Activation of Apolipoprotein AI Gene Expression by Protein Kinase A and Kinase C through Transcription Factor, Sp1*

Received for publication, January 27, 2000, and in revised form, May 9, 2000
Published, JBC Papers in Press, May 26, 2000, DOI 10.1074/jbc.M00621200

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Our previous finding that insulin induces apolipoprotein AI (apoAI) transcription points to the participation of intracellular signaling. This finding prompted us to ask whether two classical G-protein-coupled signaling pathways requiring activated protein kinase A (PKA) or kinase C (PKC) may also regulate apoAI. Therefore, human hepatoma, Hep G2 cells stably transfected with pAI.474-CAT, a reporter construct spanning −474 to −7 of apoAI DNA fused to chloramphenicol acetyltransferase (CAT) were treated with 10 μM forskolin (FSK) or 50 nM phorbol dibutyrate (PDBu) to activate PKA and PKC, respectively. Results showed that the apoAI promoter activity increased 4–5-fold following 24 h of treatment with either FSK or PDBu. Induction by either agent was blocked with actinomycin D but not the protein synthesis inhibitor, cycloheximide. The PKA inhibitor was blocked with actinomycin D but not the protein synthesis inhibitor, cycloheximide. The PKA inhibitor, PKI 14–22 amide, abrogated induction by FSK, 100 μM 8-bromo-cAMP, or 100 ng/ml cholera toxin, but it had no effect on activation via PKC. Similarly, PDBu induction was attenuated by 2 μM of the PKC inhibitor, GF109203X, but it did not affect FSK activity. Next we used deletional constructs to show that the actions of FSK and PDBu required the insulin-responsive core element (IRCE). This motif matched the consensus binding site for the transcription factor, Sp1. The binding of Sp1 to the IRCE was confirmed by gel-retardation and supershift analysis. Site-directed mutagenesis of the IRCE eliminated Sp1 action and induction by FSK or PDBu. Whereas overexpression of Sp1 enhanced basal and FSK or PDBu induced promoter activity, transfection of an antisense oligomer against Sp1 mRNA attenuated both parameters. In summary, activation of PKA or PKC increases apoAI promoter activity. The activity of both signaling pathways is mediated by the IRCE, a motif that binds the transcription factor, Sp1.

Apolipoprotein AI is the dominant and most important structural protein component of the antiatherogenic high density lipoprotein (HDL)1 particles (1, 2). This protein acts as a cofactor that enhances the activity of an enzyme, lecithin cholesterol acyltransferase. Enhanced activity of this enzyme augments a normal physiologic process, so called “reverse cholesterol transport” (RCT), whereby cholesterol is transported from extrahepatic cells to the liver for excretion in the form of bile salts or free cholesterol (3, 4). Increased RCT lowers total body cholesterol, and therefore, it is not surprising that numerous epidemiologic studies have shown an inverse relationship between plasma concentrations of HDL and the risk of coronary heart disease (5–7). Given the pivotal role of apolipoprotein AI, apoAI in the function of HDL, and the antiatherogenic properties of these particles, the identification of the mechanisms that increase levels of apoAI synthesis and HDL is of great interest and potential therapeutic importance.

Hormonal control of apoAI expression is an attractive way to manipulate apoAI gene activity, because the simple addition or removal of the agent will regulate gene activity. Therefore, many laboratories, including our own, have examined the ability of thyroid hormone, glucocorticoids, estradiol, androgens, retinoic acid, and insulin to modulate activity of the gene (8–11). Whereas the action of all of the preceding hormones except for insulin (9) is triggered by ligand binding to intracellular receptors that act within the nucleus, insulin is different in that its activity is mediated by a membrane-bound tyrosine kinase receptor. This latter finding (9) suggests new avenues for controlling apoAI gene expression and prompted us to ask whether other intracellular signal transduction pathways initiated from the cell membrane might also affect apoAI expression. To investigate this possibility, we examined the potential role of activated protein kinase A (PKA) and kinase C (PKC). These kinases play critical roles in separate classical intracellular signaling pathways regulated by G-protein-coupled receptors. Unexpectedly, the stimulatory effects of both pathways are mediated by a single transcription factor that converges on the insulin-responsive core element, IRCE (9).

