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Protein tyrosine phosphatase inhibition reduces degeneration of dopaminergic substantia nigra neurons and projections in 6-OHDA treated adult rats

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Abstract

The survival of injured adult dopaminergic substantia nigra pars compacta neurons can be promoted by various neurotrophic factors. Most neurotrophic factor receptors are activated by intracellular tyrosine phosphorylation upon ligand binding and are subsequently inactivated or dephosphorylated by protein tyrosine phosphatases. This raised the possibility that tyrosine phosphatase inhibition might improve neuronal survival. Here, we infused the stable water-soluble tyrosine phosphatase-specific inhibitor, peroxovanadium [potassium bisperoxo(1,10-phenanthroline)oxovanadate (V) (bpV(phen))], for 14 days close to the substantia nigra starting immediately after a unilateral moderate injury by injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the midbrain of adult Sprague–Dawley rats. The dopaminergic nigrostriatal neurons were identified by retrograde tracing with fluorogold 7 days prior to the injury. With infusion of 3 or 10 μM peroxovanadium, 75% of these neurons survived compared to 45% in vehicle-infused rats. Degeneration of the dopaminergic projections to the neostriatum was also reduced by 10 μM peroxovanadium. Twenty minutes after an acute injection of peroxovanadium into the substantia nigra, increased tyrosine phosphorylation in Western blots of nigral extracts was seen in the same protein bands as after injections of brain-derived neurotrophic factor (BDNF) or NT-4. This suggests that peroxovanadium enhances endogenous neurotrophic signalling resulting in improved neuronal survival. The neuroprotective effects of this small molecule protein tyrosine phosphatase inhibitor represent a proof-of-principle for a novel treatment strategy in a model for Parkinson's disease.

Introduction

The balance between phosphorylating tyrosine kinases and dephosphorylating protein tyrosine phosphatases is crucial to intracellular signalling and ultimately to the survival of a cell (Johnson & Van Vactor, 2003; Paul & Lombroso, 2003; Alonso *et al*., 2004). Upon ligand binding, most neurotrophic factor receptors are activated by phosphorylation of one or more intracellular residues to initiate intracellular signalling (Airaksinen & Saarma, 2002; Huang & Reichardt, 2003). Therefore, it is possible that decreased tyrosine receptor kinase activation by endogenous trophic factors contributes to neuronal degeneration in neurological disorders. Administration of neurotrophic factors clearly can rescue a variety of neurons in a variety of adult animal models of diseases, including those relevant to Parkinson's disease (Pezzoli *et al*., 1991; Hagg & Varon, 1993; Frim *et al*., 1994; Beck & Hefti, 1995; Tomac *et al*., 1995; Lu & Hagg, 1997; Hagg, 1998; Kordower *et al*., 2000; Lu *et al*., 2002; Quesada & Micevych, 2004). Neurotrophic factors are large proteins, making them poor pharmacological agents for delivery to the central nervous system. On the other hand, inhibition of tyrosine phosphatases with small molecules might enhance neurotrophic factor receptor activation by resident or endogenous trophic factors. Peroxovanadium is a synthetic small molecule inhibitor of protein tyrosine phosphatases (Posner *et al*., 1994) and thereby can enhance tyrosine kinase activation in multiple systems (Sekar *et al*., 1996; Ruff *et al*., 1997). That this might be the case also for TrkB-related signalling *in vivo* in the brain was suggested by our finding that brain-derived neurotrophic factor (BDNF) and tyrosine phosphatase inhibition with peroxovanadium can cooperate to rescue adult rat dopaminergic substantia nigra neurons after their axotomy (Lu *et al*., 2002). However, Parkinson's disease is characterized by the progressive degeneration of the dopaminergic nigrostriatal terminals, axons and death of their neurons (Dauer & Przedborski, 2003), which is not mimicked by the axotomy model. Therefore, we tested whether a 14-day intracerebral infusion of peroxovanadium could rescue both the neostriatal terminals and the neurons in the substantia nigra pars compacta of adult rats after a neurotoxic lesion of the dopaminergic neurons with 6-hydroxydopamine (6-OHDA). We also compared the effects of acutely injected peroxovanadium to the TrkB ligands, BDNF and NT-4, on tyrosine phosphorylation in the substantia nigra.

