Biosynthesis of prostaglandin E_2 in human skin: subcellular localization and inhibition by unsaturated fatty acids and anti-inflammatory drugs

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Abstract The biosynthesis of prostaglandin E_2 (PGE_2) from [1-^{14}C]arachidonic acid has been demonstrated in homogenates and subcellular fractions of human epidermis. This biosynthetic capacity is localized in the microsomal fraction, indicating the presence of an active prostaglandin synthetase system associated with membranes of the skin. The incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGE_2 by the microsomal fraction was enhanced by EDTA. This apparent increase in ^{14}C incorporation into PGE_2 in the presence of EDTA could be due at least in part to its chelating properties of removing the divalent cations in the homogenate that enhance the selective formation of $PGF_{2\alpha}$ and the suppression of the activity of epidermal phospholipase A, which causes the release of nonradioactive fatty acid precursors from endogenous phospholipids. This study has also demonstrated that the formation of PGE_2 from arachidonic acid by the microsomal fraction from human skin could be inhibited by polyunsaturated fatty acids, suggesting a possible regulatory role of fatty acids released from endogenous phospholipids on prostaglandin synthesis in this tissue. The inhibitory effects of some anti-inflammatory drugs on skin microsomal prostaglandin synthetase were also demonstrated in these studies. Results from these studies indicate that the skin is therefore a useful tissue for the study of mechanisms of prostaglandin biosynthesis and the mode of action of various anti-inflammatory drugs.

Supplementary key words essential fatty acid · thin-layer chromatography · polyunsaturated fatty acids · eicosatrienoic acid · triamcinolone acetonide · dexamethasone

PROSTAGLANDINS have been isolated in various amounts from tissues of several animal species, including man. The biological effects of this class of compounds are numerous and show marked species and organ specificity (3, 4). A striking uptake of tritiated PGE_1 in the dermis of the skin of adult mice was observed by Hansson and Samuelsson (5), although the biological significance of this uptake was not immediately clear. Employing a sensitive technique of gas–liquid chromatography, Jouvenaz et al. (6) found high levels of PGE_2 in rat epidermis. Kischer (7, 8) reported that prostaglandins PGE_1 and PGB_1 enhanced maturation of skin by increasing the layers of epidermal cells and causing precocious keratinization in chick skin in culture. The latter observations strongly suggest a possible role of prostaglandins in this tissue. Several studies have indicated that prostaglandins are mediators of inflammatory and allergic reactions. Intradermal injection of PGE_1, PGE_2, and $PGF_{2\alpha}$ induced vascular permeability in rat and man (9–11). PGE_2 and $PGF_{2\alpha}$ are released from sensitized lung tissue in anaphylactic shock (12) and are increased in human and rat inflammatory exudates (13–15). The inhibitory effects of the anti-inflammatory drugs aspirin and indomethacin on the biosynthesis of PGE_2 and

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Abbreviations: TLC, thin-layer chromatography. Trivial names and abbreviations are based on the systemic nomenclature proposed by Samuelsson (1) and by Nugteren et al. (2). Prostaglandin E_1 (PGE_1), 11,15-dihydroxy-9-oxoprosta-13-enoic acid; prostaglandin E_2 (PGE_2), 11,15-dihydroxy-9-oxoprosta-5,13-dienoic acid; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 9,11,15-trihydroxy-prosta-5,13-dienoic acid; prostaglandin B_1 (PGB_1), 15-hydroxy-9-oxoprosta-8(12)13-dienoic acid; prostaglandin B_2 (PGB_2), 15-hydroxy-9-oxoprosta-5,8(12)13-dienoic acid. Fatty acids are designated by number of carbon atoms:number of double bonds.

$PGF_{2\alpha}$ strongly suggest that prostaglandins may be involved at least in part in the inflammatory process (16–18).

