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Haloperidol-Induced Neuronal Apoptosis: Role of p38 and c-Jun-NH_2-Terminal Protein Kinase

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Abstract: We examined patterns and mechanisms of cell death induced by haloperidol. Cortical cell cultures exposed to 10–100 μM haloperidol for 24 h underwent neuronal death without injuring glia. The degenerating neurons showed hallmarks of apoptosis, featuring cell body shrinkage, nuclear chromatin condensation and aggregation, nuclear membrane disintegration with intact plasma membrane, and prominent internucleosomal DNA fragmentation. Neither glutamate antagonists nor antioxidants prevented the haloperidol-induced neuronal apoptosis. The c-Jun-NH_2-terminal protein kinase and p38 mitogen-activated protein kinase were activated within 1 h and were sustained over the next 3 h following exposure of cortical neurons to 30 μM haloperidol. Haloperidol-induced neuronal apoptosis was partially attenuated by 10–30 μM PD169316, a selective inhibitor of p38 mitogen-activated protein kinase. Inclusion of 1 μg/ml cycloheximide, a protein synthesis inhibitor, or 100 ng/ml insulin prevented activation of both kinases and subsequent neuronal death. The present study demonstrates that cortical neurons exposed to haloperidol undergo apoptosis depending on activation of p38 mitogen-activated protein kinase and c-Jun-NH_2-terminal protein kinase sensitive to cycloheximide and insulin. **Key Words:** Haloperidol—Apoptosis—p38—c-Jun-NH_2-terminal protein kinase—Insulin—Cycloheximide.
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Antipsychotic drugs used to treat several neuropsychiatric disorders produce extrapyramidal side effects including tardive dyskinesia (TD), an iatrogenic syndrome of hyperkinetic dyskinesia, which imposes the major limitation on the use of this class of drugs (Diederich and Goetz, 1998). However, the mechanism underlying drug-induced TD is poorly understood. Hypersensitivity of dopamine receptors (Clow et al., 1979) or a decrease in the activity of glutamic acid decarboxylase, GABA-synthesizing enzyme, following long-term administration of antipsychotic drugs may contribute to the drug-induced extrapyramidal effects (Gale, 1980; Gunne et al., 1984). However, neither dopamine antagonists nor GABA agonists showed beneficial effects in alleviating TD (Korsgaard et al., 1982; Glazer et al., 1985).

Neurotoxic effects of antipsychotic drugs have emerged as potential pathogenic events of TD. Postmortem study of TD brains showed ventricular dilatation, atrophy in several brain areas, and neurodegeneration and gliosis (Christensen et al., 1970; Mion et al., 1991; Dalgalarrondo and Gattaz, 1994). More directly, intramuscular injections of flupenthioxol or fluphenazine decanoate caused loss of striatal neurons in adult rats (Nielsen and Lyon, 1978; Jeste et al., 1992). Behl et al. (1995) demonstrated that hippocampal neurons exposed to haloperidol underwent necrotic cell death through free radical-mediated mechanisms. To the contrary, we have provided evidence that treatment with haloperidol produces neuronal apoptosis in cortical cell cultures (Noh et al., 1997). To resolve controversial results and elucidate mechanisms of haloperidol neurotoxicity, we set out experiments to examine patterns of haloperidol-induced neuronal death, turning to multiple morphological and interventional criteria. We also investigated the possibility that reactive oxygen species (ROS) and the mitogen-activated protein kinases p38 and c-Jun-NH_2-terminal protein kinase (JNK), known as mediators of cell death, mediate haloperidol neurotoxicity.

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Abbreviations used: ATF, activating transcription factor; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DCDHF-DA, dichlorodihydrofluorescein diacetate; DIV, days in vitro; GST, glutathione *S*-transferase; HINA, haloperidol-induced neuronal apoptosis; JNK, c-Jun-NH_2-terminal protein kinase; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; MEM, minimal essential medium; NAC, *N*-acetyl-L-cysteine; p38, p38 mitogen-activated protein kinase; PBN, *N*-*tert*-butyl-α-phenylnitrone; PI, phosphatidylinositol; PP2B, protein phosphatase 2B; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; TD, tardive dyskinesia; Z-VAD, *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone.

