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Comparison of the effects of dopamine D_1 and D_2 receptor antagonists on nerve growth factor mRNA expression

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Abstract

Regulation of the expression of the nerve growth factor (NGF) gene has been reported previously to be mediated by the interaction of c-*fos* with an activator protein-1 (AP-1) binding site present in the first intron on the NGF gene. Using an RNase protection assay and in situ hybridization, we examined the effects of dopamine D_1 and D_2 receptor antagonists on NGF mRNA. Haloperidol (0.1–8 mg/kg) and (−)-sulpiride (10–100 mg/kg), induced NGF mRNA in a dose-dependent fashion in the hippocampus, piriform cortex, striatum and nucleus accumbens. The haloperidol (1 mg/kg)- and (−)-sulpiride (20 mg/kg)-induced NGF mRNA expression attained a maximum level 120 min after injection and returned to control levels 24 h later. Prior administration of the protein synthesis inhibitor cycloheximide blocked the haloperidol- and (−)-sulpiride-mediated induction of NGF mRNA. In contrast, R-(−)-8-chloro-2,3,4,5-tetrahydro-3,1-methyl-5-phenyl-11-3-benzyoepine-7-ol (SCH23390) did not induce NGF mRNA expression in either a dose-dependent or time-dependent manner. Our previous studies have shown that haloperidol and (−)-sulpiride induce the expression of c-*fos* and c-*jun* mRNAs and increase their AP-1 DNA binding activities. Thus, the data suggest that neuroleptics induce NGF gene expression by increasing AP-1 DNA binding activity.

Keywords: NGF (nerve growth factor); Neuroleptic; Dopamine; Activator protein-1; Schizophrenia

1. Introduction

Nerve growth factor (NGF), a member of the family of trophic factors known as neurotrophins, promotes the survival and differentiation of specific populations of neurons (Levi Montalcini et al., 1990). In the central nervous system (CNS), the expression of NGF has been reported to be controlled by the c-*fos* proto-oncogene (Mocchetti et al., 1989; Hengerer et al., 1990). This regulation, observed in fibroblasts, is mediated via the interaction of c-*fos* with an activator protein 1 (AP-1) binding site present in the first intron of the NGF gene (Hengerer et al., 1990; D'Mello and Heinrich, 1991a). The transcriptional regulation of AP-1 responsive genes is mediated by homodimeric complexes between members of the Jun family (c-Jun, Jun B and Jun D) or by the formation of heterodimers between proteins belonging to the Jun and Fos (c-Fos, Fos B, Fra-1 and Fra-2) families (Halazonetis et al., 1988; Ryder et al., 1988, 1989; Cohen et al., 1989; Hirai et al., 1989; Rauscher et al., 1989; Smeal et al., 1989; Zerial et al., 1989). Therefore, many kinds of AP-1 complexes resulting from interactions between members of the Fos and Jun families can interact directly with AP-1 binding sites with various affinities and different transcriptional activities, a situation that provides the possibility of additional regulatory mechanisms. An important feature of these proto-oncogenes is that they are rapidly and transiently activated by a great variety of external stimuli. In the CNS, the expression of c-*fos* mRNA is induced by kainic acid, NMDA, β-adrenoceptor and dopamine D_1 receptor agonists, electrical stimulation, cerebral ischemia, and many other stimuli, as early as 30 min after stimulus onset (Morgan and Curran, 1991). Thus, cytoplasmic second messenger systems activate protein kinases that in turn phosphorylate the transcription factors. Once activated, the transcription factor can, alone or in combination with other transcription factors, bind to the regulatory regions of target genes and regulate their expression. Another important aspect of proto-oncogenes is their ability to regulate gene expression in an indirect way. These transcription factors are not usually expressed constitutively within the cell and only regulate gene expression once they themselves are transcribed and translated. This family of genes includes c-*fos*,

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fra-1, *fra*-2, *fos*B, c-*jun*, *jun*B and *jun*D. When a cell is stimulated, the first wave of gene transcription involves proto-oncogene activation. Once translated, the products of these genes re-enter the nucleus and activate other late-response genes, resulting in a delayed secondary wave of gene activity (Hughes and Dragunow, 1995).

Recent studies have shown a link between dopaminergic neurotransmission and the proto-oncogene proteins Fos, Fos-related antigens, FosB, JunB and JunD in the striatum and nucleus accumbens of the rat brain (MacGibbon et al., 1994) or in rat basal ganglia neurons (MacGibbon et al., 1995). Our previous studies have shown that the dopamine D_2 receptor antagonists, haloperidol and (−)-sulpiride, induce the expression of c-*fos* and c-*jun* mRNAs and increase their AP-1 DNA binding activity in a dose-dependent manner. In contrast, SCH23390, a dopamine D_1 receptor antagonist, does not induce these mRNAs (Ozaki et al., 1997). Haloperidol and (−)-sulpiride have been shown to induce Fos, Fos B, Fra-1, Jun and Jun D in the hippocampus, the piriform cortex, the striatum and the

