Behavioral and Neurochemical Effects of Intranigral Administration of Glial Cell Line-Derived Neurotrophic Factor on Aged Fischer 344 Rats

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ABSTRACT
To investigate the efficacy of glial cell line-derived neurotrophic factor (GDNF) in the augmentation of functional dopaminergic (DAergic) indices in aged rats, 24-month-old Fischer 344 (F344) rats received single intranigral injections of 10 μg GDNF (in 10 μl phosphate-buffered saline) or 10 μl phosphate-buffered saline. In locomotor activity tests, the GDNF-treated animals exhibited significant increases in both total distance traveled and movement speed compared with the vehicle group, 3 weeks after injections. In vivo microdialysis studies showed that basal extracellular levels of dopamine (DA) and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, were significantly increased in the striatum of the GDNF-treated rats. In addition, both potassium- (100 mM, K+) and d-amphetamine (250 μM)-induced DA overflow were augmented in the striatum and nucleus accumbens of the aged rats injected with GDNF.

Whole-tissue levels of DA and DA metabolites, as measured by high-performance liquid chromatography coupled with electrochemical detection, in the nucleus accumbens and substantia nigra were also elevated after GDNF administration. These results indicate that a single intranigral injection of GDNF is capable of augmenting locomotor behavior and DAergic function in the aged rat striatum and nucleus accumbens. This is the first report to demonstrate that a single intranigral injection of GDNF can improve the functional capacity of DAergic neurons of aged F344 rats.

Recent reports have documented the prevalence of movement disorders in the elderly population (Bennett et al., 1996; Mortimer, 1988; Richards et al., 1993; Sudarsky and Ronthal, 1983). The slowness of movements, stooped posture and shuffling gait in the elderly resemble, although to a lesser degree, the clinical features of Parkinson’s disease. A large body of scientific and clinical work has demonstrated that DA neuronal systems are severely diminished in PD, and this neuronal dysfunction is likely the major contributor to this disease (Hornykiewicz et al., 1990; Kish et al., 1992). In that PD is an age-related neurodegenerative disease, research has demonstrated a primary involvement of the nigrostriatal dopamine neuronal system in the mediation of motor behavior and has indicated its significant role in the decline of motor performance in senescence. With advancing age, there are several age-dependent degenerative alterations in the nigrostriatal DAergic pathway in both humans and animals (Burwell et al., 1995; Joseph et al., 1983; Severson et al., 1982; Watanabe, 1987), which are considered to account for many of the movement deficits observed. These age-related changes in humans include decreased cell numbers in the substantia nigra, pars compacta (McGeer et al., 1977) and alterations in the function of DA high-affinity uptake (Allard and Marcusson, 1989; Zelnik et al., 1986). Correspondingly, aged rats, 22 to 26 months old, exhibit numerous impairments within the nigrostriatal system which parallel those observed in humans, such as reductions in the number of cell bodies within the substantia nigra, pars compacta (Sabel and Stein, 1981) and deficiencies in the pool of DA available for release (Carlsson and Winblad, 1976). Additionally, age-related deficits in motor function (Altar

ABBREVIATIONS: Amp, amphetamine; AP, anterioposterior; ANOVA, analysis of variance; aCSF, artificial cerebral spinal fluid; Tc, clearance rate; DOPAC, 3,4-dihydroxyphenylacetic; DA, dopamine; DV, dorsoventral; F344, Fischer 344; GDNF, glial cell line-derived neurotrophic factor; HPLC-EC, high-performance liquid chromatography coupled with electrochemical detection; HVA, homovanillic acid; 6-OHDA, 6-hydroxydopamine; ML, mediolateral; PD, Parkinson’s disease; PBS, phosphate-buffered saline; K+, potassium; rhGDNF, recombinant human glial cell line-derived neurotrophic factor; 5-HT, serotonin; SN, substantia nigra; VTA, ventral tegmental area; NAc, nucleus accumbens; NE, norepinephrine.
and Marshall; 1988; Emerich et al., 1993; Ingram, 1988; Joseph et al., 1983) have been shown to correspond with the attenuation of stimulus-evoked overflow of DA within the striatum (Dluzen et al., 1991; Friedemann and Gerhardt, 1992; McIntosh and Westfall, 1987; Rose et al., 1986), alterations of DA reuptake systems (Allard and Marcusson, 1989; Friedemann, 1992; Marshall and Altar, 1986; Missale et al., 1986) and changes in feedback inhibition mechanisms and the sensitivity of autoreceptors regulating DA release (Govoni et al., 1977, 1980). These data suggest a decline in DA neuronal function with age in both humans and animals which may account for observed deficits in movement.

