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European Journal of Pharmacology 428 (2001) 227–233

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Antiparkinson-like effects of a novel neurotensin analog in unilaterally 6-hydroxydopamine lesioned rats

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Received 3 May 2001; received in revised form 24 July 2001; accepted 31 July 2001

Abstract

Parkinson's disease is a neuropathological disorder involving the degeneration of dopamine neurons in the substantia nigra, with the resultant loss of their terminals in the striatum. This dopamine loss causes most of the motor disturbances associated with the disease. One animal model of Parkinson's disease involves destruction of the nigrostriatal pathway with a neurotoxin (6-hydroxydopamine) injected into this pathway. In unilaterally lesioned animals, injection of D-amphetamine causes rotation towards the lesioned side, while injection of apomorphine acting upon supersensitive postsynaptic dopamine receptors causes rotation away from the lesioned side. In this study, we tested the effects of acute and subchronic injection of a neurotensin analog (NT69L) on the rotational behavior induced by D-amphetamine (5 mg/kg) or apomorphine (600 μg/kg) in unilaterally 6-hydroxydopamine lesioned rats. Pretreatment of animals with intraperitoneal injections of NT69L (1 mg/kg) resulted in a significant reduction of apomorphine-induced contralateral rotation and D-amphetamine-induced ipsilateral rotation in these lesioned rats with an ED_{50} of 40 and 80 μg/kg, respectively. After three daily injections of NT69L, its effects on this rotational behavior were unchanged, suggesting that no tolerance develops to this effect of NT69L. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Parkinson's disease; Neurotensin; 6-Hydroxydopamine

1. Introduction

Parkinson's disease is a progressive neurological disorder characterized biochemically by the degeneration of dopamine neurons in the substantia nigra, which results in the loss of the dopaminergic projections to the striatum. This loss of dopamine is responsible for most of the motor disturbances associated with the disease. Other neurotransmitter systems, including such neuropeptides as neurotensin, have been implicated in the pathophysiology of Parkinson's disease and neurotensin levels are reduced in post-mortem brains Parkinson's disease patients (Bissette et al., 1985). Neurotensin is an endogenous tridecapeptide that behaves as a neurotransmitter or neuromodulator in the central nervous system (Tyler-McMahon et al., 2000a). It interacts with serotonergic, glutamatergic, and cholinergic neurotransmitter systems (Kinkead et al., 1999), as well as other brain amines (Drumheller et al., 1990; Euler et al., 1990; Myers et al., 1986; Nemeroff et al., 1982; Quirion et al., 1992).

The association of neurotensin with central dopamine systems and their interactions anatomically, physiologically, and behaviorally have been amply studied and reviewed (Kasckow and Nemeroff, 1991). Neurotensin-containing neurons are closely associated neuroanatomically with brain dopamine systems (Drumheller et al., 1990; Kinkead and Nemeroff, 1994). Radioimmunoassay and immunohistochemical analyses of the brain distribution of neurotensin reveal that the peptide is found mainly in areas where dopamine systems are present (Palacios and Kuhar, 1981; Rostène et al., 1997). The influence of neurotensin on dopaminergic transmission in nigrostriatal and mesocortical pathways and the neurotensin/dopamine interactions kindled many studies suggesting that neurotensin might play a role in the pathophysiology of

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PII: S0014-2999(01)01260-2

several central nervous system disorders including Parkinson's disease and schizophrenia (Garver et al., 1991; Kitabgi et al., 1989; Lambert et al., 1995; Nemeroff, 1986; Rostène et al., 1992).

Early studies have shown that in Parkinson's disease, nigral neurotensin receptors as well as neurotensin receptors in other dopaminergic areas are markedly reduced (Cadet et al., 1991; Chinaglia et al., 1990; Fernandez et al., 1994; Sadoul et al., 1984; Uhl et al., 1984). This has led to the suggestion that neurotensin related agents acting centrally could possibly supplement dopaminergic agonists to augment the function of the remaining dopaminergic neurons in Parkinson's disease (Uhl et al., 1984). More recently, our laboratory showed that neurotensin receptor subtype 1 (neurotensin NTS1 receptor) mRNA is present in high levels in melanized neurons (presumably dopaminergic) of substantia nigra pars compacta and nucleus paranigralis. In this study, we showed that in Parkinson's disease brains there are markedly fewer dopaminergic neurons and correspondingly very low levels or no expression of mRNA for neurotensin NTS1 receptors (Yamada et al., 1995).

