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European Journal of Pharmacology 402 (2000) 39–44

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Comparative effects of dopamine D_1 and D_2 receptor antagonists on nerve growth factor protein induction

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Received 10 February 2000; received in revised form 5 July 2000; accepted 11 July 2000

Abstract

We previously reported that following acute administration of haloperidol or (−)-sulpiride, both dopamine D_2-receptor antagonists, to mice induced nerve growth factor (NGF) gene expression, mediated by the interaction of c-*fos* with the AP-1 binding site present in the first intron on the NGF gene. In contrast, the D_1-receptor antagonist *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. We report here immunohistochemical and Western blot analyses showing that following injection of these drugs for 14 consecutive days, the amount of NGF protein increased gradually and was induced significantly in the hippocampus, piriform cortex, amygdala, dorsal striatum, and nucleus accumbens neurons. NGF enhances the release of acetylcholine from these regions. Cholinergic innervation in the striatum and nucleus accumbens neurons is believed to be related to late-onset extrapyramidal symptoms, while in the hippocampus and piriform cortex it is involved in enhancing cognition. Thus, our data suggest that haloperidol- and (−)-sulpiride-induced NGF expression may be associated with both beneficial and adverse effects.

Keywords: Nerve growth factor (NGF); Dopamine receptor; Acetylcholine; Cognition; Extrapyramidal symptom

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 regulated via the interaction of c-*fos* with an AP-1 binding site present in the first intron of NGF gene (Hengerer et al., 1990; D'Mello and Heinrich, 1991). The expression of c-*fos* mRNA can be 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.

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 AP-1 DNA binding activity in a dose-dependent manner (Ozaki et al., 1997). 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, piriform cortex, dorsal striatum, and nucleus accumbens (Ozaki et al., 1998). These proteins comprise the AP-1 complex (Ozaki et al., 1998). Prior administration of the protein synthesis, inhibitor cycloheximide blocked the haloperidol- and (−)-sulpiride-mediated induction of NGF mRNA (Ozaki et al., 1999). SCH23390 induces Fos B, Fra-1, and Jun D in the same

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00493-3

regions and increases AP-1 DNA binding activity, but does not induce the expression of NGF mRNA (Ozaki et al., 1998). Therefore, only the increase in AP-1 activity induced by haloperidol and (−)-sulpiride correlates with the induction of NGF mRNA.

In this study, we investigated whether this induction of NGF mRNA led to translation and accumulation of the NGF protein. The present work was undertaken to clarify the link between NGF mRNA and protein. To this end, we investigated potential relationships between treatment with neuroleptics, such as haloperidol and (−)-sulpiride, and the induction of NGF protein.

2. Materials and methods

2.1. Materials

Haloperidol, (−)-sulpiride and 3,3′-diaminobenzidine were obtained from Sigma (St. Louis, USA). 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, USA). The NGF antibody was from Santa Cruz Biotechnology (Santa Cruz, USA); the production and specificity of this antibody have been

