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Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover *in vivo*

(nigrostriatal neurons/Parkinson disease/neostriatum)

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ABSTRACT **Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor (NGF)-related family of neurotrophins, promotes the survival and differentiation of cultured nigral dopamine neurons. Two-week infusions of BDNF were made above the right pars compacta of the substantia nigra in adult rats. Systemic injection of these animals with (+)-amphetamine, a dopamine-releasing drug, induced 3 or 4 body rotations per minute directed away from the nigral infusion site. Neither supranigral NGF nor neocortical BDNF infusions induced rotational behavior. Systemic injections of the postsynaptic dopamine receptor agonist apomorphine did not induce rotations in these animals, demonstrating a presynaptic dopamine neuron locus for BDNF action. In support of this, neostriatal levels of the dopamine metabolite homovanillic acid (HVA) were elevated by 28%, and the HVA/dopamine and dihydroxyphenylacetic acid (DOPAC)/dopamine ratios were elevated by 56% and 34%, respectively, in the BDNF-infused brain hemisphere. BDNF augmented striatal concentrations of HVA and DOPAC and the metabolite/dopamine ratios to even greater extents after (+)-amphetamine injection, when peak rotational effects occurred. Intrastriatal infusions of BDNF produced fewer rotations per minute (1–2.5) after (+)-amphetamine and smaller elevations in HVA and the HVA/dopamine ratio (15% and 30%, respectively) than after supranigral delivery. Neither striatal dopamine, γ-aminobutyric acid, nor acetylcholine high-affinity uptake or the synthetic enzymes for these neurotransmitters was altered by BDNF. These behavioral and neurochemical effects demonstrate an action of BDNF on dopamine neurons *in vivo* and are consistent with a potential role for BDNF in the treatment of Parkinson disease.**

It is well established that nerve growth factor (NGF) promotes the *in vitro* survival and differentiation of cultured embryonic basal forebrain cholinergic neurons (1, 2). Intracranial infusions of NGF attenuate the atrophy of these neurons following a lesion of the septohippocampal pathway (3, 4). However, the majority of central nervous system neurons tested do not respond to NGF, including dopamine neurons of the substantia nigra tested *in vitro* (5, 6) or *in vivo* (7). Because the etiology of Parkinson's disease results from the loss of these neurons, the identity of a dopamine neurotrophic factor has been an area of considerable interest. One such protein appears to be brain-derived neurotrophic factor (BDNF) (8). Deduction of a partial amino acid sequence for BDNF led to its molecular cloning (9) and production by recombinant expression systems. BDNF, like NGF, supports the survival and differentiation of cultured cholinergic neurons (10, 11) and quail dorsal root ganglia *in vivo* (12). In contrast to NGF, BDNF also enhances *in vitro* survival of embryonic dopaminergic neurons in the absence of glia and in serum-free conditions (5, 11, 13, 14). Several mitogenic growth factors, including epidermal growth factor and basic fibroblast growth factor, also promote the growth of cultured or transplanted mesencephalic dopamine neurons (15–19). However, these *in vitro* and *in vivo* effects appear to be mediated via astrocytes (15, 16).

It remains unknown whether BDNF exerts neurotrophic effects on central nervous system neurons *in vivo*. Injections of ^{125}I-labeled BDNF into the rat neostriatum (area of nigrostriatal dopamine neuron terminals) result in a pharmacologically specific retrograde transport of ^{125}I-BDNF to dopamine neuron cell bodies in the pars compacta of the substantia nigra (20). The present study investigated whether chronic infusion of BDNF above the pars compacta or into the rat neostriatum can augment functional capacities of nigrostriatal dopamine neurons. For behavioral measures, we exploited the well-known ability of systemic injections of (+)-amphetamine to induce body rotations away from the side of the brain that contains the stronger nigrostriatal dopamine system (21, 22). For neurochemical measures, dopamine and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in the neostriatum to reveal changes in dopamine turnover and metabolism (22–25).

