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Forum Minireview

Transcriptional Regulation of Neuronal Genes and Its Effect on Neural Functions: Cumulative mRNA Expression of PACAP and BDNF Genes Controlled by Calcium and cAMP Signals in Neurons

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Abstract. Although it is widely accepted that an activity-dependent gene transcription is induced by the calcium (Ca^{2+}) signals in neurons, it is still unclear how the particular mRNA moieties are transiently accumulated in response to synaptic transmission that evokes multiple intracellular signals including Ca^{2+} and cAMP ones. Promoters of the brain-derived neurotrophic factor (BDNF) and the pituitary adenylate cyclase-ativating polypeptide (PACAP) can commonly be activated through the cAMP-responsive element (CRE), to which the CRE-binding protein (CREB) predominantly bound. The activation of BDNF gene promoter I and III (BDNF-PI and -PIII, respectively) was mediated not only by the CREB but also by the upstream stimulatory factor, whereas that of PACAP gene promoter (PACAP-P) was mediated by only one CRE located at around −200. The PACAP-P was synergistically enhanced by Ca^{2+} and cAMP signals through the CRE, whereas the BDNF-PI did not show such a synergistic activation upon the stimulation with both signals. In addition, we found that the half-lives of PACAP and BDNF mRNA were prolonged by the Ca^{2+} influx into neurons but not that of Arc mRNA, indicating an activity-dependent stabilization of particular mRNA species in neurons. Thus, the activity-dependent gene expression is co-ordinately controlled by Ca^{2+} and cAMP signals not only at the transcriptional level but also at the post-transcriptional level for the cumulative mRNA expression in neurons.

Keywords: brain-derived neurotrophic factor, pituitary adenylate cyclase-activating polypeptide, calcium, transcription, mRNA degradation

Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays an important role in promoting neuronal survival, neuronal differentiation, and synaptic plasticity (1). BDNF is predominantly expressed in the nervous system, and its mRNA expression is up-regulated by neuronal activity accompanying the influx of Ca^{2+} into neurons (2). The BDNF gene consists of four short 5'-exons (exon I, II, III, and IV) and a common 3'-exon V encoding a prepro-BDNF protein (3). Four promoters, BDNF-PI, II, III, and IV, were mapped upstream of the 5'-exons, respectively, which are differentially controlled by the Ca^{2+} signals evoked via the *N*-methyl-D-aspartate glutamate receptor (NMDA-R) or L-type voltage-dependent calcium channels (L-VDCC) (4), suggesting versatile BDNF functions in the brain.

On the other hand, pituitary adenylate cyclase-activating polypeptide (PACAP) is a member of the vasoactiva intestinal polypeptide (VIP)/secretin/glucagons family and was first isolated from ovine hypothalamus.

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PACAP, which is synthesized as precursor of 175 amino acids, is processed to its bioactive forms, PACAP38 and PACAP27 (5), which bind to at least two types of receptors, PAC1 and VPAC1/2. The binding of PACAP to these receptors triggers several intracellular signalling pathways, resulting in versatile functions such as neuronal survival and synaptic plasticity. The rat PACAP gene consists of six exons including an alternative exon 1A and 1B and four exons encoding the coding region, and a variety of alternative transcripts are produced in neurons (6, 7).

It has well been established that both the expression of the BDNF and PACAP genes are activated by the Ca^{2+} signals in neurons (4, 8). However, it is still unclear how the cumulative mRNA expression of these genes responsible for synaptic plasticity is controlled transcriptionally and/or post-transcriptionally in response to synaptic transmission, which could evoke multiple intracellular signals including the Ca^{2+} and cAMP signals. In our study, we first assigned the *cis*-elements of BDNF and PACAP gene promoters responsive to the Ca^{2+} signals evoked via membrane depolarization. Since it has already been reported that the stabilization and destabilization of mRNA moieties are critical to control the level of PACAP mRNA in cells (9), we have also investigated the molecular mechanisms for the expression of BDNF and PACAP genes in neurons, particularly focusing on both the transcriptional activation and mRNA stability which can be controlled in an activity-dependent manner.