Using animal models for Parkinson's disease, researchers have measured neurotensin receptor expression and neurotensin-immunoreactivity in brain. In 6-hydroxydopamine lesioned rats, an increase in neurotensin-immunoreactivity has been found in the striatum and globus pallidus, with no changes in substantia nigra except with L-dihydroxyphenylalanine (L-DOPA) treatment (Taylor et al., 1992). More recently, in the same experimental paradigm, a marked increase in neurotensin precursor mRNA was found in the caudate (Hanson and Keefe, 1999). Lesions of the nigrostriatal pathway in rats result in the subsequent loss of a large proportion of neurotensin receptors in the substantia nigra and striatum (Palacios and Kuhar, 1981; Quirion et al., 1985). Interestingly, in 1-methyl,4-phenyl 1,2,5,6-tetrahydropyridine (MPTP)-lesioned monkeys, another animal model for Parkinson's disease, decreases in striatal neurotensin binding sites were less than expected, based on reductions in markers for dopaminergic neurons (Goulet et al., 1999). Thus, in the substantia nigra in Parkinson's disease, there may be a proportion of neurotensin receptors remaining, which may be sufficient to mediate neurotensin effects (Fernandez et al., 1995).

For neurotensin to cause its central nervous system effects, it must be delivered directly into the brain, since it is rapidly degraded by peptidases upon systemic administration. Our laboratory has been successful in developing a neurotensin analog (called "NT69L" or [*N*-methyl-Arg^{8},L-Lys^{9},L-*neo*-Trp^{11},*tert*-Leu^{12}]NT-(8–13)) that crosses the blood–brain barrier, and retains all the classic properties of neurotensin, such as hypothermia and antinociception (Tyler et al., 1999). Additionally, it blocks the haloperidol-induced catalepsy and apomorphine-induced climbing (Cusack et al., 2000), supporting the fact that NT69L interacts with dopamine neurotransmitter systems, similar to the native neurotensin. Here, we report that NT69L also blocked apomorphine-induced contralateral and D-amphetamine-induced ipsilateral turning behavior in unilaterally 6-hydroxydopamine-lesioned rats, an animal model of Parkinson's disease.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats with unilateral 6-hydroxydopamine lesions were used in these experiment. These rats were purchased from Zivic Laboratories (Zelienopole, PA) lesioned in the left nigrostriatal pathway as follows. Rats (180 g) were pretreated with 0.5 ml desipramine (12.5 mg/ml in saline) one-half to 1 h before the surgery. Under sodium pentobarbital (50 mg/kg, i.p.) anesthesia , the rats were cleaned with betadine, the interauricular area shaved and the animal mounted in a stereotaxic frame and maintained at 37 °C using a heating pad. The rats then had a small incision made and were injected with 2 μl of a 6-hydroxydopamine solution (4 mg of free base in 0.75 ml saline with 0.2 mg/ml ascorbic acid) in the left medial forebrain bundle (A: −1.5, L: 1.8, V: 7.5, with the incisor bar at +2.5) (Paxinos and Watson, 1997). The 6-hydroxydopamine solution was delivered over a period of 3 min using an infusion pump (Harvard Apparatus). The incision was closed using sterile staples. The rats were allowed to recover in individual cages and 21 days after surgery, the lesioned rats were challenged with 600 μg/kg apomorphine s.c. To assure proper lesioning, only rats showing tight head to tail contralateral rotation were used in the experiment (Taylor et al., 1992). Rats were kept in individual cages in a temperature controlled room with a 12-h dark/light cycle and were maintained on a commercial rat chow diet and water ad lib.

