Journal of Steroid Biochemistry, 1978, Vol. 9, pp. 1111–1118. Pergamon Press. Printed in Great Britain

PROSTAGLANDIN E_2-INDUCED HYDROLYSIS OF CHOLESTEROL ESTERS IN RAT ADRENOCORTICAL CELLS

V. A. HODGES, C. T. TREADWELL and G. V. VAHOUNY
The George Washington University, School of Medicine and Health Sciences, Washington, D.C. 20037, U.S.A.

(*Received* 23 *February* 1978)

SUMMARY

Adrenocortical cells were prepared from rats maintained on essential fatty acid (EFA)-deficient diet for 16 weeks, and from control litter mates. These were incubated for 2 h at 37°C in the absence and presence of ACTH, PGE_2 or $PGF_{2\alpha}$. Analyses included corticosterone output, cellular cyclic AMP levels and the content and composition of cholesteryl esters and phospholipids. The steroidogenic response of both types of cells to ACTH (0.71 nM) was comparable, although there were differences in the time at which maximal cyclic AMP levels were attained. ACTH had no effect on phospholipids in either type of cell. However, there was a marked hydrolysis of cholesteryl esters in both normal and EFA-deficient cells in response to ACTH, despite major differences in fatty acid composition.

Incubations of either normal or EFA-deficient adrenal cells with PGE_2 (30 μM) resulted in increases in cellular cyclic AMP levels which peaked at 30 min. Corticosterone output under these conditions was low, representing only 13–21% of the levels seen after incubations with ACTH. However, PGE_2 stimulated hydrolysis of total and individual cholesteryl esters in both types of cells, and to the same extent as was observed with ACTH. The results suggest that prostaglandin E_2 has a direct stimulatory effect on adenylate-cyclase of adrenocortical cells, and that hydrolysis of sterol esters is a major response to increased cyclic AMP levels. Furthermore, the findings suggest that the availability of the PGE_2 precursor, arachidonate, may not be obligatory for the normal steroidogenic and hydrolytic responses of adrenocortical cells to ACTH.

INTRODUCTION

Although there have been several reports on the potential role of prostaglandins in the regulation of adrenal steroidogenesis, the participation of these substances in the process of steroid hormone synthesis remains uncertain [1–4]. Studies with indomethacin, an inhibitor of prostaglandin synthetase, have been carried out both *in vivo* [5] and *in vitro* [6, 7]; the results suggest that endogenous prostaglandins may not be obligatory for the action of ACTH. In contrast, studies on the effects of exogenous prostaglandins have generally [7–10], but not uniformly, [11, 12], indicated that prostaglandins of the E series stimulate the formation or release of steroid hormones in various *in vitro* preparations. This *in vitro* steroidogenic effect of prostaglandins is not demonstrable *in vivo* when the anterior pituitary has been removed 24 h prior to study [5, 13]. It has therefore been suggested that the action of prostaglandins on adrenal cortex is mediated via the hypophysis [13]. In addition, the *in vitro* response of adrenal cortical tissues to prostaglandins has been attributed to the use of pharmacological levels required to elicit a response [10]; to trypsin-induced alterations in the plasma membrane during proteolytic dissociation of adrenal tissue [10]; and to the presence of "prebound" or residual ACTH associated with the tissue at the time of study [5].

The arachidonate substrate for prostaglandin synthesis in rat adrenal is present almost entirely in the cholesteryl esters [14] and phospholipids [15] of the cortical cells. With stress *in vivo*, or during incubation of adrenocortical cells with ACTH, the cholesteryl esters [4] but not phospholipids [16, 17] are extensively hydrolyzed. This is due to a direct effect on the enzyme, sterol ester hydrolase [18], which is activated by cAMP-dependent protein kinase by a mechanism involving enzyme phosphorylation [19–21]. Thus, cholesterol arachidonate in rat adrenal cortex appears to be the major source of the arachidonate required for prostaglandin synthesis and is released during incubations of adrenal cells with ACTH [22]. Furthermore, prostaglandin E_2, specifically, is synthesized under these conditions [22].

The development of essential fatty acid (EFA) deficiency in rats results in a substantial loss of the arachidonate (and linoleate) content of the sterol esters of the rat adrenal cortex [15, 23]. This is replaced largely by cholesteryl esters of 20:3 *n*-9 and 22:3 *n*-9, neither of which are known prostaglandin precursors. Thus, the cholesteryl esters in the adrenal cortex from EFA-deficient rats are essentially devoid of the normal precursors for prostaglandin E and F synthesis.

It was therefore of interest to determine the influence of EFA deficiency on the subsequent response of adrenal cells to ACTH and PGE_2 on cyclic AMP

levels, sterol ester hydrolysis and corticosterone production in adrenal cortical cells prepared from rats with essential fatty acid deficiency and from control litter mates. The results demonstrate that PGE_2 can elicit an increase in cyclic AMP in both types of cells, but this is not accompanied by the marked steroidogenic response typically observed with ACTH. The major cellular response to PGE_2 is sterol ester hydrolysis, and this is equivalent to that seen after incubations with ACTH. Furthermore, hydrolysis of cholesteryl esters in cells from EFA-deficient rats in response to stimulation by ACTH or PGE_2 is not affected by the "unusual" composition of the fatty acids of the cholesteryl esters.

