

Appendix A14.14

CaMKII Plays a Critical Role in Regulating Various Forms of Neural Plasticity: A Role in the Pathophysiology and Treatment of Severe Mood Disorders?

After binding to calmodulin and being auto-phosphorylated at multiple sites, CaMKII targets to appropriate subcellular compartments, which is critical for directing efficient and specific responses to diverse signals that may elevate calcium in discrete neuronal sub-domains (Du et al., 2004). Efforts have been made to identify CaMKII anchoring proteins (CaMKAPs), which are important for this targeting. Initial screening to identify CaMKII binding activities using a gel overlay assay detected multiple proteins in tissue extract and subcellular fractions, representing putative CaMKAPs. A protein of 180-190 enriched at postsynaptic density, termed p190 showed strongest signals. P190 interacts with α CaMKII or β CaMKII when they are autophosphorylated at Thr286 (Colbran, 2003). Other proteins showing interactions with CaMKII are NMDA receptor subunits, F-actin and α -actinin, densin-180, synGAP β CDK5 and CaMKIIN (Colbran, 2003). An important recent study demonstrated that CaMKII and the NMDA subtype of glutamate receptor form a tight complex with each other at the synapse (Lisman and McIntyre, 2001). Interestingly, this binding appears to enhance both the autophosphorylation of the kinase and the ability of the entire holoenzyme, which has twelve subunits, to become hyperphosphorylated (Lisman and McIntyre, 2001). This hyperphosphorylated state has been postulated to represent a “memory switch” which can lead to long-term strengthening of the synapse by multiple mechanisms. One important mechanism involves direct phosphorylation of the glutamate-activated AMPA receptors, which increases their conductance and translocation onto the synapses—a mechanism essential for the formation of long-term potentiation.

Numerous studies have demonstrated that CaMKII is required for the proper formation of long-term potentiation in slice preparations, and in regulating learning and memory in rodents (Fink and Meyer, 2002). In response to stimulation, CaMKII translocates to postsynaptic site, where it has two major effects on AMPA receptor activity at the post-synaptic density during the formation of LTP (Fink and Meyer, 2002). First, the AMPA single conductance is directly increased by CaMKII at Ser831 of GluR1 subunit (Derkach et al., 1999; Fink and Meyer, 2002). Second, CaMKII is required for the delivery of AMPA receptor to the synapse, which is lacking AMPA receptors (Fink and Meyer, 2002). This enhancement of synaptic GluR1 level by activation of CaMKII requires an intact C-terminal domain of GluR1, and is possibly involved in interaction with SAP97 (Hayashi et al., 2000). Protein Phosphatase 1 (PP1), which is also known to be an important modulator for learning and memory, can dephosphorylate the phosphorylation

of GluR1 at p831 site by CaMKII. CaMKII also modulates cytoskeleton protein-protein interaction with contribute to the mechanism for morphological plasticity in the dendrites. Recent study demonstrated that overexpression of β CaMKII, but not α CaMKII, regulate the movement, extension and branching of filopodia and find dendrites, and the total number of synapses.

In addition to its role as a postsynaptic modulator, CaMKII also targets to presynaptic compartment and regulates synaptic vesicle release. A form of α CaMKII tightly associated with presynaptic vesicles and phosphorylated synapsin I, promoting the dissociation of synapsin I from synaptic vesicles, thereby making more vesicles available at the active zone for synaptic vesicle release. Moreover, CaMKII has been shown to regulate exocytotic machinery in a more direct way. Auto-phosphorylated CaMKII interacts with and phosphorylates syntaxin 1A and the interaction appears to promote synaptic vesicle exocytosis. This facilitation in vesicle release also contributes an important part in the role of CaMKII in synaptic plasticity (Colbran, 2003).

Finally, CaMKII has been shown to regulate gene transcription. For example, CaMKII phosphorylates the positive regulatory site in CREB (Ser 133), as well as a dominant negative regulatory site (Ser142). Although most CaMKII holoenzymes are excluded from the nucleus because of their size, alternatively spliced variants of CaMKII α (α_B) and CaMKII γ can localized in the nucleus. Interestingly, expression of α CaMKII in the nucleus was recently shown to inhibit the neuron-like differentiation of PC12 cells. Taken together, the data suggests that certain CaMKII splicing variance are specifically targeting to the nucleus where they can participate in the regulation of gene (Colbran, 2003).

Mice deficient for the gene encoding α CaMKII provide a useful tool to link behavioral and cellular abnormalities with α CaMKII molecule. In addition to the deficits in their ability to produce LTP at the cellular level, α CaMKII knockout mice also demonstrate alterations in fear and aggression paradigms. The heterozygous knockout mouse exhibits a well-circumscribed syndrome of behavioral abnormalities, consisting primarily of a decreased fear response and an increase in defensive aggression, in the absence of any measured cognitive deficits. In addition, another transgenic mice line of mutant α CaMKII has been generated by the introduction of an aspartate to replace the Thr amino acid at 286, an important site for Ca^{2+} -dependent autophosphorylation. The CaMKII-Asp-286 mice showed impairment in spatial memory, suggesting the importance of the autophosphorylation of α CaMKII (Bach et al., 1995). In view of α CaMKII's critical roles in regulating neural plasticity, recent studies have begun to investigate its possible involvement in mood disorders, with preliminary postmortem evidence showing abnormalities (Molnar et al., 2003;).

