Kainate-Elicited Seizures Induce mRNA Encoding a CaMK-Related Peptide: A Putative Modulator of Kinase Activity in Rat Hippocampus

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ABSTRACT: **By means of differential display techniques, we have previously identified an mRNA transcript whose expression is highly induced in the rat hippocampus by kainate-elicited seizures. Here, we report the cloning of a corresponding cDNA encoding a 55-amino-acid, serine-rich peptide which contains four predicted phosphorylation sites. The peptide was designated CaMK-related peptide (CARP) as it shares significant amino acid sequence identity with part of a novel putative calcium/calmodulin-dependent kinase (CaMK-VI) that was also cloned in this study. It appears that CARP and CaMK-VI are derived from the same gene through differential splicing. Intriguingly, CARP also exhibits 64% amino acid sequence identity with the C-terminal part of human doublecortin, encoded by a recently identified gene which is mutated in patients with X-linked lissencephaly and the double-cortex syndrome. In addition, the structure of CARP resembles the autoinhibitory, serine-rich N-terminal domain of CaMK-IV, suggesting a possible modulatory role of CARP with respect to CaMK activity. Northern blot analysis and *in situ* hybridization experiments showed that CARP mRNA is specifically induced by kainate-elicited seizures in the dentate gyrus and in the pyramidal layers CA1 and CA2, but not in CA3. In contrast, kainate-induced seizures did not change the level of expression of the CaMK-VI gene. We propose that CARP induction leads to the modulation of kinase activity in specific subregions of the rat hippocampus, providing a negative feedback mechanism for seizure-induced kinases.** © 1999 John Wiley & Sons, Inc. J Neurobiol 39: 41–50, 1999

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Numerous neurological insults have been associated with changes in gene expression in the hippocampus, thus altering synaptic efficacy, neurodegeneration, and nerve sprouting. It is generally accepted that excessive activation of glutamate receptors, which is normally observed in several neurological disorders, will lead to a large increase in $[Ca^{2+}]_i$, which in turn induces the expression of immediate early genes (IEGs). For example, studies in animal models of transient forebrain ischemia and epilepsy suggest the existence of a molecular switch leading to aberrant $[Ca^{2+}]_i$ via the formation of Ca^{2+}-permeable AMPA receptors (Bennett et al., 1996; Pellegrini-Giampietro et al., 1997; Gorter et al., 1997), and numerous IEGs are induced or up-regulated in the rat hippocampus after ischemia or experimentally induced seizures (Robertson et al., 1992; Nedivi et al., 1993; Gass et al., 1994, 1997; Herdegen et al., 1993, 1997; Dragunow and Preston, 1995; Hughes et al., 1998).

Evidently, calcium-activated kinases are key candidates to link the activation of calcium-mobilizing

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glutamate receptors and the induction of IEGs in response to various brain insults. Recently it was shown that mice lacking the gene encoding c-*jun* amino-terminal kinase 3 (Jnk-3) show both resistance to kainate-induced seizures and absence of excitotoxicity-induced apoptosis in the hippocampus (Yang et al., 1997), thus suggesting a pivotal role of this kinase in such neurological insults. Furthermore, substantial, albeit circumstantial evidence has been presented in which changes in the expression of different types of kinases are associated with aberrant excitatory amino acid–mediated neurotransmission. For example, the expression of specific isozymes of the protein-kinase C (PKC) gene family has been shown to be altered after kindling epileptogenesis (Kamphuis et al., 1995) or after kainate-induced seizures in the rat hippocampus (Guglielmetti et al., 1997). In the case of calcium/calmodulin-dependent protein kinase (CaMK)-II, both mRNA levels and protein localization are changed during kindling and continuous stimulation–induced seizures (Morimoto et al., 1997; Perlin et al., 1992; Wasterlain et al., 1992). Moreover, mutations in several genes involved in neuronal CaMK pathways have been shown to cause seizures in mice (for review, see McNamara and Puranam, 1998). For example, targeted deletions in calcium channel or CaMK-II genes or aberrant splicing of their mRNAs will induce an epileptic phenotype in mice. Together, these data strongly favor a model in which aberrant calcium-activated kinase signaling affects the onset and course of various neurological insults.

Although the involvement of calcium-activated kinases in neurological insults seems evident, virtually nothing is known about how kinase (hyper)activity can be modulated or turned off. Here, we report on the identification and characterisation of a rat cDNA corresponding to mRNA that is highly induced by kainate-induced seizures in specific parts of the rat hippocampus and that encodes a small CaMK-related peptide with putative modulatory action on kinase activity.

