Corticotropin-releasing factor and vasoactive intestinal polypeptide activate gene transcription through the cAMP signaling pathway in a catecholaminergic immortalized neuron

Abstract

Corticotropin-releasing factor (CRF) and vasoactive intestinal polypeptide (VIP) are neuropeptides displaying a variety of short-term effects in the nervous system. It is shown here in transfection experiments of an immortalized noradrenergic locus coeruleus-like cell line that both CRF and VIP also trigger a signaling cascade capable of activating gene transcription. To elucidate the signaling pathway leading to transcriptional induction, cells were transfected with an inhibitor for cAMP-dependent protein kinase, targeted to the nucleus via a nuclear-localization signal. Transcriptional induction of a reporter gene by CRF and VIP was blocked in these cells, indicating that the cAMP-dependent protein kinase is required for transducing CRF and VIP generated signals into the nucleus. Additionally, transfection experiments with a reporter gene containing cAMP response elements in its regulatory region demonstrate that CRF and VIP receptor activation induce transcription through this genetic regulatory element. We conclude that long-term effects of CRF and VIP in neurons are likely to be mediated by the transcriptional regulation of CRF and VIP-responsive genes via the cAMP signaling pathway.

1. Introduction

Corticotropin-releasing factor (CRF), a neuropeptide of 41 amino acids that is highly conserved in evolution, acts as the major physiological regulator of the basal and stress-induced release of corticotropin (ACTH), β-endorphin and other proopiomelanocortin-derived peptides from the anterior pituitary gland (for review, see Owens and Nemeroff, 1991). High CRF-immunoreactivity has been found in the paraventricular nucleus of the hypothalamus but also in many extrahypothalamic regions of the brain, indicating that CRF, in addition to its endocrine activity, displays extrahypophysiotropic effects, mainly as a regulator of stress responses. Overproduction of CRF has been implicated in neuropsychiatric disorders, such as major depression, anxiety-related disorders, and anorexia nervosa (for review, see Dunn and Berridge, 1990). Vasoactive intestinal polypeptide (VIP), a regulatory peptide of 28 amino acids, is widely expressed in the central and peripheral nervous system exhibiting neurotransmitter and hormonal roles (for review, see Gozes and Brenneman, 1989). Particularly, VIP plays

Abbreviations: CRE, cAMP response element; CREB, cAMP response element binding protein; CRF, corticotropin-releasing factor; dbcAMP-N6, 2′-O-dibutyryladenosine 3′; 5′-monophosphate; GST, glutathione S-transferase; LC, locus coeruleus; NLS, nuclear localization signal; PACAP, pituitary adenylate cyclase-activating polypeptide; PKA, protein kinase A; VIP, vasoactive intestinal polypeptide.

an important role in systemic vasodilation, leading to a relaxation of cerebral blood vessels (Lee et al., 1984).

CRF and VIP mediate their actions through binding to receptors which belong to the family of seven transmembrane helix, Gs-coupled receptors (Ishihara et al., 1992; Chang et al., 1993; Chen et al., 1993; Lutz et al., 1993; Lovenberg et al., 1995). VIP additionally binds to the PAC_1 and $VPAC_1$ receptors (Sprengler et al., 1993; Rawlings et al., 1996; Harmar et al., 1998). Binding of CRF and VIP to their cognate receptors is associated with the activation of adenylyl cyclase (Chen et al., 1986, 1993; Chang et al., 1993; Ciccarelli et al., 1994; Lovenberg et al., 1995). The increased intracellular concentration of cAMP mediates the activation of cAMP-dependent protein kinase (PKA) by triggering the dissociation of catalytic and regulatory subunits of the holoenzyme. The activation of PKA is believed to transduce all known effects of cAMP in mammalian cells. PKA phosphorylates regulatory proteins in the cytoplasm such as tyrosine hydroxylase and synapsin I, and also proteins in the plasma membrane, such as ion channels and receptors (Girault et al., 1988; Moss et al., 1996; Hwang et al., 1997; Puri et al., 1997; Gray et al., 1998). In addition, the catalytic subunit of PKA translocates to the nucleus and activates gene transcription by phosphorylating transcription factors such as CREB (for review, see Montminy, 1993; Lalli and Sassone-Corsi, 1994; Vallejo and Habener, 1994). Unfortunately, in most of the studies dealing with cAMP-mediated gene transcription, membrane-penetrating cAMP analogues such as dbcAMP or unphysiological activators of adenylate cyclase like forskolin were used, and only few studies were performed with physiological activators.

In this study, the role of CRF and VIP in activating gene transcription in CATH.a cells has been investigated. From the fact that both peptides initiate the cAMP signaling pathway we suggested that one of the functions of CRF and VIP in neurons is the activation of CREB, thus leading to transcription of CREB controlled genes. One CRF responsive target gene in the pituitary was identified as the proopiomelanocortin gene. This gene, however, lacks a canonical cAMP response element (CRE). This discrepancy has been explained by the fact that CRF receptor activation induces c-*fos*, that subsequently controls proopiomelanocortin gene expression (Boutillier et al., 1991). Alternatively, a CRF responsive element has been identified in the rat proopiomelanocortin gene, together with a DNA-binding protein termed PCRH-REB-1 (Jin et al., 1994). The CRF response element and the cognate DNA-binding protein have no similarities with the CRE and the CRE-binding proteins of the bZIP familiy, respectively. VIP potentiated c-*fos* expression in cultured cortical neurons. VIP, however, did not activate the c-*fos* gene directly but rather potentiates the effect of glutamate, which activates transcription by stimulating *N*-Methyl-D-aspartate receptors (Martin et al., 1995).

To clearly elucidate the role of CRF and VIP upon CREB activation and gene transcription and other transcription factors, we used model promoters and activators to avoid interference of other transcriptional elements. In addition, we designed and used a high specific nuclear inhibitor of cAMP dependent protein kinase to verify the impact of the cAMP signaling cascade upon CRF and VIP regulated gene transcription. As a model system, to investigate the role of CRF and VIP upon gene transcription, an immortalized locus-coeruleus-like cell line, CATH.a, was used that contained both CRF, VIP and PAC_1 receptors (Duman et al., 1992; Iredale et al., 1996; Muller et al., 1997). The locus coeruleus (LC), a group of specialized neuronal cells in the brainstem, is the largest noradrenergic center in the brain, containing approximately 50% of brain noradrenergic neurons. The activation of noradrenergic LC neurons has been associated with stress due to local release of CRF (Valentino et al., 1991). In addition, the LC contains one of the highest densities of VIP binding sites in the brain (Martin et al., 1987).