Since the preceding studies strongly suggest a possible regulatory role of prostaglandins in skin function, we have examined the capacity of this tissue to synthesize prostaglandin E_2 from arachidonic acid, a known precursor for PGE_2. A recent study in this laboratory showed that indeed arachidonic acid is transformed into prostaglandin E_2 by homogenates from rat skin (19). More recently, Jonsson and Änggård (20) have demonstrated the transformation of arachidonic acid to prostaglandin E_2 by homogenates from human skin. The present study was undertaken to provide (*a*) evidence for the subcellular localization of prostaglandin biosynthesis in human epidermis obtained at surgery from amputated legs and (*b*) to examine the effects of unsaturated fatty acids and anti-inflammatory drugs on the prostaglandin synthetase enzyme system of the skin.

MATERIALS AND METHODS

Materials

[1-^{14}C]Arachidonic acid (56.6 mCi/mmole) was obtained from Dhom Products, North Hollywood, Calif. A portion of this material was methylated with diazomethane and analyzed by gas–liquid chromatography on a 3% DEGS column; 95% of the chromatographed ^{14}C was found to have the retention time of methyl arachidonate. Silica gel G was obtained from E. Merck Co. PGE_2 was a gift from Dr. John E. Pike of the Upjohn Co., Kalamazoo, Mich. The fatty acids (99%) were obtained from Lipid Organic Research, Elysian, Minn. Indomethacin and dexamethasone were gifts from Merck Sharp & Dohme Research Laboratories, Rahway, N.J. Chlorpromazine hydrochloride (Thorazine) was a gift from Smith Kline & French Laboratories, Philadelphia, Pa. Triamcinolone acetonide was a gift from E. R. Squibb & Sons Inc., New Brunswick, N.J. Reagents were of analytical grade and solvents were redistilled before use.

Methods

Preparation and incubation of subcellular fractions of human epidermis for prostaglandin synthesis. Homogenates of human skin were prepared from frozen keratome[1] strips of human epidermis obtained at surgery from amputated legs. No skin abnormalities were visible on the specimens used, and amputation was due to car accidents. 18 g of epidermal strips was minced and homogenized in 4 vol of ice-cooled potassium phosphate buffer (pH 8.0) in an ice bath with a Polytron homogenizer[2] (model PT-20, Kinematica, Lucerne, Switzerland). Unbroken cellular debris was removed by filtration of the homogenate through cheesecloth. Portions of the crude homogenate were stored in ice for incubations. The nuclei pellet was obtained by centrifugation of the homogenate at 800 *g* for 12 min at 4°C. The pellet was resuspended and homogenized in buffer and stored in ice for incubations. The supernatant fluid obtained after the removal of the nuclei pellet was centrifuged at 105,000 *g* for 60 min at 4°C in an International Equipment Co. (IEC) ultracentrifuge (model B-60) and the pellet obtained was designated the microsomal fraction. The pellet was rinsed once with buffer then resuspended and homogenized in the buffer (pH 8.0) and stored in ice for incubations. The 105,000 *g* supernatant fluid was also kept for incubations with [1-^{14}C]arachidonic acid. The protein contents of the crude homogenate, nuclei pellet, 105,000 *g* pellet, and 105,000 *g* supernatant fluid, as determined by the method of Lowry et al. (21), were 18.1 mg/ml, 2.1 mg/ml, 5.6 mg/ml, and 10.1 mg/ml, respectively.

In experiments designed to test the effects of EDTA on the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGE_2, various amounts of EDTA were added to potassium phosphate buffer (pH 8.0) used in the homogenization of the minced human epidermis before centrifugation. The optimum amount of EDTA, 30 mM, was used in all subsequent experiments with the microsomal fraction.

The effects of the unsaturated fatty acids and anti-inflammatory agents were determined by addition of the various compounds in 0.01 ml of ethanol to each incubation mixture as indicated in the text; 0.01 ml of ethanol without the compounds was added to the control vial containing active subcellular fractions. At this concentration, no inhibitory effect of ethanol on the conversion of arachidonic acid into PGE_2 was observed.