EXPERIMENTAL PROCEDURES

Materials. Trypsin (Type TLR, 180–250 units/mg), collagenase (Type I, CLS, 150–200 units/mg) and lima bean trypsin inhibitor were obtained from Worthington Biochemical Co. ACTH was purchased from the U.S. Pharmacopeia. Fatty acids, methyl esters and lipid standards were purchased from Applied Science Corp. and Supelco. Prostaglandins were kindly supplied by Upjohn Co., Kalamazoo, MI, through the courtesy of Dr John E. Pike. Reagents for determination of cyclic AMP were from Amersham–Searle.

Animals and diets. Weanling male albino rats (Wistar strain), weighing approximately 50 g, were obtained from Carworth Farms. Litters were divided into two groups, housed in individual cages and maintained on control or essential fatty acid deficient diets *ad libitum* for 16 weeks. The control animals received standard laboratory chow (Wayne Lab-blox). The pelleted experimental diet was obtained from Teklad Mills (Teklad Test Diet No. 170295, Madison, WI).

Preparation of adrenal cortical cells. The technique for preparation of rat adrenal cortical cells by collagenase dissociation of tissue minces was as described previously [17]. Each animal was sacrificed by decapitation with a minimum of handling and isolated from all other animals to be used in the study [24]. Cell counts were made using a hemocytometer and the preparations were diluted to give a suspension containing $1.25–1.50 \times 10^5$ cells/ml. Aliquots of the cell suspension (1.9 ml) were incubated in 10 ml Teflon vessels after addition of 0.1 ml Krebs–Ringer buffer, pH 7.4, or buffer containing the test substances. Prostaglandins were initially dissolved in ethanol. This was evaporated prior to suspending the compounds in the buffer. Incubations were carried out under 95% O_2–5% CO_2 for periods up to 2 h at 37°C with shaking.

Lipid extraction and analysis. For lipid analysis, aliquots (0.5 ml) of the incubation mixtures were extracted in 20 volumes of chloroform–methanol, according to the method of Folch *et al.* [25].

Prior to separation of the solvent phases of the Folch extract, 0.25 μCi [1-^{14}C]palmitic acid was added to correct for losses through the extraction, derivatization and chromatographic procedures. These have been described in detail previously [17, 26]. Major lipid classes were separated by thin layer silicic acid chromatography in a solvent system of hexane:ethyl ether:acetic acid (80:16:4 v/v), and were identified by comparison with authentic standards spotted on the same chromaplate. Areas corresponding to phospholipids, triglycerides, free fatty acids, and cholesteryl esters were individually scraped into screw-cap tubes. Triglycerides, free fatty acids and cholesteryl esters were extracted from the silicic acid with diethyl ether-methanol (3:1 v/v) according to Gartner and Vahouny [26]. These, together with the unextracted phospholipid fractions, were derivatized with boron trifluoride–methanol (14% by weight) for 2 h at 70°C according to the procedure of Morrison and Smith [27]. Under these conditions, all lipid fractions (including unextracted phospholipids) are quantitatively derivatized [26].

Fatty acid methyl esters were determined on a Beckman GC65 chromatograph equipped with a flame ionization detector, interfaced with a Hewlett–Packard 3380A integrator. Separations were accomplished on a 6 ft glass column (O.D. 4 mm × I.D. 2 mm) packed with 10% SP-2340 on 100/120 chromasorb WAW (Supelco). Column temperature was 180°C and carrier gas flow was 25 ml/min. Methyl heptadecanoate was used as internal standard for quantitation of fatty acids.

Other analyses. For corticosterone assays, 0.2 ml aliquots of the incubation mixture were extracted in 15 ml methylene chloride and corticosterone was determined fluorometrically using standards of authentic corticosterone [28]. Cyclic AMP was determined by the protein binding-displacement assay [29].

RESULTS

Diets and animals

The commercial pelletized diet deficient in essential fatty acids contained 5% fat, of which 90% was medium chain triglycerides containing predominantly octanoate and decanoate (98%). Animals maintained on this diet for 16 weeks had a mean total weight gain of 230 ± 12 g and exhibited dermatitis of the paws and tail. In contrast, litter mates on commercial rat chow gained 374 ± 7 g over the same period and were asymptomatic.

In separate studies using 10–15 rats in each group, it was determined that equivalent numbers of cortical cells were obtained by enzymatic dissociation of adrenals from both groups of animals ($1.0–1.25 \times 10^5$ cells/adrenal), and that these cells were indistinguishable when assessed by transmission electron microscopy [35].

Composition of adrenal cell lipids

The lipid content and fatty acid compositions of normal and EFA-deficient cells have been reported

Table 1. Content and composition of the fatty acids of cholesteryl esters and phospholipids of adrenal cortical cells.