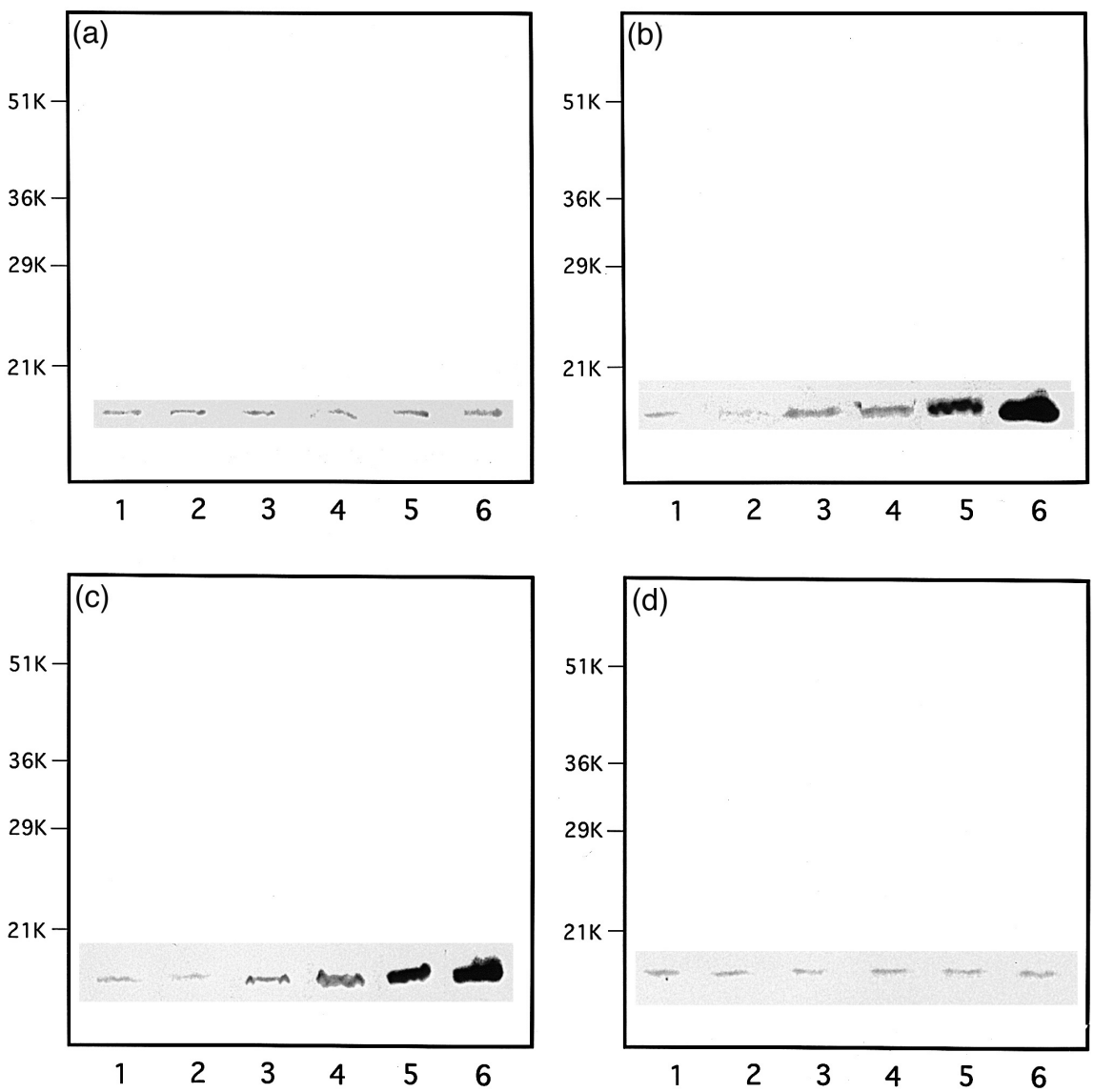

Fig. 1. Western blot analysis of NGF expression in protein extracts prepared from mice brains. This panel shows the time course following vehicle (a), haloperidol (b, 1 mg/kg), (−)-sulpiride (c, 20 mg/kg) and SCH23390 (d, 1 mg/kg) administration (lane 1: 3 h, 2: 6 h, 3: 12 h, 4: 24 h, 5: 7 days, 6: 14 days).

described previously (Clary et al., 1994). A biotinylated antibody and an avidin–biotin–peroxidase complex were purchased from Vecta Labs (Burlingame, CA, USA).

2.2. Animals and drug administration

Male ddY mice (56-day-old; body weight: 25–35 g) were purchased from Keari (Osaka, Japan). They were housed in groups of two to four under a 12-h light/dark cycle with food and water available. All animal use procedures were in strict accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Drugs were dissolved in 30 μl of 0.1% acetic acid. Doses were as follows: haloperidol at 1 mg/kg ($n = 5$), (−)-sulpiride at 20 mg/kg ($n = 5$), and SCH23390 at 1 mg/kg ($n = 5$). Control animals were given 30 μl of 0.1% acetic acid ($n = 5$). The mice were killed by decapitation at 3 h, 6 h, 12 h, 24 h following a single injection of either haloperidol (1 mg/kg), (−)-sulpiride (20 mg/kg), SCH23390 (1 mg/kg) or vehicle and were killed by decapitation at 7 and 14 days following the injection of these drugs once daily for 7 or 14 consecutive days.

