Elevated Levels of Protein Kinase C in Y1 Cells Which Express Apolipoprotein E Decrease Basal Steroidogenesis by Inhibiting Expression of P450-Cholesterol Side Chain Cleavage mRNA

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We have previously reported that steroidogenesis is dramatically reduced in mouse Y1 adrenocortical cells which express the human apolipoprotein E gene (Y1-E cells). This suppression results in part from inhibition of cAMP-mediated events. In this report we have examined the expression of protein kinase C (PKC) in the Y1-E cell lines. Total cellular PKC activity in vitro is increased 3–5-fold in the Y1-E cell lines. PKC activity in the particulate and cytosolic fractions is increased to the same relative extent. Increased PKC activity reflects increased levels of PKC mRNA, as determined by Northern blot analysis, and PKC protein, as determined by immunoblot analysis. Increased expression of PKC in the Y1-E cell lines is accompanied by a 2–3-fold increase in diacylglycerol, an in vitro activator of PKC. To determine the contribution of elevated PKC expression to the Y1-E cell phenotype, we utilized the PKC inhibitors, staurosporine and calphostin C. Upon treatment with staurosporine or calphostin C, expression of P450-cholesterol side chain cleavage mRNA is increased severalfold to a level equal to, or greater than, basal expression in the Y1-neo control cell line. Treatment with calphostin C also results in recovery of steroidogenesis in the Y1-E cells to a level comparable to the basal level observed in the Y1-neo control cell line. These results indicate that increased expression of PKC in the Y1-E cell lines decreases basal steroidogenesis by suppressing P450-cholesterol side chain cleavage mRNA expression. Inhibition of PKC, however, does not reverse the block in cAMP-stimulated steroidogenesis in Y1-E cells, suggesting that the pleiotropic effects of apoE expression are not mediated entirely through altered PKC expression.

Steroidogenic tissues represent a significant site of extrahepatic expression of apolipoprotein E. In human and monkey adrenal tissue, apolipoprotein E (apoE)1 is synthesized at a relative rate equal to, or greater than, that observed in the liver (1, 2). In the rat adrenal gland, apoE mRNA is expressed in a cell-type specific manner, being most abundant in cell types which synthesize glucocorticoids (3). The function of apoE in steroidogenic and other peripheral tissues is unclear. ApoE may facilitate local redistribution of cholesterol among cells within a tissue, or play a role in intracellular cholesterol metabolism (1, 2). ApoE may also have autocrine or paracrine functions that are independent of its role in systemic lipid transport, as indicated by the ability of exogenous apoE to modify cell-specific responses in lymphocytes and ovarian theca cells (4, 5).

Adrenocortical cells utilize cholesterol for steroid hormone biosynthesis. In vivo studies suggest a positive correlation between apoE expression and adrenal cholesterol content, while apoE expression is inversely related to adrenal steroidogenesis (6). Steroid hormone production in the adrenal gland is regulated primarily by adrenocorticotropin (ACTH) via the second messenger, cAMP. cAMP activation of cAMP-dependent protein kinase (PKA) results in both acute and chronic effects on steroid hormone synthesis. Acute effects are characterized by changes in cell shape, and mobilization and transport of cholesterol from storage sites to the inner mitochondrial membrane, the site of cholesterol side chain cleavage (7), while chronic effects include maintenance of steroid hydroxylase gene expression (8). In addition to cAMP-mediated regulation, substantial evidence suggests a role for protein kinase C (PKC) in the regulation of steroid hydroxylase gene expression and adrenal steroidogenesis. In human and bovine adrenal cells, long term treatment with phorbol 12-O-tetradecanoylphorbol-13-acetate (TPA) suppresses cAMP-stimulated steroidogenesis (9–12). In Y1 cells, TPA decreased forskolin-induced accumulation of P450-cholesterol side chain cleavage (P450-SCC) mRNA and suppressed basal expression of P450-SCC mRNA (13).

We have previously reported that expression of the human apoE gene in mouse Y1 adrenocortical cells (Y1-E cells) results in a dramatic suppression of steroidogenesis (14). This suppression is due in part to decreased expression of the rate-limiting enzyme for steroidogenesis, P450-SCC (15). Since PKC may also play a role in regulating P450-SCC mRNA expression and steroidogenesis, we asked whether PKC activity and/or expression are altered in Y1 cells that express apoE. We report here that expression of PKC is increased up to 5-fold in the Y1-E cell lines which are inhibited in steroidogenesis. This increase in PKC expression is accompanied by a 2–3-fold increase in sn-1,2-diacylglycerol (DAG) mass. We provide evidence that increased expression of PKC is responsible for decreased expression of P450-SCC mRNA and suppression of basal steroidogenesis in the Y1-E cell lines.

