Identification of Overlapping AP-2/NF-κB-responsive Elements on the Rat Cholecystokinin Gene Promoter

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In this study we evaluate both proximal and more distant transcriptional regulation of the 5′ flanking region of the rat cholecystokinin gene in transfected GH3 (rat pituitary tumor) cells. Transcriptional activity was measured on the intact (−400 to +73) 5′ flanking region of cholecystokinin (CCK), as well as with DNA constructs, which were deleted in both the conventional 5′ to 3′, as well as an unconventional 3′ to 5′ direction. Our in vivo studies indicate complex phorbol ester and forskolin interactions in the 10-base pair region between −130 and −140. We conclude, there are at least two transcriptional factors involved in regulation of the rat CCK transcription in this region. In vitro studies utilizing heterologous nuclear (HeLa) extract, as well as purified transcription factors AP-2 and NF-κB, identify overlapped AP-2- and NF-κB-responsive elements within the 17-base pair sequence between −149 and −134 of the distal 5′ flanking region. In this region complex transcriptional regulation occurs, which indicates inhibition of AP-2 CCK promoter complexing by NF-κB. Six-point mutations introduced into this sequence prevent AP-2 and NF-κB binding to CCK promoter, as well as its transcriptional activation by phorbol ester and forskolin in GH3 cells.

Cholecystokinin (CCK), a prototypical brain-gut peptide (1–5), has hemacrine (3, 6) and autocrine (7) action in the gut. In the brain CCK is a neurotransmitter (8, 9). There is a commonly accepted gut, as well as brain, CCK transcription start sites have been identified in the brain of the rat (13). Transcriptional control of CCK is regulated by multiple factors. These include phorbol ester and diacylglycerol-activated protein kinase C (28). AP-2 specifically recognizes and binds to a conserved DNA sequence motif, found in promoters of a number of genes. These include the human growth hormone, the oncogene c-myc, H-2Kb gene promoters (25), as well as in the enhancer regions of the SV40 and hepatitis B viruses (29, 30). NF-κB activation occurs as a cellular response to multiple extracellular signals. These include cytokines, phorbol esters, bacterial lipopolysaccharides, viral infection, and calcium ions, as well as physical stimuli (UV radiation and free radicals) (for review see Refs. 26, 31, and 32). NF-κB has multiple embodiments. It belongs to the Rel family of transcriptional regulatory proteins (26, 31). The p50 subunit is specifically bound to an intron enhancer site in a number of eukaryotic genes (33–36). NF-κB is involved in cross-talk with other transcription regulators. The consequence is either a synergistic activation with an AP-1 complex (36, 37) or mutual inhibition with the glucocorticoid receptor (38–40).

The purpose of this study is to further define transcriptional regulation mechanisms of the distal 5′ flanking region of the rat CCK promoter. We hypothesized that AP-2 and NF-κB would have regulatory effects on CCK transcription. We have evaluated the effect of the transcription inducers forskolin and TPA in transient transfection studies in GH3 rat pituitary tumor cells (41). Additionally, we map DNA binding in vitro using DNase I protection studies, as well as mobility shift assays. For these in vitro studies purified nuclear proteins, as well as heterologous nuclear protein extracts, were used.

Our results demonstrate that TPA and forskolin regulate transcription of the rat CCK gene in the distal 5′ flanking region. In this region we identified AP-2/NF-κB overlapping recognition sites. We additionally demonstrated a direct interaction between NF-κB and AP-2, which results in inhibition of AP-2 DNA binding.

MATERIALS AND METHODS

Cloning CCK 5′ Flanking Region and Plasmid Constructs—The cloning of the rat CCK 5′ flanking regulatory region (pCCKUER-luc) is as in the proenkephalin gene (23). This sequence binds several transcription factors including AP-1, jun, and fos homodimers, as well as cAMP-responsive element-binding protein (16, 19). Two other recognition sites on the human CCK gene are Sp1 (at −39 to −34) and E-box or upstream stimulatory factor (at −97 to −92) (16). Additionally, a negative interaction was detected between TPA-responsive element and E-box or upstream stimulatory factor-responsive element (24). TPA and forskolin activate specific nuclear proteins, which regulate gene transcription. These proteins include AP-2 (25) and NF-κB (26, 27). Transcription factor AP-2 is activated either directly or indirectly by two signal transduction pathways (25). One route involves cAMP-dependent protein kinase A. The other involves phorbol ester and diacylglycerol-activated protein kinase C (28). AP-2 specifically recognizes and binds to a conserved DNA sequence motif, found in promoters of a number of genes. These include the human growth hormone, the oncogene c-myc, H-2Kb gene promoters (25), as well as in the enhancer regions of the SV40 and hepatitis B viruses (29, 30).

