Relationship between apolipoprotein E mRNA expression and tissue cholesterol content in rat adrenal gland

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Abstract Among extrahepatic tissues the adrenal gland has one of the highest concentrations of apoE mRNA and the highest rate of apoE synthesis. In the present investigation several previously described in vivo treatments were used to assess the relationship between apoE expression and cellular cholesterol in the rat adrenal gland. Treatment of rats with 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) to lower serum cholesterol concentration and deplete adrenal gland cholesterol content decreased adrenal gland apoE mRNA concentration. These adrenal responses were blocked by dexamethasone (DEX) suggesting that the effect of 4-APP occurred indirectly via stimulation of the adrenal gland by endogenous adrenocorticotrophic (ACTH). Relative to control rats, DEX treatment increased both adrenal gland cholesterol content and apoE mRNA concentration. Concurrent ACTH and DEX administration reduced both adrenal gland cholesterol content and apoE mRNA concentration relative to DEX-treated rats. ACTH administration also rapidly decreased adrenal gland apoE mRNA concentration and cholesterol content in rats pretreated with DEX. In all the above experiments, adrenal gland cholesterol content and apoE mRNA concentration were positively correlated ($r = 0.78$, $P = 0.0001$). In contrast, aminoglutethimide treatment, which blocks adrenal gland steroidogenesis and greatly increases adrenal gland cholesterol content, was without effect on apoE mRNA concentration. ACTH administration to rats treated with DEX + aminoglutethimide resulted in decreased adrenal apoE mRNA despite greatly increased adrenal cholesterol content. This uncoupling of adrenal gland cholesterol content and apoE mRNA concentration suggests that apoE mRNA expression and cellular cholesterol are regulated independently by ACTH.—**Prack, M. M., M. Nicosia, D. L. Williams, and J. Gwynne.** Relationship between apolipoprotein E mRNA expression and tissue cholesterol content in rat adrenal gland. *J. Lipid Res.* 1991. **32:** 1611–1618.

Supplementary key words ACTH • dexamethasone • aminoglutethimide • 4-aminopyrazolo[3,4-*d*]pyrimidine

Apolipoprotein (apo) E was first identified as a component of triglyceride-rich lipoprotein particles (1, 2). ApoE is known to facilitate the removal of cholesterol-rich chylomicron remnant particles by the liver (3, 4), and also serves as ligand for the binding of some lipoprotein particles to the LDL receptor (5, 6). Although the liver appears to be the major site of apoE synthesis, a wide variety of peripheral tissues also express this protein (7–11). Steroidogenic tissues, in particular, express apoE at high levels. Human and monkey adrenal gland, for example, synthesize apoE at relative synthetic rates similar to or greater than in the liver (7, 9). ApoE mRNA concentrations in monkey and rat adrenal gland are also similar to liver apoE mRNA concentrations (10–12).

In cultured mouse peritoneal macrophages, cholesterol loading with acetylated LDL or perturbing cellular cholesterol metabolism with 25-hydroxycholesterol increases apoE synthesis and mRNA concentrations (13–15). In rat ovarian granulosa cells cultured in serum-free medium, ongoing cholesterol synthesis appears necessary for the regulation of apoE expression by protein kinase A and C pathways (16, 17). These results suggest that regulation of apoE expression and possibly apoE function are linked in some manner to cellular cholesterol metabolism. Little is known, however, about the regulation of apoE expression or the relationship between apoE expression and cholesterol metabolism in peripheral tissues in vivo.

In the current investigation we took advantage of previously established in vivo treatments (18, 19) to assess the relationship between apoE mRNA concentrations, cholesterol concentration, and steroidogenesis in rat adrenal gland. In many circumstances apoE mRNA concentration changed in parallel and was highly correlated with adrenal gland cholesterol content. However, apoE mRNA concentration could be uncoupled from adrenal

Abbreviations: apoE, apolipoprotein E; ACTH, adrenocorticotrophin; 4-APP, 4-aminopyrazolopyrimidine; DEX, dexamethasone; LDL, low density lipoprotein; AG, aminoglutethimide; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SC, subcutaneous.

gland cholesterol content, suggesting that apoE expression and cellular cholesterol metabolism are regulated independently by ACTH.