Suenaga et al., (2004) recently reported that acute and repeated (4 days), but not chronic (14 days) stress significantly increases phospho-CaMKII levels without affecting the levels of total CaMKII in hippocampus. Moreover, a selective alpha-amino-3-hydroxy-5-methyl-4-

isoxazole-propionic acid (AMPA) blocker NBQX prevented this phospho-CaMKII increase, while N-methyl-D-aspartate (NMDA) blockers MK801 and LY235959 showed no significant effects (see figure legend for a brief discussion of glutamate receptor subtypes). Together, these results suggest that the increase in intracellular Ca^{2+} concentrations by the activation of AMPA receptors may play a role in the stress-induced phospho-CaMKII and thereby regulate signaling pathways known to play critical roles in neural plasticity.

CaMKII is Also a Target for Antidepressant Drug Action

In addition to the data showing that CaMKII is regulated by stress, a growing body of data suggests that CaMKII also represents a target for antidepressant drugs. Thus, it has been found that long-term treatment with selective serotonin reuptake inhibitors (SSRI) (paroxetine and fluvoxamine) or a dual inhibitor of 5-HT and NA reuptake (venlafaxine) increased autophosphorylation and activity of CaMKII in hippocampal subcellular fraction enriched with synaptic vesicles and synaptic cytosolic fraction. Furthermore, repeated ECS or imipramine both induce a large increase in the activity of kinase contained in the total particulate fraction and a decrease in the activity of soluble kinase. Although concomitant behavioral studies weren't undertaken, the fact that different types of drugs and electroconvulsive therapy resulted in similar effects on CaMKII activity, suggested that CaMKII may play an important role in the treatment of depression or stress-related disorders (Popoli et al., 2002).

Together, the findings from Suenaga et al., (2004) and studies of antidepressant drug action suggest that CaMKII may play an important role as a molecular switch in the pathophysiology and treatment of many stress-related disorders. However, many questions still remain to be answered. For example, which isoform of CaMKII (α or β ?) is involved in stress-induced synaptic plasticity? This is an important question since α CaMKII and β CaMKII exert opposing effect on unitary synaptic strength as well as mEPSC frequency when overexpressed in neurons (Thiagarajan et al., 2002). This may be partially because that α CaMKII and β CaMKII have sharply different affinities for calmodulin. Half-maximal autophosphorylation is achieved at 130 nM of calmodulin for α CaMKII and at 15nM calmodulin for β CaMKII (Brocke et al., 1999). Due to this difference, the two isoforms have different sensitivity to Ca^{2+} signals under non-saturating levels of calmodulin. α CaMKII is selected for higher levels of Ca^{2+} signals, while β CaMKII has better sensitivity to lower levels of signal. Therefore, it is of considerable functional importance to determine the isoforms involved in the stress-induced synaptic plasticity.

References

Bach ME, Hawkins RD, Osman M, Kandel ER, Mayford M. Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. *Cell*. 1995 Jun 16;81(6):905-15.

Functional implications of the subunit composition of neuronal CaM kinase II. *J Biol Chem*. 1999 Aug 6;274(32):22713-22.

Colbran RJ, Carmody LC, Bauman PA, Wadzinski BE, Bass MA. Analysis of specific interactions of native protein phosphatase 1 isoforms with targeting subunits. *Methods Enzymol*. 2003;366:156-75.

Derkach V, Barria A, Soderling TR. Ca²⁺/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc Natl Acad Sci U S A*. 1999 Mar 16;96(6):3269-74.

Du J, Szabo ST, Gray NA, and Manji HK: CaMKII: a molecular switch in the pathophysiology and treatment of mood and anxiety disorders. *International Journal of Neuropsychopharmacology*, 7:1-6, 2004.

Fink CC, Meyer T. Molecular mechanisms of CaMKII activation in neuronal plasticity. *Curr Opin Neurobiol*. 2002 Jun;12(3):293-9. Review.

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science*. 2000 Mar 24;287(5461):2262-7.

Lisman JE, McIntyre CC. Synaptic plasticity: a molecular memory switch. *Curr Biol*. 2001 Oct 2;11(19):R788-91. Review.

Molnar M, Potkin SG, Bunney WE, Jones EG. MRNA expression patterns and distribution of white matter neurons in dorsolateral prefrontal cortex of depressed patients differ from those in schizophrenia patients. *Biol Psychiatry*. 2003 Jan 1;53(1):39-47.

Popoli M, Gennarelli M, Racagni G. Modulation of synaptic plasticity by stress and antidepressants. *Bipolar Disord*. 2002 Jun;4(3):166-82. Review.

Suenaga T, Morinobu S, Kawano K, Sawada T, Yamawaki S. Influence of immobilization stress on the levels of CaMKII and phospho-CaMKII in the rat hippocampus. *Int J Neuropsychopharmacol*. 2004 Sep;7(3):299-309. Epub 2004 Apr 26.

Thiagarajan TC, Piedras-Renteria ES, Tsien RW. alpha- and betaCaMKII. Inverse regulation by neuronal activity and opposing effects on synaptic strength. *Neuron*. 2002 Dec 19;36(6):1103-14.

