Cain, A Novel Physiologic Protein Inhibitor of Calcineurin*

(Received for publication, March 2, 1998, and in revised form, April 13, 1998)

Michael M. Lai, Patrick E. Burnett, Herman Wolosker‡, Seth Blackshaw, and Solomon H. Snyder¶¶

From the Departments of Neuroscience, Psychiatry and Molecular Sciences, and Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Calcineurin is a widely distributed protein phosphatase regulated by calcium and calmodulin. It mediates the immunosuppressive actions of drugs such as cyclosporin and FK506, and has been implicated in a number of calcium-sensitive pathways in the nervous system, including regulation of neurotransmitter release and modulation of long-term changes in synaptic plasticity. Calcineurin associates physiologically with other proteins, including calmodulin, FKBP12 (FK506-binding protein), the ryanodine receptor, and the inositol 1,4,5-trisphosphate receptor. We now report the identification, molecular cloning, and functional characterization of a novel protein, cain (calcineurin inhibitor), that interacts with and inhibits calcineurin. The full-length cain cDNA predicts a 240-kDa protein with no significant homology to any known protein. Cain associates with calcineurin both in vitro and in vivo, leading to a non-competitive inhibition of calcineurin activity. The putative calcineurin-binding domain of cain, a 38-amino acid region defined by mutational analysis, is highly basic. Like calcineurin, cain has a prominent neuronal expression and a wide tissue distribution. Cain’s expression pattern in the brain closely resembles that of calcineurin, indicating a physiologic association between the two proteins.

Calcineurin is a serine/threonine protein phosphatase activated by calcium and the calmodulin-calcium complex (1, 2). This enzyme was first purified from the brain, where it was found in high concentrations in neurons (3). Calcineurin is a heterodimer consisting of a 60-kDa catalytic subunit (CnA) and a 19-kDa calcium-binding regulatory subunit (CnB). The discovery that immunophilins cyclophilin and FKBP12 (FK506-binding protein) form inhibitory complexes with calcineurin in the presence of immunophilin-binding drugs such as cyclosporin A and FK506 (4, 5) explained the mechanism of immunosuppression by these drugs (6), and established calcineurin as a mediator of T cell activation (7–9). Calcineurin activity is necessary for the synthesis of several cytokine genes through the dephosphorylation of a family of transcription factors known as NF-AT (nuclear factor of activated T cells) (10–13). By inhibiting calcineurin activity, cyclosporin A and FK506 prevent the nuclear translocation of NF-AT secondary to dephosphorylation, thereby suppressing T cell activation (14).

Although it is expressed in many tissues, calcineurin is most concentrated in neuronal systems, and comprises over 1% of total protein in brain (2, 15). Calcineurin has diverse roles in the nervous system, where it participates in many calcium-regulated pathways. Dynamin I, whose GTPase activity regulates synaptic vesicle recycling (16, 17), is a substrate of calcineurin (18, 19). Calcineurin modulates the GTPase activity of dynamin I by altering its phosphorylation state, thereby regulating the release of neurotransmitters such as glutamate (18, 20). Calcineurin regulates the activity of N-methyl-D-aspartate (NMDA) receptor channels by both altering their ion gating properties (21) and promoting desensitization (22). Elevation of intracellular calcium concentration following opening of the NMDA receptor channel activates several downstream proteins including neuronal nitric oxide synthase, which influences neuronal cell death secondary to glutamate NMDA neurotoxicity (23, 24). The activity of nitric oxide synthase is diminished by phosphorylation so that dephosphorylation and subsequent activation of nitric oxide synthase by calcineurin implicates this phosphatase in NMDA-mediated neurotoxicity (25).

Besides mediating glutamate neurotoxicity, NMDA receptors influence synaptic plasticity. A role for protein kinases in shaping synaptic plasticity is well established (26), as multiple experimental paradigms have demonstrated a close correlation between reduction of kinase activity and impairment of long-term potentiation in the hippocampus (27–29). More recently, phosphatases, especially calcineurin, have been implicated in regulating synaptic plasticity (15). Calcineurin has diverse, sometimes contradictory, effects on synaptic plasticity (30–32). Recently, Winder and colleagues (33) discovered that calcineurin inhibits a novel, intermediate phase of long-term potentiation, which influences the transition from short-term to long-term memory (34).