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1 The abbreviations used are: apoE, apolipoprotein E; ACTH, adrenocorticotropic; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate; P450-SCC, P450-cholesterol side chain cleavage; DAG, sn-1,2-diacylglycerol; PBS, phosphate-buffered saline; EGTA, (ethylenebis(oxyethyl)tetraacetic acid.

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MATERIALS AND METHODS

Cell Culture—The Y1 adrenal cell line was obtained from the American Type Tissue Culture. The human apoE-transfected subclones (Y1-E cell lines) and the Y1-neo cell line (a control cell line transfected only with the neomycin resistance gene) have been previously described (14). Cells were maintained in Ham’s F-10 medium supplemented with penicillin (100 units/ml), streptomycin (100 ,ug/ml), 10% fetal calf serum, 12.5% (v/v) heat-inactivated horse serum, and 2.5% (v/v) heat-inactivated fetal calf serum. Stock cultures of the Y1-E cell lines were maintained in 100 ,ug/ml (active form) G418 sulfate (geneticin, Gibco). Ham’s F-10 and dibutylryl cyclic AMP were obtained from Sigma, staurosporine was from Calbiochem, and calphostin C was from Kamiya Biocchemical Co. Experiments using calphostin C were done under a fluorescent lamp. All additional cell culture reagents were obtained from Gibco.

PKC Activity in Cell Homogenates—PKC activity was measured in DEAE-purified cell homogenates as described by Yasuda et al. (16). Confluent cell monolayers (T-75 flask) were washed three times with cold phosphate-buffered saline (PBS; 11 mM KH2PO4, 27 mM KCl, 139 mM NaCl), scraped into cold buffer A (20 mM Tris, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, 25 ,ug/ml each aprotinin and leupeptin), and homogenized on ice with 15 strokes of a Dounce homogenizer. Homogenates were incubated on ice for 30 min before being pelleted at 100,000 X g for 20 min. The pellet was resuspended in 1 ml of buffer A containing Triton X-100, incubated on ice for 30 min, and cell debris was pelleted. PKC was partially purified from homogenates, and these fractions and the parent Y1 clonal cell lines were analyzed by two-dimensional gel electrophoresis (19). PKC activity was measured on a 15% (w/v) agarose gel containing 2.2 M formaldehyde, transferred to a nitrocellulose membrane (Schleicher and Schuell). Blots were saturated with 3% nonfat dry milk in PBS for 1 h at room temperature and then incubated with mouse anti-rabbit type I11 PKC monoclonal antibody (UBI 05-154; 1 pg/ml) in PBS containing 3% nonfat dry milk, 0.5 mM EDTA, 0.5 mM EGTA, 20 indoors (as Triton X-100 mixed micelles (17)). Reactions were initiated with the same samples used for enzymatic assays of PKC activity. PKC activity in the Y1-E cell lines was assayed in a total volume of 50 ,ul containing 20 mM Tris, pH 7.5, 20 mM MgCl2, 1 mM CaCl2, 20 ,um (1.25P)ATP (2000 cpm/pmol), 20 ,um acylated myelin basic protein substrate (Bethesda Research Laboratories, 1 mM TTP, and 2.8 mg/ml phosphatidyserine (as Triton X-100 mixed micelles (17)). PKC was eluted in 4 ml of cold buffer C (20 mM Tris, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM q-mepcaptoethanol, 0.2 M NaCl). PKC activity was assayed in a total volume of 50 ,ul containing 20 mM Tris, pH 7.5, 20 mM MgCl2, 1 mM CaCl2, 20 ,um (1.25P)ATP (2000 cpm/pmol), 20 ,um acylated myelin basic protein substrate (Bethesda Research Laboratories, 1 mM TTP, and 2.8 mg/ml phosphatidyserine (as Triton X-100 mixed micelles (17)). PKC activity was assayed on nitrocellulose filters (Schleicher and Schuell), cross-linked with ultraviolet light (as Triton X-100 mixed micelles (17)). PKC activity was measured using DAG kinase (21). Following the DAG kinase reaction, lipids were separated by thin layer chromatography (21) and radioactivity incorporated into phosphatidic acid was quantified by scintillation spectrometry. The mass of DAG in each sample was calculated by reference to a standard curve made from authentic sn-1,2-diacylglycerol (Avanti Lipids). DAG mass measurements were normalized to total cell protein as determined by the method of Lowry (22).