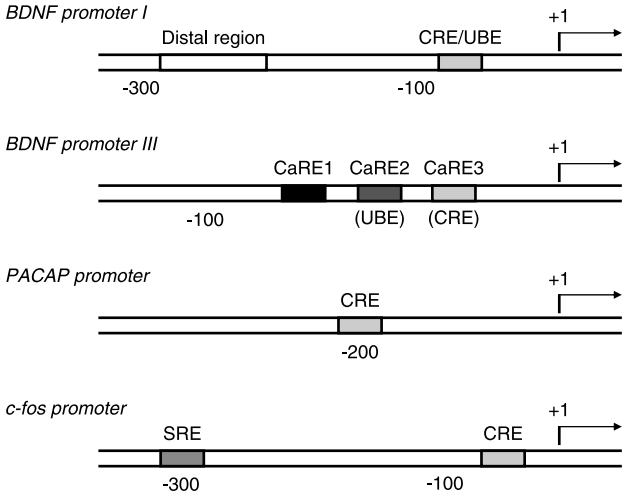

Fig. 1. Localization of *cis*-elements responsible for the Ca^{2+} signal-responsiveness in the promoters of BDNF, PACAP, and c-fos genes. The CaRE2 and CaRE3 correspond to the UBE and CRE, respectively.

Transcriptional activation of BDNF gene promoter mediated by the Ca^{2+} signals

It has already been reported that the binding of cAMP-responsive element (CRE)-binding protein (CREB) to CRE on BDNF gene promoter III (BDNF-PIII) is required for the Ca^{2+} responsiveness of BDNF-PIII (Fig. 1) (10). In addition, novel Ca^{2+} signal-responsive elements (CaREs), called CaRE1, CaRE2, and CaRE3 (or CRE), were identified within a stretch of 170-bp upstream of exon III (10). In addition to CREB, which specifically binds to CaRE3 (or CRE), a novel calcium-responsive transcription factor, which specifically binds to CaRE1, has been found to drive the neuronal specific activation of BDNF-PIII in response to the Ca^{2+} signals (Fig. 1) (11). As for the BDNF gene promoter I (BDNF-PI), we found, using rat cortical neuronal cells, that the CaREs were located in two separate (distal and proximal) regions and that the DNA sequences in the proximal region containing CRE, which is overlapped by the upstream stimulatory factor (USF)-binding element (UBE), were largely responsible for the activation of BDNF-PI (Fig. 1) (12). The CREB and USF1/2 bind to this overlapping site, depending upon their preferred sequences, which also control the magnitude of the activation. After this finding, Chen et al. (13) reported that the CaRE2 of BDNF-PIII corresponded to the UBE, to which the USF1/2 specifically bound. Thus, the two kinds of transcriptional factors, CREB and USF1/2, are commonly involved in the activation of BDNF-PI and -PIII.

Assignment of the calcium-responsive element of PACAP gene promoter

On the other hand, we found that the Ca^{2+} signal-mediated activation of the PACAP gene promoter was critically controlled by a single CRE located at around −200 nucleotide position upstream of exon I, to which the CREB predominantly bound (Fig. 1) (14). To detect the promoter activity of PACAP-P, we constructed a plasmid containing both exon IA and IB in front of a luciferase gene (pPACAP-PIAB). This promoter revealed a high responsiveness to the Ca^{2+} signals evoked via membrane depolarization about 6 – 10 h after addition of high potassium (K^+) (Fig. 2A). The promoter activity increased up to 75 mM KCl in a depolarization-dependent manner (Fig. 2B), which is mediated by the Ca^{2+} influx through both the L-VDCC and the NMDA-R (14).

To assign the *cis*-elements responsible for the Ca^{2+} signal-mediated activation of PACAP-PIAB, we constructed plasmids harboring a variety of internally deleted promoters and then examined their promoter

activities. The 5'-deletion upstream of −528 did not alter the promoter activity obtained by the full-length promoter of pPACAP-PIAB, which accommodates the nucleotide sequences up to −787, whereas the internal deletion between −265 and −184 markedly reduced the promoter activity (Fig. 3). We next examined the three different internal deletions, all of which were included between –265 and –184. As shown in Fig. 3, the 30-bp-

