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Phospholipase A Activity in the Skin

MODULATORS OF ARACHIDONIC ACID RELEASE FROM PHOSPHATIDYLCHOLINE

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The distribution of the hydrolysis of 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine and the simultaneous biosynthesis of prostaglandins by subcellular fractions from human and rat skin membrane preparations were determined. The phospholipase A2 activity was distributed among the subcellular particulate preparations with the highest specific activity in the 105000*g* particulate fraction. The activity was optimal at pH 7.5 in the presence of 1.0mM-$CaCl_2$ and was inhibited by EDTA. The hydrolysis of phosphatidylcholine by the skin 105000*g* particulate fraction was inhibited by cortisol and triamcinolone acetonide and it was stimulated by histamine, bradykinin, retinoic acid and cholera enterotoxin (freeze-dried *Vibrio cholerae*). Furthermore hydrolysis of phosphatidylcholine by the skin phospholipase A was also enhanced by low concentrations of prostaglandin E_2 and prostaglandin $F_{2\alpha}$. These last results suggest that the amplification of the hydrolysis of phosphatidylcholine by prostaglandin E_2 and prostaglandin $F_{2\alpha}$, with the consequent release of arachidonic acid (the substrate for prostaglandin synthesis) is likely a positive-feedback regulation of the arachidonic acid–prostaglandin cascade.

The physiological role of phospholipases in skin is still unclear. Some workers, however, have discussed the role of phospholipid-derived fatty acids as a source of energy (Long, 1970; Poulos *et al.*, 1973). Another noteworthy function of tissue phospholipase A2 is its regulatory role in the release of polyunsaturated fatty acids from acylglycerophosphates for prostaglandin biosynthesis (Vonkeman & Van Dorp, 1968; Kunze & Vogt, 1971; Flower & Blackwell, 1976). These latter studies have resulted in a number of reports indicating that the anti-inflammatory steroids do in part inhibit the release of precursor arachidonic acid from blood vessels, lungs and cells in tissue culture (Gryglewski *et al.*, 1975; Nijkamp *et al.*, 1976; Hong & Levine, 1976). These studies, however, did not demonstrate the direct effect of these steroid anti-inflammatory substances on the activity of isolated phospholipase A2 in these tissues.

The presence of phospholipid-hydrolysing mechanisms in animal tissues by using both endogenous and exogenous phospholipids as substrate are well known. Removal of the β-linked fatty acid from phosphatidylcholine or phosphatidylethanolamine by the action of a phospholipase A2 (phosphatide 2-acylhydrolase, EC 3.1.1.4) could be one mode of initial attack. Apart from its presence in pancreas (Hanahan, 1952; Magee *et al.*, 1962), phospholipase A2 has also been described in a variety of other tissues (Robertson & Lands, 1962; Gallai-Hatchard & Thompson, 1965; Bjornstad, 1966; Weglicki *et al.*, 1971; Long & Yardley, 1972; Nachbaur *et al.*, 1972; Kunze *et al.*, 1974; Gullis & Rowe, 1975; Scherphof & Westernberg, 1975; Wolf *et al.*, 1976).

Since the prostaglandins now appear to have a central and complex role in inflammation, these substances therefore have a pertinent role in the pathogenesis and treatment of cutaneous inflammatory diseases. Furthermore, since the intracellular concentration of arachidonic acid (which exists as ester linkage to phospholipids) is low in skin, the hydrolysis and general turnover of phospholipids in this tissue must be an initial and important step in the biosynthesis of prostaglandins in this tissue. Thus, we have in our present studies undertaken: (i) to demonstrate and localize the activity of a phospholipase A2 in rat and in human skin preparations; (ii) to ascertain whether or not the activity of this enzyme is altered by known inhibitors and activators of prostaglandin biosynthesis.