MATERIALS AND METHODS

Cell culture

Cortical cell cultures were prepared as previously described (Noh and Gwag, 1997). Neocortexes from 14- or 15-day-old fetal mice for neuron–glia cocultures and 1-day pups for glial cultures were dissociated and plated onto 24-well plates precoated with 100 μg/ml poly-D-lysine and 4 μg/ml laminin at a density of 5 hemispheres/plate, in minimal essential medium (MEM; Earle's salts) supplemented with 5% horse serum, 5% fetal bovine serum, and 21 m*M* glucose. For mixed cultures of neurons and glia, proliferation of nonneuronal cells was halted by inclusion of 10 μ*M* cytosine arabinoside at 7–9 days in vitro (DIV). After 2–3 days, cultures were fed with growth medium identical to the plating medium without fetal serum. Cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere. Animal care and treatment were in compliance with a protocol approved by our institutional animal care committee.

Experimental protocols

Cultures were exposed to various drugs in MEM supplemented with 21 m*M* glucose at 37°C. Cell death was analyzed 24 h later by measurement of lactate dehydrogenase (LDH) released into the bathing medium. The LDH values were normalized between the mean LDH values released 24 h after exposure to sham wash (=0% death) and 500 μ*M* NMDA in neuron–glia cocultures (=100% neuronal death) or 1% Triton X-100 in glial cultures (=100% glial death).

DNA damage analysis

Cortical cell cultures were incubated in 0.5 μg/ml propidium iodide for 10 min and fixed in 4% paraformaldehyde. The fluorescence image of nuclear chromatin was examined using a rhodamine filter.

To study patterns of DNA fragmentation, cultures were lysed in solution containing 0.5% Triton X-100, 5 m*M* Tris (pH 7.4), and 20 m*M* EDTA. Lysates were microcentrifuged at 14,000 *g* for 15 min at 4°C. Supernatants were extracted with phenol/chloroform/isoamyl alcohol (25:24:1), precipitated in 75% ethanol containing 300 m*M* sodium acetate, and resuspended in Tris-EDTA buffer (pH 7.4). The soluble DNA samples were subjected to electrophoresis on a 1.2% agarose gel (Gwag et al., 1997).

Transmission electron microscopy

Cultures were fixed in modified Karnovsky's fixative solution in cacodylate buffer (pH 7.4) and postfixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide. Cells were en bloc stained in 0.5% uranyl acetate, dehydrated through a graded ethanol series, and embedded in resin (Polyscience, Warrington, PA, U.S.A.). Then blocks were sectioned using a Reichert–Jung Ultracut S and stained with Ultrostain 1H and 2 (Leica, Wien, Austria). Specimens were observed and photographed under a Zeiss EM 902 A electron microscope (Leo, Oberkohen, Germany) (Sohn et al., 1998).

JNK and p38 assay

Cells were lysed in a buffer containing 50 m*M* Tris-HCl (pH 7.5), 150 m*M* NaCl, 1% Triton X-100, 0.5% deoxycholate, 10% glycerol, 1 m*M* sodium orthovanadate, and protease inhibitors (1 m*M* phenylmethylsulfonyl fluoride, 2 m*M* EDTA, 1 μg/ml pepstatin A, 10 μg/ml leupeptin). Cell lysates were centrifuged at 13,000 rpm for 10 min, the supernatants were obtained, and protein concentrations were determined using a Bio-Rad protein assay kit (Bio-Rad, CA, U.S.A.). Approximately 500 μg of protein for each condition was immunoprecipitated with 1 μg of a goat polyclonal anti-p38 antibody for 1 h at 4°C (Santa Cruz, CA, U.S.A.). Protein A-Sepharose (Sigma, St. Louis, MO, U.S.A.) was then added to the reaction mixture and reacted for 1 h at 4°C. The immunopellet was collected after a brief centrifugation at 500 *g* for 3 min and incubated in a reaction mixture containing 10 m*M* Na_3VO_4, 50 m*M* dithiothreitol, 100 m*M* $MgCl_2$, 2 m*M* HEPES (pH 7.4), 2 μCi of [γ-^{32}P]ATP, 1 μg of glutathione *S*-transferase (GST)–activating transcription factor 2 (ATF2) fusion protein, and 20 m*M* β-glycerophosphate. The reaction mixture was subjected to electrophoresis on a 10% polyacrylamide gel. The gel was dried, exposed to Kodak X-OMAT film in a standard cassette, and exposed overnight at −70°C.