Despite the abundance of calcineurin and its role in many biologically important processes, few endogenous regulators of calcineurin have been identified. Calmodulin, bound to CnA, enhances its activity, while the autoinhibitory domain of CnA competitively inhibits the phosphatase activity (35). Superoxide dismutase prevents the in vivo inactivation of calcineurin secondary to oxidative damage (36), but there is no evidence for direct binding between the two proteins. FKBP12 associates with calcineurin, even in the absence of immunosuppressive drugs (37). This interaction involves residues distinct from those mediating the FK506-dependent association of FKBP12 and calcineurin, and allows FKBP12 to function as a calcineurin anchoring protein. FKBP12 targets calcineurin to in-
tracellular calcium channels such as the ryanodine receptor (38–40) and the inositol 1,4,5-trisphosphate receptor (41). Calcineurin activity regulates the calcium flux through these channels secondary to changes in their phosphorylation states (42). Another well known anchoring protein for calcineurin is AKAP79 (A kinase anchoring protein), a scaffolding protein that also binds CAMP-dependent protein kinase and protein kinase C (43). Physical association between calcineurin and AKAP79 leads to inhibition of calcineurin activity, as well as its targeting to neuronal membranes (44).

It is unlikely that the above short list of proteins could adequately explain the diversity of calcineurin function. To better appreciate the role of calcineurin in multiple calcium-sensitive signaling pathways, we sought additional regulatory molecules for this unique phosphatase. We now report the identification and characterization of cain (calcineurin inhibitor), a novel, large protein that physiologically interacts with calcineurin and potently inhibits its catalytic activity.

**EXPERIMENTAL PROCEDURES**

**Materials**—FK506 was a gift from Dr. Joseph Steiner (Guilford Pharma) (27). Bovine calcineurin was purchased from Pharmacia Biotech. Purified human FKBP12, purified bovine calcineurin, the RII peptide (calcineurin substrate), and cAMP-dependent protein kinase were obtained from Sigma. Anti-CnA antibody was obtained from Transduction Laboratories, and anti-CnB antibody was obtained from Upstate Biotechnology Inc. The rabbit polyclonal anti-cain antibody was raised against (His)_6-cain (described below), and affinity-purified using the immunogen as described (45).

**Plasmid Constructs for Yeast Two-hybrid Analysis and in Vitro Binding Assays**—CnA was subcloned into Sall and NotI sites of pPC86/97 (46) from the full-length human calcineurin Aβ open reading frame, using the following polymerase chain reaction primers: 5′-aacgccgacccgagcagcag-3′ and 5′-ttttggcgcctagctgagatgtt-3′. Caim deletion mutants were generated from the cain fragment obtained using the following polymerase chain reaction primers and an appropriate vector primer: cain1518–1610, 5′-aacgccgacccgagcagcag-3′; cain1518–1683, 5′-ttttggcgcctagctgagatgtt-3′; cain1518–1752, 5′-ttttggcgcctagctgagatgtt-3′; cain1762–1810, 5′-aacgccgacccgagcagcag-3′; cain1715–1810, 5′-aacgccgacccgagcagcag-3′. Inserts were then digested with Sall and NotI and cloned into corresponding sites in pPC86 and pGEX-4T-2 (Amerham Pharmacia Biotech). All of the constructs were verified by sequencing.

**Yeast Two-hybrid Screen**—The full-length human FKBP12 open reading frame was cloned into yeast expression vector pCa97, containing the GAL A DNA-binding domain. This was used to screen a rat hippocampal cDNA library cloned into pC86 (47), containing the GAL A domain. The resultant 10^6 clones were screened, initially by LIAc-mediated transformation into FKBP12-deficient HF7c yeast strain, constructed as described (48). A total of 1.5 × 10^6 independent clones were screened, and positive interacting proteins were identified by selecting for His+ growth phenotype with a strict dependence on the presence of FK506. Positive clones were further evaluated for β-galactosidase expression by nitrocellulose filter lift assays as described (46).