Materials and Methods

Materials

[1-^{14}C]Arachidonic acid (sp. radioactivity 56.6mCi/mmol) was purchased from Amersham/Searle,

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Arlington Heights, IL, U.S.A. Radiopurity was ascertained as described previously by Ziboh (1973). Of the ^{14}C radioactivity, 95% was associated with methyl arachidonate. Egg lysophosphatidylcholine was purchased from Supelco Inc., Bellfonte, PA 16823, U.S.A. The purity of the lysophosphatidylcholine was verified by t.l.c. in the solvent system chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) and was found to be greater than 95%. The fatty acids (99% pure) were obtained from Lipid Organic Research, Elysian, MN, U.S.A. Triamcinolone acetonide was a gift from E. R. Squibb and Sons Inc., New Brunswick, NJ, U.S.A. Retinoic acid was a gift from Johnson and Johnson, New Brunswick, NJ, U.S.A. Cholera enterotoxin (freeze-dried *Vibrio cholerae*) was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Reagents were of analytical grade and solvents were distilled before use.

Methods

Preparation of rat liver microsomal fraction. Fresh rat liver was homogenized in 0.25M-sucrose. The unbroken cells, nuclei and mitochondria were removed by centrifugation at 22000*g* for 20min at 4°C. The supernatant fluid obtained after the removal of the pellet was centrifuged at 105000*g* for 60min at 4°C in an International Equipment Co. (IEC) ultracentrifuge (model B-60). The 105000*g* pellet obtained was designated the microsomal fraction. This material was suspended in 0.1M-Tris/HCl buffer, pH7.4, and stored in the cold until used.

Preparation of 1-acyl-2-[1-^{14}C]arachidonoyl-sn-glycero-3-phosphocholine. 1-Acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine was prepared by the acylation of 1-acyl-*sn*-glycero-3-phosphocholine with [1-^{14}C]arachidonic acid (sp. radioactivity 56.6mCi/mmol) by the procedure of Lands (1960) by using rat liver microsomal acyl transferase. Radioactive phosphatidylcholine synthesized was isolated by t.l.c. on a layer (0.5mm thick) of silica gel G by using solvent mixture chloroform/methanol/acetic acid/water (50:30:8:5, by vol.).

To determine the positional specificity of the incorporated [1-^{14}C]arachidonic acid, a portion of the biosynthesized 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine was treated with snake (*Crotalus adamanteus*) venom by the method of Robertson & Lands (1962) for 3h; the resulting hydrolysate to which unlabelled phosphatidylcholine (25μg), lysophatidylcholine (25μg) and arachidonic acid (50μg) had been added as carriers, was extracted with chloroform/methanol (2:1, v/v) and evaporated to dryness under vacuum. The lipid residue was redissolved in chloroform/methanol (1:1, v/v) and separated by t.l.c. on a layer of silica gel G in the solvent system chloroform/methanol/water (65:25:4, by vol.). Components were detected by immersion of the dried plate for a short time in I_2 vapour. The positions of the components were marked, and after evaporation of the I_2, the part of the chromatogram containing arachidonic acid was scraped and transferred into a sintered-glass funnel. The arachidonic acid and the radioactivity in this fraction were eluted with chloroform/methanol (1:1, v/v). For further identification of the radioactive product, the residue containing authentic arachidonic acid and the ^{14}C were methylated with diazomethane in diethyl ether. After drying under N_2, the methyl esters were chromatographed on silica gel G-coated plates prepared with 10% $AgNO_3$ in the solvent system diethyl ether/hexane/benzene (3:7:1, by vol.). Approx. 96–98% of the recovered radioactivity was associated with arachidonic acid. Of the recovered radioactivity, 1–3% remained with unhydrolysed phosphatidylcholine. The biosynthesized radioactive phosphatidylcholine was stored in chloroform under N_2 at −70°C.

Preparation of skin subcellular fractions. Skin specimens were removed from the shaved area of the posterior dorsum from normal or essential-fatty acid-deficient Sprague–Dawley rats as reported previously (Ziboh & Hsia, 1972). The skin specimens were minced and homogenized in 5vol. of ice-cold 0.25M-sucrose in an ice bath with a Polytron homogenizer (PT-20; Kinematica, Lucern, Switzerland). Tissue debris was removed by filtration of the homogenate through cheesecloth. Portions of the crude homogenate were stored in ice and used as enzyme source. The nuclei pellet was obtained by centrifugation of the homogenate at 900*g* for 12min at 4°C. The supernatant fluid was centrifuged at 12000*g* for 15min. The 12000*g* pellet was washed, resuspended in 0.1M-Tris/HCl buffer, pH7.5, and stored on ice. The supernatant fraction was further centrifuged at 105000*g* for 60min at 4°C in an International Equipment Co. ultracentrifuge (model B-60). The 105000*g* pellet was washed, resuspended in the same Tris/HCl buffer and stored on ice for incubations. The 105000*g* supernatant fluid was also kept for incubations. The protein contents of the various subcellular fractions were determined by the method of Lowry *et al.* (1951), with bovine albumin as standard.

