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Intranigral or intrastriatal injections of GDNF: effects on monoamine levels and behavior in rats

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Abstract

The present studies were designed to determine whether administration of recombinant human glial cell line-derived neurotrophic factor (rhGDNF) into either the substantia nigra or striatum is capable of augmenting dopamine function of the nigrostriatal pathway in normal rats. Single bolus intracranial injections of rhGDNF at either site increased locomotor activity and decreased food and water consumption and body weight in a dose-dependent manner when compared to vehicle-treated animals. These behavioral responses returned to pre-control levels within 3 weeks post rhGDNF administration. Administration of rhGDNF intranigrally increased dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels of the ipsilateral substantia nigra at 2 and 6 weeks post injection but had no augmenting effects on dopamine or its metabolites in the striatum. Administration of rhGDNF intrastriatally increased DOPAC and HVA levels of the ipsilateral striatum, although striatal dopamine levels were unchanged. Ipsilateral nigral dopamine levels were increased after intrastriatal injection of rhGDNF. The effects of intracranial rhGDNF were not specific to the nigrostriatal dopamine system, since nigrostriatal serotonin, 5-hydroxyindoleacetic acid (5-HIAA), epinephrine and norepinephrine transmitter levels were altered depending on administration route for rhGDNF and dose. Taken together, these data demonstrate long-lasting neurochemical and behavioral changes which suggest that rhGDNF can augment function in adult rat dopamine neurons. Therefore, rhGDNF may have therapeutic potential for Parkinson's disease.

Keywords: GDNF (glial cell line-derived neurotrophic factor); Substantia nigra; Striatum; Dopamine; Parkinson's disease; Monoamine

1. Introduction

Neuronal development, function and regeneration are influenced by specialized proteins referred to as neurotrophic factors. One such molecule, glial cell line-derived neurotrophic factor (GDNF), was originally isolated from the rat B49 cell line and identified as a distantly related member of the transforming growth factor-β (TGF-β) superfamily. Recombinant human GDNF (rhGDNF) was found to promote survival, morphological differentiation, and high-affinity dopamine uptake in dopaminergic neurons in dissociated cultures of rat embryo midbrain. This effect appeared to be specific to the dopaminergic system, since there was no increase in high-affinity uptake of serotonin or γ-aminobutyric acid (GABA) and no effect on the number of cultured neurons or astrocytes (Lin et al., 1993).

Since this report, non-human primate and rodent studies have demonstrated that intracranial injections of rhGDNF cause long-lasting increases in nigral dopamine levels (Hudson et al., 1995; Gash et al., 1995). However, it has not been established clearly whether rhGDNF causes any augmentation of dopamine or its metabolites in the striatum. Current therapies for Parkinson's disease, a degenerative condition of the nigrostriatal system, include L-3,4-dihydroxyphenylalanine (L-dopa) which is thought to improve motor function by increasing dopamine levels in the striatum. Thus, understanding the pharmacology of intracranially administered rhGDNF on striatal neurochemistry is of importance when considering rhGDNF as a novel therapeutic approach for Parkinson's disease.

The intranigral administration of rhGDNF to non-human primates with intact nigrostriatal systems had a pronounced increase on nigral dopamine levels but little or no effect on striatal dopamine levels (Gash et al., 1995). However, in the same study, in vivo electrochemical recordings in the ipsilateral caudate and putamen 3 weeks

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after rhGDNF administration revealed increased potassium evoked release of dopamine. In another study, normal rats showed a similar lack of effect on striatal dopamine content after intranigral injection of rhGDNF although increased striatal dopamine turnover was measured (Hudson et al., 1995).

In two rodent models of Parkinson's disease, effects on striatal neurochemistry differed depending on the route of administration of rhGDNF. Tomac et al. (1995a) found that supranigral injections of rhGDNF in mice before or after treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin of pigmented neuronal cell bodies of the substantia nigra, exerted protective and/or reparative effects in the substantia nigra, but had no effect on striatal dopamine levels. However, in the same study, intrastriatal rhGDNF in the MPTP-treated mouse increased dopamine levels in both nigral and striatal brain regions. In a related study using rats, administration of rhGDNF after lesioning the substantia nigra with 6-hydroxydopamine, an agent that causes excessive production of free radicals in dopaminergic neurons (Zigmond et al., 1989), attenuated apomorphine induced rotations and increased dopamine content of this region (Hoffer et al., 1994). However, striatal dopamine levels were not changed by rhGDNF in these studies.

Therefore, to further explore the pharmacology of rhGDNF we have compared the neurochemical effects of rhGDNF in the substantia nigra and striatum after either intranigral or intrastriatal delivery to non-lesioned rats. Locomotor activity, body weight and food and water consumption were also assessed, since an earlier report suggested that intraparenchymal rhGDNF increased locomotor activity and weight loss by a mechanism related to stimulation of the nigrostriatal system (Hudson et al., 1995). Our objectives were to clarify the neurochemical effects of rhGDNF in the striatum and identify the intracranial delivery site which leads to the more effective and selective stimulation of dopaminergic neurons. The data generated from these studies should help to explore the pharmacology of rhGDNF in the non-lesioned brain.

2. Materials and methods

2.1. Surgery and drug administration

Single intranigral or intrastriatal injections: Male Fischer 344 rats (Harlan) weighing 175–200 g were housed in cages in a temperature controlled room with a 12 h light-12 h dark cycle and were given free access to food and water ad libitum. Rats were anesthetized with 2% isoflurane + O_2 and mounted in a small animal stereotaxic frame (Kopf) with the incisor bar set at −2.5 mm. The skull was exposed and a 0.5 mm diameter burr hole was made using a dental drill. The rats received a unilateral injection of either rhGDNF (100, 10, 1, or 0.1 μg/4 μl), vehicle 4 μl (phosphate-buffered saline (PBS)) or inactive rhGDNF (130 μg/4 μl) in some studies (see Martin et al., 1995), over a 5 min period into the nigra or striatum using a 26 gauge Hamilton syringe. The syringe was left in place for an additional 5 min before removal. Injection coordinates for the striatum and the substantia nigra relative to bregma were: AP −0.3 mm, ML −3.0 mm at a depth −4.5 mm from dura; AP −4.4 mm, ML −1.3 mm at a depth −7.8 mm from dura respectively. The animals were allowed to recover once the skin was sutured. These animals were killed for central monoamine determinations at 2 and 6 weeks post rhGDNF administration.