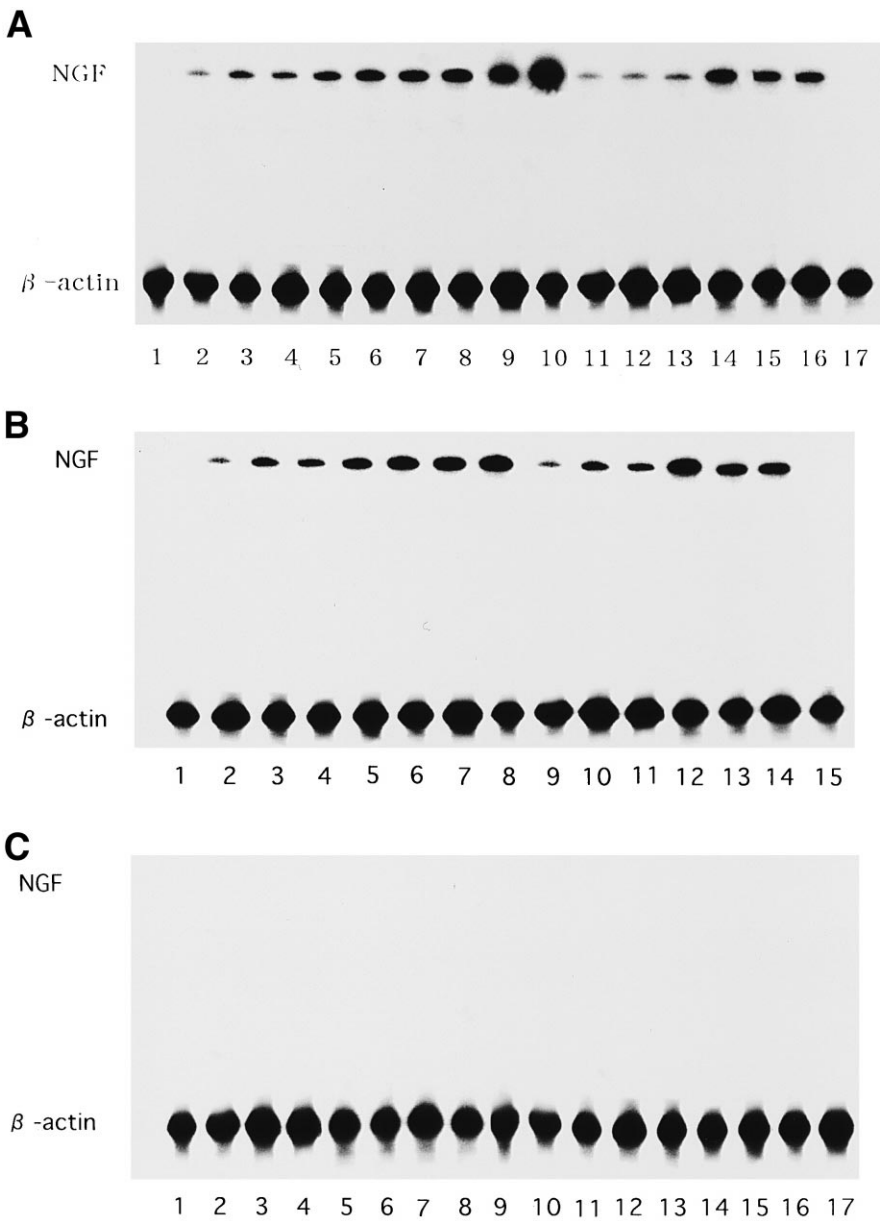


Fig. 1. Effect of treatment with haloperidol [HP, (a)], (−)-sulpiride [SP, (b)] and SCH23390 [SCH, (c)] on the induction of NGF and β-actin mRNA. This panel shows this expression at 120 min following HP administration (lane 1: sense probe, 2: control, 3: 0.1, 4: 0.2, 5: 0.4, 6: 0.5, 7: 1, 8: 2, 9: 4, 10: 8 mg/kg), SP administration (lane 1: sense pobe, 2: control, 3: 10, 4: 20, 5: 30, 6: 40, 7: 80, 8: 100 mg/kg) and SCH administration (lane 1: sense probe, 2: control, 3: 0.1, 4: 0.2, 5: 0.4, 6: 0.8, 7: 1, 8: 2, 9: 4, 10: 8 mg/kg) and the time course of this expression following HP (1 mg/kg) administration (lane 11: 15 min, 12: 30 min, 13: 60 min, 14: 120 min, 15: 180 min, 16: 360 min, 17: 24 h), SP (20 mg/kg) administration (lane 9: 15 min, 10: 30 min, 11: 60 min, 12: 120 min, 13: 180 min, 14: 360 min, 15: 24 h) and SCH (1 mg/kg) administration (lane 11: 15 min, 12: 30 min, 13: 60 min, 14: 120 min, 15: 180 min, 16: 360 min, 17: 24 h).

nucleus accumbens. These proteins comprise the AP-1 complex. SCH23390 induces Fos B, Fra-1 and Jun D in the same regions and increases the related AP-1 DNA binding activity (Ozaki et al., 1998).

The Fos/Jun heterodimer, one of the major targets of protein kinase A and protein kinase C activation (Angel et al., 1987; Bravo et al., 1987; De Groot and Sassone-Corci, 1992), could be a regulatory transcription factor for the expression of the NGF gene. The increase in AP-1 DNA binding activity has been correlated with the induction of NGF mRNA in cells derived from the CNS (Mocchetti, 1991). That c-Fos acts as a transcription factor in the induction of NGF gene expression is supported by the identification of an AP-1 consensus sequence within the exon I/intron I region of the mouse NGF gene (D'Mello and Heinrich, 1991a) which has been shown to be important for the basal and induced expression of the mouse NGF gene in fibroblasts (Hengerer et al., 1990; D'Mello and Heinrich, 1991a; Cowie et al., 1994). However, it has not been established yet whether the increase in AP-1 activity induced by haloperidol and (−)-sulpiride correlates with the induction of NGF mRNA.

The present work was undertaken to clarify the link between dopamine D_2 receptor antagonism and NGF mRNA expression. To this end, we investigated the potential relationships between the expression of members of the Fos and Jun families following treatment with neuroleptics, such as haloperidol and (−)-sulpiride, and the induction of NGF mRNA.

2. Materials and methods

2.1. Materials

Haloperidol, (−)-sulpiride, proteinase K and cycloheximide were obtained from Sigma (St. Louis, MO). We purchased the selective dopamine D_1 receptor antagonist, *R*-(−)-8-chloro-2,3,4,5-tetrahydro-3,1-methyl-5-phenyl-11-3-benzyoepine-7-ol (SCH23390), from Research Biochemicals International (Natick, MA). The synthesized NGF cDNA primers 5′-GCAGAGATAGCAACACCTGA-3′ (20 bp) and 5′-GATCCAAGCACACCGTCCCA-3′ (20 bp) were produced by Sawaday Technology (Tokyo, Japan). The reverse transcriptase was purchased from Gibco (Gaithersburg, MD, USA) and the pBluescript SK (−) plasmid vector from Stratagene (San Diego, CA, USA). Mouse β-actin cDNA (315 bp) was a generous gift from Dr. Fujii (Canser Research Institute, Kanazawa University, Japan) Amersham International. (Bucks, UK) produced the α-[^{32}P]UTP (800 Ci/mmol). T3 or T7 RNA polymerase, RNase A, RNase T1, and 5 × transcriptional buffer [200 mM Tris–HCl buffer (pH 8.0), 40 mM $MgCl_2$, 10 mM (−)-spermidine, 250 mM NaCl, 0.75 M dithiothreitol] containing γ-ATP, γ-GTP and γ-CTP were purchased from Stratagene (La Jolla, CA).

