An apolipoprotein E synthetic peptide selectively modulates the transcription of the gene for rat ovarian theca and interstitial cell P450 17α-hydroxylase, $C_{17–20}$ lyase

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Abstract **Ovarian theca/interstitial cells produce androgens in response to luteinizing hormone (LH) stimulation and apolipoprotein (apo) E exerts a selective effect on the type of steroid product made by these cells. We have identified an apoE synthetic peptide containing the low density lipoprotein (LDL) receptor binding domain, acetyl-Y(LRK LRKRLLRDADDL)$_2$C, that mimics the activity of native apoE. Depending on the concentration, the apoE synthetic peptide either enhanced or inhibited the LH-stimulated production of androstenedione with concomitant changes in the mRNA for its synthetic enzyme, P450 17α-hydroxylase, $C_{17–20}$ lyase, without any changes in progesterone production or the mRNA for its synthetic enzyme, P450 cholesterol side-chain cleavage. The apoE synthetic peptide caused changes in the rate of transcription of the mRNA for P450 17α-hydroxylase, $C_{17–20}$ lyase without altering its stability. Pretreatment of the theca/interstitial cells with receptor-associated protein, which blocks apoE binding to members of the LDL receptor superfamily, prevented the apoE synthetic peptide-mediated stimulation of androstenedione and mRNA for P450 17α-hydroxylase, $C_{17–20}$ lyase, but did not attenuate the inhibitory activity of the peptide. Thus, apolipoprotein E selectively altered the type of steroid made by ovarian theca/interstitial cells by regulating the transcription of mRNA for the gene for P450 17α-hydroxylase, $C_{17–20}$ lyase, in part through its interaction with apolipoprotein E-specific receptors of the LDL receptor superfamily.—**Zhang, G., L. K. Curtiss, R. L. Wade, and C. A. Dyer. **An apolipoprotein E synthetic peptide selectively modulates the transcription of the gene for rat ovarian theca and interstitial cell P450 17α-hydroxylase, $C_{17–20}$ lyase. *J. Lipid Res.* 1998.** 39: **2406–2414.**

Supplementary key words androgen • RT-PCR • P450 cholesterol side-chain cleavage • receptor-associated protein • LDL receptor superfamily • steroidogenesis

Steroidogenesis by the ovary is driven by gonadotropins and modulated by numerous intraovarian autocrine and paracrine agents. One postulated autocrine regulator is apolipoprotein (apo) E. ApoE is made in the ovary by theca/interstitial cells (TIC) (1) and when added to cultured TIC selectively alters the type of steroid products synthesized (2). TIC respond to luteinizing hormone (LH) and make androgens (3). Androgens are the rate-limiting substrate for follicular estrogen, which controls follicular maturation (3). Also, androgens can promote the demise of the follicle through atresia initiated by androgen-induced apoptosis in the granulosa cell compartment (4). Thus apoE modulation of TIC androgen production may play an important role in determining the fate of a maturing follicle.

ApoE selectively alters the type of steroid product made by ovarian TIC. Both rat high density lipoprotein (HDL) enriched in apoE (2) and a synthetic peptide of apoE (5) exert a biphasic effect on LH-stimulated TIC androstenedione production. At low concentrations of either form of apoE, androstenedione production is increased, whereas at higher concentrations androstenedione production is inhibited below LH-stimulated control levels.

The TIC enzymes responsible for synthesizing androgen steroids from cholesterol are P450 cholesterol side-chain cleavage (SCC), which is the rate-limiting step for progesterone production in the TIC, and P450 17α-hydroxylase, $C_{17–20}$ lyase (lyase), which converts progesterone into androstenedione. We found previously that apoE alters TIC androstenedione production without changing progesterone production (2). The apoE-mediated selec-

Abbreviations: ELOSA, enzyme-linked oligonucleotide specific assay; lyase, P450 17α-hydroxylase, $C_{17–20}$ lyase; SCC, P450 cholesterol side-chain cleavage; TIC, theca/interstitial cells; SAP, streptavidin alkaline phosphatase; RT-PCR, reverse transcription-polymerase chain reaction; RAP, receptor-associated protein.

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tive change in steroid metabolism suggested that SCC activity was unaltered but that the activity of lyase was stimulated by low apoE concentrations and inhibited by high apoE concentrations. Lyase gene expression is regulated at the level of transcription (6). Based on the changes in steroid products and given the transcriptional regulation of lyase gene expression, we postulated that apoE altered lyase gene transcription without altering SCC gene transcription. Here, we present our analysis of apoE synthetic peptide effects on TIC SCC and lyase gene expression by measuring mature mRNA levels, heterogeneous nuclear (hn) RNA levels, to assess the rate of transcription, mRNA stability, and the participation of apoE-specific receptors in mediating the synthetic peptide activities.

MATERIALS AND METHODS

ApoE synthetic peptide preparation and characterization

The apoE peptide was synthesized by the solid phase method of Merrifield on an Applied Biosystems model 430A automated peptide synthesizer and it was purified on a Waters Auto 500 preparative HPLC column as described (7). The amino acid composition was confirmed on the hydrolyzed peptide with a Beckman model 6300 high performance analyzer operated with internal standards. The peptide preparation was >91% pure and the content was >52%. The expected molecular weight of the peptide was 4011 which was confirmed by mass spectrometry. The two-carbon chain of acetic acid was covalently bound to the N-terminal of the peptide sequence, acetyl-Y$(LRKLRKRLLRDADDL)_2$C. The peptide was lyophilized and stored in a dark environment under vacuum. When used in tissue culture the synthetic peptide was reconstituted in water at a calculated concentration of 250 μm, exhaustively dialyzed in 1,000 molecular weight cut-off tubing against water, filter sterilized, and stored at −20°C.