Labeled arachidonic acid (2.8 μCi, 0.05 μmole) was dissolved in benzene and added to the incubation flask, and the solvent was evaporated by a stream of nitrogen. To the flask were added glutathione (0.65 mM) and hydroquinone (0.5 mM), as described previously (19). The incubation was initiated by the addition of 5 ml of the various subcellular enzyme suspensions to the incubation medium. The mixture was incubated aerobically

[1] The Electro-Keratome is an instrument for obtaining very thin but metabolically functional skin slices through modification of the Castroviejo Electro-Keratome (Storz Instrument Co., St. Louis, Mo.). Although it is a tool for obtaining skin specimens consisting mostly of epidermis, as in the Van Scott skin stretching method (40), it does not achieve a total separation of the epidermis from the underlying dermal tissue.

[2] The Polytron homogenizer consists of a rotating metal shaft with sharp edges fitted in a metal tube. The shaft is driven by a motor at speeds up to 22,000 rpm. The tube is immersed in the homogenizing medium cooled in crushed ice. This instrument is particularly useful for the homogenization of a tough tissue such as skin.

with shaking at 37°C for 5, 15, 30, and 60 min, respectively. Control experiments were incubated with enzyme preparations from the subcellular fractions previously boiled for 5 min. The incubations were stopped by the addition of 25 ml of chloroform–methanol 2:1 (v/v) to each incubation flask. The suspension was transferred to separatory flasks and extracted twice with 25 ml of the same solvent mixture. The combined extracts were evaporated to dryness in a rotary evaporator. The residue was dissolved in a small volume of chloroform–methanol 1:1 (v/v) and subjected to chromatography on silica gel G thin-layer plates.

Thin-layer chromatography and determination of prostaglandins. TLC on silica gel G was performed accordto Struijk, Beerthuis, and Van Dorp (22). The glass plates were coated with silica gel G and activated at 110°C for 30 min before use. The ^{14}C-labeled extract was applied to the activated plate. A small amount of reference PGE_2 was applied to a second activated plate. Both plates were developed simultaneously in the solvent system chloroform–methanol–acetic acid–water 90:6:1:0.75. Reference PGE_2 was visualized in a tank of iodine vapor. The silica gel on the plate containing the ^{14}C-labeled extract that corresponded to the reference PGE_2 was scraped into a sintered funnel and the ^{14}C-labeled material was eluted with chloroform–methanol 1:1. An aliquot of the eluate was assayed for radioactivity with a Packard Tri-Carb model 2002 liquid scintillation counter as reported previously (19), and the remaining eluate was evaporated to dryness under a stream of nitrogen. This system is effective for the separation of ^{14}C-labeled prostaglandins from [1-^{14}C]arachidonic acid and other radioactive products.

The recovered ^{14}C was dissolved in the solvent mixture chloroform–methanol 1:1 (v/v) and applied to another plate of activated silica gel G. Reference PGE_2 was similarly treated. The plates were developed in the A I system, benzene–dioxane–acetic acid 20:20:1, according to the procedure described by Gréen and Samuelsson (23). After visualizing the reference PGE_2 by iodine vapor, the silica gel corresponding to the reference PGE_2 was eluted as described above and a portion of the eluate was assayed for radioactivity. The remaining eluate was evaporated to dryness under a stream of nitrogen.

For further identification, reference PGE_2 and the remaining ^{14}C-labeled material were converted into PGB_2 by treating each sample with 3 ml of 0.5 N NaOH in 50% aqueous ethanol followed by incubation at 37°C for 30 min. A portion of the alkaline-treated PGE_2 was tested for absorbance at 278 nm. Under these conditions, the E prostaglandins form products containing the $\Delta^{8(12),13}$-9 keto chromophore by elimination of the 11-hydroxy group and isomerization of the resulting double bond (24, 25). The samples were diluted with water and