2. Experimental procedures

2.1. Reporter constructs

Plasmid 5 × GAL4OVEC has been described (Cibelli et al., 1996a). Plasmid pCgBCRE^{4}OVEC contains four copies of the CRE sequence derived from the murine chromogranin B gene and was constructed by inserting the synthetic annealed oligonucleotides 5′-TCGA-GCTGCTCCGTGACGTCAGGGCTGAG-3′ and 5′-TCGACTCAGCCCTGACGTCACGGAGCAGC-3′, comprising chromogranin B promoter sequences from −110 to −86, into the Sal I and Xho I sites of plasmid pHIVTATA-CAT (Thiel et al., 1996). This sequence was subsequently multimerized as described, excised with Sal I and Sac I and cloned into the plasmid OVEC (Westin et al., 1987).

2.2. Expression plasmids

Plasmid pGAL4-CREB, containing the human CREB cDNA fused to the DNA-binding domain of GAL4, has been described recently (Cibelli et al., 1996a). Plasmid pEBG-PKI encodes a fusion protein consisting of *Schistosoma japonicum* glutathione S-transferase (GST) and amino acids 1 to 31 of the PKA inhibitor PKI (Scott et al., 1985). This expression construct was generated by inserting a blunt ended BamHI/XbaI-fragment from plasmid pLXX-PKI(1–31) (Grove et al., 1987) into the filled in Not I site of

the eukaryotic GST-expression vector pEBGN. This vector encodes *S. japonicum* GST under the control of the EF-1α promoter. To accomplish nuclear targeting of the GST fusion protein, two synthetic, annealed oligonucleotides 5′-GATCACCTCCTAAAAAGAA-GCGCAAGGTAGGCGGAG-3′ and 5′-GATCCT-CCGCCTACCTTGCGCTTCTTTTTAGGAGGT-3′, encoding the nuclear localization sequence of the SV40 large T antigen, were inserted into the BamHI site of pEBG (Sánchez et al., 1994), a kind gift of Bruce J. Mayer, Howard Hughes Medical Institute, Harvard University, Cambridge, MA, USA.

2.3. Cell culture and transfections

The locus coeruleus-like cell line CATH.a (Suri et al., 1993) was a generous gift of Donna Chikaraishi, Neuroscience Program, Tufts University School of Medicine, Boston, MA, USA. Cells were cultured in 88% RPMI medium supplemented with 4% fetal calf serum, 8% horse serum, 100 units/ml penicillin, and 100 mg/ml streptomycin. Cells were transfected by the calcium phosphate coprecipitation procedure (Thiel et al., 1991). The β-globin reporter plasmid (5 μg), the GAL4-CREB expression plasmid (5 μg) and the internal reference plasmid ICPOref (2.5–5 μg) were transfected into cells on 100 mm plates. 24 h later, cells were treated with either 1 mM N6,2′-*O*-dibutyryladenosine 3′,5′-monophosphate (dbcAMP, Sigma), 0.5 μM ovine CRF (Serva or Calbiochem), 0.5 μM α-helical CRF9-41 (Sigma), or 1 μM VIP (Serva), respectively. N6,2′-*O*-dibutyryladenosine 3′,5′-monophosphate and VIP were dissolved in water, and CRF and α-helical CRF9-41 in 0.05 M acetic acid, before addition to the culture medium. Total cytoplasmic RNA was extracted 48 h after transfection and the β-globin mRNA was detected by RNase protection mapping (Thiel et al., 1994). COS cells were transfected using the DEAE-dextran method (Thiel et al., 1998) and whole cell extracts were prepared as described (Cibelli et al., 1996b).

2.4. Miscellaneous techniques

For Western blot analysis, proteins from transfected COS cells were separated on a 14% SDS–PAGE and transferred to nitrocellulose membranes (pore size 0.2 μm). Antibodies recognizing *S. japonicum* GST were raised in rabbits with purified recombinant GST protein. Blots were developed using an alkaline phosphatase-conjugated secondary antibody. Indirect immunofluorescence was performed as described (Cicchetti et al., 1995).