Reagents and oligonucleotides

Oligo $(dT)_{15}$ primer and dNTPs were obtained from Boehringer-Mannheim (Indianapolis, IN). PRIME RNase inhibitor was obtained from 5 Prime → 3 Prime, Inc. (Boulder, CO). Moloney murine leukemia virus reverse transcriptase (MMLV-RT) was obtained from Epicentre Technologies (Madison, WI). AmpliTaq Gold DNA polymerase was obtained from Perkin-Elmer (Branchburg, NJ). Hi-Low DNA ladder was obtained from Minnesota Molecular (Minneapolis, MN). rTth DNA polymerase was obtained from Perkin-Elmer (Branchburg, NJ). DNA BIND plates were obtained from Costar (Cambridge, MA). Streptavidin-alkaline phosphatase (SAP) was obtained from Sigma Chemical Co. (St. Louis, MO). The substrate for SAP, BluePhos, was obtained from Kirkegaard & Perry Laboratories (Gaithersburg, MD). Rat ovary total RNA was obtained from Ambion, Inc. (Austin, TX).

All oligonucleotides were obtained from Operon Technologies (Alameda, CA). The sequences for the forward, reverse oligonucleotide primers and oligonucleotide probes were: β-actin mRNA, nt 138–157 (TATGGGTCAGAAGGACTCCT) and 635–654 (ATAGCACAGCTTCTCTTTAA), and 224–243 (ATCGTCC CAGTTGGTTACAA), β-actin hnRNA, nt 256–275 (CGCGAGTA CAACCTCCTTGC) and 598–617 (AGGTGGACAGATGCGGC TTA), and 365–384 (GTGTTCCTCACGATCCTGCA) (8), SCC mRNA, nt 315–334 (AGCGACTCTCTTCTCCTGCG) and 654–673 (CCACGATCTCCTCCAACATC), and 502–521 (TGAGCTA CACCTTCCAGCAG), SCC hnRNA, nt 258–275 (GCCGTCTAC CAGATGTTC) and 482–499 (TCCTTGCTCACCAGAGAT), and 258–277 (TGGAACATCTGGTAGACGGC) (9), and lyase mRNA, nt 38–57 (CCTATTTCTTTTGGGTCAAG) and 454–473 (GC CAGCATCATATCACACAG), and 215–234 (GTGGCCGATGAT CACTGTAG), lyase hnRNA, nt 1813–1832 (TGATCATCGGC CACTATCAG) and 2392–2411 (TCGTAGGATGGCCACTCA AT), and 2038–2057 (CACCATCTTGCTGGACAAGC), respectively (10). The hnRNA reverse transcription-polymerase chain reaction (RT-PCR) was done using intron-directed reverse primers. All forward primers were 5′-biotinylated. The oligonucleotide sequences used to probe the PCR products were nested in the amplified product and were 5′-amino modified. The 5′-biotinylated oligonucleotide standards used in the enzyme-linked oligonucleotide specific assay (ELOSA) were the complement of the probe sequences.

Rat ovarian TIC preparation, RNA isolation, and analysis of steroid production

The TIC were prepared from ovaries of immature Sprague-Dawley rats that had been hypophysectomized at 21 days of age as described (2). All procedures used were in accordance with the Institutional Animal Care and Use Committee of Northern Arizona University. The TIC content was enriched by using a two-step Percoll density gradient (11) resulting in greater than 90% of the cells being identified as TIC by their 3β-hydroxysteroid dehydrogenase specific staining (3). TIC were cultured at 40,000–50,000 cells/0.25 ml of McCoy's 5a medium in a 96-well plate (Costar, Cambridge, MA) in a humidified 95% air, 5% CO_2 incubator at 37°C. The TIC were stimulated with ovine LH (NIDDK-oLH-25, 2.3 U/mg, 1 U = activity of NIH-LH-S1) which was generously provided by the National Hormone and Pituitary Program of NIDDK. After 48 h the culture supernatants were collected and stored at −20°C. At the indicated times the total RNA was isolated from cells pooled from six wells (12) reconstituted in water and stored at −80°C until analyzed in the RT-PCR–ELOSA. Progesterone and androstenedione were assayed in the culture supernatants using previously characterized antisera (2) in a standard radioimmunoassay (RIA) protocol. AssayZap (Biosoft, Ferguson, MO) was used to perform 4 parameter logistic analyses of the RIAs.

RT-PCR–ELOSA of mRNA and hnRNA

The protocol for RT-PCR of mRNA was: 6 μl of total RNA was reverse transcribed into cDNA by incubating with 50 μl of 10 mm KCl, 10 mm dithiothreitol, 1 mm each of dNTPs, 1 μg oligo $(dT)_{15}$, 2 units PRIME RNase inhibitor, and 15 units of MMLV-RT for 1 h at 37°C. The 100 μl PCR reaction mixture contained 10 μl of 10× PCR buffer (provided by Perkin-Elmer) 10 μl 25 mm $MgCl_2$, 100 pmol of each primer, 2 μl of 10 mm dNTPs, 2–10 μl cDNA, and 2.5 units of AmpliTaq Gold. After the AmpliTaq Gold was activated at 95°C, the cDNA was amplified during 25 cycles; 95°C for 30 sec, 62°C for 2 min. The lengths of the products were: β-actin = 517 bp, SCC = 359 bp, and lyase = 436 bp.