MATERIALS AND METHODS

Animals

Adult female Sprague-Dawley rats (200–250 g) were obtained from Charles River (Charles River, NY) or Taconic Farms (Germantown, NY). Experiments were conducted either at State University of New York, Stony Brook or the University of North Carolina, Chapel Hill. Rats were housed in a temperature- and light-controlled environment (12 h light/12 h dark) for 1 week prior to experiments. Rats were fed standard rat chow and water ad libitum. Since rats treated with 4-APP do not eat, in experiments including 4-APP treatment, water was available ad libitum and food was removed from cages in all treatment groups.

Rats were injected with the following agents alone or in combination as described in the results. Drugs were administered daily for 3 days between 2 and 3 h after light onset: *i*) 4-aminopyrazolopyrimidine (4-APP, Fluka) 6 mg/ml in phosphate-buffered saline, pH 2.5; 1.0 ml was injected intraperitoneally (ip); *ii*) dexamethasone-phosphate (10 mg/ml, Quaid Pharmaceuticals) 0.1 ml was injected subcutaneously (sc) daily for 1–3 days; *iii*) long-acting ACTH in gel (Acthar 40 U/ml, Armour Pharmaceutical) 20 U sc for 3 days or 8 units given in two doses at 12-h intervals; *iv*) aminoglutethimide (AG, Sigma) 40 mg/ml in phosphate-buffered saline, pH 7.5; 0.5 ml sc 30 min before ACTH. Rats were killed the following morning 2–4 h after light onset by injection of 0.5 ml Ketamine/Xylazine (3:1) (Ketaset, Avero Co., Inc.; Xylazine from Rugby Laboratories, Inc).

Preparation and analysis of apoE mRNA

Adrenals were removed, trimmed of fat and connective tissue, and frozen in liquid nitrogen. Liver was isolated and frozen in liquid nitrogen. Tissue samples were homogenized on ice in 4 M guanidine thiocyanate, 0.5% Sarkosyl, 0.1 M β-mercaptoethanol (20), and total cellular RNA was isolated by the guanidine thiocyanate-CsCl centrifugation procedure (20) followed by two phenol-chloroform extractions (21). RNA was quantified by absorbance at 260 nm. To ensure that RNA was intact, formaldehyde-treated RNA samples were analyzed by electrophoresis in 1.2% agarose followed by staining with ethidium bromide (22). Northern blot analysis demonstrated a single apoE mRNA band of approximately 1100 nucleotides that comigrated with rat liver apoE mRNA (data not shown).

A DNA excess solution hybridization assay was performed as described previously (12, 23). A cDNA fragment corresponding to nucleotide 273–497 of the coding region rat apoE mRNA (24) subcloned into bacteriophage M13 was used as a template (12). A [α^{32}P]-dCTP single-stranded probe of known specific activity was made from this template as described (10, 23). ApoE specific probe was isolated from the vector fragments by electrophoresis on a 7 M urea–5% polyacrylamide gel and recovered from the gel by electroelution (10, 23). The gel isolated probe was adsorbed to hydroxyapatite then eluted exactly as detailed previously (10). The resulting probe was 0.5–2% resistant to digestion with S_1 nuclease. Template DNA or total RNA was hybridized to completion with excess probe. S_1-nuclease-resistant hybrids were acid-precipitated, collected on glass fiber filters (Schleicher & Schuell, Keene, NH), and counted by scintillation spectrometry. ApoE mRNA values were determined by reference to a standard curve constructed with template DNA, and values were corrected for the probe size (224 nucleotides) compared to the size of rat apoE mRNA (1068 nucleotides).