2.3. Western blot analysis

Protein extracts were prepared from mice whole brain. Each brain was homogenized in 5 ml buffer (50 mM Tris-acetate (pH 7.4), 10% sucrose, 5 mM EDTA, 100 μl protease inhibitor (*p*-amidinophenyl) methanesulfonyl fluoride, benzamide, leupeptin and antipain; Sigma). Homogenates were fractionated on a sucrose gradient to enrich for plasma membranes. Crude membrane fractions

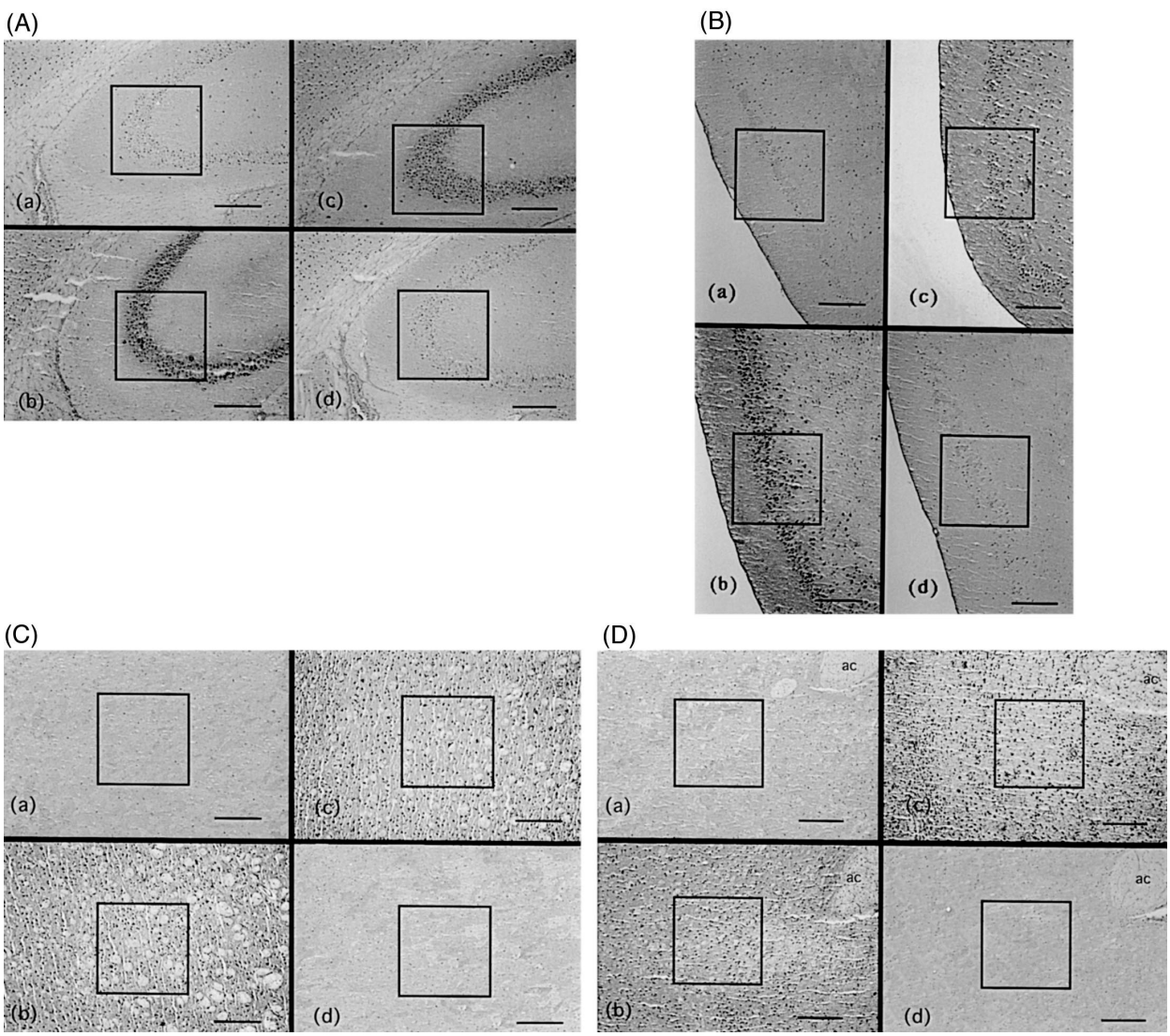

Fig. 2. Photomicrograph showing the expression of NGF protein 24 h after the last injection of a 14-day treatment with vehicle (a), haloperidol (b), (−)-sulpiride (c) or SCH23390 (d). (A) hippocampus; (B) piriform cortex; (C) striatum; (D) nucleus accumbens. Quantitative data are presented in Table 1. Each square depicts the 400 × 400-μm grid. Scale bars = 200 μm. ac: anterior commisura.

of 25 μg protein were electrophoresed on a 12–20% gradient sodium dodecyl sulfate–polyacrylamide gel, transferred to a nitrocellulose membrane, and incubated with a 1:1000 dilution of a polyclonal anti-NGF antiserum for 24 h at 4°C. Antibody binding was detected using the ECL chemiluminescence kit (Amersham, Arlington Heights, IL). Data representation and densitometric analysis were conducted using the NIH IMAGE (W. Rasband, NIMH).

2.4. Immunocytochemistry

Twenty-four hours after the last injection of the 14-day treatment with each drug, the animals were deeply anaesthetized by ether and the hearts perfused with chilled 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed rapidly, immersed in perfusion solution overnight, placed in cryoprotectant (30% sucrose in 0.1 M PBS), and then sectioned on a cryostat. Coronal sections (8 μm) were thaw-mounted on silane-coated glass slides, incubated in 1% hydrogen peroxide in methanol for 30 min (to remove endogenous peroxidase activity and enhance antibody penetration into the tissue), washed