The protocol for RT-PCR of hnRNA was: 3 μl of total RNA was added to 20 μl total RT mixture containing 2 μl of 10× RT buffer (provided by Perkin-Elmer), 2 μl 10 mm $MgCl_2$, 0.4 μl 10 mm dNTPs, 1 μl 100 pmol/μl reverse primers, 2 μl 2.5 units/μl rTth DNA polymerase, and 9.6 μl water. The RT mixture was incubated at 70°C for 15 min to synthesize cDNA that was primed by intron-specific reverse primers. The RT reaction was stopped by placing the tube on ice for 5 min. In the PCR step, 8 μl of 10× chelating buffer (provided by Perkin-Elmer), 10 μl 25 mm $MgCl_2$, 2 μl 10 mm dNTPs, and 1 μl 100 pmol/μl biotinylated forward primers and water to a final volume of 100 μl was added to the RT tube. The rTth DNA polymerase was activated at 95°C for 1 min; the cDNA was amplified during 27 cycles; 95°C for 45 sec, 60°C for 1 min. The lengths of the PCR products were: β-actin = 362 bp, SCC = 242 bp, and lyase = 599 bp.

The identities of the mRNA- and hnRNA-derived RT-PCR products were confirmed using restriction enzyme digestion done according to the manufacturer's instructions. Before the ELOSA, the PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA). The purification step was required to maintain a low background in the ELOSA. The amount of PCR product was determined by using the colorimetric-based ELOSA (13). This type of assay can be used for the relative quantitation of mRNA (14). The method used to measure hnRNA was adapted from Elferink and Reiners (15). The protocol used to measure the amount of PCR product was modified from the instructions provided by the manufacturer of the DNA-BIND plates (Costar, Cambridge, MA). Briefly, the amino-modified oligonucleotide probes were covalently attached to the DNA-BIND plates and the unreacted groups were blocked with 3.0% BSA in oligonucleotide binding buffer (50 mm Na_2PO_4, 1 mm EDTA, pH 8.5). The PCR products were denatured by boiling for 5–7 min, rapidly transferred to ice, and spun at 10,000 *g* for 2 min at 4°C. The ssDNA PCR products or standards were hybridized to the bound probe sequences for 1 h at 53°C for β-actin and SCC and 62°C for lyase for mRNA samples, and for the hnRNA samples all reactions were incubated at 57°C. The amount of bound 5′-biotinylated oligonucleotide was indicated by the bound SAP that converted the substrate into a blue color read at 600 nm on a plate reader. Initially the ELOSA conditions were optimized using rat ovary total RNA but once the TIC samples were collected, the RT-PCR–ELOSA was re-optimized for input cDNA volume in the PCR and PCR product in the ELOSA so that the reactions were in the exponential phase of amplification and color production, respectively. We optimized the semi-quantitative RT-PCR to minimize the effects of initial template content on the yield of target sequence product (16). The amount of PCR product used in the ELOSA that put it in the linear range of each assay was determined by using the oligonucleotide standards to determine the upper and lower limits of the assay. Four controls were used to assess the amount of non-specific color development; no bound probe, no PCR products, no SAP, and no BluePhos. The absorbances of the four controls were averaged and subtracted from the sample absorbance. β-Actin hnRNA/mRNA levels were determined and used to normalize all of the results for SCC hnRNA/mRNA and lyase hnRNA/mRNA to correct for differences in total RNA recovered during the isolation. As a control, hnRNA RT-PCR was run without the RT step to be certain that the hnRNA RT-PCR products were amplified from RNA and not genomic DNA.

Statistical analyses

Differences were determined by unpaired *t* test or for multiple comparisons by one-way analysis of variance. Differences were considered statistically significant at $P < 0.05$. The results represent the responses measured from 2–10 separate experiments and were the mean ± SEM from quadruplicate determinations per experiment.

RESULTS

ApoE synthetic peptide containing the LDL receptor binding domain mimicked native apoE effects on TIC androgen production

We previously reported that rat HDL enriched in apoE selectively inhibited androgen synthesis by rat TIC (2). As shown in **Fig. 1**, an apoE synthetic peptide containing the LDL receptor binding domain, amino acids 140–150 of native apoE, mimicked the effect of native apoE to suppress androstenedione production without altering progesterone production. In addition, at low concentrations of the apoE synthetic peptide, ≤100 nm, androstenedione production was significantly increased so that it was 50% greater than the amount made with only LH stimulation. At apoE synthetic peptide concentrations >300 nm, androstenedione production was significantly decreased to <25% of the response in the control cells. In contrast, progesterone production was unaltered by the apoE synthetic peptide. The stimulatory and inhibitory activities of the apoE synthetic peptide were equivalent to those of native apoE (2, 5).