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Materials and methods

Retrograde tracing, 6-OHDA lesions and supranigral infusions

Young adult female Sprague–Dawley rats (180–200 g, $n = 16$, Harlan, Indianapolis, IN) were treated in accordance to the guidelines

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of the NIH and all animal procedures were approved by the University of Louisville IACUC. For all invasive procedures, the rats were anaesthetized with an intramuscular injection of a mixture containing ketamine, acepromazine and xylazine (Emsley *et al*., 2001). To unequivocally identify surviving dopaminergic neuronal cell bodies in the substantia nigra pars compacta, all rats received bilateral injections of the rapid fluorescent neuronal tracer fluorogold into two sites per neostriatum (Emsley *et al*., 2001). One week later, all the rats received a unilateral injection of 7 μg 6-OHDA (Sigma–Aldrich, St Louis, MO) in 2 μL of saline containing 0.2% ascorbic acid into the right medial forebrain bundle at stereotaxic coordinates RC −3.8 mm, ML 2.8 mm, DV 8.7 mm, all from Bregma and with the toothbar set at −3.3 mm (Lu & Hagg, 1997). This causes a moderate injury where one-third of the neurons is expected to survive after 14 days, thus providing a window of opportunity to test neuroprotective agents. Essentially all of the dopaminergic neurons of the substantia nigra pars compacta project ipsilaterally to the neostriatum (Burt, 1993; Hagg & Varon, 1993). Therefore, the noninjected side of each rat was used as an internal control. Immediately after the 6-OHDA injections, the rats were implanted with a 0.3 mm diameter metal infusion cannula (Alzet Model 2002, Durect Corporation, CA) with the tip positioned just dorsal to the ipsilateral (right) substantia nigra at coordinates RC 4.8 mm, ML 1.7 mm, DV 7.0 mm, all from Bregma. The cannula was connected to an Alzet osmotic pump (Model 2002, 0.5 μL/h, Durect Corporation) filled with phosphate buffered saline (PBS; $n = 5$) or PBS plus 3 μM ($n = 6$) or 10 μM ($n = 5$) peroxovanadium (potassium bisperoxo(1,10-phenanthroline)oxovanadate (V) [bpV(phen)], produced and NMR-certified by Dr A. Shaver, McGill University (Posner *et al*., 1994)).

Histology and quantitative analyses

Fourteen days later, the rats were again anesthetized and killed by transcardial perfusion with ice cold PBS followed by ice cold 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were postfixed overnight and cryoprotected in 30% sucrose for 24 h. Serial 30-μm-thick coronal sections through the neostriatum (caudate-putamen) and substantia nigra were obtained using a freezing stage microtome. Starting at a random point along the rostrocaudal axis of the brain, every sixth section through the substantia nigra was mounted on glass slides and coverslipped in Fluoromount-G (Electron Microscopy Sciences, Ft Washington, PA). To visualize the nigrostriatal projections and neostriatal terminals and to evaluate the effect of peroxovanadium on the dopaminergic neurons in the ventral tegmental area, every sixth section through the neostriatum and every twelfth section through the substantia nigra was immunostained with a mouse monoclonal antibody against tyrosine hydroxylase (TH) overnight (1 : 40 000, MAB318, Chemicon, Temecula, CA) followed by an avidin-biotin-complex−3,3′-diaminobenzidine (ABC–DAB) protocol (Lu & Hagg, 1997). Sections were then mounted and coverslipped in Permount.

The total number of fluorogold-labelled neuronal cell bodies in the substantia nigra pars compacta on the ipsilateral (6-OHDA lesioned and saline- or peroxovanadium-infused) and contralateral sides were estimated separately with an unbiased optical fractionator stereological method using the Stereologer software (Systems Planning and Analysis, Alexandria, VA) and a 63× oil objective on a fully motorized Leica DMIRE2 microscope. The reference space was defined at the rostral end by the rostral end of the medial terminal nucleus of the accessory optic tract and at the caudal end by the appearance of the transverse fibers of the pons. The medial boundary was defined by the medial terminal nucleus of the accessory optic tract and the lateral side by most laterally located fluorogold-labelled neurons. The number of ipsilateral neurons was expressed as a percentage of those on the contralateral side of each individual rat. We did not count the number of dopaminergic ventral tegmental area neurons as they are not retrogradely labelled from the fluorogold injection site. We did not count the number of TH-positive neurons as TH can be down-regulated without loss of neurons (Hagg & Varon, 1993; Lu & Hagg, 1997). To estimate the presence of dopaminergic neostriatal terminals digital images of the neostriatum from three tyrosine hydroxylase-immunostained sections per side of each rat were taken with a 10× objective using a Spot RTKE camera (Diagnostic Instruments, Sterling Heights, MI). The intensity of the tyrosine hydroxylase positive fibers per side per section was estimated by subtracting the background value (regions without fibers) from the overall value, as measured by Scion Image software (Scion Corporation, Frederick, MD) on a scale of 0–255 (white − black). The ipsilateral values were calculated as a percentage of the contralateral values for each rat. The same person (AD) analysed all the substantia nigra and striatal tissues and was blind to the nature of the treatment. Statistical analyses were performed using the Student's *t*-test and Pearson's correlation. Significance was set at $P < 0.05$.