The apparent relationship between the functional integrity of the nigrostriatal DA system and movement, raises the possibility that improvements in motor performance in aged subjects might be achieved by increasing the function of DA neurons in this pathway. Several treatments for augmenting the release properties of deficient nigrostriatal DAergic neurons, which result from neurodegenerative diseases or aging, are currently under investigation. One approach involves the administration of growth factors directly or from cell lines to produce functional changes in DA nerve endings in the striatum (Dluzen et al., 1996). Studies thus far in aged rats have demonstrated that GDNF treatment can reverse motor impairment (Bowenkamp et al., 1996), increase spontaneous locomotor activity and induce DAergic and cholinergic phenotypes (Jiao et al., 1996). However, the long-term efficacy of GDNF in the augmentation of in vivo DA release in the striatum of aged animals has not been investigated.

The goal of this study was to determine whether GDNF can produce functional changes in DA nerve endings in the striatum and nucleus accumbens of aged (24-months-old) F344 rats. F344 rats this age have been shown to have dramatic deficits in both motor behavior (Emerich et al., 1993; Friedemann and Gerhardt, 1992; Joseph et al., 1983) and DAergic neuronal function (Friedemann, 1992; Friedemann and Gerhardt, 1992; Rose et al., 1986) as compared with young adult rats. First, GDNF-induced changes in motor behavior were assayed by monitoring the aged rats for their spontaneous locomotor activity and movement speed 3 weeks postadministration of a single dual-site unilateral intranigral injection of GDNF or vehicle. Second, in vivo microdialysis measurements were used to examine the effects of GDNF or vehicle administration on both basal and stimulus-evoked overflow of DA and DA metabolites. Finally, whole-tissue samples of the right and left striatum, nucleus accumbens, substantia nigra and ventral tegmental area were analyzed for changes in DA and DA metabolite levels 3 weeks after administration of GDNF or vehicle into the aged F344 rats.

Methods

Animals and GDNF injections. Adult male F344 rats, 23 months and 1 week old, weighing 400 to 450 g and obtained from the National Institute on Aging, were used for all studies. Protocols for animal use were approved by the Institutional Animal Care and Use Committee. Rats were housed two to three per cage in laminar flow units in our animal care facility, with food and water available ad libitum. On the day of surgery, the animals were anesthetized with chloral hydrate (320–350 mg/kg i.p.), placed in a stereotaxic frame and prepared for vehicle (PBS, n = 7) or GDNF (n = 7) injections. RhGDNF (Amgen Inc., Thousand Oaks, CA), expressed in Escherichia coli as described previously (Lin et al., 1993), was diluted to 1 mg/ml in PBS. All injection solutions were sterilized by filtration through polysulfone syringe filters with a 0.2-μm pore diameter (no. 4454 Gelman Sciences, Ann Arbor, MI) and stored at ~70°C until use. A slow infusion method, as described by Bowenkamp et al. (1995), was used to deliver injection fluids at a rate of 0.25 μl/min. Analogous to Hebert et al. (1996), two stereotactically guided injections, 5 μl each, were performed unilaterally at sites within the right substantia nigra (site 1: AP = 5.6 mm, ML = -1.9 mm, DV = -7.3 mm; site 2: AP = -5.6 mm, ML = -2.7 mm, DV = -6.8 mm) based on bregma (Paxinos and Watson, 1986); both injections were performed with the incisor bar positioned at -2.3 mm. The incisions were closed using stainless-steel wound clips (Stoelting, no. 59027), and animals were allowed to recover 1 week before behavioral testing. Verification of injection sites was performed upon dissection of brain tissues for HPLC-EC analysis. Injection tracts were traced from the cortex to the substantia nigra.

Behavioral measures of spontaneous locomotor activity and average movement speed. Animals were monitored for changes in spontaneous (“home-cage”) motor activity in automated activity chambers (Omnitech Instruments, model RXYZCM-8, Columbus, OH). Each monitor consisted of a 40 × 40-cm Plexiglas box with a grid of infrared beams mounted horizontally every 2.5 cm. Two tiers of beams are mounted 2 cm and 10 cm above the floor. The monitors were connected to a Digsican Analyzer (Omnitech model DCM-8, Columbus, OH) that transmitted the activity data to a computer. During operation, the pattern of beam interruptions was recorded and analyzed by the computer. For each test session, one rat was placed in the activity monitor. Activity data were collected during the light period, for six consecutive 10-min samples and summed over 60 min. Total distance traveled represents the distance traveled by an animal in a given sample period. Average movement speed is the mean distance traveled per unit time.