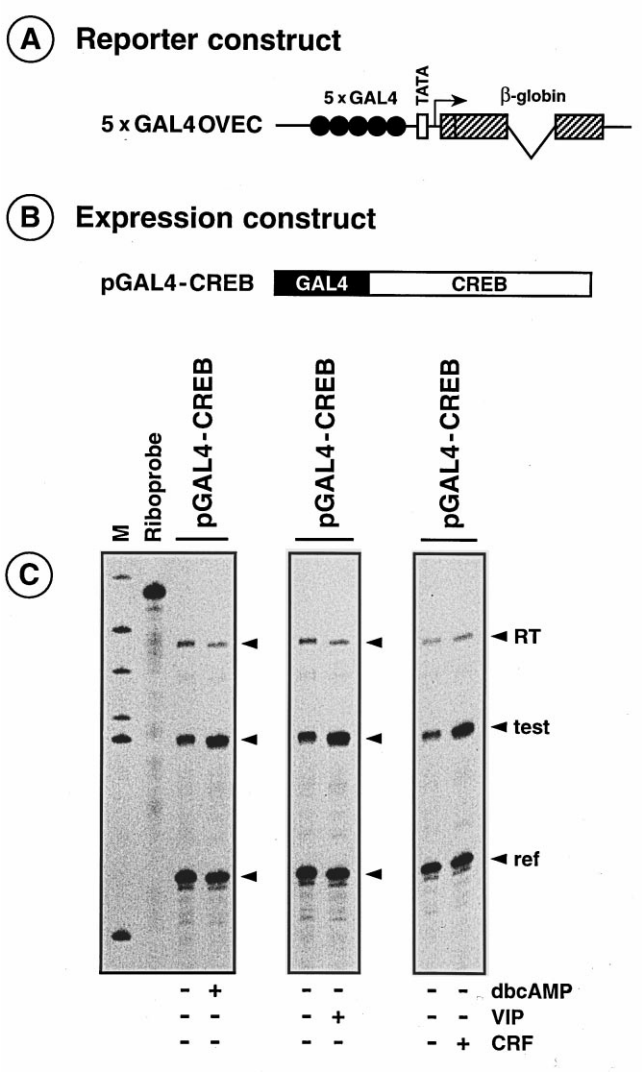

Fig. 1. VIP, CRF and dbcAMP activate gene transcription in CATH.a cells. (A) Reporter plasmid containing rabbit β-globin gene as a reporter gene, a TATA box and five copies of the GAL4 binding site upstream of the TATA box. (B) Expression vector encoding a fusion protein between the DNA-binding domain of the yeast transcription factor GAL4 and the cDNA of human CREB (plasmid pGAL4-CREB). (C) Reporter plasmid, expression plasmid and the ICPOref internal standard plasmid were introduced into CATH.a cells. Cells were incubated in the absence (−) or presence (+) of 1 mM dbcAMP, 1 μM VIP or 0.5 μM CRF for 24 h. Cytoplasmic RNA was prepared and analysed using RNase protection mapping. The bands labeled 'test' indicate correctly initiated β-globin transcripts, and the bands labeled 'ref' were generated by the internal standard plasmid ICPOref. RT: incorrectly initiated read-through transcripts of the test templates; riboprobe: aliquot of undigested cRNA; M: size marker, HaeIII digested pBR322.

3. Results

3.1. VIP and CRF induced gene transcription in a catecholaminergic LC-like cell line

The 5′-flanking regions from mammalian genes contain binding sites for many transcription regulating

proteins that exhibit effects upon transcriptional activation or repression. The tyrosine hydroxylase gene, for example, contains in its 5′-flanking region the *cis*-acting regulatory elements AP2, Ap1, E-box, POU, Sp1, and CRE showing positive and negative effects on tyrosine hydroxylase gene transcription (Lazaroff et al., 1998). To investigate whether VIP and CRF have an impact on gene transcription in CATH.a cells, model promoters and model activator proteins were used. The use of model promoters and activators allowed us to test the effect of CRF and VIP on gene transcription without influences from other promoter elements. As a model transactivator protein, a fusion protein consisting of the DNA binding domain of the yeast transcription factor GAL4 fused to the cDNA of human CREB was constructed (Fig. 1B). Since GAL4 does not bind to any known mammalian gene promoter element, interference by other competing CRE-binding proteins was avoided. As a reporter, five copies of GAL4 binding sites were inserted upstream of the β-globin reporter gene (Fig. 1A). These plasmids were introduced into the catecholaminergic cell line CATH.a, derived from a tumor developed in transgenic mice carrying the SV40 T antigen under the control of the rat tyrosine hydroxylase promoter (Suri et al., 1993). To control for variability in transfection efficiency, cells were additionally transfected with the plasmid ICPOref, containing a mutated β-globin gene under control of the herpes virus ICP0 gene promoter. Two days post-transfection, cytoplasmic RNA of the transfected cells was harvested, hybridized to a β-globin-derived cRNA probe, and analyzed for the amount of β-globin mRNA by RNase protection assay. The correctly initiated β-globin message is indicated ('test'). The internal reference plasmid ICPOref protected a shorter fragment of the β-globin riboprobe (labeled 'ref'). Fig. 1C shows that the cAMP analogue dbcAMP (left panel) and the neuropeptides VIP and CRF (middle and right panel) were able to induce transcription of the β-globin reporter gene. This experiment revealed that VIP and CRF receptor activation induced gene transcription at levels similar to that resulting from treatment of cells with a cAMP analogue. In contrast, a truncated CRF peptide, α-helical CRF9-41, that acts as a CRF antagonist, was unable to activate transcription from the reporter gene (data not shown). We conclude that receptor activation, mediated by CRF or VIP is capable of transducing a signaling cascade required for activating gene transcription.

3.2. Design and expression of a nuclear inhibitor for PKA

CRF, VIP and PAC_1 receptor activation stimulate adenylyl cyclase in CATH.a cells (Duman et al., 1992;

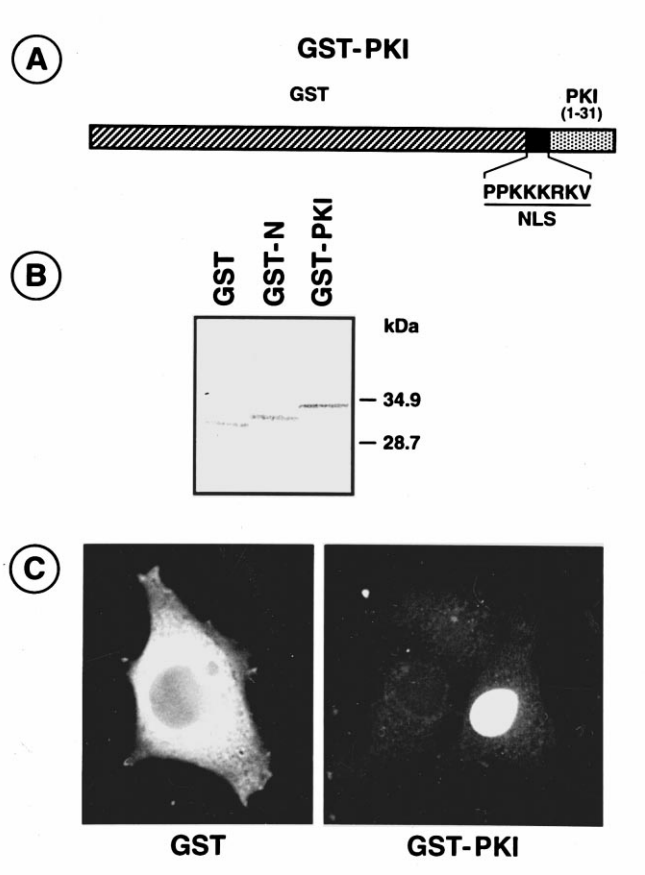

Fig. 2. Design and expression of GST-PKI, a nuclear PKA inhibitor. (A) Structure of the GST-PKI fusion protein. The GST and the PKI moieties are separated by the NLS derived from the large T antigen of SV40 virus. (B) Immunoblot analysis of GST and GST fusion proteins expressed in COS cells. Whole cell extracts of COS cells transfected with expression vectors encoding *S. japonicum* GST, a GST protein tagged with the SV40 large T antigen NLS (GST-N), and the GST-PKI fusion protein were analyzed by immunoblotting using a polyclonal antibody against GST followed by an alkaline phosphatase-labeled secondary antibody. Position of molecular markers are shown on the right. (C) Nuclear targeting of the GST-PKI fusion protein. COS cells were transfected with expression vectors encoding GST and GST-PKI and expression was monitored by indirect immunofluorescence using a polyclonal antibody against GST followed by an FITC-labeled secondary antibody.

