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Short communication

Dopamine receptor antagonists block nerve growth factor-induced hyperactivity

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Abstract

The role of dopamine receptors in mediating nerve growth factor (NGF)-induced locomotor stimulation was investigated by examining the effects of selective dopamine D_1 and D_2 receptor antagonists on the motor hyperactivity induced by NGF. A single intracerebroventricular administration of NGF (5.1 μg) increased locomotor activity immediately after injection in normal adult rats. This hyperactivity was partly blocked by the dopamine D_1 receptor antagonist SCH23390 (*R*-(+)-7-chloro-2,3,4,5-tetrahydro-3-methyl-1-phenyl-1*H*-3-benzazepine-8-ol) and by the dopamine D_2 antagonist raclopride ((*S*)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl)methyl)-2-hydroxy-6-methoxybenzamide). Effective doses of raclopride did not alter spontaneous levels of activity in control rats. These results suggest that stimulation of both subtypes of dopamine receptors is necessary for eliciting NGF-induced hyperactivity in the rat. The role of the dopamine D_2 receptor in mediating the behavioral actions of NGF appears to be more important than that of the dopamine D_1 receptor.

Keywords: NGF (nerve growth factor); Locomotion; Acetylcholine; Nicotinic receptor; Dopamine receptor antagonist

1. Introduction

Nerve growth factor (NGF) is the most extensively characterized neurotrophin, and it appears to be crucial in maintaining the function of cholinergic neurons in the central nervous system (CNS), particularly the magnocellular neurons in the basal forebrain, diagonal band and nucleus basalis (Ebendal, 1989, 1992). For example, continuous intracerebroventricular (i.c.v.) infusion of NGF can reverse the cholinergic cell body atrophy and improve retention of spatial memory in behaviorally impaired aged rats (Fischer et al., 1987). In addition to these chronic effects of NGF on cholinergic neurons, recent in vivo and in vitro studies have demonstrated that NGF also has short-term pharmacological actions on cholinergic neurons (Levi and Alema, 1991; Palmer et al., 1993; Knipper et al., 1994; Pitchford et al., 1995). We have found that a single i.c.v. administration of NGF increases locomotor activity in the rat immediately after injection (Kobayashi et al., 1997). The hyperlocomotion induced by NGF (5.1 μg) lasts for at least 3–4 h after i.c.v. injection, and it is inhibited by the central nicotinic receptor antagonist mecamylamine but not by the muscarinic receptor antagonist scopolamine. These data suggest that i.c.v.-administered NGF induces rapid acetylcholine release from cholinergic neurons, and that nicotinic receptors are involved in the NGF-induced hyperactivity (Kobayashi et al., 1997). However, the exact mechanisms by which NGF induces motor hyperactivity are not known.

Previous studies have shown that nicotine evokes dopamine release in vitro and in vivo. Nicotinic receptors appear to be located in both the somatodendritic region and the terminals of dopamine neurons (Schwartz et al., 1984; Clarke and Pert, 1985), and it is likely that nicotine induces dopamine release by stimulating nicotinic receptors located on the dopamine neurons (Wonnacott et al., 1990; Nisell et al., 1994). In addition, behavioral studies have shown involvement of dopamine receptors in the hyperactivity induced by nicotine (O'Neill et al., 1991; Damaj and Martin, 1993). It is possible, therefore, that the dopaminergic system may be indirectly involved in hyperactivity induced by NGF. To test this possibility, we have

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now examined the ability of dopamine receptor antagonists to modulate NGF-induced hyperactivity.