Additional assays

Total and unesterified cholesterol were assayed enzymatically (25) using Boehringer Mannheim High Performance Cholesterol Kit (Boehringer-Mannheim Diagnostics). Adrenal glands were extracted with chloroform–methanol (26). Extracts were dried under nitrogen and resuspended in isopropanol. Serum fluorogenic steroids were assayed following methylene-chloride extraction as described by Kowal and Fiedler (27). Protein was measured according to Lowry et al. (28) using bovine serum albumin as a standard.

Statistics

Comparisons between treatment groups were made by one-way analysis of variance followed by Tukeys test of significant difference (29). Linear regression and correlation analysis were performed using the GraphPad Instat software package, version 1.0.

RESULTS

Relationship between adrenal gland cholesterol content and apoE mRNA concentration

In order to assess the relationship between adrenal cholesterol and apoE mRNA levels, rats were treated in vivo with several agents known to alter adrenal steroidogenesis and/or cholesterol content. Previous studies have shown that 4-APP blocks hepatic lipoprotein secretion thereby reducing serum lipoprotein cholesterol concentrations (30). Adrenal gland cholesterol stores are also severely depleted in 4-APP-treated rats because ACTH-stimulated steroidogenesis occurs in the absence of sufficient serum lipoprotein cholesterol to serve as substrate for glucocorticoid production (18, 31). As shown in **Fig. 1**,

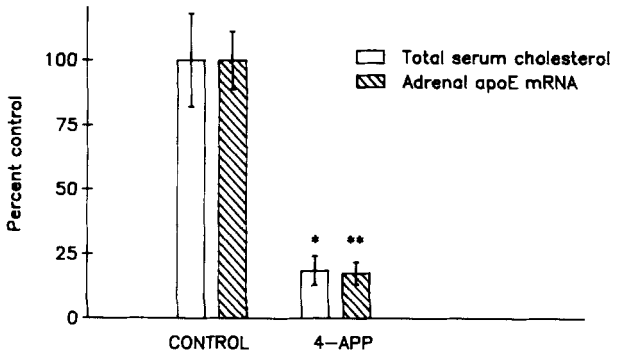

Fig. 1. Total serum cholesterol and apoE mRNA in adrenal glands from untreated rats (control) and rats injected with 6 mg 4-APP daily for 3 days. Data points represent the average ± SEM of three rats. Control rats had a serum cholesterol concentration of 44.8 mg/dl and adrenal apoE mRNA concentration of 13.6 ± 1.5 pg/μg RNA. Open bars indicate total serum cholesterol. Hatched bars indicate adrenal apoE mRNA. Significant differences are indicated: $^{*}P < 0.01$; $^{**}P < 0.002$.

treatment of rats with 4-APP for 3 days resulted in 4- to 5-fold reductions of total serum cholesterol and adrenal gland apoE mRNA concentrations.

Dexamethasone (DEX) is a synthetic glucocorticoid and a potent inhibitor of ACTH release from the pituitary gland (32, 33). By inhibiting ACTH release, DEX treatment results in a very low rate of adrenal steroid synthesis and adrenal cholesterol accumulation. A previous study has shown that the adrenal gland cholesterol depletion and enhanced steroidogenesis observed in 4-APP-treated rats does not occur when 4-APP-treated rats are given DEX (34). This suggests that adrenal cholesterol depletion is due to ACTH stimulation. To determine whether the decrease in adrenal gland apoE mRNA concentration in 4-APP-treated rats required endogenous ACTH, rats were treated with the synthetic glucocortoid DEX. The results in **Fig. 2A** show that total serum cholesterol concentration and adrenal gland cholesterol content were markedly reduced in 4-APP-treated rats as compared to DEX-treated rats. Rats treated concurrently with 4-APP and DEX also had reduced total serum cholesterol, but the depletion in adrenal gland cholesterol content was largely prevented by DEX. As shown in Fig. 2B the decrease in adrenal gland apoE mRNA concentration in 4-APP-treated rats was also prevented by DEX administration. This result suggests that the decrease in adrenal gland apoE mRNA concentration in 4-APP-treated rats was due indirectly to ACTH stimulation and not to a toxic effect of 4-APP. This result also suggests that changes in apoE mRNA concentration reflect changes in adrenal gland cholesterol content and not changes in total serum cholesterol.

