Two Separate Cis-active Elements of the Vasoactive Intestinal Peptide Gene Mediate Constitutive and Inducible Transcription by Binding Different Sets of AP-1 Proteins*

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Vasoactive intestinal peptide (VIP) gene expression is highly restricted throughout the neuroaxis and regulated by extracellular factors that activate tyrosine- or serine/threonine-directed protein kinase pathways. Cytokine, cyclic AMP, and tissue-specific response elements on the VIP gene have been characterized. Those mediating responsiveness to protein kinase C have not. The endogenous VIP gene and a 5.2-kilobase pair (kb) VIP-luciferase reporter gene, are up-regulated by phorbol 12-myristate 13-acetate (PMA) in SK-N-SH neuroblastoma cells. PMA stimulation was abolished by deletion of sequences at −1.37 to −1.28 or −1.28 to −0.904 kb, but not by removal of the single phorbol ester response element (TRE; TGACTCA) located at −2.25 kb. Mutation of sites at −1.32 or −1.20 that mediate neurotrophin responsiveness of the VIP gene (Symes, A., Lewis, S., Corpus, L., Rajan, P., Hyman, S. E., and Fink, J. S. (1994) Mol. Endocrinol. 8, 1750–1763) each reduced PMA induction in SK-N-SH cells by >50%, and double mutation abolished it. The two mutations also reduced basal VIP reporter gene transcription in SH-EP neuroblastoma cells expressing VIP constitutively. Both cis-active elements bound pre-existing AP-1 proteins in SH-EP, or PMA-stimulated SK-N-SH cell nuclear extracts. The AP-1 complex at both sites contained a Fos-related protein with c-Jun in SH-EP cells and c-Fos with a Jun-related protein in SK-N-SH cells. Recruitment of combinatorially distinct AP-1 complexes to these elements may underlie cell type-specific regulation of the VIP gene.

Vasoactive intestinal peptide (VIP)† is restricted to specific subpopulations of neurons throughout the central and peripheral nervous systems. Its expression is also tightly regulated in a temporal fashion during development. For example, VIP mRNA is expressed in a single location in the central nervous system prenatally (the ventrolateral portion of the suprachiasmatic nucleus) (1–3), and it only assumes a characteristic pattern of expression in cortex, limbic system, hypothalamus, and the remainder of the suprachiasmatic nucleus postnatally (2, 4–7). In the peripheral nervous system, VIP peptide and mRNA are detectable at embryonic day 14.5 in a substantial proportion of principal ganglion cells of the sympathetic nervous system, decreasing to less than 5% of the overall population of sympathetic neurons at birth (8, 9). In the adult rat, VIP is extensively colocalized with acetylcholine in the peripheral nervous system (10–13) but is co-expressed with catecholamines in chromaffin cells of the adrenal medulla (14) and with a variety of co-transmitters other than ACh in the central nervous system (1, 2, 6). The mechanisms by which neurotransmitter and/or neuropeptide phenotypes are encoded during development is not well understood. However, it is evident that multiple signaling pathways to the VIP gene must be utilized to allow VIP to be expressed in unique combinations with other hormones and neurotransmitters throughout the neuroendocrine axis.

VIP gene expression is in part controlled by growth factors and neurotransmitters that signal to several cis-active elements on the VIP gene, via protein kinase, calcium, and cAMP-mediated signal pathways (15–18). The molecular basis for cell-specific and inducible transcription of the VIP gene has been studied in cell lines derived from neuroblastomas, tumors of neural crest origin oncogenically transformed in the course of peripheral autonomic development. Constitutive expression of the VIP gene in SH-EP neuroblastoma cells requires the contribution of at least five distinct domains of the VIP gene 5′-flank (17, 18). These include an upstream 425-bp tissue specific element (TSE) located at about −4.3 kb from the start of transcription, a promoter-distal cAMP-responsive element (VIP-CRE), and three consecutive domains between −1.37 and −0.9 kb. Induction of VIP transcription by neurotrophic factors that activate the Jak-STAT tyrosine kinase signaling pathway requires a 180-bp cytokine-responsive element (CyRE) located at −1.33 kb in the VIP 5′-flank, containing binding sites for STAT and AP-1 proteins (19–21). The cis-active sequences responsible for VIP gene regulation by serine/threonine kinase activation and their relationship to the domains required for constitutive and neurotrophin/cytokine induction of VIP gene transcription have not yet been defined.