Miscellaneous—Fluorogenic steroids were measured as described (23) using a modification of the method of Kowal and Fielder (24). RNA was prepared by the guanidine isothiocyanate, ethanol precipitation method (25).

RESULTS

PKC Expression Is Increased in the Y1-E Cell Lines—To study the relationship between apoE expression and adrenal steroidogenesis, we have created a series of stable Y1 clonal cell lines which express the human apoE gene (14). These cell lines, which we refer to as Y1-E cells, express a range of human apoE mRNA (1-120 pg of apoE/ug of total RNA) comparable to that observed in the rat adrenal gland in vivo (3, 6). As we have previously reported, both basal and cAMP-induced steroidogenesis are suppressed in the Y1-E cell lines (14). Basal steroidogenesis is reduced up to 20-fold, while cAMP-induced steroidogenesis is reduced greater than 100-fold in some cell lines. This suppression results, at least in part, from inhibition of cAMP-regulated gene expression (15). This includes loss of acute responses to cAMP such as morphologic changes and the acute increase in steroidogenesis, as well as chronic responses such as maintenance of P450-steroid hydroxylase gene expression. Expression of P450-SCC mRNA, the rate-limiting enzyme for steroid synthesis, is decreased 6-11-fold in the Y1-E cell lines, while expression of P450-11-hydroxylase mRNA is reduced below the level of detection. The reduction in expression of P450-SCC mRNA in the Y1-E cell lines is of a magnitude comparable to the reduction observed in basal steroidogenesis. Studies from bovine and human adrenal cells, as well as from Y1 cells, suggest that in addition to cAMP-dependent regulation, PKC may also play an important role in regulating steroid hydroxylase gene expression. To determine whether PKC expression is altered in the Y1-E cell lines, we analyzed PKC activity in vitro and PKC protein and mRNA expression. PKC activity was measured in total cell homogenates from five Y1-E cell lines, the parental Y1 cell line, the Y1-neo control cell line, and four clonal Y1-neo cell lines. As seen in Table I, PKC activity is increased in the Y1-E cell lines.

| Table I |
| PKC activity in the Y1-E cell lines |
| Cell line | pmol/mg/min | Relative activity |
| Y1-neo | 64 ± 12* | 1.0 |
| Y1-E1 | 73 ± 10 | 1.1 |
| Y1-E2 | 187 ± 14 | 2.9 |
| Y1-E3 | 309 ± 77 | 4.3 |
| Y1-E10 | 298 ± 26* | 3.3 |
| Y1-E12 | 272 ± 39 | 4.3 |
| Y1-E15 | 42 ± 13* | 0.7 |

PKC activity was assayed on nitrocellulose filters (Schleicher and Schuell), cross-linked with ultraviolet light (as Triton X-100 mixed micelles (17)). PKC activity was measured on a 15% (w/v) agarose gel containing 2.2 M formaldehyde, transferred to a nitrocellulose membrane (Schleicher and Schuell). Blots were saturated with 3% nonfat dry milk in PBS for 1 h at room temperature and then incubated with mouse anti-rabbit type I11 PKC monoclonal antibody (UBI 05-154; 1 pg/ml) in PBS containing 3% nonfat dry milk, 0.5 mM EDTA, 0.5 mM EGTA, and 20 indoors (as Triton X-100 mixed micelles (17)). Reactions were initiated with the same samples used for enzymatic assays of PKC activity. PKC activity in the Y1-E cell lines was assayed in a total volume of 50 ,ul containing 20 mM Tris, pH 7.5, 20 mM MgCl2, 1 mM CaCl2, 20 ,um (1.25P)ATP (2000 cpm/pmol), 20 ,um acylated myelin basic protein substrate (Bethesda Research Laboratories, 1 mM TTP, and 2.8 mg/ml phosphatidyserine (as Triton X-100 mixed micelles (17)). PKC activity was measured using DAG kinase (21). Following the DAG kinase reaction, lipids were separated by thin layer chromatography (21) and radioactivity incorporated into phosphatidic acid was quantified by scintillation spectrometry. The mass of DAG in each sample was calculated by reference to a standard curve made from authentic sn-1,2-diacylglycerol (Avanti Lipids). DAG mass measurements were normalized to total cell protein as determined by the method of Lowry (22).