2.2. Behavioral analysis

Methods employed in these studies have been previously described in detail elsewhere (Martin et al., 1995). Briefly, rats were removed from their home cages (7:30–10:00 a.m.) and placed in cages permitting continuous monitoring of locomotor activity (continuous movement greater than 1 s). Infrared monitors were mounted on each cage and locomotor activity recorded over a 20 min period, after a 5 min acclimation period. This information was stored as files in a computer and then analyzed at a later date. Furthermore, animal groups were randomly allocated session times each day to ensure that any one group was not tested at the same time each day.

Food and water consumption was monitored prior to rhGDNF administration and then daily for the first 14 days post rhGDNF. Animal weights were also recorded on the same days as were food and water measurements. All measurements were taken between 6:45 and 7:30 a.m. each day.

2.3. Neurochemical analysis

Animals were killed (CO_2) at the times indicated above and their brains were removed rapidly onto ice-cold saline. Bilateral substantia nigra areas, cut uniformly at the level of the inferior colliculus, were taken as tissue blocks approximately 1.5–2 mm^3. After bisecting the hemispheres, the overlying cortex and corpus callosum were removed, the hippocampus was peeled away, and the striatal tissue blocks approximately 3.5–4 mm^3 were taken. Average wet weights for nigral and striatal tissue were 6–10 and 45–60 mg respectively. Tissues were transferred to 1.5 ml centrifuge tubes and frozen on solid CO_2. The samples were then weighed and stored at −70°C for future analysis.

2.4. Tissue preparation

To each tissue sample the following were added: 10 μl of ascorbate oxidase solution (10 mg ascorbate oxidase in 10 ml of mobile phase buffer), 50 μl of an internal standard 3,4-dihydroxybenzylamine (DHBA), and 400 μl

(nigral) or 500 μl (striatal) of mobile phase buffer. Tissue samples were vortexed (2–3 s) and then sonicated (5–10 s) with a Branson Sonifier cell disrupter 200.

Brain tissue levels of dopamine, L-dopa, norepinephrine, epinephrine, serotonin, homovanillic acid (HVA), 3,4-dihydroxphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with electrochemical detection. Chromatography peaks were identified by retention times and standard addition protocols. Whole tissue levels (ng/g wet tissue weight) were calculated using calibration curves for each compound and recovery using the internal standard DHBA.

2.5. Chromatographic conditions

The mobile phase contained the following: 0.07 M citrate, 0.1 M sodium acetate, 50 mg/l ethylenediaminetetraacetic acid, 650 mg/l sodium chloride, 350 mg/l octane sulfonic acid, final pH 3.9. The mobile phase was run at 85% with 15% methanol.

2.6. Statistical analysis

All data are presented as means ± S.E.M. Significant differences between groups were assessed using Student's *t*-test. Multiple group comparisons were made using analysis of variance (ANOVA) with Dunnett's or Gabriel's post hoc test. A probability value of less than 5% was considered to be statistically significant.

3. Results

3.1. Two weeks post administration of rhGDNF into the substantia nigra: substantia nigra neurochemical data

Two weeks post rhGDNF (0.1–100 μg/4 μl) administration, dopamine content of the substantia nigra was significantly increased in a dose-dependent manner ($F = 14.2$, $P < 0.001$) (Fig. 1). The highest dose of rhGDNF (100 μg/4 μl) increased dopamine content of the substantia nigra approximately 3-fold when compared to vehicle or inactive rhGDNF-treated animals (Fig. 1). The contralateral substantia nigra also showed an increase in dopamine content (Fig. 1). rhGDNF treatment ($F = 7.8$, $P < 0.01$) caused an increase in HVA levels in the substantia nigra with significant effects observed at rhGDNF doses of 0.1 ($P < 0.05$), 10 ($P < 0.01$) and 100 μg/4 μl ($P < 0.05$) compared to vehicle-treated animals (Fig. 2 and Table 1). The 1 μg/4 μl rhGDNF dose was not significantly different from vehicle-treated animals, although there was a trend for higher HVA levels. The levels of DOPAC were only significantly ($F = 5.2$, $P < 0.01$) elevated at the 100 μg/4 μl dose of rhGDNF (Table 1). rhGDNF had no significant effect on nigral serotonin, 5-HIAA, norepinephrine, epinephrine or L-dopa levels (Table 1).

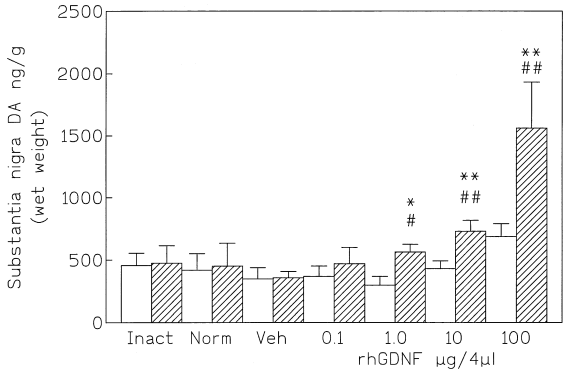

Fig. 1. Effects at 2 weeks of varying doses of intranigral rhGDNF (0.1–100 μg/4 μl), vehicle (4 μl) or inactive rhGDNF (130 μg/4 μl) on dopamine (DA) content of the ipsilateral (hatched bars) and contralateral (open bars) substantia nigra. Significant effects were seen 2 weeks post rhGDNF at the ** $P < 0.01$, and * $P < 0.05$ when compared to vehicle-treated animals after ANOVA analysis ($F = 14.2$, $P < 0.001$). Comparisons between the ipsilateral and contralateral sides were also significantly different at the $^{\#\#}$ $P < 0.01$ and $^{\#}$ $P < 0.05$. Values are mean ± S.E.M. ($n = 6–9$) per group.