In this study, candidate domains for regulation of VIP gene transcription by activation of protein kinase C have been tested using deletional and mutational analysis in the context of a VIP reporter gene that supports constitutive, tyrosine kinase-mediated, and serine/threonine kinase-mediated transcriptional regulation. Both the STAT and ncAP-1 sites of the VIP-CyRE bound AP-1-related protein complexes and were required for phorbol ester-stimulated transcription in SK-N-SH neuroblastoma cells and also for constitutive VIP gene transcription in SH-EP cells. The AP-1 complexes formed on these two ele-
ments were different, however, in SK-N-SH and SH-EP cells. The role of these two noncanonical AP-1 sites may be to recruit multiple, unique AP-1-related complexes to the transcriptional platform of the gene depending on first messenger signaling and cellular context.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cell culture reagents, LipofectAMINE, and synthetic oligonucleotides were purchased from Life Technologies, Inc. Fetal bovine serum was obtained from BioWhittaker (Walkersville, MD). Luciferase assay reagent, 5' reporter lysis buffer, and pG53 vectors were from Promega Corp. (Madison, WI). The Scotchmer system in vitro mutagenesis system was from Amersham Pharmacia Biotech (Buckinghamshire, United Kingdom). Fos and Jun antibodies were bought from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). RNA STAT-60 was from Tel-Test “B” (Friendswood, TX).

**Cell Culture and RNA Analysis**—SK-N-SH and SH-EP neuroblastoma cells were cultured as described by Wascheck et al. (16). Cells were grown in Dulbecco's modified Eagle’s medium with 4.5 g of glucose/liter, containing 10% fetal bovine serum (heat-inactivated) supplemented with glutamine (0.03%), penicillin (100 units/ml), and streptomycin (100 μg/ml). Cells were maintained in a humidified 95% air, 5% CO2 atmosphere. For Northern blot analysis, cells were grown in six-well plates to approximately 70% confluence and incubated with 50 nM PMA (or ethanol for control) for an appropriate time, and harvested for total RNA using 1.0 ml of RNA STAT-60. Three μg of total RNA isolated from SK-N-SH cells was resolved in a 1% agarose gel and transferred to Nytran membrane by electrophoresis. VIP mRNA was detected by hybridizing the membrane with [32P]-labeled human VIP cDNA for 20 h at 42 °C and exposure to Bio-Max MR film overnight. An image of the autoradiogram was acquired under ambient light using a Kodak Image Station and analyzed using 1D Image Analysis software.

**Construction of VIP-luciferase Reporter Plasmids and Site-directed Mutagenesis**—The methods used to construct VIP-luciferase plasmids were described in Ref. 18. Site-directed mutagenesis was carried out using the Sculptor mutagenesis system (Amersham Pharmacia Biotech) as described in Ref. 18. Sequences for mutagenic primers are (mutated bases are shown in lowercase type): STAT (CACTGTTAAXAAAAGATATCCGGC; ATTAAGGCCCCAGGACTCTGGC; 44-mer), Dyad (GAATACTCGGAGGGATCGTATGGAGTACGAGCTGTCA; 51-mer), and ncAP-1 (CTGATTAGAAATATGATAGCATACCGGATTAC; 38-mer). Mutations were confirmed by restriction analysis and sequencing.

**Transient Expression Assays**—SK-N-SH and SH-EP cells were transfected as described in Ref. 18. SK-N-SH and SH-EP cells were plated in 12-well Costar tissue culture plates at a density of 2.5 × 103 and 1.0 × 105 cells/well, respectively, in 1.0 ml of medium. Cells were allowed to grow to approximately 70% confluence and transfected with 0.5 μl of Lipofectamine for 5 h in serum-free Dulbecco's modified Eagle's medium. Cells were then allowed to recover in a complete medium over night and treated with 50 nM PMA (or ethanol for control). Cells were harvested 36–40 h after PMA treatment in 200 μl of reporter lysis buffer. Samples were frozen in liquid nitrogen and stored at −70 °C until ready for the assay. Luciferase assays were done using 20 μl of the lysate as described in Ref. 18. Results are expressed as a mean of duplicate readings from a single well or as a mean ± S.E. of duplicate readings from triplicate wells. Experiments were repeated at least two times.