Miscellaneous—Fluorogenic steroids were measured as described (23) using a modification of the method of Kowal and Fielder (24). RNA was prepared by the guanidine isothiocyanate, ethanol precipitation method (25).
activity in vitro is increased up to 5-fold in the Y1-E cell lines compared to Y1 or Y1-neo cells. PKC activity in the four Y1-neo clonal cell lines was comparable to that observed in the Y1-neo control cell line and Y1-parent cells (data not shown). Increased PKC activity was observed in all the Y1-E cell lines examined, with the exception of the Y1-E15 cell line, which expresses the lowest level of human apoE mRNA (<1 pg/µg total RNA) and is the least inhibited in steroidogenesis (basal steroidogenesis is 80% of control level) (15). In vivo activation of PKC involves translocation of the cytosolic protein to the membrane, where the intracellular pool of activated PKC is thought to be located (26). To determine the subcellular distribution of PKC in the Y1-E cell lines, PKC activity was measured in particulate (membrane) and cytosolic fractions from Y1-E cell homogenates. As seen in Table II, PKC activity in the Y1-E cell lines is increased to a similar extent in both the particulate and cytosolic fractions. In the Y1-E cell lines, 84–86% of the total PKC activity is in the cytosolic fraction, comparable to the 89% observed in Y1-neo cells. Although only 9–16% of the PKC activity is associated with the particulate fraction in both the Y1-E cells and Y1-neo cells, in the Y1-E cell lines, the absolute amount of PKC activity in the particulate fraction is increased about 5-fold. These data suggest that the Y1-E cell lines may contain up to 5-fold more activated PKC than either the Y1 or Y1-neo cell lines.

In vitro assays of PKC activity are done in the presence of TPA and phosphatidylserine, conditions which should result in maximal activation of the kinase. Therefore, an increase in PKC activity in vitro should be accompanied by an increase in the expression of the kinase protein. To determine if the abundance of PKC protein is increased in the Y1-E cell lines, we analyzed PKC protein levels by immunoblot analysis utilizing a monoclonal antibody specific for the catalytic subunit of the PKCα. PKCα is the only isof orm expressed in Y1 cells (27). As seen in Fig. 1, PKCα protein levels are severalfold higher in the Y1-E cell lines compared with Y1-neo cells. The single exception is the Y1-E15 cell line in which no increase in PKC activity was observed (Table I). As mentioned above, Y1-E15 cells have nearly normal levels of steroidogenesis. Immunoblot analysis of the particulate and cytosol fractions confirmed that the distribution of PKC protein in these fractions reflects the relative levels of activity in vitro (data not shown). To determine if increased PKCα protein levels correlate with increased expression of PKCα mRNA, total cell RNA from Y1-neo and Y1-E cell lines was examined for expression of PKCα mRNA by Northern blot analysis. As seen in Fig. 2, expression of PKCα mRNA is increased in all the Y1-E cell lines examined, again with the exception of the Y1-E15 cell line. These data indicate that increased PKC activity in the Y1-E cell lines results from increased PKCα mRNA.

DAG Mass Is Increased in the Y1-E Cell Lines—DAG is a potent activator of PKC and a common intermediate in inositol and choline phospholipid metabolism. To determine if increased PKC expression and activity in the Y1-E cells are accompanied by changes in the abundance of this endogenous activator of PKC, we measured DAG mass in the Y1 cells, Y1-neo cells, and four Y1-E cell lines. As seen in Table III, DAG mass is increased 2–3-fold in three of the four Y1-E cell lines examined. Neither PKC activity (Table I) nor DAG mass (Table III) was increased in the Y1-E15 cell line which expresses very low amounts of apoE mRNA (<1 pg/µg total RNA).