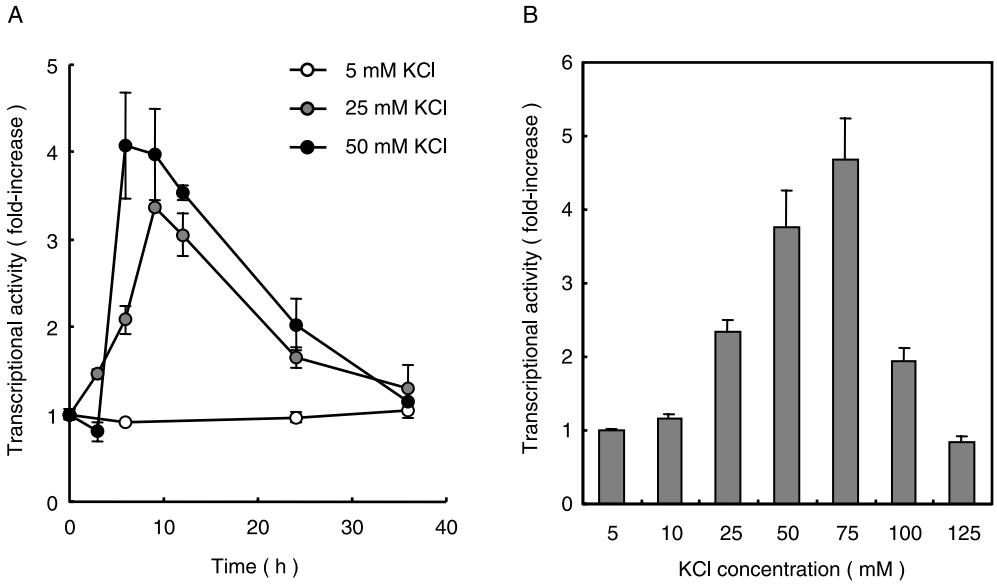

Fig. 2. The time course of the activation and the depolarization-dependent activation of PACAP gene promoter. After DNA transfection of pPACAP-PIAB to the cultured rat cortical neuronal cells and 40-h incubation, a 2 M KCl solution or vehicle was administered to adjust the KCl concentration to the indicated one. A: The cells were harvested at the indicated times (h) and subjected to the measurement of luciferase activity. B: The cells were harvested 9 h after the stimulation with the indicated concentrations of KCl.

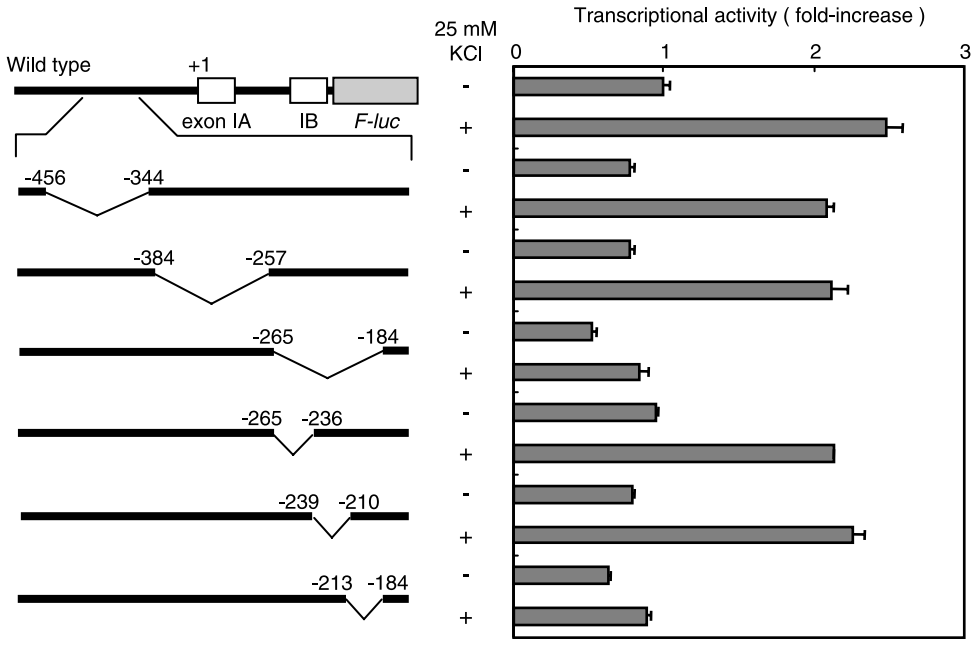

Fig. 3. Assignment of the region responsible for the activation of PACAP-P induced by depolarization. The wild type means the pPACAP-PIAB plasmid construct, which harbors both exon IA and IB. The bent line indicates the deleted region. After the stimulation of cells with 25 mM KCl for 9 h, the cells were harvested and the luciferase activity was measured.

