

Short communication

Patterns of developmental mRNA expression of neurturin and GFR α 2 in the rat striatum and substantia nigra do not suggest a role in the regulation of natural cell death in dopamine neurons

JinWhan Cho^a, Nikolai G. Kholodilov^a, Robert E. Burke^{a,b,*}

^aDepartment of Neurology, The College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA

^bDepartment of Pathology, The College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA

Accepted 18 November 2003

Abstract

We examined the mRNA expression of neurturin (NTN) and its receptor GFR α 2 in rat substantia nigra (SN) and striatum by northern analysis at ages ranging from postnatal day (PND) 2 to adult. NTN mRNA expression is developmentally regulated in striatum with a peak at PND10, but its expression in striatum is low, and less than that of SN. In SN, there is no developmental regulation. GFR α 2 was expressed most highly during the first two postnatal weeks. Like NTN, GFR α 2 mRNA was also more abundant in SN, at both PND2 and 14. Our results show that NTN expression is relatively low in the striatum, the target of dopamine (DA) neurons, and there is no apparent pattern of developmental regulation in SN. Thus these studies are not strongly supportive of a role for NTN in regulating natural cell death (NCD) in DA neurons, either as a target-derived or as a local paracrine factor.

© 2003 Elsevier B.V. All rights reserved.

Theme: Development and regeneration

Topic: Neurotrophic factors: expression and regulation

Keywords: Neurturin; GFR α 2; Natural cell death; Dopamine neuron; Substantia nigra; Striatum

Dopamine (DA) neurons of the substantia nigra (SN), like other neuronal populations, undergo a natural cell death (NCD) event during development [17,18,24]. This event is biphasic in rodents, with an initial peak at PND2 and a second at PND14. As envisioned by classic neurotrophic theory [4], the NCD event in these neurons is likely to be regulated by target interactions. In vitro studies have shown that the viability and differentiation of developing mesencephalic DA neurons are supported by striatal preparations [13,15,30]. We have shown in vivo that disruption of interactions between DA neurons and the striatal target, by target lesion [21], DA terminal lesion [22], or axotomy [10] leads to an augmentation of NCD. It is also likely, however, that other nontarget interactions may also regulate this

event, such as a local paracrine or autocrine factors, and afferent projections [2]. However, none of the endogenous, physiologic factors that regulate this event for DA neurons have yet been conclusively identified.