EXPERIMENTAL PROCEDURES

Plasmid Constructs—Construction of the reporter, pAI.474-CAT, was described previously (12). The deletional constructs; pAI.425-CAT, pAI.375-CAT, and pAI.325-CAT containing rat apoAI DNA spanning −425, −375, −325, and −235 to −7 were synthesized using the parent pAI.474-CAT as template in separate PCR as described previously (12). The wild-type IRCE (−411 to −404), GAGGCGGG, was mutated to TCTTATTG by using a primer containing these transverse mutations in a PCR. The construct containing an internal deletional of

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1 The abbreviations used are: HDL, high density lipoprotein; RCT, reverse cholesterol transport; PKA, protein kinase A; PKC, protein kinase C; IRCE, insulin-responsive core element; CAT, chloramphenicol acetyltransferase; PDBu, phorbol dibutyrate; RT-PCR, reverse transcription polymerase chain reaction; FSK, forskolin; EMSA, electrophoretic mobility shift assay; CTX, cholera toxin; PKI, PKA inhibitor.
nucleotides –208 to –193 in the apoAI promoter was created by digesting pAL474-CAT with PsI (Amer sham Pharmacia Biotech), which delets a 48-base pair insert followed by ligation to circularize the large fragment. Expression plasmids for Sp1, TrB, HNF-3, or HNF-4 were kind gifts from Drs. R. Tjian (University of California, Stanford, CA), M. Pfaller (Sidney Kimmel Cancer Center, La Jolla, CA), R. Costa (University of Illinois, Chicago, IL) and M. Sladek (University of California, Irvine, CA), respectively.


tie Culture—The human hepatoma cells, Hep G2, were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% bovine calf serum (Life Technologies, Inc.) and penicillin/streptomycin at 37 °C. Cells were cultured overnight in serum-free medium to expose to other PKA activators; 100 μM 8-bromo-cAMP (cAMP) or 100 ng/ml cholera toxin (CTX) with or without the PKA inhibitor, PKI (2 μM). The ** denotes a significant (p < 0.01) decrease of induction in the presence of the inhibitor plus activator compared with activator alone following analysis using analysis of variance.

**RESULTS**

**Activation of Either PKA or PKC Stimulates ApoAI Promoter**—The following studies were facilitated by the creation of Hep G2 cells that were stably cotransfected with pAL474-CAT and a plasmid carrying neomycin resistance. We isolated 14 colonies that were resistant to neomycin, but only 6 of these had measurable CAT activity. All 6 colonies were tested for CAT activity (9). Synthetic DNA duplexes spanning –419 to –388, ACTTTGAGCCGGGATTGTGAGT, or the recognition site for TFIID (Promega) were radiolabeled at the 5'-ends by incubating each strand separately with [γ-32P]ATP and polynucleotide kinase (A mer sham Pharmacia Biotech) prior to annealing. Each binding reaction contained 10 μM Tris-HCl, 50 mM NaCl, 0.5 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, 1.0 μg of poly(dI-dC), 1 fmol of radiolabeled probe, and 10 μM of nuclear extract. In competition studies, 200-fold excess of unlabeled homologous (–419 to –388 or Sp1 consensus, ATTCCATGGGGGGGGCCGAG) or nonhomologous competitor DNA (mutant IRCE, ACTTTTETATGATGTGAGT) was added in the reaction prior to the addition of nuclear extracts. Antibodies used in supershift experiments were added to nuclear extracts at 4 °C for 60 min prior to their use in EMSA. The antibodies against human Sp1, Sp2, and Sp3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All reactions were incubated at room temperature for 20 min and then separated on a 5% polyacrylamide nondenaturing gel (9). Electrophoresis was performed at 10 volts/cm for 3 h at 4 °C. The gel was then dried and exposed to Kodak XAR-5 film at –80 °C in the presence of intensifying screens.