Analysis of intracellular free radicals

Levels of intracellular free radicals were analyzed by measuring the fluorescent signal of dichlorodihydrofluorescein diacetate (DCDHF-DA) (Molecular Probes, Eugene, OR, U.S.A.) oxidized by ROS as described (Seo et al., 1999). Cultures grown on a glass-bottom dish were loaded with 5 μ*M* DCDHF-DA plus 2% Pluronic F-127 in HEPES-buffered control salt solution containing 120 m*M* NaCl, 5 m*M* KCl, 1.6 m*M* $MgCl_2$, 2.3 m*M* $CaCl_2$, 15 m*M* glucose, 20 m*M* HEPES, and 10 m*M* NaOH. Cultures were incubated for 20 min at 37°C, and the fluorescence signal of the oxidized products of DCDHF-DA by ROS (DCF) (E_x = 490 nm, E_m = 510 nm) was analyzed on the stage of a Nikon Diaphot inverted microscope equipped with a 100-W xenon lamp. To minimize background signal due to direct oxidation of DCDHF-DA by illumination at 490 nm, intracellular levels of ROS were analyzed within 3 s after illumination using a Quanticell 700 system (Applied Imaging, U.K.).

RESULTS

Haloperidol-induced neuronal apoptosis (HINA) in cortical cell cultures

Neuronal death by haloperidol was performed in mixed cortical cell cultures of neurons and glia at DIV 11. Continuous exposure to 10–100 μ*M* haloperidol produced dose-dependent neuronal death over the next 24 h in the absence of detectable glial toxicity (Fig. 1A). Shrinkage of cell body and condensation of nuclear chromatin were observed in degenerating neurons ~20 h following exposure to 30 μ*M* haloperidol (Fig. 1B). Electron microscope study showed aggregated condensation of nuclear chromatin and collapse of nuclear membrane, while plasma membrane remained intact (Fig. 1B). Prominent internucleosomal DNA fragmentation was observed by agarose gel electrophoresis of soluble DNA extract from cortical cell cultures exposed to 30 μ*M* haloperidol or 100 n*M* staurosporine for 16 h (Fig. 1C). The morphology and DNA damage of degenerating cortical neurons suggest that treatment with haloperidol produces apoptotic neuronal death in cortical cell cultures.

No mediation of HINA by free radicals

Recently, free radicals were shown to mediate the toxic effects of haloperidol in C6 and NCB20 cells and neurons (Behl et al., 1995). We also observed that levels of free radicals were significantly increased at 8–12 h following exposure to 30 μ*M* haloperidol (Fig. 2A). However, compared with the gradual increase in $[ROS]_i$