Phosphotyrosine analysis using Western blotting

Additional female Sprague–Dawley rats were anaesthetized and received bilateral injections of 2 μL each of either PBS (PBS, $n = 2$), peroxovanadium (0.3 μM, $n = 2$), recombinant human BDNF (1 μg, $n = 2$), peroxovanadium plus BDNF (0.3 μM plus 1 μg, $n = 2$), or recombinant human NT-4 (1 μg, $n = 2$) just above the substantia nigra (neurotrophins were a gift from Regeneron Pharmaceuticals Inc, Tarrytown, NY). The dose of peroxovanadium is similar to that which was infused over 20 min in the 14-day animals (see above). Twenty minutes later, the substantia nigras (including pars compacta and reticulata) were freshly dissected on ice from 1-mm slices through the midbrain, pooled according to treatment and lysed for protein extraction. The protein concentration of each lysate was determined with a Lowry protein assay kit (Sigma–Aldrich, St Louis, MO). Total proteins from each lysate were separated in a 7.5% protein gel under reducing conditions, transferred onto a PVDF membrane, and probed with a mouse phosphotyrosine antibody (4G10, 1 : 1,000, Cat# 05–777, Millipore Corp, Billerica, MA). The antibody was revealed by a secondary antibody and ECL reaction (Amersham Biosciences, Pittsburgh, PA) and the ECL signal documented by exposure of X-ray film. After the immunostaining, the membrane was restained with Coomassie Blue to confirm equal loading of proteins.

Results

Peroxovanadium promotes survival of dopaminergic substantia nigra neurons after a 6-OHDA lesion

Two weeks after the unilateral 6-OHDA injection into the medial forebrain bundle and a continuous infusion directly over the ipsilateral substantia nigra, the fluorogold-labelled neurons on the contralateral side were readily detectable, had several processes (Fig. 1A) and a similar morphology as we have seen before (e.g. Emsley *et al*., 2001). The morphology on the contralateral side was similar in rats treated with PBS or peroxovanadium (not shown). The neurons on the injured ipsilateral side of the animals treated with PBS were atrophic, with fewer, shorter, and poorly labelled processes (Fig. 1B). In peroxovanadium-treated rats, more ipsilateral neurons appeared to be present and their processes were more readily detectable (Fig. 1C and D). In