On the day of testing, the rats were injected with saline or NT69L (1 mg/kg) i.p. or saline and 30 min later injected with either apomorphine (600 μg/kg, s.c.) or D-amphetamine (5 mg/kg, i.p.). Rats were monitored for apomorphine-induced contralateral turning or for D-amphetamine-induced ipsilateral turning for 1 h as described by Ungerstedt and Arbuthnott (1970).

To determine the ED_{50} for the ability of NT69L to attenuate the turning behavior induced by apomorphine or amphetamine in the 6-hydroxydopamine lesioned rats, groups of four rats each were injected with varying doses of NT69L i.p. and 30 min later injected with apomorphine (600 μg/kg, s.c.) or amphetamine (5 mg/kg, i.p.) and monitored for turning behavior for 1 h. A turn consisted of a 360° rotation either ipsilateral or contralateral from the side of lesion. Turns were recorded every minute for 60 min and averaged at 10-min intervals. The 30-min average

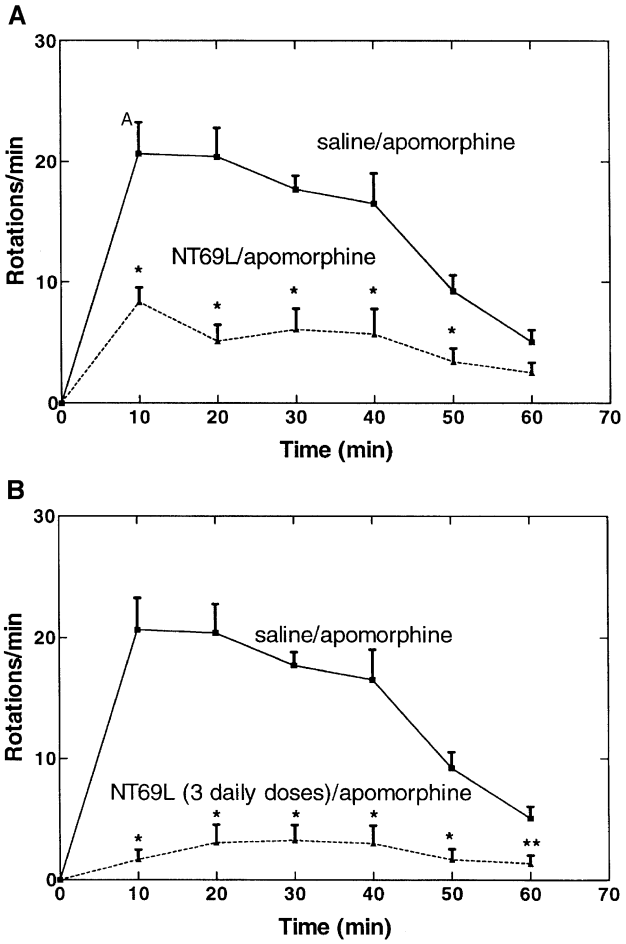

Fig. 1. Effect of a single (A) or three daily (B) injections of NT69L or saline on apomorphine-induced contralateral turning behavior in unilaterally 6-hydroxydopamine lesioned rats. In all cases, $n = 4$ and the NT69L dose was 1 mg/kg i.p. Thirty minutes after the injection of NT69L or saline, rats were injected with apomorphine (600 μg/kg s.c.) ($T = 0$). Contralateral rotation was then recorded for 1 h after apomorphine injection. * Significantly different from "saline/apomorphine" ($P < 0.05$).

was used to calculate the dose response. Rats were used only once.

For testing for tolerance, different groups of rats were injected daily for 3 days with either saline or NT69L (1 mg/kg). After the third daily injection, the rats were injected with either apomorphine or D-amphetamine at the above-mentioned doses, 30 min after saline or NT69L. The number of turns per minute was again recorded for 1 h.

