Prostaglandin synthesis in rat adrenocortical cells

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Abstract The biosynthesis of prostaglandins by isolated rat adrenocortical cells has been studied by determinations of products formed during incubations with labeled arachidonic acid and by radioimmunoassays. Analysis by thin-layer chromatographic separation of silicic acid column fractions indicated that PGE_2, PGA_2 (B_2) and $PGF_{2\alpha}$ were the predominant prostaglandins formed by rat adrenocortical cells. Approximately 75% of the incorporated isotope was associated with the prostaglandins of the PGE pathway [PGE_2 + PGA_2 (B_2)]. This was a consistent finding whether cells were incubated directly with arachidonic acid or with cells prelabeled with the substrate prior to study. ACTH did not affect the uptake or oxidation of [1-^{14}C]-arachidonate, but did significantly increase incorporation of labeled substrate into [^{14}C]prostaglandins. Of the ACTH-induced increase, 92% was accounted for by an increase in prostaglandins of the E pathway. Studies with prelabeled cells indicated that 77% of the prostaglandins synthesized in both control and ACTH-stimulated adrenocortical cells was released into the incubation medium during the 2-hr study. These had the same composition [88% PGE_2 + PGA_2 (B_2)] as did the intracellular prostaglandins. Analysis by radioimmunoassays gave comparable data on the distribution of E- and F-type prostaglandins in control cells and cells incubated with ACTH or dibutyryl cyclic AMP. Thus, with these techniques, 88–92% of the increased prostaglandin synthesis due to ACTH or cyclic AMP was produced by the PGE_2 rather than the $PGF_{2\alpha}$ pathway.

Supplementary key words arachidonic acid · radioimmunoassay · ACTH

The adrenal cortex of the rat contains high concentrations of arachidonic acid that is primarily esterified to cholesterol and in phospholipids (1–3). The hydrolysis of the sterol esters contained in lipid inclusion droplets is a well-documented response to ACTH treatment or stress (4) and this effect also occurs in rat adrenal cells exposed to ACTH (3). Adrenal phospholipids, in contrast, are not depleted under these conditions (3, 4). It has recently been shown that the sterol ester hydrolase (EC 3.1.1.13) of adrenal cortex is activated via a cyclic AMP-dependent protein kinase by a mechanism involving phosphorylation of the enzyme protein (5–7). Thus, the hormone-induced hydrolysis of adrenal sterol esters results in sustained availability of free cholesterol for steroidogenesis and the release of significant amounts of the potential prostaglandin precursor, arachidonic acid.

Studies dealing with prostaglandin synthesis and its control in adrenal cortex are limited. Flack, Jessup, and Ramwell (8) reported that ACTH caused a decrease in the prostaglandin content of superfused rat adrenals, and resulted in a greater level of prostaglandins in the tissue effluent than in the glands. Recently Laychock and Rubin (9) demonstrated the incorporation of labeled arachidonate by feline adrenal cells into $PGF_{2\alpha}$, PGE_2, and PGA. ACTH stimulated either uptake or conversion of the labeled precursor into all three prostaglandins. Comparable results were obtained by radioimmunoassay (10), and verified that the major prostaglandins in feline cortical cells were $PGF_{2\alpha}$ and PGE_2. Furthermore, ACTH produced a 128% increase in $PGF_{2\alpha}$ release, but only a 22% increase in PGE_2 (11).

In the present study, it has been shown that ACTH specifically increased the formation (and subsequent release) of PGE_2 by rat adrenocortical cells. Comparable data were obtained whether cells were incubated with the labeled precursor at the time of ACTH challenge, or were prelabeled with arachidonate prior to incubation with the tropic hormone. Additional evidence for the nature of the products, and the specificity of PGE synthesis, has been provided by radioimmunoassay techniques. A preliminary report of a portion of these studies has been presented (12).

MATERIALS AND METHODS

Materials

Trypsin, collagenase, and lima bean trypsin inhibitor were obtained from Worthington Biochemical Corp. Bovine serum albumin was from Sigma Chemical Co. Adrenocorticotropic hormone was purchased from the U.S. Pharmacopeia. Unlabeled prostaglan-

Abbreviations: PGE_2, $PGF_{2\alpha}$, PGA, PGB are prostaglandins E_2, $F_{2\alpha}$, A, and B, respectively; TLC, thin-layer chromatography; ACTH, adrenocorticotropic hormone; RIA, radioimmunoassay.

dins used as standards in isotopic and immunoassay studies were obtained through the courtesy of Dr. J. E. Pike of the Upjohn Co. [^{3}H]PGE_2 (140 Ci/mmol), [^{3}H]$PGF_{2\alpha}$ (15 Ci/mmol), and [1-^{14}C]arachidonic acid (55 Ci/mmol) were purchased from Amersham/Searle. [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (60–100 Ci/mmol) was from New England Nuclear Corp. Reagents for radioimmunoassay of PGE and $PGF_{2\alpha}$ were purchased from Clinical Assay, Inc. Lipid standards for thin-layer chromatography were from Supelco and Applied Science Corp.

