ceptor causes Na^+ and Ca^{2+} influx which with time may lead to neuronal cell swelling and cell death.[8,9] Activation of the NMDA receptor also induces PKC translocation in guinea pig cerebral synaptoneurosomes,[10] in hippocampal slices of immature rat brain,[11] in the postsynaptic density of rat brain,[12] and in cerebellum granule cells.[13]

CaMK II kinase is highly enriched in neuronal tissues.[14] CaMK II kinase regulates many cellular processes. Postsynaptic calmodulin and CaMK II kinase activities are responsible for LTP.[15] Exposure of cultured rat hippocampal neurons to glutamate stimulated CaMK II activity and translocation.[16,17] Conversely, inhibition of CaMK II activity has been observed in delayed neuronal cell death such as ischemia[18] and activation of the NMDA receptor.[19]

Little is known about the biochemical processes that are immediately activated by amphetamine administration. Because the NMDA receptor, protein kinase C (PKC), and Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) are involved in long-term potentiation (LTP),[7] they are considered as candidates in mediating early responses after amphetamine administration. The aims of the present study are therefore directed to understanding the involvement of these kinases prior to the expression of the immediate early genes, PKC and CaMK II, in cultured cortical neurons to NMDA and amphetamine. Our results suggest that amphetamine may interact with the NMDA receptor and induce long-term behavioral changes through PKC and/or CaMK II activations, because amphetamine mimics NMDA in stimulating kinase activities, while blocking the NMDA receptor by D-APV diminishes the amphetamine effect.

MATERIALS AND METHODS

Preparation of Primary Culture of Dissociated Fetal Brain Cells

Primary cultures of brain dissociated cells were prepared following the protocol of Yarom *et al.* with some modification. Briefly, rat embryos were recovered by C-section under nembutal anesthetic. Whole brain of fetal rats (16- to 18-day gestation) was removed from fetuses. The cortex was peeled back and meninges were removed from it. The pieces of cortex were treated with trypsin and incubated for 30 min at 37 °C. After trypsinization, individual cells were dissociated by trituating in Medium Eagle Medium (MEM, from Gibco) supplemented with 26.78 mM $NaHCO_3$, 22.67 mM glucose, 2 mM glutamine, and 10 μg/mL DNase using a Pasteur pipette with the tip finely fire polished. After allowing non-dispersed cells to settle out for a few minutes, the supernatants were transferred to a 50-mL tube and centrifuged for 15 min at 600 rpm. The pellets were gently resuspended in growth medium (MEM supplemented with 2 mM glutamine, 26.78 mM $NaHCO_3$, 22.67 mM glucose, 5% heat-inactivated fetal bovine serum, and 5% horse serum). Dissociated cells were plated in growth medium and were maintained in a humidified incubator in 5% CO_2 at 37 °C.

Preparation of Cell Extracts

Cells at 14 DIV (days in vitro) were treated for further experiments. At the end of incubation, cells were chilled on ice and washed 3 times with ice cold PBS. Subsequent procedures were conducted on ice unless otherwise specified. Cells were lysed by adding lysis buffer containing 10 mM Tris HCl (pH 7.5), 1 mM EGTA, 1 mM $MgCl_2$, 1 mM sodium orthovanadate, 1 mM DTT, 0.1% mercaptoethanol, 0.5% Triton X-100, and the protease inhibitor cocktails (final concentrations: 0.2 mM PMSF, 20 μg/mL aprotinin, 20 μg/mL leupeptin). Cells adhering to the plates were scraped off using a rubber policeman and stored at -70 °C for further measurements.

Fractionation of Cellular Extracts

Cells were lysed by adding homogenization buffer containing 20 mM Tris HCl (pH 7.5), 5 mM EGTA, 2 mM EDTA, 10% glycerol, 1 mM sodium orthovanadate, 1 mM DTT, and the protease inhibitor cocktails (final concentrations: 0.2 mM PMSF, 20 μg/mL aprotinin, 20 μg/mL leupeptin). Cells adhering to the plates were scraped off using a rubber policeman. After centrifuging the cell suspension at 800xg for 10 min at 4 °C, the supernatant was then subjected to centrifugation at 25000xg for 15 min at 4 °C. The supernatant represented the cytosolic fraction, and the pellet membrane fraction was resuspened in homogenization buffer.