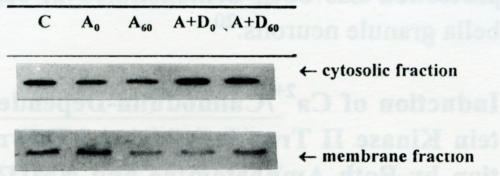
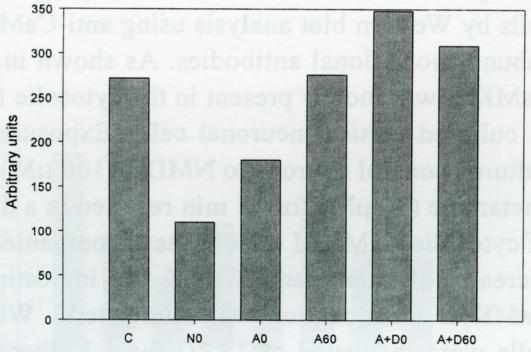
a.



b.

Cytosol



Membrane

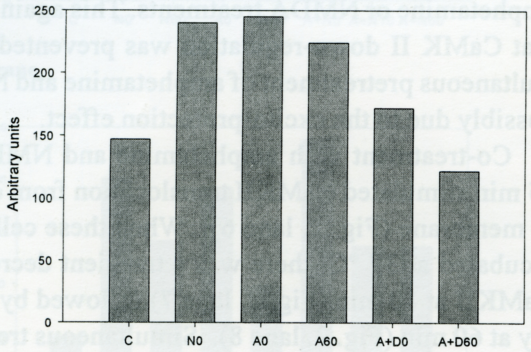


Fig. 3. AcuteNMDA-stimulated protein kinase C translocation inhibition by D-APV. D-APV, a specific antagonist to the NMDA receptor, was used to investigate whether amphetamine-induced PKC translocation is mediated through the NMDA receptor. In the membrane and cytosolic fractions of cultured cortical 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+D: amphetamine and D-APV co-treatment, Arabic numerals indicate the extra incubation periods at 37 °C after a 15-min treatment with the indicated ligands.

PKC in the membrane fraction (PKC trans-location). D-APV inhibited the amphetamine-induced decrease of cytosolic PKC and had little effect on the membrane-bound PKC activity. Chronic administration of NMDA resulted in PKC down-regulation, and this effect was partially reversed by co-addition of D-APV.

The effects of D-APV on the amphetamine-induced PKC translocation are also shown in Fig. 3. Exposure of cultured rat cortical neurons to amphetamine (30 μM) for 15 min resulted in a significant decrease of cytosolic PKC and an increase of PKC in the membrane fraction. Co-addition of D-APV completely reversed the decrease of cytosolic PKC content and had little effect on membrane PKC. When D-APV (100 μM) was given simultaneously with amphetamine administration, amphetamine-induced PKC down-regulation was prevented. The data thus support the notion that the amphetamine effect is mediated through NMDA-type glutamate receptors.

D-APV Inhibition of Amphetamine-Stimulated Ca^{2+}/CaM Kinase II Translocation and Down-Regulation

To further investigate whether amphetamine-induced CaMK II translocation and down-regulation are also mediated through NMDA-type glutamate receptors, we tested whether acute amphetamine-induced CaMK II translocation and chronic amphetamine-induced down-regulation of CaMK II in rat cortical neurons can be affected by D-APV treatment. D-APV inhibited amphetamine-induced CaMK II translocation in rat cortical neurons. Amphetamine-induced CaMK II down-regulation was also blocked by D-APV co-treatment, supporting the notion that the amphetamine-induced response was indeed mediated through NMDA-type glutamate receptors. (Fig. 4).

DISCUSSION

Repeated intermittent treatment of rats with amphetamine results in a sensitization of locomotion and stereotyped behaviors that are accompanied by transient induction of immediate early gene expression, which is dependent on postsynaptic receptor and Ca^{2+} activation.[4] Although the detailed molecular mechanisms by which these long-term behavioral changes