2.2. Striatal dopamine

Analysis of brain dopamine was done using high performance liquid chromatography with electrochemical detection (HPLC-EC, ESA, Chelmsford, MA), according to previously described procedures (Kilts et al., 1981; Krstulovic, 1982). The 6-hydroxydopamine lesioned rats were injected with either saline or NT69L (once or daily for 3 days). The rats were sacrificed 30 min after the last injection and the striatal tissue were weighed and homogenized in 10 vol. of ice-cold 0.1 N perchloric acid containing 10 mM EDTA. The tissue homogenates were centrifuged for 10 min (26,000 × g, 4 °C). Twenty microliters of the diluted supernatant was injected onto the HPLC system with MD-150/RP-C18 column (ESA). The mobile phase was 10% acetonitrile containing 75 mM sodium dihydrogen phosphate, 1.7 mM 1-octane sulfonic acid sodium salt, 0.01% triethylamine, and 25 μM EDTA, pH 3.0. The flow rate was maintained at 0.5 ml/min. The detector potentials were set at guard cell: 350 mV; E1: −175 mV and E2: 250 mV. The concentration of dopamine in each sample was quantified by comparison of peak heights between samples and external standards run on the same day.

2.3. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, using Sigma Stat software, with $P < 0.05$ being considered significant. Graphs and ED_{50} data were generated with the use of GraphPad Software (San Diego, CA). Comparisons between treatments in Fig. 5 were done by the Mann–Whitney Rank Sum Test.

3. Results

3.1. Reversal of apomorphine-induced rotation

Injection of the dopamine agonist apomorphine (600 μg/kg, s.c.) caused the 6-hydroxydopamine lesioned rats to rotate contralateral to the side of lesion. Administration of NT69L (1 mg/kg) significantly ($P < 0.01$) reduced the

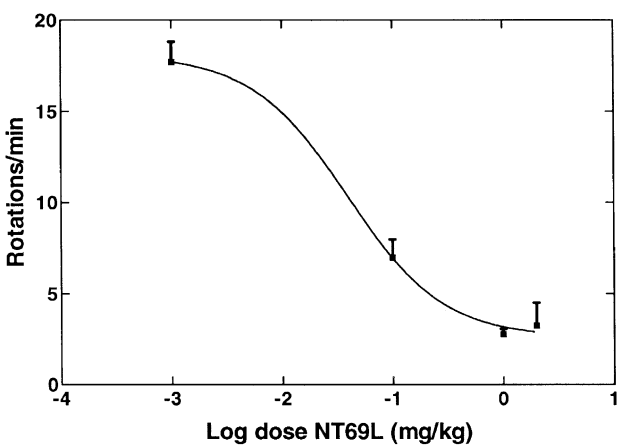

Fig. 2. Dose–response for NT69L for the reversal of apomorphine-induced contralateral turning behavior in unilaterally 6-hydroxydopamine lesioned rats. The experimental design was the same as that described in Fig. 1A, except that the dose of NT69L was varied as indicated. The time of measurement of rotation was 30 min after apomorphine injection.

number of rotations per minute (Fig. 1A) After three daily injections of NT69L (1 mg/kg) followed by an injection of apomorphine on the third day, NT69L again caused a significant reduction of contralateral rotation (Fig. 1B). With a one-time administration of NT69L, its ED_{50} for reversing the apomorphine-induced contralateral rotation at 30 min after injection of apomorphine was 40 μg/kg (determined by non-linear regression analysis, 95% confidence interval: 15 to 102 μg/kg; $R = 0.96$) (Fig. 2).

3.2. Reversal of D-amphetamine-induced rotation

Injection of D-amphetamine (5 mg/kg, i.p.) caused the unilaterally 6-hydroxydopamine lesioned rats to rotate toward the side of lesion. Administration of NT69L (1 mg/kg, i.p.) significantly ($P < 0.01$) reduced the number of rotations per minute (Fig. 3A). After three daily injections of NT69L (1 mg/kg) followed by an injection of D-amphetamine on the third day, NT69L again caused a significant reduction of D-amphetamine-induced rotation (Fig. 3B). With a one-time administration of NT69L, its ED_{50} for reversing the ipsilateral D-amphetamine-induced rotation at 30 min after injection of D-amphetamine was 80 μg/kg (determined by non-linear regression analysis, 95% confidence interval: 16 to 445 μg/kg; $R = 0.84$) (Fig. 4).