The effect of DEX on adrenal gland apoE mRNA concentration was also examined in relation to control animals. As shown in **Table 1**, apoE mRNA concentration was increased about 3-fold in DEX-treated animals in concert with increases in both adrenal gland total cholesterol and free cholesterol contents. The decrease in serum corticosteroids in DEX-treated rats indicates that ACTH release was effectively suppressed. This was also the case for the experiment shown in Fig. 2 in which serum corticosteroids (ng/ml ± SEM) were low in rats treated with DEX (58 ± 12) and DEX + 4-APP (24 ± 10) compared to rats treated with 4-APP (270 ± 20).

A time course of adrenal gland apoE mRNA concentration showed no increase at 1 day and maximal increases after 2 and 3 days of DEX treatment (data not shown). When rats were treated with DEX for 3 days and then challenged with ACTH, adrenal gland apoE mRNA concentration returned to the control level within 24 h (**Table 2**). Adrenal gland cholesterol content was also reduced to near the control level in 24 h. Concurrent administration of DEX and ACTH produced a similar result in that rats treated with DEX + ACTH had reduced adrenal gland cholesterol content and apoE mRNA concentration compared to rats treated with DEX alone (**Fig. 3**). ACTH administration to control animals also

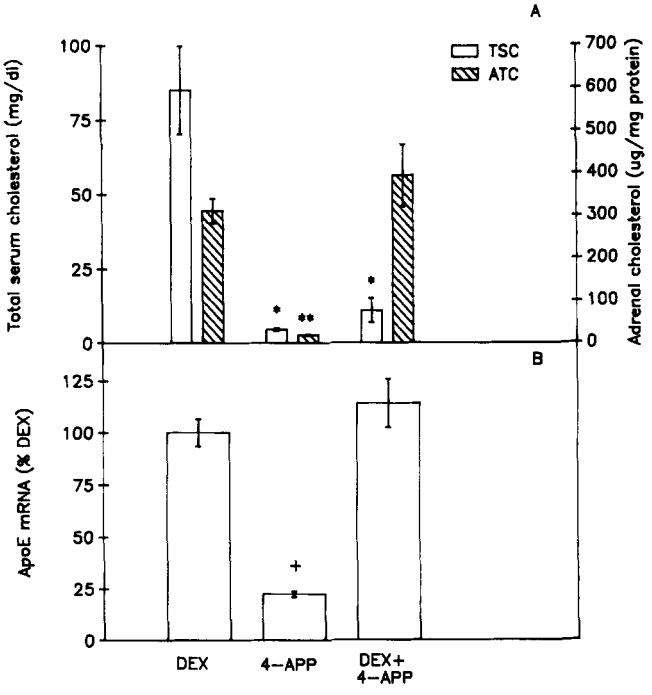

Fig. 2. Total serum cholesterol, adrenal cholesterol, and adrenal apoE mRNA in rats treated with DEX, 4-APP, or the combination of DEX + 4-APP. Rats received three daily injections of either 1 mg DEX, 6 mg 4-APP, or the combination. Data shown are the average ± SEM of three rats for each group. A) Total serum cholesterol (TSC) and adrenal total cholesterol (ATC). Significant differences are indicated. Different from DEX-treated rats $^{*}P < 0.05$; $^{**}P < 0.01$. B) Adrenal apoE mRNA. DEX-treated rats had an apoE mRNA concentration of 54.4 ± 3.0 pg/μg RNA. $^{+}$Different from DEX-treated rats $P < 0.01$, and from DEX + 4-APP, $P < 0.05$.