**In Vitro Binding Assays**—GST fusion proteins were prepared according to manufacturer’s recommendations (Pharmacia Biotech) and coupled to glutathione-Sepharose beads. (His)_6-cain construct was generated by subcloning the cain fragment isolated from the two-hybrid screen into the Sall and NotI sites of pET-28c (+) vector (Novagen). The fusion protein was prepared as per the manufacturer’s protocol. In each binding assay, 2 μg of purified (His)_6-cain and/or 2 μg of purified bovine calcineurin were added to 20 μl of the GST-fusion protein-Sepharose slurry (50% (w/v) in phosphate-buffered saline) and brought to a final volume of 200 μl with binding buffer (50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2 mM CaCl_2, 2 mM MgCl_2, 0.2% Triton X-100, 0.5 mg/ml bovine serum albumin, 0.5 mM β-mercaptoethanol). After binding was allowed to proceed at 4 °C for 1 h with gentle mixing, the samples were washed three times with binding buffer, boiled in SDS sample buffer, and separated by SDS-PAGE. Proteins were transferred onto nitrocellulose membrane and immunoblotted with anti-T7 antibody (Novagen) at 1:10,000 dilution and anti-CnB antibody at 1:1,000 dilution, followed by a 1:5,000 dilution of anti-mouse Ig antibody conjugated to horseradish peroxidase. Western blots were visualized using an enhanced chemiluminescence system (ECL, Amersham).

**Co-precipitation Assays Using Transfected Cells**—The two-hybrid cain fragment was subcloned into a mammalian expression vector with a cytomegalovirus promoter and an in-frame N-terminal myc epitope tag. This myc-cain construct was introduced into HEK293 cells by calcium phosphate-mediated transfection. Cells were harvested 48 h following transfection and lysed in lysis buffer (50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2 mM CaCl_2, 2 mM MgCl_2, 0.2% Triton X-100, 0.5 mM β-mercaptoethanol, 5 μg/ml aprotinin, 1 μg/ml leupeptin, 6 μg/ml chymostatin, 0.7 μg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride). Equal amounts of cell lysate were incubated with GST-Sepharose, GST-FKBP12-Sepharose, or calmodulin-Sepharose beads (20 μl of 50% slurry) for 1 h at 4 °C with gentle agitation. Where indicated, GST-FKBP12-Sepharose beads were preabsorbed with FK506 for 1 h at 4 °C and then washed twice in lysis buffer prior to adding to the cell lysate. FK506 was also added to the cell lysate in co-precipitation assays as indicated. Following the incubation, the Sepharose beads were washed three times with lysis buffer and boiled in SDS sample buffer. SDS-PAGE and Western blotting were performed as described above. The anti-myc antibody was used at 1:3,000 dilution, and anti-CnA antibody at 1:250 dilution.

**Tissue Co-precipitation Assays**—Adult rat brain was homogenized in lysis buffer (described above) and spun at 20,000 × g for 20 min to remove insoluble materials. The resulting lysate was divided into equal aliquots and incubated with GST-FKBP12-Sepharose beads in the same manner described above. Bound proteins were analyzed by SDS-PAGE and Western blotting as described above.

**Cloning of Full-length CAIN cDNA**—An adult rat brain cDNA library in λZAPII vector (Stratagene) was screened using the two-hybrid cain fragment, labeled with α[32P]dATP in a nick translation system (Boehringer Mannheim). A 5.4-kilobase open reading frame was identified, and a probe from the 5′-terminal sequence (nucleotides 41–1040) was generated for a second screen. A total of 4 × 10^6 clones were screened, yielding 33 overlapping inserts. 5′-Rapid amplification of cDNA ends was performed using Marathon-Ready rat brain cDNA (CLONTECH) according to manufacturer’s protocol.

