Generation of βCaMKII knock-down mouse

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Calcium/calmodulin-dependent protein kinase II (CaMKII) is an abundant protein kinase in the mammalian brain. CaMKII consists of α and β subunits, which form hetero-oligomeric complexes. In its inactive state CaMKII hetero-oligomeric complexes are associated to the actin cytoskeleton. In the presence of calcium-calmodulin, CaMKII becomes autophosphorylated and translocates from the actin cytoskeleton to the postsynaptic density. Activity dependent translocation of the kinase is believed to be a critical requirement for synaptic plasticity. Binding to the cytoskeleton is mediated by βCaMKII, which has a high affinity for F-actin. The binding site is not known but a special feature of βCaMKII is the presence of a variable domain that contains an autophosphorylation site (at Thr382). To elucidate the function of βCaMKII and of Thr382 phosphorylation, we generated mouse mutants in which the endogenous βCaMKII was mutated using ES cell-mediated gene targeting. A Neo-cassette was introduced into the 5'flanking sequence of intron 16 and simultaneously the Thr382 site was changed to code for a nonphosphorylatable Alanine. Using these genetically targeted ES cells we generated two mutants. The first mutant (βCaMKII/neo) carried both the Neo-gene and the Thr382/Ala382 point mutation. In the other mutant (Thr382/Ala382) the Neo-cassette was deleted through Cre/LoxP recombination. The presence of the Neo-gene and Thr382/Ala382 mutation in generated mice were confirmed by PCR and Southern blot. The presence of the Neo-gene appeared to cause a strong reduction in βCaMKII expression levels that was gene-dose dependent. By studying the molecular, behavioural and electrophysiological phenotype of these mutants, we hope to understand more of the role of βCaMKII in the cellular correlates of learning and memory.

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