MATERIALS AND METHODS

Production, Formulation, Stability, and Delivery of BDNF. Recombinant human BDNF (26) was at least as active as native BDNF isolated from pig brain when compared in cultures of dorsal root ganglion explants or dissociated neurons (Y. Barde, personal communication). BDNF was diluted in phosphate-buffered saline (PBS) to 1.0 mg/ml and supplemented in some cases with 0.1% bovine serum albumin (BSA) (protease-free; Boehringer Mannheim). Either formulation (1 ml) was spiked with 1 μl of ^{125}I-BDNF and loaded into Alzet 2002 osmotic pumps (flow rate, 0.5 μl/hr; Alza). Each pump was connected to an 11.2-cm piece of silated PE50 tubing (Micro-Renathane; Braintree Scientific). The pump was immersed in a glass vial that contained PBS and 0.002% sodium azide and was maintained at 37°C for 2 weeks. The effluent was collected into 400 μl of PBS at 4-day intervals. Samples were assessed for BDNF activity as described (5).

Animal Surgery. Male Sprague–Dawley rats (200–240 g; n = 6–12 per group) were treated in compliance with AALAC guidelines. Each osmotic pump was connected to a 2-cm

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Abbreviations: BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; ChAT, choline acetyltransferase; DOPAC, dihydroxyphenylacetic acid; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; HVA, homovanillic acid; NGF, nerve growth factor; TOH, tyrosine hydroxylase.

piece of tubing and a 28-G osmotic pump connector cannula 5.5 mm long for intrastriatal infusions or 6.8 mm long for supranigral infusions (Plastics One, Roanoke, VA). The pumps and flow moderators were filled with vehicle (sterile PBS with or without 0.1% BSA), BDNF (1 mg/ml), or murine NGF (1 mg/ml) to deliver 12 μg/day of either neurotrophic factor and were implanted during general anesthesia (4). This dose of BDNF was used because intracerebroventricular infusions of this dose for 2 weeks did not alter striatal dopamine or dopamine metabolite levels (unpublished observations). A 1-mm-diameter hole was drilled in the calvarium either above the right central caudate-putamen (at the following skull surface coordinates: anterior, 0.3 mm; lateral, 3.0 mm) or above the right substantia nigra (at the following interaural coordinates: anterior, 2.5 mm; lateral from midline, 2.7 mm) and the appropriate cannula was inserted. Cannulas (1.6 mm long) that delivered BDNF into the neocortex were inserted by using the supranigral infusion coordinates.

Rotational Behavior. Thirteen days after surgery, rotational behavior (21, 22) was measured with an automated rotational monitor (Rota-Count 8; Columbus Instruments, Colombus, OH) immediately after the administration of vehicle (1 ml per kg of body weight), (+)-amphetamine sulfate (3.3 mg of free base per kg of body weight, i.p.), or apomorphine HCl (0.5 mg of free base per kg of body weight, s.c.) (21, 22). Complete rotations (360°) were recorded for both clockwise (ipsiversive to the infused hemisphere) and counterclockwise (contraversive to the infused hemisphere) directions for 80 min.

Neurochemical Measurements. Sixteen days after surgery, the animals were sacrificed and the left and right caudate-putamen were dissected and homogenized in 10 vol of 0.32 M sucrose. Protein (27), catecholamines (28, 29), and the high-affinity uptake of choline, dopamine, γ-aminobutyric acid (GABA), and glutamate (30) were measured in aliquots of the sucrose homogenates. The activities of choline acetyltransferase (ChAT) (31), tyrosine hydroxylase (TOH) (32), and glutamic acid decarboxylase (GAD) (33) were measured in additional aliquots to determine the biosynthetic capacity for acetylcholine, dopamine, and GABAergic neurons, respectively.

Statistical Analysis. The statistical significance of changes in rotational behavior, and neurochemical measurements were assessed by one-way analysis of variance followed by Dunnet's *t* and Newman–Keuls post hoc comparison tests (34).