Administration of rhGDNF (0.1, 1, and 10 μg/4 μl) nigrally induced no significant changes in the HVA/dopamine ratio (0.3 ± 0.03, 0.256 ± 0.07, and 0.36 ± 0.4 respectively) when compared to vehicle (0.28 ± 0.04) injected animals. However, the high dose of rhGDNF

Table 1
Effects at 2 weeks of intranigral rhGDNF (100 μg/4 μl) or vehicle (4 μl) on ipsilateral nigral and striatal monoamine levels (ng/g wet weight)

Sampling site	DA	DOPAC	HVA	5-HT	5-HIAA	NE	EP
Nigra	1563 ±	359 ±	168 ±	843 ±	541 ±	384 ±	19 ±
rhGDNF	373 [a]	44 [a]	19 [a]	55	28	24	8
Striatum	7084 ±	1142 ±	487 ±	206 ±	209 ±	120 ±	64 ±
	1079 [b]	57	110	95	84	21	52
Nigra	354 ±	168 ±	75 ±	1040 ±	434 ±	344 ±	12 ±
Vehicle	82	28	18	53	129	77	9
Striatum	9661 ±	1392 ±	606 ±	312 ±	433 ±	207 ±	159 ±
	616	103	40	26	30	32	21

Animals were killed 2 weeks post compound administration. Statistical comparisons were made between ipsilateral rhGDNF and vehicle-treated animals. All values are mean ± S.E.M. $n = 6–9$. DA, dopamine; EP, epinephrine; 5-HT, 5-hydroxytryptamine, serotonin; NE, norepinephrine. Significant at [a] $P < 0.01$; [b] $P < 0.05$, ANOVA followed by Dunnett's test.

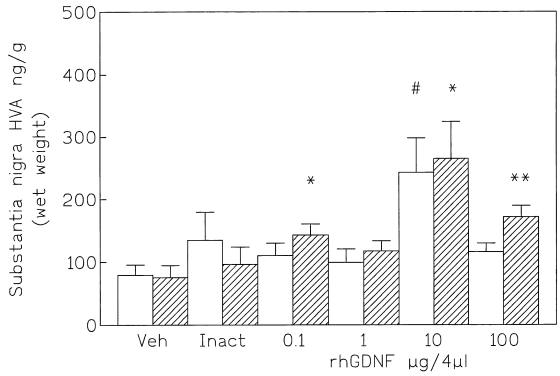

Fig. 2. Effects at 2 weeks of a single intranigral rhGDNF (0.1–100 μg/4 μl), vehicle (PBS, 4 μl) or inactive rhGDNF (130 μg/4 μl) dose on HVA levels of the ipsilateral (hatched bars) and contralateral (open bars) substantia nigra. Animals were killed 2 weeks post rhGDNF or vehicle administration. Statistical comparisons were made between vehicle and rhGDNF-treated groups (Dunnett's test after ANOVA). Values are mean ± S.E.M. ($n = 6$–9). Ipsilateral comparisons, vehicle versus rhGDNF ** $P < 0.01$, * $P < 0.05$. Contralateral comparisons, vehicle versus rhGDNF $^{\#}$ $P < 0.05$.

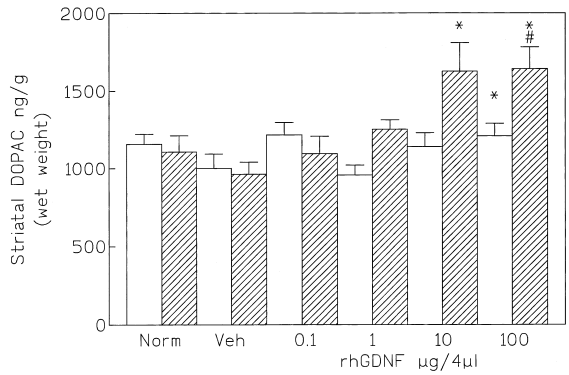

Fig. 3. Effects at 2 weeks of a single intrastriatal rhGDNF (0.1–100 μg/4 μl) or vehicle (PBS, 4 μl) dose on DOPAC levels of the ipsilateral (hatched bars) and contralateral (open bars) striatum. Animals were killed 2 weeks post rhGDNF or vehicle administration. Statistical comparisons were made between vehicle and rhGDNF-treated groups (Dunnett's test after ANOVA). Values are mean ± S.E.M. ($n = 7$). Ipsilateral comparisons, vehicle versus rhGDNF * $P < 0.05$. Contralateral comparisons, vehicle versus rhGDNF * $P < 0.05$. Ipsilateral versus contralateral comparisons $^{\#}$ $P < 0.05$.

(100 μg/4 μl) mediated a significant ($P < 0.05$) decrease in the HVA/dopamine ratio (0.1 ± 0.052) compared to the vehicle-treated animals (0.28 ± 0.04).

3.2. Two weeks post administration of rhGDNF into the substantia nigra: striatal neurochemical data

Two weeks post nigral rhGDNF administration, no significant alterations in striatal DOPAC, HVA, 5-HIAA, serotonin, norepinephrine or epinephrine levels were observed (Table 1, shows the 2-week data). Intranigral rhGDNF (100 μg/4 μl) significantly ($P < 0.05$) reduced striatal dopamine levels when compared to vehicle-treated animals (Table 1). Similarly, there was a significant ($P < 0.05$) difference between the contralateral (11 554 ± 1359 ng/g wet weight) and the ipsilateral striatum (7084 ± 1079 ng/g wet weight) ($P < 0.05$). There were no significant differences in striatal dopamine turnover (HVA/dopamine ratio) compared to vehicle-treated animals (see Table 1).

