Electrophoresis and Immunoblotting

Electrophoresis was ordinarily carried out on different percentages of SDS-polyacrylamide electrophoresis (SDS-PAGE) according to the method of Laemmli. Following electrophoresis, proteins on the gel were electrotransferred onto a nitrocellulose membrane according to the method of Twobin *et al.* After transfer, the nitrocellulose papers were washed once with PBS and twice with PBS plus 0.1% Tween 20. The nitrocellulose membranes were then blocked with blocking solution containing 1% bovine serum albumin in PBS containing 0.1% Tween 20 for 1 h at room temperature. The nitrocellulose membranes were incubated with a solution containing primary antibodies (from UBI) in the blocking buffer. Finally, the nitrocellulose paper was incubated with peroxidase-linked (or alkaline phosphatase-linked) anti-mouse IgG antibodies for 1 h and then developed using a commercially available chemiluminescence kit (from Amersham) or NBT-BCIP (from Boehringer Mannheim).

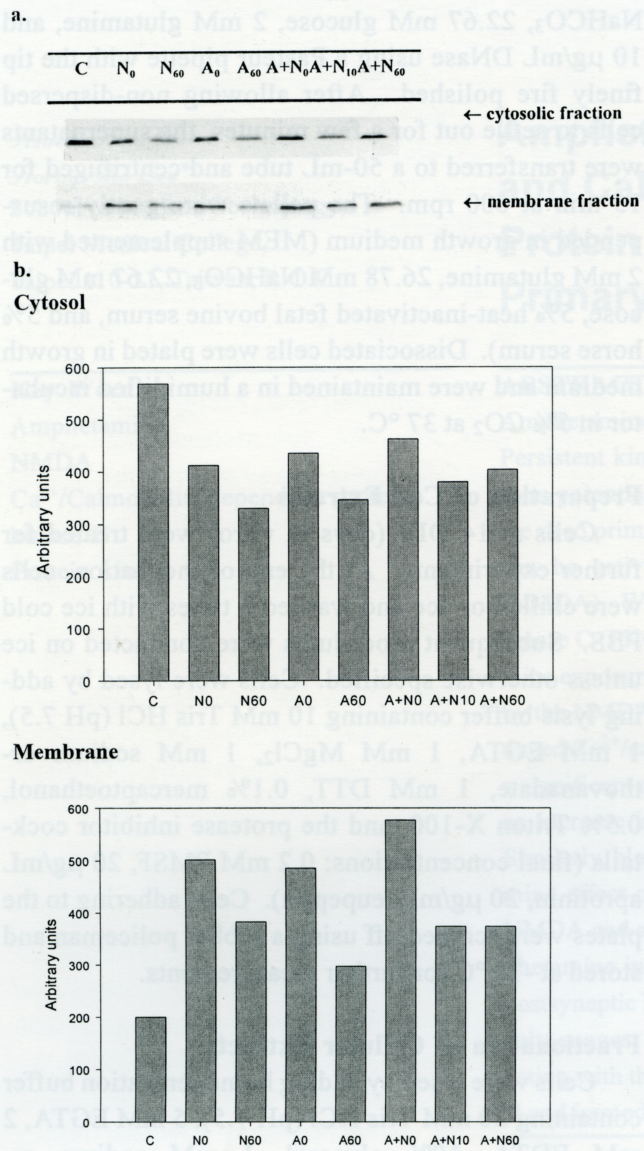

Fig. 1. Induction of protein kinase C translocation by both amphetamine and NMDA. In the membrane and cytosolic fractions of cultured cortical neuronal cells, PKC contents were examined by Western blot analysis using anti-PKC α monoclonal antibody (panel a), and then bands were analyzed with a densitometer (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.

RESULTS

Involvement of Both Amphetamine and NMDA in the Translocation and Down-Regulation of Protein Kinase C

Translocation of protein kinase C (PKC) from cytosol to membranes is an indication of PKC activation. To test the effects of amphetamine and NMDA on PKC activity, PKC contents in the membranes and cytosolic fractions of cultured cortical neurons were examined by Western blot analysis. As shown in Fig. 1, PKC is mostly present in the cytosolic fraction and only a minimal amount of PKC is present in the membrane fraction in the control cells. NMDA (100 μM) treatment for 15 min at room temperature induced a significant decrease in the amount of the enzyme in the cytosol fraction, in contrast to the increase in the membrane fraction in primary culture of cortical neurons. However, when the cells continued to incubate at 37 °C for 60 min, there was a marked reduction of PKC in both the cytosolic and the membrane fractions, suggesting that PKC down-regulation was triggered by NMDA treatment. The acute NMDA-stimulated PKC translocation and chronic NMDA-stimulated PKC down-regulation were both mimicked by amphetamine (30 μM) treatment. That PKC down-regulation is prevented by simultaneous pretreatment