Fatty acid[1]	Fatty acid composition μg/2.5 × 10^5 cells[2] Cholesteryl esters Normal	Cholesteryl esters EFA-deficient	Phospholipids Normal	Phospholipids EFA deficient
14:0	1.9 ± 0.08	1.1 ± 0.2	—	—
16:0	11.0 ± 0.6	8.7 ± 0.3	12.4 ± 0.7	6.9 ± 0.5
18:0	3.0 ± 0.2	3.5 ± 0.7	33.7 ± 1.8	20.7 ± 1.6
18:1	12.2 ± 0.5	15.4 ± 0.6	10.0 ± 0.6	23.2 ± 2.0
18:2	5.0 ± 0.08	0.5 ± 0.1	5.7 ± 0.2	—
18:3 *n*-3	—	2.7 ± 0.9	—	0.6 ± 0.2
18:3 *n*-6	3.1 ± 0.2	—	—	—
20:3 *n*-9	—	17.1 ± 0.9	—	30.1 ± 1.6
20:4 *n*-6	19.3 − 0.2	1.9 ± 0.5	28.2 ± 1.4	16.4 ± 2.6
22:3 *n*-9	—	21.3 ± 2.3	—	—
22:4 *n*-6	11.4 ± 0.3	—	1.8 ± 0.4	—
>22:4	18.5 ± 0.2	4.2 ± 0.2	—	—
Totals	86.4 ± 3.7	75.4 ± 3.5	97.3 ± 2.8	98.1 ± 1.9

[1] Fatty acids are designated by their carbon number, degree of unsaturation and position of the terminal double bond.

[2] Values represent means ± S.E.M. of 4 determinations in each group.

earlier [15]. In the present study, the content and composition of the cholesterol ester fatty acids (CEFA) and phospholipid fatty acids (PLFA) in both cell types were compared, and these data are summarized in Table 1. The major fatty acids of the cholesteryl esters in normal cortical cells included palmitate (16:0), oleate (18:1 *n*-9), arachidonate (20:4 *n*-6), adrenate (22:4 *n*-6) and fatty acids with greater degrees of polyunsaturation and chain length (22:5, 22:6, etc.). The major phospholipid fatty acids were stearate (18:0) and arachidonate with lower levels of palmitate and oleate.

In EFA-deficient cells, the essential fatty acids of the sterol esters, namely linoleate (18:3 *n*-6) and arachidonate, were almost completely absent. Also, adrenate and the more polyunsaturated analogues were also absent or markedly reduced in EFA-deficient cells. These were replaced by 18:3 *n*-3, 20:3 *n*-3 and 22:3 n-9 which comprised almost 70% of the CEFA in these cells. The major changes in PLFA due to EFA deficiency were significant reductions in the levels of palmitate, stearate and arachidonate, a marked increase in the content of oleate, and the appearance of 20:3 *n*-9 as a major (30%) component of PLFA. These CEFA and PLFA alterations induced by EFA-deficiency are comparable to those reported earlier [15, 23].

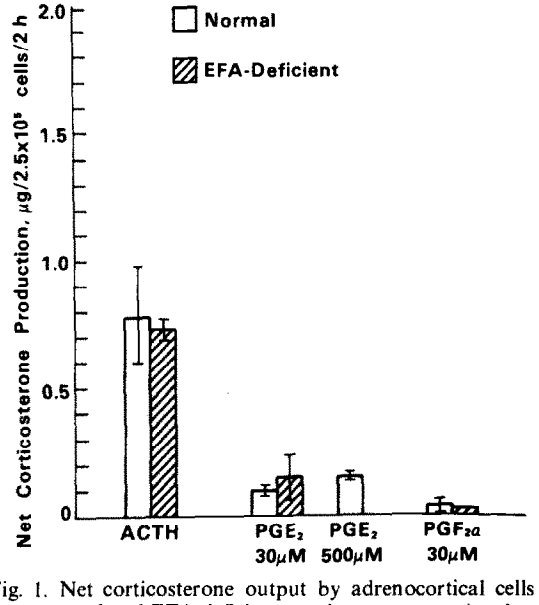

Fig. 1. Net corticosterone output by adrenocortical cells from control and EFA-deficient rats in response to incubation with ACTH, PGE_2 and $PGF_{2\alpha}$. Incubations were for 2 h at 37°C under 95% O_2–5% CO_2. Flasks contained 2 ml of cell suspension (2.5 × 10^5 cells) and ACTH (0.71 nM), PGE_2 (30 μM and 500 μM) or $PGF_{2\alpha}$ (30 μM). The results are expressed as net corticosterone output over control (2 h incubations with no addition) ± S.E.M.

Steroidogenesis and cyclic AMP levels in EFA-deficient adrenal cells

The comparative effect of ACTH and prostaglandins on net corticosterone production (120 min) by 2.5 × 10^5 adrenal cortical cells from normal and EFA-deficient rats is shown in Fig. 1. It is readily apparent that both types of cells exhibited a comparable steroidogenic response to ACTH (0.71 nM). With normal cells, the steroidogenic response to the low level of PGE_2 (30 μM), was about 13% of that obtained with ACTH. With a higher level of PGE_2 (500 μM), corticosterone production by normal cells was still only one-fifth of that observed with ACTH-stimulated cells. EFA-deficient cells also did not show a significant response to 30 μM PGE_2. Corticosterone production was equivalent to that in normal cells exposed to PGE_2 and was only about 21% of that obtained with ACTH. Prostaglandin $F_{2\alpha}$ (30 μM) did not induce a steroidogenic response in adrenal cells from either type of animal.