**RNA Preparation and RT-PCR**—Total RNA from cells was extracted using TRIReagent (Molecular Research Center, Cincinnati, OH). The RNA was reverse-transcribed with a first strand cDNA synthesis kit (Promega) with or without the PKA or PKC inhibitors: PKI and GF, respectively. A (upper panel) shows an autoradiograph of CAT activity in stable Hep G2 cells harboring the pAL474-CAT construct treated with 10 μM FSK and 50 nM PDBu for 24 h with or without the PKA or PKC inhibitors: PKI and GF, respectively. A (lower panel) shows Western blot analysis of apoAI protein in the lysates and corresponding spent media from stably transfected Hep G2 cells exposed to either FSK or PDBu as indicated below each lane. B contains a graph of the mean ± S.E., n = 6 repeats of the same studies as in A. C shows a graph of the mean ± S.E., n = 6 separate studies of cells exposed to other PKA activators; 100 μM 8-bromo-cAMP (cAMP) or 100 ng/ml cholera toxin (CTX) with or without the PKA inhibitor, PKI (2 μM). The ** denotes a significant (p < 0.01) decrease of induction in the presence of the inhibitor plus activator compared with activator alone following analysis using analysis of variance.

**Fig. 1. Induction of apoAI promoter activity by activating PKA and PKC.** A (upper panel) shows an autoradiograph of CAT activity in stable Hep G2 cells harboring the pAL474-CAT construct treated with 10 μM FSK and 50 nM PDBu for 24 h with or without the PKA or PKC inhibitors: PKI and GF, respectively. A (lower panel) shows Western blot analysis of apoAI protein in the lysates and corresponding spent media from stably transfected Hep G2 cells exposed to either FSK or PDBu as indicated below each lane. B contains a graph of the mean ± S.E., n = 6 repeats of the same studies as in A. C shows a graph of the mean ± S.E., n = 6 separate studies of cells exposed to other PKA activators; 100 μM 8-bromo-cAMP (cAMP) or 100 ng/ml cholera toxin (CTX) with or without the PKA inhibitor, PKI (2 μM). The ** denotes a significant (p < 0.01) decrease of induction in the presence of the inhibitor plus activator compared with activator alone following analysis using analysis of variance.
ApoAI is a secreted protein. Thus, abundance of the protein in culture media from cells treated with FSK or PDBu (Fig. 1A, bottom panel) were higher versus control. These findings show that both FSK and PDBu increase not only activity of the promoter but also endogenous expression of the apoAI protein.

The effect of inhibitors and other activators (Fig. 1, A–C) were tested to confirm further the role of both PKA and PKC pathways in regulating apoAI activity. 8-Bromo-cAMP and cholera toxin (CTX) are two additional activators of PKA. CAT activity (Fig. 1C) in stably transfected cells increased 4- and 3.5-fold following 24 h of exposure to 100 μM 8-bromo-cAMP or 10 ng/ml CTX, respectively (20). Induction by FSK, 8-bromo-cAMP, or CTX was inhibited in the presence of 1 μM amount of a PKA inhibitor, PKI (21, 22). Similarly, apoAI induction by PDBu was blocked in the presence of 2 μM GFX (Fig. 1B), a specific inhibitor of PKC (23). The inhibitors acted specifically on their respective pathways, because PKI did not affect induction by PDBu, and the actions of FSK were not inhibited by GF (data not shown). Additionally, promoter activity in cells exposed to both 10 μM FSK plus 50 nM PDBu was no greater than either one alone (Fig. 1, A and B). Together these findings show that activation of signaling pathways, mediated by PKA or PKC, increase apoAI promoter activity.

Rapid Induction of ApoAI Promoter by FSK and PDBu Is through Transcription Events—Next we examined the time course associated with the induction by FSK and PDBu (Fig. 2A). The results showed a significant increase in CAT activity within 3 h following exposure to either 10 μM FSK or 50 nM PDBu. The initial increase was followed by a progressive rise in CAT activity that reached submaximal levels within 24 h (Fig. 2B).

The rapid induction of apoAI promoter activity by FSK and PDBu prompted us to ask whether it required a transcriptional or post-transcriptional process. The results (Fig. 2, C and D) showed that induction by FSK and PDBu was abrogated by 1 μM actinomycin D, an inhibitor of transcription. As expected, induction of CAT activity by either FSK or PDBu was completely abolished in the presence of 10 μM cycloheximide, a protein synthesis inhibitor, because it blocked translation of CAT-mRNA. Therefore, to assess activity of pAI.474-CAT in the presence of cycloheximide, we used RT-PCR to measure the levels of CAT-mRNA. Whereas, actinomycin D blocked the induction of CAT-mRNA by either agent, cycloheximide had no effect on FSK or PDBu induction of CAT-mRNA (Fig. 2C, lower panel, and D). These observations show that FSK and PDBu increases transcriptional activity of the apoAI promoter.