The subcellular fractions of human skin epidermal tissue (obtained with informed consent at plastic surgery) were similarly prepared as described above.

Preparation of emulsions of phospholipid for the assay of skin phospholipase A activity. Phospholipase A2 activity was determined by the release of [1-^{14}C]-arachidonic acid from 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine. The procedure was based on that of Rayman & Verhagen (1970). An emulsion of the ^{14}C-labelled substrate was made as follows. Radioactive phosphatidylcholine in chloroform was transferred into a test tube and dried under

N_2. The residue was dissolved in approx. 3ml of diethyl ether. Tris/HCl buffer, 0.1M, pH7.5, was added to the desired concentration. Diethyl ether was removed by evaporation under N_2 gas. The emulsion obtained was sonicated in the test tube surrounded by ice for three periods of 1min with 30s between each period. All emulsions of phospholipid were used within 60min of preparation.

Conditions of incubation. In preliminary studies, each subcellular suspension of skin (400–600μg of protein) was incubated in 1.0ml of 0.1M-Tris/HCl buffer, pH6–9, or 0.1M-sodium acetate buffer, pH4–6, containing $CaCl_2$ (1.0mM) and [^{14}C]phosphatidylcholine (0.2μCi, 0.05μmol) at 37°C for 3min. Controls contained subcellular particulate suspension (boiled for 15min at 100°C) or appropriate buffer with $CaCl_2$ (1.0mM) and [^{14}C]phosphatidylcholine. The incubation was stopped by the addition of 5.0ml of chloroform/methanol (2:1, v/v). Unlabelled standard solutions of phosphatidylcholine (25μg), lysophosphatidylcholine (25μg), arachidonic acid (50μg), prostaglandin E_2 (25μg) and prostaglandin $F_{2\alpha}$ (25μg) were added to the incubation mixture as carriers.

In instances when non-aqueous substances were tested for effects on hydrolysis the emulsion of these substances was obtained by sonication as described for preparing the substrate.

T.l.c. and assay of radioactive products. The incubation mixture was extracted with 19vol. of chloroform/methanol (2:1, v/v) by the method of Folch *et al.* (1957). The lipid extract was evaporated to dryness under a stream of N_2 gas. The residue was dissolved in a minimum volume of chloroform/methanol (1:1, v/v) and then subjected to chromatography on silica gel G in the solvent system chloroform/methanol/acetic acid/water (90:8:1:0.8, by vol.). Lipid components were detected with I_2 vapour. After evaporating the I_2 (normally for 30min) the portion of the chromatogram containing arachidonic acid was scraped into a sintered-glass funnel and eluted with chloroform/methanol (1:1, v/v). For further characterization, the [^{14}C]arachidonic acid released was methylated with diazomethane and the fatty acid methyl ester chromatographed on a t.l.c. plate coated with a slurry of silica gel G in 10% $AgNO_3$ (Ziboh *et al.*, 1974).

The portion of the original chromatogram that contained total phospholipids (phosphatidylcholine and lysophosphatidylcholine) was eluted, dried and further fractionated by t.l.c. in the solvent system chloroform/methanol/water (65:25:4, by vol.).

To verify whether prostaglandin $F_{2\alpha}$ and prostaglandin E_2 were also products of the reaction, the portion of the chromatogram that corresponded to standards prostaglandin E_2 and prostaglandin $F_{2\alpha}$ were scraped into a sintered-glass funnel and treated with 3ml of 0.5M-NaOH in aq. 50% ethanol (Ziboh, 1973). Under these conditions, the E-prostaglandins are converted into the B-prostaglandins. The sample was diluted with water, acidified with 6M-HCl to pH2–3, extracted with dichloromethane and dried under N_2. The residue was dissolved in chloroform/methanol (1:1, v/v) and applied to a silica gel-coated t.l.c. plate. The plate was developed in the solvent system diethyl ether/acetic acid (50:1, v/v).