Methods

Preparation of suspensions of rat adrenocortical cells. The technique for preparation of adrenal fasciculata cells by collagenase dissociation of adrenal tissue minces was as reported earlier (3). For each study, 10 animals were killed by decapitation using the "quiescent sacrifice" procedure (13). The adrenals were pooled, minced, and incubated for 20 min in 10 ml of 0.25% trypsin and then in 10 ml of 0.50% trypsin in Krebs bicarbonate buffer, pH 7.4. The supernatants were discarded after each trypsin incubation. The remaining tissue fragments were incubated five times for 20 min each in fresh 10-ml aliquots of Krebs buffer containing 0.04% collagenase. The supernatants from each collagenase incubation were pooled and the cells were sedimented at 150 *g* for 30 min. The cell pellets were gently resuspended in Krebs bicarbonate buffer containing 0.2% glucose, 0.5% albumin, 7.65 mM Ca^{2+}, and 0.1% lima bean trypsin inhibitor (KRBGA buffer). Cell counts were made with a hemocytometer and, except where indicated, the preparations were diluted to give a suspension containing 8×10^6 cells/ml. Viability determined by dye exclusion (trypan blue) was consistently 93–94%.

Incubations with [1-^{14}C]arachidonic acid. In experiments on the direct effect of ACTH on arachidonate uptake and metabolism, 8 μCi of [1-^{14}C]arachidonic acid in 50 μl of acetone was added to 8 ml of the cell suspension contained in 25-ml Ehrlenmeyer flasks. One-tenth ml of Krebs buffer alone or buffer containing ACTH or dibutyryl cyclic AMP was added. The final concentration of ACTH was 7.1×10^{-10} M and of cyclic AMP was 0.5 mM. Two aliquots were removed from each flask for determination of $^{14}CO_2$ production (14).

For prelabeling studies, cells (8.0×10^6 cells/ml) were incubated with 8 μCi of [1-^{14}C]arachidonate or [^{3}H]arachidonate for 1 hr at 37°C under 95% O_2–5% CO_2. The cells were re-isolated by centrifugation at 150 *g* at 4°C. These were washed twice with 4 ml of 2% albumin in Krebs buffer and re-isolated by centrifugation for 10 min at 150 *g*. The cell pellet was gently resuspended in 8 ml of KRBGA buffer, and 0.1 ml of Krebs buffer or buffer containing ACTH was added. Aliquots were removed for determination of $^{14}CO_2$ production (14) and for extraction of lipids (15). The remaining (6 ml) cell suspensions were incubated for 2 hr at 37°C under 95% O_2–5% CO_2.

Determination of $^{14}CO_2$. Aliquots of the cell suspensions were placed in 25-ml Ehrlenmeyer flasks and the flasks were sealed with caps fitted with polyethylene cups containing 0.3 ml of Hyamine hydroxide (14). All incubations were for 2 hr at 37°C in a Dubnoff metabolic shaker. At the termination of incubation, the media in the sealed flasks containing the Hyamine were acidified with 1 ml of 6 N H_2SO_4 and incubations were continued for one additional hour at 37°C to insure complete trapping of $^{14}CO_2$ (14). The Hyamine cups were removed, wiped, and placed in scintillation vials containing 10 ml of liquid scintillant (Aquasol, New England Nuclear). Radioactivity was determined with a Beckman LS-250 scintillation spectrometer.

Extraction of cortical cell lipids. Aliquots (2 ml) of the incubation media were acidified to pH 3 and extracted in 20 volumes of chloroform–methanol 2:1 according to Folch, Lees, and Sloane Stanley (15). After separation of the phases and washing, the chloroform extract was evaporated to dryness under nitrogen and the lipid residue was redissolved in hexane. Aliquots of this extract were placed in scintillation vials and evaporated to dryness prior to adding 10 ml of liquid scintillant (Scintiverse, Fisher Scientific Co.) and the determination of radioactivity.

Extraction, separation, and identification of labeled prostaglandins. Aliquots of the contents of each incubation flask were centrifuged at 150 *g* for 30 min at 4°C. The procedures for the extraction of prostaglandins were essentially as described by Laychock and Rubin (9). The incubation medium was acidified to pH 3 and extracted twice with 20 ml of ethyl acetate. The combined extracts were evaporated to dryness under nitrogen. The cell pellets were dispersed in 4 ml of Krebs buffer (adjusted to pH 3), homogenized, and centrifuged at 4°C for 1 hr at 150 *g*. The supernatant was retained, and the pellet was rehomogenized in 4 ml of buffer and recentrifuged. The supernatants were combined and extracted twice with 20 ml of ethyl acetate–cyclohexane 2:1 (v/v). These extracts were pooled and evaporated to dryness under nitrogen.

Prostaglandins were separated by a combination of silicic acid column chromatography (16) and silicic acid thin-layer chromatography (17). The dried extracts of cells or incubation media were dissolved in 0.2 ml of benzene–ethyl acetate 60:40 and applied to the silicic acid column (16). The column was eluted with 6 ml of benzene–ethyl acetate 60:40 to obtain

