Nuclear Factor-κB Contributes to Excitotoxin-Induced Apoptosis in Rat Striatum

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ABSTRACT

Excitotoxin-induced destruction of striatal neurons, proposed as a model of Huntington's disease, involves a process having the biochemical stigmata of apoptosis. Recent studies suggested that transcription factor nuclear factor (NF)-κB may be involved in excitotoxicity. To further analyze the contribution of NFκB to excitotoxic neuronal death *in vivo*, changes in binding activities of NFκB and other transcription factors as well as the consequences of inhibiting NFκB nuclear translocation were measured after the infusion of quinolinic acid (120 nmol) into rat striatum. Internucleosomal DNA fragmentation and terminal transferase-mediated dUTP digoxigenin nick end labeling-positive nuclei appeared 12 hr later and intensified over the next 12 hr. NFκB binding activity increased severalfold from 2 to 12 hr, then gradually declined during the next 12 hr. Other transcription factor changes included AP-1, whose binding peaked about 6 hr after quinolinic acid administration, and E2F-1, which was only modestly and transiently elevated. In contrast, quinolinic acid lead to a reduction in OCT-1, beginning after 12 hr, and briefly in SP-1 binding. The NFκB, AP-1, and OCT-1 changes were attenuated both by the *N*-methyl-D-aspartate receptor antagonist MK-801 and the protein synthesis inhibitor cycloheximide. Moreover, quinolinic acid-induced internucleosomal DNA fragmentation and striatal cell death were significantly reduced by the intrastriatal administration of NFκB SN50, a cell-permeable recombinant peptide that blocks NFκB nuclear translocation. These results illustrate the complex temporal pattern of transcription factor change attending the apoptotic destruction produced in rat striatum by quinolinic acid. They further suggest that NFκB activation contributes to the excitotoxin-induced death of striatal neurons.

Neurodegenerative disorders, such as HD, are characterized by the progressive loss of specific central neurons during adult life. Although HD is caused by a polyglutamine expansion in the gene for HD, the exact process by which this abnormality triggers the death of striatal neurons is not yet known. Recent studies of postmortem tissue suggest the degenerative process may involve an apoptotic mechanism (Portera-Cailliau *et al.*, 1995).

Glutamate, which can induce oxidative stress in neuronal tissue, has been implicated in the pathogenesis of HD and other neurodegenerative disorders. Indeed, the demise of striatal neurons produced by the glutamate receptor agonist QA as well by kainic acid has been proposed as an animal model of HD (Coyle and Schwarcz, 1976). Recent observations suggest that the QA- or kainic acid -induced destruction of striatal cells occurs, at least in part, by an apoptotic mechanism (Ankarcrona *et al.*, 1993; Bonfoco *et al.*, 1995; Filipkowski *et al.*, 1995; Gillardon *et al.*, 1995; Portera-Cailliau *et al.*, 1995; Qin *et al.*, 1996; Simonian *et al.*, 1996). Although the morphological features of the apoptotic process have been well described, just how glutamatergic receptor agonists ac-

tivate cell death programs at the molecular level remains to be elucidated.

Transcription factors, including immediate early genes such as c-*jun*, E2F-1, OCT-1 and NFκB, have been increasingly implicated in the control of apoptosis (Dragunow and Preston, 1995; Grilli *et al.*, 1996; Ham *et al.*, 1995; Wang and Pittman, 1993). Induction of these regulators of gene expression typically precedes the appearance of internucleosomal DNA fragmentation (Estus *et al.*, 1994). Moreover, antisense, antibody, gene mutation, and pharmacological techniques that selectively inhibit certain transcription factors have been found to block the death of cultured cells, thus suggesting that these factors may be direct contributors to the generation of apoptotic cascades (Estus *et al.*, 1994; Ham *et al.*, 1995; Lin *et al.*, 1995a).

NFκB, a member of the *Rel* transcription factor family, participates in the regulation of a broad array of genes primarily involved in immune and stress defense mechanisms. It has also been linked to the generation of certain cancers and to the control of the cell cycle. In the central nervous system, NFκB is constitutively expressed in both neurons

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ABBREVIATIONS: HD, Huntington's disease; QA, quinolinic acid; CHX, cycloheximide; TUNEL, terminal transferase-mediated dUTP-digoxigenin nick end labeling; NMDA, *N*-methyl-D-aspartate; ANOVA, analysis of variance; IκB, inhibitor κB; NFκB, nuclear factor-κB; AP-1, activator protein 1; CREB, cAMP response element binding protein.

and glia (Kaltschmidt *et al.*, 1994). A variety of pathogenetic stimuli, including oxidative stress, ischemic insult, and β-amyloid deposition (Kaltschmidt *et al.*, 1997; Legrand-Poels *et al.*, 1995; Salminen *et al.*, 1995), can release NFκB from cytosolic sequestration sites where it is bound to a member of the inhibitory protein family, IκB (Beg and Baltimore, 1996; Brown *et al.*, 1993; Liou and Baltimore, 1993). Upon translocated to the nucleus, NFκB acts as a positive regulator of genes favoring either protective or degenerative responses, depending on genetic programs within a particular cell type (Baeuerle, 1991; Baichwal and Baeuerle, 1997; Lipton, 1997). Several NFκB target genes, including P53 and c-Myc, are well established modulators of apoptosis (Wu and Lozano, 1994).

Recent *in vitro* studies have found that stimulation of glutamate receptors strongly activates NFκB (Guerrini *et al.*, 1995; Kaltschmidt *et al.*, 1995). Subsequent reports that NFκB inhibitors such as aspirin and salicylate protect cultured neurons against glutamate-induced neuronal toxicity (Grilli *et al.*, 1996) could thus indicate that NFκB activation contributes to excitotoxic neuronal injury. Unfortunately, interpretation of these results is complicated by the fact that salicylates inhibit other transcription factors and several protein kinases in addition to having many other pharmacologic actions (Frantz and O'Neill, 1995).

To more precisely delineate the role of NFκB in excitotoxin-induced neuronal apoptosis *in vivo*, we have examined the temporal pattern of NFκB and other transcription factor alterations in relation to internucleosomal DNA fragmentation after the intrastriatal administration of the potent NMDA receptor agonist QA. We also studied the effect of inhibiting NFκB activity on QA-induced apoptosis using a cell-permeable recombinant peptide (NFκB SN50) to block NFκB nuclear translocation. The results indicate that a complex pattern of transcription factor change precedes the appearance of internucleosomal DNA fragmentation and most noticeably that the activation of NFκB may contribute to the QA-induced apoptosis of striatal neurons.