NF-κB activation occurs as a cellular response to multiple extracellular signals. These include cytokines, phorbol esters, bacterial lipopolysaccharides, viral infection, and calcium ions, as well as physical stimuli (UV radiation and free radicals) (for review see Refs. 26, 31, and 32). NF-κB has multiple embodiments. It belongs to the Rel family of transcriptional regulatory proteins (26, 31). The p50 subunit is specifically bound to an intron enhancer site in a number of eukaryotic genes (33–36). NF-κB is involved in cross-talk with other transcription regulators. The consequence is either a synergistic activation with an AP-1 complex (36, 37) or mutual inhibition with the glucocorticoid receptor (38–40).

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MATERIALS AND METHODS

Cloning CCK 5′ Flanking Region and Plasmid Constructs—The cloning of the rat CCK 5′ flanking regulatory region (pCCKUER-luc) is as

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‡ The abbreviations used are: CCK, cholecystokinin; CCKUER, 5′ flanking region of the rat CCK gene; pCCKUER-luc, plasmid of CCKUER luciferase reporter fusion construct; bp, base pair(s); FRSK, forskolin; TPA, 12-O-tetradecanoylphorbol 13-acetate; PCR, polymerase chain reaction.
CCCGCCTG 3

were cloned into the polylinker of pBL-luc, which has a for 30 s, and extension at 72 °C for 2 min). PCR-generated fragments

cification cycles (denaturation at 94 °C for 30 s, primer annealing at 58 °C (Applied Biosystems, Foster City, CA). There were twenty five ampli-

cative for the AP-2 recognition site (5'ATGTTTGGCGTCTTCCA 3'). First step PCR with Deep Vent (exo-) DNA polymerase (New England Biolabs, Inc., Beverly, MA) has thirty amplification cycles. Denaturation was at 94 °C for 1 min, annealing was at 65 °C for 1 min, and extension was at 72 °C for 1 min. PCR products were mixed with SYBR™ Green I (Molecular Probes, Inc., Eugene, OR) and loaded into 1% agarose gel. The resulting band (327 bp) was purified using a gel extraction kit (Qiagen, Chatsworth, CA). Fragments were used as the

described (13). The 5' to 3' direction nested deletions (see Fig. 1) were generated using PCR (upstream oligos were as follows: -244, 5'AGGT-

GCCAGAAAGCTTCTTAG 3'; -210, 5'ACTCATCAAAAGTAC-

CCCGCTG 3'; -170, 5'TCACTGGGCGCTTCCCTTC 3'; and -84, 5'

TGGCTACGACTGGTAAAACAG 3', and the downstream oligo was 5'

GGATGCCCCAGCCACTTACC 3'). Linearized pCCKUER (2 ng) was

mixed with 1× PCR buffer, 1 unit of AmpliTaq DNA polymerase

(Applied Biosystems, Foster City, CA). There were twenty five amplifi-
cation cycles (denaturation at 94 °C for 30 s, primer annealing at 58 °C

for 30 s, and extension at 72 °C for 2 min). PCR-generated fragments were

cloned into the polylinker of pBL-luc, which has a Photinus pyralis

luciferase reporter gene as described (13). Both strands of all PCR

fragments used in this study were sequenced to confirm CCK authen-
ticity and orientation (ABI Prism ABI50; Biotechnology Center, Utah

State University, Logan, UT). Sequence analysis was performed using

MacVector (Version 5.0; Kodak Scientific Imaging Systems). A series of

three nested deletions were transiently transfected in GH3 cells. Values were normalized to CCKUER, which has been arbitrarily assigned

the value of 100. Error bars represent S.E.; n = 4 from each experiment.