three times for 10 min with 0.01 M PBS containing 0.2% Triton X-100, incubated for 1 h in 10% normal goat serum, and were then incubated for 48 h at 4°C with rabbit polyclonal antibody to NGF protein at 1:500 dilution in immunobuffer (0.01 M PBS, 1% normal goat serum and 0.1 mg/ml bovine serum albumin). After primary antibody incubation, the sections were washed three times for 10 min in 0.01 M PBS, incubated for 60 min with a biotinylated secondary antibody (1:200 diluted in 0.01 M PBS containing 0.3% Triton X-100), followed by incubation for 60 min with an avidin–biotin–peroxidase complex (1:100 dilution in 0.01 M PBS, containing 0.3% Triton X-100), washed with 0.01 M PBS three times for 10 min, and finally placed in 50 mg/ml 3,3′-diaminobenzidine containing 0.04% hydrogen peroxide.

2.5. Data analysis

To investigate quantitative drug-induced alterations in the NGF protein level for the 14-day treatment, the numbers of immunopositive neurons in coronal sections of the hippocampus, piriform cortex, nucleus accumbens, and dorsal striatum were counted in a 400×400 μm grid using the NIH IMAGE and the corresponding number of neurons between the control and drug-administered animals was statistically analyzed by two-way analysis of variance and post hoc multiple comparison tests (Tukey test).

3. Results

Western blot analyses indicated that an increase in NGF protein level was apparent as early as 24 h after administration of either haloperidol or (−)-sulpiride. The maximal level was observed at 14 days after both haloperidol ($436 \pm 8\%$ of control, $n = 5$) and (−)-sulpiride ($345 \pm 12\%$ of control, $n = 5$) administration (Fig. 1a, b and c). Unlike the robust increase in NGF protein seen following treatment with haloperidol or (−)-sulpiride, SCH23390 administration did not have a significant effect on the NGF protein level (Fig. 1a and d).