MATERIALS AND METHODS

Animal Treatments

Young adult male Wistar rats (180–200 g) from Charles River were housed under a 12:12 h light/dark cycle with free access to food and water. Eight mg per kilogram body weight kainic acid was administered intraperitonially and the animals were observed for 3 h. Their behavior was rated as described (Sperk et al., 1983). Only rats exhibiting full limbic seizures including generalized seizures were used for this study. Control treatments consisted of administration of vehicle solution only.

Three hours after injection, the rats were decapitated and their brains were dissected and immediately frozen and stored at −80°C until use. All animal experiments were conducted according to European Communities Council Directive 86/609/EEC. The protocols were approved by the Animal Care Committee of the Faculty of Medicine, University of Leiden, The Netherlands (UDEC No. 9569).

Isolation of CARP and CaMK-VI cDNA Clones

A CARP cDNA fragment generated by differential display (DD) and corresponding to mRNA that is up-regulated by kainic acid administration (Vreugdenhil et al., 1996a) was subcloned in pGEM-T (Promega). Briefly, the CARP fragment was excised and dissociated from the noncrosslinked membrane, by boiling for 10 min in 100 μL of double-distilled water; 2 μL of this served as a template to reamplify the CARP fragment using the same PCR conditions as described (Vreugdenhil et al., 1996a). The reamplified CARP fragment was size-separated on low-melt agarose and purified by using agarase (Boehringer Mannheim). DNA sequence analysis was performed using an ABI 377 automated sequencer and a dye terminator cycle sequence kit (Perkin Elmer) according to the instructions of the manufacturer. Based on the obtained DNA sequence of a polymerase chain reaction (PCR)-based strategy called rapid amplification of cDNA ends (Frohman et al., 1988), PCR-fragments were size-separated on 1.2% agarose gels, blotted on nylon filters, and hybridized with ^{32}P-labeled oligonucleotides, designed on the basis of the internal DNA sequence of the DD fragment. Hybridizing PCR fragments were excised from agarose gels and analyzed either directly by DNA sequence analysis or after subcloning in pGEM-T (Promega). DNA sequence analysis was performed by a DNA walking approach, sequencing toward the 5′ and 3′ ends of the cDNA using synthetic oligonucleotides derived from novel DNA sequence information. This strategy resulted in the elucidation of the contiguous sequence of 4746 nucleotides. The sequence was verified by its complete amplification with synthetic oligonucleotides recognizing its 5′-most and 3′-most sequences, and by Northern blot analysis (see later). This sequence has been deposited in GenBank and is available under Accession No. AF045469.

A CaMK-VI cDNA clone with a length of 2031 nucleotides was isolated and characterized by PCR using an oligonucleotide derived from the 5′ end of the CARP cDNA (5′-CTCTGGCTCTTGGCTATTGTCAGG-3′) and an oligonucleotide corresponding to positions 2092–2115 of the Genbank entry accession number (5′-GGAGAGACACT-GTCGATCACTTGG-3′). The resulting cDNA fragment was cloned in pGEM-T and analyzed by DNA sequencing.

Northern Blot Analysis

Total RNA was isolated from rat hippocampi using TRIzol^{TM} (Gibco-BRL) according to the manufacturer's

instructions. Total RNA (approximately 20 μg/lane) was size-separated on a 1% formamide gel and blotted onto a Hybond-N filter according to standard methods (Sambrook et al., 1989). Filters were hybridized O/N at 65°C in buffer containing 0.4 *M* Na_2HPO_4, 0.1 *M* NaH_2PO_4, 7% sodium dodecyl sulfate (SDS), and 1 m*M* ethylenediaminetetraacetic acid (EDTA). They were washed at 68°C, twice in 2× SSC/0.1% SDS and once in 0.2× SSC/0.1% SDS. Each wash step was performed for 10 min. Filters were then used to expose Kodak X-Omat AR films for 1 week. To correct for differences in RNA quantity and quality, filters were reprobed with a glyceraldehyde-3 phosphate dehydrogenase (GAPDH) probe. Hybridization, washing, and exposure were performed as described above.

DNA probes were labeled with ^{32}P α-dCTP using the multiprime DNA labeling system kit (Amersham, England) according to the manufacturer's instructions. A CARP probe was prepared by PCR amplification of a part of the 3′-untranslated tail (base pairs 880–4657) with synthetic oligonucleotides 5′-CATGGAAACATGAATAAC-3′ and 5′-CGGGAGAGAGTAACATGG-3′, using hippocampal cDNA derived from a kainic acid–treated rat as a template. A CaMK-VI probe was prepared by PCR amplification of the middle part of the coding region, encompassing base pairs 769–2040 using synthetic oligonucleotides 5′-AGTCTGTGGCACCCCAACTT-3′ and 5′-GGAGAGA-CACTGTCGATCACTTGG-3′ and the cloned CaMK-VI cDNA (see above) as a template. A GAPDH probe was prepared by PCR amplification of residues 149–515 of the sequence in the EMBL database with Entry No. X02231 by using synthetic oligonucleotides 5′-GACATTGTTGCCAT-CAACGACC-3′ and 5′-TGCATTGCTGACAATCTT-GAGG-3′ and hippocampal cDNA described above as a template. All resulting DNA fragments were gel-purified and their identity was verified by DNA sequence analysis of both ends.