Rawlings and Hezareh, 1996; Muller et al., 1997), leading to an increase in the intracellular concentration of cAMP. However, not all of the effects of CRF and VIP have been attributed to a stimulation of adenylyl cyclase. The inhibitory effect of VIP on N-type calcium channels of sympathetic neurons was shown to be independent of PKA (Zhu and Ikeda, 1994). In anterior pituitary corticotrophs, it was reported that CRF and VIP increased the intracellular free calcium levels (Luini et al., 1995). Calcium activates calcium/calmodulin-dependent protein kinases and one of these kinases, calcium/calmodulin-dependent protein kinase IV, translocates into the nucleus and activates transcription, as does PKA, via the CRE/CREB pathway

(Matthews et al., 1994; Sun et al., 1994). Moreover, we found recently that CRF increased MAP kinase activity in CATH.a cells[1], suggesting that CREB could also be activated by the MAP kinase activated pp90 ribosomal S6 kinase RSK in CATH.a cells as shown for PC12 cells (Xing et al., 1998). To specify the signaling pathway leading from CRF and VIP/PACAP receptor activation to an increase in gene transcription, a nuclear inhibitor for PKA was constructed consisting of *S. japonicum* GST and amino acids 1 to 31 of the PKA inhibitor PKI. PKI, a 75 amino acid, heat-stable protein, binds with a high specificity to the catalytic subunit of PKA. The regulatory subunit of PKA and PKI bind and inhibit the catalytic subunit of PKA by analogous mechanisms. The exact physiological role of PKI is still unclear. It has been postulated that PKI fine-tunes the level of the catalytic activity of the catalytic subunit of PKA (Fantozzi et al., 1994; Seasholtz et al., 1995). The designed GST-PKI fusion protein additionally contained a nuclear localization signal to accomplish nuclear targeting (Fig. 2A). The expression of the GST-PKI protein and control proteins GST and an NLS-tagged GST, encoded by the expression vectors pEBG and pEBGN, respectively, was confirmed by transient transfection in COS cells followed by Western blot analysis using anti-GST antibodies (Fig. 2B). Indirect immunofluorescence was used to verify the intracellular distribution of the GST-PKI protein in COS cells transfected with the GST-PKI expression vector. We chose COS cells to test the expression vectors, because these cells can replicate the pEBG plasmids due to the expression of SV40 large T antigen in the cell and the presence of the SV40 replication origin in the plasmids. Thus, high expression levels of recombinant proteins can be obtained. The results show that the GST-PKI fusion protein was entirely expressed in the nuclei of the transfected cells (Fig. 2C). COS cells transfected with the expression vector for the GST protein alone, showed a clear cytoplasmic distribution (Fig. 2C).

3.3. The nuclear targeted GST-PKI fusion protein blocked transcriptional activation of a reporter gene by the CRF and VIP initiated signaling pathway

CATH.a cells were transfected with the 5 × GAL4OVEC reporter plasmid, the GAL4-CREB expression plasmid and the internal reference plasmid ICPOref. In addition, cells were transfected with an expression vector encoding GST-PKI, or, as control, the GST expression vector pEBGN. Following transfection, CATH.a cells were treated with dbcAMP, VIP or

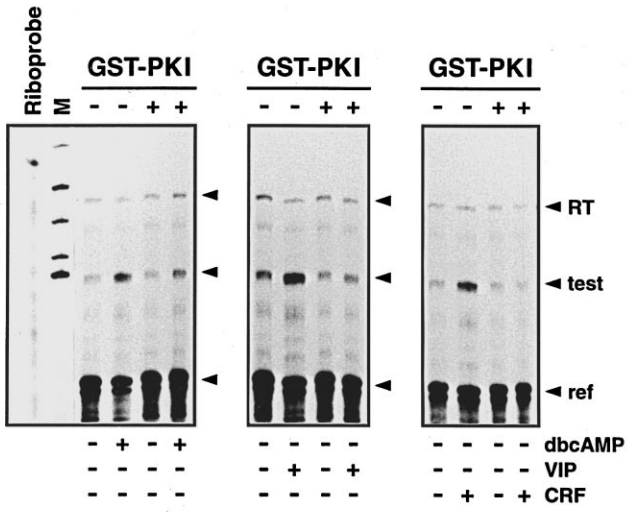

Fig. 3. The GST-PKI fusion protein inhibits VIP and CRF induced transcriptional activation in CATH.a cells. Reporter plasmid 5 × GAL4OVEC, expression plasmid pGAL4-CREB and the ICPOref internal standard plasmid were introduced into CATH.a cells together with an expression vector encoding either a nuclear targeted GST (−) or the GST-PKI fusion protein. Cells were incubated in the absence (−) or presence (+) of 1 mM dbcAMP, 1 μM VIP or 0.5 μM CRF for 24 h. Cytoplasmic RNA was analyzed for β-globin mRNA by RNase protection mapping.

CRF for 24 h. Transcription was monitored by RNase protection mapping of the β-globin mRNA. The cAMP analogue dbcAMP and the neuropeptides VIP and CRF increased transcription of the reporter gene in the absence of the PKA inhibitor (Fig. 3). Expression of the GST-PKI fusion protein blocked the transcriptional induction of the reporter gene, clearly indicating the role of PKA in the signalling cascades initiated by CRF and VIP leading to transcription. Furthermore, basal transcription of the reporter gene was impaired by GST-PKI, most likely due to an inhibition of basal PKA activity in the nucleus.