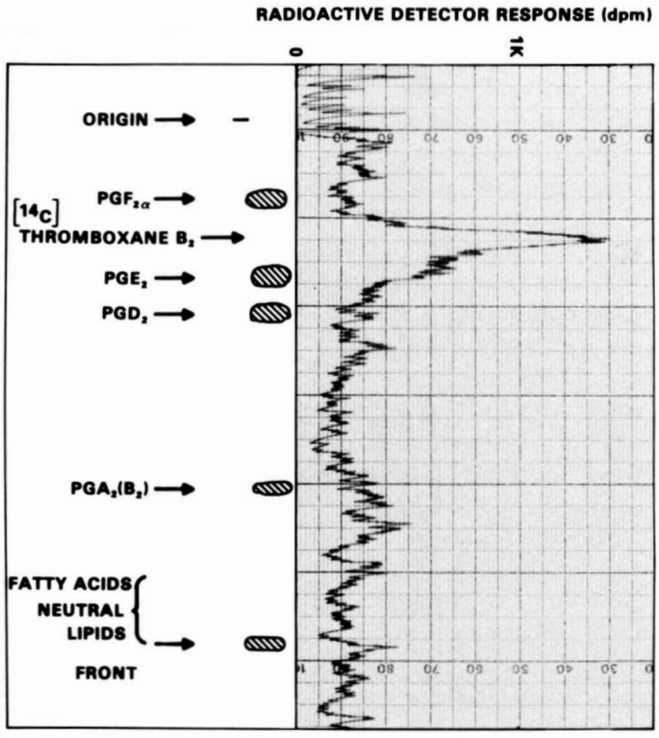

Fig. 1. Thin-layer silicic acid chromatographic separations of authentic prostaglandins $F_{2\alpha}$, E_2, D_2, and A_2 from lipids (fatty acids, triglycerides, cholesteryl esters) and radioactive thromboxane B_2. Solvent system was chloroform–methanol–acetic acid 95:5:5 (v/v/v). The prostaglandin standards were identified by iodine vapor and thromboxane B_2 was determined by radioscanning (18).

a fraction I containing PGA and PGB as well as neutral lipids and fatty acids; fraction II, containing PGE, was obtained by elution with 12 ml of benzene–ethyl acetate–methanol 60:40:2; fraction III, containing PGF, was eluted with 3 ml of benzene–ethyl acetate–methanol 60:40:20. Aliquots of each fraction were added to 10 ml of liquid scintillant for determination of radioactivity. The efficiency of separations and recoveries of prostaglandins B, E, and F were validated using labeled prostaglandins and were essentially as described earlier (9).

Thin-layer silicic acid chromatography of prostaglandins was carried out according to the method of Crowshaw (17), using chloroform–methanol–acetic acid 95:5:5. In all studies, the plates were dried and scanned for radioactivity using a Vangard Model 940 radiochromatogram scanner operated with 15% *n*-butane in helium. A representative separation of a mixture of authentic, unlabeled $PGF_{2\alpha}$, PGE_2, $PGA_2(B_2)$, PGD_2, and labeled thromboxane B_2 is shown in **Fig. 1.** The unlabeled prostaglandins were visualized after iodine sublimation and were effectively separated from one another. As is also shown by the accompanying radioscan, authentic [^{14}C]thromboxane could be distinguished from $PGF_{2\alpha}$ and PGE_2 (18).

After thin-layer chromatography of extracts of the incubation media from cells incubated with [1-^{14}C]-arachidonate, routine radioactive scanning revealed three major peaks which were associated with authentic standards of $PGF_{2\alpha}$, $PGA_2(B_2)$, and PGE_2. Further identification of these prostaglandins was obtained when thin-layer chromatography and radioscanning were carried out on the individual fractions from preliminary silicic acid column chromatography (16). Finally, the narrow silicic acid bands corresponding to authentic prostaglandins $F_{2\alpha}$, $A_2(B_2)$, and E_2 were individually scraped, extracted into ethyl acetate, and rechromatographed in the same solvent system. Radioscans of the re-isolated and rechromatographed areas corresponding to authentic $PGF_{2\alpha}$ and $PGA_2(B_2)$ are shown in **Fig. 2.**

A representative radioactive scan of PGE_2 re-isolated from thin-layer chromatoplates and rechromatographed (18) is shown in the upper panel of **Fig. 3.** The prostacyclin derivative, 6-keto prostaglandin $F_{1\alpha}$ (19) chromatographs with PGE_2 under the conditions used in these studies. However, PGE_2 is quantitatively converted to PGB_2 during alkaline isomerization (20), while 6-keto prostaglandin $F_{1\alpha}$ is not isomerized in ethanolic NaOH and its position on the chromatoplate does not change (19). Accordingly, the silicic acid zone

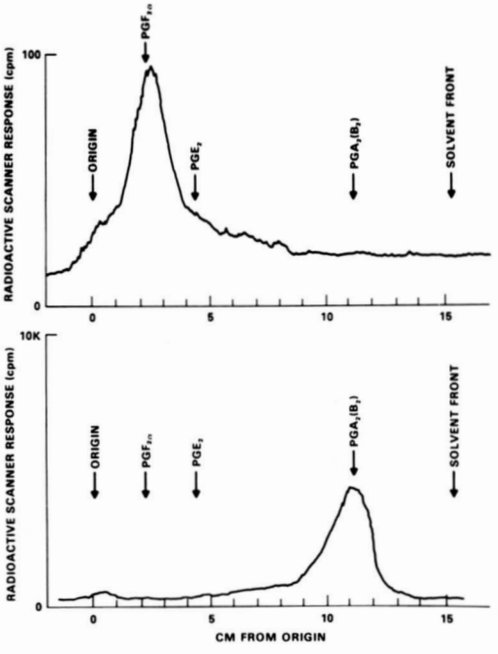

Fig. 2. Thin-layer radiochromatography scans of $PGF_{2\alpha}$ and $PGA_2(B_2)$. Extracts of adrenal cells incubated for 2 hr with [1-^{14}C]-arachidonic acid were chromatographed in a solvent system of chloroform–methanol–acetic acid 95:5:5. Radioactivity associated with authentic $PGF_{2\alpha}$ and $PGA_2(B_2)$ was extracted and individually rechromatographed in the same solvent system. Top panel, $PGF_{2\alpha}$; bottom panel, $PGA_2(B_2)$.