Materials and Methods

Animals. Sprague-Dawley rats weighing 300–350 g were purchased from Taconic. They were housed two per cage in a standard animal room with a 12-hr light/dark cycle and given free access to food and water. All procedures were conducted in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Drug treatment. To study the time course of QA-induced DNA fragmentation, rats were unilaterally infused with QA (120 nmol) or saline into the striatum (7) and killed 2, 6, 12, or 24 hr later. Whole brains were used for tissue sections for TUNEL assay or striata were dissected for the extraction of genomic DNA.

To examine the time course of QA-induced changes in transcription factor binding activity, rats were infused with QA (120 nmol) and killed 2, 6, 12, or 24 hr later. Their striata were dissected on a cold plate for nuclear protein extraction.

To evaluate the effect of NMDA receptor blockade on QA-induced alterations in transcription factors, animals were given MK-801 (dizocilpine; RBI, Natick, MA) intraperitoneally. The first MK-801 dose (2.0 mg/kg) was injected 15 min before and the second (2.0 mg/kg) and third doses (1.0 mg/kg) 3 and 6 hr after intrastriatal QA infusion. Control rats received either intraperitoneal injections of vehicle (0.9% NaCl) plus intrastriatal QA or intraperitoneal injections of MK-801 plus intrastriatal vehicle (0.9% NaCl). Animals were

killed 12 hr after striatal QA or vehicle infusion, and their striata were dissected for nuclear protein extraction.

To study the effect of a protein synthesis inhibitor on QA-induced transcription factor alterations, rats were injected intrastriatally with CHX (480 nmol in 2 μl of 0.9% NaCl; Sigma, St. Louis, MO). Fifteen minutes later, QA was injected intrastriatally as previously described. Control animals received either intrastriatal vehicle plus intrastriatal QA or intrastriatal CHX plus intrastriatal vehicle. Animals were killed 12 hr after striatal QA or vehicle administration, and their striata were dissected for nuclear protein extraction.

To assess the effect of a cell permeable recombinant peptide that inhibits NFκB nuclear translocation (NFκB SN50; Biomol, Plymouth Meeting, PA) on QA-induced NFκB activation and internucleosomal DNA fragmentation, rats received either a single intrastriatal injection of NFκB SN50 (20 μg) 15 min before QA treatment, or two injections of NFκB SN50 (10 or 30 μg/injection) 15 min before and 8 hr after QA treatment. Animals were killed 12 or 24 hr after QA administration, and their striata were dissected for nuclear protein and genomic DNA extraction. The effect of NFκB SN50 on QA-induced striatal cell death was examined in animals given a single injection of NFκB SN50 (20 μg) 15 min before QA treatment, or two injections of NFκB SN50 (10 or 30 μg/injection) 15 min before and 8 hr after QA treatment. Animals were killed 10 days later, and brain sections were processed for receptor autoradiography and *in situ* hybridization histochemistry.

Genomic DNA isolation and electrophoresis. Genomic DNA was isolated (Qin *et al.*, 1996), and 20 μg were electrophoresed on 2% agarose gel (NuSieve 3:1) for 3 hr. DNA fragments were detected with a UV transilluminator after staining with ethidium bromide.

TUNEL. Brain sections (12 mm) were cut on a cryostat and thaw-mounted onto gelatin-coated microslides. Sections were fixed in phosphate-buffered 10% formalin for 10 min and then rinsed three times in phosphate-buffered saline, pH 7.4. DNA fragmentation was evaluated in individual cells using an apoptosis detection kit (ApopTag; Oncor, Gaithersburg, MD) according to the manufacturer's protocol. DNA fragmentation was disclosed by fluorescence microscopy and photographed.

Nuclear protein preparation and gel shift assay. Nuclear proteins were extracted by a modification of a previously described procedure (Ogita and Yoneda, 1994) and dialyzed using microdialyzers (Daigger, Wheeling, IL). Double-stranded oligodeoxynucleotide containing consensus sequences for different transcription factors were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Promega (Madison, WI). Double-stranded DNA probes were labeled with [^{32}P]ATP by T4 polynucleotide kinase (Promega). Nuclear protein (5–14 μg) were incubated with radioactively labeled DNA probes (about 40,000 cpm) for 15 min at room temperature in a binding buffer containing 5 mM MgCl$_2$, 2.5 mM EDTA, 25 mM dithiothreitol, 250 mM NaCl, 50 mM Tris·HCl, pH 7.5, 0.25 mg/ml poly(dI·dC) and 20% glycerol (Promega). Nuclear proteins were mixed with 1/10 volume of a loading buffer containing 250 mM Tris·HCl (pH 7.5), 0.2% bromphenol blue, 0.2% xylene cyanol, and 40% glycerol and then electrophoresed on 4% polyacrylamide gel (monoacrylamide/bisacrylamide, 80:1) with 0.5 × Tris/borate/EDTA (1× = 89 mM Tris base, 89 mM boric acid, and 2 mM Na$_2$-EDTA). Autoradiograms were developed by exposing vacuum-dried gels to x-ray film at −80° with intensifying screens for 12–48 hr. The specificity of transcription factor binding to DNA probes used in the gel shift assay was tested by assessing the affinity of the nuclear proteins to individual ^{32}P-labeled transcription factor consensus sequences in the presence of an excess of the unlabeled probes, or by mutation of DNA-protein binding motifs in DNA probes. The specificity of NFκB binding was also confirmed by supershift assay using p65 (NFκBp65, sc-109; Santa Cruz Biotechnology) and p50 (NFκBp50, sc-1190, Santa Cruz Biotechnology) antibodies. Autoradiographic results were semiquantitatively evaluated by means of an image analyzer (Image 1.42; National Institutes of Health). A standard template was used to ensure that the binding signal from each

sample was measured in the same sized area. Nonspecific binding was determined by adding a 60-fold excess of unlabeled DNA probes to the assay. Specific binding was calculated by subtracting nonspecific binding from total binding.