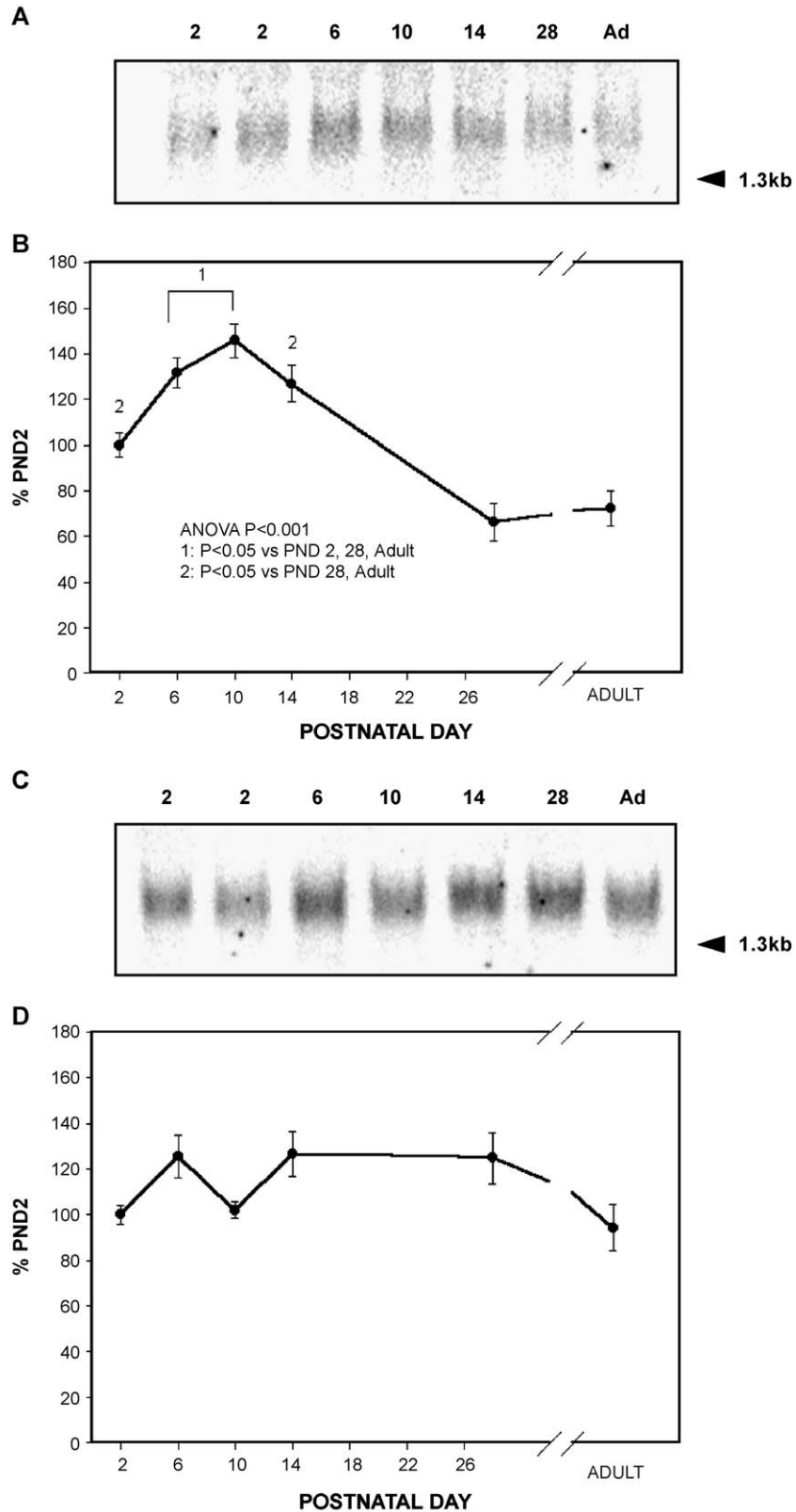
The glial cell line-derived neurotrophic factor (GDNF) family members play a significant role as trophic factors, supporting viability of neurons during development. Considerable evidence now suggests that GDNF, in particular, may serve as a striatal target-derived neurotrophic factor for SN DA neurons. Its mRNA is highly expressed in striatum during the postnatal NCD event [6,8,9,28,29], and its receptor, GFR α 1, and signaling kinase, Ret, are highly expressed in the SNpc [31,32]. We have shown that GDNF suppresses apoptotic cell death in DA neurons during the postnatal period both in vitro [7] and in vivo [25], and that intra-striatal injection of neutralizing antibodies to GDNF augments the NCD event in vivo [25]. We have also shown that sustained overexpression of GDNF in striatum in double transgenic mice increases the surviving number of DA neurons after the first phase of NCD (Kholodilov et al., submitted).

* Corresponding author. Department of Neurology, The College of Physicians and Surgeons, Columbia University, Room 308, Black Building, 650 West 168th Street, New York, NY 10032, USA. Tel.: +1-212-305-7374; fax: +1-212-305-5450.

E-mail address: rb43@columbia.edu (R.E. Burke).

Neurturin (NTN), another member of the GDNF receptor ligand family of proteins, has been considered to possibly be a neurotrophic factor for DA neurons. It was originally purified and cloned by virtue of its ability to support the

survival of sympathetic neurons in culture [20]. NTN exhibits potent effects on the protection and restoration of mid-brain DA neurons both in vitro and in vivo [1,16,20,23]. If NTN is a limiting, striatum-derived neurotrophic factor for