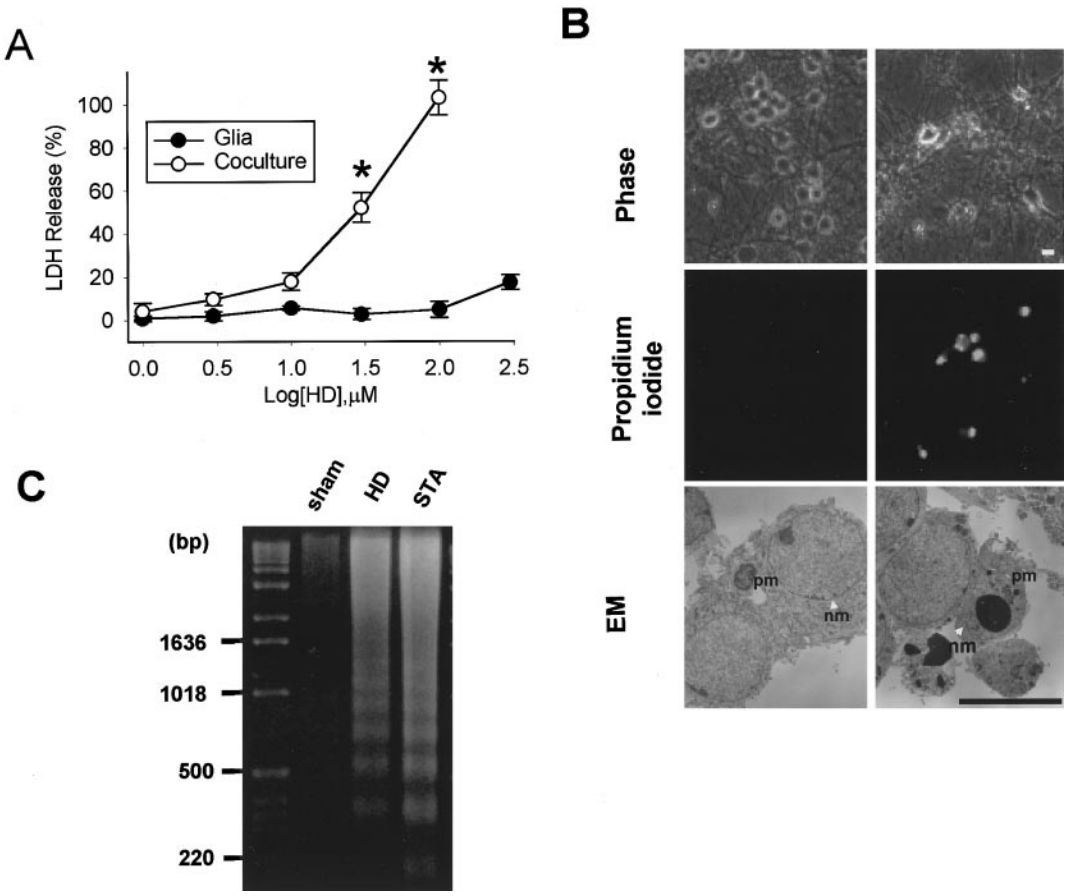

FIG. 1. Haloperidol induces neuronal apoptosis. **A:** Mixed cortical cultures of neurons and glia (DIV 11) or cultures of glia (DIV 11) were exposed to indicated doses of haloperidol (HD). LDH in the bathing medium was measured 24 h later (mean ± SEM, n = 8 culture wells/condition), scaled to the mean LDH value corresponding to the near-complete neuronal or glial death induced by 24-h exposure to 500 μM NMDA (=100% neuronal death) in mixed culture or 1% Triton X-100 in glial culture (=100% glial death). *$p < 0.05$, significant difference from the sham control, using analysis of variance and Student–Newman–Keuls test. **B:** Phase contrast (**upper panels**), fluorescence (**middle panels;** staining with propidium iodide), and transmission electron photomicrographs (**bottom panels**) of mixed cortical cell culture (DIV 11) taken 20 h after exposure to a sham wash (**left**) or 30 μM haloperidol (**right**). Note the shrunken cell body and condensed nuclear chromatin in neurons treated with haloperidol. nm or pm indicates nuclear or plasma membrane, respectively. Bars = 20 μm. **C:** Agarose gel electrophoresis of soluble DNA samples extracted immediately after 16-h exposure to a sham wash (sham), 30 μM haloperidol (HD), or 100 nM staurosporine (STA).

following administration of Fe^{2+}, cortical neurons treated with haloperidol produced slight and transient increases (~14%) in $[ROS]_i$. To examine if free radicals are required for execution of HINA, several antioxidants such as Trolox, *N*-acetyl-L-cysteine (NAC), *N-tert*-butyl-α-phenylnitrone (PBN), and vitamin C were included during exposure to haloperidol. Whereas these antioxidants attenuate neurotoxicity induced by free radical-inducing agents such as Fe^{2+} or menadione (data not shown), none of them attenuated HINA (Fig. 2B). This implies that the slight and transient production of ROS by haloperidol is not necessary for execution of HINA. Neither inclusion of 10 μM MK-801, an NMDA receptor antagonist, nor 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainate receptor antagonist, interfered with neuronal death following exposure to 30 μM haloperidol (Fig. 2B).

Activation of p38 and JNK during HINA

Extensive evidence indicates that p38 and JNK are activated to execute apoptosis induced in PC12 cells and sympathetic neurons deprived of nerve growth factor (Xia et al., 1995; Kummer et al., 1997; Eilers et al., 1998). In cortical cell cultures exposed to 30 μM haloperidol, activity of p38 and JNK was increased within 1 h, lasted until 3 h, and then returned to the control level by 4 h (Fig. 3A and B). Concurrent addition of 10–30 μM PD169316, a specific inhibitor of p38 kinase, partially prevented HINA, suggesting a causative activation of this kinase in the process of HINA (Fig. 3C–E).

Haloperidol-induced activation of p38 and JNK as well as HINA attenuated by cycloheximide or insulin

Postmitotic neurons deprived of appropriate growth factors undergo apoptosis in a way sensitive to inhibitors