RESULTS

Delivery of BDNF from Osmotic Pumps. The delivery of BDNF from Alzet osmotic pumps maintained for 2 weeks at 37°C was verified by scintillation spectometry, reversed-phase HPLC, and biological activity using cultured dorsal root ganglia. The recovery of ^{125}I-BDNF radioactivity at 1–4, 6–10, or 11–14 days was 77–83% in the absence of BSA and 72–85% in the presence of BSA. Reversed-phase HPLC revealed little or no degradation products in BDNF solutions compared with the initial BDNF material. BDNF recovered from the pumps showed at least 80% of the activity and potency in inducing outgrowth of neurites from explanted dorsal root ganglia when compared with the stock solutions of BDNF that had been stored at 4°C or at −80°C.

Supranigral BDNF. Histological analysis revealed very few cannulas that were placed >1.5 mm from the target and only minor decreases in TOH immunoreactivity that was adjacent to the cannula tract. Two weeks of chronic BDNF infusion into the neostriatum or above the substantia nigra, daily observation, and, in several cases, objective behavioral scoring of videotaped intervals (35) revealed no alteration in spontaneous turning or postural bias, stretching, yawning, hindlimb scratching, sniffing, limb flicks, jumping, or snout contact with the cage surface. There was a transient lowering of body weight that lasted 2–4 days after surgery in several groups of animals that received BDNF into the striatum and, to a lesser extent, the substantia nigra. The body weights of the BDNF-treated animals increased over the next 2 weeks at a rate equal to that of vehicle-treated animals and exceeded their preoperative body weight but remained 10–15% (supranigral delivery) or 15–20% (striatal delivery) below vehicle-infused animals.

Animals receiving supranigral infusions of BDNF produced an average of 3 or 4 contraversive rotations per min and nearly 3 net contraversive rotations per min during the 80 min after (+)-amphetamine administration (Fig. 1) and up to 4 or 5 contraversive rotations per min during 10-min intervals from 20 to 80 min after (+)-amphetamine injection (Fig. 2). Supranigral infusions of vehicle (Fig. 1) or murine NGF or neocortical infusions of vehicle or BDNF (data not shown) did not induce rotational behavior after (+)-amphetamine injection. A subcutaneous dose of the postsynaptic dopamine receptor agonist apomorphine (0.5 mg/kg) failed to induce rotations in rats that received supranigral infusions of vehicle or BDNF, yet (+)-amphetamine given 2 days later to these animals induced contraversive rotations (Fig. 3) as observed in the four prior experiments.

HPLC revealed that supranigral BDNF infusions produced a 28% elevation in striatal HVA concentrations of the infused hemisphere (Fig. 4). The HVA/dopamine and DOPAC/dopamine ratios were elevated 56% and 34%, respectively, by BDNF over the vehicle-infused group, and DOPAC concentrations did not change. The statistically nonsignificant 20% decrease in striatal dopamine concentrations contributed to the more robust elevations of the metabolite/dopamine ratios.

An injection of vehicle or (+)-amphetamine was made 30 min before sacrifice in additional animals that had received 2-week supranigral infusions of vehicle or BDNF. After vehicle injection, BDNF pretreatment was again associated with an elevation in basal HVA (23%) and the HVA/dopamine ratio (20%; Fig. 5). (+)-Amphetamine injections produced greater elevations in the concentrations of HVA