2.2. Animals and drug administration

Fifty-six-day-old male ddY mice (body weight: 25 to 35 g) were purchased from Keari (Osaka, Japan). They were housed individually and maintained for 15 days on a 12 h:12 h light/dark cycle with free access to food and water. Drugs were dissolved in 30 μl of 1% acetic acid and injected intraperitoneally. Doses used were as follows: haloperidol at 0.1, 0.2, 0.4, 0.8, 1, 2, 4 or 8 mg/kg ($n = 5$), (−)-sulpiride at 10, 20, 30, 40, 80 or 100 mg/kg ($n = 5$), and SCH23390 at 0.1, 0.2, 0.4, 0.8, 1, 2, 4 or 8 mg/kg ($n = 5$). The animals were killed at 15, 30, 60, 120, 180, 360 min or 24 h following the injection of either haloperidol (1 mg/kg), (−)-sulpiride (20 mg/kg) or SCH23390 (1 mg/kg). To establish whether the induction of NGF mRNA was dependent upon the de novo synthesis of AP-1 after the injection of haloperidol or (−)-sulpiride, we blocked drug-induced synthesis of AP-1 by using the protein-synthesis inhibitor, cycloheximide (10 mg/kg).

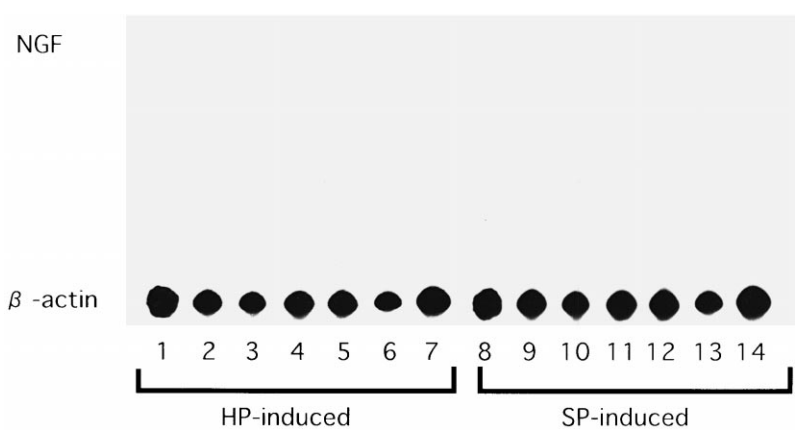


Fig. 2. This panel shows the time course of NGF and β-actin mRNA expression following pretreatment with cycloheximide (10 mg/kg) 1 h after haloperidol (1 mg/kg) or (−)-sulpiride (20 mg/kg) administration (lane 1: 15 min, 2: 30 min, 3: 60 min, 4: 120 min, 5: 180 min, 6: 360 min, 7: 24 h, 8: 15 min, 9: 30 min, 10: 60 min, 11: 120 min, 12: 180 min, 13: 360 min, 14: 24 h).