Before vehicle or GDNF injections, the animals were tested to establish habituated base-line activity and separated into treatment groups that had equivalent average scores for all activity measures. Habituation to the testing environment occurred after four trials. After the injections, the animals were monitored once a week for 3 weeks. Vehicle- and GDNF-treated animals were run simultaneously during each testing period. Spontaneous activity and average movement speed measures were analyzed by an independent, repeated measures ANOVA.

In vivo microdialysis measurements. Microdialysis methods, similar to Church and Justice (1987), were used to study the extracellular levels of basal and stimulated (K+ and d-amphetamine) overflow of DA and DA metabolites in the rat striatum and nucleus accumbens ipsilateral to vehicle or GDNF injections. The microdialysis probes were constructed from 300 μm outside diameter hollow cellulose fibers (ENKA AG, Germany; molecular weight cutoff, 11,500), with an active recording area of 4 mm. Before use, the probes were rinsed with ETOH (50%) for 24 hr and distilled water for 1 hr. A computerized multisyringe pump (World Precision Instruments, Sarasota, FL) fitted with 1000 μl Hamilton (no. 1001) gas-tight syringes was used to perfuse the probes at a flow rate of 1.0 μl/min. All probes were tested for recovery in vitro before use (Robinson and Whishaw, 1988) and all in vivo dialysate samples were
corrected for recovery. Recoveries were measured at 37°C in aCSF (123 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 1 mM NaHCO₃, 1 mM NaH₂PO₄, 5.9 mM glucose) containing 0.1 M DA, 0.1 M DOPAC and 1 µM HVA. Probe recoveries ranged from 14% to 22% for DA and averaged 17 ± 1% (n = 16).

For the in vivo recordings, the rats were anesthetized with urethane (1.0 g/kg i.p.) and placed in a stereotaxic apparatus. Rats remained anesthetized throughout the experiment with core temperature maintained at 37°C by using a heating pad connected to a rectal probe. The skull was exposed and a hole drilled to allow implantation of the dialysis probe into the striatum and nucleus accumbens ipsilateral to the GDNF or PBS injection. With the incisor bar positioned at −2.3 mm, the probe was placed at the following coordinates with respect to bregma: AP +1.5 mm and ML −2.3 mm (Paxinos and Watson, 1986). With the tip of the probe lowered to a depth of 8.0 mm below the dural surface, the active sampling area of the probe (4 mm) spanned the dorsal and ventral striatum, including the nucleus accumbens. Implanted probes were perfused (1.0 µl/min) with aCSF; brain perfusates were collected every 20 min and assayed for DA and the primary metabolites DOPAC and HVA with use of HPLC-EC methods (1–15 pg detection limit per compound). Measurements were obtained as the dialysis experiment progressed, and no sample storage was necessary.

Basal levels of DA, DOPAC and HVA were obtained from an hour of base-line samples. The first 20-min perfusate was discarded, because this fraction typically contains excess amounts of DA caused by damage during probe implantation. Stimulus-evoked DA overflow was performed by dialyzing with either high-potassium CSF (23 mM NaCl, 100 mM KCl, all other reagents in concentrations identical to those for aCSF) or d-amphetamine (aCSF containing 125 µM d-amphetamine sulfate). The stimulus solutions were applied continuously for 20 min each. A 60-min washout with aCSF was performed between successive stimuli. All perfusion solutions were gassed with 95% O₂/5% CO₂, adjusted to pH 7.4 and contained 500 µM ascorbic acid.

Dialysates were quantified by HPLC-EC as described previously (Hall et al., 1989). Samples were manually injected through a 50-µl loop (Altec x 210A) onto a Hypersil C18 column (Keystone Scientific, 4.6 x 100 mm, 3-µm particles). Mobile phase (2.0 ml/min flow rate) consisted of a citrate-acetate buffer solution (pH 4.1); 6% MeOH, 0.14 g octane-sulfonic acid, 27.6 g sodium acetate, 28 g citric acid monohydrate and 0.1 g EDTA in 2 liters of ultrapure H₂O. The separated compounds were detected by a dual-electrode coulometric electrochemical detector (ESA, Inc. model 5100 with a 5011 analytical cell). An oxidation potential of +400 mV was applied to the first detector, and a reduction potential of −250 mV was applied to the second detector. DA and DOPAC were quantified from the second or reducing detector, and HVA levels were calculated based on signals obtained from the first or oxidizing detector. The concentrations of the compounds were calculated based on retention times and peak heights relative to known concentrations of each compound. The detection limits of the assay were calculated as three times the standard deviation of the noise, and were <1 pg per injection of DA and DOPAC and <15 pg per injection of HVA.