**Calcineurin Activity Assay**—The assay was carried out as described by Hubbard and Klee (49) except where otherwise indicated. A peptide corresponding to the phosphorylation site on the RII subunit of cAMP-dependent protein kinase was phosphorylated and used as substrate. Assays were performed in 50-μl aliquots containing 40 mM Tris-HCl, pH 7.4, 100 mM NaCl, 6 mM MgCl_2, 0.1 mM CaCl_2, 0.1 mg/ml bovine serum albumin, 0.5 mM dithiothreitol, 100 mM calmodulin, and 25 mM cain (calcineurin). After 1 h preincubation with (His)_6-cain at 4 °C, reactions were started by the addition of 2–150 μM substrate peptide. Assays were performed at 25 °C for 8 min and terminated by the addition of 5% trichloracetic acid in 0.1 mM K_HPO_4.

**Northern Blot Analysis**—A commercial rat multiple tissue Northern blot (CLONTECH) was hybridized according to the manufacturer’s recommendations with the N-terminal cain probe described above.

**Multiple Tissue Western Analysis**—Tissues from an adult male rat were homogenized in ice-cold phosphate-buffered saline (+ 5 μg/ml aprotinin, 1 μg/ml leupeptin, 6 μg/ml chymostatin, 0.7 μg/ml pepstatin, and 1 mM phenylmethylsulfonyl fluoride). Each homogenate was centrifuged at 16,000 × g for 20 min and the pellet discarded. Equal amounts (40 μg of proteins) from all samples were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membrane. Immuno blotting was performed using affinity-purified polyclonal anti-cain antibody, followed by anti-rabbit Ig at 1:5,000 dilution and detected with ECL reagents.

In situ Hybridization—RNA probes for FKBP12, CnA, and cain, and cain were generated by subcloning each open reading frame from pC86 or pCa97 into the Sall and NotI sites of pBS/SK+1+ (Stratagene). Brain slices for in situ hybridization were prepared and probed as described (50).

**RESULTS**

**Identification of CAIN, a Novel Calcineurin-binding Protein**—In initial experiments we utilized the yeast two-hybrid system to identify proteins that selectively bind FKBP12 in the presence of the drug FK506. We screened an intact hippocampal cDNA library and identified a 300-amino acid protein fragment without homology to any known protein sequence. This fragment constitutes the C-terminal portion of cain (calcineurin-inhibitor).

In the yeast two-hybrid analysis, wild-type HF7c and FKBP12-deficient HF7c yeast strains were used to assay pro-
tein interactions based upon the activation of two reporters, β-galactosidase expression and the more sensitive histidine prototrophy. In yeast containing endogenous FKBP12 (wild-type HF7c), we detected a weak interaction between FKBP12 and CnA in the presence of 1 μM FK506 (HIS3+, β-gal+) (Table I, rows 1 and 2). We reasoned that the endogenous yeast FKBP12 may compete with the human FKBP12 bait protein in the two-hybrid system; accordingly, we assayed the interaction between FKBP12 and CnA in the FKBP12-deficient yeast and observed a strong interaction (HIS3+, β-gal+) (Table I, rows 3 and 4).

Using the FKBP12-deficient yeast, we found that cain interacted with FKBP12 only in the presence of FK506 (Table I, rows 5–8). However, the weakness of this interaction (HIS3+, β-gal+) suggested that the association between cain and FKBP12/FK506 may be mediated by a third protein. Since calcineurin binds tightly to FKBP12 in the presence of FK506, proteins that associate with calcineurin as substrates or regulators may indirectly interact with FKBP12/FK506. Using CnA as the bait protein in the two-hybrid system, we found a robust interaction (HIS3+, β-gal+) with cain (Table I, rows 9–12). The strength of this interaction was unaltered in the absence of FK506 and endogenous FKBP12, indicating a direct association between calcineurin and cain.

Experiments utilizing purified proteins substantiate a direct interaction between cain and calcineurin. Affinity purified (His)_6-cain fragment bound GST-FKBP12/FK506 resin only in the presence of calcineurin, establishing that there is no direct interaction between cain and FKBP12 (Fig. 1A). This conclusion is supported by binding experiments utilizing GST-cain affinity resin and purified calcineurin. The direct association between calcineurin and cain was unaltered by the addition of FK506 and FKBP12 (Fig. 1B).