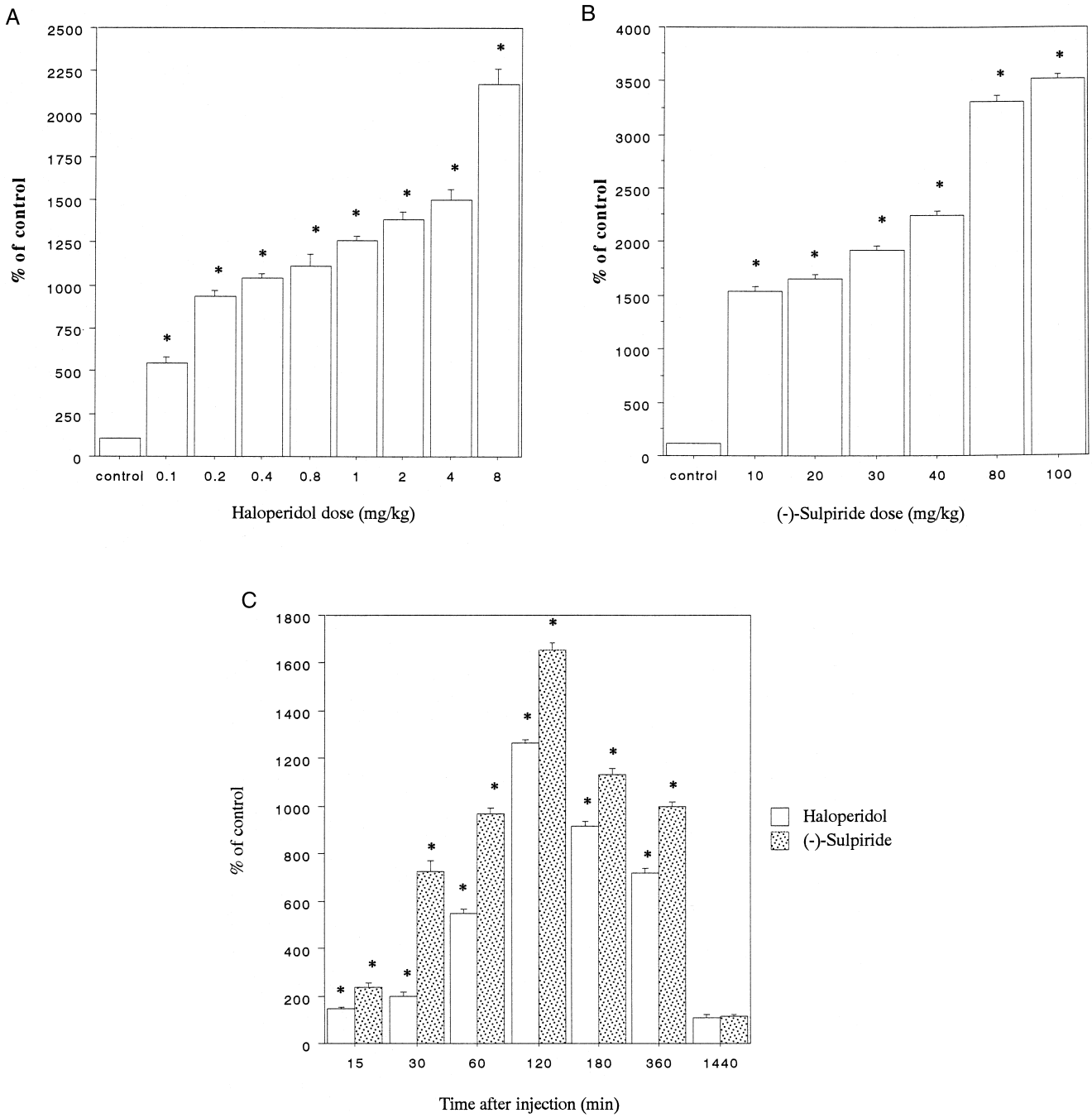
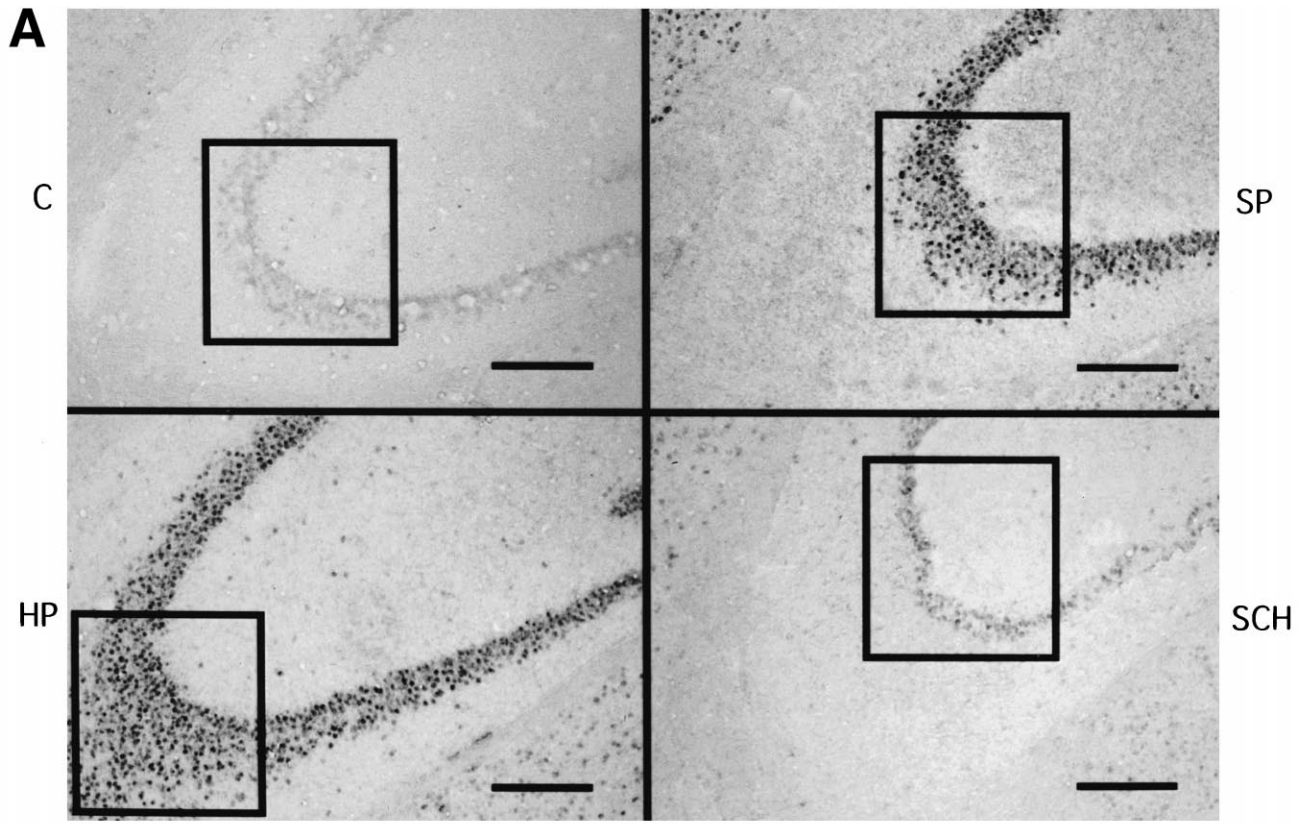


Fig. 3. (a, b, c) Haloperidol- or (−)-sulpiride-induced NGF mRNA expression as described in Fig. 1a,b was quantitatively analyzed with a Macintosh image analysis program (NIH IMAGE, W. Rasband, NIMH) and expressed as a percentage of the basal level of NGF mRNA seen in control groups. Data are presented as means ± standard error. * $P < 0.01$ compared with control.

Cycloheximide was given 1 h prior to the drug injections. Control animals were given 30 μl of 1% acetic acid ($n = 5$) and killed at the same time points. Animals were immediately decapitated and the whole brains were dissected. Total RNA was extracted by a single-step method (Chomczynski and Sacchi, 1987). These doses are more