Actions of FSK or PDBu Require the IRCE—The preceding observation raises the question of how two separate intracel-
IRCE increased in cells treated with either agent but that bound to the TFIID motif was the same in control and treated cells (Fig. 4A, lanes 4–9). The IRCE binding activity was specific, because formation of the protein-DNA complexes (Fig. 4B) was completely abolished by including either excess of nonradioactive IRCE or consensus Sp1 binding motif; but the mutant IRCE failed to affect formation of the complexes. There are at least three isoforms belonging to the Sp family of proteins. To determine the specific isoform bound to the IRCE, antibodies against Sp1, Sp2, and Sp3 were added to separate EMSA reactions. Whereas, Sp1 antibody supershifted the complexes, neither Sp2 nor Sp3 (data not shown) affected their mobility. The preceding studies show that the transcription factor, Sp1, binds to the IRCE.

Sp1 Stimulates ApoAI Promoter and Augments Induction by FSK and PDBu—In addition to binding to Sp1 to the IRCE, it should also exert a functional effect. To examine the functional role of Sp1, we transfected an expression vector that overexpressed Sp1 into three (numbers 2, 8, and 10) separate colonies of stable Hep G2 cells. Enhanced Sp1 expression increased basal apoAI promoter activity in all three clones by 2-fold (Fig. 5, data for one clone) compared with cells transfected with empty vector. If Sp1 mediated the effects of FSK and PDBu, then enhanced expression of the factor should augment apoAI induction by these agents. To test this hypothesis, we treated stable Hep G2 cells that overexpressed Sp1 with submaximal doses of FSK (2 μM) or PDBu (10 nM). Either agent alone caused a 2–2.5-fold increase of CAT activity in stable cells without exogenous Sp1. In contrast, CAT activity increased 6–7-fold in cells that overexpressed Sp1. These findings show that Sp1 overexpression increases apoAI promoter activity and augments its induction by FSK and PDBu.

Decreasing Sp1 Attenuates Induction by FSK or PDBu—If Sp1 plays an important role in mediating the actions of FSK and PDBu, then the converse of the above approach, i.e., decreasing levels of Sp1, should attenuate the actions of these agents. To test this hypothesis, we lowered intracellular levels of Sp1 using an antisense approach (17, 18). Results showed that in the presence of antisense Sp1, basal CAT activity in stably transfected cells decreased by 60% relative to control cells (Fig. 6, A and B). In contrast, the sense oligomer that targets the same sequence in Sp1 mRNA had no effect on CAT activity. Furthermore, induction of apoAI promoter activity by either FSK or PDBu was abrogated in cells containing Sp1 antisense, but not sense, oligonucleotides (Fig. 6, C and D).

Next we used Western blot analysis to confirm that Sp1 antisense oligonucleotides did indeed reduce levels of the protein. Results (Fig. 6, E and F) showed a 65–68% reduction in the level of Sp1 protein in cells containing Sp1 antisense but not sense oligonucleotides compared with nontransfected cells. Furthermore, neither FSK nor PDBu had a significant effect on the levels of Sp1 protein in these stably transfected cells. Together these findings show that Sp1 plays a key role in mediating FSK and PDBu induction of apoAI promoter activity.

IRCE Acts Independently—The following studies examine whether the actions of the IRCE may be linked to adjacent cis-acting elements. To address this question we performed separate transfections with vectors that enabled the stable cells to overexpress the thyroid hormone receptor α (TRα), HNF-3β, or HNF-4. These factors modulate the actions of sites A, B, and C, respectively, in the apoAI promoter (reviewed in Ref. 8). CAT activity in the transfected cells was compared with the untreated control. Results (Table I) showed that FSK and PDBu induction of CAT activity in the transfected with TRα, HNF-3β, or HNF-4 cells were not different from that in the control cells exposed to these agents (compare induced values
This observation suggests that IRCE-mediated induction by FSK and PDBu was not affected by adjacent elements A, B, and C. Next we wondered whether the consensus Sp1 motif (−220 to −211) located 3′ to the IRCE contributed to activity of the promoter. To investigate this possibility, we created a construct that contained an internal deletion from −208 to −193. CAT activity in cells transiently transfected with the deletional mutant was activated 5.6 ± 0.8- and 6.5 ± 0.9-fold by FSK or PDBu, respectively, compared with control. This response is similar to that of the wild type promoter (“None” column in Table I). Together these findings show that IRCE activity was not influenced by adjacent cis-acting elements A, B, and C. In addition, removal of the Sp1 site 3′ to the IRCE played an insignificant role in the FSK and PDBu induction of apoAI promoter activity.