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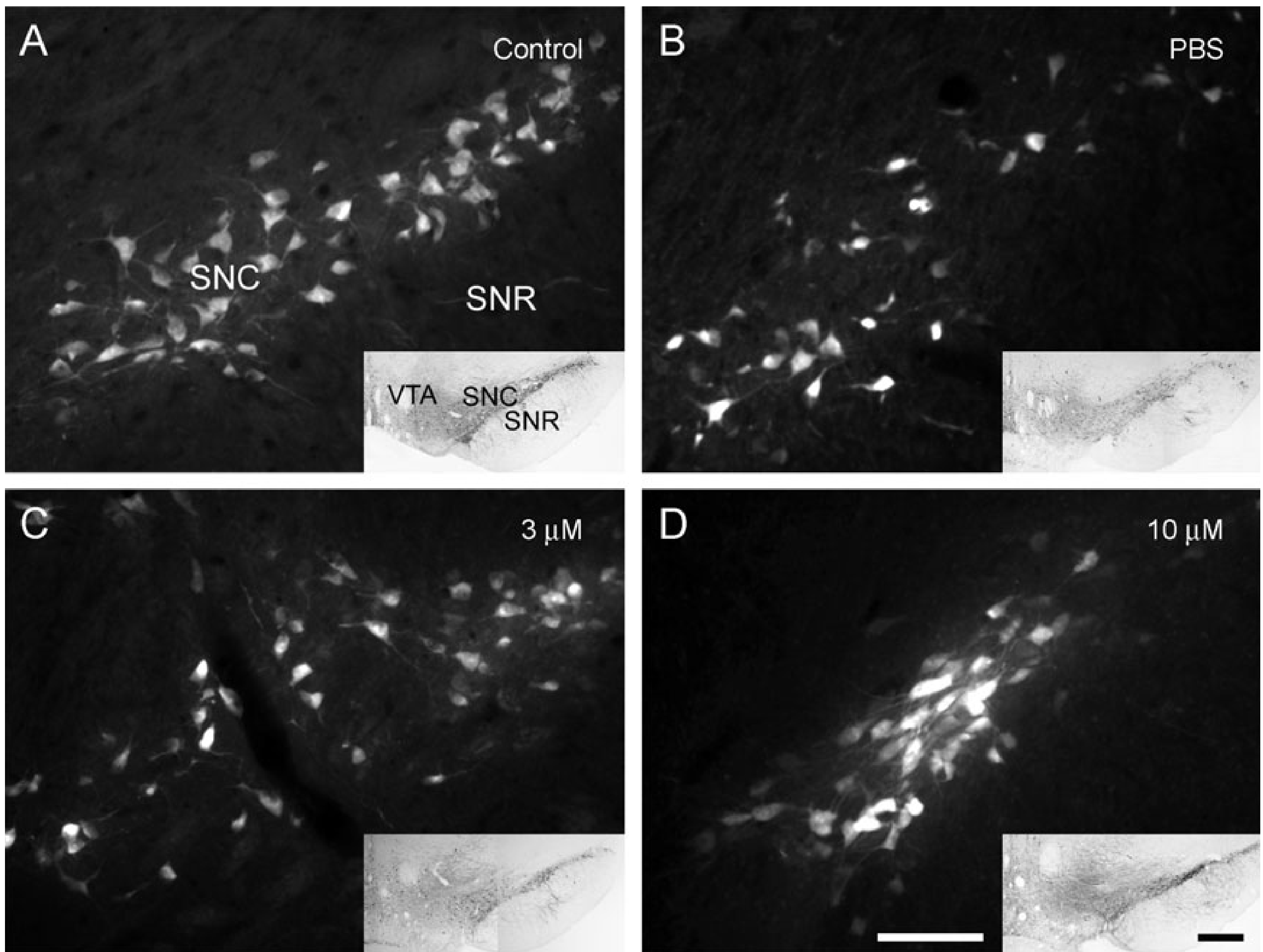

FIG. 1. Protein tyrosine phosphatase inhibition promotes survival of dopaminergic substantia nigra pars compacta neurons after a unilateral 6-OHDA lesion. Neurons were retrogradely traced with fluorogold injected in the neostriatum 1 week before the lesion. Compared to the uninjured side (A), the side that received a supra-nigral infusion of control PBS (B) showed a reduction in the number and size of neuronal cell bodies. With 3 μM (C) or 10 μM (D) peroxovanadium infusion more fluorogold labelled neurons were present, indicating that protein tyrosine phosphatase inhibition can promote neuronal survival. Scale bar, 100 μm (in D for A–D). Insets show adjacent sections stained for tyrosine hydroxylase to confirm the presentation of equal rostrocaudal levels. Scale bar, 500 μm (in the inset of D for all insets). SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata; VTA, ventral tegmental area.

PBS-infused lesioned rats, 45 ± 5% [± standard error of the mean (SEM)] of the fluorogold-labelled neurons were present in the ipsilateral substantia nigra pars compacta. With infusion of 3 or 10 μM peroxovanadium, 72 ± 8% ($P < 0.05$) or 75 ± 8% ($P < 0.01$) of the neurons were present, respectively. In three of the peroxovanadium-treated rats, over 90% of the neurons were present. The treatment seemed to have failed in one rat infused with 3 μM peroxovanadium for an unknown reason. The two peroxovanadium groups were not statistically different.

Tissue sections stained for tyrosine hydroxylase showed that lesioned rats infused with PBS had an overall reduction in the number of neurons in the substantia nigra pars compacta and the length of their dendritic processes in the pars reticulata (Fig. 2B), compared to the uninjured contralateral side (Fig. 2A). The ventral tegmental area also showed decreased tyrosine hydroxylase-staining in neurons (Fig. 3B vs. A). With infusion of 3 or 10 μM peroxovanadium more tyrosine hydroxylase-stained neurons with longer processes seemed to be present than with the PBS infusion in the substantia nigra (Fig. 2C and D) and ventral tegmental area (Fig. 3C and D). Overall, the tyrosine hydroxylase-staining in the 3 and 10 μM peroxovanadium-treated rats appeared similar to that of the uninjured contralateral side.