To ascertain whether the interaction between cain and calcineurin takes place in intact mammalian cells, we utilized HEK293 cells transfected with myc-cain (Fig. 2A). We monitored co-precipitation of myc-cain and the endogenous calcineurin onto a GST-FKBP12/FK506 affinity resin. Both myc-cain and calcineurin bound the resin only in the presence of FK506. The interaction between calmodulin and calcineurin also allows for purification of calcineurin by a calmodulin affinity resin. Using this resin, we evaluated the ability of myc-cain to co-purify with calcineurin (Fig. 2B). In the presence of calmodulin-Sepharose but not GST-Sepharose, both myc-cain and calcineurin bound the calmodulin resin in a FK506-independent manner.

We next examined whether cain and calcineurin associate physiologically in tissues. Using adult rat brain homogenate, we bound endogenous calcineurin onto GST-FKBP12/FK506 affinity resin and looked for co-precipitating cain proteins using an anti-cain antibody. The amount of incubation time was minimized in order to detect association of endogenous proteins, not secondary associations that occur after lysis. We found that cain and calcineurin co-precipitated onto GST-FKBP12 columns in the presence of FK506, and that the amount of precipitated cain varied in accordance with the amount of bound calcineurin (Fig. 2C). The association between cain and calcineurin could also be detected similarly in liver tissue (data not shown). In addition, this experiment revealed that the endogenous cain protein has an apparent molecular mass of 230 kDa (more details below).

**Cloning and Mutational Analysis of Cain.—**Utilizing nucleotide probes derived from the cain sequence, we screened a rat

---

**TABLE I**

| Protein in pC86 | Protein in pC97 | Endogenous yeast FKBP12 | Presence of FK506 (1 μM) | Activation of HIS3 reporter | Activation of β-galactosidase reporter |
|-----------------|-----------------|--------------------------|---------------------------|------------------------------|----------------------------------------|
| 1 FKBP12        | Calcineurin A   | +                        | +                         | +                            | +                                      |
| 2 FKBP12        | Calcineurin A   | +                        | +                         | +                            | +                                      |
| 3 FKBP12        | Calcineurin A   | +                        | +                         | +                            | +                                      |
| 4 FKBP12        | Calcineurin A   | –                        | +                         | +                            | +                                      |
| 5 Calcineurin   | FKBP12          | –                        | –                         | –                            | –                                      |
| 6 Calcineurin   | FKBP12          | –                        | +                         | +                            | +                                      |
| 7 p70S6K        | Calcineurin A   | –                        | +                         | +                            | +                                      |
| 8 p70S6K        | Calcineurin A   | –                        | +                         | +                            | +                                      |
| 9 Calcineurin   | Calcineurin A   | –                        | +                         | +                            | +                                      |
| 10 Calcineurin  | Calcineurin A   | –                        | +                         | +                            | +                                      |
| 11 Calcineurin  | Calcineurin A   | –                        | +                         | +                            | +                                      |
| 12 Calcineurin  | Calcineurin A   | –                        | +                         | +                            | +                                      |

**FIG. 1.** CAIN AND CALCINEURIN ASSOCIATE IN VITRO. A, cain binding to GST-FKBP12 requires both FK506 and calcineurin. Calcineurin and/or (His)_6-cain are incubated with GST-FKBP12 affinity resin, and the results of binding visualized by Western analysis. Calcineurin binds GST-FKBP12 in a FK506-dependent manner (lanes 1–4), while (His)_6-cain only binds GST-FKBP12 in the presence of both FK506 and calcineurin (lanes 5–12). B, cain associates directly with calcineurin. Purified bovine calcineurin is incubated with GST-cain affinity resin and binding analyzed by Western blot. The difference in intensities between CnA and CnB bands reflects sensitivity differences of the two antibodies, not the stoichiometry of the two subunits.
brain cDNA library and obtained 33 overlapping clones. A
5'-rapid amplification of cDNA ends polymerase chain reaction
yielded no additional upstream sequence (data not shown). The
full-length cDNA of cain assembled from overlapping clones
contains 7272 nucleotides. The starting methionine was as-
signed to the first in-frame AUG codon (nucleotide 332). This
AUG is preceded by two in-frame stop codons and exists in the
context of a Kozak translation initiation consensus (51). The
6.5-kilobase open reading frame codes for a protein of 2182
amino acids with a predicted molecular mass of 243 kDa (Fig.
3). A search of sequence data bases revealed no significant
homology with any previously characterized protein.