3.3. Six weeks post administration of rhGDNF into substantia nigra: substantia nigra neurochemical data

Table 2 shows that ipsilateral nigral dopamine levels were still significantly ($F = 18.03$, $P < 0.01$) elevated over the corresponding vehicle control at 6 weeks after intranigral injection of rhGDNF. Although there appeared to be an increase in the contralateral nigral dopamine content in the rhGDNF-treated animals this effect was not significant when compared to vehicle-treated animals. Furthermore, the dopamine metabolites HVA and DOPAC were significantly increased in the ipsilateral nigra ($P < 0.01$). The contralateral nigra also had significant ($P < 0.01$) increases in DOPAC levels compared to the contralateral

Table 2
Effects at 6 weeks of intranigral rhGDNF (100 μg/4 μl) or vehicle (4 μl) on nigral monoamine levels (ng/g wet weight)

Sampling site Nigra	DA	DOPAC	HVA	5-HT	5-HIAA	NE
Ipsilateral	968 ±	320 ±	140 ±	783 ±	491 ±	315 ±
rhGDNF	113 [a,c]	40 [a]	11 [a]	68	35 [b]	35 [b]
Contralateral	618 ±	309 ±	128 ±	797 ±	601 ±	324 ±
	48	40 [d]	11	66	34 [e]	31 [d]
Ipsilateral	351 ±	82 ±	79 ±	689 ±	244 ±	200 ±
Vehicle	41	11	4	81	32	33
Contralateral	472 ±	135 ±	85 ±	701 ±	290 ±	199 ±
	28	13	34	20	21	23

Animals were killed 6 weeks post compound administration. All values are mean ± S.E.M. $n = 7$–10. Statistical comparisons were made between the following: ipsilateral nigral rhGDNF and vehicle-treated animals ([a] $P < 0.01$; [b] $P < 0.05$); ipsilateral and contralateral nigral tissue of rhGDNF or vehicle-treated animals ([c] $P < 0.05$); and between the contralateral nigras of rhGDNF and vehicle-treated animals ([d] $P < 0.01$; [e] $P < 0.05$) ANOVA followed by Dunnett's test.

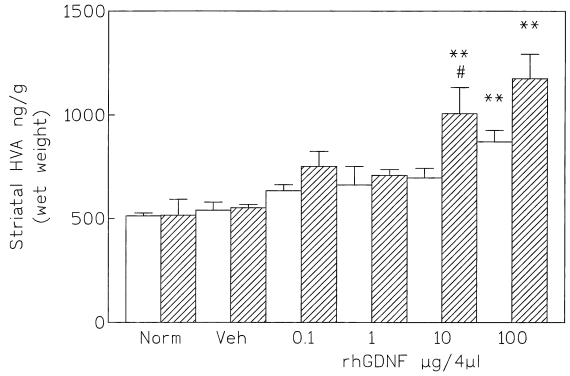

Fig. 4. Effects at 2 weeks of a single intrastriatal rhGDNF (0.1–100 µg/4 µl) or vehicle (PBS, 4 µl) dose on HVA levels of the ipsilateral (hatched bars) and contralateral (open bars) striatum. Animals were killed 2 weeks post rhGDNF or vehicle administration. Statistical comparisons were made between vehcile and rhGDNF-treated groups (Dunnett's test after ANOVA). Values are mean ± S.E.M. ($n = 7$). Ipsilateral comparisons, vehicle versus rhGDNF ** $P < 0.01$. Contralateral comparisons, vehicle versus rhGDNF ** $P < 0.01$. Ipsilateral versus contralateral comparisons $^{\#}$ $P < 0.05$.

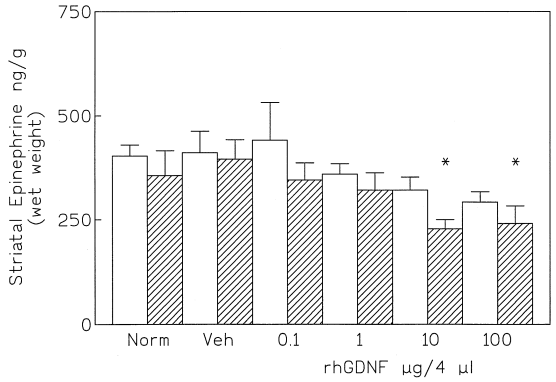

Fig. 5. Effects at 2 weeks of a single intrastriatal rhGDNF (0.1–100 µg/4 µl) or vehicle (PBS, 4 µl) dose on epinephrine levels of the ipsilateral (hatched bars) and contralateral (open bars) striatum. Animals were killed 2 weeks post rhGDNF or vehicle administration. Statistical comparisons were made between vehcile and rhGDNF-treated groups (Dunnett's test after ANOVA). Values are mean ± S.E.M. ($n = 7$). Ipsilateral comparisons, vehicle versus rhGDNF * $P < 0.05$.

vehicle controls. The contralateral nigral HVA levels were increased by 62% (Table 2). As with the 2-week study the 6-week study showed a decrease in the HVA/dopamine ratio for rhGDNF (100 µg/4 µl): 0.14 ± 0.025 (vehicle 0.23 ± 0.04) ($P < 0.05$). No differences in serotonin levels were observed in these studies (Table 2). The ipsilateral and contralateral nigra had significantly elevated norepinephrine and 5-HIAA levels in rhGDNF-treated compared to vehicle-treated animals (Table 2).

3.4. Six weeks post administration of rhGDNF into the substantia nigra: striatal neurochemical data

In the 6-week study intranigral rhGDNF had no significant effect on ipsilateral striatal monoamines or monoamine metabolites when compared to vehicle-treated animals (data not shown).

3.5. Two weeks post administration of rhGDNF into the striatum: striatal neurochemical data

rhGDNF treatment had a significant ($F = 4.19$; $P < 0.01$) effect on increasing striatal DOPAC levels (Fig. 3).