**Electrophoretic Mobility Shift Assays**—SK-N-SH and SH-EP cell nuclear extracts were prepared as in Ref. 18 with minor modifications. Buffer A contained a phosphatase inhibitor NaVO4 (100 μM), in addition to protease inhibitors pepstatin A (1 μg/ml), leupeptin (10 μg/ml), benzamidine (0.5 mM), and aprotinin (10 μg/ml). Buffer C contained additional phosphatase inhibitors β-glycerophosphate (50 mM) and NaF (25 mM) in addition to NaVO4 and protease inhibitors. SK-N-SH cells were treated with 50 nM PMA (or ethanol for control) for an appropriate time before cells were harvested. Synthetic oligonucleotides were annealed and labeled using [γ-32P]ATP and T4 polynucleotide kinase. Approximately 100,000 cpm of labeled oligonucleotides were mixed with 5–30 μg of nuclear extract in a total volume of 10 μl containing 50 mM HEPES (pH 7.9), 8% glycerol, 40 mM NaCl, 40 mM KCl, 25 mM potassium phosphate buffer (pH 7.9), 1.5 mM dithiothreitol, 1.2 mM EDTA, 100 μg/ml poly(dI-dC). Binding reactions were carried out at room temperature for 20 min, and samples were resolved on 5% non-denaturing PAGE gels in 0.5% TBE at 4 °C. For supershift assays, antibodies were added to the reaction before the addition of labeled oligonucleotides, and reactions were extended for an additional 10 min at room temperature. The manufacturer’s (Santa Cruz Biotechnology) catalog numbers for antibodies (TransCruz Gel Supershift reagent) are SC-253 for pan-Fos (K-25), SC-52G for c-Fos, SC-45G for FosB, SC-605 for Fra-1, SC-171 for Fra-2, SC-44 for Jun (D-G), SC-45G for c-Jun, SC-46G for JunB, and SC-74G for JunD. Fos (K-25) and Jun (D-G) are referred to as pan-Fos and pan-Jun, respectively, in this report. The c-Jun, JunB, Fos, FosB, Fra-1, Fra-2, and FosB antibodies are reported to specifically recognize the single Jun- or Fos-related protein against which they are raised, and not other known members of either family, under gel supershift/neutralization conditions.

**RESULTS**

5.2 kb of the VIP gene 5'-flank (Fig. 1A) contains sequences necessary for constitutive expression in SH-EP and SH-IN neuroblastoma cells, and for PMA and forskolin induction in SY-5Y and SK-N-SH neuroblastoma cells (16, 17). Progressive deletions from this construct were used to pinpoint the cis-active sequences responsible for PMA induction of the VIP gene in SK-N-SH cells. Deletion of the VIP gene from −5.2 to −2.5 kb removes the upstream TSE, causing a decrease in basal expression in SK-N-SH cells as reported for SH-EP and SH-IN VIP-expressing neuroblastoma cell lines (17) but did not decrease the -fold induction of gene transcription by PMA (Fig. 1B). Further deletion from −2.5 to −1.37 kb caused no further reduction in fold stimulation of reporter gene expression by PMA compared with the VIP5.2 construct, indicating that the single consensus TRE in the VIP gene located at −2.25 kb (TGACTCA; see Fig. 1A) does not mediate PMA-stimulated transcription in SK-N-SH cells. Removal of sequences from −1.37 to −1.28 kb, however, abolished PMA stimulation, indicating that this region (domain c in Fig. 1A) contains sequences important for PMA induction in SK-N-SH cells. Removal of domain d from VIP gene constructs in which domain c was retained also abolished PMA stimulation, which was restored by adding back a 5′ portion of domain d between −1.28 and −0.904 kb (Fig. 1C). Thus, deletional analysis suggests that domains c and d′ are both required for PMA-inducible expression of the VIP gene in SK-N-SH cells.

Mutational analysis of the VIP-luciferase reporter gene was employed to determine which cis-active sites within domains c and d′ of the VIP gene are required for PMA-stimulated transcription in SK-N-SH cells. We have previously reported that domains c and d′, acting in combination with other elements in the gene, separately contribute positive and negative regulatory functions required for neuroendocrine cell-specific expression of the VIP gene and its silencing in non-VIPergic cells (18). Domain c contains a highly conserved dyad symmetry sequence (22), likely to be functionally significant. The junction between domains c and d also contains a 180-bp CyRE, which, when fused to a heterologous promoter, confers up-regulation of transcription by cytokines of the CNTF family in NBF1 neuroblastoma cells (20). The CyRE contains separate STAT and AP-1 protein binding sites, both required for CNTF regulation of gene transcription (20).