Hybridization signals were quantified using an Olympus image analysis system (Paes, The Netherlands) equipped with a Cue CCD camera. The film background was subtracted after shading correction.

In Situ Hybridization

In situ hybridization experiments were performed as previously described (Meijer and de Kloet, 1994). Briefly, serial coronal sections (20 μm) were cut through different brain regions. The sections were thaw-mounted on poly-L-lysine–coated slides and stored at −80°C. Hybridization was performed using the CARP-specific 180-bp cDNA fragment identified by DD. This fragment corresponds to base pairs 3268–3443 and does not overlap with CaMK-VI. The fragment was polished using *PfuI* DNA polymerase (Stratagene) and ligated into the *SrfI* site of the vector pScript (Stratagene). Both sense and antisense cRNA probes were generated using ^{35}S-labeled UTP and T7 or T3 polymerase, respectively. For hybridization, 100 μL of probe with a concentration of 2.5×10^7 cpm/mL was used. The sections were exposed to Kodak X-OMAT AR film for 7 days.

RESULTS

We have previously used differential display (DD) to identify kainate-responsive genes which are under the control of corticosteroids (Vreugdenhil et al., 1996a,b). Analysis of kainate–up-regulated genes by *in situ* hybridization indicated that one gene is specifically induced in subregions of the hippocampus during kainate-induced seizures (see below) but not by administration of subthreshold doses of kainate (data not shown). We have cloned the corresponding cDNA encompassing 4746 bp with a stretch of 41 adenosine residues at the 3′ end and a classical polyadenylation signal AATAAA 20 bp upstream of the polyA tail. Northern blot analysis of hippocampal total RNA derived from a kainic acid–treated rat resulted in the identification of a major 4950-base transcript. Therefore, we may have cloned a near-full-length cDNA. Open-reading frame (ORF) analysis demonstrated the presence of many small potential peptide/protein sequences. Cloning of the homologous cDNA in mouse (data not shown), and comparison of all potential ORFs of mouse and rat revealed that the only conserved ORF is located at the extreme 5′ end and encodes a peptide of 55 amino acids with a calculated pI of 4.69 [Fig. 1(A)]. A remarkable feature of this peptide is the presence of 12 serine residues and seven proline residues. No apparent consensus signal peptide or transmembrane regions could be identified. A search for posttranslational modification sites with the PROSITE (Bairoch, 1993) program revealed phosphorylation sites at amino acids Thr 17, Ser 20, and Ser 33 by PKC, and Ser45 by casein kinase (Fig. 1), implicating the predicted peptide as a target in cellular signal transduction pathways.

Database searches did not reveal significant sequence similarity of the 3′-most 4600 bp to any known sequence. However, in complete contrast to this, the remaining 146 base pairs at the 5′ end were 100% identical to candidate plasticity gene 16 of rat (cpg16; GenBank Accession No. U78857) and residues 53–146 were 93% identical to human KIAA0369 (GenBank Accession No. AB002367), a cDNA clone which exhibits a high amount of sequence identity with cpg16 at its 3′ end (Nagase et al., 1997) [Fig. 2(B)]. As yet, no literature reporting the structure of the protein encoded by cpg16 is available. To verify the sequence of cpg16 and to study its possible relationship with our cDNA, we cloned a 2031-bp cDNA clone by PCR called CaMK-VI (see below) using cpg16-specific oligonucleotides (see Materials and Methods). DNA sequence analysis confirmed the cpg16 sequence except for an insertion of an addi-