Receptor autoradiography and *in situ* hybridization histochemistry. Receptor autoradiography was performed as previously described (Qin *et al.*, 1994a). Briefly, brain sections were incubated in 50 mM Tris·HCl buffer, pH 7.4, containing 2 nM [^{3}H]SCH-23390 (NEN, Boston, MA) and 80 nM ketanserin for 1 hr at room temperature. Nonspecific binding was determined by incubating adjacent sections in the buffer with 2 μM SCH-23390 added. Sections were rinsed, air-dried, and exposed to x-ray film (Hyperfilm, [^{3}H] Sensitive, Amersham) for 10 days. The density of D$_1$ dopamine receptors was determined using an image analyzer. Three brain sections (bregma 1.7 to −0.3) from each treated animal were analyzed. *In situ* hybridization histochemistry was performed as described previously with minor modifications (Qin *et al.*, 1994b). A 36-mer oligonucleotide probe (5′-GCT AAA CCA ATG ATA TCC AAA CCA GTA GAG AGC TGG-3′) complimentary to rat GAD mRNA encoding a 67-kDa GAD protein was synthesized by Genosys. Oligonucleotide probes were labeled with [^{33}P]dATP using terminal deoxynucleotidyl transferase and purified by filtration chromatography (Chroma Spin-10, Clontech). Formalin-fixed sections were incubated with labeled probes in a hybridization cocktail (Amresco, Solon, OH) at 37° for 18 hr. After hybridization, sections were washed, dehydrated , and exposed to x-ray film (Hyperfilm β Max; Amersham) for 10 days. The results were quantitatively assessed with an image analyzer. Three brain sections (bregma 1.7 to −0.3) from each treated animal were analyzed.

Results

Temporal pattern of QA-induced DNA fragmentation. DNA fragmentation first became detectable on ethidium bromide-stained agarose gels 12 hr after QA administration. DNA fragmentation was further increased 24 hr after QA treatment. The size of the DNA fragments approximated multimers of 180–200 base pairs and thus formed typical DNA ladders (Fig. 1). Internucleosomal DNA fragmentation was not observed in the contralateral striatum or in the vehicle injected striatum (data not shown).
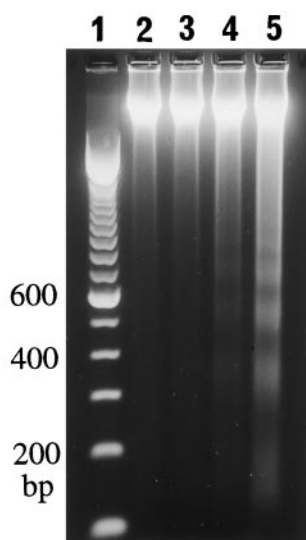
Fig. 1. Internucleosomal DNA fragmentation detected by agarose gel electrophoresis in rat striatum. Rats were treated with intrastriatal injections of QA (120 nmol) and killed 2, 6, 12, or 24 hr later. *Lane 1*, 100-base pair DNA maker; *lanes 2*, *3*, *4*, and *5*, QA treatment for 2, 6, 12 and 24 hr, respectively.

The number of TUNEL-positive nuclei seen under light microscopy in the QA infused striatum increased steadily after excitotoxin administration (Fig. 2). From 2 to 6 hr after QA treatment, only a few TUNEL-positive nuclei were observed at the injection sites. At 12 hr, more TUNEL-positive nuclei were found in a wider area surrounding the injection sites. Twenty-four hours after QA treatment, numerous TUNEL-positive nuclei could be seen throughout 50–65% of the QA injected striatum. Many of these TUNEL-positive nuclei had fragmented into small clumps (Fig. 2, G and H). There were no TUNEL-positive nuclei in the uninjected striatum (Fig. 2E) and only a few positive nuclei were found at injection sites in the vehicle-treated striatum (Fig. 2F).

Temporal pattern of QA-induced changes in striatal transcription factor binding. The intrastriatal administration of QA significantly altered binding activities of four of the seven transcription factors studied (Figs. 3 and 4). QA induced a rapid rise in AP-1 binding, which peaked 6 hr after excitotoxin injection and then slowly returned to basal levels. NFκB binding, which remained low in the vehicle-treated striatum, increased markedly in the QA-treated striatum. The maximal increase occurred 12 hr after QA infusion. Subsequently, NFκB binding gradually diminished, although continuing to be significantly elevated 24 hr after QA treatment. A small transient rise in E2F-1 binding activity was observed 6 hr after the QA treatment. In contrast, QA induced a gradual decline in OCT-1 binding, with the decrement reaching statistical significance 12 hr after excitotoxin exposure. SP-1 binding activity also decreased, although only briefly 12 hr after QA administration. There was no appreciable change in CREB or Myc-Max binding activity.

NMDA receptor antagonist and protein synthesis inhibitor effects on QA-induced alterations in transcription factor binding. Given alone, systemically administered MK-801 significantly inhibited SP-1 binding activity ($p < 0.001$), but had no effect on the other striatal transcription factors studied. MK-801 co-administration completely blocked the QA-induced increases in AP-1 ($p < 0.05$) and NFκB ($p < 0.05$) binding found 12 hr after intrastriatal excitotoxin infusion. MK-801 also tended to reverse the QA-induced decrease in OCT-1 binding. Although MK-801 had an inhibitory effect on SP-1, there was no additive effect on the QA-induced decline in SP-1 binding activity when it was co-administered with QA (Fig. 5).

CHX, when given alone, increased AP-1 ($p < 0.05$) and NFκB binding ($p < 0.01$) slightly, although significantly, whereas it reduced SP-1 binding activity. CHX co-administration attenuated rather than potentiated the QA-induced increases in NFκB binding ($p < 0.05$). A tendency for CHX to diminish the QA-induced rise in AP-1 binding and reverse the decrement in OCT-1 binding activity did not quite attain statistical significance. CHX had no effect on the QA-induced reduction in SP-1 binding activity (Fig. 6).