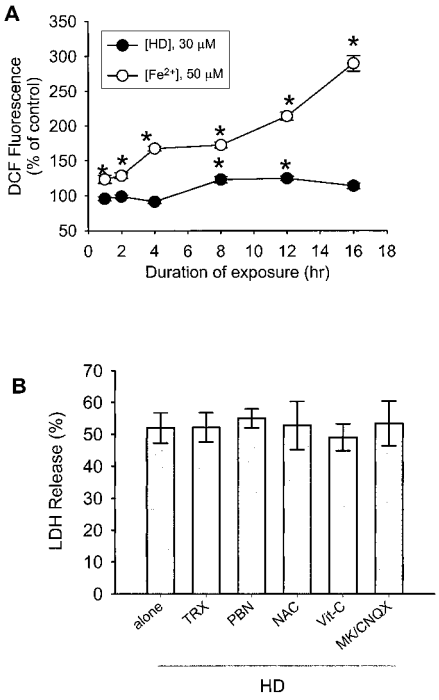

FIG. 2. ROS does not mediate HINA. **A:** $[ROS]_i$ values in cortical neuron (DIV 11) at indicated times after exposure to 30 μM haloperidol (HD) or 50 μM Fe^{2+} were analyzed by measuring fluorescence intensity of oxidized DCDHF-DA (mean ± SEM, n = 25 neurons randomly chosen from three culture wells for each condition), scaled to mean neuronal $[ROS]_i$ following a sham control (=100). *$p < 0.05$, significant difference from $t = 0$, using analysis of variance and Student–Newman–Keuls test. **B:** Cocultures of neurons and glia (DIV 11) were exposed to 30 μM haloperidol, alone or with the following additions: 100 μg/ml Trolox (TRX, a vitamin E analogue), 3 mM PBN, 100 μM NAC, 300 μM vitamin C (Vit-C), or 10 μM MK-801 plus 50 μM CNQX (MK/CNQX). Neuronal death was analyzed 24 h later by measuring LDH in the bathing medium (mean ± SEM, n = 8 culture wells/condition), as described above.

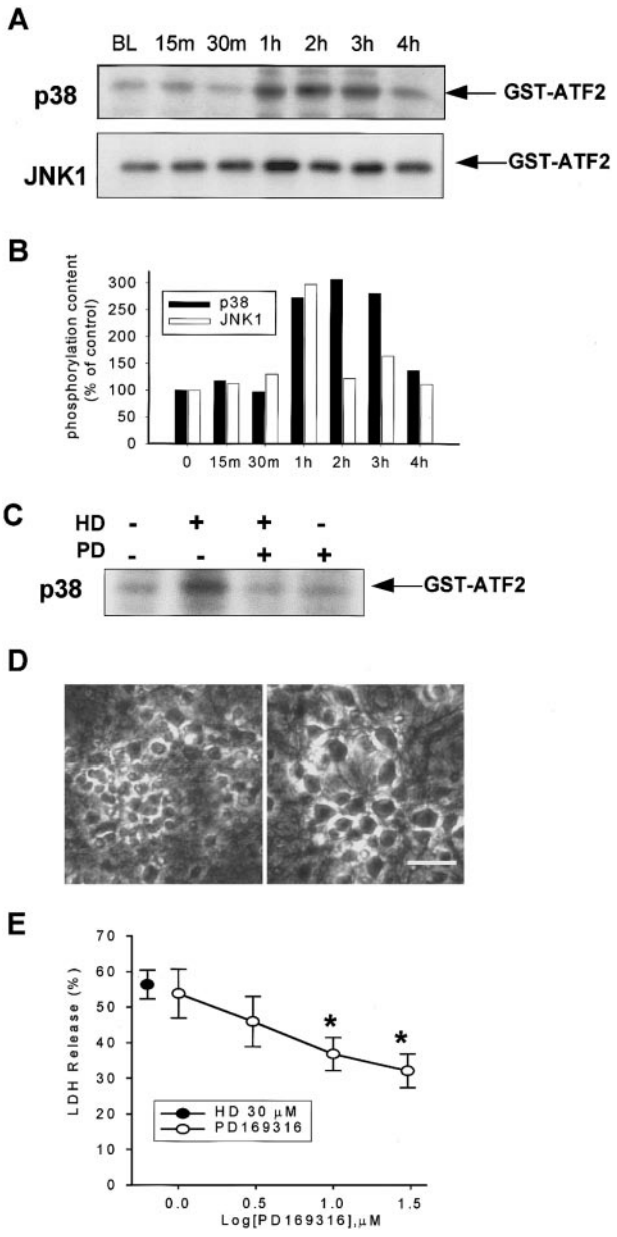

FIG. 3. Activation of p38 and JNK during HINA. **A** and **B:** Cortical cell cultures (DIV 11) were exposed to 30 μM haloperidol for the indicated points of time. Cells were lysed immediately after treatment, immunoprecipitated with anti-p38 or anti-JNK1 antibody, and the resulting immunoprecipitates incubated in a reaction mixture containing [γ-^{32}P]ATP and GST-ATF2. Reaction mixtures were subjected to polyacrylamide gel electrophoresis, and x-ray images for activity of p38 and JNK were visualized (A) and analyzed (B). BL, basal value. **C:** Cultures were exposed to a sham control, 30 μM haloperidol (HD), 30 μM haloperidol + 30 μM PD169316, or 30 μM PD169316 for 1 h. Cultures were then subjected to assay of p38 kinase as described above. **D:** Phase contrast photomicrographs of mixed cortical cell culture (DIV 11) taken 20 h after exposure to 30 μM haloperidol, alone (**left**) or with 30 μM PD169316 (**right**). Bar = 30 μm. **E:** Sister cultures were exposed to 30 μM haloperidol, alone or with indicated dose of PD169316. Neuronal death was analyzed 24 h later by measuring LDH in the bathing medium (mean ± SEM, n = 8 culture wells/condition). *$p < 0.05$, significant difference from $t = 0$, using analysis of variance and Student–Newman–Keuls test.