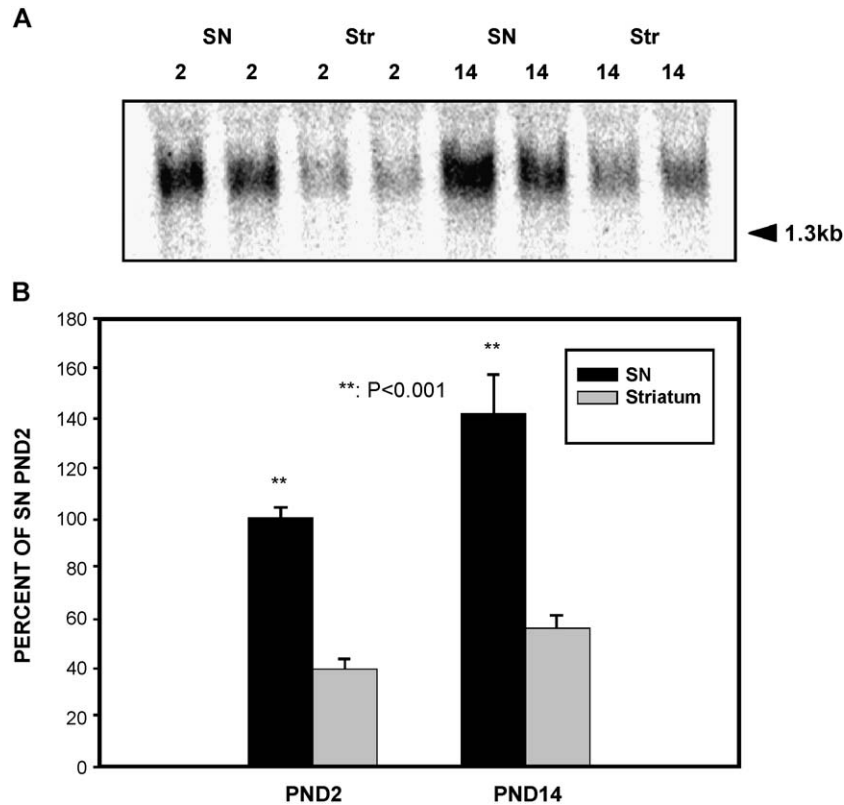


Fig. 2. Relative levels of NTN expression in striatum and SN at the two peaks of NCD in SN DA neurons. (A) Representative Northern analysis of NTN mRNA expression in striatum and SN at PND2 ($N=2$ for each region) and PND14 ($N=2$ for each region). (B) Quantitative analysis of NTN mRNA expression in striatum and SN at the two phases of NCD in SN ($N=8$ for each condition). NTN mRNA was more highly expressed in SN during both periods of maximal NCD in DA neurons ($p<0.001$).

mesencephalic DA neurons, then it would be expected that, like GDNF, its expression should be relatively abundant in striatum, the expression of its receptor should be relatively abundant in SN, and expression should be regulated in phase with NCD events. We have therefore examined the time course, and the relative levels of striatal and SN expression of mRNAs for NTN, and its putative receptor GDNF family receptor (GFR) $\alpha 2$ [3,19,27].

Timed pregnant Sprague–Dawley rats (Charles River Labs, Wilmington, MA) were used to obtain striatum and SN at PND2, 6, 10, 14, 28, and adult. The day of delivery was defined as PND1. SN and striatum tissue were obtained by microdissection as previously described [11]. The tissues were then frozen on dry ice and stored at -80°C until RNA isolation. This protocol has been approved by the Institutional Animal Care and Use Committee at Columbia–Presbyterian Medical Center.

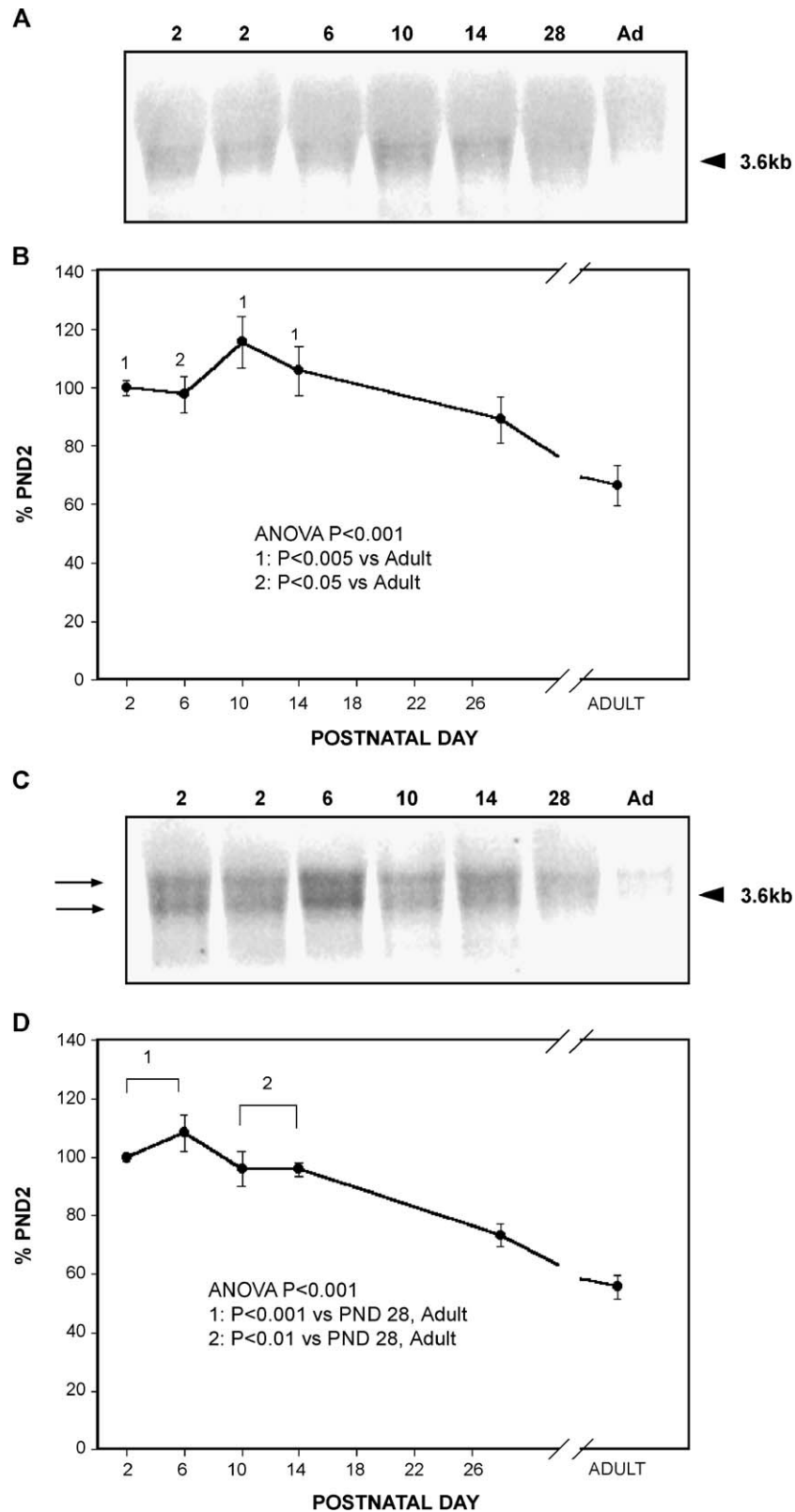
With a database search, we found three partial sequences for NTN. Two of them (GenBank accession no: AF184922 and AB032562) represent all of the coding sequence except the 5'-end. The third sequence, available through LabOn-Web (Compugen), represents the 5'-end of NTN cDNA. Based on these three known rat NTN sequence sources, we constructed a predicted rat NTN full coding sequence (646-bp) and designed primers. We were unable to obtain a complete product by RT PCR using RNA from several tissues. Therefore, we amplified three fragments of rat NTN cDNA (bps: 1–227, 206–385, and 365–671) and annealed them. PCR was then performed on the annealed fragments, and the full size coding region was successfully amplified. It was cloned into a pGEM-T vector and the sequence confirmed (GenBank accession no: AY190603).