Mutations in either the STAT site or the AP-1 binding site (ncAP-1 site; see Fig. 1A) reduced the absolute level of expression after PMA treatment by 88 and 89%, respectively, and fold induction by PMA by 62 and 77%, respectively (Fig. 2A). A STAT and ncAP-1 double mutation completely abolished PMA induction, indicating that these two cis-acting sequences play a critical role in PMA-inducible expression of the VIP gene. Mutations in the dyad symmetry sequence had little effect on the level of expression after PMA compared with untreated control cells and did not reduce fold induction by PMA.
Both the STAT and ncAP-1 sites are located within the domains of the VIP gene (domains c and d', respectively; see Fig. 1A) that are important for cell-specific constitutive expression of VIP in SH-EP cells (18). We therefore tested the effect of mutations in these sites on constitutive expression of the VIP-luciferase reporter gene in SH-EP cells (Fig. 2B). For these experiments, the VIP1.55-TSE construct, containing all five domains of the VIP gene necessary for cell-specific and inducible expression (domains a, b, c, d, and e; see Fig. 1A) was used. The VIP1.55-TSE construct was expressed at a 26-fold higher level than the VIP1.55 construct in SH-EP cells. Mutation of the dyad symmetry, STAT, and ncAP-1 sites resulted in decreases of 40, 38, and 74%, respectively, in the level of the VIP1.55-TSE reporter gene in SH-EP cells (Fig. 3). Thus, the STAT and ncAP-1 sites of the VIP gene are involved both in PMA-stimulated up-regulation of the gene in SK-N-SH cells.
and its constitutive expression in SH-EP cells, while the dyad symmetry element is required only for basal expression of the VIP gene.

Gel mobility shift assays were performed to identify nuclear proteins binding to the STAT and ncAP-1 sites in SK-N-SH cells after PMA treatment. Nuclear extracts from SK-N-SH cells treated with PMA exhibited binding of specific protein complexes to both the ncAP-1 and STAT sites, absent from untreated cells (Fig. 3). Complex formation was observed in extracts from cells treated with PMA for 2 h, was maximum in cells treated for 4 h, and persisted, with decreasing intensity, for up to 72 h (data not shown).

PMA-inducible binding of SK-N-SH nuclear proteins to the labeled ncAP-1 probe (Fig. 3A) was completely blocked with excess unlabeled ncAP-1 oligonucleotide or AP-1 consensus oligonucleotide. An oligonucleotide containing the consensus TRE (TGACTCA) at 2.25 kb in the VIP gene also competed with labeled ncAP-1 probe for binding of the SK-N-SH nuclear proteins. Mutated ncAP-1 (ncAP-1/m3) and a STAT binding consensus sequence (acute phase response element or interleukin-6-response element) of the rat α₂-macroglobulin gene (23) failed to compete with the labeled probe. Pan-Fos and pan-Jun antibodies broadly react with c-Fos, FosB, Fra-1, and Fra-2 and with c-Jun, JunB, and JunD, respectively.

**Fig. 2.** STAT and ncAP-1 sites of the VIP gene are required for both PMA-inducible expression in SK-N-SH cells and constitutive expression in SH-EP cells. A, a transient expression assay was performed in SK-N-SH cells using the VIP1.55 construct in which luciferase expression is driven by 1.55 kb of the VIP gene 5'-flank with or without the following mutations. The Dyad construct contains a 7-bp substitution of the 25-bp dyad symmetry sequence in domain c. STAT and ncAP-1 constructs contain 4- and 3-bp substitutions and are located in domains c and d', respectively (see Fig. 1A). The STAT + ncAP construct contains mutations in both the STAT and ncAP-1 sites. The mean and S.E. for triplicate paired determinations from a single experiment are shown. In the inset, data are expressed as fold induction in the presence of 50 nM PMA compared with basal expression in the absence of PMA. B, a transient expression assay was done in SH-EP cells using VIP1.55-TSE constructs with or without mutations in the dyad symmetry, STAT, or ncAP-1 sequences. The mean and S.E. for triplicate paired determinations from a single experiment are shown.

**Fig. 3.** AP-1 proteins are stimulated by PMA to bind both the ncAP-1 and STAT sites in SK-N-SH cells. Gel shift assays were performed using 32P-labeled ncAP-1 probe (A) or STAT probe (B). Nuclear extracts were prepared from SK-N-SH cells treated with 50 nM PMA (or ethanol for control) for 5 h. For competition and supershift assays, a molar excess of unlabeled oligonucleotides or specific antibodies were mixed with the nuclear extract before the addition of the probe. The ncAP-1/m3 and STAT/m4 oligonucleotides contain three and four mutated bases, respectively. VIP-TRE is the oligonucleotide spanning the TRE consensus sequence of the VIP gene located at −2.25 kb. APRE, represents acute phase response element (or interleukin-6-response element) of the rat α₂-macroglobulin gene (23). The AP-1 consensus oligonucleotide is from the human collagenase gene (25). Pan-Fos and pan-Jun antibodies broadly react with c-Fos, FosB, Fra-1, and Fra-2 and with c-Jun, JunB, and JunD, respectively.