3.4. CRF and VIP induced transcription of a reporter gene containing cAMP response elements in its regulatory region

The experiments described so far have shown that CRF and VIP receptor activation leads to changes in gene transcription and, additionally, that PKA is necessary for this activity. These data suggest that endogenous CREB or related bZIP proteins are activated by CRF and VIP via PKA to increase transcription of those genes containing CREB binding sites, such as the CRE sequence, in their regulatory regions. To demonstrate that VIP and CRF induced transcriptional activity can, in fact, be mediated by the CRE sequence, a reporter gene containing in its regulatory region four copies of the CRE derived from the chro-

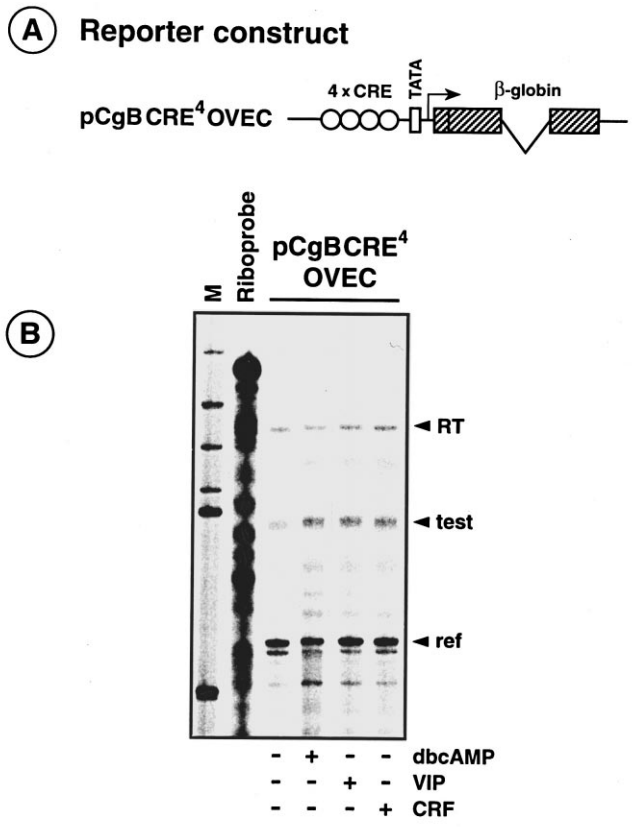

Fig. 4. VIP and CRF activate transcription in CATH.a cells via the cAMP response element (CRE). (A) Reporter plasmid pCgBCRE^{4}OVEC containing the rabbit β-globin gene as reporter gene, a TATA box and four copies of the CRE sequence derived from the murine chromogranin B gene upstream of the TATA box. (B) Reporter plasmid pCgBCRE^{4}OVEC and the ICPOref internal standard plasmid were introduced into CATH.a cells. Cells were incubated in the absence (−) or presence (+) of 1 mM dbcAMP, 1 μM VIP or 0.5 μM CRF for 24 h. Cytoplasmic RNA was analyzed for β-globin mRNA by RNase protection mapping.

mogranin B gene, was constructed (plasmid pCgBCRE^{4}OVEC, Fig. 4A). Chromogranin B, a member of the granin family, is an acidic secretory protein found in secretory granules of most peptidergic neuroendocrine cells and neurons, and is expressed in CATH.a cells as determined by Northern blot analysis[2]. The CRE of the chromogranin B promoter is a binding site for CREB and functions as a cAMP-inducible enhancer in neuronal and neuroendocrine cells (Jüngling et al., 1994). The reporter plasmid pCgBCRE^{4}OVEC and the internal standard plasmid ICPOref were introduced into CATH.a cells by transient transfection. Cells were treated with either dbcAMP, VIP or CRF for 24 h and transcription of the reporter gene was determined by RNase protection mapping (Fig. 4B). The cAMP analogue dbcAMP, as well as the peptides VIP and CRF, increased transcription of the β-globin gene in CATH.a cells, indicating that these neuropeptides are, in fact, able to generate signalling cascades which result in transcriptional activation via the CRE sequence.

4. Discussion

A challenging problem in neurobiology is the elucidation of molecular mechanisms by which neurons adapt to changes in their environment. Extracellular signaling molecules such as neurotransmitters and neuromodulators have been shown to initiate rapid and short-lived changes following activation. Such short-term responses are alterations of the ion permeability of the membrane, changes in the current of ion channels, protein–protein interactions and post-translational modifications. In some cases, neuronal stimulation is also believed to result in physiological changes that can persist for weeks and months, suggesting that neurotransmitters and neuromodulators may also trigger a signaling cascade that ultimately results in an alteration of the genetic program of the postsynaptic neuron. Thus, gene regulation in the nervous system is likely to be important for long-term cellular phenotypic alterations, and form the molecular basis for phenomena such as synaptic plasticity, learning and memory (for review, see Armstrong and Montminy, 1993). Changes in gene transcription have been monitored following the activation of the *N*-Methyl-D-aspartate and D1/D2 dopamine receptors, respectively (Gerfen et al., 1990; Bading et al., 1993). Furthermore, the cAMP signaling pathway, through its effects on gene transcription, is thought to play an important role in memory consolidation (for review, see Stevens, 1994; Bailey et. al., 1996; Abel et al., 1998).

Here, the role of CRF and VIP upon gene transcription has been studied. Two different types of model reporter genes were used throughout the analysis. The first reporter gene contained binding sites for the yeast transcription factor GAL4 in its regulatory region. Transcriptional activation of the β-globin reporter gene was dependent on the activation state of the expressed GAL4-CREB fusion protein. Using a battery of neuronal cell lines, it was reported that this protein is activated by a rise of the intracellular cAMP concentration (Cibelli et al., 1996a). The second model promoter contained four CRE sequences immediately upstream of the rabbit β-globin TATA box. Gene transcription of the reporter required the recruitment of an endogenous, active CREB or related proteins. The analysis of both model promoters in CATH.a cells, which resemble the noradrenergic neurons of the

LC, revealed that both CRF and VIP receptor activation set off a signaling cascade that results in gene transcription of the β-globin reporter gene. Moreover, the signaling pathway required PKA activity in the nucleus, as shown in an experiment using a nuclear PKA inhibitor.

Further clarification of the role of CRF and VIP in regulating gene transcription will be possible through an identification of those genes that are induced by these stimuli in different neuronal populations. Based on the data presented in this study, the most promising candidate genes should contain a CRE sequence in their regulatory regions. Recently, the macrophage migration-inhibitory factor (MIF) gene was identified as CRF-responsive in anterior pituitary cells. The CRF-mediated increase in transcription was executed by a CRE within the 5-regulatory sequence of the MIF gene (Waeber et al., 1998). Another likely CRF-responsive candidate gene is the gene encoding tyrosine hydroxylase. This enzyme catalyzes the reaction from L-tyrosine to 3,4-dihydroxy-L-phenylalanine which is the rate limiting reaction in catecholamine biosynthesis. Interestingly, repeated administration of CRF increased, as does chronic stress, the level of tyrosine hydroxylase in LC neurons (Melia and Duman, 1991). Moreover, it has been reported that transcription of a tyrosine hydroxylase promoter/chloramphenicol acetyltransferase gene could be induced in CATH.a cells by adding dbcAMP to the cells. The CRE was shown to be responsible for >80% of basal (uninduced) transcription of the reporter gene (Lazaroff et al., 1995). Furthermore, when this study was in progress, it was reported that VIP was able to induce transcription of a tyrosine hydroxylase promoter/chloramphenicol acetyltransferase gene in CATH.a cells (Muller et al., 1997). It would be of interest to know whether CRF exhibits a similar effect.