GH3 Cell Culture and Cationic Lipid-mediated Transfection—GH3 rat pituitary tumor cells (ATCC, Manassas, VA) were cultured and transfected using Lipofectamine (Life Technologies, Inc.) as described (13). Two transcriptional enhancers were evaluated, forskolin and TPA (both from Sigma). Samples were reconstituted in dimethyl sulfoxide and further diluted in sterile water (molecular biology grade; Whittaker, Walkersville, MD) to a final concentration of 10 μM. Fresh solutions of these transcriptional enhancers were prepared for each transfection. Aqueous solutions (5 μl) of 10 μM solutions of phorbol ester and/or forskolin were added to the medium following 6 h of transfection. In preliminary studies we determined that 100 nM forskolin, as well as TPA, was the optimal concentration (data not presented). 12 h later cells were harvested, lysed, and assayed for luciferase activity as described (13). To compare data from different experiments, values were normalized to intact CCKUER, arbitrarily assigned the value of 100. Data is presented as mean ± S.E.

DNase I Protection Assay—DNase protection assays were performed as described (13). The only variation was the final binding buffer for the NF-κB experiments (10 mM HEPES (pH 7.9), 0.2 mM EDTA, 50 mM KCl, 2.5 mM dithiothreitol, 10% glycerol, and 0.05% Nonidet P-40). The transcription factors studied were AP-2, as well as NF-κB (p50 subunit) (all from Promega, Madison, WI). The digestion products were analyzed on denaturing polyacrylamide gels. The markers are sequenced single-stranded M13mp18 bacteriophage (T7 Sequenase, Version 2.0, DNA sequence kit; Amersham Pharmacia Biotech). A

PCR-Site-directed Mutagenesis—PCR-site-directed mutagenesis was performed as described (42). This particular method of mutagenesis requires two sequential PCR reactions. The upstream primer is muta-
genic for the AP-2 recognition site (5'CCTTCTATCTCGGATCCACT-

TCGATCGG 3'). The downstream primer recognizes a sequence from the luciferase reporter (5' ATGTTTGGCGTCTTCCA 3'). First step PCR with Deep Vent (exo-) DNA polymerase (New England Biolabs, Inc., Beverly, MA) has thirty amplification cycles. Denaturation was at 94 °C for 1 min, annealing was at 65 °C for 1 min, and extension was at 72 °C for 1 min. PCR products were mixed with SYBR™ Green I (Molecular Probes, Inc., Eugene, OR) and loaded into 1% agarose gel. The resulting band (327 bp) was purified using a gel extraction kit (Qiagen, Chatsworth, CA). Isolated fragment was then used as the
mutagenic primer in the second PCR reaction, where it was mixed with the 20-mer forward primer (20 pmol, 5′ TCACGGCCATGTCATTGTGG 3′). The conditions in the second PCR reaction were identical to those used in the first. The resulting PCR product was religated into pBL-luc. Mutated plasmids contained an additional BamHI restriction site, which had been introduced by the mutagenesis. Successful mutagenesis and the correct orientation were confirmed by restriction digest mapping, as well as by DNA sequencing (Biotechnology Center, Utah State University, Logan, UT).

**Mobility Shift Assay**—The fragment to be radiolabeled was PCR-generated using linearized pCCKUER as a template with primers (5′ TCACGGCCATGTCATTGTGG 3′ and 5′ AGTCATCTGTTACCCAGT-GCTGAC 3′). Obtained fragment (113 bp) was then dephosphorylated using calf intestine phosphatase (Promega, Madison, WI). The termini of this fragment were labeled with [γ-32P]ATP using T4 polynucleotide kinase (Promega, Madison, WI). Labeled, double-stranded DNA (50,000 cpm, 0.01 pmol) was incubated for 30 min at room temperature. Titrations were performed with increasing amounts of HeLa cell nuclear extract (see Fig. 5, panel A), AP-2 protein extract (see Fig. 5, panel B), or NF-κB (p50) protein (see Fig. 6). Following incubation, samples were mixed with loading buffer and analyzed on a 4% (80:1 acrylamide: bisacrylamide) non-denaturing polyacrylamide gel in 0.5 × Tris borate, EDTA. The resulting gel was dried prior to autoradiography. Following exposure, the bands of interest were excised and quantified in a scintillation counter (Beckman LS 7000).

**RESULTS**

The effect of TPA and forskolin were evaluated both independently and in combination. We used the full-length CCKUER (473 bp), as well as four 5′ to 3′ direction deletions of the 5′ flanking region of the rat CCK gene (Fig. 1, left panel). These constructs were then stimulated with TPA and forskolin (Fig. 1, right panel). Both TPA and forskolin enhance CCKUER-luc expression. Deletion to −170 results in loss of forskolin-induced expression. By contrast, TPA-induced expression is maintained until deletion proceeds to −84. Thus, these data identify a TPA-activated transcription factor-responsive element between −84 and −170 bp.