NFκB antagonist effects on QA-induced internucleosomal DNA fragmentation and striatal cell death. Co-administration of the NFκB inhibitor, NFκB SN50, markedly reduced the QA-induced activation of NFκB ($p < 0.001$), but did not alter QA-induced increases in AP-1 binding. NFκB SN50 had no significant effect on QA-induced reductions in OCT-1 and SP-1 binding (Fig. 7). Treatment with two doses of NFκB SN50 (10 or 30 μg/dose) attenuated QA-induced internucleosomal DNA fragmentation 24 hr after QA treat-

Fig. 2. TUNEL detection of QA-induced DNA fragmentation in rat striatum. Rats were treated with intrastriatal injections of QA (120 nmol) and killed 2, 6, 12, or 24 hr later. A, B, C, and D, QA 2, 6, 12, and 24 hr, respectively. E, uninjected striatum. F, Vehicle injected (24 hr) striatum. G and F, Higher magnitude to show fragmented nuclear clumps; *arrowheads*, fragmented nuclei. Bar = 50 μm.

ment in a dose-dependent manner. NFκB SN50 alone did not produce appreciable internucleosomal DNA fragmentation (Fig. 8A). A single dose of NFκB SN50 (20 μg) also substantially inhibited QA-induced internucleosomal DNA fragmentation (Fig. 8B). Similarly, a single injection of NFκB SN50

(20 μg) significantly attenuated the QA-induced decrease in striatal D_1 dopamine receptor binding sites ($p < 0.001$, Fig. 9A). *In situ* hybridization histochemistry further confirmed that a single dose of NFκB SN50 (20 μg) reduced the QA-induced decrement in striatal GAD_{67} mRNA levels ($p <$
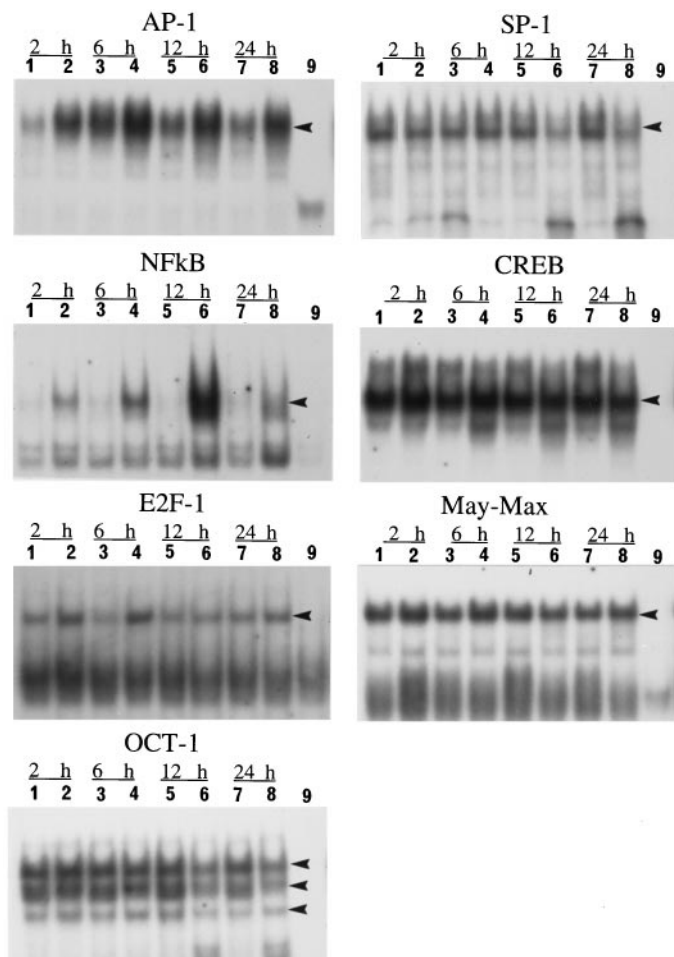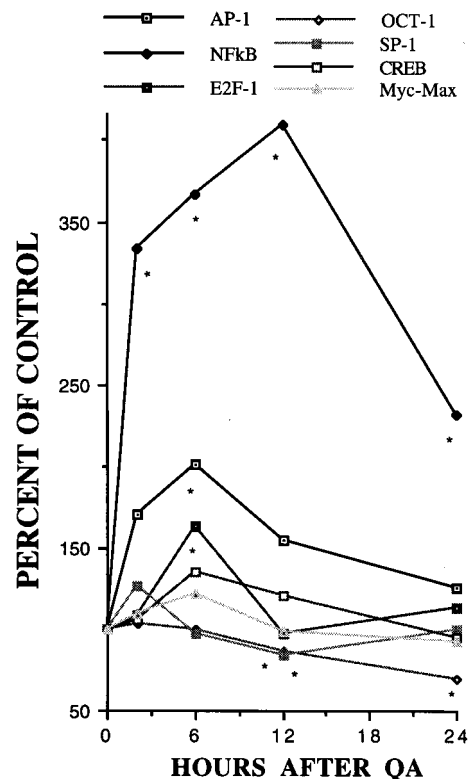
Fig. 3. Effect of QA on transcription factor binding activities in rat striatum. Rats received an intrastriatal injection of QA (120 nmol) or vehicle and were killed 2, 6, 12, or 24 hr later. A representative autoradiogram of each transcription factor is presented; *arrows*, specific binding analyzed. In each assay, *lanes 1, 3, 5,* and *7,* control animals; *lanes 2, 4, 6,* and *8,* QA-treated animals; *lane 9,* a 60-fold excess of unlabeled probes was added to define nonspecific binding.

Fig. 4. Quantitative analysis of the effect QA on transcription factor binding activity. Rats were treated as described in legend to Fig. 3. Binding activity was quantified using an image analyzer. Results are expressed as percent of vehicle-treated animals at each time point. Statistical comparisons were carried out by one group *t* test. ∗, $p < 0.05$ (compared with vehicle-treated animals, $n = 4$).

0.001, Fig. 9B). However, a second injection (20 μg) of NFκB SN50 failed to provide additional protection against QA-induced striatal cell death as indicated both by receptor autoradiography and *in situ* hybridization histochemistry.

Discussion

The present results document the complex temporal relation between acute excitotoxin exposure and transcription factor induction in rat striatum. After QA administration, AP-1 and NFκB rapidly increased, whereas OCT-1 and SP-1 gradually declined. These transcription factor alterations, detected by a highly sequence-specific mobility shift assay, are consistent with previously reported changes in immediate early gene expression (including c-*fos*, *fos B*, c-*jun*, *jun B*) as well as alterations in AP-1 and NFκB binding activities in neurons as a consequence of glutamate receptor activation (Coyle *et al.*, 1989; Bading *et al.*, 1993; Dure *et al.*, 1995; Guerrini *et al.*, 1995; Kaltschmidt *et al.*, 1995).