Northern analysis was performed as previously described [11]. Briefly, RNA was isolated using Qiagen RNAeasy Mini

Fig. 1. Developmental time course of NTN mRNA expression in striatum and in SN. (A) Representative Northern analysis of NTN mRNA expression in striatum from PND2 to adult. Twenty micrograms of total RNA was loaded per each lane, and the blot was probed with a riboprobe generated from 306 bp cDNA of NTN. The blot revealed one major transcript, at 1.4 kb. (B) Developmental time course of NTN mRNA expression in striatum ($N=16$ for PND2 [$N=2$ per blot, 8 blots were examined]; $N=8$ for all other time points [$N=1$ per blot]). NTN mRNA expression is developmentally regulated in striatum. Higher levels of NTN expression were observed during the early developmental period, with the highest level in the PND10 ($p<0.05$). (C) Representative Northern analysis of NTN mRNA expression in SN from PND2 to adult. (D) Developmental time course of NTN mRNA expression in SN. The temporal pattern of NTN mRNA expression does not show a significant difference at any age, within the limits of the sampling interval used.

kit. The RNA concentration of each sample was determined by measuring absorption at 260 nm on a GenQuant spectrophotometer (Amersham Pharmacia Biotech, Piscataway, NJ). Twenty micrograms of RNA was electrophoresed in

1.4% agarose-formaldehyde gel and transferred onto an Immobilon membrane (Millipore, Bedford, MA). To create probes for Northern analysis, oligonucleotide primers were designed based on our sequence for NTN (GenBank acces-



sion no: AY190603) and accession no. U97143 for GFR α 2. Primers were synthesized by GenLink (Hawthorne, NY). The forward and reverse primers were as follows: for NTN forward primer was 5'-AACCGTCCTGTTCCGCTACT-3' and the reverse primer was 5'-CGAGTTCACAC-TTTATG-3'; for GFR α 2 forward primer was 5'-GATC-TACTGGAGCATCCATC-3' and the reverse primer was 5'-TGTCATATCAAACCCAATCATG-3'. One microgram of the SN or striatum RNA was used for cDNA synthesis with a Promega reverse transcription system using the recommended conditions. RT-PCR was performed in a Mastercycler (Eppendorf Scientific) with Roche Taq polymerase. PCR products were cloned in pGEM-T vector (Promega), and the sequence confirmed. Plasmids were used for generation of a 32 P-labeled antisense riboprobe with an *in vitro* transcription system.