ApoE specific receptors were required for the stimulation but not the inhibition of androstenedione production that was mediated by the apoE synthetic peptide

Receptor-associated protein (RAP) binds to all members of the LDL receptor superfamily and at 100 nm blocks apoE binding (17). RAP alone (100 nm) caused a

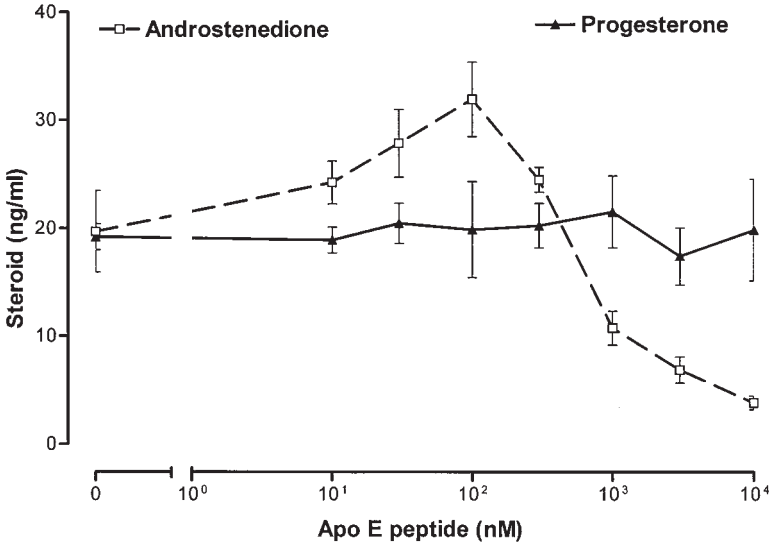

Fig. 1. ApoE peptide had a biphasic effect on TIC androstenedione production. TIC (50,000 cells/0.25 ml medium/well) were stimulated with LH (1.0 ng/ml) ± increasing concentrations of the apoE peptide. After 48 h the culture supernatants were collected and stored at −20°C until the amounts of androstenedione and progesterone were determined in specific RIAs. The individual data points are the mean of 4 replicates ± SEM and are representative of 10 individual experiments.

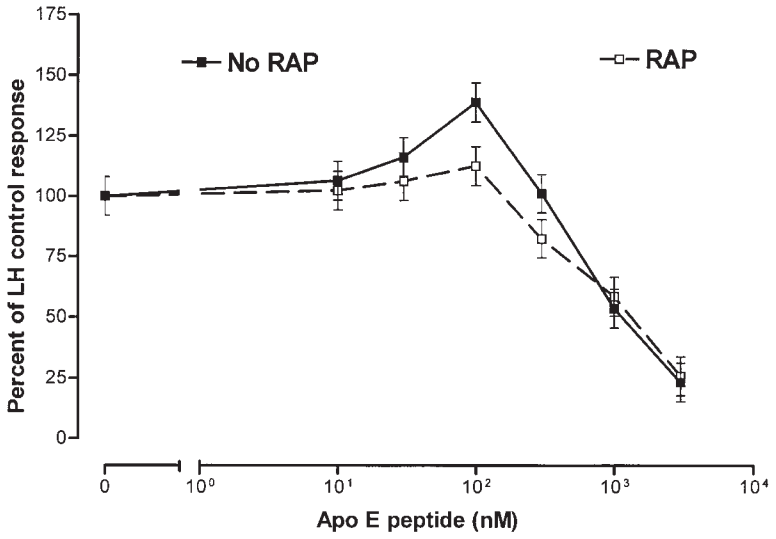

Fig. 2. RAP suppressed the apoE peptide-mediated increase in androstenedione production. TIC (50,000 cells/0.25 ml medium/well) were stimulated with LH (1.0 ng/ml) and increasing apoE peptide concentrations ± RAP (100 nm). After 48 h the culture supernatants were collected and stored at −20°C until the amount of androstenedione was determined by specific RIA. The data are expressed as the percentage of the LH or LH + RAP control conditions. The data are the mean of 4 replicates ± SEM and are representative of 4 individual experiments.

significant decrease from 16.2 ± 1.1 to 9.64 ± 0.1 ng/ml in LH-stimulated androstenedione production in the control cells. Because RAP alone altered the response to LH, the results shown in **Fig. 2** were expressed as the percent response of the control treatments: LH or LH + RAP. RAP pretreatment of the TIC eliminated the apoE synthetic peptide-mediated increase in androstenedione production. RAP changed the shape of the response curve, ablating the enhancement of androstenedione production without changing the inhibition of androstenedione production observed at higher apoE synthetic peptide concentrations. RAP (100 nm) had no effect on progesterone production in either the control cells or those with apoE synthetic peptide present (data not shown).

ApoE selectively altered the level of mRNA for lyase

We suspected that the apoE synthetic peptide-mediated changes in androstenedione production reflected equivalent changes in the level of TIC mRNA for lyase. To test this postulate we developed a new semiquantitative RT-PCR–ELOSA to analyze the changes in lyase and SCC mRNA levels in TIC. First, to validate the RT-PCR–ELOSA, we analyzed the changes in TIC lyase and SCC mRNA in response to increasing LH concentrations. In preliminary experiments we determined that the optimum time to collect TIC total RNA to measure both SCC and lyase mRNA was after 14–18 h of LH stimulation (data not shown). As shown in **Fig. 3A**, **3B**, both progesterone and androstenedione production paralleled the changes in mRNA levels of SCC and lyase, respectively, as a function of LH concentration. Progesterone and SCC mRNA reached a plateau at LH concentrations ≥3.0 ng/ml. In contrast, androstenedione and lyase mRNA levels peaked at 3.0 ng/ml of LH and at greater concentrations of LH both outcomes returned to levels observed in unstimulated TIC.

Using the RT-PCR–ELOSA we found that the apoE synthetic peptide selectively altered the mRNA for lyase without changing the mRNA for SCC (open symbols, **Fig. 4A**, **4B**). There was no pattern to the changes in the mRNA levels for SCC over the full range of apoE synthetic peptide concentrations. In contrast, apoE synthetic peptide at concentrations ≤100 nm caused significant increases in lyase mRNA and, at concentrations >300 nm, caused significant decreases in lyase mRNA.