of macromolecule synthesis (Martine et al., 1992). Consistent with this, inclusion of 1 μg/ml cycloheximide, a protein synthesis inhibitor, protected cortical neurons from apoptosis induced following exposure to 30 μM haloperidol (Fig. 4A and B). HINA was also attenuated by inclusion of 100 ng/ml insulin or 100 μM *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (Z-VAD), a broad-spectrum inhibitor of caspases, that preferentially prevented apoptotic neuronal death in cortical cell cultures (Fig. 4A and B) (Ryu et al., 1999). Although neither cycloheximide nor insulin alone influenced activity of p38 and JNK, haloperidol-induced activation of p38 and JNK was diminished in the presence of cycloheximide or insulin (Fig. 4C and D). This implies that cycloheximide and insulin likely prevent apoptosis by interfering with activation or upstream signals of p38 and JNK. Activity of p38 and JNK was increased within 2 h after exposure to haloperidol and returned to basal level 4 h later. Interestingly, late application of cycloheximide 8 h after exposure to haloperidol when the activity of p38

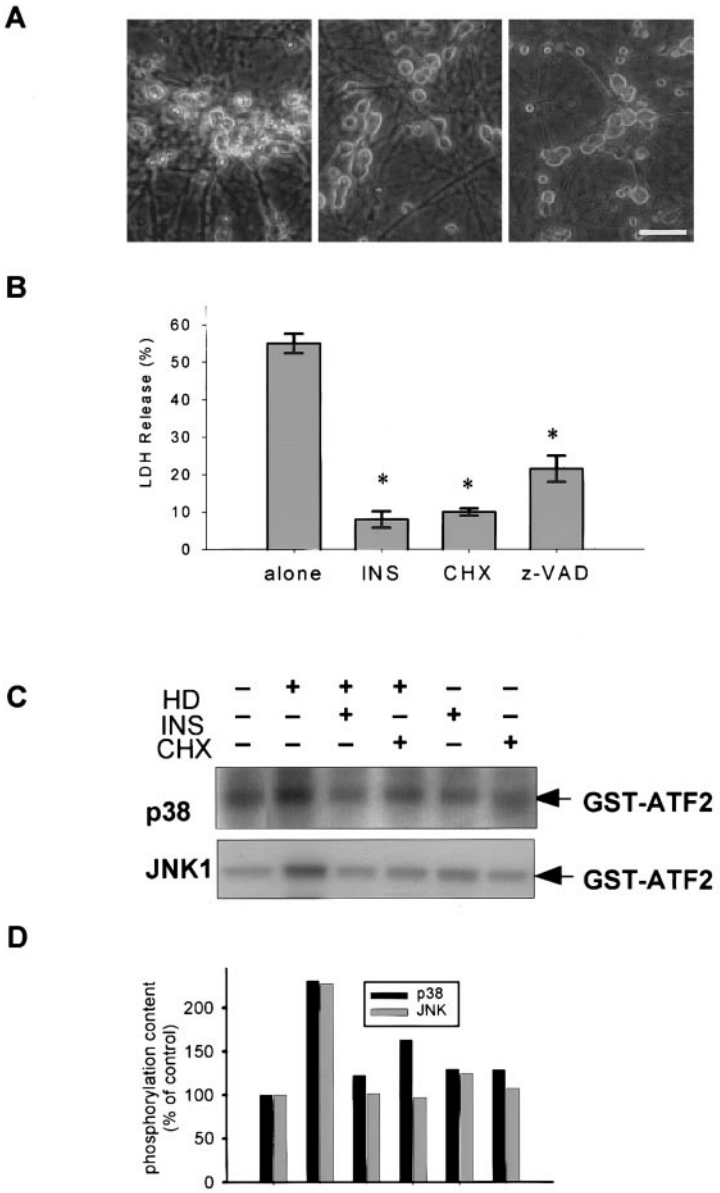

FIG. 4. Attenuation of HINA by cycloheximide or insulin. **A:** Phase contrast photomicrographs of cortical cell cultures (DIV 11) were taken 20 h after exposure to 30 μM haloperidol, alone (**left**) or with 1 μg/ml cycloheximide (**middle**) or 100 ng/ml insulin (**right**). Bar = 30 μm. **B:** Sister cultures were exposed to 30 μM haloperidol, alone or with 100 ng/ml insulin (INS), 1 μg/ml cycloheximide (CHX), or 100 μM z-VAD. Neuronal death was analyzed 24 h later by measuring LDH in the bathing medium (mean ± SEM, n = 8 culture wells/condition). $^*p < 0.05$. **C** and **D:** Cells exposed to various conditions for 1 h were lysed, immunoprecipitated with anti-p38 or anti-JNK1 antibody, and processed for analysis of p38 and JNK as described for Fig. 3. **E:** Sister cultures were exposed to 30 μM haloperidol, alone or with delayed addition of 1 μg/ml cycloheximide at indicated points of time. Neuronal death was analyzed 24 h later by measuring LDH in the bathing medium (mean ± SEM, n = 8 culture wells/condition). $^*p < 0.05$, significant difference from the sham control, using analysis of variance and Student–Newman–Keuls test.

and JNK returned to the basal level significantly protected cortical neurons from HINA, suggesting that cycloheximide attenuates HINA without interfering with activation of p38 and JNK (Fig. 4E).

DISCUSSION

The antipsychotic drug haloperidol causes apoptotic neuronal death. Neither excitotoxicity nor free radicals mediate HINA. Transient activation of p38 and JNK following exposure to haloperidol appears to be necessary for neuronal apoptosis. Cycloheximide and insulin attenuate HINA possibly via pathways linked to activation of p38 and JNK.