CRF receptor activation leads to an increase in proopiomelanocortin transcription in the pituitary. This effect was attributed to an activation of the transcription factor c-*fos* that subsequently induces proopiomelanocortin gene expression (Boutillier et al., 1991). However, a proopiomelanocortin promoter/chloramphenicol acetyltransferase gene lacking the major exonic *fos* binding site was still CRF responsive (Boutillier et al., 1995). Alternatively, a DNA-binding protein termed PCRH-REB-1 (Jin et al., 1994) might trigger transcriptional induction of the proopiomelanocortin gene via a CRF response element in its regulatory region. PCRH-REB-1 and the CRF response element exhibit no similarities with the CRE-binding proteins of the bZIP family or the CRE sequence, respectively. However, PCRH-REB-1 contains nine possible PKA phosphorylation sites suggesting that the CRF-induced activation of PKA might have an impact on PCRH-REB-1 activity via phosphorylation. Although PKA induces gene transcription in a variety of genes by phosphorylating bZIP proteins such as CREB, PKA is also able to phosphorylate other transcription factors in the nucleus and change their activities (Rohlff, 1997; Zhong et al., 19987).

Further work will be needed to elucidate the connection between CRF receptor activation and PCRH-REB-1 activity. Moreover, a nonclassical CRE was recently identified in the human proopiomelanocortin gene that functioned as a binding site for CREB and conferred cAMP responsiveness to a reporter gene (Kraus and Hölt, 1995). Interestingly, the genes encoding the neuropeptides CRF and VIP contain CRE sequences in their regulatory regions and activation of the cAMP signaling pathway increased CRF and VIP gene transcription (Tsukada et al., 1987; Dorin et al., 1993; Guardiola-Diaz et al., 1994; Itoi et al., 1996). Therefore, CRF and VIP might control the expression of their own genes by inducing the cAMP signaling pathway.

In conclusion, using a catecholaminergic neuronal cell line, it is shown here that CRF and VIP induce gene transcription mediated by the cAMP signaling pathway. Future work to identify CRF and VIP responsive genes, quite likely mediated by CRE sequences in their regulatory regions, appears promising. The identification of such target genes will help to clarify the molecular basis for the long-term effects of these neuropeptides in the nervous system.

References

Armstrong, R.C., Montminy, M.R., 1993. Transsynaptic control of gene expression. Annu. Rev. Neurosci. 16, 7–29.

Abel, T., Martin, K.C., Bartsch, D., Kandel, E.R., 1998. Memory suppressor genes: inhibitory constraints on the storage of long-term memory. Science 279, 338–341.

Bading, H., Ginty, D.D., Greenberg, M.E., 1993. Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 260, 181–186.

Bailey, C.H., Bartsch, D., Kandel, E.R., 1996. Towards a molecular definition of long-term memory storage. Proc. Natl. Acad. Sci. USA 93, 13445–13452.

Boutillier, A.L., Monnier, D., Lorang, D., Lundblad, J.R., Roberts, J.L., Loeffler, J.P., 1995. Corticotropin-releasing hormone stimulates proopiomelanocortin transcription by cFos-dependent and -independent pathways: characterization of an AP1 site in Exon 1. Molecular Endocrinology 9, 745–755.

Boutillier, A.L., Sassone-Corsi, P., Loeffler, J.P., 1991. The protooncogene c-fos is induced by corticotropin-releasing factor and stimulates proopiomelanocortin gene transcription in pituitary cells. Molecular Endocrinology 5, 1301–1310.

Chang, C-P., Pearse II, R.V., O'Connell, S., Rosenfeld, M.G., 1993. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron 11, 1187–1195.

Chen, F.M., Bilezikjian, L.M., Perrin, M.H., Rivier, J., Vale, W., 1986. Corticotropin releasing factor receptor-mediated stimulation of adenylate cylase activity in the rat brain. Brain Res. 381, 49–57.

Chen, R., Lewis, K.A., Perrin, M.H., Vale, W., 1993. Expression cloning of a human corticotropin-releasing-factor receptor. Proc. Natl. Acad. Sci. USA 90, 8967–8971.

Cibelli, G., Jüngling, S., Schoch, S., Gerdes, H-H., Thiel, G., 1996a. Identification of a functional cAMP response element in the secretogranin II gene. Eur. J. Biochem. 226, 171–179.

Cibelli, G., Schoch, S., Pajunk, H., Brand, I.A., Thiel, G., 1996b. A (G + C)-rich motif in the aldolase C promoter functions as a constitutive transcriptional enhancer element. Eur. J. Biochem. 237, 311–317.

Ciccarelli, E., Vilardaga, J-P., De Need, P., Di Paolo, E., Waelbroeck, M., Bollen, A., Robberecht, P., 1994. Properties of the VIP-PACAP type II receptor stably expressed in CHO cells. Reg. Peptides 54, 397–407.

Cicchetti, P., Ridley, A.J., Zheng, Y., Cerione, R.A., Baltimore, D., 1995. 3BP-1, an SH3 domain binding protein, has GAP activity for Rac and inhibits growth factor-induced membrane ruffling in fibroblasts. EMBO. J. 14, 3127–3135.

Dorin, R.I., Zlock, D.W., Kilpatrick, K., 1993. Transcriptional regulation of human corticotropin releasing factor gene expression by cyclic adenosine 3′,5′-mono-phosphate: differential effects at proximal and distal promoter elements. Mol. Cell. Endocrinol. 96, 99–111.

Duman, R.S., Terwilliger, R.Z., Nestler, E.J., 1992. 'Locus coeruleus-like' cell line: a model system for studies of opiate and antidepressant drug action. Soc. Neurosci. Abstr. 18, 816.