RAP pretreatment of the TIC attenuated the apoE synthetic peptide-mediated increase in TIC mRNA for lyase without altering mRNA for SCC (solid symbols, Fig. 4A, 4B). RAP caused a significant decrease in mRNA for lyase when it was present with apoE synthetic peptide concentrations 1–30 nm and 300–3000 nm. RAP alone caused a significant decrease in control levels of mRNA for lyase without changing levels of SCC mRNA in control cells.

ApoE synthetic peptide altered lyase mRNA levels at the level of gene transcription

The previous observations were based on the analysis of steady state levels of mature mRNA for lyase and SCC. We next asked whether the changes in lyase mature message levels were due to changes in the rate of gene transcription and/or message stability. To measure the rate of transcription for lyase and SCC mRNA, we modified the RT-PCR–ELOSA to measure heterogeneous nuclear RNA (15). It was necessary to use this surrogate assay for the in vitro transcription run-on assay because the number of nuclei required to perform a standard nuclear run-on assay was not practical when using primary TIC preparations (18). To validate this assay, we measured hnRNA levels for SCC and lyase made in response to increasing LH concentrations. Again, β-actin hnRNA was measured and used to normalize for differences in total RNA recovery. The amount of hnRNA present at a given time is a function of the rate of transcription of the target gene and the rate of processing of the immature hnRNA into mRNA (15). If the rate of processing of the hnRNA is constant and not changed by the experimental treatment, then the changes in the hnRNA levels can be attributed to transcription. As a control, hnRNA RT-PCR was run without the RT step to prove that the hnRNA RT-PCR products were amplified from RNA and not genomic DNA. This control was used in the hnRNA measurements and

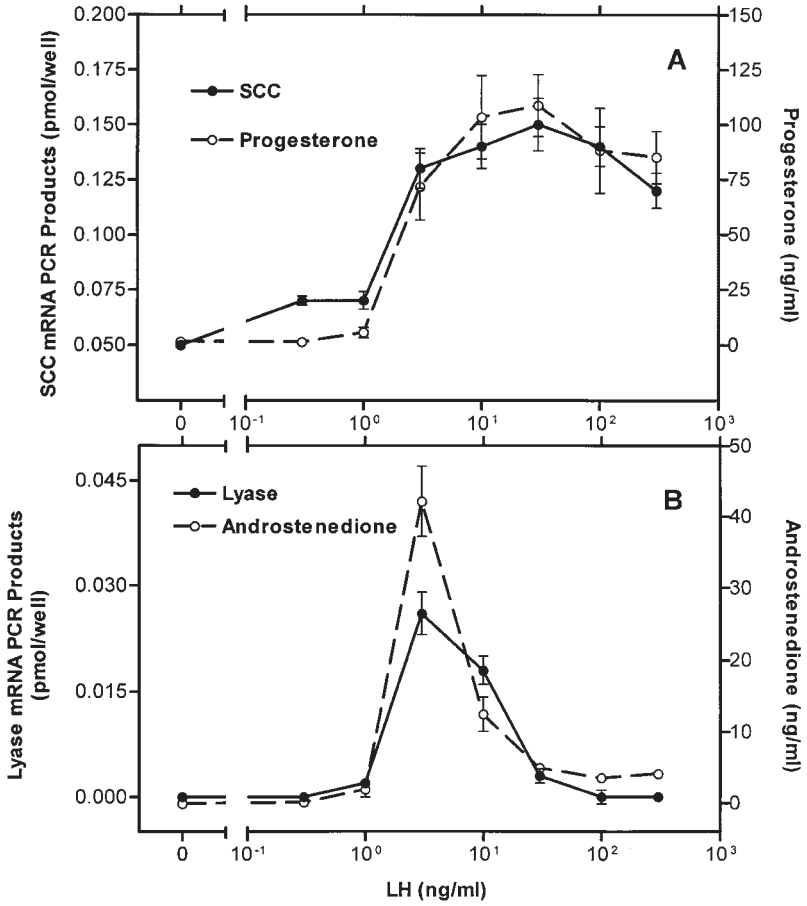

Fig. 3. SCC and lyase mRNA levels paralleled the change in steroid products stimulated by increasing LH concentrations. TIC (50,000 cells/0.25 ml medium/well) were cultured in the presence of increasing LH concentrations. After 18 h the total RNA was extracted from the TIC pooled from 6 wells and the levels of mRNA for SCC and lyase were measured by the RT-PCR–ELOSA. Androstenedione and progesterone were measured by RIA in the culture supernatants collected at 48 h from a duplicate culture plate. The data are the mean of 4 replicates ± SEM and are representative of 5 individual experiments.

PCR products were never observed when the RT step was left out.

HnRNA for SCC and lyase increased with increasing LH concentration (**Fig. 5**). As before, with increasing concentrations of LH, the amount of hnRNA for lyase was at first increased and then decreased, whereas hnRNA for SCC was increased and sustained at higher LH concentrations. The same shape and relative position of the response curves of SCC and lyase hnRNA as a function of LH concentration were observed when the levels of SCC and lyase mRNA were determined from the same samples (data not shown). Therefore, the rate of hnRNA processing was constant and the level of hnRNA was representative of the rate of transcription of the target gene.