Alternatively, we evaluated the effect of TPA and forskolin treatment of transfected GH3 cells for a series of 3′ to 5′ nested deletions (Fig. 2, left panel). In our preliminary studies we have determined that by removing the dominant signal generated at the conventional transcription initiation site as a result of 3′ to 5′ direction of deletion, the rate of basal transcription would be significantly declined resulting in decrease of luciferase signal (13). This enabled us to isolate the effect of more distal 5′ regulatory elements associated with upstream alternative start sites of the rat CCK gene. Both TPA and forskolin induced expression until CCKUER was deleted to −130 (Fig. 2, right panel, lanes −50 and −130). When the next 10 bp were deleted TPA induction was lost (Fig. 2, right panel, lane −140). In contrast, the same deletion construct (−140) still exhibits AP-2/NF-κB elements on CCK promoter. Single end-labeled DNA fragments from the coding strand of the 400-bp 5′ flanking region of the rat CCK gene were combined with purified AP2 or NF-κB (p50) proteins either alone as described (0, 1, or 2 footprint units as indicated) or in combination (MIX = 1:1 footprint units as indicated). Limited DNase I digestion was followed by polyacrylamide gel electrophoresis and autoradiography.

![Fig. 3. TPA and forskolin inhibit each other within the sequence between −140 and −130 bp of the 5′ flanking region of the rat CCK gene.](image)

![Fig. 4. Binding AP2 and NF-κB to the 5′ flanking region of the rat CCK gene.](image)
Overlapped AP-2/NF-κB Elements on CCK Promoter

bition is maintained for the first two deletion constructs studied (CCK −50 and CCK −130). However, when deletion proceeds an additional 10 bp, to CCK −140, the inhibition was lost, and signal increased up to 3-fold. Forskolin, but not TPA, remains active at CCK −140 when deletion proceeds to −140 (Fig. 2). These data corroborate our initial observation that there is a major site of transcriptional regulation in the −130 to −140 region of the 5′ flanking region of the rat CCK gene.

Sequence analysis suggests AP-2, as well as NF-κB, binding sites in this region of −130 to −140 (see Fig. 9). Accordingly, DNase protection assays with purified AP-2 and NF-κB transcription factors were performed (Fig. 4). Purified AP-2 protects the coding strand in two regions, between −37 and −48 and between −139 and −149. Purified NF-κB (p50) protects the coding strand between −134 and −145. Thus, both NF-κB and AP-2 bind in the sequence between −130 and −140 of the 5′ flanking region of the rat CCK gene, which is regulated by TPA and forskolin stimulation (Figs. 2 and 3).

Binding to this −130 to −140 region was then evaluated in a mobility shift assay. HeLa cell nuclear extract, which contains AP-2, was used. As the HeLa cell nuclear extract amount is increased there is linear increase in signal (Fig. 5, panel A, lanes 5, 10, and 20). Next a competitive inhibition study was performed using 1- and 10-fold molar excess of “cold” wild type CCK fragment between −57 and −170 (Fig. 5, panel A, lanes 5 and 6 from the left). As the amount of cold CCK is increased, the radiolabeled signal band (cpm) decreases by 70% (data not presented). This indicates competition by the wild type CCK for the fraction of the HeLa cell nuclear extract, which binds to the radiolabeled fragment of CCK. The same experiment was then repeated using the unlabeled 113-bp mutated (Δ) CCK fragment between −57 and −170 (Fig. 5, panel A, lanes 7 and 8 from the left). There were six bp mutations introduced in the AP-2 consensus recognition sequence located between −130 and −140 (see “Materials and Methods”). These mutations result in only 7% decrease in binding (cpm data not presented).

This competitive inhibition by the wild type cold CCK demonstrates the specificity of the interaction between HeLa cell nuclear extract with wild type AP-2 cis-element on the CCK gene. Panel B, a DNA-protein binding study performed with increasing amounts (1.4, 2.8, and 4.2 μg) of purified AP-2 protein and the [γ-32P]ATP-labeled fragment of CCK (−170 to −57).