To ascertain the region of cain that interacts with cal-
cineurin, we generated different deletion mutants by truncat-
ing the 900-base fragment detected in the initial yeast two-
hybrid analysis (Fig. 4A). Utilizing the yeast two-hybrid
system, we found two overlapping deletion mutants of cain
that retain the ability to interact with calcineurin (Fig. 4A).
This was verified by in vitro binding studies using purified cal-
cineurin and cain deletion mutants coupled to GST-Sepharose
beads (Fig. 4B). These analyses indicate that all of the calcineurin binding activity of cain can be ascribed to a 38-amino acid region near the C terminus (Fig. 4A). This sequence is notable in that the calculated pI for this 38-amino acid fragment is 10.3, while that of the full-length is 5.7.

**Cain is a Potent, Non-competitive Inhibitor of Calcineurin**—To evaluate influences of cain on calcineurin catalytic activity, we assayed calcineurin activity in the presence of purified (His)_6-cain. Cain potently inhibited calcineurin activity in vitro with half-maximal inhibition evident at about 0.4–0.5 μM and a calculated Ki value of 0.44 ± 0.07 μM (mean ± S.E., n = 4) (Fig. 5A). A Dixon plot indicates that the inhibition is non-competitive (Fig. 5B).

To investigate the possibility that the inhibition of calcineurin activity by cain may be explained by its binding to calmodulin, we performed our inhibition studies in the presence of varying concentrations of calmodulin. We found that the inhibition of calcineurin by cain is independent of calmodulin concentration. Even in the presence of a 50-fold increase in calmodulin concentration, the inhibition curve was essentially unaltered (Fig. 5C).

**Cain Has a Wide Tissue Distribution**—Using Northern analysis, we detected a 7.5-kilobase transcript of cain enriched in brain, kidney, and testis but which also can be detected in all other tissues examined (Fig. 6A). This size transcript is consistent with our predicted full-length cDNA. In addition, a smaller and less abundant transcript, possibly resulting from alternative splicing, can be detected in all tissues examined.

Western analysis using affinity purified polyclonal anti-cain antibody revealed a discrete band at approximately 230 kDa. The mobility of this band on SDS-PAGE is consistent with the predicted molecular weight of full-length cain. Several smaller...
immunoreactive species were also prominent in cerebellum and liver, possibly representing tissue-specific isoforms or proteolytic fragments of the full-length protein. All of these bands were eliminated by pre-absorbing the antibody with (His)$_6$-cain (data not shown). Protein expression matched closely the mRNA levels suggested by Northern analysis, particularly in regard to the low abundance of cain in skeletal muscle and heart (Fig. 6B). Subcellular fractionation experiments indicate that cain is predominantly cytosolic (data not shown).

**Cain Expression Closely Resembles Calcineurin in the Brain**—Calcineurin and FKBP12 share highly similar distribution patterns in the brain, supporting a functional interaction between these two proteins (52). *In situ* hybridization of cain, calcineurin, and FKBP12, revealed a striking similarity between cain and calcineurin expression (Fig. 7A). Both display low expression in the brainstem, but are prominent in the olfactory bulb, caudate, nucleus accumbens, olfactory tubercle, zona reticulata of the substantia nigra, and all layers of the hippocampus and dentate gyrus. The co-localization of cain and calcineurin transcripts appears even greater than that of FKBP12 and calcineurin. In the olfactory epithelium, where FKBP12 is virtually undetectable, both cain and calcineurin are present in great abundance (Fig. 7B). FKBP12 is localized predominantly to cerebellar Purkinje cells and not to granule cells. While cain and calcineurin are both enriched in granule cells, calcineurin also occurs in Purkinje cells (Fig. 7C).