2. Materials and methods

2.1. Animals and drugs

We used male Sprague-Dawley rats (Alab, Sweden), weighing 190–220 g at the beginning of the experimental procedures and housed in air-conditioned rooms with a regular light-dark cycle (light on at 07:00 h and off at 18:00 h). β-NGF was purified from male mouse submandibular glands as previously described (Mobley et al., 1976; Ebendal et al., 1984). The following dopamine receptor antagonists were used: SCH23390 (*R*-(+)-7-chloro-2,3,4,5-tetrahydro-3-methyl-1-phenyl-1*H*-3-benzazepine-8-ol, Research Biochemicals International, USA), a dopamine D_1 receptor antagonist (O'Neill et al., 1991; Damaj and Martin, 1993) and raclopride ((*S*)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl)methyl)-2-hydroxy-6-methoxybenzamide, Astra Arcus, Sweden), a dopamine D_2 receptor antagonist (Ögren et al., 1986). The antagonists were dissolved in 0.9% saline, and all systemic injections were subcutaneous into the scruff of the neck in volumes of 1.0 ml/kg.

2.2. Surgical procedures

Rats were anesthetized with halothane and 26-gauge guide cannulae (3.8 mm in length, Single cannula system, Plastics One, USA) for i.c.v. injection were implanted over the right lateral ventricle (coordinates: 1.2 mm posterior to bregma, 1.4 mm lateral to the midline). All cannulae were embedded with dental cement and anchored to the skull with steel screws.

2.3. Locomotor activity testing

Seven to 10 days after cannula implantation, spontaneous locomotor activity was measured immediately after i.c.v. administration of NGF. The animals were habituated for 60 min in the test room. A dopamine receptor antagonist or 0.9% saline was then injected systemically 15 min prior to i.c.v. administration of NGF or cytochrome *c*. Cytochrome *c* was chosen in the control group because its molecular weight is similar to that of neurotrophins while having no known extracellular actions. Mouse β-NGF (5.1 μg in 10 μl Ringer solution) or cytochrome *c* (5 μg in 10 μl Ringer solution) was injected into the ventricle through a 33-gauge internal injection cannula (4.3 mm in length) connected via poly-propylene tubing to a microsyringe. This could be inserted manually into the fixed guide cannula without significantly disturbing the behavior of awake animals. The rats were restrained gently by hand while the drug was injected at a rate of 5 μl/min, with the internal cannula left in place for an additional 1 min before retracting it.

Immediately after i.c.v. injection, the animals were placed individually in 12 locomotor cages ($25 \times 40 \times 30$ cm) for a 2 h period during the morning. The cages were equipped with arrays of horizontal and vertical photocell detectors. Motility, locomotion and rearing (vertical activity) were simultaneously recorded by a computerized system using infrared photocells to detect movements of the rats (Ögren et al., 1979). Motility is defined as a movement covering a distance of 4 cm in any direction, and locomotion is defined as a movement covering 8 photocells or 32 cm. Photocells located 13 cm over the cage floor detected the number of times the rat stood on its hind limbs (rearing). All experiments were based on independent subject design. Each subject was used once only. After completion of the experiment, the location of the cannula tip was confirmed by histological examination of serial brain sections. Animals were excluded from further analysis if the cannula failed to hit the ventricle.

2.4. Statistical analysis

The results obtained in the study of locomotor activity were analyzed by analysis of variance (ANOVA) followed by Fisher protected least-significant difference (PLSD) multiple comparison test. The criterion set for statistical significance was $P < 0.05$.

3. Results

Figs. 1 and 2 show the accumulated counts of photocell beam interruptions during a 30–120 min period after i.c.v. injection of NGF or cytochrome *c*. This time interval was chosen because our previous studies demonstrated that the most pronounced difference in locomotor activity occurred during this period (Kobayashi et al., 1997). NGF produced a significant increase in both motility and locomotion ($F(5,42) = 4.996$ and 3.834, respectively; $P < 0.01$) (Fig. 1A and B). In contrast, the NGF group showed a mean decrease in rearing, although this difference was not statistically significant (Fig. 1C).