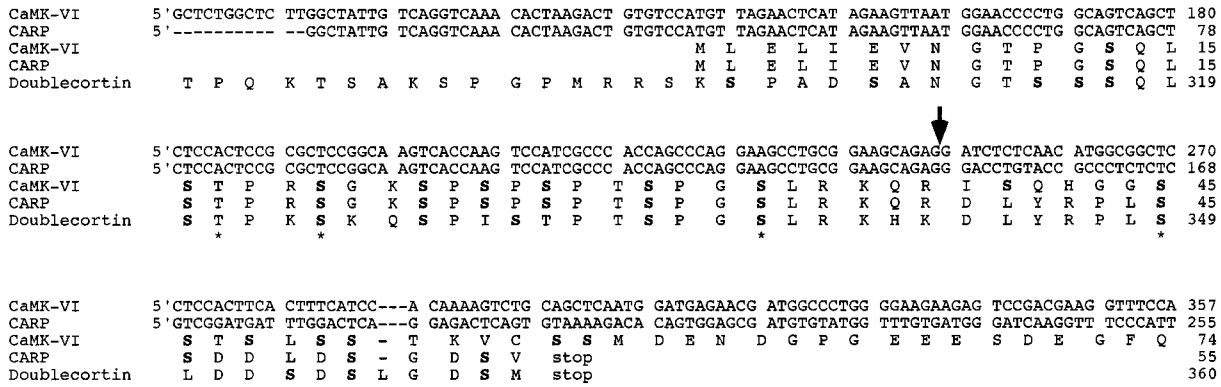

Figure 1 Alignment of the 5′ part of CARP and CaMK-VI cDNA sequences and alignment of the deduced amino acid sequences with the C-terminus of human doublecortin. The potential exon–exon boundary is indicated by a closed arrow. Serine residues are shown in bold. Putative phosphorylation sites are shown with asterisks.

tional 74 nucleotides in the CaMK-VI sequence at position 1273 of the original cpg16 clone. This insertion caused a frame shift and an in-frame stop codon 130 nucleotides downstream of the point of insertion, and thus resulted in a completely different C-terminus with respect to the one predicted in the cpg16 cDNA clone. As KIAA0369 predicts a protein which is identical to the C-terminus predicted by our CaMK-VI clone, we conclude that CaMK-VI clone is authentic and represents a transcript that is actually expressed in the rat hippocampus.

CaMK-VI contains an ORF of 1299 bp encoding a 422–amino acid protein [Fig. 2(A)] which contains all the amino acids and motifs which are characteristic for the superfamily of calcium/calmodulin-dependent protein kinases (Pearson and Kemp, 1991). The 267 amino acids encompassing the catalytic domain exhibit 40–45% sequence identity with the catalytic domains of other CaMKs, while the putative regulatory domains are less conserved (10–35% amino acid sequence identity). These figures are well in line with those reported for other members of the CaMK family (Hanson and Schulman, 1992; Haribabu et al., 1995; Nairn and Picciotto, 1994). In particular, the structural organization of CaMK-VI is reminiscent of the CaMK-IV protein, with a putative catalytic kinase domain preceded by a serine-rich N-terminal extension of approximately 60 amino acids. Although the overall sequence identity in this extension is low (18%), 17 serine residues are present in CaMK-VI, five of which can be aligned with serine residues of CaMK-IV [Fig. 2(A)]. Although definite proof must await functional analysis, we conclude that CaMK-VI is a putative novel member of the CaMK family.

Alignment of the CaMK-VI cDNA clone with the cDNA clone identified by DD, encoding the 55–amino acid peptide discussed above, revealed identical sequences of the first 147 bp, while no identical regions were found in the remaining parts (Fig. 1). The sequences diverged at a DNA motif resembling exon–exon boundaries (AGGA and AGGG, respectively), suggesting that the corresponding transcripts were derived by alternative splicing of the same gene. At the protein level, the predicted amino-terminal 38 amino acids were identical; of the remaining 17 amino acids, only four were identical, three of which were serine residues. We will refer to the 55–amino acid peptide as CaMKVI-related peptide (CARP).

In addition to its strong resemblance to the CaMK-VI N-terminus, CARP strikingly exhibits 70% sequence identity with the extreme C-terminus of human doublecortin. The latter protein is encoded by a brain-specific gene which has recently been implicated in X-linked lissencephaly and the double-cortex syndrome, and the doublecortin is thought to function as a signaling protein (des Portes et al., 1998; Gleeson et al., 1998). Two of the four putative phosphorylation sites, i.e., Thr 17 and Ser 30, are conserved between CARP and doublecortin. Interestingly, the C-terminal 17 amino acids of CARP that are nonidentical to CaMK-VI share a high degree of amino acid sequence identity (82%) with doublecortin. Moreover, the length of the the C-terminal 17 amino acids of CARP can be aligned precisely with the C-terminal 17 amino acids of doublecortin, suggesting a common evolutionary origin of the last coding exon for CARP and doublecortin.