The hybridization was performed overnight at 68 °C in Ultrahyb buffer from Ambion (Austin, TX). The membrane was then exposed to phosphor imager cassettes, scanned and analyzed by Image Quant software (Molecular Dynamics, Indianapolis, IN). For the developmental studies, all developmental ages were represented by a single animal on each membrane, except PND2 which was represented by two. Eight independent hybridizations were performed, for an $N=16$ for PND2, and $N=8$ at all other developmental time points. Radioactive bands were expressed as a relative percentage of the radioactivity at PND2 on each membrane. We used quantitative analysis of total RNA and inspection of ethidium bromide stained gels to ensure equal loading of lanes. We have previously shown that GAPDH mRNA expression, often used as an indicator of total mRNA loading, is developmentally regulated [11], so that normalization by GAPDH mRNA levels results in a spurious, decreased apparent level of expression of the mRNA of interest between postnatal weeks 2 through 4. Similarly, α - and β -tubulin mRNA are developmentally regulated [5]. For these reasons, we did not normalize mRNA determinations by the mRNA for these genes. For the studies of the relative levels of expression in SN and striatum at the two peaks of NCD (PND2 and 14), two animals representing each region and each time point were represented on each blot. Four independent hybridizations were performed, for an $N=8$ for each region at PND2 and 14.

Northern blot analysis showed that NTN mRNA was detected in both striatum and SN as a single transcript of about 1.4 kb, somewhat larger than the 1.0 kb reported for mouse [20]. In striatum, NTN mRNA expression was

developmentally regulated. The level was highest during the early developmental period; it began to increase after PND2 and reached its peak level at PND10, just preceding the second phase of NCD (Fig. 1A,B). Its expression thereafter rapidly declined, and the lowest levels were observed in the PND28 and adult at about 45% of levels observed at PND10. In SN, however, there was no developmental regulation (Fig. 1C,D).

Separate experiments were performed to compare the relative levels of NTN mRNA expression in SN and striatum at the two peaks of NCD at PND2 and 14. Unlike what would be anticipated for a striatal target-derived neurotrophic factor, NTN mRNA was more highly expressed in SN, at more than twice the levels of expression observed in striatum, during both periods of maximal NCD in DA neurons at PND2 and 14 (Fig. 2A,B).

GFR α 2 mRNA was detected in SN as a doublet of two mRNA species, approximately 3.1 and 4.0 kb, as previously reported [27]. However, in striatum, where levels of expression were much lower than in SN (see below), the doublet was more difficult to discern. In striatum, GFR α 2 was developmentally regulated, with the highest levels during the first 2 weeks (Fig. 3A,B). In SN, there also was developmental regulation, with high levels in the first two postnatal weeks, and the highest levels achieved during the first postnatal week (Fig. 3C,D). Thus, in SN, the GFR α 2 receptor for NTN obtains the highest levels of expression prior to the peak level of expression for NTN in the striatal target.

In separate experiments to directly compare the relative abundance of GFR α 2 in SN and striatum at PND2 and 14 on the same blot, we found that like NTN, GFR α 2 mRNA was also more abundant in SN than striatum (at about 55% of levels observed in SN), at both PND2 and 14 ($p<0.001$) (Fig. 4).

NTN has been proposed to possibly serve as a target-derived neurotrophic factor for SN DA neurons. This possibility has been suggested by its ability to support embryonic ventral mesencephalic neurons in culture [1,16], its mRNA expression in developing striatum [1,16], and its ability to both protect and restore adult SN DA neurons in lesion models [1,16,23]. The late developmental expression of NTN in striatum has been interpreted to mean that it may play a sequential role with GDNF, providing support during a later phase of development [1]. Our results are comparable to those of Akerud et al. [1] in that they show a highest level of NTN mRNA expression in striatum at PND10. However, unlike what would be expected for a target-derived neuro-

Fig. 3. Developmental time course of GFR α 2 mRNA expression in striatum and in SN. (A) Representative Northern blot of GFR α 2 mRNA expression in striatum. Twenty micrograms of total RNA was loaded per lane, and the blot was probed with a riboprobe generated from 597 bp cDNA of GFR α 2. (B) Developmental time course of GFR α 2 mRNA expression in striatum ($N=16$ for PND2 [$N=2$ per blot, 8 blots were examined]; $N=8$ for all other time points [$N=1$ per blot]). Note that GFR α 2 is expressed during the first two postnatal weeks, with the highest level at PND10 and a significant decrease at PND28 and the adult time points ($p<0.05$). (C) Representative Northern analysis of GFR α 2 mRNA expression in SN from PND2 to adult. The blot revealed two major transcripts, at 3.1 and 4.0 kb (arrows). (D) Developmental time course of GFR α 2 mRNA expression in SN ($N=16$ for PND2 [$N=2$ per blot, 8 blots were examined]; $N=7-8$ for all other time points [$N=1$ per blot]). Note that expression of GFR α 2 is abundant during the first postnatal week with a peak at PND6, and then it progressively decreases after PND 14 ($p<0.01$).