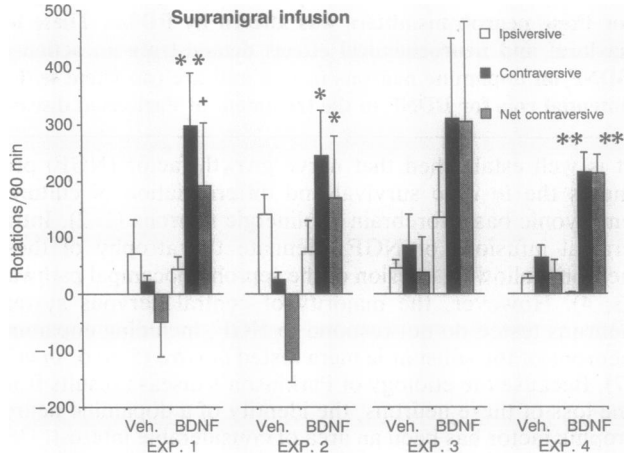

FIG. 1. (+)-Amphetamine-induced rotational behavior in animals receiving supranigral infusions of vehicle or BDNF is shown for four separate experiments. Male rats (n = 7–11 per group) received 2-week infusions of vehicle (0.5 μl/hr) or BDNF (0.5 μg/hr) above the right pars compacta and were then injected i.p. with (+)-amphetamine (3.3 mg/kg). Complete rotations toward (ipsiversive) or away from (contraversive) the infused side were measured for 80 min. Net contraversive rotations reflect the difference between contraversive and ipsiversive rotations. Values are means ± SEM. **, $P < 0.01$; *, $P < 0.05$; +, $P < 0.07$ vs. vehicle-infused group; Dunnett's *t* test.

(33%), DOPAC (64%), HVA/dopamine (49%), and DOPAC/dopamine (72%) compared to (+)-amphetamine-treated animals that had received supranigral vehicle infusions (Fig. 5).

Analysis of the TOH and ChAT activities in striatal homogenates after supranigral infusion of vehicle revealed enzyme activities of 19 ± 0.6 fmol per 8 min per μg of protein and 71 ± 2.7 pmol per hr per μg of protein, respectively, in reasonable agreement with previous reports (36, 37). Neither TOH nor ChAT activities were altered relative to the vehicle-infused groups after supranigral infusions of BDNF. The high-affinity striatal uptake of dopamine or GABA was 41.1 ± 2 and 33.9 ± 7.3 fmol per μg of protein per 10 min, respectively, in vehicle-infused rats and was unchanged following supranigral or intrastriatal administration of BDNF. Analysis of GAD activity in the substantia nigra showed that 34.6 ± 2.7 pmol of glutamate was converted each 2 hr per μg of protein, in approximate agreement with previous findings (38). GAD levels in the substantia nigra were unchanged after supranigral infusions of vehicle or BDNF compared with the intact hemisphere (data not shown).

