Stimulation of Anterior Pituitary Prostaglandin E Content and Somatotropin (Growth Hormone) Synthesis by Phospholipase A

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The prostaglandin E content of dispersed rat anterior pituitary glands was found to increase in the presence of phospholipase A or arachidonic acid. The increases were abolished by the addition of indomethacin. Similarly, the rate of somatotropin (growth hormone) synthesis was increased by these two agents, and the increases were again abolished by indomethacin. Phospholipase A also stimulated somatotropin release. The stimulation of prostaglandin E accumulation was a specific response to those fatty acids that are precursors for prostaglandin synthesis. One such precursor, [^3H]arachidonic acid, was incorporated by rat anterior pituitary glands *in vitro*, and found to be associated mainly with phosphatidylethanolamine-like material. It is concluded that the intracellular concentration of prostaglandin E is limited by the availability of precursor fatty acids and that this can be increased by the addition of exogenous precursors or by the action of exogenous phospholipase A on the cellular phospholipid. Factors that increased prostaglandin E concentrations also increase the rate of synthesis of somatotropin, providing further evidence for the concept that prostaglandin E is involved in modulation of the rate of synthesis of this hormone.

The anterior pituitary gland synthesizes and secretes a number of polypeptide hormones, the production of which is primarily under the control of the hypothalamus. The action of hypothalamic hormones in regulating synthesis and secretion of the corresponding pituitary hormones has been studied in some detail, and cyclic nucleotides and prostaglandins have both been proposed as mediators of this action (Labrie *et al.*, 1976).

Prostaglandins have been shown to stimulate the synthesis and secretion of several pituitary hormones *in vitro*, including somatotropin (Schofield, 1970; MacLeod & Lehmeyer, 1972). Luliberin, a hypothalamic factor that controls the secretion of lutropin and follitropin, stimulates prostaglandin E concentrations in dispersed rat anterior-pituitary cells (Barden & Betteridge, 1977), whereas insulin, at concentrations that decrease the rate of somatotropin biosynthesis, lowers the prostaglandin E content of such cells (Betteridge & Wallis, 1977). It thus seems possible that modulation of prostaglandin E concentrations in the anterior pituitary plays a role in the regulation of hormone synthesis and release, though whether such concentrations are changed by alteration of prostaglandin E synthesis or degradation remains to be established.

Treatment of some tissues with phospholipase A increases prostaglandin formation (Bartels *et al.*, 1970) by releasing prostaglandin precursors from membrane phospholipid (Kunze & Vogt, 1971). This

suggests that in some tissues, at least, prostaglandin concentrations are limited by availability of precursors. In the present paper we describe studies on dispersed anterior-pituitary cells, designed to determine whether a similar limitation may apply here also. The effects of phospholipase A and various fatty acids were assessed on the intracellular prostaglandin E content and the effect of phospholipase A on somatotropin biosynthesis and secretion was also measured.

Experimental

Materials

L-[4,5- ^3H]Leucine (sp. radioactivity 40–50 Ci/mmol), [5,6,8,9,11,12,14,15- ^3H]arachidonic acid (sp. radioactivity 135 Ci/mmol) and [5,6,8,11,12,14,15- ^3H]prostaglandin E₂ (sp. radioactivity 150 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Phospholipase A (specific activity 1220 units/mg; 1 unit will hydrolyse 1 μmol of L- α -phosphatidylcholine/min at 37°C, pH 8.5), various fatty acids and Amido Black were obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K., and phospholipid standards were from Lipid Products, Redhill, Sussex, U.K. Prostaglandins were a gift from Upjohn Pharmaceuticals, Crawley, Sussex, U.K. Tissue culture media, sera and additions were from Gibco,

NY, U.S.A. Rat somatotropin (growth hormone), (NIAMDD rat GH-B-3 and NIAMDD rat GH-RP-1) and monkey anti-(rat somatotropin) (NIAMDD-Anti-rat GH-serum) were gifts from Dr. A. Parlow and the Rat Pituitary Hormone Program of the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD, U.S.A.

Dispersed pituitary cells

Anterior-pituitary glands were taken from 2–3-month-old male Sprague–Dawley rats. They were treated with collagenase and hyaluronidase and then with pancreatic extract (Viokase-Gibco) as described by Vale *et al.* (1972). The dispersed-cell preparation obtained was washed several times by centrifugation with Dulbecco's modified Eagle's medium and then plated out in the same medium containing 10% (v/v) horse serum, 2.5% (v/v) foetal calf serum, non-essential amino acids and antibiotics (Vale *et al.*, 1972) into sterile tissue-culture dishes (3.5 cm diameter, Falcon Plastics). Three dishes were obtained from each pituitary gland used. The dishes of cells were then incubated at 37°C for 4 days in a humidified atmosphere of CO₂/air (1:19). The dishes were then washed with fresh Dulbecco's modified Eagle's medium (without sera) to remove unattached cells and the cells were preincubated in the same medium (1.5 ml/dish) for 1 h. The cells were incubated in Dulbecco's modified Eagle's medium with the various additions for 15 min where prostaglandin E content was to be measured, or for 5 h in the presence of 10 μ Ci of [³H]leucine/ml where somatotropin synthesis was to be measured.