**DISCUSSION**

The pivotal role of apoAI in HDL particles, and their function in mediating RCT (3, 4), underlies our interest to investigate the expression of this gene. Increased abundance of apoAI...
The top 31752 (treatment group. A schematic diagram showing the relative location of the mean and S.D. for at least three repetitions of each transfection and function of sites A, B, and C, respectively. Each value in the table shows express TR
the introduction of no additional plasmid (none) or ones that over
transfected Hep G2 cells containing the reporter pAI.474-CAT following
5–7,
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with transcriptional activity (reviewed in (8)), we recently
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that examine hormonal regula-

Table I, shows FSK or PDBu induction of CAT activity in stably
apoAI promoter
that examine hormonal regula-

Our previous studies (9) show that insulin stimulates apoAI
gene transcription. Insulin regulation of the apoAI promoter
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of apoAI by a family of small lipophilic ligands including
apoAI promoter
that examine hormonal regula-

enhances RCT, thereby lowering the levels of cholesterol in the
body. Decreased levels of cholesterol reduce the risk of cardio-
vascular disease. Therefore, a better understanding of the
mechanism(s) underlying the induction of the apoAI gene will
help us find new ways to decrease heart disease.

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shows that insulin, a peptide hormone, also activates the
gene. This finding prompted us to ask whether other intracel-
ular signaling pathways may also regulate apoAI expression.
Therefore, we examined the potential involvement of two clas-
sical signaling pathways mediated by PKA and PKC.

To perform these studies, we created Hep G2 cell stably
transfected with the apoAI reporter, pAI.474-CAT. CAT activity
in these cells increased up to 4–5-fold following treatment
with FSK or PDBu to activate PKA and PKC, respectively. In
the same cells, endogenous expression of apoAI protein was
noted in both lysates and media (Fig. 1). That activation of PKA
stimulates apoAI promoter activity is supported by four inde-
dent lines of evidence (Fig. 1): 1) FSK, a direct activator of
adenyl cyclase, raises cAMP levels thereby activating PKA
and enhances activity of the apoAI promoter; 2) CTX, an irre-
versible activator of Goα, also increases cAMP and stimulates
activity of the promoter and parallels the actions of FSK; 3)
8-bromo-cAMP, a direct activator of PKA via its binding to the
enzyme’s regulatory subunit, like cAMP, increases apoAI ac-
tivity and mimics the actions of FSK or CTX; and 4) the PKA
inhibitor, PKI (21, 22), reverses the effects of all three activa-
tors. PDBu specifically activates PKC by binding directly to
this enzyme. The ability of PDBu to stimulate promoter activity
was blocked by the PKC inhibitor, GF109203X, that unlike
staurosporin (often used for PKC inhibition) does not affect
tyrosine kinase activity. Moreover, it is important that the PKC
inhibitor, GF109203X (23), did not diminish the stimulatory

Table I
Effect of adjacent cis-acting sites on FSK and PDBu induction of the
apoAI promoter

| Treatment | Transfection | None | TRα | HNF-3β | HNF-4 |
|-----------|--------------|------|-----|--------|--------|
| FSK (10 μM) | – | 1 | 0.6 ± 0.2 | 2.3 ± 0.6 | 1.8 ± 0.3 |
| | + | 4.6 ± 0.5 | 4.8 ± 0.8 | 4.8 ± 0.7 | 4.3 ± 0.8 |
| PDBu (50 nM) | – | 1 | 0.6 ± 0.2 | 2.3 ± 0.6 | 1.8 ± 0.3 |
| | + | 5.1 ± 0.6 | 5.4 ± 0.9 | 5.2 ± 0.8 | 5.0 ± 0.7 |