Radioactivity in released arachidonic acid, and in fractions containing prostaglandin E_2, prostaglandin $F_{2\alpha}$, lysophosphatidylcholine and unmetabolized [^{14}C]phosphatidylcholine, were determined by scraping silica gel from the respective t.l.c. plates into scintillation vials containing Econofluor pre-mix scintillation solution (New England Nuclear, Boston, MA, U.S.A.) and then counting radioactivity in the vials in a Packard Tri-Carb model 2002 liquid-scintillation counter. Radioactivity was corrected to 100% efficiency by the use of external standard.

Results

Effect of pH on the hydrolysis of 1-acyl-[1-^{14}C]-arachidonoyl-sn-glycero-3-phosphocholine by rat skin subcellular fractions

Because different pH optima have been associated with the activity of phospholipases in different subcellular fractions (Nachbaur *et al.*, 1972), we examined the pH optimum for the hydrolysis of the [^{14}C]phosphatidylcholine in our various subcellular fractions. As shown in Fig. 1, the optimal pH of phospholipase A activity in both the 12000*g* and 105000*g* particulate fractions ranged between 7.5 and 8.0. Optimal pH for the supernatant fraction was approx. 5. Because of these observations, subsequent incubations to assay for the phospholipase A activity associated with prostaglandin biosynthesis in the skin (maximal at pH7.5) by the method of Ziboh (1973) were carried out at pH7.5.

Distributions of the hydrolysis of [^{14}C]phosphatidylcholine by rat and human skin subcellular preparations

The distribution of hydrolysis of 1-acyl-2-[1-^{14}C]-arachidonoyl-*sn*-glycero-3-phosphocholine by skin phospholipase A in subcellular fractions is shown in Table 1. Maximal release of [^{14}C]arachidonic acid at pH7.5 was associated with the 105000*g* pellet, although some activity was associated with the 12000*g* pellet. Only minor radioactivity was detected in the supernatant fraction. These results indicate the presence of a membrane-bound phospholipase A2 activity in the skin. Since the substrate used in the present study was a 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine, we examined the amount of ^{14}C in the lysophosphatidylcholine fraction after incubation with the 105000*g* particulate fraction for possible skin phospholipase A1 activity. Negligible radioactivity (<0.1%) was associated with

the lysophosphatidylcholine fraction, suggesting that most of the activity in the 105000*g* preparation was probably due to a skin phospholipase A2. Furthermore, examination of the t.l.c. plates revealed the presence of ^{14}C-labelled prostaglandins formed by particulate fractions, particularly the 105000*g* particulate fraction. Incubation for a longer period (10min) resulted in a significant increase in the conversion of the released [^{14}C]arachidonic acid into prostaglandins, thus confirming the presence of the prostaglandin-synthesizing enzymes as reported previously (Ziboh, 1973).

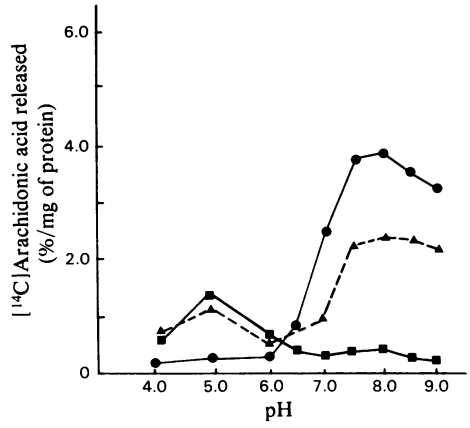

Fig. 1. *Hydrolysis of* 1-*acyl*-2-[1-^{14}*C*]*arachidonoyl-sn-phosphocholine and biosynthesis of prostaglandins by subcellular preparations from normal rat skin*

Each 1ml of subcellular fraction (600μg of protein) in 0.1M-Tris/HCl buffer (pH6–9) or 0.1M-sodium acetate buffer (pH4–6) contained $CaCl_2$ (1.0mM) and [^{14}C]phosphatidylcholine (0.2μCi, 0.05μmol). Each mixture was incubated at 37°C for 3min. The ^{14}C radioactivity in arachidonic acid was determined after separation by t.l.c. as described in the Materials and Methods section. Results are expressed as a percentage of [^{14}C]arachidonic acid released from [^{14}C]phosphatidylcholine/mg of subcellular protein after correction for the value for the boiled subcellular control. Each data point represents a mean for duplicate determinations from three different experiments and the values agreed within 10% of the means. Symbols: ▲, 12000*g* pellet; ●, 105000*g* pellet; ■, 105000*g* supernatant.