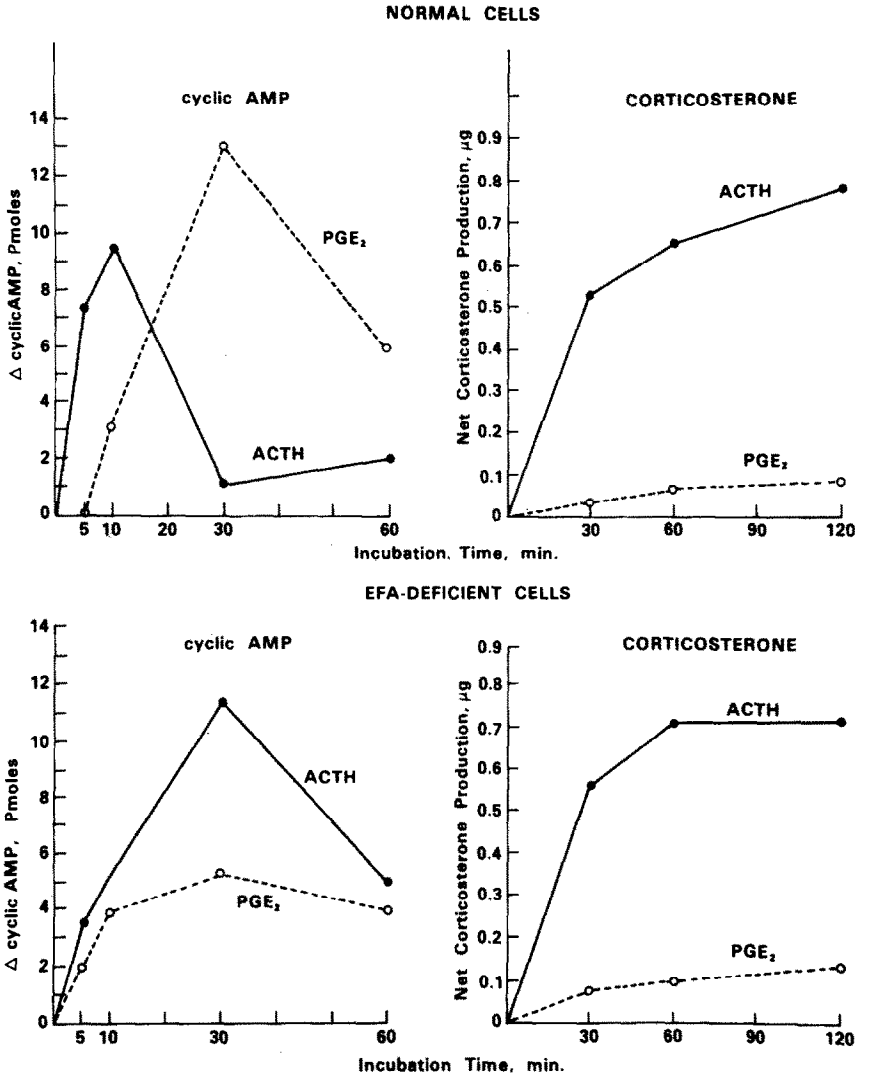

Fig. 2. The effect of ACTH (0.71 nM) and PGE_2 (30 μM) on cyclic AMP levels and corticosterone production by 2.5×10^5 adrenocortical cells from normal and EFA-deficient rats. The results are expressed as net changes over levels in control incubations with no additions.

Temporal changes in cellular cyclic AMP levels and corticosterone production were determined in two separate studies and are shown in Figs. 2A and 2B. Incubation of normal adrenal cells with ACTH resulted in a rapid and transient increase in cellular cyclic AMP levels which peaked at 10 min of incubation (Fig. 2A). Corticosterone production by these cells was maximal during the first 30 min of incubation. Incubations with PGE_2 also resulted in a marked increase in cyclic AMP levels, but only after a 5 min lag period. These levels attained a maximum after 30 min and were 138% of the maximal levels obtained with ACTH. Despite these high cyclic AMP levels, there was only a minor steroidogenic response to PGE_2 (see also Fig. 1).

Cyclic AMP and corticosterone production by EFA-deficient cells is shown in Fig. 2B. Incubations with ACTH produced a delayed but more pronounced increase in cellular cyclic AMP than was observed with normal cells. Moreover, both the rate and extent of corticosterone production under these conditions were comparable to that by normal cells incubated with ACTH. Prostaglandin E_2 also produced a slow increase in cyclic AMP which, in these cells, did not attain a distinct maximum. At 30 min, this level was only 47% of that observed with ACTH, and was only 39% of the cyclic AMP levels observed in normal cells incubated with PGE_2. As with normal cells, PGE_2 had only a minor steroidogenic effect in EFA-deficient cells.