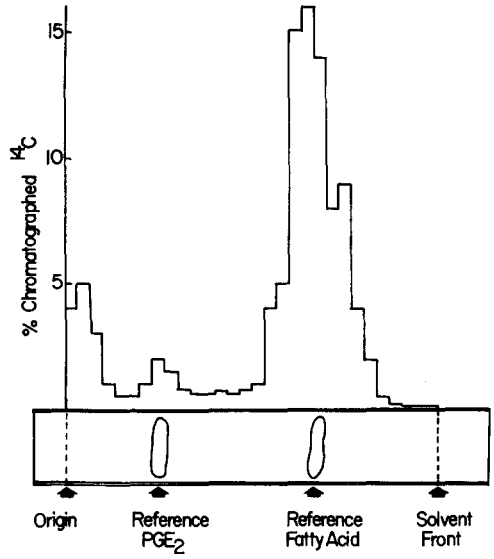

FIG. 1. Thin-layer chromatography of PGE_2 and the product obtained after incubating [1-^{14}C]arachidonic acid with homogenate of human epidermis. [1-^{14}C]Arachidonic acid (2.8 μCi, 0.05 μmole) was incubated with a homogenate of human epidermis (90.5 mg of protein) containing 0.65 mM glutathione and 0.55 mM hydroquinone. Details of incubation conditions and the TLC system are described under Methods. The heights of the open bars from the base line to the top indicate the percentages of chromatographed ^{14}C; the spots on the plate represent reference PGE_2 and arachidonic acid.

then acidified with 6 N HCl to pH 2–3. The acidified extracts were extracted three times with 10 ml of dichloromethane, and the combined extracts were evaporated to dryness under nitrogen. The samples were dissolved in a minimal amount of chloroform–methanol 1:1 and applied to activated silica gel G plates. The plates were developed in the solvent system ether–acetic acid 100:2. After visualizing the PGB_2 with iodine vapor, the corresponding area of silica gel on the plate containing the ^{14}C extract was scraped off and eluted with chloroform–methanol 1:1. The ^{14}C in the eluate was assayed by liquid scintillation counting as described above.

RESULTS

Intracellular localization of PGE_2 biosynthesis from [1-^{14}C]arachidonic acid in human epidermis

Incubations of [1-^{14}C]arachidonic acid with subcellular fractions obtained from human epidermis resulted in the formation of radioactive products with chromatographic mobilities similar to reference PGE_2 (Fig. 1). After further purification of the radioactive PGE_2 in solvent system A I (23), the eluted ^{14}C-labeled material was treated with sodium hydroxide as described under Methods. Final identification of radioactive PGB_2 was achieved after chromatography in the solvent

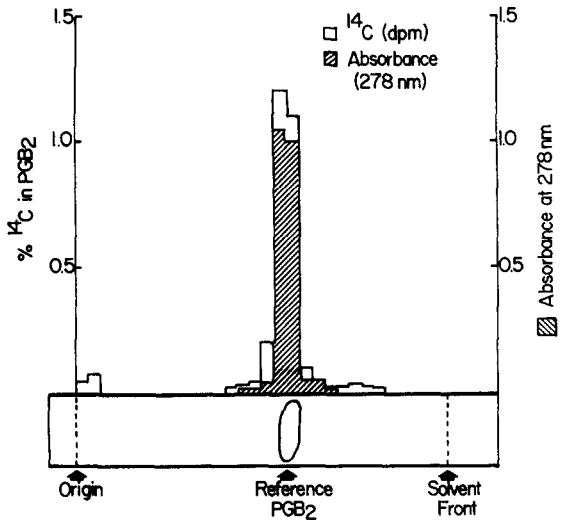

FIG. 2. Thin-layer chromatography of reference PGB_2 and the product obtained after treatment of radioactive fractions in Fig. 1 with 0.5 N NaOH. The heights of the open bars from the base line to top indicate the percentage of ^{14}C in PGB_2; the striped bars indicate the absorbance of PGB_2 at 278 nm.

system ether–acetic acid 100:2. A typical chromatogram is shown in Fig. 2.