long deletion between −213 and −184 markedly reduced the activation of promoter activity. In this restricted region, we found one CRE located at around −200. The introduction of an exchange of all the nucleotide sequences entirely covering the CRE resulted in the marked reduction of the promoter activation induced by depolarization, glutamate, or forskolin (Fig. 4).

As described above, the BDNF gene promoters have other *cis*-elements like UBE other than CRE for their Ca^{2+} signal responsiveness. The c-*fos* has the serum-response element (SRE) (Fig. 1), which is also responsible for the promoter activation mediated by the Ca^{2+} signals (our unpublished data). In the activation of the promoter of nitric oxide synthase (NOS) gene, whose

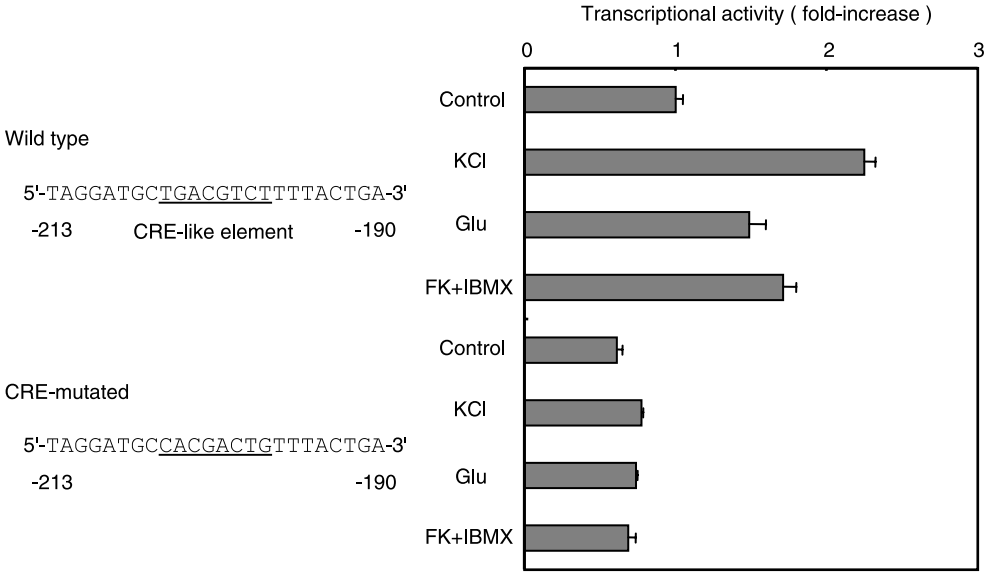

Fig. 4. Effect of the mutation of CRE on the activation of PACAP-P induced by high KCl, glutamate, and forskolin. The eight nucleotides covering the CRE were changed and used for DNA transfection to measure the promoter activity. IBMX: isobutylmethylxanthine.