Effects of ACTH and PGE_2 on adrenal cholesteryl esters

Changes in the levels of cholesteryl esters and phospholipids in normal and EFA-deficient adrenal cells under various incubation conditions are summarized in Fig. 3. With either type of cell preparation, incubation for 2 h at 37°C in the absence of any stimuli

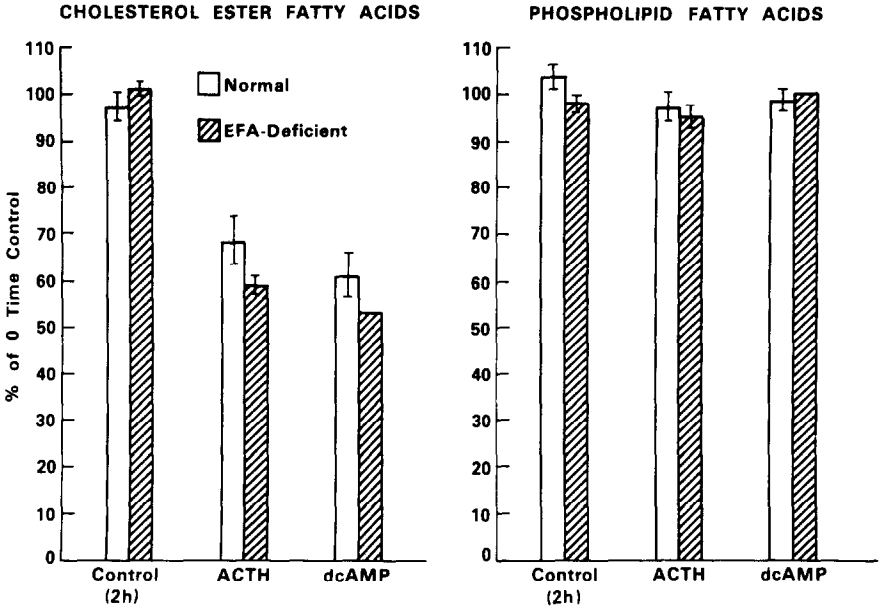

Fig. 3. The effect of ACTH (0.71 nM) and PGE_2 (30 μM) on the levels of cholesteryl esters and phospholipids in adrenocortical cells from control and EFA-deficient rats. Incubations (2.5×10^5 cells) were for 2 h at 37°C under 95% O_2–5% CO_2. The results are expressed as percentages of preincubation levels ± S.E.M.

did not alter the preincubation levels of CEFA and PLFA. As we have reported earlier [17], this is the case only when the treatment and sacrifice of animals is carried out with extreme care to avoid "pre-stimulation" of the gland [24]. Also, as has been shown previously [17], incubation of adrenocortical cells from control rats with ACTH resulted in a significant and reproducible hydrolysis (31–39%) of sterol esters without affecting tissue PLFA levels. As seen in Fig. 3, this was also the case when adrenal cells from EFA-deficient rats were incubated with ACTH (41–48% hydrolysis of cholesteryl esters). Of considerable interest was the finding that incubation with PGE_2 (30 μM) alone resulted in highly significant depletion of sterol esters in both types of cells, and that these levels of hydrolysis were comparable to those observed with ACTH. Also, as in the case of ACTH incubation, adrenal PLFA were not significantly altered during incubations with PGE_2.

The comparative effects of ACTH and PGE_2 on the esterified cholesterol level and fatty acid composition of the cholesteryl esters in normal adrenocortical cells is presented in Table 2. Both substances had similar effects on hydrolysis of individual cholesteryl esters of the cells. Thus, the majority of the total CEFA depletion resulting from either ACTH or PGE_2 incubations with normal cells (26.5 and 27.6 μg, respectively) was accounted for by hydrolysis of the arachidonate, oleate, linoleate, adrenate and palmitate esters of cholesterol.

Comparative data on the overall effects of ACTH and PGE_2 on the cholesteryl esters of EFA-deficient cells are summarized in Table 3. Again, incubations with either substance resulted in extensive hydrolysis of sterol esters (31.3 and 35.4 μg, respectively), despite the unusual CEFA composition. Under these conditions, about 40% of the net decrease was accounted for by the hydrolysis of the 22:3 *n*-9 ester, and an additional 25–29% was due to hydrolysis of the oleate and 20:3 *n*-9 esters of cholesterol.

DISCUSSION

In the present study, rats were fed an essential fatty acid-deficient diet for a period sufficient (16 weeks)

Table 2. Effect of ACTH and PGE_2 on the total and individual fatty acids of cholesterol esters in adrenal cortical cells from normal rats

Measurement[1]	Incubations, 2 h Control	ACTH	PGE_2
Cholesterol ester fatty acids			
Total, μg	85.7 ±3.3	59.2 ±4.4	58.1 ±8.6
Individual, μg			
14:0	2.1	1.1	1.1
16:0	8.1	5.8	5.4
18:0	4.1	4.1	4.2
18:1	12.7	8.9	8.2
18:2	5.7	3.9	2.5
18:3	1.9	—	—
20:3	6.7	2.1	3.7
20:4	21.5	15.0	13.7
22:4	14.9	11.7	12.4
>22:4	8.0	6.6	6.8

[1] All determinations are expressed in terms of means ± SEM for 4 studies. Cells (2.5×10^5) were incubated for 2 hours under 95% O_2–5% CO_2 in the absence and presence of 0.71 nM ACTH and 30 μM PGE_2.

