Supplementary Material

Results

I-κB can be phosphorylated by CaMKII, a major protein kinase in the PSD

The fact that NF-κB is present in the PSD and that the phosphorylated form of I-κB is enriched in the PSD (Fig. 4) suggests that the PSD may be a site at which NF-κB is preferentially activated in the synapse. Others have shown that activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which is enriched in the PSD fraction (1), is necessary for the activation of neuronal NF-κB by synaptic activity (2). Although phosphorylation of I-κBα is believed to be carried out almost exclusively by the I-κB kinase (IKK), we could not detect IKK in the PSD fraction (data not shown). Therefore, we hypothesized that CaMKII may phosphorylate I-κBα and thus directly activate NF-κB in the PSD. To test this hypothesis, we performed an *in vitro* kinase assay using recombinant I-κBα and CaMKII purified from adult rat forebrains. We found that CaMKII is able to phosphorylate I-κBα on serines 32 and 36 (Figure S1). These findings suggest a model in which activation of neuronal NF-κB by synaptic activity is directly mediated by CaMKII via phosphorylation of I-κB in response to stimulation of glutamate receptors in the PSD and subsequent influx of calcium into the dendritic spine. Whether CaMKII phosphorylates I-κB *in vivo*, or acts through IKK (3) to mediate the activation of NF- κB remains an open question.

Materials and Methods

CaMKII kinase assay

CaMKII was purified from adult rat forebrains as described in Bennett et al. (4). Phosphorylation of recombinant full-length human GST-tagged I-κBα (sc-4094, Santa Cruz Biotechnology) by purified CaMKII was carried out in a reaction mix containing 50 mM Tris-HCl (pH 8.0), 10 mM magnesium chloride, 0.4 mM EGTA, 10 mM DTT, 30 µM ATP, 0.7 mM calcium chloride, 10 µg/ml calmodulin, 3 µg of CaMKII, and 3 µg of I-κBα. Phosphorylation was initiated by addition of CaMKII and ATP to a 30 µl reaction mix prewarmed to 30°C for 2 min. The negative control reaction contained the same reagents, except that calcium chloride and calmodulin were omitted. The reaction was carried out for 2 min, stopped by addition of SDS-PAGE sample buffer, and processed for immunoblotting as described above.

References

1 Kennedy, M.B., Bennett, M.K. and Erondu, N.E. (1983) Biochemical and immunochemical evidence that the "major postsynaptic density protein" is a subunit of a calmodulin-dependent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 7357-7361.

2 Meffert, M.K., Chang, J.M., Wiltgen, B.J., Fanselow, M.S. and Baltimore, D. (2003) NF-κ B functions in synaptic signaling and behavior. *Nat. Neurosci.*, **6**, 1072-1078.

3 Hughes, K., Edin, S., Antonsson, A. and Grundstrom, T. (2001) Calmodulin-dependent kinase II mediates T cell receptor/CD3- and phorbol ester-induced activation of IkappaB kinase. *J. Biol. Chem.*, **276**, 36008-36013.

4 Bennett, M.K., Erondu, N.E. and Kennedy, M.B. (1983) Purification and characterization of a calmodulin-dependent protein kinase that is highly concentrated in brain. *J. Biol. Chem.*, **258**, 12735-12744.

Legends to Figures

Figure S1: NF-κB can be directly activated by CaMKII. Representative immunoblot (n=2) of products of *in vitro* kinase reactions performed with purified CaMKII as kinase and recombinant I-κBα as substrate in the presence (+) and absence (-) of calcium/calmodulin (Ca^{2+}/CaM). On the left; total I-κBα (red) and I-κBα-pS32 (green); on the right; I-κBα doubly phosphorylated on serine 32 and 36 (I-κBα-pS32+pS36, red) and I-κBα-pS32 (green).

Figures

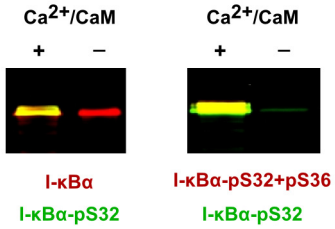

Figure S1