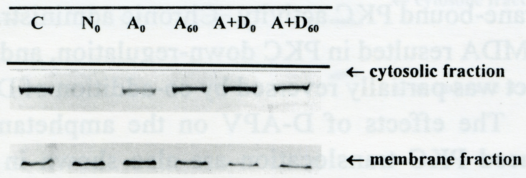
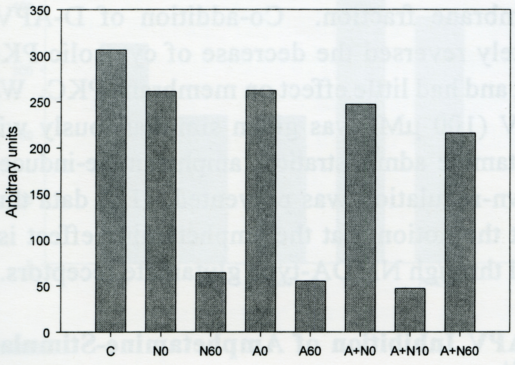
a.



b.

Cytosol



Membrane

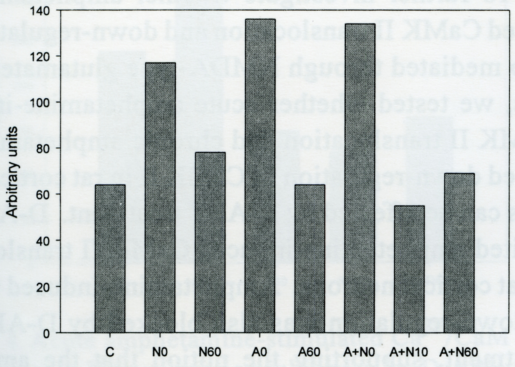


Fig. 2. Induction of Ca^{2+}/calmodulin-dependent protein kinase II translocation by both amphetamine and NMDA. In the membrane and cytosolic fractions of cultured cortical neuronal cells, CaMK II contents were examined by Western blot analysis using anti-CaMK II β-subunit monoclonal antibody (panel a), and then bands were analyzed with a densitometer (panel b). C: control; N: NMDA; A: amphetamine; A+N: amphetamine and NMDA co-treatment. Arabic numerals indicate the extra incubation periods at 37 °C after a 15-min treatment with the indicated ligands.

of amphetamine and NMDA possibly due to excito-protection has been demonstrated in cultured cerebella granule neurons.[20]

Induction of Ca^{2+}/Calmodulin-Dependent Protein Kinase II Translocation and Down-Regulation by Both Amphetamine and NMDA

We next examined the effects of amphetamine and NMDA on CaMK II distribution in the membranes and cytosolic fractions of cultured cortical neuronal cells by Western blot analysis using anti-CaMK II β-subunit monoclonal antibodies. As shown in Fig. 2, CaMK II was mostly present in the cytosolic fraction in cultured cortical neuronal cells. Exposure of the cultured cortical neurons to NMDA (100 μM) or amphetamine (30 μM) for 15 min resulted in a decrease of cytosolic CaMK II which was accompanied by an increase of membrane CaMK II, indicating that CaMK II translocation was stimulated. When the cells were incubated at 37 °C for 1 h, there was a marked reduction of total CaMK II, suggesting that CaMK II down-regulation was triggered by prolonged amphetamine or NMDA treatments. This again shows that CaMK II down-regulation was prevented by simultaneous pretreatment of amphetamine and NMDA possibly due to the excito-protection effect.

Co-treatment with amphetamine and NMDA for 15 min stimulated CaMKII translocation from cytosol to membrane (Fig. 2, lane 6). When these cells were incubated at 37 °C, there was a transient decrease of CaMKII at 10 min (Fig. 2, lane 7) followed by recovery at 60 min (Fig. 2, lane 8). Simultaneous treatment with amphetamine and NMDA possibly results in rapid degradation or redistribution of CaMKII, followed by the re-synthesis or reappearance of CaMKII in cytosol and membrane fractions. Whether CaMKII is sequestered in other compartments or degraded requires further investigation.

APV Inhibition of Acute Amphetamine-Stimulated Protein Kinase C Translocation

D-APV, a specific antagonist to the NMDA receptor, was used to investigate whether amphetamine-induced PKC translocation is mediated through NMDA -type glutamate receptors. As shown in Fig. 3, exposure of cortical neurons to NMDA (100 μM) resulted in a decrease of the cytosolic PKC and an increase of