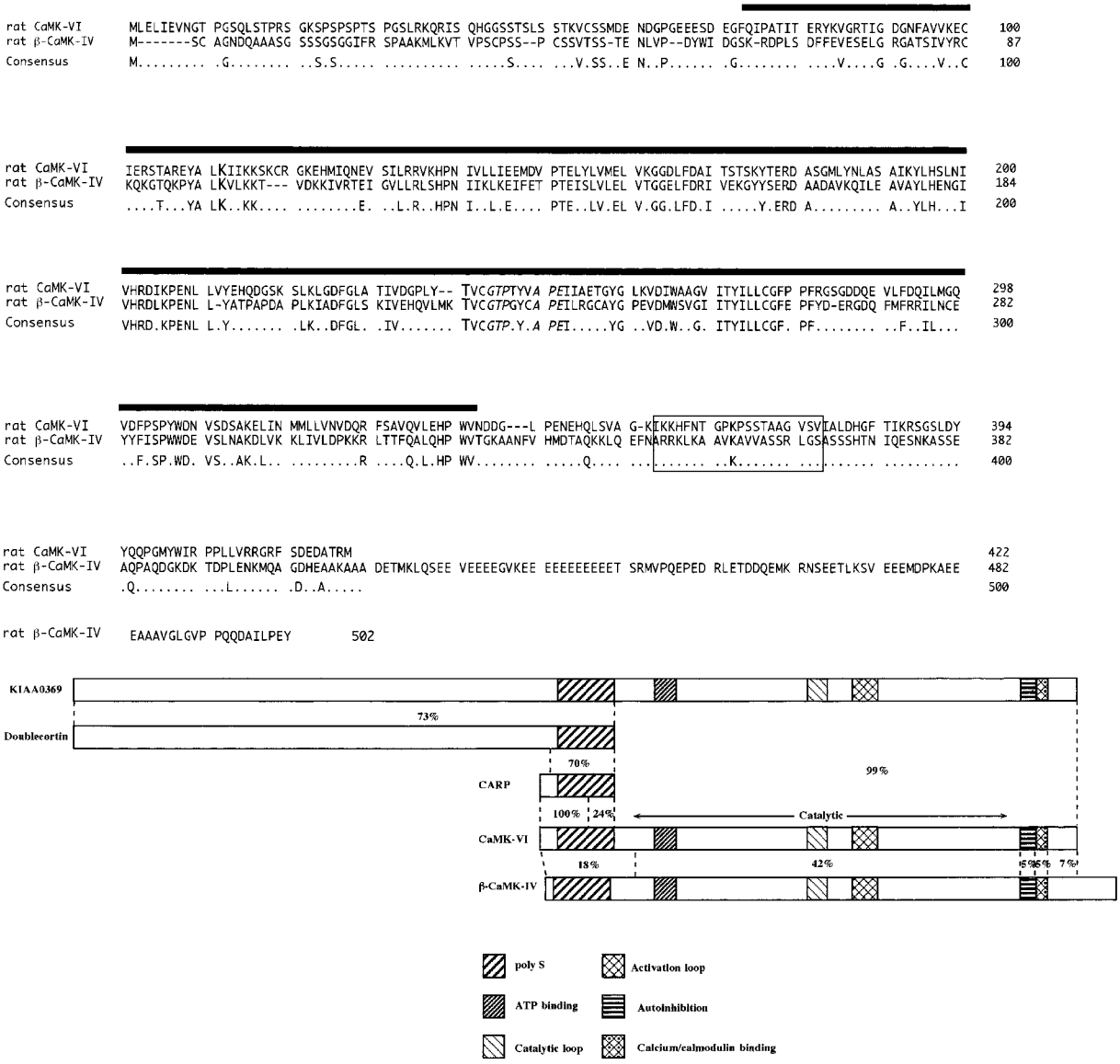

Figure 2 Structure and comparison of the predicted rat CaMK-VI, CaMK-IV, CARP, and human doublecortin and KIAA0369 proteins. (A) Alignment of the predicted primary structure of rat CaMK-VI and β-CaMK-IV proteins. The putative catalytic domain is indicated with a solid line above the sequences; the regulatory domain is boxed. Lys 99 of CaMK-IV and Lys 112 of CaMK-VI involved in ATP binding, and Thr 224 of CaMK-IV and the corresponding Thr 239 of CaMK-VI, which are thought to be crucial for activation of the kinases by phosphorylation, are shown in bold. The GTPXXXAPE motif characterizing Ser/Thr kinases is shown in italics and can be found at position 227–234 in CaMK-IV and position 242–250 in CaMK-VI. (B) Schematic representation and comparison of CARP, CaMK-VI, CaMK-IV, doublecortin, and KIAA0369. The different functional domains are boxed and indicated in the lower part. Amino acid sequence identities are indicated between the molecules. For further details, see text.