Measurement of prostaglandin E

After incubation the medium was removed from the dishes and the cells were scraped off in ethanol/0.9% (w/v) NaCl (1:1) (1 ml per dish) and then sonicated for 5 s at 2 A (Soniprobe; Dawe, London W.3, U.K.). The prostaglandins were then extracted with 2 ml of ethyl acetate and the extract was dried *in vacuo* and the residue was redissolved in phosphate-buffered saline (Jaffe & Behrman, 1974) for use in the radioimmunoassay as described elsewhere (Barden & Betteridge, 1977). The antibody used was fairly specific for prostaglandins of the E-series (less than 1% cross-reaction by prostaglandins F_{1 α} or F_{2 α} ; less than 8% cross-reaction by prostaglandins A₁ or A₂), but did not discriminate between prostaglandins E₁ and E₂. Therefore results are expressed in terms of prostaglandin E (E₁ and E₂ combined), but the possibility of interference by significant quantities of prostaglandins A₁ or A₂ cannot be excluded. [³H]Prostaglandin E₂ was used as the radioactive ligand and synthetic prostaglandin E₂ as standard.

Measurement of the rate of somatotropin synthesis

After incubation the cells were scraped from their dishes and sonicated in their medium as above. Samples of the sonicates were subjected to polyacrylamide-gel electrophoresis (Davis, 1964) with rat somatotropin (25 μ g/gel) as a marker. The gels were stained in 0.01% (w/v) Amido Black in 7.5% (v/v) acetic acid. The somatotropin-containing band (Lewis *et al.*, 1965; Betteridge & Wallis, 1973) was then excised and treated for scintillation counting (Betteridge & Wallis, 1973).

Measurement of somatotropin secretion

Samples of incubation medium were diluted (usually between 1:5 and 1:25) and assayed for somatotropin content with a radioimmunoassay kit obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases. The procedure used was that described in the instructions accompanying the reagents, except that the antibody-hormone complex was precipitated with poly(ethylene glycol), as described by Desbuquois & Aurbach (1971). The somatotropin contents of the samples were estimated from the standard curve and expressed in terms of ng of NIAMDD-GH-RP1 standard/ml of incubation medium.

Expression of results

The protein contents of the cells in each dish were measured by subjecting samples of the sonicate to the method of Lowry *et al.* (1951). The prostaglandin E content of the cells was expressed as pmol of prostaglandin E per mg of protein in each dish, and the rate of somatotropin synthesis was expressed as c.p.m. of [³H]leucine incorporated into somatotropin-like material/mg of protein in each dish. The mean and s.e.m. values from several experiments were obtained and compared for statistical differences between experimental and control values by using Student's *t* test.

Incorporation of [³H]arachidonic acid into lipids

Anterior pituitary glands from two rats were incubated in 1 ml of Dulbecco's modified Eagle's medium containing 20 μ Ci of [³H]arachidonic acid. At the different time points, the pituitaries were removed from the medium and homogenized in 1 ml of 0.9% (w/v) NaCl. The homogenate was extracted with 3 vol. of chloroform/methanol (9:1, v/v). The phases were separated by centrifugation and the organic phase was dried *in vacuo* and redissolved in a small volume of chloroform/methanol (9:1) for t.l.c. on Eastman Chromagen silica-gel sheets. The chromatograms were developed in chloroform/

hexane/acetic acid (65:35:2, by vol.) or chloroform/methanol/water (65:35:4, by vol.) (Waite & Van Deenen, 1967). A variety of phospholipids and neutral lipids was used as markers and the spots were identified with I_2 vapour. The radioactivity in spots that ran with similar R_F values to the standards was determined by scintillation counting. The results are expressed as the radioactivity in any region of the chromatogram given as a percentage of the amount of radioactivity recovered from that sample.

Results

Effects of phospholipase A and arachidonic acid on prostaglandin E content

Anterior-pituitary cells were incubated in Dulbecco's modified Eagle's medium, either alone or containing phospholipase A (10 units/ml), arachidonic acid (10 μ g/ml) or indomethacin (10 μ g/ml) or various combinations of these, for 15 min. The medium was then removed and the prostaglandin E content of the cells was measured (Table 1). Phospholipase A alone caused a 70% increase ($P < 0.01$) in the prostaglandin E concentration within the cells, which was largely abolished by the presence of indomethacin. Similarly, arachidonic acid caused a 90% increase ($P < 0.02$), which was also largely abolished by indomethacin.