Table 3. Effect of ACTH and PGE_2 on the total and individual fatty acids of cholesterol esters in adrenal cortical cells from EFA-deficient rats

Measurement[1]	Incubations, 2 h Control	ACTH	PGE_2
Cholesterol ester fatty acids			
Total, μg	75.4 ±3.5	44.1 ±2.3	40.0 ±4.5
Individual, μg			
14:0	1.4	1.7	1.2
16:0	8.9	4.8	4.8
18:0	3.7	1.8	—
18:1	15.5	11.7	10.8
18:3 *n*-3	3.6	2.2	2.0
20:3 *n*-9	15.3	10.9	9.7
22:3 *n*-9	22.8	9.9	10.4
>22:3	4.1	2.6	2.0

[1] All determinations are expressed in terms of means ± S.E.M. for 4 studies. Cells (2.5×10^5) were incubated for 2 h under 95% O_2-5% CO_2 in the absence and presence of 0.71 nM ACTH and 30 μM PGE_2.

to extensively deplete adrenal cholesteryl esters of their linolenate and arachidonate contents. Under these conditions, cellular phospholipids retained over half of their normal content of arachidonate. Moreover, as shown in the present study and earlier ones [17, 22], phospholipids are not hydrolyzed during ACTH stimulation of adrenocortical cells. Thus, it appears that adrenal phospholipids are not a significant source of the fatty acid substrates for prostaglandin synthesis in adrenal cortex. Thus, adrenocortical cells from EFA-deficient rats, in which the linolenate and arachidonate esters of cholesterol are either absent or markedly reduced, may be a unique model for studies on the role of endogenous prostaglandin synthesis in ACTH-induced steroidogenesis. With these cells, the need to employ prostaglandin synthetase inhibitors or prostaglandin antagonists might be circumvented.

An important finding in these studies is that EFA-deficiency, at least to the extent developed in these rats, does not alter the overall functional responsiveness of adrenal cells to a single dose of ACTH. Although there were temporal differences in the rates of cyclic AMP production, cells from EFA-deficient adrenals were comparable to adrenal cells from normal animals with respect to both corticosterone production and hydrolysis of sterol esters. It would appear, therefore, that EFA-deficiency does not markedly alter either cell membrane receptors for ACTH or the subsequent steps leading to corticosterone output. Also, it appears that prostaglandin synthesis from arachidonate (or γ-homolinolenate) is not obligatory for the short-term steroidogenic response of adrenal cells to ACTH (see also Refs 5, 10, 30). While it is possible that the 20:3 *n*-9 in EFA-deficient cells may represent a potential substrate for prostaglandin synthetase, and result in formation of a "pseudo-prostaglandin", there is no evidence to date that this can occur.

The present study on the effects of exogenous PGE_2 on the two types of rat adrenocortical cells clearly demonstrates that the prostaglandins stimulate adenylate cyclase activity by a mechanism independent of the presence of ACTH. In normal cells, the rate of cyclic AMP production was slower than that observed after the ACTH challenge, suggesting either a separate membrane receptor for PGE_2 [31] or a difference in the rate or mechanism of adenylate cyclase activation. However, while PGE_2 produced elevations of cellular cyclic AMP levels, the net output of corticosterone by both the normal and EFA-deficient adrenal cells was low compared to that observed with ACTH.

Previous studies [7–11] on the "direct" effects of exogenous prostaglandins on adrenal steroidogenesis *in vitro* have been inconsistent. This may be due, in part, to the high concentrations of exogenous prostaglandins required to elicit a response [10] or to a possible requirement of pre-bound ACTH in order to demonstrate this "direct" steroidogenic response to added prostaglandins [5]. It is generally recognized [5] that techniques of animal care and sacrifice can markedly alter subsequent responses of adrenal tissue preparations. In our laboratory, it has been repeatedly observed that the depletion of cellular sterol ester levels during 2 h control incubations is a sensitive indicator of factitious pre-activation of adrenal cells. This can occur even in the absence of corticosterone release, and is the likely explanation of the low levels of cholesteryl esters reported by others [32, 33] in rat adrenal cell preparations. In the present studies, the levels of cholesteryl esters (Tables 2 and 3) in the isolated cells were comparable to those in intact rat adrenal [14] and were not altered during control incubations at 37°C (Fig. 3); this suggests that there was no pre-activation of the hydrolase and no pre-bound ACTH. Our data show that, under these conditions, a major cellular response to exogenous PGE_2, but not $PGF_{2\alpha}$, is extensive hydrolysis of adrenal sterol esters. This response was observed in both normal and EFA-deficient cells, was equivalent to the stimulation of cholesteryl ester hydrolysis by ACTH, and produced a similar effect on individual cholesteryl esters to that obtained with ACTH.