Over the course of the experiments reported here, we observed that the TIC sensitivity to LH concentration varied from one cell preparation to another. For instance, in the experiment shown in Fig. 3B, the LH concentration that caused the greatest production of androstenedione and lyase mRNA was 3.0 ng/ml, whereas, in Fig. 5, the LH concentration that caused the greatest enhancement of both hnRNA and mRNA for lyase was 0.3 ng/ml. The variation in the concentration of LH that stimulated the maximum SCC and lyase hn/mRNA response, which always co-varied, differed from preparation to preparation and also reflected differences in the maximum levels of androstenedione and progesterone produced by each TIC isolation. Importantly, in spite of these changes in absolute levels, the pattern of SCC hnRNA-mRNA/progesterone and lyase hnRNA-mRNA/androstenedione responses to LH concentration always had the same characteristic shape curve.

The apoE synthetic peptide had no effect on the levels of hnRNA for SCC stimulated by increasing LH concentrations 0.3, 1.0, 3.0 ng/ml (**Fig. 6**). In contrast, the apoE synthetic peptide up to 100 nm caused increased lyase hnRNA which returned to baseline levels at greater apoE synthetic peptide concentrations (**Fig. 7**). The same pattern of response to apoE synthetic peptide was observed in the mRNA for SCC and lyase measured in the same samples, indicating that there was no change in the rate of hnRNA processing (data not shown) and the hnRNA levels represented the rate of transcription.

ApoE synthetic peptide did not alter the stability of SCC and lyase mRNA

The half-lives of SCC mRNA and of lyase mRNA were determined after actinomycin D was used to inhibit new mRNA synthesis (19). The rates of degradation of SCC mRNA and lyase mRNA were measured using the RT-PCR–ELOSA. At 1.0 ng/ml of LH, the half-life of SCC was 3.68 ± 0.05 h and was 0.49 ± 0.01 h for lyase mRNA. The apoE synthetic peptide, from 10–3000 nm concentrations, had no effect on the half-life for either SCC mRNA, 3.58 ± 0.11 h, or lyase mRNA, 0.47 ± 0.13 h, respectively.

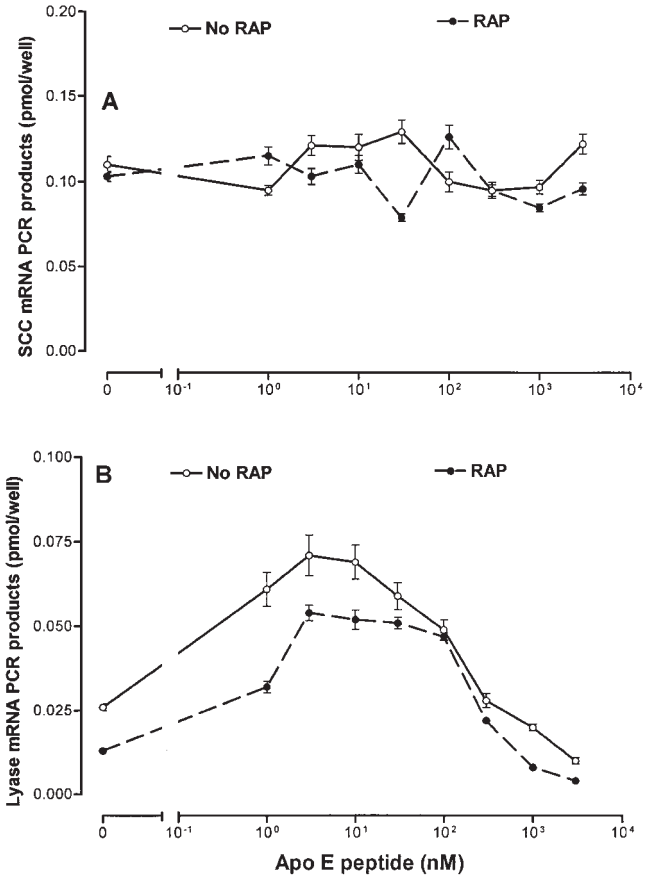

Fig. 4. RAP suppressed the apoE peptide-mediated increase in mRNA for lyase. TIC (50,000 cells/0.25 ml medium/well) were stimulated with LH (1.0 ng/ml) and treated with increasing concentrations of apoE peptide ± RAP (100 nm). After 18 h of culture the total RNA was extracted from the TIC pooled from 6 wells and the levels of mRNA for SCC and lyase were measured by the RT-PCR–ELOSA. The data are the mean of 4 replicates ± SEM and are representative of 2 individual experiments.

DISCUSSION

The apoE synthetic peptide containing the LDL receptor binding domain mimicked native apoE and selectively altered TIC androgen production. Because TIC were cultured in the absence of serum, the apoE synthetic peptide was active in the absence of lipoproteins and thus appeared to exert its effects independent of exogenous cholesterol supply. The apoE synthetic peptide acted at the level of lyase gene expression by altering the rate of transcription in a biphasic manner. Low concentrations of apoE synthetic peptide stimulated lyase gene transcription mediated via a pathway that was dependent on one or more members of the LDL receptor superfamily. Higher concentrations of apoE synthetic peptide inhibited lyase gene transcription but this activity was not mediated by a known apoE-specific receptor. The transcription of SCC gene and production of the steroid product, progesterone, were not altered by any concentration of the apoE synthetic peptide.