The incorporation of ^{14}C from arachidonic acid into PGB_2 in the various subcellular fractions is shown in Table 1. The results indicate that the microsomal fraction (105,000 *g* pellet) prepared from human epidermis is most active in the biosynthesis of PGE_2 from arachidonic acid. These results are consistent with other published reports which indicate that the microsomal fraction is the site of prostaglandin biosynthesis (22, 26–28). Although the 105,000 *g* supernatant fluid had little or no activity, it inhibited the incorporation of ^{14}C from arachidonic acid into PGE_2 when added to the microsomal fraction. The nature of this inhibition is not clear, and further experiments are necessary to clarify this point.

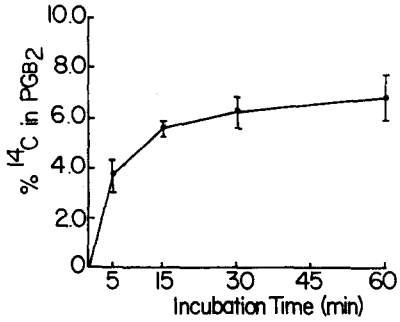

FIG. 3. Time course of conversion of [1-^{14}C]arachidonic acid into PGB_2. [1-^{14}C]Arachidonic acid (2.8 μCi, 0.05 μmole) was incubated with a microsomal fraction (28.0 mg of protein) obtained from epidermis homogenized in 0.1 M potassium phosphate buffer (pH 8.0) containing 30 mM EDTA. The incubation mixture contained 0.6 mM glutathione and 0.55 mM hydroquinone. Conditions of incubation and identification of radioactive products are described under Methods. The results are expressed as percentages of added substrate recovered in the PGB_2 fractions. The data are averages of duplicate experiments; vertical bars indicate ranges.

TABLE 1. Intracellular localization of PGE_2 biosynthesis in human epidermis

Fractions	Protein	^{14}C in PGB_2
	mg	%
Homogenate	90.5	1.7
800 *g* pellet	10.5	0.1
105,000 *g* pellet	28.0	2.8
105,000 *g* pellet + supernate	10.1	2.1
105,000 *g* supernate	50.5	0.1

The incubation mixtures consisted of 5 ml of subcellular fraction obtained from human epidermis homogenized in 0.1 M potassium phosphate buffer (pH 8.0), 0.65 mM glutathione, 0.55 mM hydroquinone, and [1-^{14}C]arachidonic acid (2.8 μCi, 0.05 μmole). The incubation was at 37°C for 15 min. Identification of radioactive products is described in the text. Results are expressed as percentages of added substrate recovered in PGB_2 fraction after correction for boiled enzyme control. The data are averages of duplicate experiments. The ranges of values were on the order of those shown in the figures.

Time course of ^{14}C incorporation from [1-^{14}C]-arachidonic acid into PGE_2 by microsomal fraction

Since the preceding experiments showed that the biosynthesis of PGE_2 from arachidonic acid occurs in the microsomal fraction, all subsequent incubations were carried out with this preparation. The time course of the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGB_2 is shown in Fig. 3. The enzymatic activity of this fraction was rapid and approached maximum in approximately 15 min.

Effect of increasing microsomal protein on the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGE_2

The relationship between percentage conversion of labeled arachidonic acid into PGB_2 and the amount of protein from the microsomal fraction is shown in Fig. 4. The amounts of ^{14}C incorporated into PGB_2 increased with increased amounts of microsomal protein and was linear up to 28 mg of protein. On the basis of these results, this amount of microsomal protein was used in subsequent incubations.