After the experiment, the animals were sacrificed by decapitation, and the brains were dissected to verify the probe placements. Gross anatomical visualization revealed that all probes were correctly positioned in the striatum, spanning the nucleus accumbens. Microdialysis data were analyzed by a repeated measures ANOVA design and a two-tailed Student’s t test. The average value of the three points before stimulation was taken to represent basal dialysate levels for each animal.

Whole-tissue HPLC measurements. After in vivo microdialysis recordings of DA overflow, the animals were decapitated and the brains quickly removed and placed in ice-cold saline. Tissue samples of both the right and left striatum, SN, VTA and the ventrocaudal region of the NAc were dissected, weighed and frozen at −70°C until HPLC-EC measurements were performed. During the dissections the placements of the microdialysis probes were anatomically verified.

The dissection began by placing the brain ventral surface upward. To dissect the cell body regions of the SN and the VTA, coronal cuts were made at the anterior and posterior boundaries of the mammillary nucleus. Localization of the two regions was facilitated by removing the mammillary nucleus on the ventral surface and overlying cortex on the dorsal surface. Distinct lateral aspects of the remaining tissue block contained the SN. Tissue regions with a height and width of approximately 1.5 mm were excised from this lateral area of the right and left hemispheres (see fig. 38, Paxinos and Watson, 1986). White matter was removed from these areas leaving discrete oyster-shaped cell body regions defined as the SN. The VTA sections were dissected by taking bilateral regions, with a height of 1.25 mm, whose horizontal boundaries were defined by the midline and the medial border of the SN. After removing white matter, the cell bodies of the VTA were also distinct and resembled small cubes.

The area of the brain anterior to the mammillary nucleus contains the striatum and NAc regions. To dissect the NAc, the anterior commissure which lies lateroventral to the NAc, was located. This was done by slowly removing brain tissue, beginning at the ventral surface of the brain and moving dorsally. Once located, the anterior commissure was lifted away and the NAc regions, positioned dorso-medial to this, were removed. All white matter surrounding the distinct right and left NAc regions was discarded. To dissect the striatum, a hemisection of the remaining frontal cortex was performed. Because the corpus callosum provides a “e-like” boundary of the striatum, removing it exposes the striatum. The striations of the striatum made it easy to identify and separate from the remaining tissue. This was done bilaterally.

To prepare the tissues for HPLC-EC analysis, the frozen brain samples were sonicated in cold mobile phase (pH 4.1), containing dihydroxybenzylamine as an internal standard. After centrifuging the samples at 16,000 × g for 10 min, 50 µl of supernatant was directly injected into the HPLC coupled with dual-coulometric electrochemical detectors as described previously (Hall et al., 1989). The levels of DA, DOPAC, HVA, NE and 5-HT in the right and left striatum, NAc, SN and VTA of vehicle- and GDNF-treated 24-month-old F344 rats were calculated as total nanograms per gram wet weight (ng/g) of tissue. The detection limits of our system were <1 ng/g tissue wet weight. Statistical comparisons between hemispheres and across groups were made with a two-tailed Student’s t test.

Results

Locomotor behavior. The effects of GDNF administration on spontaneous activity and average movement speed on the 24-month-old F344 rats were assessed. Before and at 1, 2 and 3 weeks after GDNF injections, comparisons were made between the vehicle-treated (n = 7) and the GDNF-treated (n = 7) groups. Small differences in locomotion between the two groups were observed 2 weeks after intracranial injections; however, highly significant increases (P < .001**) in total distance traveled (fig. 1) and average movement speed (fig. 2) were not noticed until the 3-week time point. Total distance traveled was increased by nearly 50% in the rats treated with GDNF (3067 ± 134 cm) 3 weeks postinjection compared with those treated with vehicle (2145 ± 122 cm). Similarly, movement speed was greatly increased in the GDNF-treated group (8.58 ± 0.04 cm/sec) 3 weeks posttreatment, when compared with the vehicle group (7.72 ± 0.09 cm/sec). A measure of clockwise and counterclockwise rotations indicated that the rats treated with GDNF or PBS did not spontaneously circle in either direction. No significant differences were found between pre- and post-treatment body.
weight measurements that could have accounted for the observed changes in locomotion. Thus, unilateral GDNF injections into the SN were seen to increase the spontaneous activity and the average movement speed of aged rats, with the most significant differences occurring 3 weeks after a single injection.