TABLE 1. Serum corticosteroids, adrenal cholesterol, and adrenal apoE mRNA concentrations in untreated and in dexamethasone-treated rats

Treatment	Adrenal ApoE mRNA	Adrenal Total Cholesterol	Adrenal Free Cholesterol	Serum Corticosteroids
	pg/μg RNA	*μg/mg protein*	*μg/mg protein*	*ng/ml*
Control	14.6 ± 2 (8)	178 ± 18 (7)	28.3 ± 5 (3)	560 ± 100 (4)
Dexamethasone	45.9 ± 4 (10)	517 ± 43 (13)	69.5 ± 9 (10)	42.7 ± 7 (10)

Data shown are the average ± SEM for untreated (control) rats or rats given three daily 1-mg dexamethasone injections. Number of rats indicated in parentheses.

decreased adrenal gland total cholesterol content but yielded only a small decrease in apoE mRNA concentration that was not statistically different from the control (**Fig. 4**).

The experiments presented above suggest that adrenal gland apoE mRNA concentration varies in proportion to adrenal gland cholesterol content in animals treated with DEX, 4-APP, ACTH, or combinations of these agents. Least square linear regression between adrenal apoE mRNA and adrenal cholesterol was computed for each experiment. The correlation coefficients for total cholesterol content versus apoE mRNA concentration ranged between 0.92 and 0.98. When data from all experiments were combined, and the correlation was computed for all data points, the correlation coefficient was 0.78 (**Fig. 5**; n = 32; P = 0.0001). The treatments used in these experiments produce parallel changes in adrenal gland total cholesterol and free cholesterol contents (Table 1 and data not shown). Free cholesterol ranged between 3.6 and 138 μg cholesterol/mg protein and was also highly correlated with adrenal apoE mRNA (r = 0.91; n = 22; P = 0.0001). Consequently, the relationship between adrenal gland apoE mRNA concentration and cholesterol content is similar whether presented as free, total, or esterified cholesterol.

TABLE 2. ACTH-mediated reversal of adrenal apoE mRNA and adrenal cholesterol accumulation in dexamethasone-treated rats

Group	Adrenal ApoE mRNA	Adrenal Cholesterol
	pg/μg RNA	*μg/mg protein*
DEX	108 ± 21	491 ± 39
DEX + saline	106 ± 26	576 ± 53
DEX + ACTH	31 ± 4[a]	302 ± 37
Saline	38 ± 1[a]	265 ± 34[a]

Data shown are the average ± SEM for four rats in each group. Rats received 1 mg dexamethasone (DEX) or an equal volume (100 μl) saline daily for 3 days. One day after the third DEX injection, rats received 8 IU ACTH (DEX + ACTH) given in two 4-IU doses at 12-h intervals or an equal volume of saline (100 μl, DEX + saline).

[a]Different from DEX and DEX + saline ($P < 0.05$).

Uncoupling of adrenal gland cholesterol content and apoE mRNA concentration

Aminoglutethimide inhibits cholesterol side chain cleavage by mitochondrial P450-cholesterol side chain cleavage enzyme thereby blocking steroidogenesis and promoting cholesterol accumulation in rat adrenal gland (35). The results in Fig. 4 show that treatment of rats for 3 days with aminoglutethimide increased adrenal gland total cholesterol content about 3-fold but had no effect on apoE mRNA concentration. Concurrent treatment with ACTH and aminoglutethimide partially inhibited the increase in cholesterol content but was without effect on apoE mRNA concentration. In DEX-treated rats aminoglutethimide completely blocked the ACTH-mediated decrease in adrenal gland total cholesterol content (Fig. 3). However, aminoglutethimide did not inhibit the ACTH-mediated decrease in apoE mRNA concentration. These data suggest that adrenal gland apoE mRNA concentration is insensitive to increases in cholesterol content elicited by aminoglutethimide. In addition, the ACTH-