The neuroleptic drug haloperidol was shown to produce cytotoxicity in various cell types including primary neurons (Behl et al., 1995; Sagara, 1998). Cell body swelling and random DNA fragmentation have been observed in the course of haloperidol toxicity, suggesting that haloperidol causes necrotic cell death (Behl et al., 1995). In the present study, however, we found that treatment with haloperidol at micromolar concentrations produced apoptosis of cortical neurons accompanied by shrinkage of the cell body, prominent DNA ladders, aggregation and condensation of nuclear chromatin, early disruption of nuclear membrane, and sensitivity to antiapoptosis agents. Although this conflict pattern of cell death by haloperidol may be attributable to differences in types of cells, the culture condition appears to be more relevant. In experiments reporting occurrence of necrosis by haloperidol, cultures were exposed to halo-

peridol in medium containing insulin, which possesses antiapoptotic but prooxidant properties (Behl et al., 1995; Ryu et al., 1999).

ROS have been proposed as a key mechanism underlying haloperidol neurotoxicity. ROS were generated in degenerating cells after administration of haloperidol (Post et al., 1998; Sagara, 1998). Vitamin E, melatonin, and overexpression of bcl-2, known to protect cells against free radical toxicity, prevented haloperidol-induced cell death (Behl et al., 1995; Lezoualc'h et al., 1996; Post et al., 1998). We also observed transient increase in $[ROS]_i$ following exposure to haloperidol. However, this increase was not enough to mediate HINA as none of the antioxidants Trolox, NAC, PBN, or vitamin C prevented HINA. Therefore, HINA appears to move on through other routes irrespective of ROS.

Signaling molecules of cell death have been extensively investigated to delineate mechanisms of apoptosis. Among these, extensive evidence demonstrates that two subgroups of MAPKs, p38 and JNK, play an essential role in transduction of cell death. Overexpression of mitogen-activated/extracellular response kinase kinase kinase 1 (MEKK1), an upstream activator of JNK/stress-activated protein kinase (SAPK), induces apoptosis in mouse fibroblasts (Johnson et al., 1996). PC12 cells or Jurkat T cells undergo apoptosis following overexpression of mitogen-activated protein kinase (MAPK) kinase 3 or MAPK kinase 6, upstream kinases of p38 (Xia et al., 1995; Huang et al., 1997). COS7 cells and *Xenopus* embryos that are allowed to overexpress apoptosis signal-regulating kinase 1 or transforming growth factor-β-activated kinase 1 for activation of JNK/SAPK and p38 undergo apoptosis (Ichijo et al., 1997; Shibuya et al., 1998). Moreover, maneuvers interfering with activity of JNK/SAPK or p38 attenuate apoptosis induced by tumor necrosis factor-α, ultraviolet C and gamma radiation, or deprivation of nerve growth factor (Xia et al., 1995; Chen et al., 1996; Verheij et al., 1996). These results imply that activation of JNK/SAPK and p38 is required for execution of apoptosis. In the present study, we observed that p38 was activated as a causative mediator necessary for apoptosis of cortical neurons treated with haloperidol. It remains to be determined whether activation of JNK contributes to HINA in a synergistic fashion with that of p38.