The selective dopamine D_1 receptor antagonist SCH23390 tended to reduce NGF-induced motility and locomotion in a dose-dependent manner (Fig. 1A and B). The 0.01 mg/kg dose, but not the 0.001 mg/kg dose of SCH23390 significantly reduced the NGF-induced increase in motility and locomotion ($P < 0.05$ for both motility and locomotion). These doses of SCH23390 did not significantly reduce locomotor activity in the cytochrome *c* group, although the 0.01 mg/kg dose had suppressive effects on all aspects of locomotor activity.

The 0.015 mg/kg dose of raclopride, the selective dopamine D_2 receptor antagonist, had no significant ef-

fects in either the NGF or the cytochrome *c* group (Fig. 2). The 0.05 mg/kg dose of raclopride tended to reduce NGF-induced increases of motility, although not significantly (Fig. 2A). The 0.05 mg/kg dose of raclopride significantly reduced NGF-induced effects on locomotion

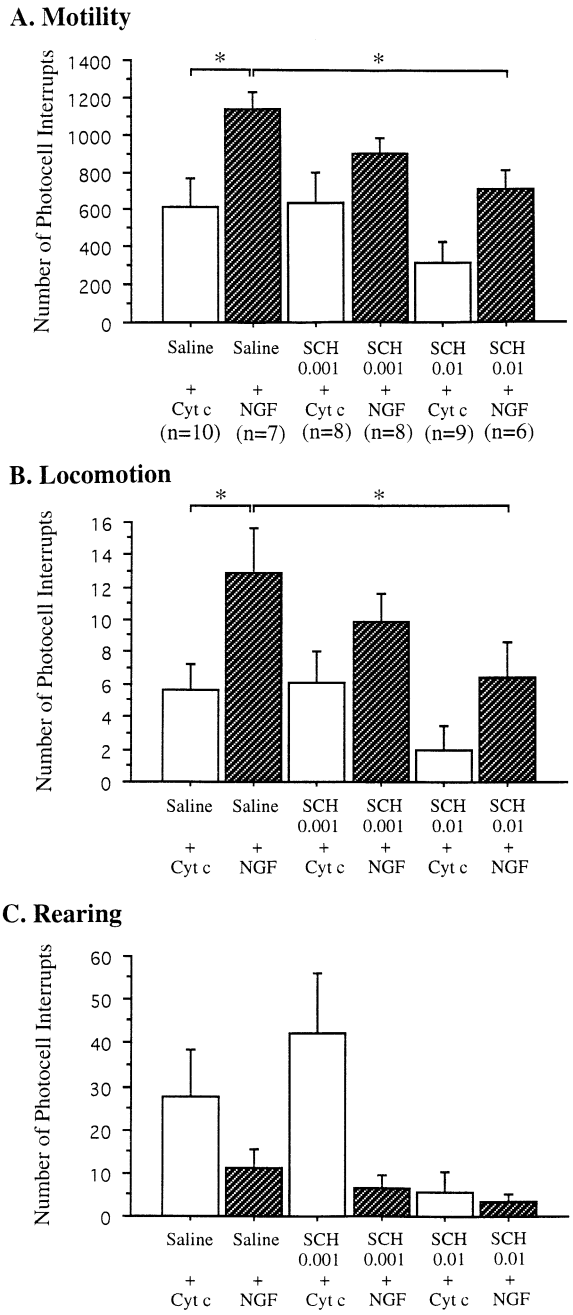

Fig. 1. Effect of SCH23390 on hyperlocomotion induced by NGF. (A) motility, (B) locomotion, (C) rearing. Open columns show data for animals receiving i.c.v. injections of cytochrome *c*, hatched columns show data for animals receiving i.c.v. injections of NGF. Data show the accumulated mean values and standard errors for the 90 min measurement period (from 30 to 120 min after i.c.v. injection of NGF or cytochrome *c*) ($n = 6$–10). Symbols indicate significant difference as revealed by Fisher PLSD multiple comparison test after ANOVA. $^{*}P < 0.05$.