Peroxovanadium also promotes the maintenance of dopaminergic projections to the neostriatum

The tyrosine hydroxylase immunostaining was consistently and uniformly intense in the contralateral neostriatum irrespective of the treatments (Fig. 4A). On the ipsilateral side of PBS-treated rats the staining was markedly reduced, particularly in the central and lateral regions (Fig. 4B). In animals treated with 10 μM peroxovanadium, but less so with 3 μM, the intensities were distinguishably greater, with a more even distribution of stained fibers in the neostriatum (Fig. 4C and D). The tyrosine hydroxylase immunostaining intensities of the PBS-infused group was 42 ± 7% of contralateral (ipsilateral vs. contralateral, $P < 0.001$, paired *t*-test), and 55 ± 14% or 63 ± 6% ($P < 0.05$

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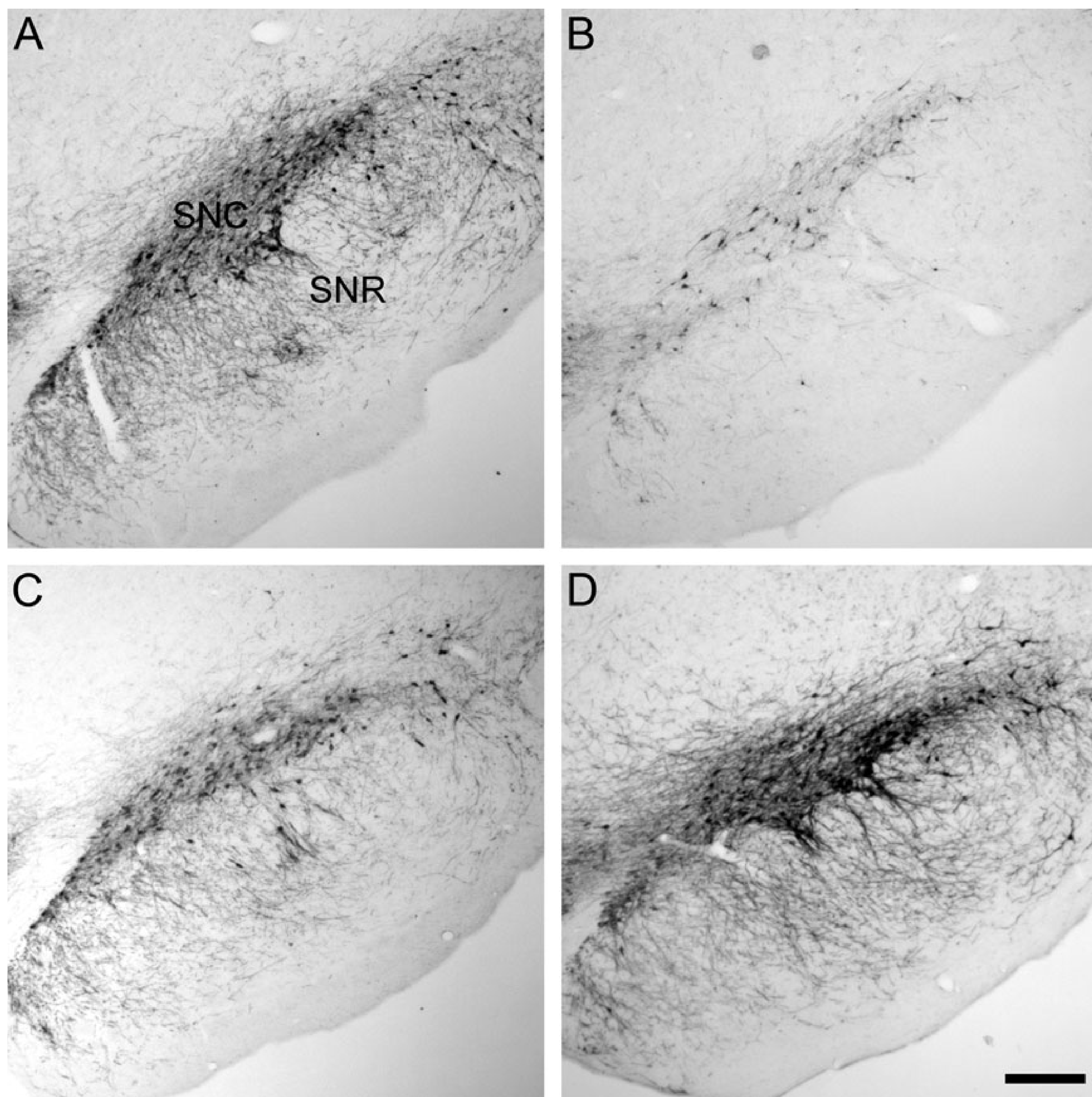