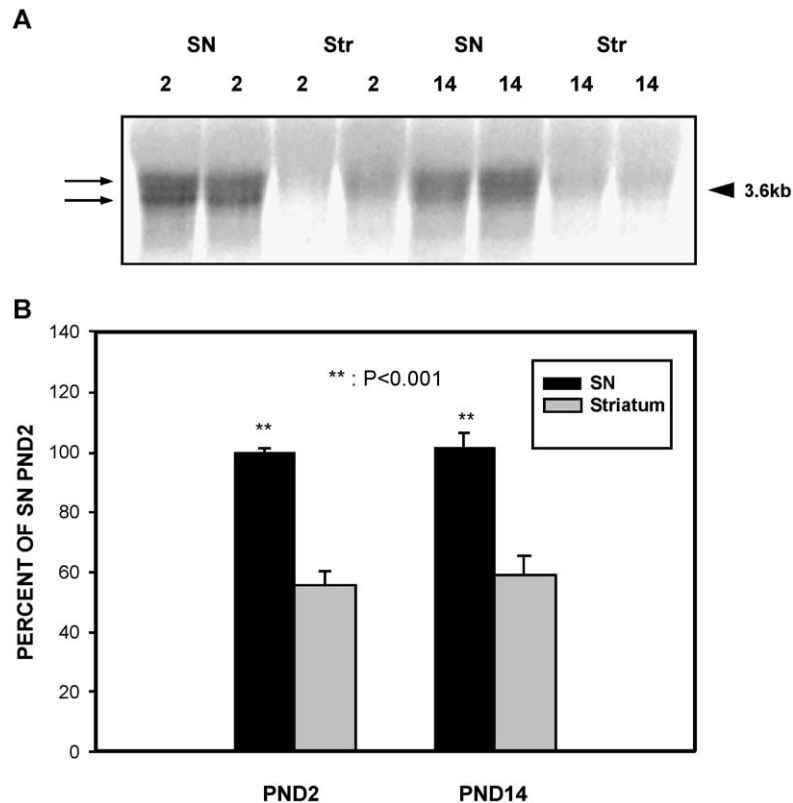


Fig. 4. Relative levels of GFR α 2 expression in striatum and SN at the peaks of NCD in SN DA neurons. (A) Representative Northern analysis of GFR α 2 mRNA expression in striatum and SN at PND2 and PND14. (B) Quantitative analysis of GFR α 2 mRNA expression in striatum and SN ($N=8$ for each condition) at the two phases of NCD in SN. GFR α 2 mRNA was more abundant in SN, at both PND2 and 14 ($p<0.001$).

trophic factor, NTN was more abundantly expressed in SN than striatum during both of the major phases of NCD. Thus, in its regional expression pattern, NTN is quite unlike GDNF, which, as substantial evidence indicates, probably plays a role as a striatal target-derived neurotrophic factor during the first phase of NCD at PND2 in DA neurons (reviewed in Ref. [25]). During the first phase of NCD, GDNF mRNA levels in striatal target are at their peak [8] and are much more abundant than the levels in SN (unpublished observation). The higher level of expression of NTN mRNA in SN would be more compatible with a local autocrine or paracrine role in supporting DA neurons. However, against this possible role during development is our observation that there is no developmental regulation of SN expression.

Expression of GFR α 2 mRNA in the ventral midbrain has previously been demonstrated by in situ hybridization in adult mice [12,16]. However, whether it is expressed during development has been less clear. Golden et al. [12] detected mRNA in the ventral midbrain of embryonic day 18 mice by in situ hybridization, but Widenfalk et al. [31] and Yu et al. [32] were unable to detect mRNA by in situ hybridization in SN at PND7 in mouse and rat, respectively. We have been able to detect GFR α 2 transcripts by Northern analysis in microdissected ventral mesencephalon and find that it is

developmentally regulated, with highest levels in the first postnatal week.

However, against the possibility that NTN acts as a neurotrophic factor for DA neurons through the GFR α 2 receptor is the observation of Horger et al. [16] that GFR α 2 expression does not co-localize with that of SN DA neurons identified by tyrosine hydroxylase immunohistochemistry. This study was performed in adult animals, and while the cellular pattern of expression may be different in the developmental setting, such a possibility at present is unknown. We therefore conclude that our studies, and those of others, of the patterns of mRNA expression for NTN and its putative receptor GFR α 2 are not strongly supportive of a developmental neurotrophic role, either as a target-derived factor or as a local paracrine factor. This conclusion is supported by reports of homozygous null mice for NTN [14] and for GFR α 2 [26], which show no effect on the mature number of SN DA neurons.

