrate used was phosphatidyl choline-1-α-1-palmitoyl-2 arachidonoyl [arachidonoyl-1-^{14}C]

Results are expressed as percentage inhibition found in dexamethasone supernatants versus control supernatants.

found on the inhibition of PGE_2 secretion which decreases linearly with the concentration of the inhibitor, (in this case, the concentration of phospholipase A_2 in the assay is assumed to be constant).

Our experimental data have shown that the biological activity (as measured by the inhibition of PGE_2 secretion in untreated cells over a 40 min incubation period) is destroyed by treatment with proteolytic enzymes and/or by boiling (data not shown) strongly suggesting that this factor is a protein. Furthermore, our previous findings (Russo-Marie *et al.*, 1979), had demonstrated that the inhibitory effect of dexamethasone on PGE_2 secretion was prevented either by cycloheximide or by actinomycin D. Both these arguments allow us to conclude that this factor is a protein.

Further purification of this protein determined its physical properties, the isoelectric point and the molecular weight. In these experiments, the biological activity was measured by determining the inhibition of PGE_2 secretion in untreated cells.

Determination of the isoelectric point using chromatofocusing separation

Supernatants from control and dexamethasone-treated cells were analysed on a PB 94 chromatofocusing column (Figure 2).

The upper part of the figure shows the separation performed with a polybuffer exchange (PBE) allowing a 6–9 linear pH gradient. Two zones of inhibition can be detected, one corresponding to an isoelectric point of about 8.3 and one corresponding to a zone below 6. The lower part of the figure shows the separation performed with a PBE allowing a 7–4 linear pH gradient. Two zones of inhibition are found, one corresponding to an isoelectric point of about 5.8 and one corresponding to an isoelectric point above 7 (i.e., the 8.3 zone detected in the other PBE).

Determination of the molecular weights by gel permeation

Supernatants from control and dexamethasone-treated cells were separated by gel permeation on an ultrogel AcA 54 column (Figure 3). The fractions showing biological activity (i.e. inhibition of PGE_2 secretion over a 40 min incubation period in untreated cells) were eluted from supernatants derived from dexamethasone-treated cells and were found in two zones corresponding to apparent molecular weights of 15,000 and 30,000 daltons.

Discussion

The results of the present study demonstrate that dexamethasone, a powerful anti-inflammatory steroid, induces the synthesis and the release in rat renomedullary interstitial cells of two proteins exhibiting anti-phospholipase like properties. We had previously shown (Russo-Marie, Paing & Duval, 1979), that in this model of interstitial cells in culture,