Effects of EDTA on the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGE_2

Homogenization of epidermis with 0.1 M potassium phosphate buffer containing EDTA markedly increased the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGB_2 by the microsomal fraction (Fig. 5). This

TABLE 2. Effects of fatty acids on biosynthesis of PGE_2 by microsomal fraction from human epidermis

Fatty Acid	Concentration	^{14}C in PGB_2	Inhibition
	mM	%	%
None		5.9	
Palmitic acid (16:0)	1	5.7	3
	2	5.5	5
Palmitoleic acid (16:1, n − 7)	1	4.8	19
	2	4.2	29
Linoleic acid (18:2, n − 6)	1	3.0	49
	2	1.3	78
Linolenic acid (18:3, n − 3)	1	1.8	70
	2	0.8	87
Eicosatrienoic acid (20:3, n − 3)	1	1.3	78
	2	0.2	96

[1-^{14}C]Arachidonic acid (2.8 μCi, 0.05 μmole) was incubated with 5 ml of microsomal fraction (28.0 mg of protein) obtained from epidermis homogenized in 0.1 M potassium phosphate buffer (pH 8.0) containing 30 mM EDTA. Incubation mixture contained 0.65 mM glutathione, 0.55 mM hydroquinone, and fatty acids dissolved in 0.01 ml of ethanol. Incubation was at 37°C for 15 min. Identification of radioactive products is described in the text. Results are expressed as percentages of added substrate recovered in PGB_2 fraction after correction for boiled enzyme control. The data are averages of duplicate experiments. The ranges of values were on the order of those shown in the figures.

finding was in harmony with previous reports describing the enhancement of the biosynthesis of prostaglandin E_2 by EDTA in homogenates of rat stomach (29, 30).

Effects of fatty acids on the biosynthesis of PGE_2 from arachidonic acid

The effects of fatty acids on the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGB_2 by the microsomal fraction of human epidermis is shown in Table 2. A marked inhibition occurred with free polyunsaturated fatty acids, particularly linolenic acid (18:3, n − 3) and eicosatrienoic acid (20:3, n − 3). No inhibition was observed at two concentrations of palmitic acid. Preincubation of the microsomal fraction for 5 min with the above inhibitors before the addition of [1-^{14}C]arachidonic acid inhibited the incorporation of ^{14}C into PGB_2 even at a lower concentration (Fig. 6). The addition of higher concentrations of [1-^{14}C]arachidonic acid did not reverse the inhibitory effects of 18:3, n − 3 and 20:3, n − 3. It appears from these latter experiments that linolenic acid and eicosatrienoic acid may compete with arachidonic acid for the prostaglandin synthetase.

Effects of anti-inflammatory drugs on the biosynthesis of PGE_2 from arachidonic acid

The inhibitory effect of anti-inflammatory drugs such as indomethacin and aspirin on prostaglandin synthesis has been reported (29–31). Experiments were therefore

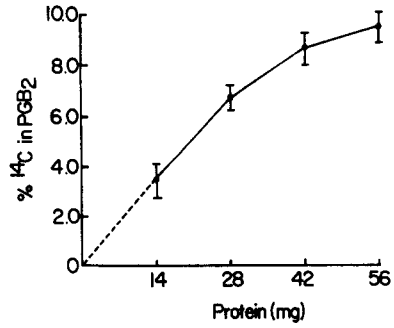

FIG. 4. Effect of increasing microsomal protein on the conversion of [1-^{14}C]arachidonic acid into PGB_2. [1-^{14}C]Arachidonic acid (2.8 μCi, 0.05 μmole) was incubated for 15 min with increasing amounts of microsomal protein obtained from EDTA-treated homogenate. Details of incubation and identification of radioactive products are described in the text. The results are expressed as percentages of added substrate recovered in the PGB_2 fraction. The data are averages of duplicate experiments; vertical bars indicate ranges.

carried out with various known anti-inflammatory drugs on the activity of prostaglandin synthetase in the skin. The results of the incubation of the microsomal fraction with anti-inflammatory drugs are shown in Table 3. Indomethacin and acetylsalicylic acid inhibited the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGB_2. On the other hand, no appreciable inhibitory effect could be demonstrated by triamcinolone acetonide and dexamethasone at the concentrations employed in these studies.