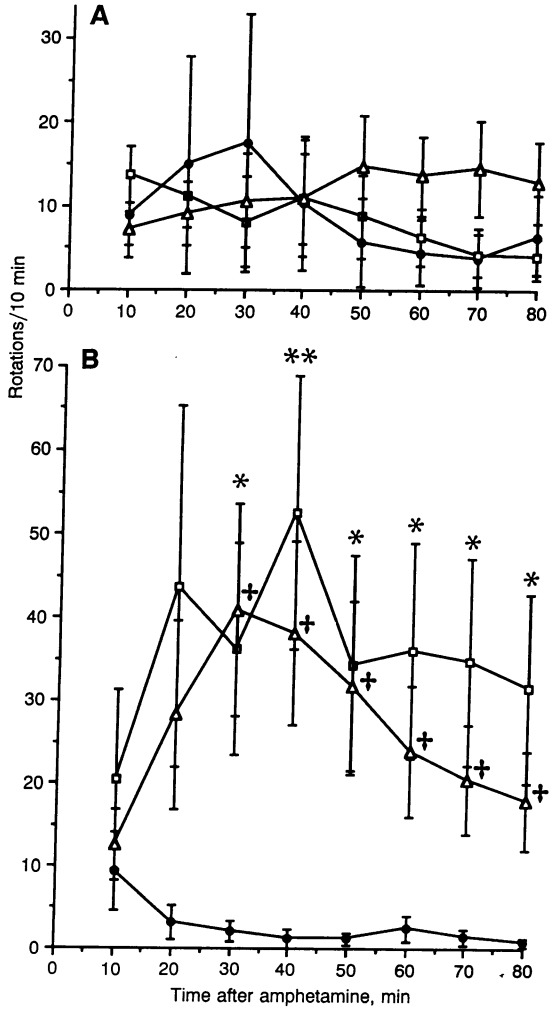

FIG. 2. Ipsiversive (*Upper*) and contraversive (*Lower*) rotations per 10-min interval by rats infused with vehicle or BDNF as described in Fig. 1 and challenged with (+)-amphetamine; $n = 8$ per group (supranigral) or 6–12 per group (intrastriatal). **, $P < 0.01$; * and †, $P < 0.05$ vs. vehicle-treated group; Student's t test. The (+)-amphetamine rotation data for rats that received intrastriatal vehicle closely resembled that of the group that received supranigral vehicle and are not shown. □, Supranigral BDNF; △, neostriatal BDNF; ●, supranigral vehicle.

Intrastriatal BDNF. Compared with supranigral BDNF, intrastriatal BDNF treatments were associated with fewer (1–2.5 per min) and statistically less significant contraversive rotations after (+)-amphetamine over four experiments. The group of rats that received intrastriatal BDNF and that showed the strongest rotational response is plotted in Fig. 2. Intrastriatal infusions of vehicle were not associated with a rotational bias after (+)-amphetamine injections. Intrastriatal BDNF produced small but statistically significant elevations in striatal HVA (15%) and the HVA/dopamine ratio (30%) but did not change dopamine or DOPAC concentrations or the DOPAC/dopamine ratio (Fig. 4). Neostriatal levels of TOH or ChAT, or the high-affinity uptake of dopamine, GABA, choline, or glutamate were unaltered by intrastriatal infusions of BDNF.

Supranigral NGF and Intracortical BDNF. Supranigral infusion of NGF for 2 weeks failed to induce contraversive rotations for 80 min after (+)-amphetamine injection (187 ± 75 per min) compared with vehicle-infused animals (85 ± 55 per min) ($P > 0.05$; $n = 8$ per group). A 2-week intracortical infusion of BDNF also failed to induce contraversive rotations during the 80 min following (+)-amphetamine administration, producing only 5 ± 2 contraversive rotations compared with 30 ± 24 for animals that received neocortical infusions of vehicle. Neither net contraversive nor ipsiversive rotations were altered by cortical BDNF or supranigral NGF compared with the vehicle-treated group.

DISCUSSION

The present results demonstrate that central nervous system administration of BDNF can produce a pharmacologically