**DISCUSSION**

In the present study we have identified a novel protein, cain, which physiologically interacts with and inhibits calcineurin. Multiple experimental paradigms including the yeast two-hybrid system and several binding studies reveal that the interaction occurs both *in vitro* and *in vivo*. We also detected physiologic association of cain and calcineurin in both brain and liver tissues. Perhaps the most impressive evidence establishing a physiologic link between cain and calcineurin is their strikingly similar localizations in the brain, where both pro-
tein occurs exclusively in neurons. Previously, we reported a close similarity between localizations of calcineurin and FKBP12 in the brain, supporting a physiological relationship between the two proteins (52). In situ hybridization reveals an even greater similarity between calcineurin and calcineurin expression than that between calcineurin and FKBP12.

Calcineurin inhibits calcineurin with a Kₘ value of about 0.4 μM, and appears to be the most potent known endogenous inhibitor of calcineurin. AKAP79, a well established calcineurin inhibitor, is about 0.2% as potent in this regard (44). Whether the inhibitory action of cain on calcineurin is exerted upon cain itself is also bind kinases. Whether the kinase and phosphatase activ-

idues (44). AKAP79 binds cAMP-dependent protein kinase and the binding site contains a preponderance of basic amino acid res-

content: **Cain**

**REFERENCES**

1. Stewart, A. W., Ingelfrisen, T. S., Manalan, A., Klee, C. B., and Cohen, P. (1982) *FEBS Lett.* 137, 80–84

2. Klee, C. B., Draetta, G. F., and Hubbard, M. J. (1988) *Adv. Enzymol. Relat. Areas Mol. Biol.* 61, 149–200

3. Klee, C. B., Creutz, T. H., and Krinks, M. H. (1979) *Proc. Natl. Acad. Sci. U. S. A.* 76, 6270–6273

4. Liu, J., Farmer, J. D., Jr., Lane, W. S., Friedman, J., Weissman, I., and Schreiber, S. L. (1991) *Cell* 66, 807–815

5. Friedman, J., and Weissman, I. (1991) *Cell* 66, 799–806

6. Schreiber, S. L., and Crabtree, G. R. (1992) *Immuno. Today* 13, 136–142

7. Clipstone, N. A., and Crabtree, G. R. (1992) *Nature* 357, 695–697

8. O'Leary, S. J., Tamura, J., Kincade, R. L., Toeci, M. J., and O'Neill, E. (1992) *Nature* 357, 692–694

9. Fruman, D. A., Klee, C. B., Bierer, B. E., and Burakoff, S. J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 3686–3690

10. Jain, J., McCaffrey, P. G., Miner, K., Kerpold, T. K., Lambert, J. N., Verdine, G. L., Curran, T., and Rao, A. (1993) *Nature* 365, 352–355

11. Luo, C., Burgeon, E., Carew, J. A., McCaffrey, P. G., Badalian, T. M., Lane, W. S., Hogan, P. G., and Rao, A. (1996) *Mol. Cell. Biol.* 16, 3955–3966

12. Loh, C., Shaw, K. T., Carew, J., Bierer, B. E., and Burakoff, S. J. (1992) *J. Biol. Chem.* 267, 1274–1247

13. Rao, A., Luo, C., and Hogan, P. (1997) *Annu. Rev. Immunol. 15*, 707–747

14. Yekel, J. L. (1997) *Trends Pharmacol. Sci.* 18, 124–134

15. Tsume, R., and Takeda, K. (1999) *Neuron* 16, 2478–2487

16. Urrutia, R., Henley, J. R., Cook, T., and McNeen, M. A. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 377–384

17. Nichols, R. A., Suplitsch, G. R., and Brown, J. M. (1994) *J. Biol. Chem.* 269, 25817–25823

18. Liu, J., Sim, A. T., and Robinson, P. J. (1994) *Science* 265, 970–973

19. Siha, T. S., Nairn, A. C., Kloppeogen, P., Lin, Z., and Pouzat, C. (1995) *Biochem. Biophys. Res. Commun.* 212, 609–616