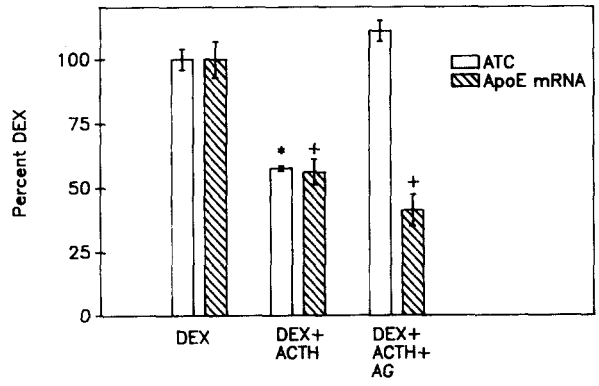

Fig. 3. The effects of ACTH and AG on adrenal cholesterol and apoE mRNA concentrations in dexamethasone-treated rats. All rats received three daily injections of 1 mg DEX alone or with ACTH (20 IU) or AG (20 mg). Data shown are the average ± SEM for three rats in each group. Adrenal cholesterol concentration for DEX-treated rats was 670 μg/mg protein and apoE mRNA concentration was 53.5 ± 3.7 pg apoE mRNA/μg RNA. Open bars indicate adrenal total cholesterol concentration (ATC) and hatched bars indicate adrenal apoE mRNA. Differences from DEX group are indicated: •$P < 0.01$.

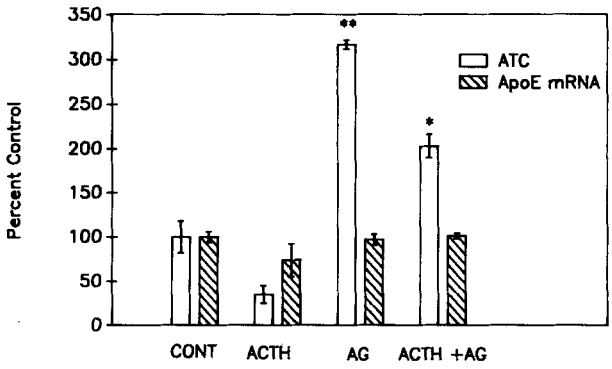

Fig. 4. The effects of ACTH and aminoglutethimide (AG) on adrenal cholesterol and apoE mRNA. Rats received three daily injections of ACTH (20 IU), AG (20 mg), or both drugs. Data (average ± SEM, n = 3) shown are expressed as percent of the concentration measured in adrenal glands from untreated (control) rats that had 138 ± 27 μg cholesterol/mg protein and 19.4 ± 1.2 pg apoE mRNA/μg RNA. Open bars indicate adrenal total cholesterol concentration (ATC) and hatched bars indicate adrenal apoE mRNA concentration. Differences from control group are indicated: $^{*}P < 0.05$; $^{**}P < 0.001$.

mediated decrease in apoE mRNA concentration in DEX-treated rats occurred in the presence of aminoglutethimide even though adrenal cholesterol content is increased. No correlation between adrenal gland apoE mRNA concentration and cholesterol content was seen when aminoglutethimide-treated animals were analyzed.

Hepatic apoE mRNA concentrations

Hepatic apoE mRNA concentrations were measured in several experiments to assess the effects of experimental treatments on a tissue other than the adrenal gland. None of the treatments that altered adrenal gland apoE mRNA concentration and cholesterol content had a substantial effect on hepatic apoE mRNA concentration (**Fig. 6**) or hepatic total cholesterol content (data not shown).

DISCUSSION

The results of this study show that adrenal gland apoE mRNA concentration is highly regulated by experimental treatments that also regulate adrenal cholesterol metabolism and steroidogenesis. Treatment of control rats with 4-APP or ACTH administration to DEX-pretreated rats elicits adrenal cholesterol depletion, increased steroidogenesis, and results in decreased apoE mRNA concentrations in adrenal gland but not in liver. Conversely, treatment of rats with DEX to block endogenous ACTH release allows adrenal cholesterol content to increase and results in increased apoE mRNA concentration. Treatment with dexamethasone or ACTH both increase circulating glucocorticoid concentration; however, administration of these two agents yields contrasting effects on adrenal apoE. This suggests that high circulating glucocorticoids alone do not regulate adrenal apoE mRNA. Under these conditions, therefore, apoE mRNA concentrations changed in direct proportion to adrenal gland cholesterol content (Fig. 5) and in inverse proportion to adrenal steroid production. Adrenal gland free cholesterol content and esterified cholesterol content both were altered by these treatments. Consequently, no distinction can be made as to whether apoE mRNA concentrations are more closely related to free or esterified cholesterol content in adrenal cells.