Ovarian TIC do not have basal androgen production (2). Low levels of LH stimulate lyase gene expression, whereas higher LH concentrations similar to those that occur during the LH surge in vivo suppress lyase gene expression (20–23). ApoE production and secretion by cultured TIC is increased with increasing LH concentration (data not shown). Therefore, during follicular development when LH concentration and apoE production are low, the combined result would be enhancement of androgen production by TIC. At high LH concentrations and greater apoE production, the combined result would be to suppress TIC androgen production. Thus, apoE may function as an autocrine modulator of LH-stimulated TIC androgen production.

Lyase gene expression is regulated at the level of transcription primarily via cAMP-mediated signal transduction through the protein kinase A pathway (10). There are species-specific and tissue-specific cAMP-dependent *cis*-acting response elements that control lyase gene expression in the adrenal and testes (10, 24–26). However, the tissue-specific regulation of lyase gene expression in the TIC has not been examined. It is unclear whether the regulatory elements identified in the adrenal and testes will be equally important for the regulation of ovarian expression of the lyase gene.

ApoE expression suppresses steroidogenesis of Y1 cells (27). Y1 cells are an adrenal cell line that normally does not express apoE. However, the expression of a transfected human apoE gene completely suppresses Y1 cell steroidogenesis. In Y1 cells, apoE exerts pleiotropic effects including suppression of basal and cAMP-stimulated steroidogenesis and elevation of protein kinase C activity that together suppress basal steroidogenesis (28). The apoE synthetic peptide altered TIC steroidogenesis at a point after SCC expression and activity, well after the point at which apoE suppresses Y1 cell steroidogenesis. Therefore, the mechanisms responsible for inhibition of steroidogenesis in TIC and Y1 cells are not likely to be the same.

It was unexpected that RAP, in the absence of added apoE, suppressed lyase mRNA without altering SCC mRNA. TIC have abundant mRNA for apoE (1) which is up-regulated when the whole animal is treated with hCG (29). In addition, cultured TIC produce and secrete increasing amounts of apoE with increasing concentrations of LH (data not shown). Thus, RAP may have suppressed lyase mRNA by blocking the apoE-specific receptors that mediate the stimulation of lyase gene expression by native, endogenous apoE secreted by the TIC. These data suggest that TIC-derived apoE participates in the cells' response to hormone stimulation as an autocrine/paracrine modulator.

RAP attenuated the apoE synthetic peptide-mediated increase in lyase mRNA and androstenedione production, indicating that one or more of the LDL receptor gene superfamily members was involved in the increased TIC response. There are six candidate receptors all expressed in the whole ovary, gp330 or megalin (30), LDL receptor-related protein (LRP) (30), LR11 (31), LR7/LR8 (32, 33), very low density lipoprotein (VLDL) receptor (34),

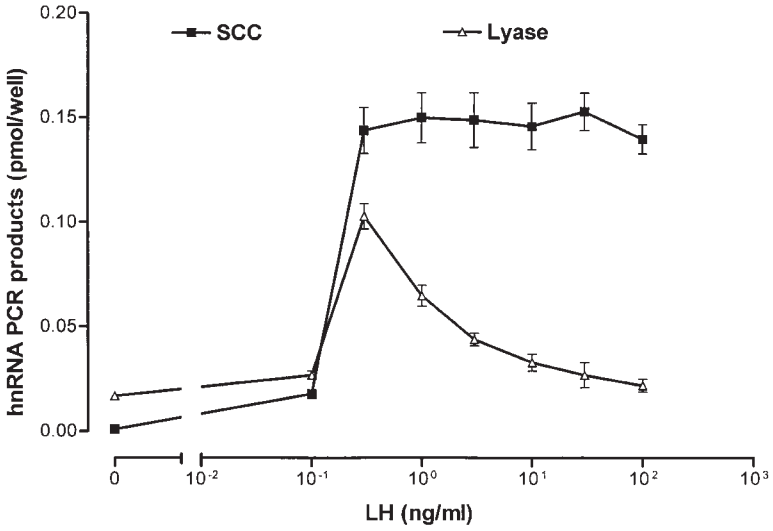

Fig. 5. Increasing LH concentrations altered the rate of transcription for SCC and lyase. TIC (50,000 cells/0.25 ml medium/well) were stimulated with increasing LH concentrations. After 14 h of culture the total RNA was extracted from the TIC pooled from 6 wells and the levels of hnRNA for SCC and lyase were measured by the RT-PCR–ELOSA. The data are the mean of 4 replicates ± SEM and are representative of 3 individual experiments.

and the LDL receptor. Direct binding of the peptide to LDL receptors on fibroblasts has been observed (7). However, which receptors and when in the estrus cycle any or all are expressed by TIC has not been determined. It is unclear whether the apoE synthetic peptide acts via these receptors. If it does, it could initiate a signal upon binding and/or after it is endocytosed and released into the cytoplasm from the lysosomal compartment. Either means would represent a novel alteration of gene expression by apoE.

The amount of mRNA for SCC was always several-fold greater than the level of mRNA for lyase. The greater abundance of SCC mRNA could have resulted from more being expressed per cell or more cells expressing SCC mRNA. The second possibility is most likely as both theca and interstitial cells express SCC mRNA (35), whereas, only theca cells of developing follicles express lyase mRNA (36). The LH receptor is expressed by both theca and interstitial cells (3). Moreover, in response to stimulation, both cell types would make SCC mRNA and progesterone, whereas only the theca cells would make lyase mRNA and androstenedione, probably providing an explanation for the abundance of SCC mRNA and equal or greater progesterone production relative to lyase mRNA and androstenedione production.