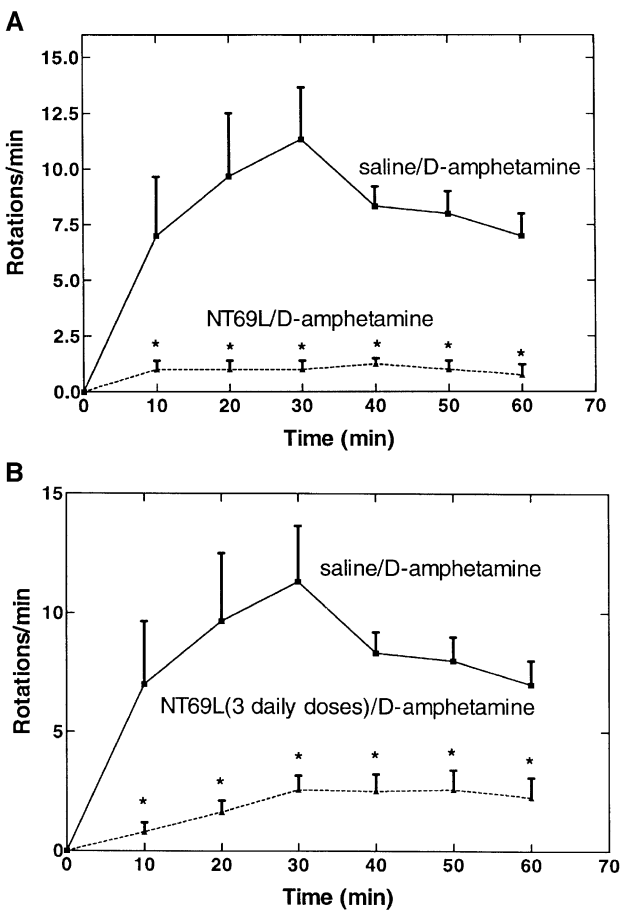

Fig. 3. Effect of a single (A) or three daily (B) injections of NT69L or saline on D-amphetamine-induced ipsilateral turning behavior in unilaterally 6-hydroxydopamine lesioned rats. In all cases, $n = 4$ and the NT69L dose was 1 mg/kg i.p. Thirty minutes after the injection of NT69L or saline, rats were injected with D-amphetamine (5 mg/kg i.p.) ($T = 0$). The ipsilateral rotation was then recorded for 1 h after D-amphetamine injection. * Significantly different from "saline/D-amphetamine" ($P < 0.05$).

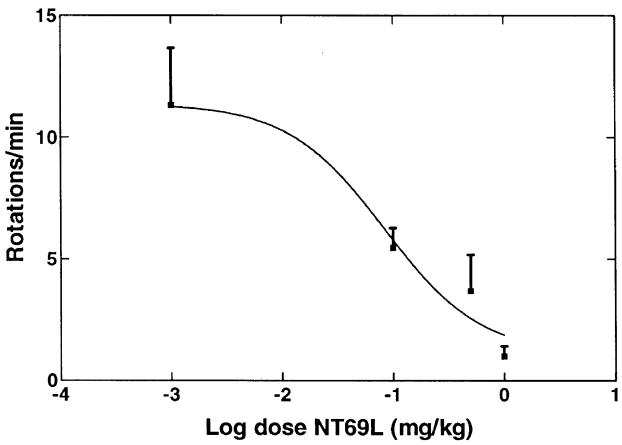

Fig. 4. Dose–response for NT69L and the reversal of D-amphetamine-induced ipsilateral turning behavior in unilaterally 6-hydroxydopamine lesioned rats. The experimental design was the same as that described in Fig. 3A, except that the dose of NT69L was varied as indicated. The time of measurement of rotation was 30 min after D-amphetamine injection.