The excitotoxin-induced effects on striatal transcription factors observed in this study were probably mediated by glutamate receptors of the NMDA subtype, because QA, at the doses used, acts selectively at these receptors. Moreover, the noncompetitive NMDA receptor antagonist MK-801, in amounts previously found to block QA-induced cell death by a process having the hallmarks of apoptosis, totally prevented QA associated changes in AP-1, NFκB, and OCT-1 binding. Our results with MK-801, which inhibits calcium permeability when bound to NMDA channels, are thus consistent with earlier reports suggesting that enhanced calcium influx, as a consequence of NMDA channel activation, contributes to the observed alterations in transcription factor binding (Dure *et al.*, 1995).

The observed QA-induced increases in NFκB and AP-1 were attenuated by the protein synthesis inhibitor CHX. Previously, we have reported that CHX, under the conditions used in this study, diminishes QA-induced internucleosomal DNA fragmentation (Qin *et al.*, 1996). Thus although not all types of apoptosis depend on new protein synthesis (Milligan *et al.*, 1994), the present results suggest a genetic program, involving certain transcription factor inductions, at least in part, attends the excitotoxic death of striatal neurons. However, CHX only modestly, although significantly, attenuated QA-induced internucleosomal DNA fragmentation and NFκB activation in our studies. This may reflect the fact that a single dose of CHX does not completely inhibit new protein synthesis, or that newly synthesized proteins contribute little to the apoptotic process. In the case of the NFκB family, presynthesized proteins in association with an inhibitory
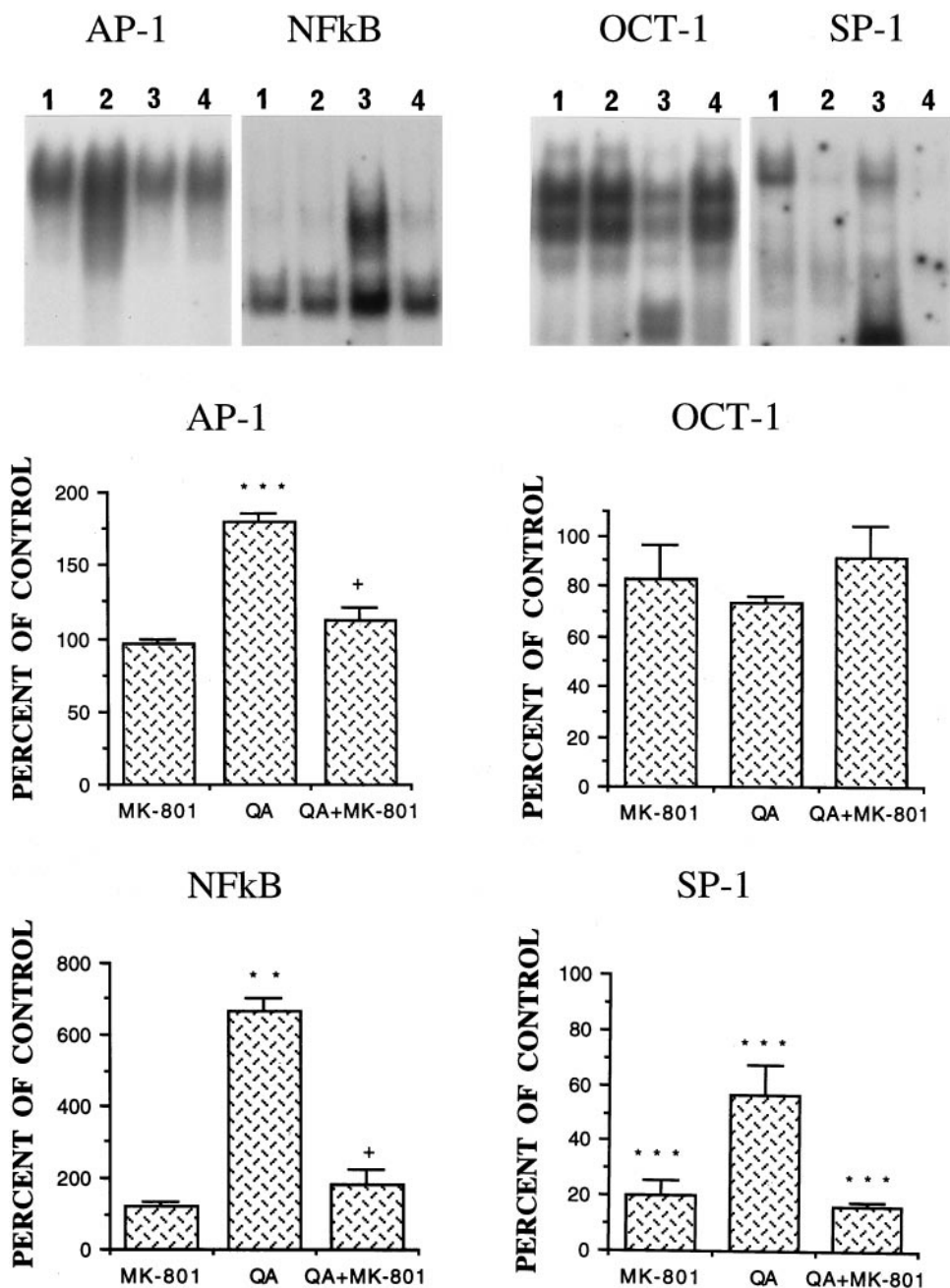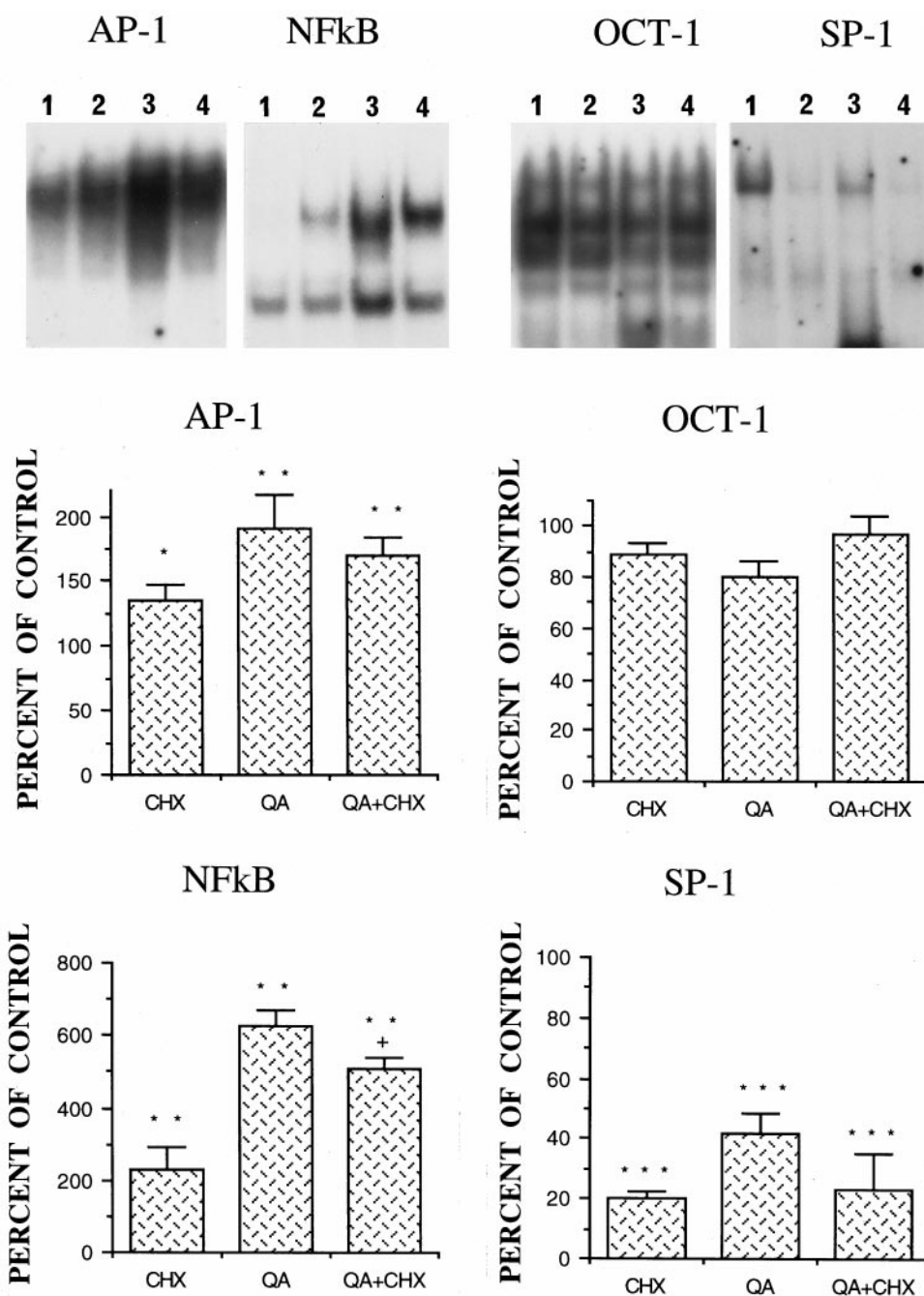
Fig. 5. Effect of MK-801 on QA-induced alterations in transcription factor binding activity. Rats were treated with MK-801 and QA as described in text. AP-1: *lane 1*, vehicle only; *lane 2*, vehicle + QA, 120 nmol; *lane 3*, MK-801 + vehicle; *lane 4*, MK-801 + QA, 120 nmol. NFκB, OCT-1, and SP-1: *lane 1*, vehicle only; *lane 2*, MK-801 + vehicle; *lane 3*, vehicle + QA, 120 nmol; *lane 4*, MK-801 + QA, 120 nmol. Results were quantitatively analyzed using an image analyzer and are expressed as percent of control (animals treated with intraperitoneal injections of vehicle plus intrastriatal injections of vehicle). Statistical comparisons were carried out by ANOVA followed by a Dunnett t test. ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$ (compared with vehicle-treated animals, $n = 6$). +, $p < 0.05$ (comparison between QA + vehicle and QA + MK-801-treated animals).