corresponding to PGE_2 was scraped and extracted into ethyl acetate. Aliquots were treated with ethanolic NaOH (20), acidified to pH 3, re-extracted into ethyl acetate, and chromatographed in the same solvent system. As shown in the lower panel of Fig. 3, there was a complete shift in the radioactivity originally chromatographing with authentic PGE_2 to a silicic acid area corresponding to authentic $PGA_2(B_2)$. This confirmed the original identification of PGE_2 and suggests the absence of prostacyclin formation from arachidonate in rat adrenal cortex.

Radioimmunoassay of prostaglandins.[1,2] The extraction of prostaglandins and removal of protein was carried out on 0.5 ml of the incubation medium according to the procedure of Jaffe and Behrman (22). The procedure for radioimmunoassay of prostaglandin $F_{2\alpha}(F_{1\alpha})$ is a modification of those reported earlier (22–24). At 50% binding of $PGF_{2\alpha}$ with the PGF_2 antiserum, cross-reaction with the PGE's is reported to be 1:5000. This was confirmed by using authentic standards of PGE_2 and PGA_2. The antiserum cross reacts with $PGF_{1\alpha}$ and, therefore, results are expressed as PGF equivalents. These were obtained by comparing competitive binding by unknown samples with standard curves for authentic $PGF_{2\alpha}$ ranging from 9 pg to 25 ng.

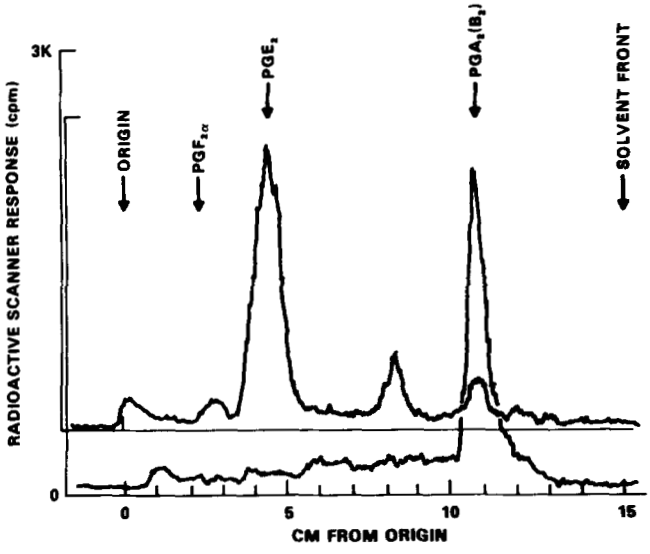

Fig. 3. Thin-layer chromatography of [^{14}C]PGE_2 before (upper panel) and after (lower panel) alkaline isomerization (21). Conditions were as described in Fig. 2.

[1] Clinical Assays, Inc., Cambridge, MA, Protocol CA 503. [^{3}H] Prostaglandin $F_{2\alpha}$ Radioimmunoassay Kit for the Quantitative Measurement of Prostaglandin $F_{2\alpha}$ in Plasma or Tissue Extracts. Feb. 14, 1975.

[2] Clinical Assays, Inc., Cambridge, MA, Protocol CA-501. [^{3}H] Prostaglandin E Radioimmunoassay Kit for the Quantitative Measurement of Prostaglandin A and E in Serum or Tissue Extracts. Feb. 14, 1975.

TABLE 1. Uptake and utilization of [1-^{14}C]arachidonate by rat adrenocortical cells

Additions to Incubation Medium[a]	Uptake	[1-^{14}C]Arachidonic Acid		
		Oxidation to $^{14}CO_2$	In Cell Lipids[b]	Conversion to Prostaglandins[c]
	%	% of uptake		
None	7.1 ± 0.8	9.3 ± 3.8	87.8 ± 2.6	3.0 ± 0.6
ACTH (7.1×10^{-10} M)	8.4 ± 2.6	5.8 ± 0.5	92.1 ± 0.7	4.3 ± 0.3
Significance	N.S.	N.S.	N.S.	$P < 0.05$

[a] Incubation flasks contained 1.25×10^5 cells/ml in Krebs bicarbonate buffer, pH 7.4, containing 0.2% glucose, 0.5% albumin, and 7.65 mM Ca^{2+}. Incubations were for 2 hr at 37°C under 95% O_2–5% CO_2 in sealed flasks. The techniques for determination of $^{14}CO_2$ production are as described previously (14). Figures represent means ± SEM for 3–5 experiments.

[b] Incorporation into cell lipids was determined after extraction according to Folch et al. (15).

[c] Radioactivity in prostaglandins represents the sum of radioactivity associated with prostaglandins $F_{2\alpha}$, E_2, and $A_2(B_2)$.