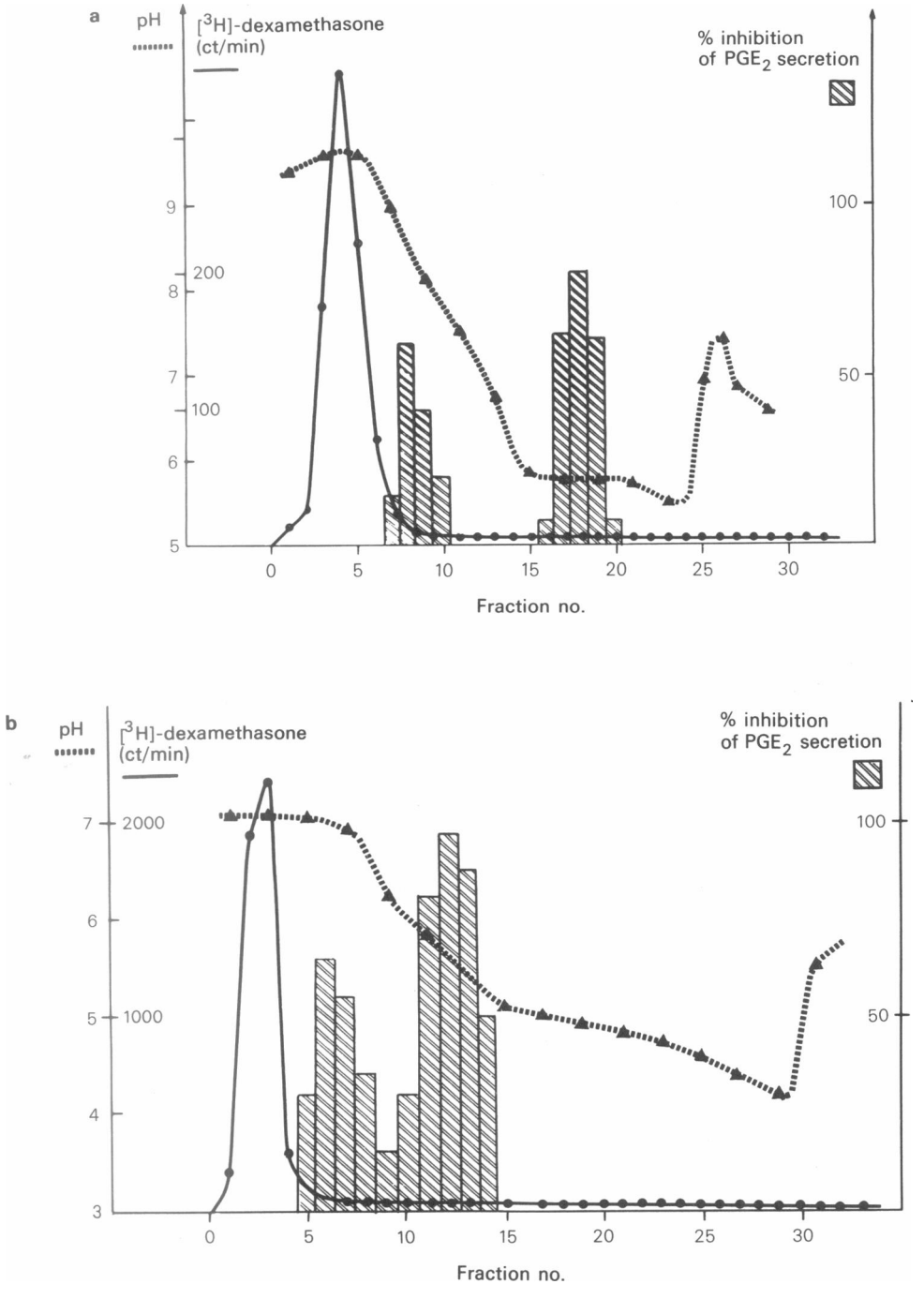

Figure 2 PB94 chromatofocusing separation of supernatants derived from control cells and cells treated with dexamethasone 10^{-6} M for 24 h. In (a) is shown the separation performed with a polybuffer exchange (PBE) allowing a 6–9 linear pH gradient. In (b) is shown the separation performed with a PBE allowing a 7–4 linear pH gradient. The radioactivity (●) and the pH (▲) were measured. The inhibition of PGE_2 secretion on a 40 min incubation period in untreated cells is shown as a histogram (hatched columns).

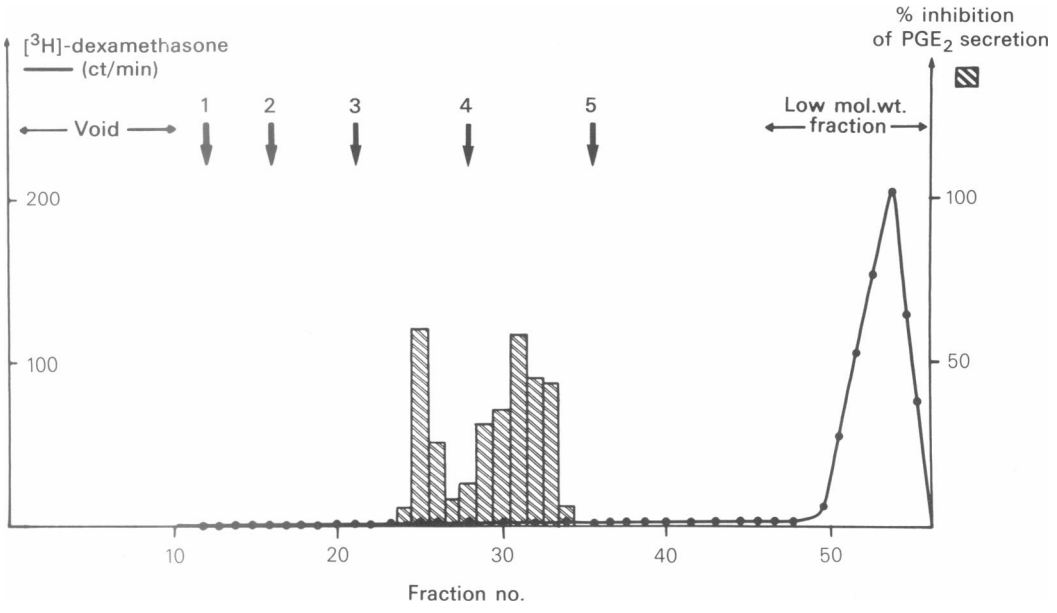

Figure 3 Gel permeation experiments on an ultrogel AcA column of supernatants derived from control cells and cells treated with dexamethasone 10^{-6} M for 24 h. Void volume was determined by the elution of dextran blue, dexamethasone eluted with low molecular weight fractions. The column was calibrated by measuring the elution volume (Ve) of bovine serum albumin (1), haemoglobin (2), ovalbumin (3), α-chymotrypsin (4) and cytochrome C (5). The radioactivity (●) of each fraction was measured. The inhibition of PGE_2 secretion on a 40 min incubation period in untreated cells is shown as a histogram (hatched columns).