Fig. 4. Haloperidol (HP)-, (−)-sulpiride (SP)- and SCH23390 (SCH)-mediated regulation of NGF expression in the hippocampus (A), the piriform cortex (B), the striatum (C) and nucleus accumbens (D). Photomicrographs show NGF mRNA after treatment with HP, SP or SCH. The level of NGF mRNA observed under basal conditions in the controls (C) was increased after HP or SP, but not after SCH. Quantitative data are presented in Table 1. Each square depicts the 400 × 400 μm grid. Scale bars = 200 μm.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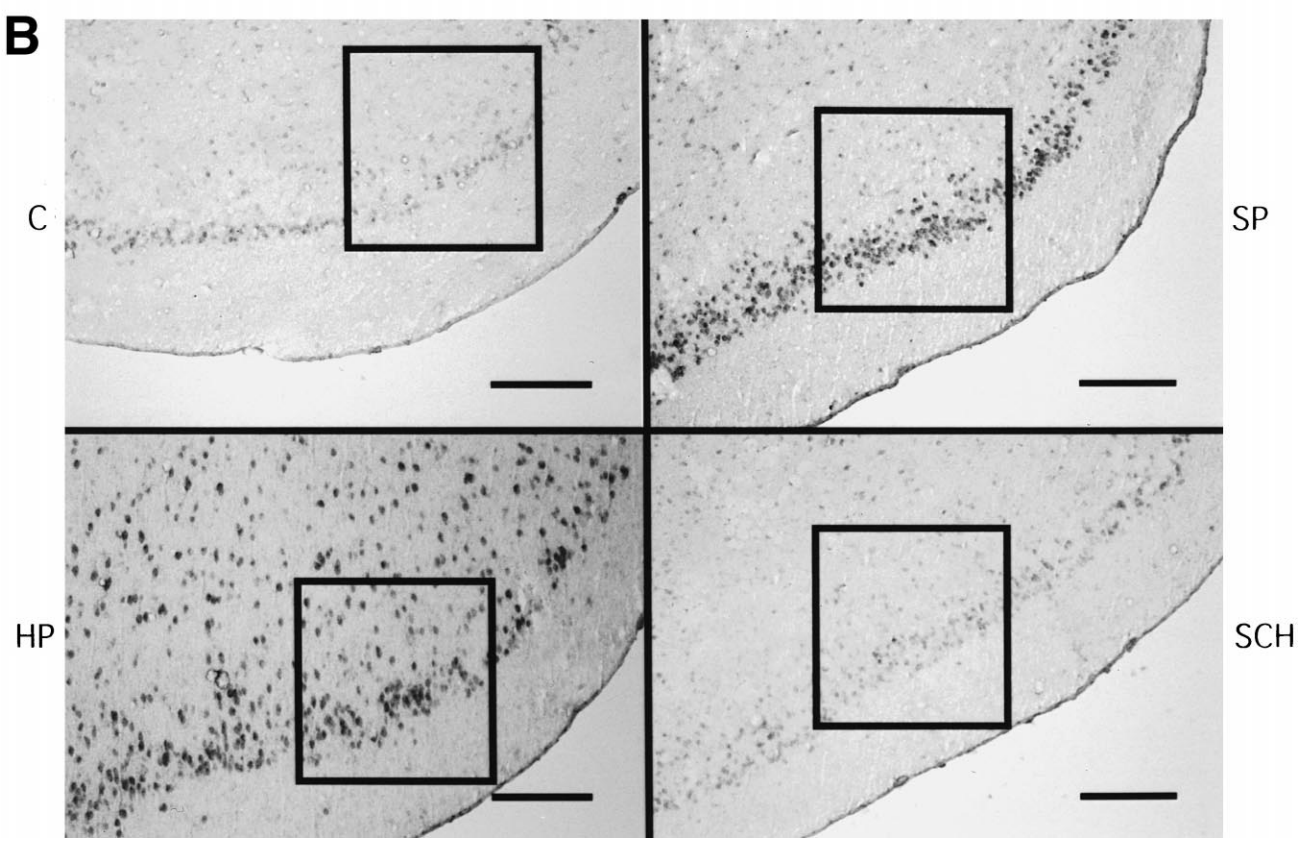
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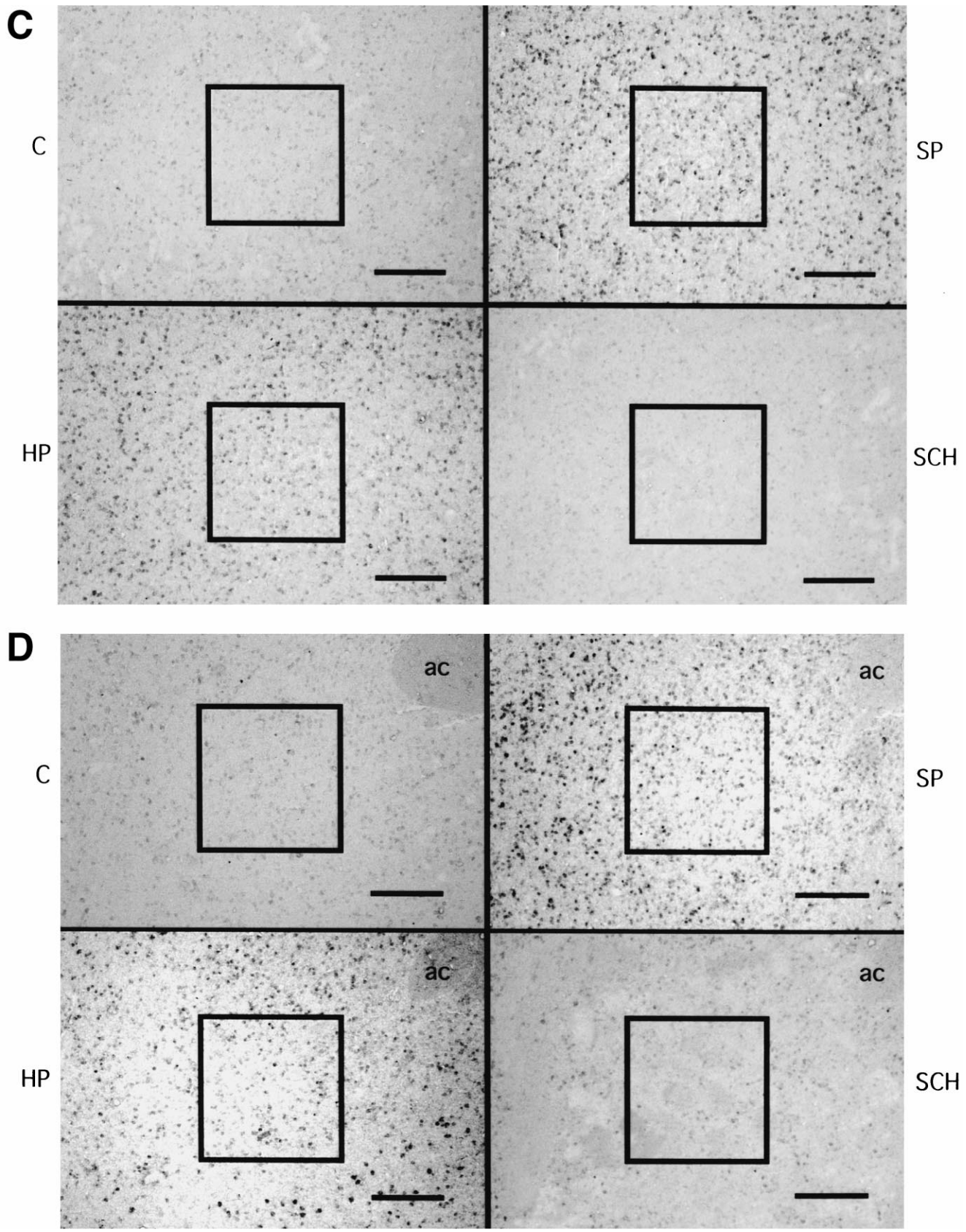