FIG. 2. Protein tyrosine phosphatase inhibition protects expression of tyrosine hydroxylase in the 6-OHDA-lesioned substantia nigra. Compared to the uninjured contralateral side (A), immunostaining of tyrosine hydroxylase in the ipsilateral substantia nigra pars compacta (SNC; B) is much reduced, showing fewer cell bodies and shorter dendritic processes in the substantia nigra pars reticulata (SNR). After infusion of 3 μM (C) or 10 μM peroxovanadium (D) more neurons and longer dendritic processes are seen. Scale bar, 250 μm (in D for A–D).

compared to PBS, $P < 0.001$ compared to contralateral, paired *t*-test) in the groups treated with 3 or 10 μM peroxovanadium, respectively (Fig. 5B).

When the results of all rats were combined, irrespective of the treatment the rats received, there was a correlation between the numbers of fluorogold labelled substantia nigra pars compacta neurons and the tyrosine hydroxylase immunostaining intensities in the neostriatum ($r = 0.43$, $P < 0.05$, $n = 16$, Fig. 5C). The values for the peroxovanadium-treated rats were shifted to the upper right, indicating the combined effect on cell body survival and protection of terminals in the neostriatum.

Peroxovanadium induces tyrosine phosphorylation in the substantia nigra similar to neurotrophins

To identify the potential molecular mechanism underlying the peroxovanadium-induced neuronal survival, we analysed the effect of acutely injected peroxovanadium on protein tyrosine phosphorylation

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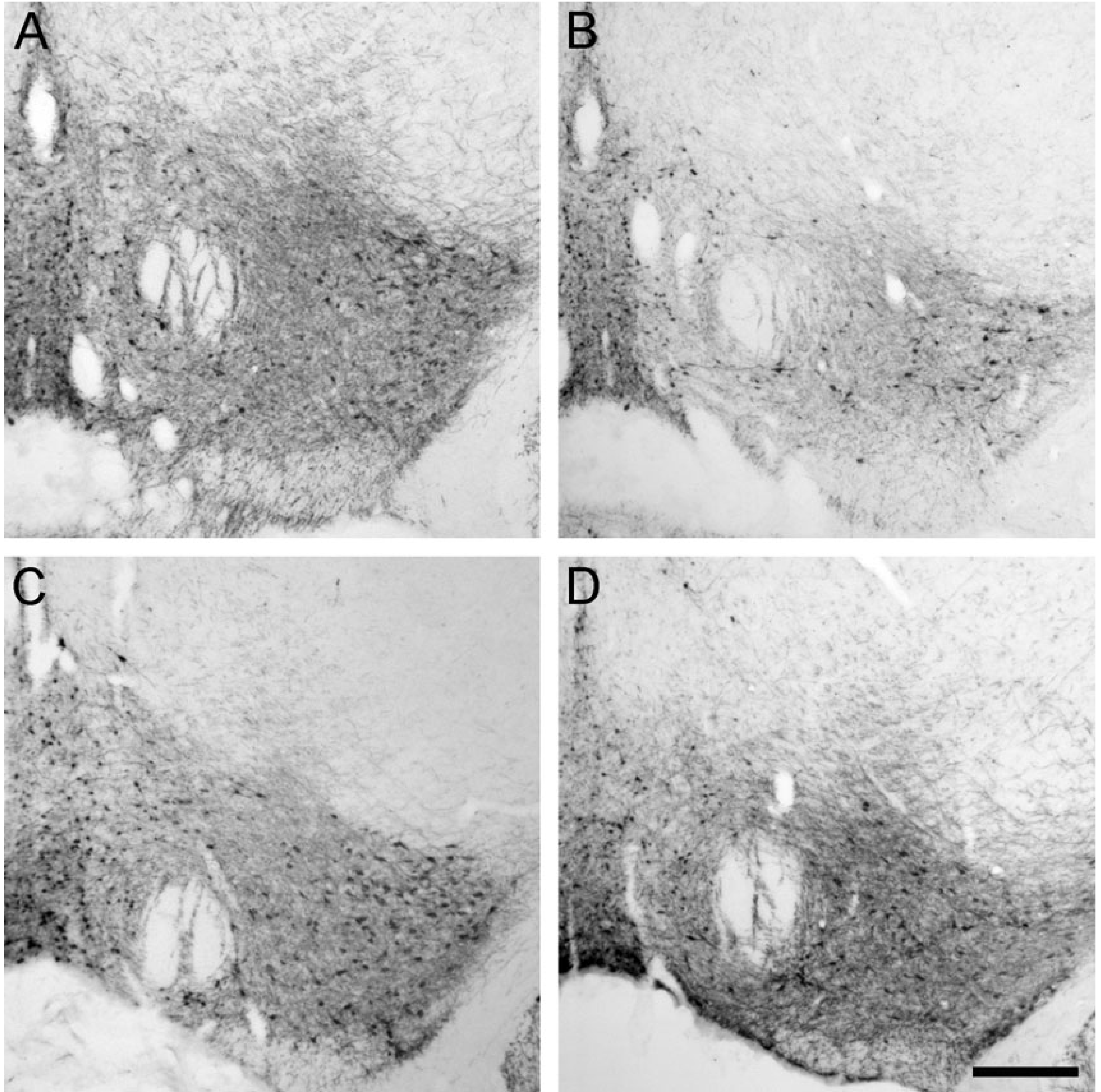