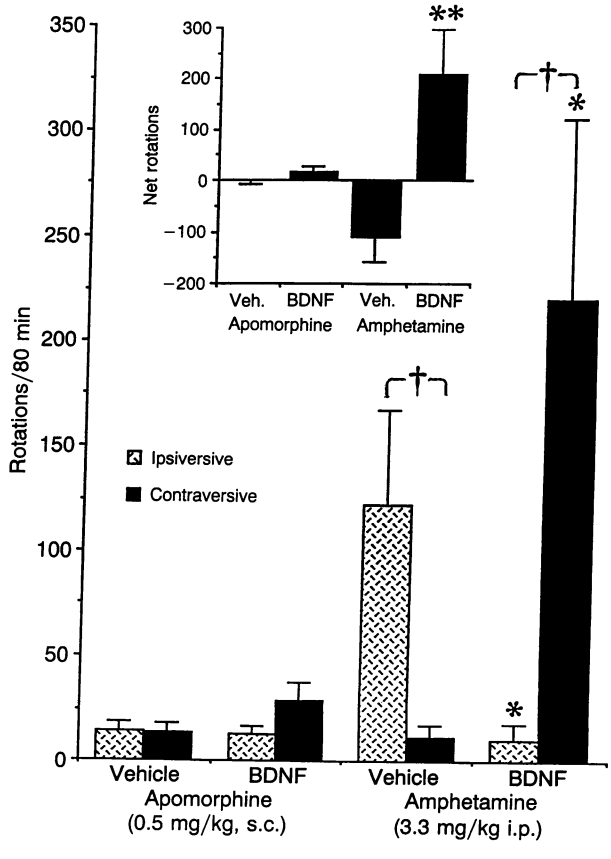

FIG. 3. Ipsiversive, contraversive, and net contraversive rotations (*Inset*) after 0.5-mg/kg apomorphine or 3.3-mg/kg (+)-amphetamine injections of vehicle (Veh.)- or BDNF-infused rats ($n = 8$ per group). Apomorphine failed to induce rotations in either group, whereas (+)-amphetamine induced contraversive and net contraversive rotations in BDNF-treated rats. *, $P < 0.05$; **, $P < 0.01$ vs. vehicle-treated group; †, $P < 0.05$ vs. ipsiversive rotation.

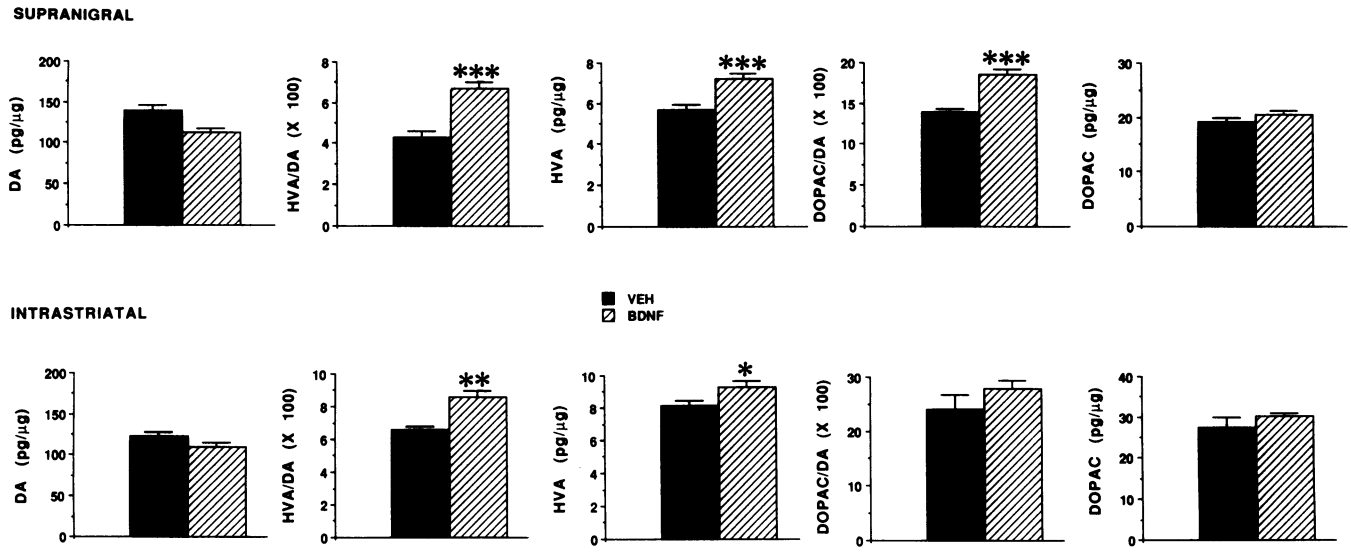

FIG. 4. Dopamine (DA), HVA, and DOPAC, and HVA/DA and DOPAC/DA ratios in the neostriata of rats that received 2-week supranigral ($n = 31$) or intrastriatal ($n = 28$) vehicle (solid bars) or BDNF (hatched bars). Values are means ± SEM of pooled values from four experiments conducted 4 weeks apart; HVA and HVA/DA ratio were significantly elevated in each of the four experiments for either BDNF delivery route. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$ vs. vehicle group. Values in the uninfused hemisphere did not vary between vehicle and BDNF-treated animals in any experiment (data not shown).

and anatomically specific increase in the behavioral and neurochemical output of the nigrostriatal dopamine pathway. These findings represent an extension to the intact animal of the neurotrophic effects of BDNF on cultured embryonic dopamine neurons observed *in vitro* (5, 13, 14, 39). Rotational behavior was not induced by (+)-amphetamine in animals that received supranigral infusions of NGF, intracortical infusions of BDNF, or striatal or supranigral infusions of the vehicle, which contained a carrier protein, BSA.