FIG. 6. Decreasing Sp1 reduces the actions of FSK and PDBu. A shows an autoradiograph of CAT activity in stable cells treated without
(lane 1) and with an antisense (lane 2) or sense (lane 3) Sp1 phosphoribonucleotide. B is a graph of relative CAT activity in cells treated with
conditions noted at the bottom of each bar (mean ± S.E., n = 4; ** denotes a significant difference: p < 0.01, between the group of control cells and
the group treated with antisense). C shows an autoradiograph of CAT activity in the cells exposed to 50 nM PDBu (lanes 2–4) or 10 μM FSK (lanes
5–7). Cells were pretreated with either an antisense (lanes 3 and 6) or sense (lanes 4 and 7) Sp1 phosphoribonucleotide as indicated at the top of
each lane. D shows a graph of relative CAT activity in cells treated with conditions noted at the bottom of each bar (mean ± S.E., n = 4; ** indicates
p < 0.01). E shows a Western blot probed with a Sp1 antibody. The lanes contain cell lysate from Hep G2 cells treated as per conditions noted at the
top. F is a graph of Sp1 protein in cells treated with conditions noted at the bottom of each bar (mean ± S.E., n = 4; ** indicates p < 0.01).
effect of activated PKA, nor does the PKA inhibitor, PKI, affect stimulation of promoter activity by PKC activation. Additionally, the combined action of both FSK and PDBu was no greater than either one alone. These observations clearly show that apoAI promoter activity and endogenous expression of the protein are stimulated by activated PKA or PKC. Furthermore, the specificity of the inhibitors for their respective kinases suggests that these signaling pathways operate in parallel to up-regulate the apoAI gene expression.

Although the preceding results show that PKA and PKC increase apoAI gene activity, the mechanism(s) underlying this induction remains unknown. A variety of mechanism(s) may explain the stimulatory actions of PKA and PKC. To address this question, we used actinomycin D or cycloheximide to block gene transcription and translation, respectively, in the stable Hep G2 cells. The outcome of these studies show that activated PKA or PKC enhances apoAI gene transcription. Therefore, the apoAI promoter should contain cis-acting element(s) that mediate the action of PKA or PKC.

To identify the motif(s) that mediated the stimulatory actions of PKA or PKC, we measured CAT activity in deletional mutants of the promoter following exposure to FSK and PDBu, respectively. Results showed that a critical element was situated between nucleotides −425 and −376 of the gene. Our previous studies showed that the same fragment contained the IRCE, a motif that mediated the stimulatory actions of insulin (9). Therefore, we postulated that the actions of either or both FSK and PDBu were mediated by the IRCE. This hypothesis was tested using a reporter containing a mutated IRCE. Unexpectedly, neither FSK nor PDBu stimulated activity of the mutant construct. This finding suggests that the actions of both agents were mediated by a single cis-acting element, the IRCE.

The pivotal role of the IRCE in mediating PKA and PKC activation of the apoAI expression prompted us to identify the transcription factor(s) that binds to this motif. There were two clues that helped us find a potential candidate that interacted with the IRCE. The first one being that the IRCE was GC-rich, and second, when the motif was scanned using TFSEARCH program (25), it was identical to the recognition sequence bound by the transcription factor, Sp1 (26, 27). To demonstrate the binding of Sp1 to the IRCE, we performed EMSA analysis using nuclear extract from Hep G2 cells. Results show that IRCE binding activity increases in cells treated with FSK or PDBu. This binding is specific, because formation of IRCE complex is inhibited in the presence of excess unlabeled IRCE or authentic Sp1 recognition site but not IRCE oligomer containing the mutated motif. That the IRCE binding activity is indeed Sp1 comes from supershift studies. Whereas, Sp1 antibody supershifted the protein-IRCE complex, neither Sp2 nor Sp3 antibody affected mobility of the band. The role of Sp1 was examined by altering levels of the protein in Hep G2 cells. Overexpression of Sp1 in stable Hep G2 cells augmented the induction of apoAI promoter activity in the presence of FSK or PDBu. Transfection of a Sp1 antisense oligonucleotide into Hep G2 cells to reduce Sp1 protein caused a decrease in the basal activity of the promoter and blocked its induction by either FSK or PDBu. These studies show that Sp1 binds to the IRCE, and this transcription factor mediates apoAI promoter activation by two separate signaling pathways requiring either PKA or PKC.