FIG. 3. Protein tyrosine phosphatase inhibition protects expression of tyrosine hydroxylase in the 6-OHDA-lesioned ventral tegmental area. Compared to the uninjured contralateral side (A), immunostaining of tyrosine hydroxylase in the ventral tegmental area is reduced, showing fewer cell bodies and processes (B). After infusion of 3 μM (C) or 10 μM peroxovanadium (D) more neurons and densely distributed processes are seen than with infusion of PBS. Scale bar, 250 μm (in D for A–D).

in the naïve substantia nigra compared to the effects of the TrkB ligands BDNF and NT-4. Twenty minutes after supranigral injection of peroxovanadium, noticeable increases in tyrosine phosphorylation were seen at approximately 140–150 kDa and in two bands between 50 and 75 kDa, as shown by Western blotting (Fig. 6). The increase at these positions was similar after injection of the TrkB receptor ligands BDNF or NT-4. The combination of peroxovanadium and BDNF did not seem to increase the tyrosine phosphorylation further, suggesting that both are at maximally effective concentrations. The clearly increased tyrosine phosphorylation seen after NT-4 at ∼140–150 kDa most likely represents TrkB, which migrates at 145 kDa. In separate experiments (not shown) we have found that the levels of detectable TrkB are very low in the substantia nigra, perhaps explaining the low amount of signal seen here.

Discussion

The current study shows that chronic infusion with the small molecule protein tyrosine phosphatase inhibitor, peroxovanadium, close to the