Characteristics of the hydrolysis of 1-*acyl*-2-[1-^{14}*C*]-*arachidonoyl-sn-glycero*-3-*phosphocholine by rat skin* 105000*g particulate fraction*

Since the preceding experiments showed that hydrolysis of [^{14}C]phosphatidylcholine was maximal with the 105000*g* particulate fraction, all subsequent incubations were carried out with this fraction. The time course of the hydrolysis of [^{14}C]phosphatidylcholine is shown in Fig. 2. The enzymic activity was rapid and maximal in approx. 3min. A longer incubation for 10min resulted in diminished activity. The nature of this inhibitory effect has not been determined. Nonetheless, possible explanations could be either the accumulation of lysophosphatidylcholine, which has been reported to inhibit phospholipase A activity in human seminal plasma (Kunze *et al.*, 1974) or the transformation of released arachidonic acid into prostaglandins.

The relationship between the hydrolysis of [^{14}C]-phosphatidylcholine (expressed as the percentage of [^{14}C]arachidonic acid released) and the increasing amounts of 105000*g* particulate fraction was determined. The amounts of [^{14}C]arachidonic acid released increased with increasing amounts of particulate protein up to approx. 0.6mg.

The requirement of Ca^{2+} for the optimal activity of phospholipase A2 has been reported in a variety of tissues (Bjornstad, 1966; Smith & Winkler, 1968;

Table 1. *Hydrolysis of* 1-*acyl*-2-[1-^{14}*C*]*arachidonoyl-sn-glycero*-3-*phosphocholine and biosynthesis of prostaglandins by subcellular preparations from rat and human skin*

Each 1ml of subcellular suspension of skin preparation (600μg of protein) in 0.1M-Tris/HCl buffer, pH7.5, contained $CaCl_2$ (1.0mM) and [^{14}C]phosphatidylcholine (0.2μCi, 0.05μmol). Each mixture was incubated at 37°C for 3min. The ^{14}C in arachidonic acid, and total prostaglandins were determined after separation by t.l.c. as described under 'Methods'. Results are expressed as a percentage of [^{14}C]arachidonic acid released or [^{14}C]prostaglandins formed per mg of subcellular protein after correction for boiled subcellular control. Values are means ± S.E.M. for the numbers of determinations indicated in parentheses. The percentage of [^{14}C]arachidonic acid released is equal to the amount of ^{14}C-labelled non-esterified fatty acid released after hydrolysis by skin 105000*g* particulate fraction divided by the total recovered ^{14}C after incubation, multiplied by 100. Abbreviation used: ND, not detectable.

	^{14}C in arachidonic acid (%/mg of protein)		^{14}C in prostaglandins (%/mg of protein)	
Particulate fraction	Rat	Human	Rat	Human
Homogenate	2.6±0.19 (4)	6.8±0.58 (4)	0.4±0.04 (4)	1.5±0.11 (4)
12000*g* pellet	2.0±0.15 (4)	4.5±0.32 (4)	0.4±0.03 (4)	1.3±0.09 (4)
105000*g* pellet	3.8±0.18 (4)	8.2±0.42 (4)	1.0±0.04 (4)	2.4±0.19 (4)
105000*g* supernatant	0.3±0.06 (4)	0.5±0.06 (4)	ND (4)	ND (4)

Waite *et al.*, 1969; Gullis & Rowe, 1975). We therefore tested the requirement of Ca^{2+} by the rat skin 105000*g* particulate fraction for maximal activity. The phospholipase A activity was enhanced from approx. 0.7% to approx. 3.8% by added concentrations of $CaCl_2$. Optimal concentrations of added Ca^{2+} showed a broad plateau from 1 to 10mM. This stimulatory effect was inhibited by EDTA (3mM).