Acknowledgements

This work was supported by the Parkinson's Disease Foundation, the Lowenstein Foundation and by NINDS grants NS26836, NS38370.

References

- [1] P. Akerud, J. Alberch, S. Eketjäll, J. Wagner, E. Arenas, Differential effects of glial cell line-derived neurotrophic factor and neurturin on developing and adult substantia nigra dopaminergic neurons, *J. Neurochem.* 73 (1999) 70–78.
- [2] M.A. Alonso-Vanegas, J.P. Fawcett, C.G. Causing, F.D. Miller, A.F. Sadikot, Characterization of dopaminergic midbrain neurons in a DBH:BDNF transgenic mouse, *J. Comp. Neurol.* 413 (1999) 449–462.
- [3] R.H. Baloh, M.G. Tansey, J.P. Golden, D.J. Creedon, R.O. Heuckeroth, C.L. Keck, D.B. Zimonjic, N.C. Popescu, E.M.J. Johnson, J. Milbrandt, TrnR2, a novel receptor that mediates neurturin and GDNF signaling through Ret, *Neuron* 18 (1997) 793–802.
- [4] Y.A. Barde, Trophic factors and neuronal survival, *Neuron* 2 (1989) 1525–1534.
- [5] B. Bhattacharya, C. Mandal, S. Basu, P.K. Sarkar, Regulation of alpha- and beta-tubulin mRNAs in rat brain during synaptogenesis, *Brain Res.* 388 (1987) 159–162.
- [6] M. Blum, C.S. Weickert, GDNF mRNA expression in normal postnatal development, aging, and in weaver mutant mice, *Neurobiol. Aging* 16 (1995) 925–929.
- [7] R.E. Burke, M. Antonelli, D. Sulzer, Glial cell line-derived neurotrophic growth factor inhibits apoptotic death of postnatal substantia nigra dopamine neurons in primary culture, *J. Neurochem.* 71 (1998) 517–525.
- [8] J. Cho, N.G. Kholodilov, R.E. Burke, The developmental time course of glial cell line-derived neurotrophic factor (GDNF) and GDNF receptor alpha-1 mRNA expression in the striatum and substantia nigra, *Ann. N. Y. Acad. Sci.* 991 (2003) 284–287.
- [9] D.L. Choi-Lundberg, M.C. Bohn, Ontogeny and distribution of glial cell line-derived neurotrophic factor (GDNF) mRNA in rat, *Dev. Brain Res.* 85 (1995) 80–88.
- [10] B.F. El-Khodori, R.E. Burke, Medial forebrain bundle axotomy during development induces apoptosis in dopamine neurons of the substantia nigra and activation of caspases in their degenerating axons, *J. Comp. Neurol.* 452 (2002) 65–79.
- [11] B.F. El-Khodori, N.G. Kholodilov, O. Yarygina, R.E. Burke, The expression of mRNAs for the proteasome complex is developmentally regulated in the rat mesencephalon, *Brain Res. Dev. Brain Res.* 129 (2001) 47–56.
- [12] J.P. Golden, R.H. Baloh, P.T. Kotzbauer, P.A. Lampe, P.A. Osborne, J. Milbrandt, E.M.J. Johnson, Expression of neurturin, GDNF, and their receptors in the adult mouse CNS, *J. Comp. Neurol.* 398 (1998) 139–150.
- [13] L.M. Hemmendinger, B.B. Garber, P.C. Hoffmann, A. Heller, Target neuron-specific process formation by embryonic mesencephalic dopamine neurons in vitro, *Proc. Natl. Acad. Sci. U. S. A.* 78 (1981) 1264–1268.
- [14] R.O. Heuckeroth, H. Enomoto, J.R. Grider, J.P. Golden, J.A. Hanke, A. Jackman, D.C. Molliver, M.E. Bardgett, W.D. Snider, E.M.J. Johnson, J. Milbrandt, Gene targeting reveals a critical role for neurturin in the development and maintenance of enteric, sensory, and parasympathetic neurons [see comments], *Neuron* 22 (1999) 253–263.
- [15] P.C. Hoffmann, L.M. Hemmendinger, C. Kotake, A. Heller, Enhanced dopamine cell survival in reaggregates containing target cells, *Brain Res.* 274 (1983) 275–281.
- [16] B.A. Horgor, M.C. Nishimura, M.P. Armanini, L.C. Wang, K.T. Poulsen, C. Rosenblad, D. Kirik, B. Moffat, L. Simmons, E.J. Johnson, J. Milbrandt, A. Rosenthal, A. Bjorklund, R.A. Vandlen, M.A. Hynes, H.S. Phillips, Neurturin exerts potent actions on survival and function of midbrain dopaminergic neurons, *J. Neurosci.* 18 (1998) 4929–4937.