In vivo microdialysis. Three weeks after nigral injections, in vivo microdialysis measurements were used to assess potential presynaptic changes in DA function in the ipsilateral striatum and nucleus accumbens. A dual-site unilateral intranigral injection of GDNF significantly augmented basal levels of DA and DA metabolites (fig. 3). A two-tailed Student’s t test revealed that basal DA levels were significantly increased and averaged 0.11 ± 0.01 μM (n = 21) in rats treated with GDNF as compared with 0.07 ± 0.01 μM (n = 21) in vehicle-treated rats (P < .05). In addition, the basal extracellular levels of DOPAC were significantly augmented averaging 2.45 ± 0.09 μM (n = 21) in the GDNF-treated group as compared with 1.99 ± 0.09 μM (n = 21) for the control group (P < .01). Moreover, basal levels of HVA were also significantly increased in the GDNF-treated rats and averaged 3.39 ± 0.11 μM (n = 21) versus 2.21 ± 0.11 μM (n = 21) in the vehicle-treated F344 rats (P < .001). Thus, GDNF was seen to significantly augment basal extracellular levels of DA, DOPAC and HVA 3 weeks after a single intranigral injection.

The potential functional effects of GDNF treatment on potassium- and d-amphetamine-evoked overflow of DA in the striata of the 24-month-old F344 rats were also determined by microdialysis methods (fig. 4). Repeated measures ANOVA revealed that DA overflow was significantly increased (P < .001) in the GDNF-treated rats under both stimulus conditions versus the vehicle-treated group. Potassium-stimulated overflow led to a DA release of 1.29 ± 0.07 μM (n = 7) in the GDNF group as compared with 0.63 ± 0.01 μM (n = 7) in the vehicle-treated group of 24-month-old F344 rats. Similarly, d-amphetamine-evoked DA overflow in the same animals receiving GDNF was 0.69 ± 0.07 μM (n = 7).
and DA metabolite levels in the NAc and SN bilaterally. GDNF treatment was seen to significantly increase DA levels bilaterally and contralaterally in the aged F344 rats. Thus, GDNF treatment was seen to significantly increase DA levels and DA metabolite levels in the NAc and SN bilaterally.

HPLC studies of DA and DA metabolites in whole-tissue samples. HPLC-EC methods were used to determine whether the differences in extracellular DA concentrations, measured by in vivo microdialysis, were related to alterations in the storage of DA within the cell bodies or terminal fields of dopaminergic neurons in rats injected with GDNF. DA and DA metabolite levels were measured in tissue samples of striatum, NAc, SN and VTA dissected from rats at the completion of the microdialysis experiments. Table 1 summarizes the average whole-tissue levels of DA, DOPAC, HVA, NE and 5-HT in the vehicle- and GDNF-treated rats. Because of the complexity of the VTA dissection, the averages for this region represent an n of 5 for each hemisphere. All significance values are given for right hemisphere of vehicle-injected rats compared with right hemisphere of GDNF-treated animals, and vice versa. As observed previously in young adult rats, there was a small, but significant decline in the levels of DA, DOPAC and HVA in the striatum ipsilateral to the GDNF injections (Hebert et al., 1996; Hudson et al., 1995). Conversely, there were increases in DA levels in both the ipsilateral and contralateral NAc and SN. Although the GDNF was administered unilaterally, the effects were significant in both hemispheres of the aged F344 rats. Thus, GDNF treatment was seen to significantly increase DA levels and DA metabolite levels in the NAc and SN bilaterally.

Discussion

In the present study, a single unilateral injection of rh-GDNF into the SN of aged F344 rats was shown to significantly enhance spontaneous locomotor activity, basal and stimulus-evoked DA overflow and whole-tissue neurochemical levels 3 weeks after administration. The behavioral studies indicate that GDNF treatment leads to increases in average movement speed and total distance traveled. In vivo microdialysis measurements in the ipsilateral striatum and NAc 3 weeks after intranigral injections detected significantly increased basal levels, in addition to potassium- and d-amphetamine-evoked release of DA in those animals receiving the trophic factor. Whole-tissue levels of DA and DOPAC were found to be significantly higher in the ipsilateral and contralateral SN of GDNF-treated rats as compared with controls. Similarly, significant bilateral increases in DA and HVA were detected in the NAc of rats injected with GDNF compared with those injected with PBS. These results suggest that acute GDNF treatment can produce long-lasting changes in the functional capacity of dopaminergic neurons in aged F344 rats.

Unilateral, intranigral administration of the putative DA neurotrophic factor, GDNF, into aged F344 rats elicited increases in both total distance traveled and average movement speed. Differences in motor activity between the vehicle- and GDNF-treated groups were noticed 2 weeks after treatment and became highly significant at 3 weeks. The time course of development of the GDNF-induced effects on movement speed resembles that observed in young F344 rats (Hebert et al., 1996). However, unlike the young animals treated with GDNF, the 24-month-old animals treated with GDNF exhibited progressive increases in total distance traveled and average movement speed.