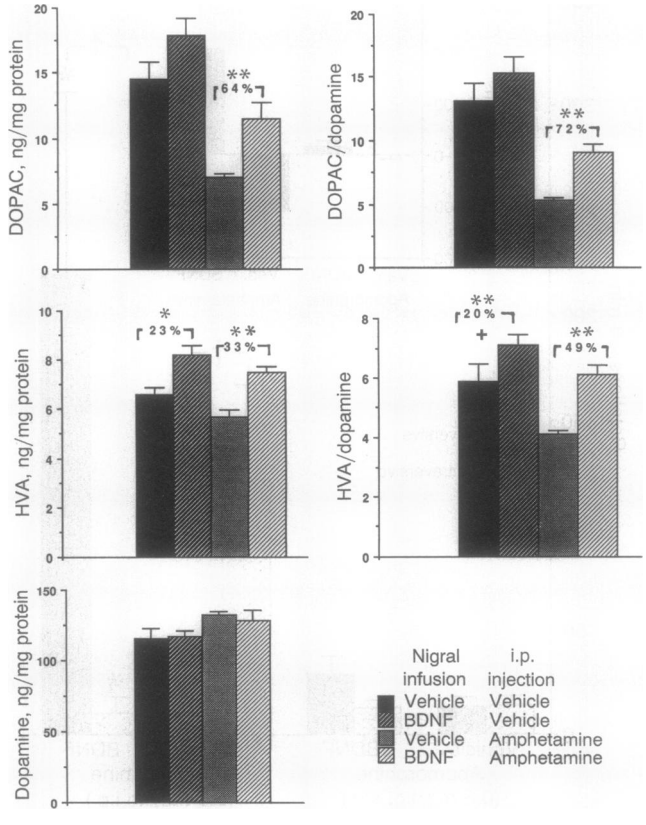

FIG. 5. Potentiation by (+)-amphetamine (3.3 mg/kg, i.p.; 30 min before sacrifice) of striatal dopamine metabolite and metabolite/dopamine ratios by supranigral infusion of BDNF. +, $P < 0.06$; *, $P < 0.05$; **, $P < 0.01$ vs. nigral vehicle infusion group; Dunnett's t test; n = 8–10 per group. Numbers between brackets refer to percentage by which BDNF-infused group exceeded the vehicle-infused group.

The contraversive rotational behavior induced by (+)-amphetamine in BDNF-treated rats resembled in appearance, but were opposite in direction, the 5–10 ipsiversive rotations that typically occur after (+)-amphetamine injections to animals with extensive unilateral nigrostriatal lesions (21, 22). Rotational behavior results from a greater dopamine release or greater effect of postsynaptic dopamine receptor stimulation in the striatum opposite the direction of turning. The inability of apomorphine to induce rotation in these animals suggests that BDNF may produce an augmented response by nigrostriatal neurons to amphetamine rather than an enhanced sensitivity of postsynaptic targets of these neurons. In support of this, BDNF infusions elevated the turnover and/or release of dopamine, as evidenced by elevations in striatal levels of HVA and DOPAC/dopamine and HVA/dopamine ratios. That high-affinity striatal dopamine uptake or TOH content was unchanged in these animals indicates that dopamine turnover and/or release, rather than terminal sprouting, was enhanced in the BDNF-infused hemisphere. HVA/dopamine or DOPAC/dopamine ratios are elevated after at least a 30% lesion of the nigrostriatal pathway but do so because HVA and DOPAC levels are diminished less than is dopamine (22, 24). Importantly, the metabolite/dopamine ratio increases, and the induction of rotation in the present study, were obtained in the absence of dopamine nerve terminal damage. In fact, the striatal contents of HVA and, in several experiments, DOPAC, were elevated above values in the vehicle or uninfused brain hemispheres. Thus, the elevation in dopamine metabolism after BDNF is not like that obtained after partial nigrostriatal lesions (22, 24), nor is it like that observed after *in vivo* inhibition of dopamine neuron impulse conduction, which results in equivalent increases in the dopamine metabolite levels (particularly DOPAC) and dopamine (23).