3.3. Dopamine levels in the striatum

Treatment with 6-hydroxydopamine on one side of the rat brain resulted in a greater than 90% reduction of

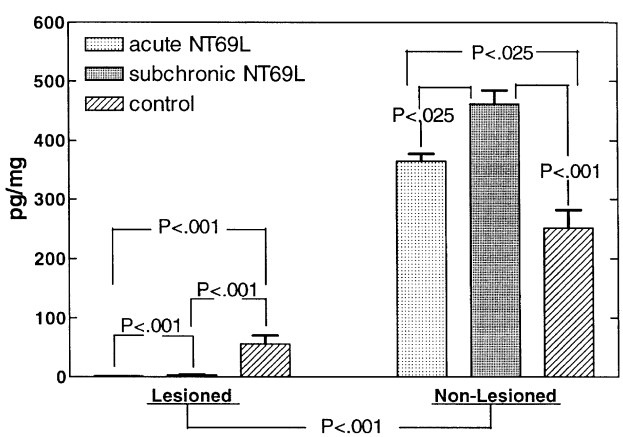

Fig. 5. Effect of 6-hydroxydopamine lesioning and NT69L on striatal dopamine in the lesioned and the non-lesioned side. 6-Hydroxydopamine lesioned rats were injected with saline (control), NT69L (1 mg/kg i.p.) once (acute) or NT69L (1 mg/kg i.p.) or saline daily for 3 days (subchronic). Rats were sacrificed 30 min after the last injection and brains were harvested on ice and kept at −80 °C until assayed. The striatal tissue was homogenized, centrifuged and assayed on HPLC-ED. Comparisons between treatments were done by the Mann–Whitney Rank Sum Test and the level of significance is indicated. For each respective comparison between lesioned and non-lesioned sides, the difference was significant ($P < 0.001$), as indicated below the horizontal axis.

dopamine in the ipsilateral striatum, compared to the levels in the non-lesioned side (Fig. 5). Interestingly, acute or subchronic NT69L significantly increased dopamine levels on the non-lesioned side, while these treatments significantly decreased levels on the lesioned side. Thus, dopamine levels on the non-lesioned side after the third daily injection were significantly greater than those after a single injection of NT69L compared to control levels or to levels after a single dose of NT69L (Fig. 5). Additionally, on the lesioned side the levels of dopamine were significantly greater after three daily injections, compared to those after a single injection.

4. Discussion

NT69L is an analog of neurotensin-(8-13), the active part of the tridecapeptide. The affinity of NT69L is high for both rat and human neurotensin NTS1 receptor, with equilibrium dissociation constants of 1.55 and 0.83 nM, respectively (Cusack et al., 2000). When given intraperitoneally, it causes hypothermia and antinociception (Tyler et al., 1999), similar to effects of native neurotensin, when it is directly injected into the brain. NT69L also blocks apomorphine-induced climbing and haloperidol-induced catalepsy (Cusack et al., 2000), and reduces body weight in Sprague–Dawley and obese Zucker rats (Boules et al., 2000).

In the present study, we tested the effects of acute and subchronic injection of NT69L on the rotational behavior induced by apomorphine or D-amphetamine in unilaterally 6-hydroxydopamine lesioned rats. 6-Hydroxydopamine lesioned rats share the major neuropathological abnormality of Parkinson's disease, i.e. degeneration of the dopamine neurons of the substantia nigra, with the resulting loss of their terminals in the striatum. We showed this to be the case indirectly in Fig. 5 using HPLC-ED analysis, where the lesioned side had significantly lower dopamine levels as compared to those of the non-lesioned side. This loss of nigrostriatal dopaminergic neurons is responsible for most of the motor disturbances seen in Parkinson's disease. While unilaterally lesioned rats behave quite normally, they rotate toward the side of the lesion (ipsilateral rotations) when injected with D-amphetamine, whereas they rotate away from the lesion (contralateral turning) when injected with apomorphine (Ungerstedt and Arbuthnott, 1970). The functional imbalance between the dopaminergic nigrostriatal pathways on the two sides of the brain results in the rotational behavior, such that the animal will turn away from the side of higher dopaminergic activity (Hudson et al., 1993). D-amphetamine is causing increased synaptic dopamine on the non-lesioned side, while apomorphine is acting on supersensitive postsynaptic dopamine receptors on the lesioned side.