Expression of CARP and CaMK-VI in Rat Hippocampus

As the 5′-most parts of the CARP and CaMK-VI cDNAs are identical, it is highly likely that the corresponding transcripts are derived from the same gene. The CARP and CaMK-VI–specific transcription units might therefore share a common promotor, in which case their transcription regulation should be similar during kainate-induced seizures. To study this

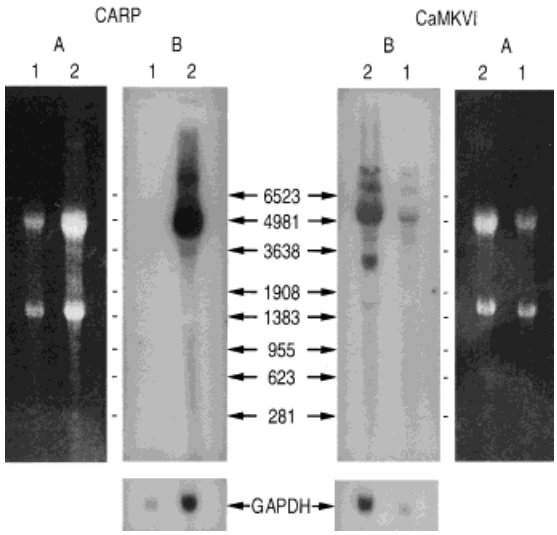

Figure 3 Expression of CARP and CaMK-VI in the rat hippocampus as determined by Northern blot analysis. Total RNA was isolated from the hippocampus of a vehicle-treated rat (lane 1) or a kainic acid–treated rat (lane 2), size-separated by gel electrophoresis, and visualized by ethidium bromide staining (A). The blots were probed with ^{32}P-labeled CARP [(B), left] or CaMK-VI [(B), right] DNA probes. To correct for differences in RNA quantity and quality, blots were reprobed with a GAPDH cDNA probe (bottom). The position of RNA markers and their molecular sizes (in bases) are indicated with arrows.

and to characterize the length and expression level of CARP and CaMK-VI transcripts, Northern blot analysis was performed using total RNA derived from hippocampi of vehicle-treated animals and animals with kainate-induced seizures. Virtually no CARP transcript was detectable in vehicle-treated animals (Fig. 3, panel CARP B, lane 1). However, a dramatic 30-fold induction of a 4.9-kb CARP RNA species (Fig. 3, panel CARP B, lane 2) was found in animals with kainate-induced seizures. In addition, two minor transcripts of approximately 8.3 and 4.0 kb were observed. However, the expression of CaMK-VI differed from that of CARP in that no clear up-regulation was seen after kainate treatment. One major CaMK-VI–specific transcript with a length of 5.0 kb and five minor transcripts (8.3, 7.0, 4.0, 3.4, and 1.4 kb) were detected in both vehicle-treated and kainate-treated animals (Fig. 3, panel CaMK-VI B, lanes 1 and 2; see also Table 1). Ethidium bromide staining of the RNA gel revealed differences in the amount of RNA loaded in the different lanes [Fig. 3(A)] which were confirmed by hybridization with a GAPDH-specific control probe (Fig. 3, bottom). After correction for these variations, no clear difference was observed in the expression levels of five of the six CaMK-VI transcripts after kainate treatment (Table 1). However, the 3.4-kb CaMK-VI transcript was up-regulated after kainate-induced seizures by a factor of 2.5 (Table 1). In conclusion, these experiments show that at least six different CaMK-VI transcripts are present in the rat hippocampus, one of which is differentially regulated by kainate treatment. In contrast, CARP transcript levels are very low in the hippocampus under normal conditions, while kainate treatment gives rise to a dramatic induction of a 4.9-kb CARP transcript in particular.

To study the localization of CARP and CaMK-VI mRNA expression and to confirm the Northern blot analysis, we used *in situ* hybridization. In accordance with the Northern blot analysis, CARP mRNA was not detected in the brain of vehicle-treated animals (Fig. 4). Kainate-induced seizures, however, led to induction of CARP mRNA in the hippocampus within 3 h, but not in any other part of the brain (Fig. 4). The induced CARP mRNA was prominently present in the dentate gyrus and, to a lesser extent, in the pyramidal layers CA1 and CA2; no CARP expression was detected in CA3. A CARP mRNA induction such as observed here is in line with the DD results (Vreugdenhil et al., 1996a). In accordance with the Northern blot analysis, CaMK-VI was expressed at similar levels in both vehicle-treated animals and animals with kainate-induced seizures. The highest expression was found in the dentate gyrus; the signal was weaker in CA1 and CA2. In contrast to the situation for CARP, CaMK-VI mRNA was also detected in the CA3 region.