The observation that GDNF can dramatically affect the locomotor behavior of aged rats is significant. Many reports have documented age-related disturbances in balance and coordination, decreases in motor function and locomotion (Gage et al., 1984; Martin et al., 1983; Wallace et al., 1980; Willig et al., 1987) and deficits in movement speed (Birren et al., 1979; Emerich et al., 1993; Welford et al., 1969). Present evidence indicates that many of the behavioral abnormalities that occur during the normal aging process result from a reduced capacity for neurotransmission (Joseph et al., 1983; Morgan and Finch, 1988). The deficits in motor performance of aged rats have been considered to be mediated by age-associated alterations in the striatal DA system (Friedemann et al., 1992; Marshall and Berrios, 1979; Sanberg et al., 1979).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Region</th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
<th>NE</th>
<th>5-HT</th>
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<tr>
<td>Striatum</td>
<td>Vehicle (n = 6)</td>
<td>L-STR</td>
<td>9951 ± 352</td>
<td>2850 ± 197</td>
<td>1463 ± 83</td>
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<td></td>
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<td>R-STR</td>
<td>10091 ± 234</td>
<td>2963 ± 484</td>
<td>1437 ± 233</td>
<td>134 ± 12</td>
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<td>GDNF (n = 7)</td>
<td>L-STR</td>
<td>9531 ± 667</td>
<td>2146 ± 239</td>
<td>903 ± 144</td>
<td>108 ± 10</td>
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<td></td>
<td></td>
<td>R-STR</td>
<td>8574 ± 577*</td>
<td>1822 ± 249*</td>
<td>839 ± 145*</td>
<td>111 ± 16</td>
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<td>NAc</td>
<td>Vehicle (n = 6)</td>
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<td>2821 ± 171</td>
<td>511 ± 13</td>
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<td>R-NAc</td>
<td>847 ± 24</td>
<td>2441 ± 175</td>
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<td></td>
<td>GDNF (n = 7)</td>
<td>L-NAc</td>
<td>1283 ± 37***</td>
<td>2610 ± 142</td>
<td>644 ± 59*</td>
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<td></td>
<td></td>
<td>R-NAc</td>
<td>1502 ± 48***</td>
<td>2768 ± 180</td>
<td>612 ± 26**</td>
<td>224 ± 31</td>
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<td>273 ± 39</td>
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<td>R-SN</td>
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<td>242 ± 22</td>
<td>50 ± 5</td>
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<td>171 ± 10*</td>
<td>48 ± 2</td>
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<td>431 ± 17***</td>
<td>146 ± 13**</td>
<td>47 ± 4</td>
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<td>R-VTA</td>
<td>102 ± 24**</td>
<td>126 ± 47</td>
<td>65 ± 23</td>
<td>354 ± 75*</td>
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*Shown are the mean values ± S.E.M. reported as ng/g wet weight of tissue. Values are significant at * P < .05, ** P < .01, *** P < .001. L, left, noninjected side; R, right, injected side; STR, striatum. END OF AUTHOR QUERIES
al., 1987; Welford, 1982), involving both the mesolimbic and nigrostriatal components (Dunnett and Robbins, 1992). We suggest that the effects of GDNF on basal and stimulus-evoked DA overflow in the dorsal striatum and NAc may be responsible for the increases observed in spontaneous locomotor activity seen in the present study. We have shown that intranigral GDNF treatment in normal young F344 rats produces increases in spontaneous locomotor activity, which corresponds to an increase in stimulus-evoked DA release (Hebert et al., 1996) and tyrosine-hydroxylase immunoreactivity (Hudson et al., 1995). Interestingly, the aged rats treated with GDNF, in this study, exhibited open-field measurements for total distance traveled and average movement speed that were not significantly different than those scores previously reported for young (6-month-old) F344 rats (Hebert et al., 1996). Emerich and colleagues (1996) demonstrated that aged (20-month-old) rats implanted with encapsulated GDNF-producing fibroblasts exhibit locomotor activity akin to control young (5-month-old) rats, as early as 4 weeks after the surgery. The unusual augmentation of activity measures in aged rats to the levels measured in young adult rats suggests that GDNF treatment may restore functional capacity to dopaminergic terminals that are responsible for locomotion.