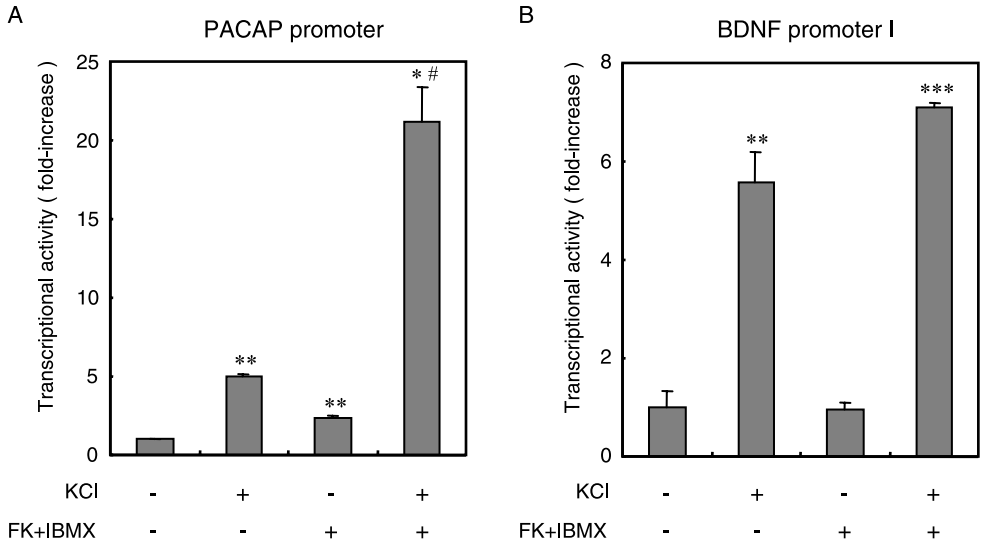

Fig. 5. The effect of co-administration of Ca^{2+} and cAMP signals on the activation of PACAP-P and BDNF-PI. Forty hours after the DNA transfection with PACAP-P (A) or BDNF-PI (B), the cells were stimulated with depolarization (50 mM KCl) and/or forskolin plus isobutylmethylxanthine (IBMX). *P<0.05, **P<0.01, and ***P<0.001 versus the control (5 mM KCl). $^{\#}P$<0.05 versus the sample stimulated with KCl or forskolin plus IBMX.

mRNA can be controlled in an activity-dependent manner, two CREs, which are separated by three nucleotides, are responsible for the responsiveness to the Ca^{2+} signals (15). Thus, the PACAP gene promoter is peculiar in terms of the pinpoint usage of a single CRE for the Ca^{2+} signal-mediated activation. In any case, it seems likely that CREB plays a key role in inducing the activity-dependent gene expression in neurons.

Synergistic activation of the promoter attained by the treatment of cells with Ca^{2+} and cAMP signals in neurons

Using a CRE-reporter plasmid vector, Impey et al. (16) reported that a synergistic activation of CREB-dependent transcription was attained by cross-talk between ERK/MAP kinase and PKA in PC12 and rat hippocampal neuronal cells, which can be induced by depolarization and forskolin. This synergism has also been suggested to contribute to the formation of long-lasting neuronal plasticity and memory storage (17). In our study, we detected the synergistic activation of PACAP promoter induced by the Ca^{2+} and cAMP signals evoked via depolarization and forskolin, depending upon the CRE (Fig. 5A). In good agreement with the synergistic activation of the promoter, a high expression level of PACAP mRNA was obtained by the administration of both signals (14). The synergistic activation of the promoter was completely repressed by co-administration of U0126 and H89 or KT5720 but incompletely by either, suggesting a requirement of co-ordinated activation of ERK/MAP kinase and PKA pathways for the synergistic promoter activation. Impey et al. (16) already demonstrated that the PKA activation induced by forskolin was required for the Ca^{2+} signal-induced nuclear translocation of ERK. This coupling of the ERK/MAP kinase and PKA pathways may involve Rap1 (*Ras proximate* 1), a member of the Ras super-family of GTP-binding proteins, which is activated by PKA (18) and has been demonstrated to be involved in neuronal plasticity (17). On the other hand, the promoter activity of BDNF-PIII was activated by depolarization but not by forskolin, whereas the level of activation induced by depolarization was slightly enhanced by the co-administration of Ca^{2+} and cAMP signals, compared to the activation induced by depolarization only (Fig. 5B). The different reponses of PACAP and BDNF gene promoters to the administration of both signals may be due to the different combination and localization of CaREs on the promoters between PACAP and BDNF genes.

Ca^{2+} signal-mediated stabilization of PACAP mRNA and its cumulative expression

Since it was considered that the Ca^{2+} and cAMP signals could affect the stability of PACAP and BDNF mRNA, we examined the effect of depolarization and/or forskolin on the stability of PACAP mRNA by adding actinomycin D to stop the mRNA synthesis in neurons.