Dunn, A.J., Berridge, C.W., 1990. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res. Rev. 15, 71–100.

Fantozzi, D.A., Harootunian, A.T., Wen, W., Taylor, S.S., Feramisco, J.R., Tsien, R.Y., Meinkoth, J.L., 1994. Thermostable inhibitor of cAMP-dependent protein kinase enhances the rate of export of the kinase catalytic subunit from the nucleus. J. Biol. Chem. 269, 2676–2686.

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma Jr, F.J., Sibley, D.R., 1990. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250, 1429–1432.

Girault, J.A., Shalaby, I.A., Rosen, N.L., Greengard, P., 1988. Regulation by cAMP and vasoactive intestinal peptide of phosphorylation of specific proteins in striatal cells in culture. Proc. Natl. Acad. Sci. USA 85, 7790–7794.

Gozes, I., Brenneman, D.E., 1989. VIP: Molecular biology and neurobiological function. Mol. Neurobiol. 3, 202–235.

Gray, P.C., Scott, J.D., Catterall, W.A., 1998. Regulation of ion channels by cAMP-dependent protein kinase and A-kinase anchoring proteins. Curr. Opin. Neurobiol. 8, 330–334.

Grove, J.R., Price, D.J., Goodman, H.M., Avruch, J., 1987. Recombinant fragment of the protein kinase inhibitor blocks cyclic AMP-dependent gene transcription. Science 238, 530–533.

Guardiola-Diaz, H.M., Boswell, C., Seasholtz, A.F., 1994. The cAMP-responsive element in the corticotropin-releasing hormone gene mediates transcriptional regulation by depolarization. J. Biol. Chem. 20, 14784–14791.

Harmar, A.J., Arimura, A., Gozes, I., Journot, L., Laburthe, M., Pisegna, J.R., Rawlings, S.R., Robberecht, P., Said, S.I., Sreedharan, S.P., Wank, S.A., Waschek, J.A., 1998. International union of pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. Pharmacol. Rev. 50, 265–270.

Hwang, O., Park, S., Kim, K.S., 1997. Protein kinase A coordinately regulates both basal expression and cyclic AMP-mediated induction of three catecholamine-synthesizing enzyme genes. J. Neurochem. 68, 2241–2247.

Iredale, P.A., Terwilliger, R., Widnell, K.L., Nestler, E.J., Duman, R.S., 1996. Differential regulation of corticotropin-releasing factor$_1$ receptor expression by stress and agonist treatment in brain and cultured cells. Mol. Pharmacol. 50, 1103–1110.

Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K., Nagata, S., 1992. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. Neuron 8, 811–819.

Itoi, K., Horiba, N., Tozawa, F., Sakai, Y., Sakai, K., Abe, K., Demura, H., Suda, T., 1996. Major role of 3′,5′-cyclic adenosine monophosphate-dependent protein kinase A pathway in corticotropin-releasing factor gene expression in the rat hypothalamus in vivo. Endocrinology 137, 2389–2396.

Jin, W.D., Boutillier, A-L., Glucksman, M.J., Salton, S.R.J., Loeffler, J-P., Roberts, J.L., 1994. Characterization of a corticotropin-releasing hormone-responsive element in the rat proopiomelanocortin gene promoter and molecular cloning of its binding protein. Mol. Endocrinol. 8, 1377–1388.

Jüngling, S., Cibelli, G., Czardybon, M., Gerdes, H-H., Thiel, G., 1994. Differential regulation of chromogranin B and synapsin I gene promoter activity by cAMP and cAMP-dependent protein kinase. Eur. J. Biochem. 226, 925–935.

Kraus, J., Höllt, V., 1995. Identification of a cAMP-response element on the human proopiomelanocortin gene upstream promoter. DNA Cell. Biol. 14, 103–110.

Lalli, E., Sassone-Corsi, P., 1994. Signal transduction and gene regulation: the nuclear response to cAMP. J. Biol. Chem. 269, 17359–17362.

Lazaroff, M., Patankar, S., Yoon, S.O., Chikaraishi, D.M., 1995. The cyclic AMP response element directs tyrosine hydroxylase expression in catecholaminergic central and peripheral nervous system cell lines from transgenic mice. J. Biol. Chem. 270, 21579–21589.

Lazaroff, M., Qi, Y., Chikaraishi, D.M., 1998. Differentiation of a catecholaminergic CNS cell line modifies tyrosine hydroxylase transcriptional regulation. J. Neurochem. 71, 51–59.

Lee, T.J., Saito, A., Berezin, I., 1984. Vasoactive intestinal polypeptide-like substance: The potential transmitter for cerebral vasodilation. Science 224, 898–901.

Lovenberg, T.W., Liaw, C.W., Grigoriadis, D.E., Clevenger, W., Chalmers, D.T., De Souza, E.B., Oltersdorf, T., 1995. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc. Natl. Acad. Sci. USA 92, 836–840.

Luini, A., Lewis, D., Guild, S., Corda, D., Axelrod, J., 1995. Hormone secretagogues increase cytosolic calcium by increasing cAMP in corticotropin-secreting cells. Proc. Natl. Acad. Sci. USA 82, 8034–8038.

Lutz, E.M., Sheward, W.J., West, K.M., Morrow, J.A., Fink, G., Harmar, A.J., 1993. The VIP2 receptor: molecular characterization of a cDNA encoding a novel receptor for vasoactive intestinal peptide. FEBS Lett. 334, 3–8.

Martin, J-L., Dietl, M.M., Hof, P.R., Palacios, J.M., Magistretti, P.J., 1987. Autoradiographic mapping of [mono[125I]iodo-

Tyr10,MetO17]-vasoactive intestinal peptide binding sites in the rat brain. Neuroscience 23, 539–565.

Martin, J-L., Gasser, D., Magistretti, P.J., 1995. Vasoactive intestinal peptide and pituitary adenylate cyclase–activating polypeptide potentiate c-fos expression induced by glutamate in cultured cortical neurons. J. Neurochem. 65, 1–9.

Matthews, R.P., Guthrie, C.R., Wailes, L.M., Zhao, X., Means, A.R., McKnight, G.S., 1994. Calcium/calmodulin-dependent protein kinase types II and IV differentially regulate CREB-dependent gene expression. Mol. Cell. Biol. 14, 6107–6116.