Effects of phospholipase A and arachidonic acid on somatotropin synthesis

The rate of incorporation by dispersed anterior-pituitary cells of [3 H]leucine into somatotropin-like material, over a 5 h period, was measured in the presence or absence of phospholipase A (10 units/ml), arachidonic acid (10 μ g/ml) or indomethacin (10 μ g/ml) (Table 2). Both phospholipase A and arachidonic acid caused significant increases ($P < 0.01$ and $P < 0.02$ respectively) in the rate of somatotropin biosynthesis. Indomethacin was able to abolish completely the stimulation by arachidonic acid, and largely the effect of phospholipase A.

Effect of phospholipase A on somatotropin release

In addition to its effects on somatotropin synthesis, phospholipase A was tested for its ability to influence the rate of somatotropin secretion by dispersed anterior pituitary cells. The dishes of cells were incubated for 15 min in Dulbecco's modified Eagle's medium containing 0–20 units of phospholipase A/ml. The medium was removed and its somatotropin content was measured by radioimmunoassay (Fig. 1). All concentrations tested (1–20 units/ml) significantly ($P < 0.05$) stimulated somatotropin secretion from these cells. Phospholipase A (10 units/ml) (the concentration used in the experiments described

Table 1. *Effects of phospholipase A, arachidonic acid and indomethacin on prostaglandin E content*

Dispersed anterior-pituitary cells were incubated for 15 min in Dulbecco's modified Eagle's medium, either alone or containing phospholipase A (10 units/ml), arachidonic acid (10 μ g/ml) or indomethacin (10 μ g/ml). The medium was removed and the prostaglandin content of the cells was measured as described in the text. The values shown are the mean $\pm$ S.E.M. from six separate observations.

Additions to medium	Prostaglandin E content (pmol/mg of protein)	Statistical difference from control
None (control)	44.2 $\pm$ 3.9	—
Phospholipase A	74.0 $\pm$ 5.1	$P < 0.01$
Phospholipase A + indomethacin	9.5 $\pm$ 1.0	$P < 0.001$
Arachidonic acid	85.5 $\pm$ 13.2	$P < 0.02$
Arachidonic acid + indomethacin	17.1 $\pm$ 3.0	$P < 0.01$
Indomethacin	6.7 $\pm$ 1.7	$P < 0.001$

Table 2. *Effects of phospholipase A, arachidonic acid and indomethacin on somatotropin synthesis*

Dispersed anterior-pituitary cells were treated as described in Table 1, except that all the media contained 10 μ Ci of [3 H]leucine/ml and the incubations lasted for 5 h. The amount of [3 H]leucine incorporated into somatotropin-like material was measured as described in the text. The values shown are the mean $\pm$ S.E.M. from six separate observations.

Additions to medium	$10^{-3} \times$ Somatotropin synthesis (c.p.m./mg of protein)	Statistical differences from control
None (control)	19.8 $\pm$ 0.8	—
Phospholipase A	25.0 $\pm$ 0.9	$P < 0.01$
Phospholipase A + indomethacin	21.6 $\pm$ 1.2	$P > 0.05$
Arachidonic acid	27.5 $\pm$ 1.9	$P < 0.02$
Arachidonic acid + indomethacin	19.4 $\pm$ 1.1	$P > 0.05$
Indomethacin	19.0 $\pm$ 1.1	$P > 0.05$

Fig. 1. Effect of phospholipase A on somatotropin release

Dishes of cells were incubated for 15 min in Dulbecco's modified Eagle's medium containing various concentrations of phospholipase A. The media were removed and frozen. After thawing, samples of the media were analysed for somatotropin content by using a specific radioimmunoassay as described in the text. The values shown are the mean $\pm$ S.E.M. from four separate observations.

previously) caused a 5-fold increase in somatotropin release.

Effects of various fatty acids on the prostaglandin E content of the cells

It was thought possible that the effect of arachidonic acid on prostaglandin E concentrations and/or somatotropin synthesis could be a non-specific effect caused by the addition of an unsaturated fatty acid

to the cells. Therefore a variety of fatty acids (all at a final concentration of $10 \mu\text{g/ml}$) were tested for their effects on the prostaglandin E content of anterior-pituitary cells after 15 min of incubation (Table 3). Of the fatty acids tested only arachidonic (the precursor of prostaglandin E_2) and eicosa-8,11,14-trienoic (the precursor of prostaglandin E_1) acids were able to stimulate the prostaglandin E contents of the cells. All the other fatty acids tested were ineffective including eicosa-11,14,17-trienoic acid, which is not a prostaglandin precursor.