Table 3
Effects at 2 weeks of intrastriatal rhGDNF (100 µg/4 µl) or vehicle (4 µl) on striatal monoamine levels (ng/g wet weight)

Striatal treatment	DA	5-HT	5-HIAA	NE
rhGDNF	12776 ± 1691	521 ± 48	499 ± 45 [a]	182 ± 28
Vehicle	12959 ± 707	497 ± 24	380 ± 15	210 ± 10

Animals were killed 2 weeks post compound administration. Statistical comparisons between ipsilateral rhGDNF and vehicle-treated animals. Values are mean ± S.E.M. $n = 7$. Significant at [a] $P < 0.05$; ANOVA.

Fig. 3 shows the dose-response effect of rhGDNF on striatal DOPAC levels. Doses of 10 or 100 µg/4 µl rhGDNF significantly ($P < 0.05$) increased striatal DOPAC levels compared to vehicle-treated animals. rhGDNF had a significant ($F = 3.33$, $P < 0.05$) contralateral effect on striatal DOPAC levels (Fig. 3). Striatal HVA levels were significantly elevated ($F = 7.82$, $P < 0.01$) and a comparison between vehicle and rhGDNF-treated animals showed significant ($P < 0.01$) differences for the 10 and 100 µg/4 µl rhGDNF dose groups (Fig. 4). The contralateral striatum also had a significant rhGDNF treatment effect ($F = 4.49$, $P < 0.01$); animals treated with the 100 µg/4 µl rhGDNF dose mediated a significant ($P < 0.01$) increase in striatal HVA levels (Fig. 4).

Striatal epinephrine levels were significantly ($F = 3.28$, $P < 0.05$) decreased by rhGDNF treatment (Fig. 5). A

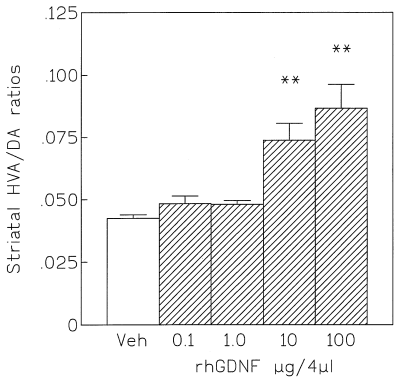

Fig. 6. Effects at 2 weeks of a single intrastriatal rhGDNF (0.1–100 µg/4 µl; hatched bars) or vehicle (PBS, 4 µl; open bars) dose on HVA/dopamine (HVA/DA) ratios of the ipsilateral striatum. Animals were killed 2 weeks post rhGDNF or vehicle administration. Statistical comparisons were made between vehicle and rhGDNF-treated groups (Dunnett's test after ANOVA). Values are mean ± S.E.M. ($n = 7$). Ipsilateral comparisons, vehicle versus rhGDNF ** $P < 0.01$.

Table 4
Effects at 2 weeks after intrastriatal rhGDNF (100 μg/4 μl) or vehicle (4 μl) administration on ipsilateral nigral monoamine levels (ng/g wet weight)

Treatment	DA	DOPAC	5-HT	5-HIAA	NE
rhGDNF	1489 ± 174 [a]	308 ± 80	900 ± 90 [b]	478 ± 86	360 ± 60 [b]
Vehicle	463 ± 79	250 ± 21	533 ± 145	517 ± 32	216 ± 12

Animals were killed 2 weeks post compound administration. Statistical comparisons were made between ipsilateral rhGDNF and vehicle-treated animals. Values are mean ± S.E.M. $n = 7$. Significant at [a] $P < 0.01$; [b] $P < 0.05$, ANOVA followed by Dunnett's test.

comparison between vehicle and rhGDNF-treated animals showed significant ($P < 0.05$) differences at the 10 and 100 μg/4 μl rhGDNF dose groups. No significant contralateral effect was observed. Administration of rhGDNF into the striatum had no effect on striatal dopamine, serotonin, or norepinephrine when compared to vehicle-treated animals (Table 3). However, there was a significant rhGDNF treatment effect on ipsilateral 5-HIAA levels ($F = 3.09$, $P < 0.05$; Table 3). L-Dopa levels were not determined in these studies.

Two weeks following rhGDNF injection, dopamine turnover (HVA/dopamine ratio) was significantly ($P < 0.01$; $F = 10.96$) elevated. Comparisons between dose groups revealed significant ($P < 0.01$) differences for the 10 and 100 μg/4 μl rhGDNF groups compared to the vehicle-treated animals (Fig. 6).

3.6. Two weeks post administration of rhGDNF into the striatum: substantia nigra neurochemical data

We also determined certain monoamine levels in the substantia nigra after striatal administration of rhGDNF (2 weeks post administration). Table 4 shows the levels of various monoamines for vehicle and 100 μg/4 μl rhGDNF-treated rats. The ipsilateral substantia nigra dopamine levels were significantly ($P < 0.01$) increased compared to vehicle-treated animals (Table 4). Increased ($P < 0.05$) levels of nigral serotonin and norepinephrine were also observed 2 weeks after striatal rhGDNF (100 μg/4 μl) administration compared to vehicle-treated rats (Table 4). Striatal rhGDNF had no effect on substantia nigral DOPAC or 5-HIAA levels. The contralateral substantia nigra had similar monoamine levels as vehicle-