Over the last few years, several publications have described the participation of Sp1 in the PKA or PKC regulation of specific genes. For example, in doxorubicin-resistant HL-60 leukemia cells, activated PKA phosphorylated Sp1 and enhanced its ability to bind DNA (28). In addition, resistance of breast carcinoma cells to multiple drugs caused by PKA induction of the MDR1 gene also required Sp1 (29). Similarly, Sp1 also mediated the actions of PKC on specific genes. The stimulation of Sp1-mediated vascular permeability factor/vascular endothelial growth factor transcription required an interaction between Sp1 and PKC-ζ (30). More recently, Sp1 was shown to be essential in the 12-O-tetradecanoylphorbol-13-acetate stimulation of human lysosomal acid lipase gene activity in monocytes (31). The preceding report clearly set a precedent for the participation of Sp1 in the actions of either PKA or PKC. Moreover, these findings help us identify the novel aspects of our results. First, in the case of the apoAI gene, Sp1 is required for the actions of both PKA and PKC pathways. We are unaware of any other models where induction of gene activity by these separate signaling pathways is mediated by the transcription factor, Sp1. Second, the ability of Sp1 to mediate actions of both PKA and PKC may be limited to liver cells, because other reported studies were done in extra-hepatic models. Together these novel aspects of our results suggest an increasing role for Sp1 in signal transduction.

Sp1 is not the only transcription factor that can be regulated by both PKA and PKC signaling pathways. Previous studies showed that the transcription factor, AP-2, can also be activated by PKA and PKC following exposure to FSK or phorbol ester, respectively (32, 33). These findings have largely challenged the classical signaling model in regard to specificity and interaction of intracellular signaling pathways.

The ability of Sp1 to mediate the actions of PKA and PKC raises the possibility that this protein may have consensus amino acid motifs recognized by both kinases. Therefore, we scanned the amino acid sequence of Sp1 using the PhosphoSite database program (34) for putative phosphorylation motifs. This search revealed several potential phosphorylation sites for a variety of protein kinases including cAMP- or cGMP-dependent kinase, PKC, mitogen-activated protein kinase (Erk), casein kinase II, and many others. In support of this idea, recent studies suggest that Sp1 is phosphorylated by Erk2 (35), PKC-ζ (30), casein kinase (36), or PKA (29). The phosphorylation modification of Sp1 protein may lead to either an increase (e.g. Erk2) or decrease (e.g. casein kinase II) in its DNA binding activity. Given the presence of PKA and PKC target sites in Sp1, we speculate that the stimulatory effects of these kinases on apoAI promoter may require phosphorylation of Sp1. This topic will be the focus of future studies.

There is increasing evidence to show that GC-rich sequences can bind to more than one member of the Sp family (26, 27). The binding sequence for Sp1 is highly homologous to the one for Sp3. In the case of the IRCE, our studies show that it binds to only Sp1. Within the context of the rat apoAI promoter, the IRCE is not the only motif that interacts with Sp1. Previous studies (36) in the human apoAI promoter show a Sp1 binding site is located proximal to the IRCE. The corresponding motif in the rat promoter spans −220 and −211 of the gene. However, this latter site does not appear to mediate the actions of either PKA or PKC because deletion or mutation of the IRCE abolished the enhancing actions of Sp1 and a deletional mutant lacking the proximal Sp1 site retained its response to FSK and PDBu. The question of why this 3′ Sp1 site does not function in the same manner as the IRCE is beyond the scope of this report.

It is of interest that hormones such as adrenalin and glucagon, which activate hepatocyte adenyl cyclase, and therefore potentially augment apoAI gene transcription, are traditionally thought of as agents that are “counter-regulatory” for insulin action. The implications of our findings are that in the case of apoAI, the effects of insulin would be in parallel or additive with the effects of glucagon and adrenalin. Similarly, by activating Gαq, hormones such as noradrenalin (α1-adreno-
Regulation of ApoAI Promoter by PKC and PKA

In summary, our studies show that activation of PKA and PKC in the human hepatoma cells, Hep G2 increases apoAI promoter activity and the actions of these signaling pathways require the transcription factor, Sp1. Sp1 induces apoAI promoter activity and the actions of these signaling pathways require the transcription factor, Sp1. These findings are not only novel for apoAI regulation, but also for the transcription factor Sp1 in regards to its pivotal role in mediating the actions of both the PKA and PKC.

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