protein IκB normally reside in the cytoplasm. Upon appropriate stimulation, IκB is degraded and NFκB can then translocate to the nucleus and regulate gene expression.

Transcription factor changes found in this study presumably relate to the excitotoxic induction of an apoptotic cascade in striatal neurons. Although a significant reactive glia contribution to these transcription factor alterations cannot be excluded, previous investigations have suggested that the QA-induced death of medium spiny neurons, which account for more than 90% of nerve cells in rat striatum, involves an NMDA receptor-mediated apoptotic mechanism. For example, QA has not only been observed to produce many of the hallmarks of apoptosis, including internucleosomal DNA fragmentation, chromatin condensation, and nuclear fragmentation (Qin *et al.*, 1996), but also to induce proteins, such

as p53 and Bax, known to be directly involved in the apoptotic process (Hughes *et al.*, 1996). Using the same QA administration technique, we now find a marked increase in the number of TUNEL-positive nuclei and clear laddering of DNA fragments on agarose gels 12–24 hr after excitotoxin exposure. These fragments were of a size, multimers of 180–200 base pairs, expected from DNA cleavage by endonuclease during apoptosis. Moreover, all apoptotic stigmata appeared in close temporal relation to the observed transcription factor alterations: maximal induction of AP-1 and NFκB binding activity occurred 6–12 hr after QA treatment, thus preceding internucleosomal DNA fragmentation, whereas OCT-1 binding had significantly declined when DNA fragmentation peaked. In addition, MK-801 and CHX doses that inhibited QA-induced changes in transcription factors were the same
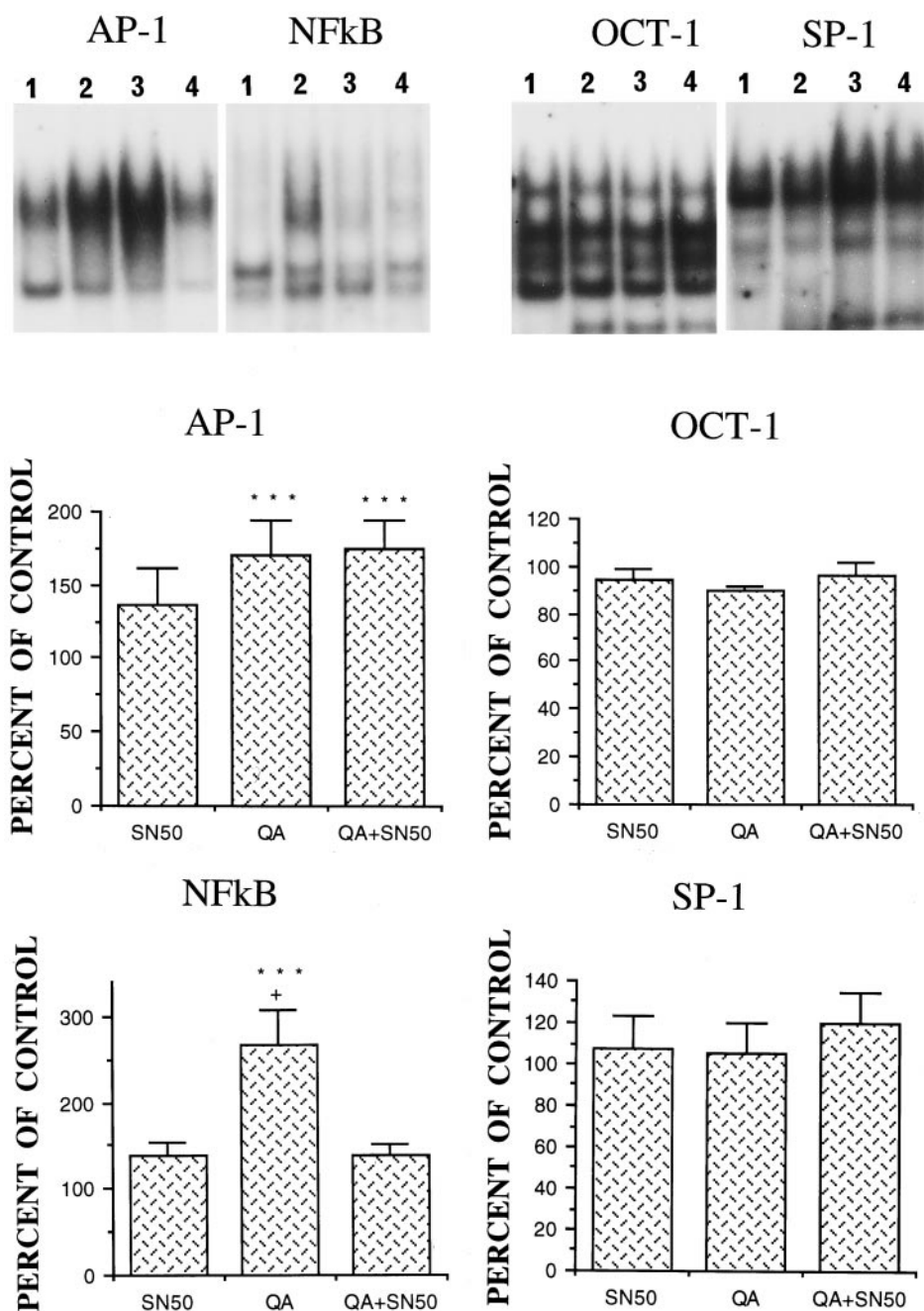
Fig. 6. Effect of CHX on QA-induced alterations in transcription factor binding activity. Rats were treated with CHX and QA as described in the text. *Lane 1*, vehicle only; *lane 2*, CHX, 480 nmol + vehicle; *lane 3*, vehicle + QA, 120 nmol; *lane 4*, CHX, 480 nmol + QA, 120 nmol. Results were quantitatively analyzed using an image analyzer and are expressed as percent of control (animals treated with vehicle only). Statistical comparisons were carried out by ANOVA followed by a Dunnett t test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (compared with vehicle-treated animals, $n = 6$). +, $p < 0.05$ (comparison between QA + vehicle and QA + CHX-treated animals).

as those that reduced apoptosis in our earlier study (Qin *et al.*, 1996).

The present results further suggest that NFκB plays an important role in QA-induced apoptosis. The functional consequences of preventing NFκB activation were evaluated by means of NFκB SN50, which interferes with NFκB nuclear translocation. This recombinant peptide contains the nuclear localization signal of the transcription factor NFκB p50 and the hydrophobic region of Kaposi fibroblast growth factor. The Kaposi fibroblast growth factor hydrophobic region confers cell permeability, whereas the nuclear localization signal inhibits translocation of the NFκB complex from the cytoplasm to the nucleus. Lin *et al.* (1995b) have demonstrated that this peptide inhibits nuclear translocation of NFκB *in vitro* in a dose-dependent manner. In the present studies,

co-administration of the recombinant cell-permeable peptide selectively inhibited both QA-induced NFκB activation and internucleosomal DNA fragmentation. Moreover, the decrement in internucleosomal DNA fragmentation was associated with a reduction in striatal cell death, as indicated by both receptor autoradiography and *in situ* hybridization histochemistry. It should be noted, however, that inhibition of NFκB activation failed to protect all striatal neurons against QA toxicity. Conceivably, additional NFκB-independent apoptotic cascades exist or both apoptosis and necrosis contribute to excitotoxic neuronal destruction. Alternatively, a basal level of NFκB activity may be required for the maintenance of normal cell function, although support for this possibility is somewhat weakened by the fact that NFκB SN50 alone can cause tissue damage.

Fig. 7. Effect of NFκB SN50 on QA-induced alterations in transcription factors. Rats were treated with NFκB SN50 and QA as described in the text. *Lane 1*, vehicle only; *lane 2*, vehicle + QA, 120 nmol; *lane 3*, NFκB SN50 + QA, 120 nmol; *lane 4*, NFκB SN50 + vehicle. Results are expressed as percent of control (animals treated with vehicle only). Statistical comparisons were carried out by ANOVA followed by Dunnett *t* test. ∗∗∗, $p < 0.001$ (compared with vehicle-treated animals, $n = 5$–6). +, $p < 0.001$ (comparison between QA + vehicle- and QA + NFκB SN50-treated animals).

The participation of transcription factors in neuronal apoptosis has been studied in various experimental systems. Proteins that bind to AP-1 consensus sequences include the Fos and Jun families; increases in both c-jun and c-fos proteins or mRNAs have been described during the apoptotic death of neurons (Dure *et al.*, 1995; Estus *et al.*, 1994; Tong and Perez-Polo, 1995). Similarly, intrastriatal injections of QA increase the expression of c-fos mRNA in areas which degenerate, and especially in medium spiny neurons (Aronin *et al.*, 1991). A study showing that anti-c-Jun antibodies block apoptosis in cultured sympathetic neurons provides relatively direct evidence of transcription factor involvement in the apoptotic process (Estus *et al.*, 1994). Binding activity of the OCT-1 transcription factor family, implicated in the regulation of various housekeeping genes and certain cell-specific genes, reportedly also decline in ischemia-induced brain damage and nerve growth factor deprivation-induced apoptosis in cultured cells (Wang and Pittman, 1993).