![Fig. 5. HeLa cell nuclear extract and AP-2 protein binding to the 5′ flanking region of the rat CCK gene. Panel A, the 113-bp fragment between −170 and −57 was labeled with [γ-32P]ATP. The control is the labeled fragment incubated in buffer without any nuclear protein extract. In the next three lanes, the labeled fragment was incubated with increasing amounts (5, 10, and 20 μg) of HeLa cell nuclear extract. In the next two lanes, the labeled 113-bp fragment was incubated with 10 μg of HeLa nuclear extract in the presence of either 1- or 10-fold molar excess of a 113-bp cold fragment of the rat CCK gene (−170 to −57). In the last two lanes, the labeled 113-bp fragment was incubated with 10 μg of HeLa nuclear extract in the presence of either 1- or 10-fold molar excess of a 113-bp cold mutated fragment ΔCCK (−170 to −57). This competitive inhibition by wild type cold CCK demonstrates the specificity of the interaction between HeLa cell nuclear extract with wild type AP-2 cis-element on the CCK gene. Panel B, a DNA-protein binding study performed with increasing amounts (1.4, 2.8, and 4.2 μg) of purified AP-2 protein and the [γ-32P]ATP-labeled fragment of CCK (−170 to −57).]
site. This region contains, in part, AP-2 and NF-κB (p50) consensus recognition sequences (see Fig. 4).

**DISCUSSION**

In this study we identify hitherto undescribed transcription regulatory sites in the 5' flanking region of the rat CCK gene. The region of most interest in this study was a 10-bp sequence located between −130 and −140. In the course of this study we demonstrate that this region is responsive to both TPA and forskolin. In addition, we determine the complex relationship between these two transcription enhancers in the same region.

**FIG. 6.** Mutations in the AP-2/NF-κB consensus recognition sequence reduces binding by NF-κB (p50). The 113-bp fragments CCK, −170 to −57 (three lanes on the left), and the mutated ΔCCK, −170 to −57 (three lanes on the right), were labeled with [γ-32P]ATP and incubated with increasing amounts (0.13 and 0.26 μg) of NF-κB (p50) protein.

**FIG. 7.** Combination of NF-κB and AP-2 prevents AP-2/wild type CCK complexing. The fragment between −170 and −57 labeled with [γ-32P]ATP was incubated with increasing amounts (0.13 and 0.26 μg) of purified NF-κB and 1.4 and 2.8 μg of purified AP-2 both individually and subsequently in combination (center lane). When both proteins are studied in combination, the AP-2 DNA complex, previously identified, is completely lost (center lane, see arrow on the right, indicating AP-2 DNA complex migration).

**FIG. 8.** Mutation in AP-2/NF-κB consensus recognition sequence eliminates transcriptional activation of CCK by both TPA and forskolin. Open column, intact pCCKUER. Hatched column, mutated pCCKUER. The mutations are identified by *.

**FIG. 9.** The sequence of 5' flanking region of the rat CCK gene. The consensus transcription initiation site is indicated by an arrow and is assigned the number 0. Brain ATIS* is the alternative initiation site (located at −41) identified in the cerebral cortex in the rat (13). cAMP-responsive element/TPA-responsive element (AP-1, between −87 and −79) and upstream stimulatory factor cis-elements were previously identified in both human and rat CCK DNA sequences (16, 23). NF-κB and AP-2 cis-elements are identified in this study. Sp1 cis-elements were determined by DNase I protection assay.2 These are boxed and marked.

**TABLE 1**

| Sequence                                |
|------------------------------------------|
| ACTGATCTG TGAAGGTCC CGGTTTCT CACAGGGCTT |
| ACGTCTCAGA TGATGATCGA GCTTCCTCAG       |
| TCTCGGGAAG TGGGCGTGAC AAAGAAGACG        |
| CGGAAACGCG GGCAAGAAC AAGACCTAGT         |
| AGAAGCGCC CTGAGGCGCA ATCTTCGCA          |
| GGGGCGGG AAACTGACTG CGGGCGCGG CTGGTCTAG |
| Sp1                                      |
| Sp1                                      |
| Sp1                                      |
| Sp1                                      |
| Sp1                                      |
| Sp1                                      |