Thus, the overall effects of exogenous PGE_2 on adrenal cortical cells from both normal and EFA-deficient rats are increased cellular cyclic AMP levels, extensive hydrolysis of cholesteryl esters, and negligible steroidogenesis. It is unlikely that the lack of a marked steroidogenic response to PGE_2 is related to the rate of cyclic AMP production. Under these conditions, cyclic AMP levels peaked after 30 min of incubation. This conclusion is based on the data obtained during incubations of EFA-deficient cells with ACTH; this study demonstrated that there was a "delayed" peak for cyclic AMP levels in response to ACTH, but despite this, corticosterone production

in these cells was comparable to that in cells from control animals. There are several possible explanations for these results. The cyclic AMP levels induced by ACTH and PGE_2 challenges may be responses to separate receptors [31] and are elevated in unique cellular compartments [4]. It is also possible that the total pathway from cholesterol ester to steroid hormone output involves one or more additional factors, in addition to cyclic AMP (i.e. protein synthesis), and that these are not affected by PGE_2 alone. Finally, it has been suggested that the production of cyclic AMP is not *sine qua non* for the steroidogenic action of ACTH [4]. Perchellet *et al.* [34] have recently reported that submaximal and supramaximal concentrations of ACTH did not cause detectable changes in cyclic AMP levels of isolated adrenal cells during the first 30 min, whereas there was an increase in cyclic GMP levels and an accompanying increase in protein phosphorylation and steroidogenesis. Thus, it appears possible that cyclic GMP may be the physiological mediator of increased steroidogenesis from available free cholesterol [34], while cyclic AMP represents the "second messenger" for the relatively slow activation of adrenal sterol ester hydrolase [20]. This latter response is responsible for continued availability of free cholesterol from the extensive stores of esterified cholesterol in adrenal cortex.

The finding that PGE_2 induces extensive hydrolysis of cholesteryl esters in the absence of a marked steroidogenic response clearly demonstrates that activation of the protein kinase-dependent hydrolase is under independent hormonal control (i.e. not a result of increased steroidogenesis), as has been suggested previously [18–21]. Furthermore, the very low levels of corticosterone output by both types of cells exposed to PGE_2 probably represents an indirect effect, resulting from increased levels of free cholesterol within the cell.

Overall, PGE_2 generally mimics the responses of adrenocortical cells to ACTH with respect to cyclic AMP levels and activation of sterol ester hydrolase. PGE_2 does not, however, elicit the significant levels of corticogenesis typically seen with ACTH. This difference should provide a unique opportunity to determine additional requirements for the utilization of the free cholesterol, produced by hydrolysis of cholesteryl esters in lipid droplets, by the mitochondria for pregnenolone production.