Fig. 4 (continued).

than the ED_{50} value of either haloperidol, (−)-sulpiride or SCH23390.

2.3. Cloning of mouse NGF genes and synthesis of riboprobes

Total RNA was prepared from mouse brain using the single-step method (Chomczynski and Sacchi, 1987). CDNA synthesized from total RNA was treated with DNase I treatment (0.1 U/μg RNA for 10 min at 37°C) after which 0.5 μg RNA was incubated with 500 μM dNTP, 40 ng random hexamer primer, 2 U RNase inhibitor (Promega, Madison, WI, USA), and 400 U Moloney murine leukemia virus reverse transcriptase. The incubation was performed in a solution of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 5 mM dithiothreitol for 60 min at 37 and then heated at 95°C for 5 min to inactivate the enzyme. cDNA prepared from 0.1 μg RNA was used in the polymerase chain reaction (PCR). PCR amplifications were performed as follows: denaturation at 94 for 1 min, annealing at 55°C for 1 min and extension at 72 for 1 min with 30 cycles, using primers designed from the region involving the AP-1 consensus sequence of the mouse NGF gene (Selby et al., 1987). The PCR product (620 bp) was subsequently subcloned into the deoxy-thymidine triphosphate-tailed pBluescript plasmid, which possesses the T7 and T3 promoters for RNA synthesis, and then sequenced using a 7-diazza Sequenase DNA sequencing kit (USB, Cleveland, OH, USA). Transcription of the NGF (antisense and sense) riboprobe was performed in the presence of 50 μCi α-[^{32}P]UTP or digoxigenin-UTP, 10 units of T3 or T7 RNA polymerase and 5 × transcriptional buffer.

2.4. Tissue preparation

One hundred-twenty minutes after injection of either haloperidol (1 mg/kg), (−)-sulpiride (20 mg/kg), SCH23390 (1 mg/kg) or vehicle, the animals were deeply anesthetized with ether, and their brains rapidly removed, frozen on dry ice, and cut into 12-μm sections on a cryostat. Sections were thaw-mounted on silan-coated slides, fixed for 30 min in a 4% paraformaldehyde–0.1 M phosphate buffer (pH 7.4), and incubated for 10 min in a fresh solution of 0.25% acetic anhydride in 0.1 M triethanolamine and 0.9% saline (pH 8.0). The slide-mounted sections were then dehydrated, defatted for 10 min in chloroform, dehydrated, and air-dried.

2.5. RNase protection assay

The RNase protection assay was performed according to the method of Zinn et al. (1983). Total RNA (10 μg) and the labeled (antisense or sense) riboprobe (8 × 104 c.p.m.) were mixed. The concentration of ammonium acetate was adjusted to 0.5 M in the mixture, and a 2.5-fold volume of ethanol was then added. Following centrifugation at 18 000 × *g* for 20 min, the precipitated RNA was dissolved in 20 μl of hybridization buffer (80% deionized formamide, 100 mM sodium citrate, 300 mM sodium acetate and 1 mM EDTA). The mixture was incubated at 45°C for 18 h for hybridization between the probe and the complementary mRNA. One microliter of the RNase mixtures, consisting of 250 units/ml of RNase A and 10 000 units/ml of RNase T1 in glycerol solution, was added to the above mixture and then incubated at 37°C for 15 min. Five microliters of proteinase K and 20 μl of 10% sodium dodecyl sulfate were then added, and the mixture was incubated under the same conditions. Following the addition of a phenol-chloroform mixture (1:1, V/V) and ethanol, the digestion products were centrifuged at 18 000 × *g* for 20 min. The pellets obtained were then resuspended in 8 μl of loading buffer (80% deionized formamide, 0.1% xylene cyanol, 0.1% bromophenol blue and 2 mM EDTA) and loaded on a 5% polyacrylamide gel containing 8 M urea. After the gel was run at 300 V for 1.5 h, it was transferred to chromatography paper, covered with plastic wrap, and exposed to X-ray film at −80°C for 48 h. The protected fragments of NGF mRNA were quantified with a densitometer.