Inhibition of Protein Kinase C Partially Overcomes the Block in Steroidogenesis—The data reported above suggest that increased PKC expression may be responsible for suppression of P450-SCC mRNA expression in the Y1-E cell lines. To
explore this possibility, we have utilized the PKC inhibitors, staurosporine (28) and calphostin C (29). Y1-neo and Y1-E12 cells were grown in the presence of staurosporine or calphostin C and P450-SCC mRNA expression was analyzed. As seen in Fig. 3A, expression of P450-SCC mRNA increased severalfold after treatment with staurosporine. The maximal level of P450-SCC mRNA in the Y1-E12 cells is equal to, or greater than, the basal level of expression of P450-SCC mRNA in the Y1-neo cell line. Incubation with staurosporine resulted in a similar increase in P450-SCC expression in the Y1-E2 and Y1-E10 cell lines (data not shown). Although staurosporine is a very potent inhibitor of PKC, it is a relatively nonspecific protein kinase inhibitor. To confirm that increased expression of P450-SCC mRNA results from inhibition of PKC, we utilized calphostin C, a highly specific PKC inhibitor. As seen in Fig. 3B, incubation of Y1-E cells with calphostin C results in an increase in P450-SCC mRNA similar to that observed with staurosporine. These results indicate that increased expression of PKC is responsible for inhibition of P450-SCC mRNA expression in the Y1-E cell lines.

In the presence of ample substrate, conversion of cholesterol to pregnenolone by P450-SCC is the rate-limiting step in steroid hormone production. To determine if inhibition of PKC activity results in recovery of steroidogenesis, Y1-neo, Y1-E10, and Y1-E12 cells were grown for 24 h in the presence of calphostin C, and steroid production was measured. As seen in Table IV, incubation with calphostin C results in a 5-fold increase in steroid production by the Y1-E10 and Y1-E12 cell lines. This increase approximates the increase in P450-SCC mRNA expression observed (Fig. 3), indicating that P450-SCC mRNA expression is rate-limiting for basal steroidogenesis in the Y1-E cell lines. Similar results were obtained with staurosporine (data not shown).

We have previously reported that the Y1-E cell lines are defective in various aspects of cAMP-mediated signal transduction (15). Given the evidence that PKC and PKA pathways converge to regulate some aspects of steroidogenesis, one possibility is that elevated expression of PKC in the Y1-E cells results in suppression of cAMP-mediated signaling. To address this question, we examined whether inhibition of PKC activity by calphostin C results in recovery of cAMP responsiveness in Y1-E12 cells. Cells were grown for 24 h under fluorescent light. Calphostin C (25 nM) or calphostin C (25 nM) + dibutyryl cAMP (2 mM) was included as indicated. Production of fluorescent steroid was determined as described under "Materials and Methods." Basal steroid production was set at 100% for the Y1-neo cell line. Calphostin C and calphostin C + cAMP values are shown as a percent of basal level in the Y1-neo cell line. Values represent the average of two experiments which differed by less than 20%.

**DISCUSSION**

We have previously reported that basal and ACTH or cAMP-stimulated steroidogenesis are dramatically reduced in mouse Y1 adrenocortical cells which express the human apoE gene (14). This reduction is due in part to suppression of cAMP-regulated events required for steroid hormone synthesis (15). Expression of the rate-limiting enzyme for steroid synthesis, P450-SCC, is reduced up to 11-fold in these cell lines; a decrease comparable in magnitude to the reduction in basal steroidogenesis. This suggests that reduced P450-SCC expression is responsible for inhibition of basal steroidogenesis in the Y1-E cell lines. Expression of P450-SCC is regulated in a positive fashion by ACTH via cAMP. In addition, substantial evidence implicates PKC as a negative regulator of this gene in adrenal and other steroidogenic cells. In the

![Fig. 3. Inhibition of PKC increases expression of P450-SCC mRNA. Panel A, top: Y1-E12 cells (lanes 1-4) or Y1-neo cells (lanes 5-8) were incubated for 24 h with increasing concentrations of staurosporine, RNA was prepared as described under "Materials and Methods," and expression of P450-SCC mRNA was analyzed. Lanes 1 and 5, no staurosporine; lanes 2 and 6, 10 nM; lanes 3 and 7, 50 nM; lanes 4 and 8, 100 nM. Panel b, bottom: ethidium bromide-stained agarose gel to show equal RNA loading. Panel B, top: Y1-E12 cells were incubated for 48 h without (lane 1) or with (lane 2) 50 nM calphostin C. Panel b, bottom: ethidium bromide-stained agarose gel to show equal RNA loading.](attachment:fig3.png)

**Table IV**

| Cell line | Calphostin C |
|-----------|--------------|
|           | 0 nM | 10 nM | 50 nM |
| Y1-neo    | 0.65 ± 0.04 | 0.84 ± 0.02 | 2.61 ± 0.15 |
| Y1-E10    | 0.15 ± 0.04 | 0.25 ± 0.10 | 0.72 ± 0.01 |
| Y1-E12    | 0.12 ± 0.06 | 0.18 ± 0.07 | 0.63 ± 0.07 |