Unilateral lesions of dopamine cell bodies not only cause modifications in dopamine release and binding, but also result in alterations in striatal neurotensin immunoreactive material, with an ipsilateral increase in striatal neurotensin immunoreactive material content and a marked decrease in [^{125}I]neurotensin binding site densities in substantia nigra and striatum (Masuo et al., 1990). Similar results were reported by others (Cadet et al., 1991) in unilaterally lesioned rats, but in this latter study the changes occurred bilaterally, suggesting an interdependence between the two dopaminergic pathways. The fact that 6-hydroxydopamine-induced destruction of dopaminergic cell bodies induces marked decreases in neurotensin binding sites indicates that a significant proportion of neurotensin receptors are co-localized with dopamine neurons. However, intraventricular administration of neurotensin reduces rigidity and tremors induced by bilateral 6-hydroxydopamine lesions in rats (Jolicoeur et al., 1991; Rivest et al., 1991), results that further support the involvement of this neuropeptide in the attenuation of Parkinson's disease symptoms.

Brains from Parkinson's disease patients show a decrease in neurotensin binding sites and in mRNA for neurotensin NTS1 receptor and an increase in neurotensin-immunoreactivity levels in the substantia nigra (Cadet et al., 1991; Chinaglia et al., 1990; Fernandez et al., 1994; Sadoul et al., 1984; Yamada and Richelson, 1995). Instead of an increase in neurotensin-immunoreactivity, others report only a hippocampal decrease in the concentration of neurotensin-immunoreactivity (Bissette et al., 1985). The increase in neurotensin-immunoreactivity levels may be an attempt to activate the reduced dopamine nigrostriatal pathway activity in Parkinson's disease patients (Fernandez et al., 1995), since neurotensin has long been regarded as a modulator of the dopaminergic system.

In our study, the administration of the neurotensin analog, NT69L, significantly reduced the apomorphine- and D-amphetamine-induced rotation in 6-hydroxydopamine lesioned rats with an ED_{50} of 40 and 80 μg/kg, respectively. These ED_{50} values are much lower than those for NT69L's hypothermic, antinociceptive, and anticataleptic (against haloperidol) effects (260 to 400 μg/kg) (Cusack et al., 2000; Tyler-McMahon et al., 2000b), demonstrating highly potent effects of NT69L on the pathological effects of dopamine receptor agonists in an animal model of Parkinson's disease.

Tolerance develops to NT69L's effects on body temperature, pain perception, haloperidol-induced muscle rigidity, and food intake after a single dosage (Boules et al., in preparation; Boules et al., 2000). Importantly, three daily injections of NT69L did not diminish its inhibitory effects on dopamine receptor agonist-induced rotations in unilaterally 6-hydroxydopamine lesioned rats. In addition, three daily injections of NT69L did not diminish its effects on striatal dopamine levels in the lesioned and non-lesioned sides. In fact, the effect of NT69L on elevating dopamine levels on the non-lesioned side was significantly greater after three daily dosages, than after a single injection (Fig.

5). In microdialysis studies with freely-moving, normal rats (Warrington et al., submitted for publication), we showed that a single injection of NT69L markedly enhanced dopamine turnover.

Several animal behavioral studies suggest that activation of brain neurotensin receptors inhibits or attenuates the effects of pharmacological agents that activate brain dopamine receptors (Ervin et al., 1981; Jolicoeur et al., 1983; Jolicoeur et al., 1985). Our previous results with NT69L (Cusack et al., 2000) and the present results support this notion. Furthermore, NT69L can attenuate or block behaviors mediated not only by dopamine receptor agonists, but also dopamine receptor antagonists. Thus, NT69L seems to work on both presynaptic, as well as postsynaptic dopamine receptors. However, it is also possible that NT69L is working on postsynaptic neuronal systems that can attenuate the effects of these compounds.

Acknowledgements

This work was funded by grant MH 27692 from the National Institute of Mental Health, the Forrest C. Lattner Foundation and by the Mayo Foundation for Medical Education and Research.

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