Melia, K.R., Duman, R.S., 1991. Involvement of corticotropin-releasing factor in chronic stress regulation of the brain noradrenergic system. Proc. Natl. Acad. Sci. USA 88, 8382–8386.

Montminy, M.R., 1993. Control of transcription and cellular proliferation by cAMP. In: Karin, M. (Ed.), Gene Expression: General and Cell-type-specific. Birkhuser, Boston, pp. 76–92.

Moss, S.J., McDonald, B.J., Rudhard, Y., Schoepfer, R., 1996. Phosphorylation of the predicted major intracellular domains of the rat and chick neuronal nicotinic acetylcholine receptor α 7 subunit by cAMP-dependent protein kinase. Neuropharmacol. 35, 1023–1028.

Muller, A., Monnier, D., Rene, F., Larmet, Y., Koch, B., Loeffler, J-P., 1997. Pituitary adenylate cyclase-activating polypeptide triggers dual transduction signaling in CATH.a cells and transcriptionally activates tyrosine hydroxylase and c-fos expression. J. Neurochem. 68, 1696–1704.

Owens, M.J., Nemeroff, C.B., 1991. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol. Rev. 43, 425–473.

Puri, T.S., Gerhardstein, B.L., Zhao, X.L., Ladner, M.B., Hosey, M.M., 1997. Differential effects of subunit interactions on protein kinase A- and C-mediated phosphorylation of L-type calcium channels. Biochemistry 36, 9605–9615.

Rawlings, S.R., Hezareh, M., 1996. Pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP/vasoactive intestinal polypeptide receptors: actions on the anterior pituitary gland. Endocr. Rev. 17, 4–29.

Rohlff, C., Ahmad, S., Borellini, F., Lei, J., Glazer, R.I., 1997. Modulation of transcription factor Sp1 by cAMP-dependent protein kinase. J. Biol. Chem. 22, 21137– 21141.

Sánchez, I., Hughes, R.T., Mayer, B.J., Yee, K., Woodgett, J.R., Avruch, J., Kyriakis, J.M., Zon, L.I., 1994. Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-jun. Nature 372, 794–798.

Seasholtz, A.F., Gamm, D.M., Ballestero, R.P., Scarletta, M.A., Uhler, M.D., 1995. Differential expression of mRNAs for protein kinase inhibitor isoforms in mouse brain. Proc. Natl. Acad. Sci. USA 92, 1734–1738.

Scott, J.D., Fischer, E.H., Takio, K., Demaille, J.G., Krebs, E.G., 1985. Amino acid sequence of the heat-stable inhibitor of the cAMP-dependent protein kinase from rabbit skeletal muscle. Proc. Natl. Acad. Sci. USA 82, 5732–5736.

Sprengler, D., Waeber, C., Pantaloni, C., Holsboer, F., Bockaert, J., Seeburg, P.H., Journot, L., 1993. Differential signal transduction by five splice variants of the PACAP receptor. Nature 365, 170–175.

Stevens, C.F., 1994. CREB and memory consolidation. Neuron 13, 769–770.

Sun, P., Enslen, H., Myung, P.S., Maurer, R.A., 1994. Differential activation of CREB by Ca^{2+}/calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. Genes Dev. 8, 2527–2539.

Suri, C., Fung, B.P., Tischler, A.S., Chikaraishi, D.H., 1993. Catecholaminergic cell lines from the brain and adrenal glands of tyrosine hydroxylase-SV40 T antigen transgenic mice. J. Neurosci. 13, 1280–1291.

Thiel, G., Greengard, P., Südhof, T.C., 1991. Characterization of tissue-specific transcription by the human synapsin I gene promoter. Proc. Natl. Acad. Sci. USA 88, 3431–3435.

Thiel, G., Lietz, M., Cramer, M., 1998. Biological activity and modular structure of RE-1-silencing transcription factor (REST), a repressor of neuronal genes. J. Biol. Chem. 273, 26891–26899.

Thiel, G., Petersohn, D., Schoch, S., 1996. pHIVTATA-CAT, a versatile vector to study transcriptional regulatory elements in mammalian cells. Gene 168, 173–176.

Thiel, G., Schoch, S., Petersohn, D., 1994. Regulation of synapsin I gene expression by the zinc finger transcription factor zif268/egr-1. J. Biol. Chem. 269, 15294–15301.

Tsukada, T., Fink, J.S., Mandel, G., Goodman, R.H., 1987. Identification of a region in the human vasoactive intestinal polypeptide gene responsible for regulation by cyclic AMP. J. Biol. Chem. 262, 8743–8747.

Valentino, R.J., Page, M.E., Curtis, A.L., 1991. Activation of noradrenergic locus coeruleus neurons by hemodynamic stress is due to local release of corticotropin-releasing factor. Brain Res. 555, 25–34.

Vallejo, M., Habener, J.F., 1994. Mechanisms of transcriptional regulation by cAMP. In: Conaway, R.C., Conaway, J.W. (Eds.), Transcription: mechanisms and regulation. Raven Press, Ltd, New York, pp. 353–368.

Waeber, G., Thompson, N., Chautard, T., Steinmann, M., Nicod, P., Pralong, F.P., Calandra, T., Gaillard, R.C., 1998. Transcriptional activation of the macrophage migration-inhibitory factor gene by the corticotropin-releasing factor is mediated by the cyclic adenosine 3′,5′-monophosphate responsive element-binding protein CREB in pituitary cells. Mol. Endocrinol. 12, 698–705.

Westin, G., Gerster, T., Mller, M.M., Schaffner, G., Schaffner, W., 1987. OVEC, a versatile system to study transcription in mammalian cells and cell-free extracts. Nucleic Acids Res. 17, 6787–6798.

Xing, J., Kornhauser, J.M., Xia, Z., Thiele, E.A., Greenberg, M.E., 1998. Nerve growth factor activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways to stimulate CREB serine 133 phosphorylation. Mol. Cell. Biol. 18, 1946–1955.

Zhong, H., SuYang, H., Erdjument-Bromage, H., Tempst, P., Ghosh, S., 1997. The transcriptional activity of NF-κB is regulated by the IκB-associated PKAc subunit through a cyclic AMP-independent mechanism. Cell 89, 413–424.

Zhu, Y., Ikeda, S.R., 1994. VIP inhibits N-type Ca2+ channels of sympathetic neurons via a pertussis toxin-insensitive but cholera toxin-sensitive pathway. Neuron 13, 657–669.

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