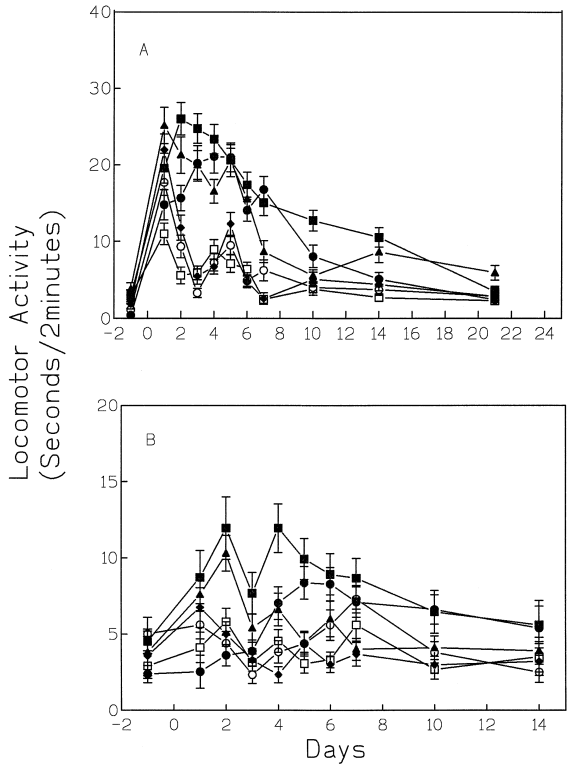

Fig. 7. Effects of intranigral (A) or intrastriatal (B) rhGDNF or vehicle on locomotor activity. All injections occurred on day 0, and locomotor activity was recorded the day prior to injection and on given days post injection. Locomotor activity was also determined for naive normal animals (rats that received no surgical or treatment manipulations). Values are mean ± S.E.M. ($n = 7$). Locomotor activity is expressed as s/2 min. Open and closed symbols represent the following: (○) vehicle (PBS 4 μl); (□) naive normal animals; rhGDNF (●) 100, (■) 10, (▲) 1 and (♦) 0.1, μg/4 μl.

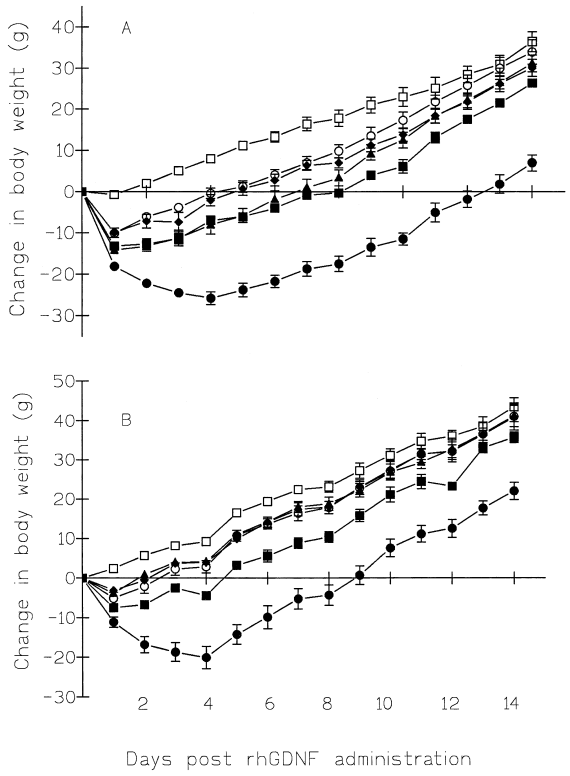

Fig. 8. The effects of a single bolus intranigral (A) or intrastriatal (B) rhGDNF dose on rat body weight. Body weight was also determined for naive normal animals (rats that received no surgical or treatment manipulations). Values are mean ± S.E.M. ($n = 7$ for each group). Open and closed symbols represent the following: (○) vehicle (4 μl); (□) naive normal animals; rhGDNF (●) 100, (■) 10, (▲) 1 and (♦) 0.1, μg/4 μl.

treated animals (data not shown) indicating that administration of rhGDNF striatally had no effect on the contralateral nigral monoamine content. L-Dopa, HVA or epinephrine were not determined in these studies.

3.7. Six weeks post administration of rhGDNF into the striatum: striatal neurochemical data

Six weeks following striatal rhGDNF injection, striatal dopamine turnover was back to vehicle control values as were all monoamine levels (data not shown).

3.8. Six weeks post administration of rhGDNF into the striatum: substantia nigra neurochemical data

Substantia nigra monoamine levels were back to vehicle control values at 6 weeks post striatal rhGDNF administration (data not shown).

3.9. Locomotor activity

Nigral administration of rhGDNF increased locomotor activity. A 2-way analysis of variance was performed to test group (dose-response) and day effects. Both were highly significant ($P = 0.0001$). Multiple comparisons indicated that the 10 μg/4 μl group had significantly ($P < 0.05$) higher numbers of large movements than the 1 or 100 μg/4 μl groups. The number of movements in the latter groups was significantly increased compared to vehicle treatment ($P < 0.05$). In addition, the number of large movements in general increased with time, but was back to vehicle control levels by day 21 (Fig. 7A).

Striatal administration of rhGDNF significantly ($P = 0.005$) increased locomotor activity, although compared to nigral administration for rhGDNF, the magnitude of the response was less robust (Fig. 7B). Similar to the nigral data, the 10 μg/4 μl group had significantly ($P < 0.05$) higher numbers of large movements compared to the other treatment groups. It must be noted that the 1 and 100 μg/4 μl rhGDNF dose groups were significantly ($P < 0.05$) different from the vehicle-treated group. Prior to and 2 weeks after rhGDNF, locomotor activity was similar to that of animals given vehicle intrastriatally (Fig. 7B).