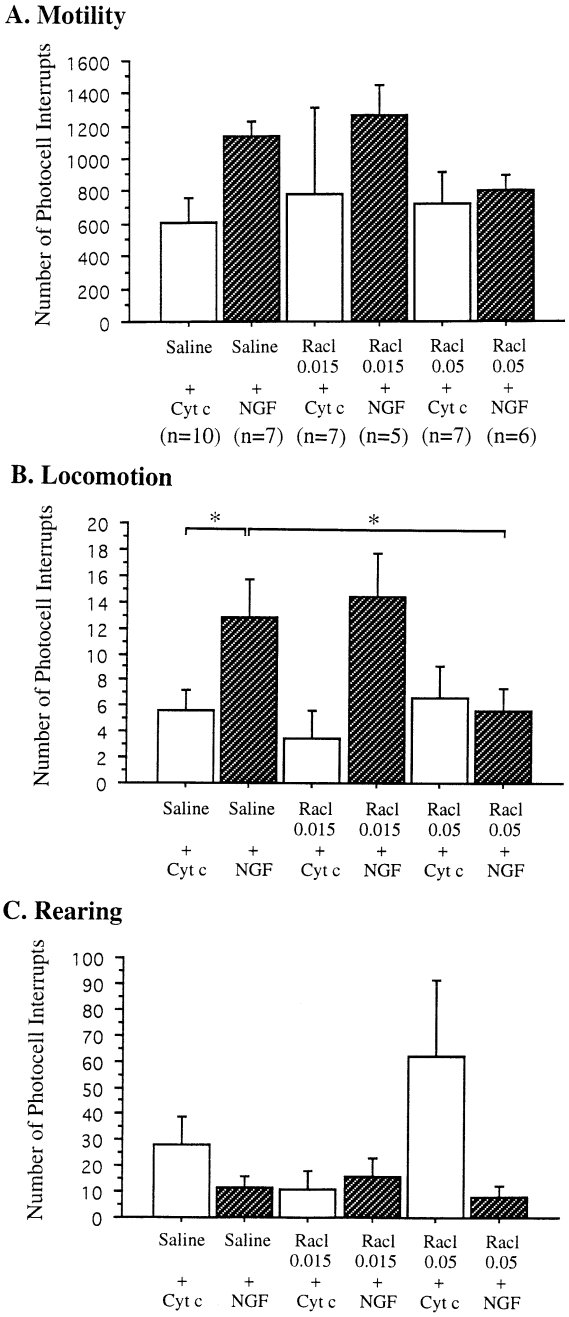

Fig. 2. Effect of raclopride on hyperlocomotion induced by NGF. (A) motility, (B) locomotion, (C) rearing. Open columns show data for animals receiving i.c.v. injections of cytochrome *c*, hatched columns show data for animals receiving i.c.v. injections of NGF. Data show the accumulated mean values and standard errors for the 90 min measurement period (from 30 to 120 min after i.c.v. injection of NGF or cytochrome *c*) ($n = 5$–10). Symbols indicate significant difference as revealed by Fisher PLSD multiple comparison test after ANOVA. $^{*}P < 0.05$.

($F(5,36) = 3.352$, $P < 0.05$) (Fig. 2B). This dose of raclopride had no significant effects on locomotor activity in the cytochrome *c* group.

4. Discussion

In the present studies, we have demonstrated that the pretreatment with SCH23390, a selective dopamine D_1 receptor antagonist, and raclopride, a selective dopamine D_2 receptor antagonist, significantly reduced hyperactivity induced by NGF delivered into the cerebroventricular cerebrospinal fluid (CSF). These results imply that the stimulatory effects of NGF on locomotor activity involve activation of both D_1 and D_2 receptors. We have previously demonstrated that the effects of NGF on ambulation are blocked by nicotinic receptor antagonists but not by muscarinic receptor antagonists. Taken together, these findings suggest that i.c.v.-administered NGF induces acute acetylcholine release which results in stimulation of nicotinic receptors located on the dopamine neurons. Nicotinic receptor activation then causes release of dopamine stimulating both the D_1 and the D_2 subtypes of dopamine receptors leading to increased locomotor activity.