2.6. Digoxigenin in situ hybridization

All solutions were treated with diethyl pyrocarbonate and autoclaved before use. All histochemical processes were performed with minimal shaking to reduce the possibility of sections floating off the slides. The NGF (antisense or sense) riboprobe at a concentration of approximately 0.1 μg/ml was added to the hybridization buffer (50% deionized formamide, 20 mM Tris–HCl [pH 8.0], 5 mM EDTA, 1.8% NaCl, 100 mM sodium phosphate buffer [pH 8.0], 1 × Denhardt, salmon sperm DNA [200 μg/ml], yeast tRNA [500 μg/ml] and 0.2% sarcosyl). Sections were incubated at 55°C for 18 h, washed at 65°C for 30 min in washing buffer (0.3 M NaCl/0.03 M sodium citrate, 50% formamide and 0.1 M dithiothreitol) and treated with 20 μg/ml RNase A in RNase buffer comprised of 10 mM Tris–HCl (pH 8.0), 1 mM EDTA and 0.5 M NaCl. After the RNase treatment, sections were washed in the above washing buffer under the same conditions. To detect the hybridized probe, the hybridized slides were incubated for 60 min at room temperature with a blocking buffer (pH 7.5, 0.1 M Tris base and 9% NaCl) containing 0.3% (W/V) Tween 20. The slides were then incubated for 12 h at room temperature with diluted anti-digoxigenin alkaline phosphatase conjugate (1:500, Boehringer Mannheim, Meylan, France) in the above blocking buffer. Unbound antibody conjugate was removed by washing three times in the same buffer. The colored product was visualized using nitro blue tetrazolium and 5-bromo-4-chlor-3-indolyl-phosphate as substrate, and the slides were incubated for 72 h at room temperature. The slides were mounted with glycerin jelly and kept at 4°C.

2.7. Data analysis

The levels of NGF mRNA were calculated from densitometric analysis of autoradiograms using a Macintosh image analysis program (NIH IMAGE, W. Rasband, NIMH) and expressed as a percentage of the basal level of NGF mRNA seen in control groups. The difference in the NGF mRNA levels between the control and drug-treated animals was statistically analyzed by a two-way analysis of variance (ANOVA) and post hoc multiple comparison tests (Tukey test). To investigate quantitative drug-induced alterations in neuronal NGF mRNA, the number of NGF mRNA-positive neurons in coronal sections of the hippocampus, piriform cortex, nucleus accumbens and striatum was counted in 400×400 μm grids using the NIH program and compared to the total number of cells. The difference in the number of mRNA-positive neurons between the control and drug-treated animals was statistically analyzed by a two-way ANOVA and post-hoc multiple comparison tests (Tukey test).

3. Results

3.1. RNase protection assay

The expression of NGF mRNA was examined at various times following the administration of single doses of either haloperidol (1 mg/kg), (−)-sulpiride (20 mg/kg) or SCH23390 (1 mg/kg). As shown in Fig. 1A,B, an increase in NGF mRNA was apparent as early as 15 min after administration of either haloperidol or (−)-sulpiride. The maximal level was observed at 120 min after both haloperidol ($1232 \pm 6\%$ of control, $n = 5$) and (−)-sulpiride ($1542 \pm 12\%$ of control, $n = 5$) administration. The induction of NGF mRNA was maintained until 360 min after both haloperidol and (−)-sulpiride. Thereafter, levels declined to control levels by 24 h. Multiple comparison tests showed significant increases in NGF mRNA in both the haloperidol- and (−)-sulpiride-treated mice at 15, 30, 60, 120, 180 and 360 min (Fig. 3C, all $P < 0.01$). Prior administration of cycloheximide blocked the haloperidol- or (−)-sulpiride-mediated induction of NGF mRNA at 24 h (Fig. 2). Unlike the robust increase in NGF mRNA seen following treatment with haloperidol or (−)-sulpiride, SCH23390 administration did not have significant effect on the level of NGF mRNA (Fig. 1C). The expression of NGF mRNA examined 120 min after injection of various doses of haloperidol or (−)-sulpiride showed a dramatic dose-dependent increase (Fig. 1A,B and Fig. 3A,B, all $P < 0.01$). The maximal level was observed at a dose of 8 mg/kg haloperidol ($2118 \pm 39\%$ of control, $n = 5$) and 100 mg/kg (−)-sulpiride ($3283 \pm 16\%$ of control, $n = 5$). However, no dose of SCH23390 induced an increase in the expression of NGF mRNA (Fig. 1C). Only faint background hybridization was observed with the sense control riboprobe.

3.2. In situ hybridization

As shown in Fig. 4A and Table 1, NGF mRNA expression was significantly higher in the hippocampus (CA 2 and CA 3) after treatment with either haloperidol ($1020 \pm 8\%$ of control, $n = 5$) or (−)-sulpiride ($9733 \pm 8\%$ of control, $n = 5$) compared to vehicle. In other regions of the hippocampus, both haloperidol and (−)-sulpiride induced greater NGF mRNA expression than did the vehicle (data not shown). In the piriform cortex (Fig. 4B), (−)-sulpiride ($475 \pm 6\%$ of control) administration significantly increased the number of NGF mRNA- positive neurons as did haloperidol ($475 \pm 7\%$ of control, $n = 5$) administration (Table 1). Additionally, haloperidol-induced NGF mRNA expression was increased significantly in both the striatum ($607 \pm 8\%$ of control, $n = 5$) and the nucleus accumbens ($803 \pm 12\%$ of control, $n = 5$) (Fig. 4C,D and Table 1). Fig. 4C,D and Table 1 show that a significant increase in (−)-sulpiride-induced NGF mRNA expression was also observed in both the striatum ($575 \pm 10\%$ of control, $n = 5$) and the nucleus accumbens ($825 \pm 13\%$ of control, $n = 5$).

In contrast, SCH23390 treatment did not increase NGF mRNA expression in the hippocampus (Fig. 4A), piriform cortex (Fig. 4B), striatum (Fig. 4C), nucleus accumbens (Fig. 4D) or any other brain region examined. In vehicle-

Table 1
NGF mRNA-positive nuclei in four regions in animals treated with either vehicle or drug injection

Region	Control (vehicle)	Haloperidol (1.0 mg/kg)	(−)-Sulpiride (10 mg/kg)	SCH23390 (1.0 mg/kg)
Hippocampus	18.0 ± 1.58	183.6 ± 2.41[a]	175.2 ± 3.03[a]	19.0 ± 0.71
Piriform cortex	24.4 ± 1.14	116.0 ± 3.54[a]	115.8 ± 3.77[a]	26.2 ± 1.30
Striatum	22.4 ± 1.14	136.0 ± 3.81[a]	128.8 ± 5.07[a]	21.2 ± 1.30
Nucleus accumbens	15.4 ± 1.67	123.8 ± 4.21[a]	127.0 ± 4.53[a]	13.8 ± 1.92

Mice were treated with either vehicle, haloperidol (1 mg/kg body weight), (−)-sulpiride (20 mg/kg body weight) or SCH23390 (1 mg/kg body weight). Then 120 min after drug administration the animals were anesthetized and the brains were rapidly removed.
Values present the mean ± standard error of the number of NGF mRNA-containing neurons per 400 μm^2.
The data were analyzed by ANOVA followed by multiple comparison tests (Tukey test), $n = 5$/group.
[a] $P < 0.01$ compared with control.

treated mice, few NGF mRNA-positive neurons were detected in the cortex (data not shown), hippocampus (Fig. 4A), piriform cortex (Fig. 4B), striatum (Fig. 4C) and nucleus accumbens (Fig. 4D). Only faint background hybridization was observed with the sense control riboprobe.