Table 1 Effect of Kainic Acid Administration on CARP and CaMK-VI mRNA Levels

CARP		CaMK-VI	
Transcript Species (kb)	Fold Induction	Transcript Species (kb)	Fold Induction
8.3	nd	8.3	0.8
4.9	30	7.0	0.6
4.0	nd	5.0	0.6
		4.0	0.2
		3.4	2.5
		1.4	0.6

Hybridization signals were quantified using an image analysis system equipped with a Cue CCD camera. Variations in CARP and CaMK-VI signals owing to differences in RNA loading were corrected for by relating them to signals obtained with a GAPDH probe. Numbers given represent the degree of induction by kainate treatment versus vehicle treatment and were obtained by dividing the autoradiographic signals for these two conditions. As we could not detect a signal for the 8.3- and 4.0-kb CARP transcripts in the vehicle-treated animals, their induction was not determined (nd).

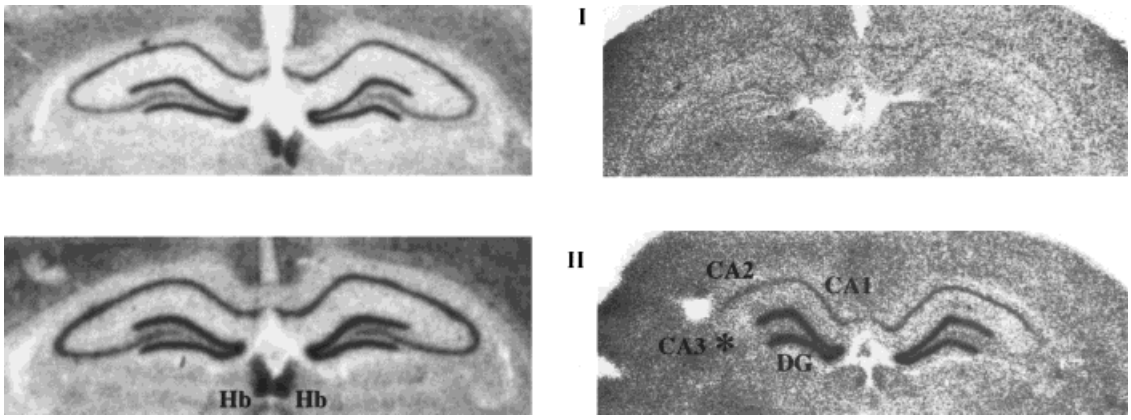

Figure 4 *In situ* hybridization analysis of CARP and CaMK-VI transcripts in the rat hippocampus. The two sections shown on the left were hybridized with a CaMKVI-specific probe; the two sections on the right were hybridized with a CARP-specific probe. (I) Section of a vehicle-treated animal. (II) Brain section obtained from an animal with kainate-induced seizures. Asterisk in (II) indicates the CA3 region lacking CARP expression. Further indicated are the pyramidal layers CA1 and CA2 and the dentate gyrus (DG). Cells of the habenula (Hb) express CaMKVI but not CARP. Control experiments using radiolabeled sense RNA probe did not give a signal above background (data not shown).

In conclusion, our Northern blot and *in situ* hybridization experiments showed differential regulation of CARP and CaMK-VI transcripts during kainic acid–induced seizures. CARP mRNA expression is normally below detection level but is dramatically induced by seizure activity in the hippocampus. In contrast, CaMK-VI expression is found under normal conditions, and this expression is not affected by seizure activity.

DISCUSSION

We isolated and characterised a cDNA clone encoding a 55–amino acid, serine-rich peptide, CARP, which is highly induced by kainate-induced seizures. As CARP contains several putative phosphorylation sites, is related to a putative CaMK, and is induced during experimentally evoked seizures, it may be involved in a kinase-mediated signal transduction pathway associated with epileptic attacks.

Furthermore, a large part of CARP, 48 of 55 amino acids, is highly similar to the C-terminal part of the recently characterized human doublecortin protein. Mutations in the doublecortin gene give rise to X-linked lissencephaly and the double-cortex syndrome (des Portes et al., 1998; Gleeson et al., 1998). These disorders are caused by a migrational arrest of cortical neurons, preventing them from arriving at their destination and leading to malformation of the cortex, and are further characterized by severe mental retardation and epileptic seizures (Dobyns and Truwit, 1995). As doublecortin is a likely substrate for members of the MAP kinase family, it was suggested that doublecortin transduces signals which are critical for migrating neurons (Gleeson et al., 1998). The similarity of CARP to the C-terminal part of doublecortin and the conservation of some of the phosphorylation sites suggest a role for CARP in neuronal plasticity. Seizure activity has been shown to result in massive reorganization of synaptic contacts and neurogenesis and affects neuronal networks in the hippocampus—in particular, in the dentate gyrus (Sloviter, 1992; Buckmaster and Dudek, 1997; Mathern et al., 1996; Parent and Lowenstein, 1997; Parent et al., 1997; von Campe et al., 1997). Our finding that CARP mRNA is specifically induced in the hippocampus after kainate-induced seizures, and that this induction is most pronounced in the dentate gyrus, is well in line with a function of CARP in neuronal plasticity.