Androgen production by the ovary is essential for estrogen production and consequently controls follicular development (3). Follicular maturation is controlled by lyase gene expression that provides adequate substrate for estrogen production by the granulosa cells of the growing follicle (3). In contrast, too much androgen results in the demise of the follicle by causing the granulosa cells to become apoptotic (4). Thus, the fate of the follicle is a function of the balance of androgen production which can be modulated by apoE.

ApoE knockout mice that have had the apoE gene deleted by homologous recombination are fertile. However, apoE knockout male mice, when compared to wild-type mice of the same genetic background, have significantly reduced testes weight relative to body weight, a greater degree of apoptosis in their germ cells, and immature germ cells mixed with mature germ cells, in the tail of the epididymis. As the apoE knockout male mice age, they im-

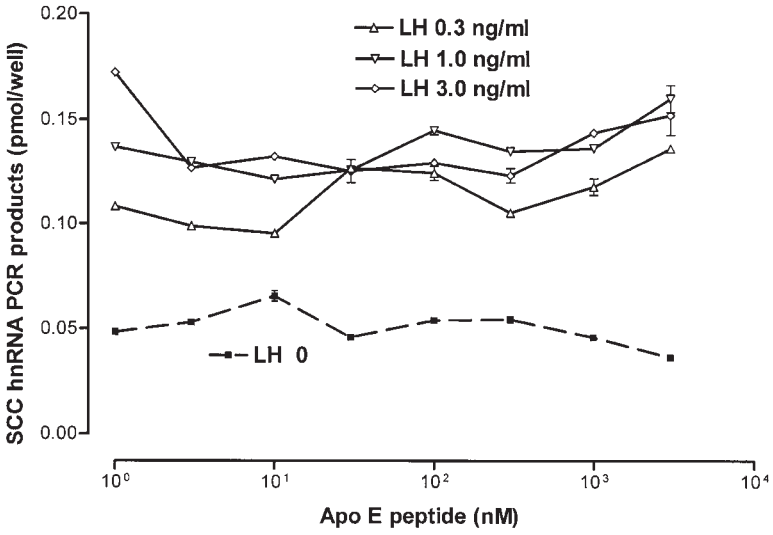

Fig. 6. The rate of SCC gene transcription was unchanged by treatment with the apoE peptide. TIC (50,000 cells/0.25 ml medium/well) were cultured without LH, or stimulated with LH of 0.3, 1.0, 3.0 ng/ml in the presence of increasing apoE peptide. After 14 h of culture the total RNA was extracted from the TIC pooled from 6 wells and the level of hnRNA for SCC was measured by the RT-PCR–ELOSA. The data are the mean of 4 replicates ± SEM and are representative of 2 individual experiments.

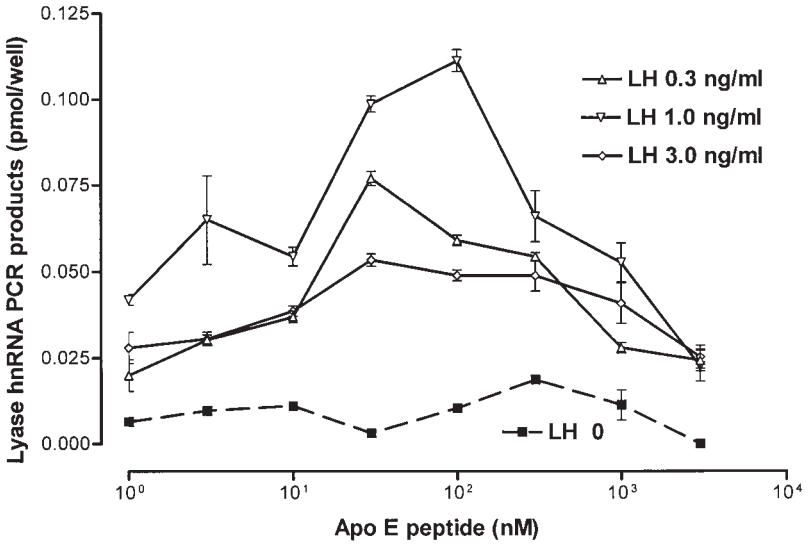

Fig. 7. The rate of lyase gene transcription was dependent on the apoE peptide concentration. TIC (50,000 cells/0.25 ml medium/well) were cultured without LH, or stimulated with LH of 0.3, 1.0, 3.0 ng/ml in the presence of increasing apoE peptide. After 14 h of culture the total RNA was extracted from the TIC pooled for 6 wells and the level of hnRNA for lyase was measured by the RT-PCR–ELOSA. The data are the mean of 4 replicates ± SEM and are representative of 2 individual experiments.

pregnate fewer wild-type female mice and have lower reproductive efficiency than their normal counterparts (37). Comparable measures of reproductive function in female apoE knockout mice have not been reported. Thus, apoE is not essential to reproduction but necessary, because when it is absent male mouse reproductive function is significantly compromised.

ApoE's role in cell biology and pathology has grown due to the recent appreciation of its pivotal involvement in atherosclerosis (38, 39) and Alzheimer's disease (40). In these diseases, as in the TIC, apoE acts to alter the phenotype of select cell populations via mechanisms that are independent of its role as a cholesterol transport protein (41, 42). Further understanding of how apoE acts to change lyase gene expression will likely be applicable to understanding how apoE acts to alter cell function and phenotype in atherosclerosis and Alzheimer's disease.

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