REFERENCES

1. Flack J. D.: The hypothalamus-pituitary-endocrine system. In *The Prostaglandins* (Edited by Ramwell P. W.), pp. 327–345 (1973). Plenum Press, New York.
2. Shaw J. E. and Tillson S. A.: Interactions between the prostaglandins and steroid hormones. In *Advances in Steroid Biochemistry and Pharmacology*, Vol. 4 (Edited by Briggs M. H. and Christie G. A.), pp. 189–207 (1974). Academic Press, London.
3. Batta S. K.: Effect of prostaglandins on steroid biosynthesis. *J. Steroid Biochem.* **6** (1975) 1075–1080.
4. Halkerson I. D.: Cyclic AMP and adrenocortical function. In *Advances in Cyclic Nucleotide Research*, Vol. 6 (Edited by Greengard P. and Robison G. A.), pp. 99–136 (1975). Raven Press, New York.
5. Gallant S. and Brownie A. C.: The *in vivo* effect of indomethacin and prostaglandin E_2 on ACTH and DBcAMP-induced steroidogenesis in hypophysectomized rats. *Biochim. Biophys. Res. Commun.* **55** (1973) 831–836.
6. Laychock S. G. and Rubin R. P.: Indomethacin-induced alterations in corticosteroid and prostaglandin release by isolated adrenocortical cells of the cat. *Br. J. Pharmac.* **57** (1976) 273–278.
7. Honn K. V. and Chavin W.: Prostaglandin modulation of the mechanism of ACTH action in the human adrenal *Biochem. Biophys. Res. Commun.* **73** (1976) 164–170.
8. Flack J. D., Jessup R. and Ramwell P. W.: Prostaglandin stimulation of rat corticosteroidogenesis. *Science* **163** (1969) 691–692.
9. Saruta T. and Kaplan N. M.: Adrenocortical steroidogenesis: The effects of prostaglandins. *J. clin. Invest.* **51** (1969) 2246–2251.
10. Warner W. and Rubin R. P.: Evidence for a possible prostaglandin link in ACTH-induced steroidogenesis. *Prostaglandins* **9** (1969) 83–95.
11. Blair-West J. R., Coghlan J. P., Denton D. A., Funder J. W., Scoggins B. A. and Wright R. P.: Effects of prostaglandin E_1 on the steroid secretion of the adrenal of the sodium deficient sheep. *Endocrinology* **88** (1971) 367–371.
12. Lowry P. J., McMartin C. and Peters J.: Properties of a simplified bioassay for adrenocorticotrophic activity using the steroidogenic response of isolated adrenal cells. *J. Endocrinology* **59** (1973) 43–55.
13. Peng T. C., Six K. M. and Munson P. L.: Effects of prostaglandin E_1 on the hypothalamo-hypophyseal-adrenocortical axis in rats. *Endocrinology* **86** (1970) 202–206.
14. Goodman D. S.: Cholesterol ester metabolism. *Physiol. Rev.* **45** (1965) 747–839.
15. Walker B. L.: The fatty acids of adrenal lipids from essential fatty acid-deficient rat. *J. Nutr.* **100** (1970) 355–360.
16. Riley C.: Lipids of human adrenals. *Biochem. J.* **87** (1963) 500–507.
17. Vahouny G. V., Chanderbhan R., Hinds R. W., Hodges V. A. and Treadwell C. R.: ACTH-induced hydrolysis of cholesterol esters in rat adrenal cells. *J. Lipid Res.* **19** (1978) 44–51.
18. Behrman H. R. and Greep R. O.: Hormonal dependence of cholesterol ester hydrolase in the corpus luteum and adrenal. *Horm. Met. Res.* **4**, (1972) 206–209.
19. Naghshineh S., Treadwell C. R., Gallo L. and Vahouny G. V.: Activation of adrenal sterol ester hydrolase by dibutyryl cAMP and protein kinase. *Biochem. Biophys. Res. Commun.* **61** (1974) 1076–1082.
20. Beckett G. J. and Boyd G. S.: Purification and control of bovine adrenal cortical cholesteryl ester hydrolase and evidence for the activation of the enzyme by phosphorylation. *Eur. J. Biochem.* **72** (1977) 223–233.
21. Naghshineh S., Treadwell C. R., Gallo L. L. and Vahouny G. V.: Protein kinase-mediated phosphorylation of a purified sterol ester hydrolase from bovine adrenal cortex. *J. Lipid Res.* **19** (1978) 35–43.
22. Chanderbhan R., Treadwell C. R., Hodges V. A. and Vahouny G. V.: ACTH-induced synthesis of prostaglandin E_2 in adrenal cortical cells. *Fed. Proc.* **36** (1977) 674.
23. Gidez L. I.: Occurrence of a docosatrienoic acid in the cholesterol esters of adrenals of rats on essential fatty acid deficient diets. *Biochem. Biophys. Res. Commun.* **14** (1964) 413–418.

24. Bergon L., Gallent S. and Brownie A. C.: Cholesterol side-chain cleavage activity and levels of high-spin cytochrome P-450 in adrenal regeneration hypertension. *Endocrinology* **94** (1974) 336–345.
25. Folch J., Lees M. and Stanley G. H. S.: A simple method for isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226** (1957) 497–509.
26. Gartner S. L. and Vahouny G. V.: Effects of epinephrine and cyclic 3',5'-AMP on perfused rat hearts. *Am. J. Physiol.* **222** (1972) 1121–1124.
27. Morrison W. R. and Smith L. M.: Preparation of methyl esters and dimethylacetals from lipids with boron trifluoride. *J. Lipid Res.* **5** (1964) 600–608.
28. Silber R. H., Busch R. D. and Oslapes R.: Practical procedure for estimation of corticosterone or hydrocortisone. *Clin. Chem.* **4** (1958) 278–285.
29. Steiner A. L., Kipnes D. M., Utiger R. and Parker C.: Radioimmunoassay for the measurement of adenosine 3',5'-cyclic phosphate. *Proc. Natn. Acad. Sci. U.S.A.* **64** (1969) 367–373.
30. Laychock S. G. and Rubin R. P.: Regulation of steroidogenesis and prostaglandin formation in isolated adrenocortical cells. The effects of pregnenolone and cyclohexamide. *J. Steroid Biochem.* **8** (1977) 663–667.
31. Dazord A., Morera A. M., Bertrand J. and Saez J. M.: Prostaglandin receptors in human and ovine adrenal glands. Binding and stimulation of adenyl cyclase in subcellular preparations. *Endocrinology* **95** (1974) 352–359.
32. Sharma R. K., Hashimoto K. and Kitabchi A.: Steroidogenesis in isolated adrenal cells of rat. III. Morphological and biochemical correlation of cholesterol and cholesterol ester content in ACTH and N^6-2'-O-dibutyryladenosine-3',5'-monophosphate activated adrenal cells. *Endocrinology* **91** (1972) 994–1003.
33. Brecher P., Braga-Illa F. and Chobanian A. V.: The metabolism of cholesterol esters in rat adrenal suspension. *Endocrinology* **95** (1973) 1163–1172.
34. Perchellet J., Shanker G. and Sharma R. K.: Regulatory role of guanosine 3',5'-monophosphate in adrenocorticotropin hormone-induced steroidogenesis. *Science* **199** (1978) 311–312.
35. Vahouny G. V., Hodges V. A. and Treadwell C. R.: Essential fatty acid deficiency and adrenal cortical function *in vitro*. *J. Lipid Res.* In press.