20. Lieberman, D. N., and Mody, I. (1994) *Nature* 369, 235–239

21. Tong, G., Shepherd, D., and Jahr, C. E. (1995) *Science* 267, 1510–1512

22. Dawson, V. L., Bredt, D. S., Fotuhi, M., Hwang, P. M., and Snyder, S. H. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 7739–7801

23. Choi, D. W. (1992) *Science* 258, 241–243

24. Dawson, T. M., Steiner, J. P., Dinerer, J. L., Uhl, G. R., and Snyder, S. H. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 8808–8812

25. Retkox, D. S., English, J. D., and Sweet, J. D. (1996) *Learn. Mem.* 3, 1–24

26. Abel, T., Nguyen, P. V., Barad, M., Deuel, T. A. S., Kandel, E. R., and Bourjoulou, C. (1997) *Cell* 88, 1–11

27. Mayford, M., Maney, I. M., Muller, R. U., and Kandel, E. R. (1997) *Learn. Mem.* 3, 74–85

28. Hashimoto, Y., Perrino, B. A., and Soderling, T. R. (1999) *J. Biol. Chem.* 263, 1924–1947

29. Wang, X., Cudotta, V. C., and Klee, C. B. (1996) *Nature* 383, 434–437

30. Cardenas, E. M., Hemeenwaay, C., Mui, R. S., Ye, R., Fiorentino, D., and Heitman, J. (1994) *EMBO J.* 13, 5844–5947

31. Jayaraman, T., Brillantes, A. M., Timmerman, A. P., Fleischer, S., Erdjument-Bromage, H., Tempst, P., and Marks, A. R. (1992) *J. Biol. Chem.* 267, 9474–9477

32. Timmerman, A. P., Oegunbunnui, E., Freund, E., Wiedrechter, G., Marks, A. R., and Fleischer, S. (1993) *J. Biol. Chem.* 268, 22992–22999

33. Brailis, A. B., Ondrisz, L., Scott, A., Kobrinsky, E., Onorizoe, E., Moschella, M. C., Jayaraman, T., Landers, M., Ehrlich, B. E., and Marks, A. R. (1994) *Cell* 77, 521–533

34. Cameron, A. M., Steiner, J. P., Sabatini, D. M., Kaplin, A. I., Walewnsky, D., and Snyder, S. H. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 1784–1788

35. Cameron, A. M., Steiner, J. P., Rokas, A. J., Ali, S. M., Ronnett, G. V., and Snyder, S. H. (1995) *Cell* 83, 463–472

36. Klauck, T. M., Faux, M. C., Labuda, K., Langeberg, L. K., Jaken, S., and Scott, J. D. (1996) *Science* 271, 1589–1592

37. Coghill, V. M., Perrino, B. A., Howard, R. K., Hicks, J., Gallatian, W. M., and Scott, J. D. (1995) *Science* 267, 108–111

38. Sharp, A. H., McPherson, P. S., Dawson, T. M., Aoki, C., Campbell, K. P., and Snyder, S. H. (1993) *J. Neurosci.* 13, 2519–2524

39. Gueldner, U., Heck, S., Fielder, T., Reihmann, J., and Hegemann, J. H. (1996) *Nucleic Acids Res.* 24, 2519–2524

40. Hubbard, M. J., and Klee, C. B. (1991) in *Molecular Neurobiology (Wheat, H., and Chad, J., eds)* pp. 133–137, Oxford Press, Oxford

41. Blackshaw, S., and Snyder, S. H. (1997) *J. Neurosci.* 17, 8074–8082

42. Kozak, M. A. (1989) *J. Cell Biol.* 108, 229–241

43. Dawson, T. M., Steiner, J. P., Sabatini, D. M., Fotuhi, M., Blue, M. S., and Snyder, S. H. (1994) *Neuroscience* 62, 569–580

44. Kissing, C. R., Parge, H. E., Theis, K., Pelletier, L. A., Bourtchouladze, R. (1997) *Science* 271, 507–522

45. Griffith, J. P., Kim, J. L., Kim, E. E., Sintchak, M. D., Thomson, J. A., Fitzgibbon, M. J., Fleming, M. A., Caron, P. P., Hsiao, K., and Navia, M. A. (1995) *Cell* 82, 507–522