The procedure for radioimmunoassay of PGE was modified from earlier methods (25, 26) and is dependent on the quantitative conversion of PGE_2 and PGA_2 to PGB_2 by alkaline isomerization (20). Prostaglandins A_2 and A_1 are considered to occur factitiously during the course of the extraction and separation of prostaglandins (9). Also, the procedure does not differentiate PGB_1 and PGB_2. Therefore, the results are expressed as prostaglandin E equivalents and were derived by comparison to standard curves obtained with 10–2000 pg of PGB_1. At 50% binding of PGB_2 with PGB_1 antisera, cross-reaction with $PGF_{1\alpha}$ was less than 1:400. At the dilutions employed in the present study (100–200 pg of PGE equivalents), interference by PGF's was less than 0.1%.

Other analysis. For corticosterone assay, 0.2-ml aliquots of the incubation mixtures were extracted in 15 ml of methylene chloride. Corticosterone was determined fluorimetrically (27) using standards of authentic corticosterone.

RESULTS

Uptake and metabolism of [1-^{14}C]arachidonate by adrenocortical cells

Experiments on the effect of ACTH on the cellular uptake and utilization of labeled arachidonate are summarized in **Table 1.** These data, obtained after 2-hr incubations of cells with [1-^{14}C]arachidonate, show that while there is a significant uptake of the fatty acid by adrenocortical cells, this is unaffected by ACTH. Macho and Saffron (28) previously reported

TABLE 2. Distribution of radioactivity after thin-layer chromatography of lipid extracts of adrenocortical cells incubated with [1-^{14}C]arachidonic acid

Thin Layer Fraction[a]	% of Cellular Radioactivity		
	Control	ACTH	ACTH/Control
Origin	25.7	19.8	0.77
$PGF_{2\alpha}$	0.75	0.62	0.83
PGE_2	0.66	1.13	1.71
$PGA_2(B_2)$	1.77	2.55	1.44
Neutral lipids and unesterified fatty acids	71.2	75.8	1.06

[a] Adrenocortical cells (2.5×10^5/2 ml) were incubated with 2 μCi of [1-^{14}C]arachidonic acid in the absence and presence of 7.1×10^{-10} M ACTH. Incubations were at 37°C for 2 hr under 95% O_2–5% CO_2. Cells were re-isolated by centrifugation at 150 *g* for 10 min and the cell pellets were extracted according to Folch et al. (15). Thin-layer silicic acid chromatography was performed as described by Crowshaw (17).

that ACTH (400 milliunits/100 mg) decreased the apparent incorporation of labeled palmitate into rat adrenal. However, Brecher, Braga-Illa, and Chobanian (29) have subsequently shown that 100 milliunits of ACTH had no effect on the uptake of [1-^{14}C]oleic acid by rat adrenal cells.

Production of $^{14}CO_2$ over the 2-hr period (Table 1) also indicated that ACTH had no significant effect on the oxidation of the labeled fatty acid. Similar results were obtained with cells prelabeled with arachidonate and washed with 2% albumin prior to study. With these cells, 2.5 ± 0.3% of the cellular radioactivity in control incubations was oxidized to $^{14}CO_2$ over the 2-hr period. During incubations with ACTH, 2.3 ± 0.4% of the cellular radioactivity was recovered as $^{14}CO_2$. These data complement an earlier report (28) that ACTH has no effect on oxidation of labeled palmitate by rat adrenal in vitro.

As is shown in Table 1, approximately 90% of the arachidonate uptake by control adrenocortical cells was recovered as cell lipids and this was not affected by ACTH. A similar finding had also been observed by Brecher et al. (29).

Formation of labeled prostaglandins

In the preliminary studies shown in Table 1, chloroform–methanol extracts of re-isolated cells were subjected to thin-layer chromatography for separation of prostaglandins (17). Narrow silicic acid bands corresponding to authentic prostaglandins $F_{2\alpha}$, E_2, and $A_2(B_2)$ were individually scraped into scintillation vials containing 10 ml of liquid scintillant. The origin (phospholipids) and the areas containing unesterified fatty acids and neutral lipids (triglycerides, cholesteryl esters) were also scraped and counted.

As shown in Table 1, 3% of the radioactivity taken up in control cells was cochromatographed with the three prostaglandins. After incubations with ACTH, incorporation of cellular radioactivity into prostaglandins was increased to 143% of that in control cells. The distribution of this radioactivity is shown in **Table 2.** Of the radioactivity associated with prostaglandins in control cells, 25% was cochromatographed with $PGF_{2\alpha}$ and the remainder was in prostaglandins of the E pathway. In cells incubated with ACTH, all of the increase in radioactivity incorporated into prostaglandins was accounted for by increases in labeled PGE_2 and $PGA_2(A_2)$.

Corroborative data on the distribution of labeled prostaglandins in control cells and on the effect of ACTH and incorporation of radioactivity into prostaglandins were obtained using techniques of extraction and chromatography specific for prostaglandins (see Materials and Methods).

In the first approach, adrenocortical cells were incubated for 2 hr with labeled arachidonate in the presence and absence of ACTH. Ethyl acetate–chloroform extracts were subjected to preliminary column separations prior to thin-layer chromatography. In four separate studies comparable to that shown in **Fig. 4***A*, prostaglandins of the E pathway contained 75.6 ± 0.1% of the total labeled prostaglandins. Incubations with ACTH resulted in a 44.4% ± 3.6% increase in prostaglandin synthesis and, of this increase, 92.2% ± 4.8% was attributable to $PGE_2 + PGA_2(B_2)$. Thus, in adrenocortical cells incubated with ACTH, $PGF_{2\alpha}$ represented only 15.1% ± 0.6% of the total radioactivity in prostaglandins.