- [17] V. Jackson-Lewis, M. Vila, R. Djaldetti, C. Guegan, G. Liberatore, J. Liu, K.L. O'Malley, R.E. Burke, S. Przedborski, Developmental cell death in dopaminergic neurons of the substantia nigra of mice, *J. Comp. Neurol.* 424 (2000) 476–488.
- [18] E. Janec, R.E. Burke, Naturally occurring cell death during postnatal development of the substantia nigra of the rat, *Mol. Cell Neurosci.* 4 (1993) 30–35.
- [19] R.D. Klein, D. Sherman, W.H. Ho, D. Stone, G.L. Bennett, B. Moffat, R. Vandlen, L. Simmons, Q. Gu, J.A. Hongo, B. Devaux, K. Poulsen, M. Armanini, C. Nozaki, N. Asai, A. Goddard, H. Phillips, C.E. Henderson, M. Takahashi, A. Rosenthal, A GPI-linked protein that interacts with Ret to form a candidate neurturin receptor, *Nature* 387 (1997) 717–721.
- [20] P.T. Kotzbauer, P.A. Lampe, R.O. Heuckeroth, J.P. Golden, D.J. Creedon, E.M.J. Johnson, J. Milbrandt, Neurturin, a relative of glial-cell-line-derived neurotrophic factor, *Nature* 384 (1996) 467–470.
- [21] A. Macaya, F. Munell, R.M. Gubits, R.E. Burke, Apoptosis in substantia nigra following developmental striatal excitotoxic injury, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 8117–8121.
- [22] M.J. Marti, C.J. James, T.F. Oo, W.J. Kelly, R.E. Burke, Early developmental destruction of terminals in the striatal target induces apoptosis in dopamine neurons of the substantia nigra, *J. Neurosci.* 17 (1997) 2030–2039.
- [23] Y. Oiwa, R. Yoshimura, K. Nakai, T. Itakura, Dopaminergic neuroprotection and regeneration by neurturin assessed by using behavioral, biochemical and histochemical measurements in a model of progressive Parkinson's disease, *Brain Res.* 947 (2002) 271–283.
- [24] T.F. Oo, R.E. Burke, The time course of developmental cell death in phenotypically defined dopaminergic neurons of the substantia nigra, *Dev. Brain Res.* 98 (1997) 191–196.
- [25] T.F. Oo, N. Kholodilov, R.E. Burke, Regulation of natural cell death in dopaminergic neurons of the substantia nigra by striatal GDNF in vivo, *J. Neurosci.* 23 (2003) 5141–5148.
- [26] J. Rossi, K. Luukko, D. Poteryaev, A. Laurikainen, Y.F. Sun, T. Laakso, S. Eerikainen, R. Tuominen, M. Lakso, H. Rauvala, U. Arumae, M. Pasternack, M. Saarma, M.S. Airaksinen, Retarded growth and deficits in the enteric and parasympathetic nervous system in mice lacking GFR alpha2, a functional neurturin receptor, *Neuron* 22 (1999) 243–252.
- [27] M. Sanicola, C. Hession, D. Worley, P. Carmillo, C. Ehrenfels, L. Walus, S. Robinson, G. Jaworski, H. Wei, R. Tizard, A. Whitty, R.B. Pepinsky, R.L. Cate, Glial cell line-derived neurotrophic factor-dependent RET activation can be mediated by two different cell-surface accessory proteins, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 6238–6243.
- [28] D.G. Schaar, B.A. Sieber, C.F. Dreyfus, I.B. Black, Regional and cell specific expression of GDNF in rat brain, *Exp. Neurol.* 124 (1993) 368–371.
- [29] I. Stromberg, L. Bjorklund, M. Johansson, A. Tomac, F. Collins, L. Olson, B. Hoffer, C. Humpel, Glial cell line derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons in vivo, *Exp. Neurol.* 124 (1993) 401–412.
- [30] Y. Tomozawa, S.H. Appel, Soluble striatal extracts enhance development of mesencephalic dopaminergic neurons in vitro, *Brain Res.* 399 (1986) 111–124.
- [31] J. Widenfalk, C. Nosrat, A. Tomac, H. Westphal, B. Hoffer, L. Olson, Neurturin and glial cell line-derived neurotrophic factor receptor-beta (GDNFR-beta), novel proteins related to GDNF and GDNFR-alpha with specific cellular patterns of expression suggesting roles in the developing and adult nervous system and in peripheral organs, *J. Neurosci.* 17 (1997) 8506–8519.
- [32] T. Yu, S. Scully, Y. Yu, G.M. Fox, S. Jing, R. Zhou, Expression of GDNF family receptor components during development: implications in the mechanisms of interaction, *J. Neurosci.* 18 (1998) 4684–4696.