As shown in Fig. 2a and Table 1, the number of NGF-positive neurons was significantly higher in the hippocampus (CA 2 and CA 3) after 14-day treatment with either haloperidol ($527 \pm 17\%$ of control, $n = 5$) or (−)-sulpiride ($569 \pm 20\%$ of control, $n = 5$) compared to vehicle. In other regions of the hippocampus, both haloperidol and (−)-sulpiride induced more NGF protein than did the vehicle (data not shown). In the piriform cortex (Fig. 2b), (−)-sulpiride ($406 \pm 10\%$ of control, $n = 5$) administration significantly increased the number of NGF-positive neurons, as did haloperidol ($451 \pm 17\%$ of control, $n = 5$) administration (Table 1). Additionally, haloperidol-induced NGF protein was increased significantly in both the dorsal striatum ($511 \pm 10\%$, $n = 5$) and the nucleus accumbens ($495 \pm 13\%$, $n = 5$) (Fig. 2c, d and Table 1). Fig. 2c, d and Table 1 show that a significant increase in (−)-sulpiride-induced NGF protein expression was also observed in both the dorsal striatum ($467 \pm 8\%$ of control, $n = 5$) and nucleus accumbens ($507 \pm 16\%$ of control, $n = 5$).

In contrast, the 14-day treatment with SCH23390 did not increase the number of NGF-positive neurons in the hippocampus (Fig. 2a), piriform cortex (Fig. 2b), dorsal striatum (Fig. 2c), nucleus accumbens (Fig. 2d), or any other brain region examined. In vehicle-treated mice, a few NGF-positive neurons were detected in the cortex (data not

Table 1
NGF-positive neurons in four regions from animals treated with vehicle or each drug

Region	Control	Haloperidol (1.0 mg/kg)[a]	(−)-Sulpiride (20 mg/kg)[a]	SCH23390 (1.0 mg/kg)
Hippocampus	22.6 ± 1.8	119.0 ± 3.7	128.7 ± 4.6	24.8 ± 2.9
Piriform cortex	27.8 ± 1.5	125.3 ± 4.6	112.9 ± 3.6	31.2 ± 2.2
Striatum	28.4 ± 2.4	145.1 ± 2.8	132.7 ± 2.3	25.8 ± 2.9
Nucleus accumbens	24.1 ± 2.9	119.4 ± 3.2	122.3 ± 3.8	25.7 ± 2.9

Mice were treated with either the vehicle, haloperidol (1 mg/kg body weight), (−)-sulpiride (20 mg/kg body weight), or SCH23390 (1 mg/kg body weight) once daily for 14 consecutive days. Then, 120 min after the last administration, the animals were anesthetized and the brains were rapidly removed. Values presented are the mean ± standard error of the number of NGF-positive neurons in a 400 × 400-μm grid. The data were analyzed by two-way analysis of variance, followed by multiple comparison tests (Tukey test), $n = 5$/group.

[a] $P < 0.01$ compared with control.

shown), hippocampus (Fig. 2a), piriform cortex (Fig. 2b), dorsal striatum (Fig. 2c), and nucleus accumbens (Fig. 2d).

4. Discussion

Our previous studies have shown a transcriptional regulatory pathway induced by the blockade of dopamine receptors (Ozaki et al., 1997). Induction by haloperidol and (−)-sulpiride of c-*fos* and c-*jun* mRNA expression in mouse brain is a direct effect of dopamine D_2-receptor antagonism. Furthermore, haloperidol and (−)-sulpiride induce the immediate early gene products (*Fos*, *Fos* B, *Fra*-1, *Jun* and *Jun* D) in the hippocampus, piriform cortex, dorsal striatum, and nucleus accumbens (Ozaki et al., 1998). Several reports suggest that the AP-1 transcription factor complex, consisting of DNA-binding proteins (homo- or heterodimers of the Fos–Jun family), binds with high affinity to corresponding regulatory elements (TGACTCA) in the promoter region of many genes, thereby altering their expression (Halazonetis et al., 1988). The NGF genomic sequence includes an AP-1 consensus sequence in the first intron of the gene. Our previous study has demonstrated that following injection of the drugs, a significant increase in AP-1 DNA binding activity leads to haloperidol- and (−)-sulpiride-induced NGF mRNA expression in the hippocampus, piriform cortex, dorsal striatum, and nucleus accumbens (Ozaki et al., 1999). Furthermore, after chronic injection for 14 consecutive days, NGF protein was increased significantly in the hippocampus, piriform cortex, dorsal striatum, and nucleus accumbens (Fig. 2a, b, c, and d). In contrast, SCH23390 did not induce either NGF mRNA or protein, even though AP-1 DNA binding activity was significantly increased (Ozaki et al., 1997, 1999). These results suggest that the transcription of NGF mRNA was not induced, even though an AP-1 complex composed of Fos B, Fra-1, and Jun D does bind to the AP-1 consensus sequence (Ozaki et al., 1998). The expression of c-*fos* mRNA 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). That the mechanism is thought to involve the induction of the c-*fos* gene as a result of NMDA receptor activation (Sonnenberg et al., 1989; Dragunow et al., 1990; Aronin et al., 1991) is supported by the fact that 2-amino-5-phosphonovalerate, a NMDA-receptor antagonist, has been reported to block haloperidol-mediated Fos induction (Aronin et al., 1991). Thus, we suggest that both NGF mRNA and protein induced by haloperidol or (−)-sulpiride are increased by a pharmacological interaction between an increase in glutamate concentration in the synaptic cleft and AP-1 DNA binding activity; glutamate appears to induce Fos- and Jun-related proteins via a mechanism involving the NMDA receptor.