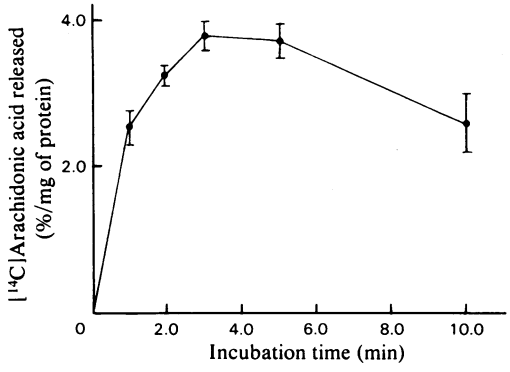

Fig. 2. *Time course of the hydrolysis of* 1-*acyl*-2-[1-^{14}C]-*arachidonoyl-sn-glycero*-3-*phosphocholine by* 105000*g particulate fraction from rat skin*

Each 1ml of 105000*g* particulate fraction (600μg of protein) was incubated as described in the legend to Table 1. Identification and estimation of radioactive products are as described in the Materials and Methods section. Results are expressed as the percentage of [^{14}C]arachidonic acid released from [^{14}C]phosphatidylcholine/mg of particulate protein. Each data point represents a mean ± S.E.M. for duplicate determinations from three different experiments.

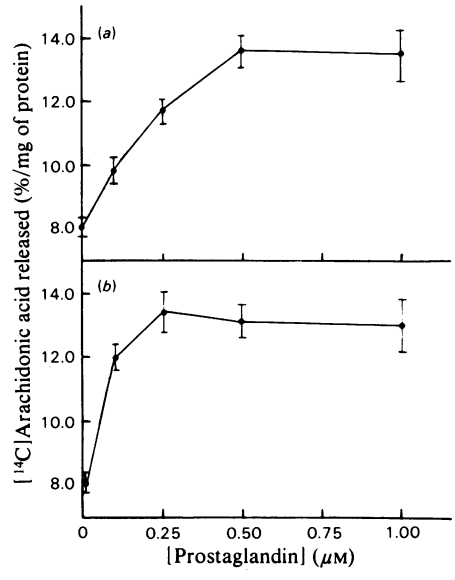

Fig. 3. *Effects of prostaglandin* E_2 *and prostaglandin* $F_{2\alpha}$ *on the hydrolysis of* 1-*acyl*-2-[1-^{14}C]*arachidonoyl-sn-glycero*-3-*phosphocholine by rat skin* 105000*g particulate fraction*

Various amounts of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ dissolved in buffer were incubated with 105000*g* particulate fraction (600μg of protein) as described in the legend to Table 1. Identification and estimation of radioactive products are as described in the Materials and Methods section. Results are expressed as the percentage of [^{14}C]arachidonic acid released from [^{14}C]phosphatidylcholine/mg of particulate protein. Each data point represents a mean ± S.E.M. for duplicate determinations from three different experiments.

Table 2. *Chemical activators of the hydrolysis of* 1-*acyl*-2-[1-^{14}C]*arachidonoyl-sn-glycero*-3-*phosphocholine by* 105000*g particulate fraction from normal rat skin*

Each test substance was incubated with 105000*g* particulate fraction (600μg of protein) as described in the legend to Table 1. The control contained no substances. Identification and determination of radioactive products are as described in the Materials and Methods section. Values are means for duplicate experiments and the values agreed within 10% of the means.

Additions	Concentration (μM)	^{14}C in arachidonic acid (%/mg of protein)	Stimulation (%)
None	—	4.2	—
Histamine	10	6.7	59
Histamine	50	10.8	157
Bradykinin	10	8.9	112
Bradykinin	50	12.4	195
Retinoic acid	10	6.5	54
Retinoic acid	50	11.8	281
Cholera enterotoxin (freeze-dried *V. cholerae*)	(1μg)	21.6	414
Cholera enterotoxin (freeze-dried *V. cholerae*)	(2μg)	40.2	957

Table 3. *Anti-inflammatory-steroid inhibition of the hydrolysis of* 1-*acyl*-2-[1-^{14}*C*]*arachidonoyl-sn-glycero*-3-*phosphocholine by* 105000*g particulate fraction from normal rat skin*

Various concentrations of anti-inflammatory steroids were incubated with 105000*g* particulate fraction (600 μg of protein) as described in the legend to Table 1. Controls contained no steroids. Identification and estimation of radioactive products are as described in the Materials and Methods section. Values are means ± S.E.M. for the numbers of determinations indicated in parentheses.