Protein phosphatases have been proposed as target molecules for cellular action of antipsychotics. Administration of haloperidol results in direct inhibition of the protein phosphatase 2B (PP2B) (Gong et al., 1996). Cyclosporine A, an immunosuppressant known to selectively inhibit PP2B, produced apoptosis of cultured cortical neurons and oligodendrocytes (McDonald et al., 1996). Cyclosporine A induced transient activation of JNK and p38 in cortical neurons in a similar fashion to haloperidol (Fig. 5). Therefore, activation of p38 and JNK subsequent to inhibition of PP2B may mediate the proapoptotic action of haloperidol.

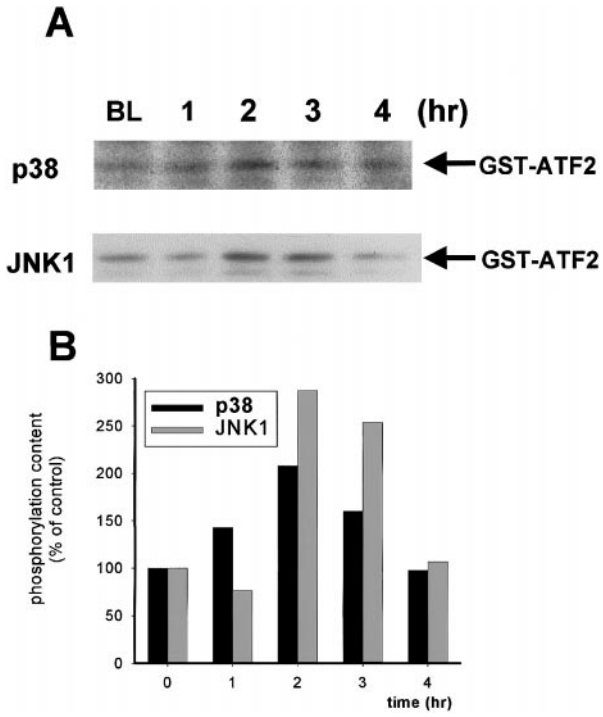

FIG. 5. Cyclosporine A activates p38 and JNK. X-ray images (**A**) and densitometric analysis (**B**) show activity of p38 and JNK in cortical cell cultures (DIV 11) exposed to a sham control (BL) or 20 μM cyclosporine A for the indicated periods of time.

The antiapoptotic action of insulin has been well documented in various types of cells (Estevez et al., 1995; Jung et al., 1996; McDonald et al., 1996) and appears to be exerted through inhibition of p38 (Kummer et al., 1997). Treatment with insulin induces activation of phosphatidylinositol (PI) 3-kinase in cortical neurons, which was necessary for preventing apoptosis of cortical neurons deprived of serum (Ryu et al., 1999). Activated PI3-kinase would interfere with activation of JNK and p38 (Berra et al., 1998; Shimoke et al., 1999). It is conceivable that insulin attenuates HINA by PI3-kinase-mediated blockade of JNK and p38.

Selective inhibitors of protein synthesis prevented apoptosis by blocking activation of JNK and p38 (Shifrin and Anderson, 1999), raising the possibility that activation of the MAPKs during apoptosis depended on protein synthesis. This was further supported by the present study that cycloheximide attenuated haloperidol-induced activation of JNK and p38. However, delayed application of cycloheximide that did not influence activation of p38 or JNK resulted in decreased but substantial neuroprotection against HINA (Fig. 4E). This suggests that HINA should have a biphasic process of death depending on protein synthesis: a rapid process leading to activation of JNK and p38 and a slowly evolving death process after activation of the MAPKs. Activated JNK and p38 MAPKs can induce mitochondrial cytochrome *c* release or expression of Fas ligand that triggers activation of

caspases for execution of apoptosis (Hatai et al., 2000; Zhuang et al., 2000).

Accumulating evidence suggests that haloperidol can cause brain damage and TD in psychotic patients after chronic administration that results in accumulation of the drug in brain (Sunderland and Cohen, 1987). In this study, we have demonstrated that haloperidol produces neuronal apoptosis through activation of p38 and JNK as a necessary step. Insulin and other antiapoptotic agents possess reasonable promise to treat undesirable neurotoxic effects of haloperidol and other antipsychotic drugs.

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