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Gel mobility shift assays were performed to identify nuclear proteins binding to the STAT and ncAP-1 sites in SK-N-SH cells after PMA treatment. Nuclear extracts from SK-N-SH cells treated with PMA exhibited binding of specific protein complexes to both the ncAP-1 and STAT sites, absent from untreated cells (Fig. 3). Complex formation was observed in extracts from cells treated with PMA for 2 h, was maximum in cells treated for 4 h, and persisted, with decreasing intensity, for up to 72 h (data not shown).

PMA-inducible binding of SK-N-SH nuclear proteins to the labeled ncAP-1 probe (Fig. 3A) was completely blocked with excess unlabeled ncAP-1 oligonucleotide or AP-1 consensus oligonucleotide. An oligonucleotide containing the consensus TRE (TGACTCA) at −2.25 kb in the VIP gene also competed with labeled ncAP-1 probe for binding of the SK-N-SH nuclear proteins. Mutated ncAP-1 (ncAP-1/m3) and a STAT binding consensus sequence (acute phase response element or interleukin-6-response element) of the rat α₂-macroglobulin gene (23) failed to compete with the labeled probe. Pan-Fos and pan-Jun antibodies broadly recognize Fos and Jun transcription factor family members, respectively, completely blocked binding of SK-N-SH nuclear proteins to the ncAP-1 oligonucleotides in antibody supershift/competition assays. When antibodies that specifically recognize individual members of Fos and Jun families were used in supershift assays, anti-c-Fos antibodies showed strong blockade of complex formation, with smaller
effects of FosB and Fra-1 and Fra-2 antibodies. None of the antibodies to specific members of the Jun family gave complete blockade of ncAP-1 complex formation. Thus, the AP-1 complex binding to the ncAP-1 site of the VIP gene is probably a dimer of c-Fos and a Jun-related protein in SK-N-SH cells.

Similar results were obtained when the STAT oligonucleotide was used as a probe (Fig. 3B), indicating that although this cis-active element binds STAT proteins in response to cytokine treatment (19), it functions as an AP-1 binding site after exposure to PMA.

PMA induction of endogenous VIP mRNA and gel shift complex formation on the ncAP-1 oligonucleotide were both unaffected by blockade of new protein synthesis with cycloheximide, and both were blocked by treatment with H-7, a broad specificity serine-threonine protein kinase inhibitor (Fig. 4). Thus, the c-Fos and Jun-related components of the AP-1 complex regulating VIP transcription in SK-N-SH cells are activated via a post-translational mechanism involving protein phosphorylation and are not produced by de novo biosynthesis, following treatment with PMA.

Gel shift assays were also performed with SH-EP cell nuclear extracts to identify candidate trans-acting factors operating on the STAT and ncAP-1 sites to mediate constitutive VIP expression. Specific binding of nuclear proteins to the ncAP-1 and STAT oligonucleotides was readily detected in extracts from untreated SH-EP cells (Fig. 5). Specific complex formation on both ncAP-1 and STAT oligonucleotides was observed with SH-EP cell nuclear extracts and was inhibited by the addition of unlabeled AP-1 consensus or VIP-TRE oligonucleotides in molar excess (Fig. 5). Both pan-Jun and pan-Fos antibodies interacted with the complex formed by SH-EP cell nuclear proteins on the ncAP-1 and STAT oligonucleotides (Fig. 5). Supershift analyses revealed that the AP-1 complex formed on both the STAT and ncAP-1 elements in SH-EP cell nuclear extracts consisted of a dimer of c-Jun and Fra-1 or Fra-2 (Fig. 5). The STAT and ncAP-1 sites of the VIP gene are therefore occupied by different AP-1 complexes in SH-EP and PMA-stimulated SK-N-SH neuroblastoma cells.