Additions	Concentration (μM)	^{14}C in arachidonic acid (%/mg of protein)	Inhibition (%)
None	—	3.6 ± 0.17 (4)	—
Cortisol	10	3.3 ± 0.29 (4)	7
Cortisol	50	2.1 ± 0.11 (3)	40
Cortisol	100	1.7 ± 0.05 (3)	52
Triamcinolone acetonide	10	3.2 ± 0.29 (3)	10
Triamcinolone acetonide	50	1.8 ± 0.29 (3)	50
Triamcinolone acetonide	100	1.3 ± 0.06 (3)	62

The effect of essential-fatty acid deficiency on the ability of the rat skin 105000*g* particulate fraction to hydrolyse 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine was determined in a number of experiments. Full deficiency was induced as reported previously (Ziboh & Hsia, 1972). The phospholipase A activity was approx. 80% higher in the skin of the essential-fatty acid-deficient rats than in the normal fed control rats.

Effects of some activators of arachidonic acid and prostaglandin release on the hydrolysis of 1-*acyl*-2-[1-^{14}*C*]*arachidonoyl-sn-glycero*-3-*phosphocholine by rat skin* 105000*g particulate fraction*

Since the hydrolysis and release from membrane phospholipid is the rate-limiting step that provides precursor fatty acid for the biosynthesis of prostaglandins, we tested the effects of a number of substances on the hydrolysis of [^{14}C]phosphatidylcholine by skin 105000*g* particulate fraction. These substances have been reported to enhance the release of arachidonic acid and the formation of prostaglandins (McGiff *et al.*, 1972; Ferreira *et al.*, 1973; Ziboh *et al.*, 1975). Histamine, bradykinin and retinoic acid at a concentration of 50 μM effectively stimulated the hydrolysis of the phosphatidylcholine (Table 2). Commercial cholera enterotoxin, a substance previously reported to induce severe diarrhoea and gastrointestinal inflammatory reactions (Finck & Katz, 1972), also markedly enhanced the skin phospholipase A activity.

Effects of prostaglandin E_2 *and prostaglandin* $F_{2\alpha}$ *on the hydrolysis of* 1-*acyl*-2-[1-^{14}*C*]*arachidonoyl-sn-glycero*-3-*phosphocholine by rat skin* 105000*g particulate fraction*

To determine whether the primary prostaglandins would exert a feedback control on the release of arachidonic acid from phosphatidylcholine we tested the effects of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ at various concentrations on the hydrolysis of 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine by rat and human skin 105000*g* particulate fractions. Results in Fig. 3 demonstrated that both prostaglandins stimulated the hydrolysis and release of arachidonic acid from phosphatidylcholine in a dose-dependent manner. In particular, the results showed a maximal stimulatory effect of prostaglandin $F_{2\alpha}$ at a concentration of 0.25 μM, whereas the maximal stimulatory effect of prostaglandin E_2 was at 0.5 μM.

Effects of anti-inflammatory corticosteroids on the hydrolysis of 1-*acyl*-2-[1-^{14}*C*]*arachidonoyl-sn-glycero*-3-*phosphocholine by rat skin* 105000*g particulate fraction*

To ascertain whether the reported (Voorhees *et al.*, 1977) decrease in arachidonic acid release from human skin epidermis after treatment with glucocorticoids was related to its effect on skin phospholipase A activity, we tested the effects of two commonly used steroid anti-inflammatory drugs on the hydrolysis of 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine by rat skin 105000*g* particulate fraction. Cortisol and triamcinolone at the low concentration of 10 μM had little or no effect on the hydrolysis of the phosphatidylcholine by skin 105000*g* particulate fraction. However, at 50 μM the inhibitory effects of these steroid anti-inflammatory substances ranged from 40–60% (Table 3). ^{14}C in prostaglandins was also decreased in these experiments (results not shown).