In vivo microdialysis techniques were used to measure the extracellular basal levels of DA and DA metabolites, as well as K⁺ and d-amphetamine-induced overflow of DA, 3 weeks after GDNF administration. The results of these experiments indicate that both DA release and metabolism in the striatum and NAc of aged rats were augmented by GDNF treatment. The observed GDNF-induced alterations in the aged rats have been compared with changes seen in young adult (6-month-old) rats injected with GDNF (Hebert et al., 1996). GDNF was shown to produce a significant increase in basal levels of DA, DOPAC and HVA in the aged rats, which was similar to basal levels found in normal young adult rats. This suggests that perhaps intranigral GDNF treatment in aged rats can restore the functional capacity of striatal DAergic neurons. This hypothesis is further supported by data from the stimulus-evoked DA release experiments. Both K⁺-depolarization of the DA terminals and d-amphetamine-induced displacement of DA resulted in highly significant increases in DA release in the striatal region ipsilateral to GDNF treatment. The degree of augmentation observed in stimulus-evoked release of DA in GDNF-treated aged rats resembles that seen in young adult rats injected with GDNF (Hebert et al., 1996). At both ages, DA release in the groups treated with trophic factor was 2-fold greater than control. When comparing the responses of aged (24-month-old) and young (6-month-old) rats to GDNF it must be noted that DA release in the GDNF-treated aged rats exceeded that measured in young controls, but not that measured in young rats treated with GDNF. These observations indicate that intranigral injections of GDNF can augment the functional capacity of DA neurons in aged rats to the same degree as shown in young rats (Hebert et al., 1996).

Because both calcium-dependent (K⁺) and non-calcium-dependent (d-amphetamine) evoked overflow were augmented in GDNF-treated aged rats, we suggest that growth factor administration results in trophic enhancement of the release machinery and/or the release mechanism (Knipper et al., 1994). Enhanced release could be the consequence of a change in one condition or the combination of several events. For example, an increased percentage of DA vesicles in the “readily releasable pool” (Gillis et al., 1996; Rosenmund and Stevens, 1996) may result in augmented quantal content or quantal size in rats receiving GDNF. Similarly, the time to replenish the pool of releasable quanta may be decreased in the striatum and NAc of GDNF-treated aged rats (Stevens and Tsujimoto, 1995). Likewise, the vesicular docking and fusion mechanism may be altered, such that the equilibrium between vesicular release and cytoplasmic content is changed (Bark and Wilson, 1994). Further studies are needed to elucidate the cellular alterations or possible neuronal regeneration resulting from GDNF treatment that produce alterations in DAergic regulation.

Although in vivo microdialysis is a valuable measure of dopamine release and metabolism, it can only provide information about the “readily releasable” pools of DA (Arbuthnott et al., 1990; Justice et al., 1988). In contrast, HPLC-EC techniques allow us to measure GDNF-induced effects on inter- and intratransmitter stores of neurotransmitters. Consequently, whole-tissue levels of DA, DA metabolites, NE and 5-HT were measured within the striatum, SN, NAc and VTA by use of HPLC-EC methods. It was determined that GDNF administration produced significant increases in the levels of DA within both the ipsilateral and contralateral SN and NAc. The bilateral increase in DA within the SN parallels studies in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys (Gash et al., 1996), MPTP-treated mice (Tomac et al., 1995), 6-OHDA-lesioned rats (Bowenkamp et al., 1995; Hoffer et al., 1994) and normal young rats (Hebert et al., 1996). Conversely, small but significant decreases in DA were observed within the ipsilateral striatum and VTA of GDNF-treated rats compared with controls. The GDNF-induced decrease in striatal DA has been observed previously in normal young adult F344 rats (Hebert et al., 1996; Hudson et al., 1995). DA metabolite levels in the regions assayed were either significantly elevated or reduced in rats injected with GDNF compared with controls. It was also determined that both DA metabolites, DOPAC and HVA, were reduced in the ipsilateral striatum of GDNF-treated animals. A bilateral increase in HVA was observed in the NAc and a bilateral decrease in DOPAC was found in the SN of rats receiving the trophic factor.

The apparent distinction between the whole tissue and dialysate measures of DA and DA metabolites within the striatum and NAc provides insight into the particular actions of GDNF in these regions. The HPLC-EC data suggest that the GDNF-induced increases in both basal and evoked release of DA within the striatum/NAc region is not caused by an increased concentration of stored striatal DA. Instead, the observed effects may be a result of the increased storage of DA within the NAc and/or a direct affect of GDNF on the regulation of DA available for release. Likewise, enhanced DA release may be a result of a regulation process that senses increased storage within the cell bodies. Additional experiments are necessary to determine the relationship between static neurochemical levels within the cell bodies and terminal fields and the observed augmented DA release seen within the dorsal striatum and NAc.

One of the most profound findings from this study is the dramatic alterations in the mesolimbic dopaminergic system that result from a single unilateral intranigral injection. Recently, numerous reports have indicated the substantial
effects of GDNF on the nigrostriatal dopaminergic system; this is, however, the first report of GDNF-induced alterations in the storage and synthesis of DA within the NAc and VTA brain regions. The data suggest that GDNF administration unilaterally in the SN can alter the capacity of DA neurons within the mesolimbic (A10) system bilaterally.