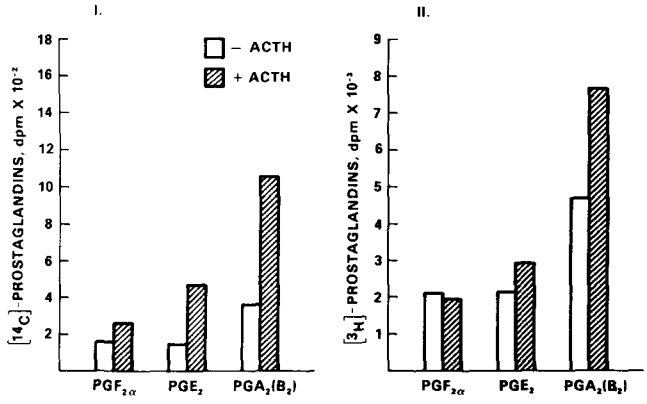

Fig. 4. Biosynthesis of prostaglandins in rat adrenocortical cells. *Left panel,* cells were incubated for 2 hr with [1-^{14}C]arachidonate in the absence and presence of 7.1×10^{-10}M ACTH; *right panel,* cells were prelabeled with [^{3}H]arachidonate by incubation for 1 hr at 37°C. Cells were re-isolated and washed twice with 2% albumin in Krebs buffer. Aliquots of these resuspended cells were incubated for 2 hr in the absence and presence of 7.1×10^{-10}M ACTH. In both studies, re-isolated cells were extracted with ethylacetate–chloroform. The extracts were subjected to silicic acid column chromatography and each column fraction was further purified by thin-layer chromatography.

The results of a similar analytical approach applied to cells prelabeled with [^{3}H]arachidonate and washed with 2% albumin prior to incubations with and without ACTH are shown in Fig. 4*B*. In this study, 73% of the radioactivity associated with cellular prostaglandins following the prelabeling period was recovered with PGE_2 and $PGA_2(B_2)$. During a 2-hr control incubation in the absence of ACTH, additional conversion of endogenous arachidonate radioactivity occurred and this was due entirely to an increase in $PGA_2(B_2)$. Incubation with ACTH resulted in a further 30% increase in prostaglandin radioactivity, all of which was associated with prostaglandins of the E pathway. For three similar studies, incubations with ACTH resulted in a 30–270% increase in radioactivity associated with prostaglandins. This was accounted for by the variable levels of prostaglandin synthesis in cells during control incubations. However, during incubations with ACTH, 5.4% ± 0.6% of the total cellular radioactivity was converted to prostaglandins. Of these increases due to ACTH, 92 ± 4.8% was due to increased radioactivity in prostaglandins of the E pathway, and this is identical to the distributions obtained during direct incubation with arachidonate.

The distribution of radioactive prostaglandins in the cells and cell-free incubation media is shown in **Fig. 5.** These data were obtained by thin-layer chromatography of silicic acid column fractions following separate extractions of postincubation cell pellets and cell-free incubation media. Collectively, ACTH resulted in a 61% increase in prostaglandin radioactivity, of which 96% was attributed to prostaglandins in the E pathway. These distributions were reflected both in the cells and in the cell-free media. In control cells, 72% of the total prostaglandin radioactivity was released into the incubation medium,

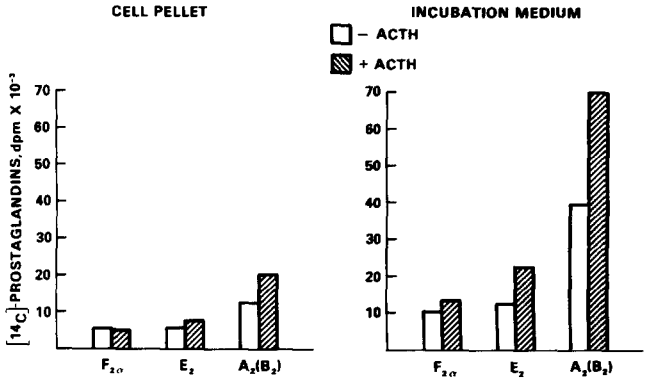

Fig. 5. Synthesis and release of radioactive prostaglandins by adrenocortical cells prelabeled with [^{14}C]arachidonate prior to incubations (2 hr, 37°C) in the absence and presence of ACTH. Re-isolated cells and incubation media were extracted separately (9), and extracts were subjected to silicic acid column and thin-layer chromatography for separation of prostaglandins.