NGF can promote the survival of cholinergic neurons after experimental injury (Morse et al., 1993; Koliatos et al., 1994; Venero et al., 1994); NGF can also promote the sprouting of intact cholinergic neurons (Heisenberg et al., 1994) and chronic administration in vivo or in vitro enhances synthesis, storage, and release of acetylcholine (Lapchak et al., 1992) and high-affinity choline uptake (Pelleymounter et al., 1996). NGF enhances the release of acetylcholine from synaptosomes of rat hippocampus and piriform cortex, and NGF rapidly increases high-affinity choline transport into synaptosomes (Knipper et al., 1994). Alterations in NGF levels in the hippocampus and piriform cortex may alter the activity of cholinergic neurons and contribute to the behavioural effects of nicotine on attention and related tasks.

NGF can modify behaviours that are related to the activity of the cholinergic system. NGF treatment of cognitively impaired rats increases performance in a Morris water maze task (Gage et al., 1988). An anti-NGF antibody produces perseveration and inflexible behaviour in Morris water maze consecutive extinction trials (Van der Zee et al., 1995). A reciprocal interaction between NGF and acetylcholine may, therefore, represent a mechanism for controlling synaptic efficiency in the cholinergic cortico-hippocampal system, with the consequence of enhanced cognition.

Decreased dopaminergic tone, induced by neuroleptic agents, might lead to glutamatergic predominance, increased striatal activity, and reduced sensory input reaching the cortex via the projection pathways from nucleus accumbens and, thus, to a state like that induced by tonic electrical stimulation of the striatum. The striatum is generally assumed to regulate motility; the dorsal striatum is often thought to be involved in motor functions, which would predict extrapyramidal side effects (Carlsson et al., 1997). Therefore, in the dorsal striatum and nucleus accumbens, NGF enhances the cholinergic innervation related to late-onset extrapyramidal side effects.

Based on our current findings, we suggest that the high concentrations of NGF protein induced by haloperidol and (−)-sulpiride in the hippocampus, piriform cortex, dorsal striatum, and nucleus accumbens, in association with enhanced cognition may represent a mechanism for controlling synaptic efficiency in the cholinergic cortico-hippocampal system, and may be responsible for late-onset extrapyramidal side effects that predominate in the striatum and nucleus accumbens (Carlsson et al., 1997).

In conclusion, Haloperidol and (−)-sulpiride induced c-*fos* and c-*jun* mRNAs and the transcriptional products of these genes by inhibition of dopamine D_2 neurons (Ozaki et al., 1997). Finally, these neuroleptics induced NGF protein by increasing AP-1 DNA binding activity in the hippocampus, piriform cortex, dorsal striatum, and nucleus accumbens. Our results suggest that in the hippocampus and piriform cortex, these neuroleptics induced NGF, which is required for the development and maintenance of neurons, having the consequence of enhancing cognition. On the other hand, induced NGF in the striatum and nucleus accumbens causes late-onset extrapyramidal side effects.

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