**FIG. 4. Inhibition of PKC does not result in recovery of cAMP responsiveness in Y1-E12 cells.** Cells were incubated under fluorescent light for 24 h with the indicated concentration of calphostin C. Production of fluorescent steroid was determined as described under "Materials and Methods" and normalized to total cell protein. Numbers represent the average of two experiments ± half the range.
present study we have examined the expression of PKC in the Y1-E cell lines. Our data shows that increased expression of PKC in the apoE-expressing Y1 cell lines accounts for the reduced level of P450-SCC mRNA expression and the suppression of basal steroidogenesis.

Expression of PKCa is increased up to 5-fold in the Y1-E cell lines, with the exception of the Y1-E15 cell line. This cell line expresses the least amount of apoE and is only slightly inhibited in steroidogenesis. No quantitative correlation between apoE mRNA expression and PKC activity was observed, perhaps reflecting a threshold of apoE expression above which maximal PKC expression is observed. The magnitude of the increase in PKC expression is comparable in the particulate and cytosol fractions, suggesting that in Y1-E cells the pool of the activated, or membrane-associated, form of PKC is increased. Consistent with the idea that Y1-E cells have elevated PKC activity in vivo, is the observation that endogenous DAG levels are also increased 1.8–3-fold.

As one of the few cell lines described which overexpress endogenous PKC, Y1-E cells provide a unique model to study PKC-mediated signal transduction. Our data show that overexpression of PKCa in Y1 cells results in suppression of steroid hydroxylase gene expression. Inhibition of PKC activity in vivo with either staurosporine or calphostin C results in an increase in P450-SCC mRNA to a level which is comparable to, or greater than, the basal level of expression in Y1-neo cells. Likewise, incubation with calphostin C results in recovery of steroidogenesis to a level comparable to parental cells, but not Y1-E cells. Thus, increased expression of PKC appears to account for part of the phenotype we observe in the Y1-E cells, however, PKC independent events are also likely to be involved. In addition, the failure to recover cAMP-stimulated steroidogenesis when basal levels of P450-SCC are restored by inhibitors of PKC suggests that the block in cAMP-induced steroidogenesis in the Y1-E cells is independent of the elevated level of PKC expression. Alternatively, chronic inhibition of PKC may be required for recovery of cAMP responsiveness in these cell lines.

The mechanism underlying increased PKC expression in Y1-E cells is unknown. One possibility is that expression of apoE alters lipid metabolism resulting in activation of PKC, or of an activator of PKC. We have found that DAG mass is increased severalfold in the Y1-E cell lines which are inhibited in steroidogenesis. Increased PKC expression and DAG mass have also been observed in ras transformed fibroblasts (33). In this situation, down-regulation of PKC blocks the ability of ras to elevate DAG and phosphatidylcholine hydrolysis, suggesting that phosphatidylcholine hydrolysis may be the source of DAG (33). Although a mechanism such as this could explain chronic activation of PKC in Y1-E cells in vivo, the basis for the increased expression of PKC mRNA and protein we describe is unclear. One possibility is that PKC is a positive regulator of the expression of its own gene, or PKC expression is under negative regulation by the cAMP-PK pathway. Alternatively, apoE or a mediator regulated by apoE could result in increased expression of PKC. In this regard, two other reported functions are suggestive of an ability of apoE to alter signal transduction. Dyer and Curtiss (4) have shown in rat ovarian theca/interstitial cells that exogenous apoE inhibits induction of androgen synthesis by luteinizing hormone. apoE also inhibits mitogen stimulated lymphocyte activation (5). In both cases, apoE must be present prior to the hormonal or mitogenic signal to block the response.

The phenotype of the Y1-E cell lines appears to reflect pleiotropic manifestations of apoE expression including suppression of basal and cAMP-stimulated steroidogenesis, and alterations in cholesterol ester storage and free cholesterol efflux (34). Results of the present study suggest that PKC is responsible for the suppression of basal steroidogenesis, but probably not for the suppression of cAMP-stimulated steroidogenesis in the Y1-E cell lines. Taken together, our studies and others suggest that PKC and cAMP-dependent signal transduction pathways have both dependent and independent roles in regulating adrenal steroidogenesis.

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