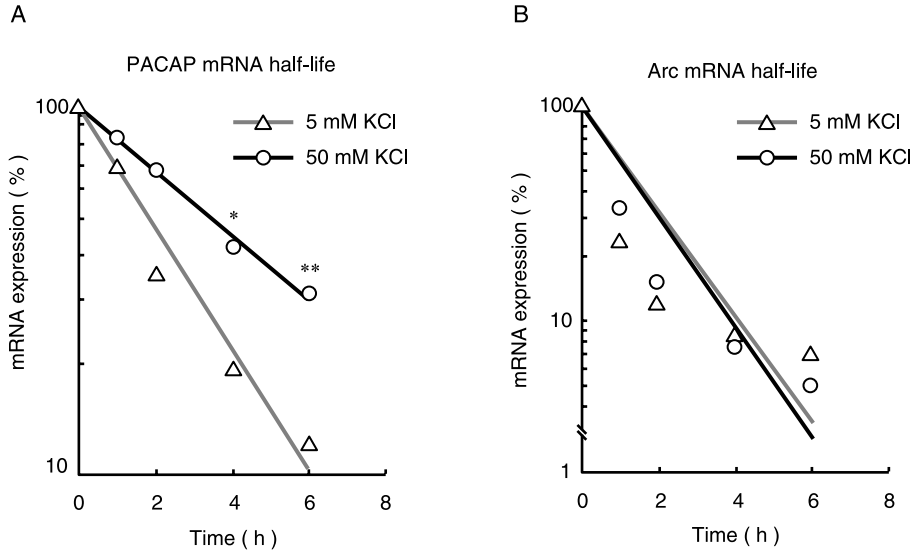

Fig. 6. Effect of depolarization on the half-life of PACAP and Arc mRNA. Two hours after the cells were treated with 50 mM KCl or vehicle, 10 μg/ml of actinomycin D was added, and the incubation was carried out to collect the total RNA at the indicated times (h) for the measurement of PACAP (A) and Arc (B) mRNA by real time RT-PCR. *P<0.05 and **P<0.001 versus 5 mM KCl.

As the result, we found that the half-life of PACAP mRNA was prolonged from about 1.8 to 3.4 h by depolarization (Fig. 6A), which was inhibited by the blockade of Ca^{2+} influx into neurons with nicardipine and DL-amino-5-phosphonovalerate (APV), indicating that the Ca^{2+} signals are responsible for the prolongation of the half-life of PACAP mRNA. The same prolongation of stability was also detected with BDNF mRNA (data not shown) but not with Arc (activity-regulated cytoskeleton-associated protein) mRNA (Fig. 6B), whose protein products can be selectively recruited to active regions of the dendritic arbor and be translated there (19). These observations clearly indicate an activity-dependent stabilization of particular mRNA species in neurons, which can be controlled by Ca^{2+} signals.

On the other hand, the amount of PACAP mRNA rapidly decreased in the presence of cAMP signals evoked via forskolin (14), the mechanism for which is still unknown. Such a decrease in the content of mRNA was not detected with the BDNF mRNA expression (data not shown). Despite this reduction, however, it was clearly observed that co-administration of depolarization with forskolin restored the cellular content of PACAP mRNA to its highest level (14). This restoration does not seem to be solely attributable to the synergistic activation of the PACAP promoter because the half-life of the transcripts was prolonged from 1.82 to 3.13 h by depolarization even in the presence of forskolin (14), suggesting that the restoration of mRNA degradation should, at least in part, contribute to the effective accumulation of transcripts. This fact also indicates a dominant effect of Ca^{2+} signals over the cAMP signals on the regulation of stabilization and de-stabilization of PACAP mRNA in neurons.

Since not only the activity-dependent gene transcription but also the activity-dependent dendritic targeting of BDNF mRNA has been reported (20), the metabolism of particular mRNA species might be dynamically regulated in neurons in an activity-dependent manner. Although such an activity-dependent mRNA stabilization had not been reported, the mechanisms for this might be related to the AU-rich elements (AREs) located in the 3' untranslated region (UTR) of mRNAs (21), which are commonly found in mRNAs with short half-lives and prominently in the group of early-response gene products, including cytokines and lymphokines (22). The PACAP gene can be clarified as an early response gene and we found one ARE in the 3' UTR of the PACAP mRNA (data not shown).