**DISCUSSION**

AP-1 is composed of dimers of members within the 

The jun and fos gene families (c-Fos, FosB, Fra-1, and Fra-2; for a review see Ref. 24). AP-1 binds to a cis-active element present on many genes (TRE; TGA(C/G)TCA) defined by its ability to mediate phorbolester-dependent induction of transcription (25). This report identifies two elements contained within the VIP gene capable of recruiting distinct AP-1 complexes in a cell context-dependent fashion, to achieve both stimulus-conditional and constitutive expression in neuroendocrine cells. Despite the fact that neither are consensus sequences for AP-1, binding of AP-1-related proteins to these relatively closely positioned sites (STAT and ncAP-1 sites at −1.32 and −1.20 kb, respectively) is apparently required for both PMA-inducible expression in SK-N-SH cells and constitutive expression in SH-EP cells. Interestingly, a single TRE consensus sequence in the VIP gene (at −2.25 kb), although capable of binding AP-1, is apparently not involved in either PMA-inducible expression in SK-N-SH cells or constitutive expression in SH-EP cells (18). Thus, the cis-activation potential of a given AP-1 site may depend both on its position in the gene and on proteins binding to neighboring sequences that enhance or forbid access to co-activators and other proteins that make up the pretranscriptional platform. Members of the Ets family of transcription factors, for example, are in some cases required for transcription activation by AP-1, binding to an adjacent site and cooperating with AP-1 in the recruitment of additional proteins to the gene (26–29). The VIP gene contains Ets-like core sequences (AGGA(A/T)) several bases downstream of the STAT and ncAP-1 sites that could function in this manner. On the other hand, the AP-1 consensus site at −2.25 kb may exert some AP-1-dependent function in another VIPergic cellular context, in which cis-active elements neighboring it recruit appropriate AP-1-cooperative trans-activating proteins.

In SK-N-SH cells, PMA caused an activation of pre-existing AP-1 (c-Fos and JunB or related proteins) in a protein phos-
phorylation-dependent manner without requiring new protein synthesis. How this c-Fos-Jun complex acquires the ability to bind both the nAP-1 and STAT sites of the VIP gene after stimulation by PMA is a subject of current investigation. Several distinct mitogen-activated protein kinase cascades have been implicated in the control of AP-1 activity in response to various extracellular stimuli (for a review, see Ref. 30). One type of cascade, initiated by growth factors, culminates in the activation of extracellular stimulus-responsive kinase, phosphorylation and activation of the transcription factor Elk-1, and increased transcription of the c-fos gene, leading to increased levels of c-Fos protein (31). Another type of cascade, initiated by stimuli such as tumor necrosis factor or UV irradiation leads to the activation of the Jun N-terminal kinase and the Fos-regulating kinase, which phosphorylate c-Jun and c-Fos, respectively, allowing binding to AP-1 sites and transactivation of target genes (32–35). A protein synthesis-independent mitogen-activated protein kinase pathway, like that involving activation of pre-existing Jun and Fos proteins, may be utilized in PMA-inducible expression of the VIP gene in SK-N-SH cells.

AP-1 binding to the STAT and nAP-1 sites of the VIP gene in SH-EP cells is likely to be a Fra-c-Jun dimer rather than a c-Fos-Jun-related protein dimer as in SK-N-SH cells. Signaling pathways leading either to induction of AP-1 protein synthesis or post-translational activation of AP-1 proteins could be responsible for activation of AP-1-dependent VIP gene transcription in SH-EP cells, depending on whether VIP expression in these cells is driven by continuously produced autocrine factors or constitutively activated signal transduction components within the cell, respectively.

While PMA induction of VIP mRNA does not require new protein synthesis in SK-N-SH cells, it does depend on new protein synthesis in primary cultured bovine adrenal chromaffin cells. Thus, while PMA induction of the VIP gene is mediated by post-translational modification and activation of pre-existing AP-1 in SK-N-SH cells, it is likely to be mediated by newly synthesized AP-1 (i.e. immediate early gene induction) in bovine chromaffin cells. Thus, differences in both the levels of AP-1 protein and the availability of pre-existing AP-1 for binding to DNA as a function of phosphorylation state play a critical role in determining the activity and inducibility of the VIP gene in SH-EP, SK-N-SH, and adrenal chromaffin cells. All of these cells are of neural crest origin, differing in their relative state of differentiation relative to sympatho-adrenal development. As such, the recruitment of distinct sets of AP-1 complex proteins to the VIP gene in these cells may reflect underlying mechanisms for cell-specific VIP expression throughout the neuroaxis in the intact animal. Changes in the level (or composition) of AP-1 proteins and their status of activation, determined by extracellular signals and the potency of the signal transduction pathways activated by them, are to be critical for cell-specific VIP gene regulation throughout the neuroendocrine axis.

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