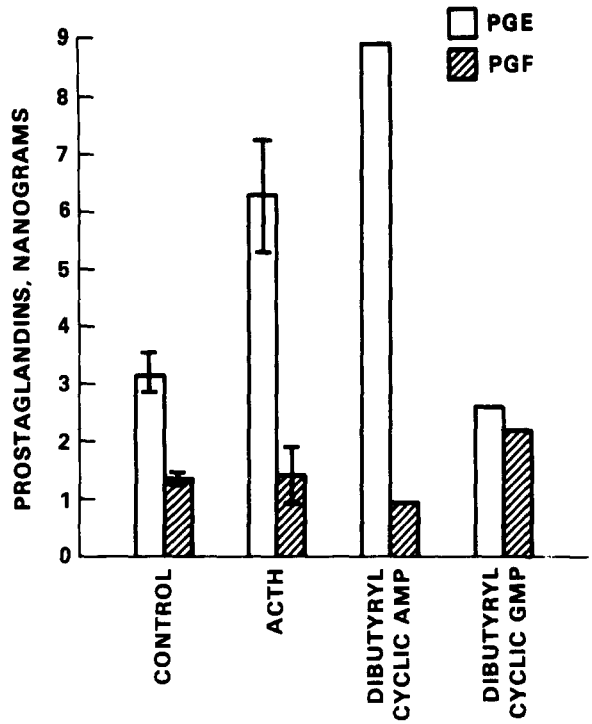

Fig. 6. Radioimmunoassays of PGE and PGF in rat adrenocortical cells (2.5×10^5 cells) incubated for 10 min in the absence (control) and presence of ACTH (7.1×10^{-10} M), dibutyryl cyclic AMP (0.5 mM), or dibutyryl cyclic GMP (0.5 mM). Data are expressed as PGE and PGF equivalents ($n = 4$ for control and ACTH; $n = 2$ for the dibutyryl derivatives of cyclic nucleotides). Preincubation levels of PGE and PGF were 3.2 ± 0.4 ng/2.5×10^5 cells and 1.3 ± 0.1 ng/2.5×10^5 cells, respectively, and these were not significantly altered during control incubations.

while with ACTH-stimulated cells, 76% of the total prostaglandin radioactivity was found in the cell-free medium. For three similar studies, 77.3 ± 3.4% of prostaglandins synthesized in control and ACTH-stimulated adrenocortical cells was released from the cells into the incubation medium.

Radioimmunoassay for prostaglandins F and E

It was determined in temporal studies that incubations of adrenocortical cells with ACTH resulted in maximal levels of prostaglandin E(A) within 10 min. The data in **Fig. 6** summarize the levels (ng/2.5×10^5 cells) of PGF and PGE equivalents determined after 10-min incubations of adrenocortical cells with no additions or with ACTH, dibutyryl cyclic AMP (0.5 mM), or dibutyryl cyclic GMP (0.5 mM). Prior to any incubations, total prostaglandins were 4.5 ± 0.2 ng/2.5 $\times 10^5$ cells, of which 70% was PGE equivalents (compared to 81.7% determined by isotope assays). During control incubations, the absolute levels of PGE (3.2 ± 0.4 ng) and PGF (1.3 ± 0.1 ng) did not change. During incubations with ACTH, total prostaglandins were increased to 8.4 ± 1.2 ng. It is readily apparent

that there was no significant change in PGF levels under these conditions. Thus, 88 ± 12% of the increase in prostaglandins was as PGE equivalents, and this figure compares favorably with the ACTH-induced stimulation of PGE_2 synthesis determined by isotope experiments (92.2 ± 4.8%).

In a separate experiment, the effects of dibutyryl derivatives of cyclic AMP and cyclic GMP on prostaglandin synthesis were compared with that of ACTH. Although these are preliminary studies, the data (Fig. 6) indicate that the cyclic AMP had effects comparable to ACTH with respect to specific stimulation of PGE synthesis. This was in marked contrast to the results obtained during incubations with cyclic GMP. In this latter case, the total prostaglandin level (4.8 ng) was not increased over that in control cells (4.5 ng). However, it appears that, under these conditions, the levels of PGE and of PGF were comparable.

DISCUSSION

In recent years, there have been extensive efforts made to define the pharmacological and physiological effects of prostaglandins on adrenocortical function (see 30–32). However, studies on prostaglandin synthesis in this tissue have been limited. In 1975, Laychock and Rubin (9) demonstrated the formation of labeled prostaglandins by cat adrenocortical cells incubated with [^{14}C]arachidonic acid. It was reported that ACTH stimulated arachidonate incorporation into PGF and PGE and that, under these conditions, prostaglandin release from cells was increased by 25%. It was subsequently shown by radioimmunoassays (10) that $PGF_{2\alpha}$ is the predominant PGF, and PGE_2 is the predominant PGE in cat adrenal cells. Furthermore, the mean percentage increases in $PGF_{2\alpha}$ and PGE_2 formation in response to ACTH were about equal (96% and 70%, respectively).

During our studies on the hormone-sensitive sterol ester hydrolase of adrenal cortex (5, 7) and the metabolism of cholesteryl esters in adrenocortical cells (3), we became interested in the fate of the fatty acid moiety released during ACTH-induced hydrolysis of cholesteryl ester. The majority of these esters contained polyunsaturated fatty acids, and particularly arachidonate (1). Furthermore, cholesteryl arachidonate is extensively hydrolyzed during stress (33) or ACTH administration (see 32) in vivo and in adrenocortical cells incubated with ACTH or dibutyryl cyclic AMP (3).

The present studies were designed to determine *a*) the effect of ACTH on the uptake and oxidation of arachidonic acid by rat adrenocortical cells; *b*) the levels of cellular prostaglandins of the E and F series; and *c*) the effect of ACTH on the conversion of arachidonate to PGE_2 and $PGF_{2\alpha}$.