Discussion

The present study has demonstrated the presence of a Ca^{2+}-activated phospholipase A activity that catalyses the hydrolysis of 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine in rat and human skin membrane preparations. Because most

of the radioactivity released from our substrate 1-acyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine was associated with arachidonic acid and not with the lysophosphatidylcholine, we conclude that most of the enzyme activity in our preparation was due to skin phospholipase A2 rather than phospholipase A1. The pH optimum for the phospholipase A2 activity in the particulate preparations as shown in Fig. 1 is approx. 7.5. We therefore suggest that it is the skin phospholipase A2, which is active at pH 7.5, that plays a regulatory role in the release of polyunsaturated fatty acids from skin phosphatidylcholine (the major skin phospholipid) for prostaglandin biosynthesis. This view is consistent with data in Table 1 that demonstrated that the incubation of phosphatidylcholine with 105000*g* particulate fractions prepared from rat and human skin specimens resulted in the release of non-esterified arachidonic acid and the formation of prostaglandins. A similar release of arachidonic acid and the formation of prostaglandins after the treatment of phosphatidylcholine with a preparation from snake venom (*C. adamanteus*) had been reported (Vonkeman & Van Dorp, 1968). These experiments demonstrate that phospholipids (at least in skin) form an important source of substrate for prostaglandin biosynthesis. The small hydrolytic activities at pH 5.0 demonstrated in both the 12000*g* particulate and 105000*g* supernatant fractions could be due to skin lysosomal acylhydrolases, which may be consistent with reported observations (Nachbaur *et al.*, 1972) in rat hepatocytes.

The present results also suggest that skin phospholipase A2 activity is associated almost exclusively with the particulate or membrane fractions (Table 1). Although phospholipase A2 activity had previously been reported in acetone-powder preparations from cow snout epidermal homogenate (Long & Yardley, 1972), the subcellular localization and properties of this enzyme had not been fully reported. The present results have extended these studies and localized the activity of this enzyme in rat and human skin particulate fractions.

The rat and human skin phospholipase A2 activity is sensitive to $CaCl_2$ concentration and the optimal stimulatory effect by $CaCl_2$ was attained at 1–10mM. This $CaCl_2$-stimulatory effect was inhibited by EDTA. The inhibition produced by EDTA could be reversed by adding Ca^{2+} ions in excess (results not shown). Thus it appears that the inhibitory effect of EDTA on the activity of the phospholipase A2 is by complexing endogenous Ca^{2+} that is required for the enzyme activity, rather than exerting a direct effect on the enzyme.

Essential-fatty acid deficiency increased the activity of the skin phospholipase A2 in the scaly and hyperproliferative skin tissue. It is noteworthy that markedly increased amounts of arachidonic acid and 12-hydroxyeicosa-5,8,11,14-tetraenoic acid were found in the hyperproliferative epidermis of human psoriatic plaques (Hammerstrom *et al.*, 1975). It is likely that this increased degradation and turnover of skin phospholipids may contribute at least in part to the increased cutaneous permeability characteristic of these hyperproliferative skin tissues.

The mechanism of action of the variety of substances previously reported to enhance the release of arachidonic acid and/or prostaglandins (McGiff *et al.*, 1972; Ferreira *et al.*, 1973; Ziboh *et al.*, 1975; Su Chen & Levine, 1976) on the phospholipase A activity and now tested in our particulate fraction (Table 2) still remains unclear. The present results do, however, suggest that the enhancement of phospholipase A2 activity may be a possible mode of action for these substances. Furthermore, the mechanism of the prostaglandin stimulatory effect on skin phospholipase A2 activity as shown in Fig. 3 is presently unclear. It is noteworthy, however, that prostaglandin E_2 and prostaglandin $F_{2\alpha}$ have been reported to facilitate the release of Ca^{2+} from uterine microsomal preparations in a manner similar to ionophores by forming Ca^{2+} complexes (Carsten & Miller, 1977). Since the rat skin phospholipase A2 activity is sensitive to Ca^{2+} concentration, it is likely that the stimulatory effects of prostaglandins E_2 and $F_{2\alpha}$ on the activity of the phospholipase A2 as observed in the present studies may be due to their effects in mobilizing Ca^{2+} for the enzyme activity. On the other hand, the inhibition of phospholipase A2 activity by cortisol and triamcinolone acetonide as shown in Table 3 may explain at least in part the inhibition of the release of arachidonic acid and the formation of its metabolites by anti-inflammatory steroids previously reported in skin epidermis (Voorhees *et al.*, 1977) and in other systems such as in MC5-5 fibroblasts (Su Chen & Levine, 1976).

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