Co-ordinated regulation of the cumulative mRNA expression controlled by Ca^{2+} and cAMP signals in neurons

It is of particular interest that the rapid increase in the PACAP mRNA expression was attained by the Ca^{2+} signals even in the presence of cAMP signals because

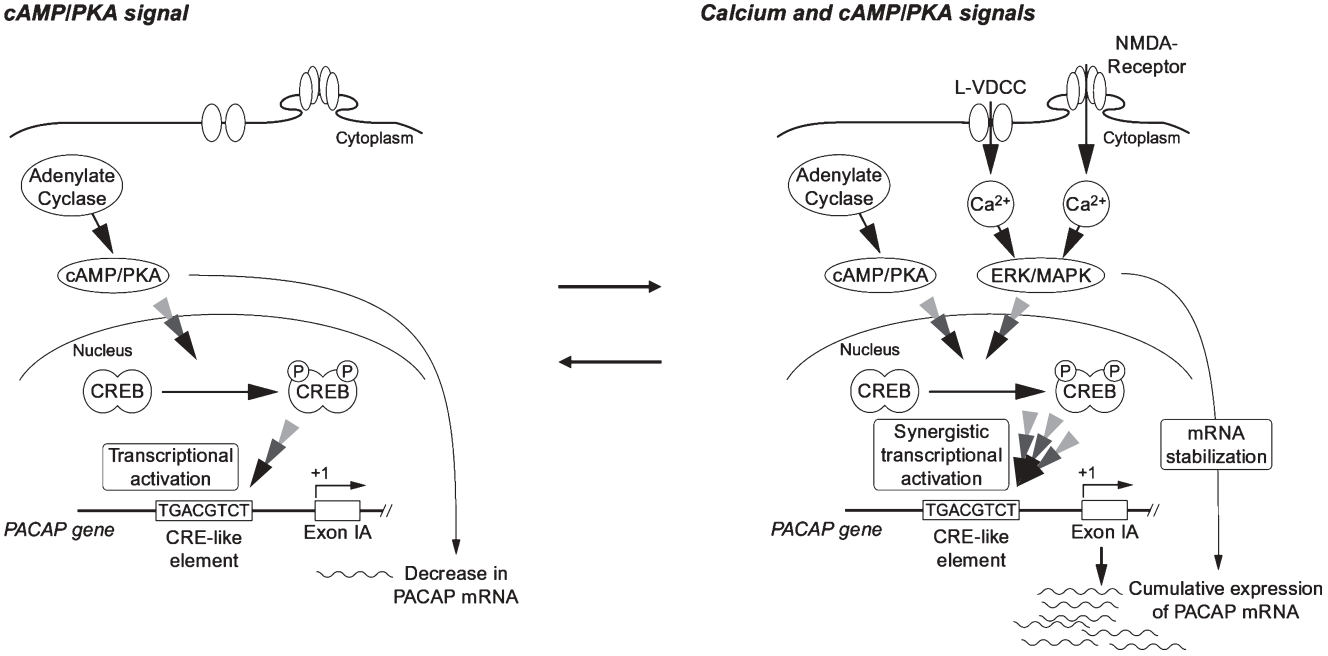

Fig. 7. Schematic representation for the coordinated PACAP mRNA expression in neurons receiving synaptic inputs that can evoke the Ca^{2+} and/or cAMP signals.

these phenomena indicate that the cellular content of PACAP and BDNF mRNA can be dynamically regulated not only at the level of transcription but also at that of mRNA degradation in an activity-dependent manner (Fig. 7). Once neurons are deprived of the Ca^{2+} signals in the presence of cAMP signals, the accumulated PACAP mRNA would immediately be degraded, probably giving rise to a temporal expression of PACAP mRNA in neurons in response to the Ca^{2+} signals. Based on these observations, it can be considered that the coordination of activity-dependent transcriptional activation and mRNA stabilization, which is mainly controlled by Ca^{2+} signals but also modulated by cAMP signals, plays an important role in acutely changing the cellular content of specific mRNA species in response to synaptic transmission, such as glutamatergic and adrenergic inputs, possibly leading to the formation of long-lasting neuronal plasticity.

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