2 Manucript in preparation.
of CCK promoter.

Four from six previously described 3’ to 5’ direction deletion constructs (13) were utilized in this study (Fig. 2). We additionally generated four constructs, which were deleted in the more conventional 5’ to 3’ direction (Fig. 1). Mutational analysis was performed using six bp mutations, which were inserted between −130 and −140. These constructs permitted a more detailed evaluation of the TPA- and forskolin-responsive regions, which had been identified during the course of the study. Deleted, as well as mutated, constructs were transiently transfected into GH3 cells. We had previously used GH3 (rat pituitary tumor) cells in our studies with CCK (13). GH3 cells produce neither endogenous CCK mRNA nor any CCK peptides. However, GH3 cells self-evidently have the transcriptional mechanisms that induce transcription of the 5’ flanking region of the rat CCK gene in the transient transfection studies that we performed. The data from cell culture show complex transcriptional regulation of the 5’ flanking region of the rat CCK gene as a result of TPA and forskolin treatment (Figs. 1–3 and Fig. 8). However, pathways of TPA and forskolin action remain to be fully defined in GH3 cells.

In this study, in addition to the role of TPA and forskolin, we show that two transcription factors have overlapping recognition sites in the 17-bp region located between −134 and −149. These two factors are AP-2 and NF-κB. We speculate that in GH3 cells forskolin induction of AP-2 may well occur. Preliminary data determine AP-2 expression of in GH3 cells (data not presented).

The unconventional 3’ to 5’ direction of deletion, which we use, shows that when deletion proceeds to −140, forskolin, but not TPA, is still stimulatory (Fig. 2). In contrast, the conventional 5’ to 3’ deletion constructs support that TPA is still stimulatory in the fragment that contains this −134 to −149 region, and forskolin induction is lost between −170 and −210 (Fig. 1). The deletion construct (−170) contains the AP-2/NF-κB binding sites identified in this study. A possible explanation for this paradox may lie in the presence of other forskolin-responsive elements associated with alternative initiation sites (13) in the distal region of CCK promoter. These elements are deleted as a result of 5’ to 3’ deletion but are still present in the 3’ to 5’ deletion constructs. Our preliminary data indicate, for example, previously unreported AP-1 binding sequences, which we identify between −354 and −387 (data not presented).

As a consequence of our data, we hypothesize that forskolin may activate binding to the identified AP-2, as well as some other distal responsive elements, possibly, including the distal AP-1 site in GH3 cells. This hypothesis will be determined in subsequent study.

There is inter-species sequence conservation in the proximal 5’ flanking region of CCK genes. The first 100 bp 5’ to the conventionally accepted transcription initiation site in rat (10, 23, mouse (11), and man (16) have 80% homology. Sp1 consensus sequence has been described in the human CCK promoter between −37 and −48 (16). In the rat gene, we were unable to demonstrate Sp1 binding to this sequence (data not presented). However, we have demonstrated that AP-2 binds in a homologous sequence (−37 to −48) in the 5’ flanking region of rat CCK gene (Fig. 4). In the more distal 5’ flanking regulatory region of rat, mouse, and man CCK DNA sequence homology falls to ∼40%. In this study we identify an AP-2/NF-κB binding site in the rat (−134 to −149). By contrast, we find no analogous AP-2/NF-κB binding region in a sequence analysis of the 5’ flanking region of man or mouse. A similar 17-bp sequence in mouse CCK gene (11) contains 3-bp mismatches and a 4-bp mismatches in the human CCK gene (16). These data imply that the proximal 5’ flanking region is of more significance in basal CCK transcription. The distal upstream 5’ flanking regulatory region may be of greater relevance in species-specific regulation of CCK transcription.

Both the human, as well as the rat, CCK promoter have a highly conserved cAMP-responsive element/TPA-responsive element binding region (−79 and −87) (16, 29). A three-point mutation within this site eliminates transcriptional activation by both TPA and forskolin (16). We confirm a homologous region in the rat gene (−79 and −87) (Fig. 9) and an additional (−42 to −46), which is protected from DNase I digestion by AP-1 (data not presented). Our data indicate a complex inhibition of transcription by TPA and forskolin (Figs. 1–3 and Fig. 8). We additionally show that in this region of inhibition are overlapped responsive elements of AP-2 and NF-κB, characterized by complex negative interaction of each other (Fig. 7). NF-κB and a glucocorticoid receptor mutually inhibit transcriptional activation (38–40). However, to our knowledge, these data are the first that demonstrate transcriptional inhibition involving NF-κB and AP-2. Studies of the mechanism involved in the interactions by which inhibition of these nuclear factors is mediated should be addressed in the future.

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