4. Discussion

Previous research has revealed a transcriptional regulatory pathway induced by the binding of dopamine to the receptor (Ozaki et al., 1997). The expression of c-*fos* and c-*jun* mRNA expression induced by haloperidol and (−)-sulpiride is a direct effect of dopamine D_2 receptor antagonism. Therefore, haloperidol and (−)-sulpiride induce the transcriptional products of these genes (Fos, Fos B, Fra-1, Jun and JunD) in the hippocampus, piriform cortex, striatum and nucleus accumbens (Ozaki et al., 1998). These proteins bind to each other to form a hetero- or homodimeric nucleoprotein complex that possesses a high affinity for the DNA consensus sequence (TGACTCA) of transcription factors, such as the AP-1 complex. Moreover, the NGF cDNA sequence includes the AP-1 consensus sequence in the first intron of the gene. Therefore, our study has demonstrated that a significant increase in AP-1 DNA binding activity leads to haloperidol- and (−)-sulpiride-induced NGF mRNA expression in the hippocampus, piriform cortex, striatum and nucleus accumbens. In contrast, SCH23390 did not induce NGF mRNA expression although AP-1 DNA binding activity was significantly increased (Ozaki et al., 1997). This leads us to speculate that the transcription of NGF mRNA was not induced, although the AP-1 complex composed of Fos B, Fra-1 and Jun D binds to the AP-1 consensus sequence (Ozaki et al., 1998). The c-*fos* mRNA expression induced by haloperidol and (−)-sulpiride has been reported to be a direct effect of dopamine D_2 receptor antagonism (Dragunow et al., 1990; Robertson et al., 1992). The mechanism is thought to involve the induction of the c-*fos* gene as a result of NMDA receptor activation (Aronin et al., 1991; Dragunow et al., 1990; Sonnenberg et al., 1989). This is supported by the finding that MK-801, a NMDA receptor antagonist, has been reported to block haloperidol-mediated Fos induction. Therefore, we suggest that haloperidol- and (−)-sulpiride-induced NGF mRNA expression is increased by a pharmacologic interaction between an increase in the glutamate concentration in the synaptic cleft and AP-1 DNA binding activity. A previous study has shown that the related neurotrophin brain-derived neurotrophic factor (BDNF) is induced as an immediate early gene following NMDA receptor activation (Hughes et al., 1993). We speculate that, in this study, focal injury, which increased the extra- and intra-cellular glutamate concentration, leading to neuron damage, resulted in the induction of BDNF mRNA as a neuroprotective mechanism. Our results suggest that haloperidol and (−)-sulpiride increase the glutamate concentration only in the synaptic cleft, and that NGF mRNA is induced in order to stabilize neuronal function.

Nerve growth factor is a neurotrophin synthesized in dentate gyrus granule cells and pyramidal neurons of the hippocampal formation. These neurons receive excitatory glutamatergic afferents from the entorhinal cortex via the angular bundle/perforant path. The levels of NGF mRNA can be regulated in vitro and in vivo in the hippocampal formation by events associated with pharmacological activation of glutamate receptors. In previous studies, the level of NGF mRNA in the hippocampal formation was examined following an intrahippocampal injection of fluorocitrate, which temporarily inhibits astrocyte metabolism activity in vivo. Fluorocitrate treatment significantly increased glutamate levels in the dentate gyrus. The increased ratio of glutamate to glutamine was followed by a significant increase in NGF mRNA expression selectively in dentate gyrus granule cells. The effects of increasing glutamate levels were blocked by pretreatment with 2-amino-5-phosphonovalerate, a competitive antagonist that acts at the NMDA receptor (Gwag et al., 1997). These findings suggest that NGF mRNA expression is regulated, in part, by changes in endogenous glutamate levels, partially through enhanced excitatory neurotransmission through NMDA receptors.

The interaction of glutamate and dopamine in the hippocampus is suggested to be of some relevance to the pathogenesis of schizophrenia. Several groups (Kovelman and Scheibel, 1984; Bogerts et al., 1985; Altsschuler et al., 1990; Benes et al., 1991; Kerwin and Murray, 1992; Akbarian et al., 1993; Squires et al., 1993) have demonstrated the possible involvement of the hippocampal formation in schizophrenia. Theories of schizophrenia have included a functional role for the hippocampus in the generation of psychotic symptoms (Weinberger, 1987; Kerwin and Murray, 1992; Krieckhaus et al., 1992). Krieckhaus et al. (1992) have suggested that CA2 hyperactivity may be a cause of some of the positive symptoms of schizophrenia, because dopamine D_2 receptor antagonists decrease hippocampal neuronal firing, thereby ameliorating the symptoms. Deakin et al. (1989), Carlsson and Carlsson (1990), Kerwin et al. (1988), Harrison et al. (1991), Sherman et al. (1991), and Ishimaru et al. (1994) have postulated aberrant glutamate function, particularly in the temporal lobe, as a possible mechanism for schizophrenia.

In conclusion, haloperidol and (−)-sulpiride increase glutamate concentrations in the synaptic cleft by inhibition of dopamine D_2 neurons which suppress glutamate neurons. High concentrations of glutamate in the synaptic cleft induce NGF mRNA expression via glutamate receptors and NGF is required for the development and maintenance of neurons which have aberrant function as a possible mechanism for schizophrenia in the limbic system including the hippocampus, the piriform cortex, the striatum and the nucleus accumbens.

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