ApoE mRNA concentrations are increased in mouse peritoneal macrophages loaded with cholesterol or treated with 25-hydroxycholesterol (13–15). Rat ovarian granulosa cells cultured in serum-free medium appear to require ongoing cholesterol synthesis to permit increased apoE mRNA levels to occur in response to cAMP (17). These data have been presented as evidence that cholesterol or a metabolite is directly involved in the positive regulation of apoE mRNA levels in these cells in much the same way that cholesterol is believed to be directly involved in the negative regulation of LDL receptor and HMG-CoA reductase mRNA concentrations (36). Is cholesterol directly involved in the regulation of apoE mRNA levels in rat adrenal gland? While the relationship between adrenal gland cholesterol content and apoE mRNA concentration (Fig. 5) is consistent with this possibility, the uncoupling of cholesterol content and apoE mRNA concentration in aminoglutethimide-treated rats argues against a direct role of cholesterol in regulating apoE mRNA concentrations. Adrenal gland cholesterol content was increased in aminoglutethimide-treated rats as much or more so than in DEX-treated rats (Figs. 3 and 4), yet apoE mRNA concentrations were low,

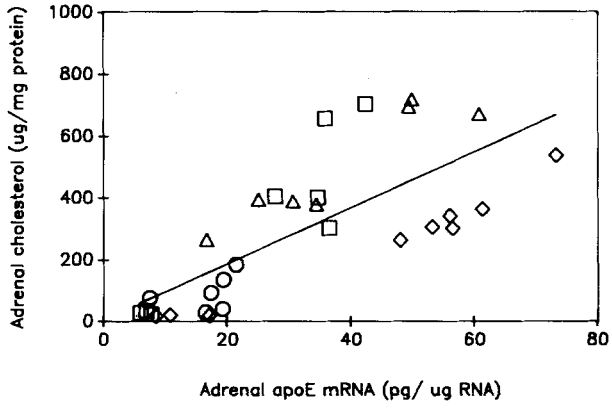

Fig. 5. Correlation between adrenal apoE mRNA and adrenal cholesterol in untreated rats and rats treated with DEX, 4-APP, ACTH, or combination of these drugs. Data points represent the values from each of the animals in four independent experiments with the same symbol used for each rat in that experiment. Data from all experiments were pooled to obtain the regression line shown. The correlation coefficient for the regression line, $r = 0.78$ is significant at the $P < 0.0001$ level (n = 32).

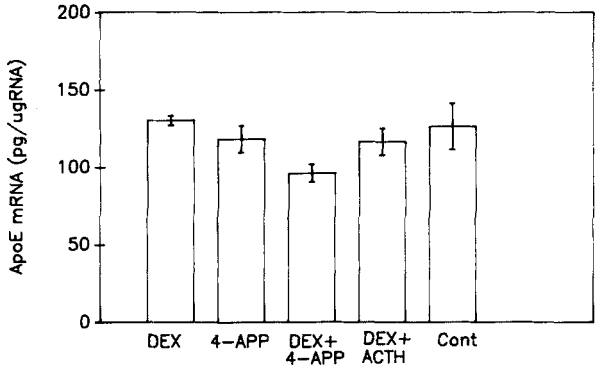

Fig. 6. The effects of treatments on hepatic apoE mRNA concentration. Rats were treated with saline (Control), DEX, 4-APP, DEX + 4-APP, or DEX + ACTH for 3 days as described for Figs. 2 and 3. Data shown are the average ± SEM for three or four rats in each group.

similar to the concentration measured in ACTH-treated rats. This result is clearly inconsistent with cellular cholesterol being a primary regulatory influence for adrenal gland apoE mRNA concentrations. Two additional points are relevant to this argument.