NFκB is well known to be involved in regulating important cellular functions including programmed cell death. Although somewhat inconsistent results have been reported, NFκB activation generally inhibits apoptosis, particularly when induced by tumor necrosis factor (Beg *et al.*, 1993; Wu and Lozano, 1994). Although convincing evidence in support of the participation of NFκB in apoptotic mechanisms within neuronal tissues has not been previously reported, NFκB has been implicated in the programmed death of cultured cells (Grimm *et al.*, 1996; Lin *et al.*, 1995a). The present study provides the first *in vivo* results indicating that NFκB may contribute to excitotoxin-induced apoptosis of striatal me-
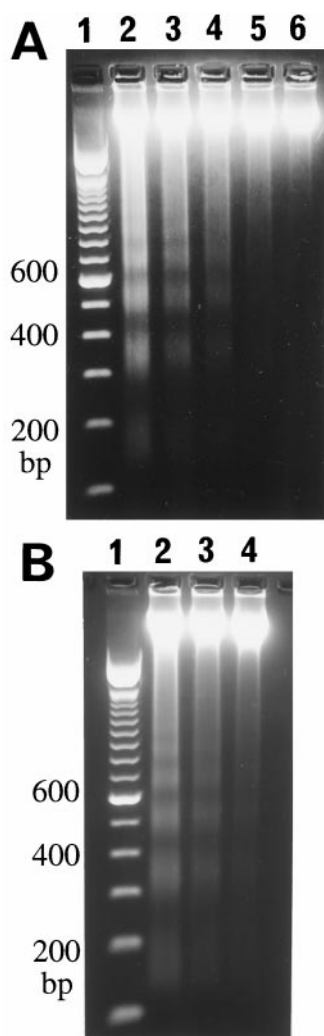
Fig. 8. Effect of NFκB SN50 on QA-induced internucleosomal DNA fragmentation. Rats were treated with NFκB SN50 and QA as described in the text. A, Rats were treated with two doses of NFκB SN50. *Lane 1*, 100-base pair DNA marker; *lane 2*, QA, 120 nmol + vehicle; *lane 3*, NFκB SN50, 10 μg + QA 120 nmol; *lane 4*, NFκB SN50 30 μg + QA, 120 nmol; *lane 5*, NFκB SN50 30 μg + vehicle; *lane 6*, vehicle only. B, Rats were treated with a single injection of NFκB SN50. *Lane 1*, 100-base pair DNA marker; *lane 2*, QA, 120 nmol + vehicle; *lane 3*, NFκB SN50, 20 μg + QA 120 nmol; *lane 4*, NFκB SN50, 20 μg + vehicle.

dium spiny neurons. Conceivably, NFκB influences apoptotic mechanisms in neurons differently than in other cells. Whether the apparently pro-apoptotic role of NFκB we observed here in medium spiny neurons can be generalized to other types of neurons or to other apoptotic triggers remains to be determined. Nevertheless, the results of this study, taken together with recent findings suggesting that aspirin and sodium salicylate protect cultured cerebellar granule cells and hippocampal neurons against glutamate toxicity by a mechanism possibly involving NFκB inhibition (Grilli *et al.*, 1996), could have important implications for the treatment of striatal neurodegenerative disorders.

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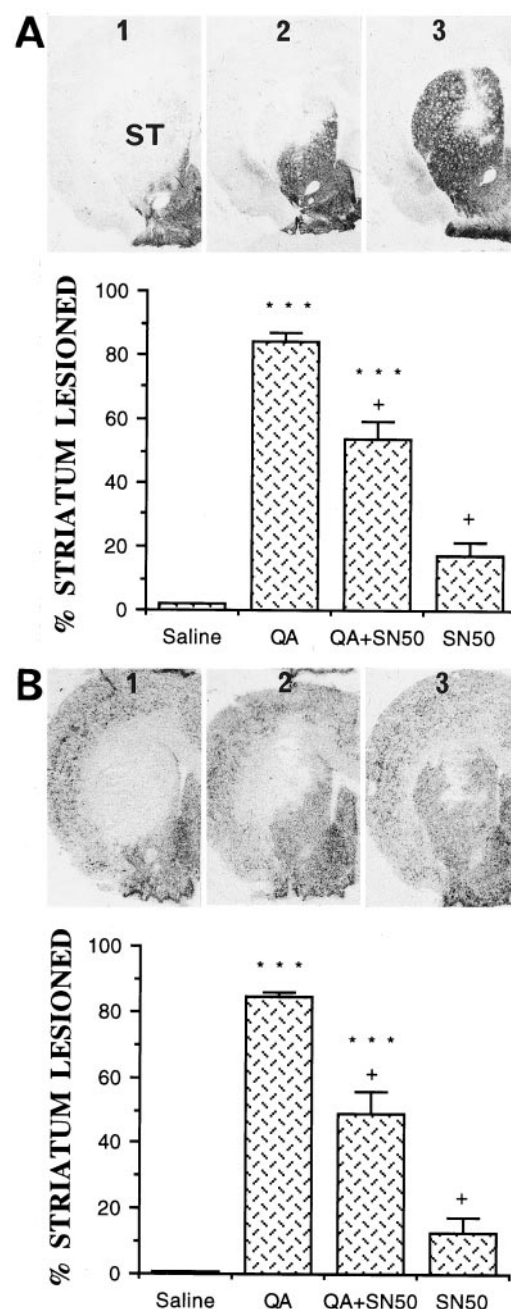
Fig. 9. Effect of NFκB SN50 on QA-induced striatal cell death. Rats were treated with NFκB SN50 and QA as described in the text. A, Representative receptor autoradiography of D_1 dopamine receptors and quantitative analysis. B, Representative *in situ* hybridization histochemistry of GAD_{67} mRNA and quantitative analysis. Results are expressed as percent of striatum lesioned (lesioned area/intact striatum $\times$ 100%). Statistical comparisons were carried out by ANOVA followed by Dunnett *t* test. *1*, QA, 120 nmol + vehicle; *2*, QA + NFκB SN50, 20 μg; *3*, NFκB SN50, 20 μg + vehicle. ***, $p < 0.001$ (compared with vehicle-treated animals, $n = 6$). +, $p < 0.001$ (comparison between QA + vehicle- and QA + NFκB SN50-treated animals). *ST*, striatum.

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