The dopamine D_1 and D_2 receptors are subdivided based on the ability to activate adenylate cyclase (D_1), or the ability to attenuate the activity of this enzyme (D_2) (Kebabian and Calne, 1979). Previous reports suggest that D_1 and D_2 receptors interact in either synergistic or opposing fashion. Dopamine D_1 and D_2 receptors interact synergistically to stimulate locomotion and to induce stereotypy (Arnt et al., 1987). It is likely that the concomitant stimulation of D_1 receptors is essential for the full expression of D_2 receptor-mediated motor events (Starr and Starr, 1989).

The hyperactivity induced by NGF was blocked by raclopride at a dose that did not affect spontaneous levels of activity in the cytochrome *c* group. On the other hand, the 0.01 mg/kg dose of SCH23390 had suppressive effects in the cytochrome *c* group, although it significantly reduced hyperactivity induced by NGF. These results suggest that the role of the dopamine D_2 receptor in mediating the behavioral action of i.c.v.-administered NGF is more important than that of the dopamine D_1 receptor.

The brain areas where NGF acts to bring about behavioral changes following i.c.v. delivery have not been identified. Our immunohistochemical studies demonstrated that NGF diffused readily from the ventricular space into brain parenchyma after an i.c.v. injection (Kobayashi et al., 1997). NGF should have direct access to striatum via its medial surface facing the lateral ventricle. Intrastriatal cholinergic neurons give rise to a dense cholinergic innervation. Therefore, it is not unlikely that striatum would play a major role in the stimulatory effect of NGF on locomotor activity. However, examination of locomotor activity immediately after NGF administration directly into the dorsal striatum revealed no significant effects on locomotor activity within 4 h after injection, although immunohistochemical data suggested diffuse and widespread distribution of NGF in the entire body of striatum 1 h after injection (Kobayashi et al., in preparation). These results do not support the hypothesis that the behavioral effects of NGF are mediated directly by striatum.

NGF produced significant increases of horizontal ambulatory components of locomotor activity (motility and locomotion) but reductions in vertical movement (rearing). Previous reports suggest that locomotor effects are mediated primarily via the mesolimbic dopaminergic systems, whereas the stereotyped behaviors are mediated mainly via the nigrostriatal system (Asher and Aghajanian, 1974; Kelly et al., 1975). Therefore, it appears likely that the mesolimbic dopaminergic system is involved in the NGF-induced locomotor hyperactivity. In addition, recent reports strongly suggest that the ventral tegmental area may be involved in the mediation of nicotine-induced hyperactivity. Microinjection of nicotine into the ventral tegmental area containing the cell bodies of the mesolimbic dopaminergic system can increase locomotor activity in rats (Reavill and Stolerman, 1990). Neurotoxin lesions of the mesolimbic dopamine pathway, on the other hand, can attenuate or abolish the locomotor activity of nicotine (Clarke et al., 1988). Taken together, our data suggest that NGF delivered into the CSF may act on nicotinic receptors in the mesolimbic dopamine neurons more potently than on receptors in the nigrostriatal dopamine neurons.

In conclusion, we have demonstrated that NGF-induced hyperactivity is partly blocked by the selective dopamine D_1 receptor antagonist SCH23390 and by the selective dopamine D_2 receptor antagonist raclopride. These results suggest that stimulation of both subtypes of dopamine receptors is necessary for eliciting NGF-induced hyperactivity. Taken together with our previous results, we now suggest that i.c.v. administration of NGF leads to acetylcholine release which via nicotinic receptors on mesolimbic dopamine neurons causes release of dopamine eliciting the behavior responses.

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