Site of arachidonic acid storage in anterior-pituitary cells

The ability of phospholipase A to increase the prostaglandin E content of these cells suggests that phospholipids may be an important store of prostaglandin precursors. Therefore, anterior-pituitary glands were incubated with [^3H]arachidonic acid and the distribution of the radioactivity between various lipid fractions was measured by t.l.c. (Table 4). After 1 h 50% of the [^3H]arachidonic acid on the chromatograms was found to co-run with phosphatidylethanolamine and most of the rest appeared to be incorporated into other phospholipids. At the end of a 24 h incubation, 60% of the radioactivity was associated with material that behaved like phosphatidylethanolamine and all but 5% was found associated with phospholipid-like material.

Discussion

The fact that arachidonic acid stimulates prostaglandin E accumulation suggests that it is able to enter the cells fairly easily and act as substrate for the intracellular prostaglandin synthase; this is confirmed by the observation that the effect can be prevented by indomethacin, a known inhibitor of the synthetase. The ability of exogenous phospholipase A to increase the intracellular prostaglandin E concentration could be explained if it was able to interact with the cellular phospholipids to increase the release of endogenous prostaglandin precursors. Whether it would have to enter the cell to do this is not clear. This suggests that the availability of prostaglandin precursors must be limiting on their rate of synthesis and these precursors can be provided either exogenously, or endogenously by the action of phospholipase A. The ability of prostaglandins A_1 and A_2 to cross-react, to some extent, in the radioimmunoassay may mean that some of the increases measured are due to these, but this would not affect the conclusions. Agents such as insulin or luliberin, which alter the concentrations of prostaglandin E in pituitary cells, may do so by altering phospholipase A activity and hence changing the availability of prostaglandin precursors.

The similarity of the results obtained when

Table 3. *Effects of various fatty acids on the prostaglandin E content of the cells*

Dishes of dispersed anterior-pituitary cells were incubated for 15 min in Dulbecco's modified Eagle's medium containing various fatty acids (all at 10 µg/ml.). The medium was removed and the prostaglandin E content of the cells was measured as described in the text. The values shown are the mean $\pm$ S.E.M. from six separate observations.

Addition to medium	Prostaglandin E content of cells (pmol/mg of protein)	Statistical differences from control
None	49.0 $\pm$ 8.9	—
Arachidonic acid	94.7 $\pm$ 7.1	$P < 0.01$
Eicosa-8,11,14-trienoic acid	97.5 $\pm$ 9.3	$P < 0.01$
Eicosa-11,14,17-trienoic acid	47.7 $\pm$ 7.8	$P > 0.05$
Arachidic acid	61.9 $\pm$ 8.2	$P > 0.05$
Linolenic acid	63.3 $\pm$ 6.1	$P > 0.05$

Table 4. *Incorporation of [3 H]arachidonic acid into anterior-pituitary lipids*

Anterior-pituitary glands were incubated for 1 h or 24 h in 1 ml of Dulbecco's modified Eagle's medium containing 20 µCi of [3 H]arachidonic acid. The tissue was then homogenized in 0.9% (w/v) NaCl and the lipids were extracted as described in the text. Samples were separated by t.l.c. and the spots were detected and the radioactivity in each spot was determined as described in the text. The amount of radioactivity in each spot is expressed as a percentage of the total radioactivity recovered from the chromatogram.

Time of incubation (h) ... Lipid	Percentage of total recovery	
	1	24
Phosphatidylcholine	23.5	21.8
Phosphatidylethanolamine	52.7	61.8
Phosphatidylinositol	21.8	11.3
Phosphatidylserine		
Other lipids	2.0	5.1

measuring prostaglandin E concentrations and the rate of somatotropin synthesis further supports the idea that intracellular prostaglandin E concentrations are involved in the control of the rate of somatotropin biosynthesis (Betteridge & Wallis, 1977). The effects of phospholipase A and arachidonic acid on somatotropin synthesis were less pronounced than those on prostaglandin E content, but our previous observations have suggested (A. Betteridge & M. Wallis, unpublished work) that changes in prostaglandin E concentration only cause changes in the rate of somatotropin biosynthesis after a 2–3 h time-lag.

Phospholipase A also markedly stimulates somatotropin release. Clearly, this effect could be due to the enzyme causing loss of membrane (and cellular) integrity, but the fact that somatotropin biosynthesis is also increased by phospholipase A treatment suggests that such cell damage is not occurring.

The inability of fatty acids, other than prostaglandin precursors, to alter the prostaglandin E content of the cells confirms that the effects of the prostaglandin E precursors arachidonic and eicosa-8,11,14-trienoic

acids are specific effects on the rate of prostaglandin synthesis. Our results also suggest that prostaglandin precursors are stored mainly in phospholipid, and at least in the case of arachidonic acid in the rat anterior-pituitary gland, mainly in phosphatidylethanolamine.

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