3.10. Weight loss

Nigral administration of rhGDNF produced weight loss during the first week after administration ($P < 0.001$, re-

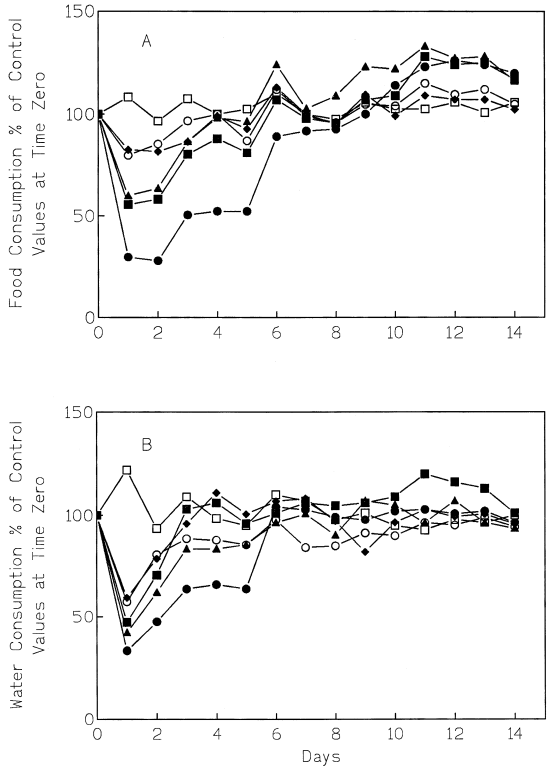

Fig. 9. The effects of a single bolus intranigral administration of rhGDNF ((●) 100, (■) 10, (▲) 1 and (◆) 0.1, μg/4 μl) or (○) vehicle (PBS, 4 μl) on food (A) and water (B) consumption in rats. Animals were housed 7 per cage, values represent total food and water consumption each day and expressed as a percentage of pre-treatment levels. Food and water consumption was also determined for (□) naive normal animals (rats that received no surgical or treatment manipulations).

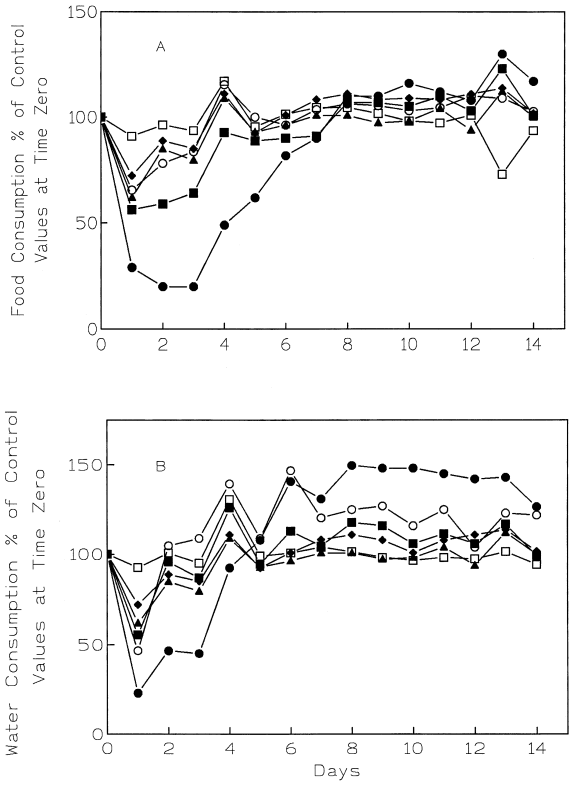

Fig. 10. The effects of a single bolus intrastriatal administration of rhGDNF ((●) 100, (■) 10, (▲) 1 and (◆) 0.1, μg/4 μl) or (○) vehicle (PBS, 4 μl) on food (A) and water (B) consumption in rats. Animals were housed 7 per cage, values represent total food and water consumption each day and expressed as a percentage of pre-treatment levels. Food and water consumption was also determined for (□) naive normal animals (rats that received no surgical or treatment manipulations).

peated measures ANOVA). Little or no effect was seen with the 0.1 or 1 μg/4 μl injections when compared to vehicle-treated animals, but significant ($P < 0.05$) changes were observed after the 10 or 100 μg/4 μl, with 100 μg/4 μl eliciting a larger response than 10 μg/4 μl (Fig. 8A).

Administration of rhGDNF into the striatum produced a dose-dependent loss in body weight compared to vehicle-treated animals ($F = 10.2$, $P < 0.001$). rhGDNF (100, 10 or 1 μg/4 μl) mediated weight changes that were significantly ($P < 0.05$) different from vehicle. Fig. 8B shows the time-course of changes in body weight in vehicle and rhGDNF-treated animals. Body weights returned to pre-rhGDNF values within 7–13 days (Fig. 8B).

rhGDNF injected into the nigra or striatum mediated a dose-dependent decrease in food and water consumption (Figs. 9 and 10). Food and water consumption returned to vehicle control levels at 6–8 days post injection of rhGDNF. Vehicle treatment caused a transient effect on weight loss and food and water consumption.

4. Discussion

The most striking new findings of the present study were the long-lasting increases in dopamine content of the substantia nigra at 6 weeks after intranigral injection of rhGDNF and the effectiveness of the striatum as a delivery site for increasing substantia nigra dopamine levels after rhGDNF injection. The latter effect implicates possible retrograde transport of rhGDNF from axonal terminals to cell bodies, which has recently been demonstrated after intrastriatal injection (Tomac et al., 1995b).

The increases in dopamine content of the substantia nigra after intranigral or intrastriatal rhGDNF administration were very pronounced, whereas dopamine content of the striatum was reduced after the former or did not change after the latter. The increase in substantia nigra dopamine is probably due to an effect on dopamine neurons intrinsic to the substantia nigra. These neurons have been shown to alter neuronal transmission both locally and at the striatal level (Cheramy et al., 1981). Furthermore, there is substantial evidence indicating that dopamine release from the dendrites of these intrinsic neurons plays an important role in regulating motor function (Robertson and Robertson, 1989; Double and Crocker, 1995). Thus, the upregulation of these neurons by rhGDNF may have important implications in regulating motor function in Parkinson's disease.

The small reduction in striatal dopamine levels after intranigral rhGDNF administration seen at 2 weeks is probably a compensatory response due to the large increases of dopamine observed in the substantia nigra (Cheramy et al., 1981). The reduction in striatal dopamine content has been reported for other neurotrophins. For example, animals treated with brain derived neurotrophic factor had a 20% decrease in striatal dopamine content which is similar to that reported in this study for rhGDNF (Altar et al., 1992). Another possibility is that a local necrotizing effect of the high dose of rhGDNF may have resulted in a partial reduction in the afferent innervation of the ipsilateral striatum (Hoffer et al., 1994).