Whatever its mode of action, the effects of GDNF on mesolimbic neurons in aged F344 rats is not only surprising but also important in the field of aging research. Our laboratory has previously shown that DA neurons within the NAc of F344 rats show significant deficiencies in stimulus-evoked release as early as 18 months (Friedemann and Gerhardt, 1992). At this same time point, striatal neurons show very little age-related deficits. In fact, it is not until 24 months before deficits in DA uptake and release are apparent in the striatum. The significant GDNF-induced changes in the mesolimbic system of the rats observed in this study suggest that this factor could be used not only as a restorative agent but perhaps as a prevention against age-induced degeneration of dopaminergic neurons.

GDNF may be acting via several different mechanisms to elicit the observed response. GDNF may diffuse from the SN to interact directly with receptors for GDNF on VTA cell bodies; however, the presence of GDNF receptors within the rat VTA remains to be elucidated. It has been demonstrated that the physiological responses to GDNF require a formation between the GDNFα receptor (Jing et al., 1996; Treanor et al., 1996) and the orphan tyrosine kinase receptor Ret (Takahashi et al., 1993), thereby inducing its tyrosine phosphorylation (Treanor et al., 1996). Evidence does suggest that rat embryonic ventral midbrain and adult SN neurons express high levels of mRNA for the GDNFα receptor (Treanor et al., 1996) and the c-RET receptor (Trupp et al., 1996). If receptors for GDNF are not present in the VTA, then the observed effects within the A10 system may arise from indirect actions of GDNF on other types of neurons by way of signaling cascades.

GDNF or its downstream effectors may cause an alteration in the dopaminergic afferents themselves or in other neurotransmitter circuits in the brain that may affect the functioning of DAergic neurons terminating in the striatum and NAc. Results from our laboratory and others confirm the nondopaminergic effects of GDNF in the central nervous system (Beck et al., 1996; Hoffer et al., 1994; Hudson et al., 1995; Tomac et al., 1995). Two interesting observations from this study included the significant increase in 5-HT within the ipsilateral NAc, and the significant decrease in NE levels within the ipsilateral SN and VTA. Several other studies (Arenas et al., 1995; Beck et al., 1996) have reported alterations in the serotonergic and noradrenergic systems with GDNF administration. Alternatively, accumulating evidence has indicated that the DAergic tone of the nigrostriatal system is modulated by cholinergic (Chesselet, 1984; Joseph and Roth, 1988; Joseph et al., 1988) and glutamatergic inputs (Shimizu et al., 1990; Trussell and Fischbach, 1989; Wu et al., 1993). Experiments involving intraventricular infusions of GDNF into aged F344 rats have yielded increases in choline acetyltransferase activity in the striatum of aged rats (Jiao et al., 1996). Although, GDNF was originally characterized based on its DAergic selectivity (Lin et al., 1993, 1994), this trophic factor may enhance both dopaminergic and nondopaminergic systems which may interact to produce the observed GDNF-related neurochemical and behavioral changes in these aged rats.

With a few exceptions, the present study yielded behavioral, stimulus-evoked release and neurochemical data which paralleled results reported in young rats (Hebert et al., 1996). Increases in movement speed were observed at 3 weeks posttreatment. Additionally, 2-fold increases in both K+ - and d-amphetamine-induced DA release were observed in both experiments. Whole-tissue levels of DA in the ipsilateral striatum of GDNF-treated animals were found to be reduced in both studies. However, in the present study we observed significant increases in DA levels within the SN of aged animals 3 weeks postinjection, which was not observed at the same time point in young animals.

In conclusion, these results indicate that a single GDNF administration can greatly augment DA function in the basal ganglia of aged F344 rats. The treatment-induced changes include increases in spontaneous activity, basal and stimulus-evoked DA overflow and whole-tissue neurochemical alterations. This is the first report to show changes in the functional capacity of dopaminergic neurons of aged Fischer 344 rats resulting from a single intranigral injection of GDNF. The implications of a trophic factor treatment enhancing DA release are profound, in that deficits in the dynamic properties of the nigrostriatal and mesolimbic system have been observed in aged rats and monkeys (Burwell et al., 1995; Friedemann and Gerhardt, 1992; Gerhardt et al., 1995; Joseph et al., 1978; Rose et al., 1986). Although additional experiments are needed to investigate the direct actions of GDNF which may act to restore function to DAergic cell bodies and terminals within the striatum and NAc of aged animals, the combined data suggest that GDNF may be beneficial in restoring function to the nigrostriatal and mesolimbic systems of aged animals.

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References


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