At the predicted protein level, CARP overlaps with CaMK-VI at the extended, serine-rich N-terminus, but lacks a putative catalytic or regulatory domain. This suggests a function of CARP which is related to that of the serine rich N-terminus of CaMK-VI. In this respect, the structural similarity of CaMK-VI to CaMK-IV is of interest. In contrast to other CaMKs, rat α- and β-CaMK-IV also contain an extended, serine-rich N-terminus (Means et al., 1991; Sakagami and Kondo, 1993). Full activation of CaMK-IV involves at least three steps: first, calcium/calmodulin

binding will lead to exposure of the catalytic domain to a CaMK-IV kinase; second, phosphorylation of Thr 214 induces kinase activity; and third, autophosphorylation of several serine residues in the N-terminus occurs (for review, see Means et al., 1997). Mutation of both Ser 12 and Ser 13 into alanine residues in α-CaMK-IV completely abolishes kinase activity. Moreover, removal of the N-terminal 20 amino acids results in a kinase with wild-type CaMK-IV properties. This suggests an autoinhibitory function of the N-terminus which can be relieved by autophosphorylation (Chatila et al., 1996). The structural resemblance of CaMK-VI and CaMK-IV throughout the two proteins, including the extended serine-rich N-terminus, and the presence of several putative phosphorylation sites in CaMK-VI suggest a similar three-step activation mechanism for CaMK-VI as described for CaMK-IV, implying that phosphorylation of N-terminal located serine residues is crucial for full activity. Accordingly, CARP may function as an modulator of CaMK-VI and/or other kinases that become hyperactivated during seizures.

CARP is likely to be derived from a CaMK gene which gives rise to a number of splice variants encoding different peptides and/or proteins involved in kinase-mediated signal transduction pathways. Besides CARP, transcription of this CaMK gene leads to the putative CaMK that we call CaMK-VI. Over its entire 422–amino acid length, CaMK-VI shares 99% amino acid sequence identity with the C-terminal part of a predicted 750–amino acid–long human protein encoded by a cDNA clone called KIAA0369 [Fig. 2(B)]. Thus, it appears that the predicted KIAA0369 protein is an extended human homologue of CaMK-VI, which suggests that the gene encoding CARP/CaMK-VI may also generate a transcript encoding an extended CaMK-VI protein. Indeed, a rat DNA sequence encoding amino acid sequence which is highly similar with the amino-terminus of human KIAA0369 has been deposited in GenBank (Accession No. AA818566). In addition, the multiple CaMK-VI transcripts observed in our Northern blot analysis fit with this notion. Interestingly, the predicted 360–amino acid–long doublecortin protein shares a high degree of amino acid sequence identity (70%) with N-terminal part of KIAA0369, but lacks the catalytic kinase domain (des Portes et al., 1998; Gleeson et al., 1998) [Fig. 2(B)]. Thus, at least two related genes are present in human, i.e., doublecortin and KIAA0369, which encode proteins involved in kinase-mediated signal transduction. As both doublecortin and CARP are associated with epileptic seizures, it suggests a pivotal role of this gene family in epilepsy-related neuronal plasticity.

CARP mRNA is induced by kainate-elicited seizures, whereas CaMK-VI is not. CARP-specific induction might be the result of a negative feedback loop aimed at turning off kinase activity. It is conceivable that calcium overload in a cell—triggered by kainate-induced seizures—leads to activation of different CaMKs and/or other kinases. Enhanced kinase activity may induce CARP expression, which subsequently acts as a modulator of (hyper)activated kinase activity, e.g., by acting as a substrate or by irreversible binding to activated kinases. Whether such a kinase feedback system occurs must await the functional analysis of CARP and related proteins.

In conclusion, we cloned and characterized a cDNA clone encoding a 55–amino acid, serine-rich peptide called CARP, which is specifically up-regulated by kainate-induced seizures. CARP transcripts are probably derived from a putative CaMK gene that also gives rise to a novel calcium/calmodulin-dependent protein kinase which we also cloned in this study. Expression of CARP may result in the modulation of kinase activity. Our findings further indicate an important role for kinase-mediated signal transduction pathways in the course of epileptic seizures. We postulate a model in which calcium overload leads to overactivation of kinases and the subsequent induction of CARP expression which attenuates kinase activity through a negative feedback action. The cloning of CARP and related cDNAs opens up the possibility of testing such a hypothesis.

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