First, HMG-CoA reductase activity is decreased (37) and acyl-CoA:cholesterol acyltransferase activity is increased (38) in adrenal glands from aminoglutethimide-treated rodents. Similarly, HMG-CoA reductase activity is reduced and cholesterol esterification is enhanced in murine Y-1 adrenocortical cells treated with aminoglutethimide (39). These results indicate that the increase in adrenal gland free cholesterol resulting from aminoglutethimide treatment is sufficient to perturb the regulatory pool of cholesterol or a cholesterol metabolite. Nevertheless, apoE mRNA concentrations are unchanged. Second, since the adrenal gland is composed of different cell types, one could argue that the increase in adrenal gland cholesterol in aminoglutethimide-treated rats does not occur in the same cells that make apoE. This is not the case, however, since in situ hybridization analysis showed that apoE mRNA is similarly distributed in zona fasciculata and zona reticularis cells in control and aminoglutethimide-treated rats (40). Markedly enhanced oil red O staining in these cells in aminoglutethimide-treated rats confirmed that cholesterol accumulated in the same cells in which apoE mRNA concentration was unchanged.

These points argue that the cellular content of free or esterified cholesterol is not a primary regulator of apoE mRNA concentration in rat adrenal gland. On the basis of the current data, it is more likely that ACTH is a primary negative regulator of apoE mRNA levels in rat adrenal gland. ApoE mRNA concentration is increased when endogenous ACTH release is blocked by DEX treatment, and apoE mRNA concentration is decreased by 4-APP treatment and by ACTH administration to DEX- and DEX + aminoglutethimide-treated rats. With the exception of aminoglutethimide treatment, apoE mRNA concentration and adrenal gland cholesterol content are regulated in parallel but probably independently of each other.

The function of apoE synthesized in adrenal gland is not known. The present results show that adrenal gland apoE mRNA expression is high in DEX-treated rats in which LDL receptor activity is low and the demand for lipoprotein cholesterol for steroidogenesis is minimal (19). Thus, it is unlikely that apoE is secreted by adrenocortical cells to facilitate lipoprotein uptake by the LDL receptor pathway. Additionally, it is unlikely that apoE is made to facilitate selective cholesteryl ester uptake from HDL since apoE expression is low in 4-APP-treated rats in which selective cholesteryl ester uptake is greatly increased (41).

The present results show that adrenal gland apoE mRNA expression is inversely related to the level of adrenal steroidogenesis. Recent work by Reyland and coworkers (42) suggests, in addition, that apoE may play a role in determining the level of adrenal steroidogenesis possibly by regulating the availability of cholesterol to the mitochondrion or by modulating cholesterol side chain cleavage activity. In this study, mouse Y1 adrenal cells (which do not express apoE) were used to generate stable cell lines expressing the human apoE protein. The apoE-expressing cell lines showed greatly reduced basal steroidogenesis and reduced ACTH- or cAMP-stimulated steroidogenesis (42). Since apoE expression occurred constitutively in these cell lines and was not decreased by ACTH or cAMP, it is possible that elevated levels of apoE expression limit steroidogenic activity in some manner not yet understood. In the present study, the inverse relationship between adrenal gland apoE expression and the level of steroidogenic activity is consistent with the idea that apoE may play a role in regulating steroidogenesis in the adrenal gland in vivo.

The pattern of apoE regulation seen in the present study and the possibility that apoE may play a role in regulating adrenal steroidogenesis raise important questions about the fate of the apoE synthesized by adrenal cortical cells. While apoE appears to be a typical secretory protein, little is known about the post-translational processing, secretion, or re-uptake of apoE by adrenal cells. A recent immunocytochemical study identified apoE in rat liver peroxisomes (43) and in apparently cytoplasmic locations near the bile canaliculus in addition to the expected cellular locations for this protein in the secretory and endocytic pathways (43). This result suggests that apoE may have intracellular roles not previously appreciated and possibly involved with intracellular cholesterol movement (7). It will be important to determine whether apoE occurs in similar intracellular locations in adrenocortical cells and how this may relate to its function in these cells.

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