in order to inhibit prostaglandin secretion, protein synthesis was necessary, involving specific glucocorticoid receptor occupancy and mRNA synthesis. We also demonstrated that the protein involved in this regulatory system was not an enzyme of the arachidonic acid cascade (Russo-Marie & Duval, 1982). This induced protein inhibited the deacylating step of arachidonic acid from phospholipids, because its action was prevented by addition of exogenous arachidonic acid (Russo-Marie & Duval, 1982). Therefore, the fact that our cells release in the supernatant a factor whose biological activity (i.e. inhibition of prostaglandin secretion in untreated cells) is destroyed either by boiling or by proteolytic enzymes but whose activity is preserved after heating 5 min at 70°C and that these same supernatants are able to inhibit phospholipase A_2 activity favours the concept that our cells release proteins exhibiting anti-phospholipase A_2 like properties similar to either macrocortin (Blackwell *et al.*, 1980; Blackwell, Carnuccio, DiRosa, Flower, Langham, Parente, Persico, Russel-Smith & Stone, 1982) or lipomodulin (Hirata *et al.*, 1980). These induced proteins are very unlikely to be similar to other proteins affecting the prostaglandin system by reducing prostaglandin synthetase activity (Saeed, McDonald-Gibson, Cuthbert, Copas, Schneider, Gardiner, Butt & Collier, 1977; Moore & Hoult, 1980) for two reasons: (1) in our system, the inhibition of prostaglandin secretion is reversed by addition of exogenous arachidonic acid (Russo-Marie & Duval, 1982); (2) in our cells in culture, we have also shown (Russo-Marie, Seillan & Duval, 1980) that prostaglandin synthetase activity is not reduced by corticosteroids but increased. This increase is probably a regulatory mechanism due to the lower availability of arachidonic acid for cyclooxygenase.

Other arguments favour the hypothesis that these proteins are similar to macrocortin and/or lipomodulin. They share the same biological properties (inhibition of phospholipase A_2 activity, inhibition of prostaglandin synthesis) and after separation on gel chromatography, we find that the biological activity is found in two zones, one in the 15 k mol.wt. and one in the 30 k mol.wt. Macrocortin has first been reported as having a 15 k mol.wt. (Blackwell *et al.*, 1980) although further studies have demonstrated that the biological activity was also found in other mol.wt. zones. Hirata *et al.* (1980) have reported that rabbit polymorphs release a protein, lipomodulin, of mol.wt. 40 k, but in the absence of protease inhibitors lipomodulin decomposed to various fragments including 15 k and 25 k peptides.

Our proteins have been found in the supernatants

of our cells in culture, in presence of foetal calf serum, where proteases are present and we cannot exclude the possibility that we are dealing with moieties of one protein of a mol.wt. of 40k. A possible hypothesis would be that in our system, the proteins which are released in the supernatant of our cells are of lower mol.wt. than the protein which is synthesized inside the cell and might represent proteolytic fragments of a bigger one. Experiments are now in process to test such an hypothesis.

Anti-inflammatory steroids have been shown to induce the synthesis of proteins (macrocortin, lipomodulin, renocortins) in various cell types. All these proteins cause the anti-phospholipase-like effects of glucocorticoids. The variety of the cells synthesizing such proteins involving the glucocorticoid function is in favour of a more physiological phenomenon. In addition, recent data have shown that the synthesis of anti-phospholipase A_2 proteins occurs in rats not only following dexamethasone treatment but also when animals are treated with ACTH (Blackwell *et al.*, 1982). A tempting explanation could prolong the hormonal pathway described up to now: cRH (ACTH releasing factor) would induce ACTH synthesis. ACTH would induce glucocorticoid formation which would induce the synthesis of antiphospholipase proteins: the chronologically formed products becoming more specific for a certain biological activity.

The fact that macrophages and neutrophils, both types of cells involved in the inflammatory process, do secrete anti-phospholipase proteins under anti-inflammatory steroid treatment is in favour of a role of these antiphospholipase proteins in the control of the inflammatory reaction. These proteins probably do participate in the anti-inflammatory action of steroids (Blackwell *et al.*, 1982) but their exact role in this process is not known, apart from their action on the arachidonic acid cascade. This hypothesis seems to have a pathophysiological relevance since it has been found that patients with rheumatic diseases have auto-antibodies for lipomodulin in their serum (Hirata, Del Carmine, Nelson, Axelrod, Schiffmann, Warabi, De Blas, Nirenberg, Manganiello, Vaughan, Kumagai, Gran, Decker & Steinberg, 1981).

The synthesis of proteins exhibiting anti-phospholipase like properties in renal cells, not involved in the inflammatory process under anti-inflammatory steroid treatment may be an argument for a more general physiological mechanism as has been discussed before. However, we cannot exclude the hypothesis that the proteins induced by dexamethasone in renal cells might also play a specific local role, in relation to this target organ of steroid hormones. This hypothesis can be questioned from recent results, indicating that systemic lupus erythematosus patients present disorders of arachidonic acid metabolism in the kidney, namely an increased production of renal thromboxane and PGE_2 (Ciabattoni, Patriganani, Filabozzi, Pierucci, Simonetti, Cinotti, Pinca, Gotti, Remuzzi and Patrono, 1982).

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