Although intrastriatal rhGDNF had no effect on striatal dopamine levels it did increase dopamine turnover, evidenced by the increased HVA/dopamine ratio at the 2-week time point. This was similar to that demonstrated in a previous study (Hudson et al., 1995). The increases in the HVA/dopamine ratio are reflective of an increase in HVA levels in striatal tissue, rather than a decrease in dopamine. HVA is a product of both extra- and intraneuronal dopamine metabolism, and hence, may reflect the extent of dopamine transmission. This increase in HVA/dopamine ratio has been reported for other neurotrophic factors. For example, continuous unilateral supranigral infusion of brain derived neurotrophic factor 12 μg/day for 14 days, produced a 50–60% increase in HVA/dopamine ratio compared to vehicle-treated animals in the striatum (Altar et al., 1992).

In these studies rhGDNF was not selective for the dopaminergic system since norepinephrine, 5-HIAA and epinephrine levels were also altered. These effects were consistent with previous studies, that rhGDNF can influence other neuronal populations besides the nigrostriatal system, suggesting that rhGDNF may not be specific to dopamine related pathways (Schaar et al., 1993; Martin et al., 1995; Buj-Bello et al., 1995; Henderson et al., 1994; Oppenheim et al., 1995; Yan et al., 1995). Furthermore, rhGDNF has been shown to upregulate dopamine in other dopaminergic pathways (Martin et al., 1996).

Administration of rhGDNF nigrally demonstrated no significant increases in nigral HVA/dopamine ratios; however, at the 100 μg dose, the ratio decreased. This effect is in contrast to an earlier study in which intranigral rhGDNF (10 μg) increased the nigral HVA/dopamine ratio, suggesting an increase in dopamine transmission (Hudson et al., 1995). The decrease in the HVA/dopamine ratio was probably a reflection of the large increases in local dopamine levels, versus only modest increases in HVA. The physiological significance of this is not apparent and needs to be addressed further.

The finding that rhGDNF elicits increased locomotor activity after a single injection supports a previous report that rhGDNF administration into the substantia nigra (Hudson et al., 1995) or intracerebroventricular (Miller et al., 1994; Martin et al., 1996) increases locomotor activity. This behavior is consistent with upregulation of dopaminergic activity, and is similar to those produced by indirect dopamine receptor agonists such as amphetamine or methylphenidate (Goodman and Gilman, 1990). The increase in locomotor activity was more pronounced in studies where rhGDNF was administered intranigrally rather than intrastriatally. This may reflect the greater neurochemical

changes that occurred in the substantia nigra compared to the striatum. The dose of rhGDNF appeared to be important in locomotor activity. For example the 100 μg/4 μl dose was clearly less effective than the 10 μg/4 μl dose of rhGDNF on locomotor activity. Although this may seem odd, other investigators have reported similar instances where cytokines and neurotrophic factors were only effective within narrow dose ranges (Rothwell and Hopkins, 1995; Gurney et al., 1992; Schults et al., 1995). For example, lower doses of rhGDNF were more effective in attenuating amphetamine-induced rotations and increasing tyrosine hydroxylase immunoreactive neurons in the substantia nigra (Schults et al., 1995).

Animals that received rhGDNF striatally or nigrally lost weight in a dose-dependent manner. This effect of rhGDNF is not unusual since several neurotrophic factors (such as brain derived neurotrophic factor, neurotrophin 3, nerve growth factor and ciliary neurotrophic factor) given intracranially or peripherally cause weight loss (Altar et al., 1992; Williams, 1991; Espat et al., 1996; Martin-Iverson et al., 1994). It is notable that no sustained reduction in body weights occurred in the present study for the vehicle, or in a previous study for inactive rhGDNF (Martin et al., 1996). These results are also consistent with weight loss in recent studies in which primates received bolus intracranial injections of rhGDNF (Gash et al., 1995).

Several mechanistic possibilities exist. For example, increased locomotor activity could contribute to the decreases in body weight, due to increased energy demands. However, the l00 μg dose of rhGDNF caused greater weight loss but less locomotor activity than the 10 μg dose of rhGDNF. This may represent some neurotoxicity from this protein at high doses when administered locally, as previously indicated (Hoffer et al., 1994; Gash et al., 1995). Alternatively, the loss in body weight is consistent with an upregulation of dopaminergic activity in the target structures, and similar effects have been observed using indirect dopamine agonists (Goodman and Gilman, 1990). Furthermore, intranigrally administered rhGDNF directly or indirectly affects monoamine levels of the hypothalamus, which in turn can modulate weight gain (Miller et al., 1994). It is also possible that changes in other monoamines besides dopamine will affect weight gain (Blundell, 1984). Moreover, rhGDNF mediated weight loss is probably a combination of the above affects.

rhGDNF has shown promising potential in animal models of Parkinson's disease. Initial studies evaluated the efficacy of a single intracranial injection of rhGDNF 4 weeks after a 6-hydroxydopamine lesion to the substantia nigra. rhGDNF attenuated apomorphine-induced rotations and increased the dopamine content, the number of dopaminergic neurons and the size of dopaminergic neurons in the lesioned substantia nigra pars compacta (Schults et al., 1995; Hoffer et al., 1994). Furthermore, intracranial injection of rhGDNF prior to or after an acute 6-hydroxydopamine or 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine lesion protects against the neurochemical, behavioral and cell loss mediated by these lesions (Hoffer et al., 1994; Kearns and Gash, 1995; Opacka-Juffry et al., 1995; Miller et al., 1995; Tomac et al., 1995a; Gash et al., 1996).

In conclusion, based on previous studies and on the studies presented here, a single central rhGDNF injection induces a significant upregulation of mesencephalic dopamine neurons which lasts for several weeks in the adult rat. Thus, rhGDNF shows considerable promise for continued investigation as an agent to restore function to the compromised dopaminergic system in Parkinson's disease.

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