DISCUSSION

The present studies have demonstrated that human epidermis can transform arachidonic acid into PGE_2. This finding confirms the earlier studies by Jonsson and Änggård (20), who demonstrated a similar transforma-

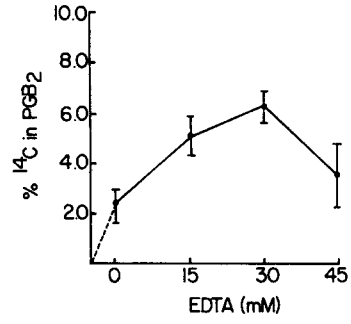

FIG. 5. Effect of EDTA on conversion of [1-^{14}C]arachidonic acid into PGB_2. [1-^{14}C]Arachidonic acid (2.8 μCi, 0.05 μmole) and microsomal fraction (28.0 mg of protein) were incubated for 15 min as indicated under Fig. 2 with increasing amounts of EDTA. Details of incubation and identification of radioactive products are described in the text. The results are expressed as percentages of added substrate recovered in the PGB_2 fraction. The data are averages of duplicate experiments; vertical bars indicate ranges.

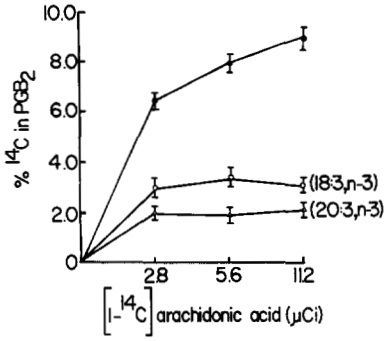

FIG. 6. Inhibition of [1-^{14}C]arachidonic acid conversion to PGB_2 by linolenic acid (18:3, n − 3) and eicosatrienoic acid (20:3, n − 3). The microsomal fraction (28.0 mg of protein) was incubated for 5 min with 0.5 mM linolenic acid and eicosatrienoic acid, respectively. Increasing amounts of substrate [1-^{14}C]arachidonic acid were added to the incubation mixture and incubated for 15 min as described under Methods. Identification of radioactive products is described in the text. Results are expressed as percentages of added substrate recovered in the PGB_2 fraction. The data are averages of duplicate experiments; vertical bars indicate ranges.

TABLE 3. Effects of anti-inflammatory drugs on biosynthesis of PGE_2 by microsomal fraction from human epidermis

Drug	Concentration	^{14}C in PGB_2	Inhibition
	μM	%	%
None		6.3	
Indomethacin	3	5.1	19
	6	4.2	33
Acetylsalicylic acid	30	4.8	24
	60	3.3	47
Triamcinolone acetonide	30	5.9	6
	60	5.6	11
Dexamethasone	30	6.0	5
	60	5.8	8

[1-^{14}C]Arachidonic acid (2.8 μCi, 0.05 μmole) was incubated with 5 ml of microsomal fraction (28.0 mg of protein) obtained from epidermis homogenized in 0.1 M potassium phosphate buffer (pH 8.0) containing 30 mM EDTA. Incubation mixture contained 0.65 mM glutathione, 0.55 mM hydroquinone, and anti-inflammatory drugs dissolved in 0.01 ml of ethanol. Incubation was at 37°C for 15 min. Identification of radioactive products is described in the text. Results are expressed as percentages of added substrate recovered in PGB_2 fraction after correction for boiled enzyme control. The data are averages of duplicate experiments. The ranges of values were on the order of those shown in the figures.