The increases in HVA and the HVA/dopamine ratio in the present study were generally greater than those for DOPAC and DOPAC/dopamine. DOPAC primarily reflects intraneuronal metabolism of dopamine, whereas HVA is a product of intra- and extraneuronal metabolism of released dopamine

(23, 25). A direct study of dopamine release by measurements of neostriatal 3-methoxytyramine (25, 40), the unique extracellular metabolite of dopamine, or *in vivo* microdialysis of extracellular dopamine in animals treated with BDNF will help identify whether the increases in dopamine metabolites observed with supranigral BDNF result from elevations in dopamine release, metabolism, or both. A relative inability of dopamine biosynthesis to replenish dopamine that is depleted by chronically augmented release could also explain the trend for decreased dopamine levels that we observed.

Intrastriatal injections of ^{125}I-BDNF result in retrograde transport and accumulation of ^{125}I-BDNF in TOH-positive nigrostriatal cell bodies of the pars compacta (20). High-affinity ^{125}I-neurotrophin 3 binding sites that can be displaced by BDNF (41) and the mRNA for BDNF (40) are in the pars compacta. The greater behavioral and neurochemical effects found here with supranigral infusions of BDNF indicate that more nigrostriatal neurons may be affected by BDNF by this route compared with the intrastriatal route. Supranigral infusions of BDNF may reach more nigrostriatal neurons, by virtue of the close proximity of their cell bodies in the pars compacta than infusions into the larger and more diffuse striatal terminal field. BDNF also confers trophic effects on cholinergic (10, 11) and GABAergic (13) neurons *in vitro*. However, neither striatal cholinergic, GABAergic, or glutamatergic terminals, cholinergic or catecholaminergic enzyme activities, nor nigral GABAergic neuron GAD levels were altered by supranigral or intrastriatal BDNF delivery. It is possible that alterations in the release of GABA, glutamate, or acetylcholine could account for these effects. However, *in vivo* microdialysis of the caudate-putamen of freely moving animals infused with BDNF according to the present protocol reveals no effect on basal glutamate or GABA release.

Basic fibroblast growth factor (FGF), acidic FGF (17–19), and epidermal growth factor (42) exert a modest and apparently glial-dependent (15, 16) attenuation of nigrostriatal neuron damage *in vivo* and have not been shown to affect intact nigrostriatal neurons. Unlike epidermal growth factor or basic FGF (15, 16), the *in vitro* effects of BDNF (5, 13, 39) are obtained in low density and glial-free cultures. BDNF can also attenuate the death of cultured dopamine neurons exposed to the dopamine neurotoxins 6-hydroxydopamine or 1-methyl-4-phenylpyridine (MPP^+) (13, 39). The protective effects of BDNF may be mediated by an antioxidant-promoting capacity, since BDNF increases the glutathione reductase activity of dopaminergic SH-SY5Y neuroblastoma cells 2-fold and prevents the 5-fold elevation in oxidized glutathione normally associated with 6-hydroxydopamine toxicity (39). BDNF infusions to the pars compacta before and after nigrostriatal injury thus may also reverse behavioral or neurochemical deficits associated with the lesion, either by attenuating dopamine neurodegeneration or by potentiating the function of surviving dopamine neurons.

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