Uptake and oxidation of arachidonic acid by rat adrenocortical cells

Under the conditions employed in these studies, ACTH was found to have no stimulatory effect on either the uptake or oxidation of arachidonic acid by rat adrenocortical cells. This lack of effect on oxidation was demonstrated during direct incubations with free arachidonate in the incubation medium, or after incorporation of labeled arachidonate into cellular lipids. From these and previous studies (28, 29), it appears that oxidation is not a major metabolic fate of sterol ester fatty acids and is not significantly altered during increased energy requirements associated with enhanced steroidogenesis.

Prostaglandin levels and synthesis in adrenal cells

The occurrence of prostaglandins in rat adrenocortical cells was confirmed by using antisera specific for PGF and PGE (determined as PGB). In control cells, prostaglandins of the F series were determined to be 1.35 ± 0.15 ng PGF equivalents, and PGE equivalents were 3.15 ± 0.35 ng/2.5 × 10^5 cells. Thus, by RIA, PGE's represent approximately 70% of total cellular prostaglandins. It has been reported that, in cat cortical cells, basal prostaglandin levels are about 700 pg/2.5 × 10^5 cells, of which approximately 70% occurs as $PGF_{2\alpha}$ (11). However, the levels in cat cells have been found to be variable with respect to total prostaglandin content and individual levels of PGE_2 and $PGF_{2\alpha}$ (10).

Prostaglandin synthesis in rat adrenocortical cells was documented using direct incubation with [1-^{14}C]- or [^{3}H]arachidonate, and with cells whose lipids were prelabeled with arachidonate prior to control 2-hr incubations. Although the levels of prostaglandin synthesis were variable in control cells, about 75% of the radioactive prostaglandins formed in control incubations were accounted for by prostaglandins of the E pathway ($E_2 + A_2$), while only 25% was recovered as $PGF_{2\alpha}$. These data are in complete accord with the distribution of PGE and PGF in control cells as determined by radioimmunoassay.

The ACTH stimulation of prostaglandin synthesis in adrenocortical cells was demonstrable by isotope analysis and by radioimmunoassay techniques. During incubations of adrenal cells with labeled arachidonate, ACTH produced a 40–50% increase in radioactive PG synthesis, 92% of which was attributable to prostaglandins of the E pathway. This increased incorpora-

tion was not a result of increased arachidonate uptake by the cells (Table 1). With cells in which lipids had been prelabeled with arachidonate, about 5% of the cellular radioactivity was incorporated into prostaglandins in the presence of ACTH and, again, 92% of this increase was recovered as PGE_2 and $PGA_2(B_2)$.

Based on radioimmunoassay, incubations with ACTH resulted in a 90% increase in total prostaglandin synthesis, of which 88% was in the E fraction. Comparable results were obtained with cells incubated with dibutyryl cyclic AMP, but not with dibutyryl cyclic GMP. Thus, stimulation of prostaglandin synthesis by ACTH, and the specific effect on the synthesis of PGE, was a consistent observation. Also, with both control and ACTH-stimulated cells, about 75% of the labeled prostaglandins synthesized from radioactive arachidonate was released from the cells over the 2-hr period of study.

The results of the present study are, with one major exception, comparable to those obtained with cat adrenal cells (9–11). It appears that, with cat cells, basal $PGF_{2\alpha}$ levels are equal to or greater than PGE_2 levels (11). While ACTH caused a 60–70% increase in PG synthesis (11), the large variations in these studies preclude differentiating between specific effects on PGE_2 and $PGF_{2\alpha}$. Finally, the data on cat adrenal cells suggest a preferential release of $PGF_{2\alpha}$ from cells incubated with ACTH (11). The reason for this difference from that in the present study is not clear except for the species employed.

The prostaglandin endoperoxide, PGG_2 is the common intermediate for the PGE_2 isomerase and PGF_2 reductase (31). The specific increase in synthesis of PGE_2 in the present study might be attributed to differences in the K_m values of the PGE isomerase and PGF reductase, or to a greater rate of degradation of $PGF_{2\alpha}$.

The present study clearly shows that prostaglandin E_2 synthesis in rat adrenocortical cells is stimulated by ACTH or dibutyryl cyclic AMP, but not by dibutyryl cyclic GMP. We have also recently reported that the tropic hormone or its second messenger stimulates hydrolysis of endogenous cholesteryl esters in these cells (3) via activation of sterol ester hydrolase (7). Cyclic GMP is also ineffective in these respects (34).

Although the physiological actions of PGE_2 in adrenal cortex have not been elucidated, there is suggestive evidence that this prostaglandin may modulate adenyl cyclase activity (35–37). Addition of PGE_2 (30 μM) to the incubation medium of adrenocortical cells mimics the action of ACTH in that there result increased levels of cellular free cyclic AMP and increased hydrolysis of endogenous cholesteryl esters (37). However, unlike ACTH, there is little or no stimulation of corticosterone release (37). In contrast, $PGF_{2\alpha}$ addition caused none of these responses (37) but did result in a transient increase in free cyclic GMP levels.[3]

The evidence to date supports the hypothesis (35) that E-type prostaglandins, whose synthesis is stimulated by the tropic hormone or cyclic AMP, may, in turn, modulate adenyl cyclase activity or the resulting levels of endogenous cyclic AMP. However, the finding that exogenous PGE_2 also causes marked increases in cellular cyclic AMP levels resulting in activation of sterol ester hydrolase without eliciting a corticosterone response (37) suggests a more complex relationship than is currently proposed. Some of these interrelationships are currently under investigation (37).

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