tion by human skin. The present studies have also demonstrated that this biosynthetic activity is localized in the microsomal fraction. It was of particular interest to observe that the addition of the 105,000 *g* supernatant fluid to the microsomal fraction suppressed the transformation of arachidonic acid into PGE_2. Although the nature of this inhibitory effect is not clear at the moment, the supernatant fraction may contain sufficient amounts of divalent cations that form metal ligand complexes which serve in the selective formation of F prostaglandins (31). On the other hand, homogenization of the epidermal tissue with EDTA enhanced the incorporation of ^{14}C from [1-^{14}C]arachidonic acid into PGE_2 by the microsomal fraction (Fig. 5). A possible explanation for this increase in prostaglandin synthesis in the presence of EDTA may be due to its chelating properties to remove the metal ions and the suppression of the activity of epidermal phospholipase A (phosphatide acylhydrolase EC 3.1.1.4). EDTA has been demonstrated to inhibit the activity of phospholipase A in the myocardial tissue (32). The activity of phospholipase A has also been demonstrated in cow snout epidermis (33). It is therefore likely that the increased incorporation of ^{14}C into PGE_2 observed in the present studies may be due to the inhibitory effect of EDTA on the release of nonradioactive arachidonic acid from endogenous phospholipids produced during homogenization (20, 29, 30). Clarification of the mechanism of the EDTA enhancement of ^{14}C incorporation from arachidonic acid into PGE_2 by skin must await further studies.

This study has also shown that the biosynthesis of PGE_2 from arachidonic acid is strongly inhibited by polyunsaturated fatty acids (Table 1). Marked inhibition occurred particularly with linolenic and eicosatrienoic acids, which are not normally converted to prostaglandin-like compounds. At concentrations of 2 mM, both fatty acids inhibited the formation of PGE_2 from arachidonic acid by more than 80%. These observations are significant in view of the accumulation of certain polyunsaturated fatty acids, particularly eicosatrienoic acid, in tissues of essential fatty acid-deficient animals (34–37). It is possible that the accumulation of these polyunsaturated fatty acids may regulate the biosynthesis of prostaglandins in tissues. Further studies are, however, necessary to test this possibility, particularly with 20:3 (n − 9) eicosatrienoic acid, which accumulates in the tissues of essential fatty acid-deficient animals. Preincubation of the microsomal fraction with linolenic and eicosatrienoic acids for 5 min before the addition of labeled arachidonic acid resulted in inhibition at an even lower concentration (Fig. 6). Further increase of the radioactive substrate in the presence of the inhibitors failed to reverse the inhibitory effects. Under the conditions of the present studies, linolenic and eicosatrienoic acids irreversibly inhibit the biosynthesis of PGE_2 from arachidonic acid. A similar inhibitory effect of linolenic acid on the biosynthesis of PGE_2 from arachidonic acid by homogenates from rat stomach has been reported (38). However, the nature of the inhibitory effects of the polyunsaturated acids on the skin prostaglandin synthetase could not be ascertained by these experiments. Since trace amounts of radioactive substrate were used in the presence of an undetermined pool of nonradioactive substrate, no reliable stoichiometry could be

obtained between substrate and amount of inhibitor used in these experiments. Full information on whether linolenic and eicosatrienoic acids inhibit the biosynthesis of PGE_2 by the skin competitively or noncompetitively must await further studies. It is interesting, however, that the inhibitory effects of linoleic acid and linolenic acid on the prostaglandin synthetase from sheep vesicular tissue were reversed after incubation of the inhibitors with arachidonic acid for several hours (39). A notable feature of the vesicular tissue is the transformation of the unsaturated fatty acids into hydroxy fatty acids.

Indomethacin and aspirin both inhibited the biosynthesis of PGE_2 from arachidonic acid by the microsomal fraction from human epidermis. The results indicate that indomethacin is approximately eight times more potent than aspirin. The steroid anti-inflammatory drugs had minor, if any, inhibitory effects. These data indicate that the anti-inflammatory steroids used in these studies do not inhibit the conversion of arachidonic acid into PGE_2 in vitro. The anti-inflammatory effects of these steroid compounds in vivo are probably exerted at some other steps in the biosynthetic pathway of prostaglandins. That human skin is a useful organ for the study of the mechanisms of prostaglandin biosynthesis has been demonstrated by these studies. This tissue can be obtained readily and therefore can serve as a useful tool for the study and screening of various compounds for their anti-inflammatory properties.

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