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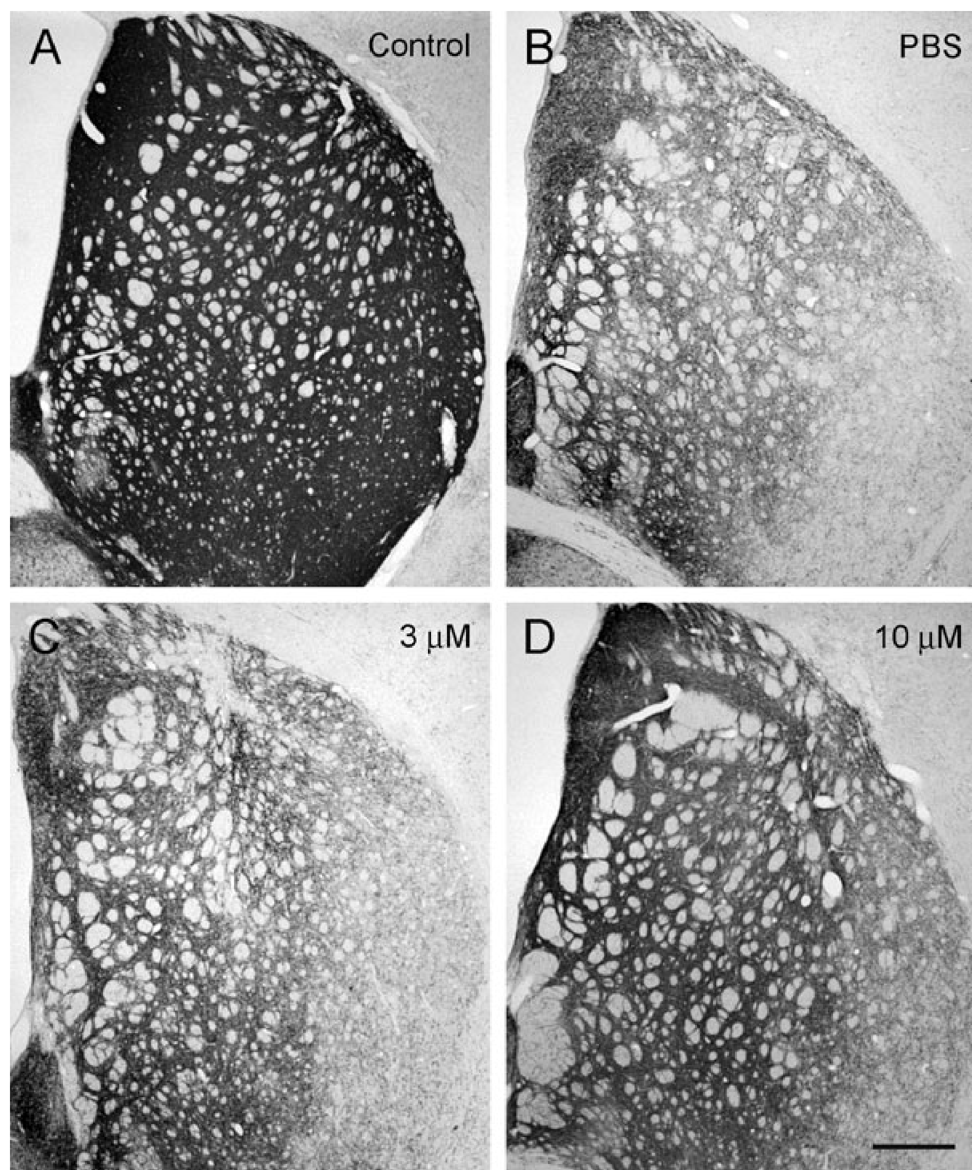

FIG. 4. Dopaminergic fibers in the neostriatum are protected by inhibition of protein tyrosine phosphatases in the substantia nigra. Compared to the uninjured contralateral side (A), immunostaining for tyrosine hydroxylase, a marker for the dopaminergic fibers in the neostriatum is largely absent in 6-OHDA lesioned rats infused with PBS over the substantia nigra (B). With infusion of 3 μM (C) or 10 μM (D) peroxovanadium much more dopaminergic innervation is present throughout the neostriatum. Scale bar, 500 μm (in D for A–D).

substantia nigra can rescue the dopaminergic nigrostriatal neurons as well as their projections to the neostriatum after a moderate 6-OHDA-induced lesion in adult rats. In several of the peroxovanadium-treated rats more than 90% of the neurons had survived. The extent of protection of ~30% over controls is similar to what we have seen before in peroxovanadium-treated rats after axotomy of the dopaminergic pathway (Lu *et al*., 2002) and similar to that achieved with glial cell line derived neurotrophic factor (GDNF) using the same 6-OHDA lesion (Lu & Hagg, 1997). These results are a proof-of-principle that tyrosine phosphatase inhibition can be as potent a neuroprotective strategy as neurotrophic factors such as GDNF. It should be noted that the therapeutic efficacy in the 6-OHDA model and the similar effect to GDNF do not necessarily predict efficacy in humans with Parkinson's disease. One advantage of this strategy is the use of a water-soluble small molecule, which ensures good diffusion through the brain tissue. We have previously observed effects of 100 μM peroxovanadium in the contralateral substantia nigra (Lu *et al*., 2002), suggesting a diffusion of at least 5 mm. This is farther than the 1 mm that peroxovanadium had to reach into all directions to cover the entire ipsilateral substantia nigra in the current study. The half-life (T1/2) of this peroxovanadium (produced by Dr A. Shaver, McGill University) is 10 days at 37 °Celsius when dissolved in PBS (Cerovac *et al*., 1999), as it would be in the Alzet pump. That study also showed that tyrosine phosphorylation is not affected by the peroxovanadium metabolites. Thus the substantia nigra would have been treated with fresh peroxovanadium during the entire 14-day period. We do not know the stability in the tissue but are unaware of chemical reactions in the tissue that would cause a shorter half-life once the peroxovanadium is released from the cannula into the substantia nigra. Whether peroxovanadium would be effective when

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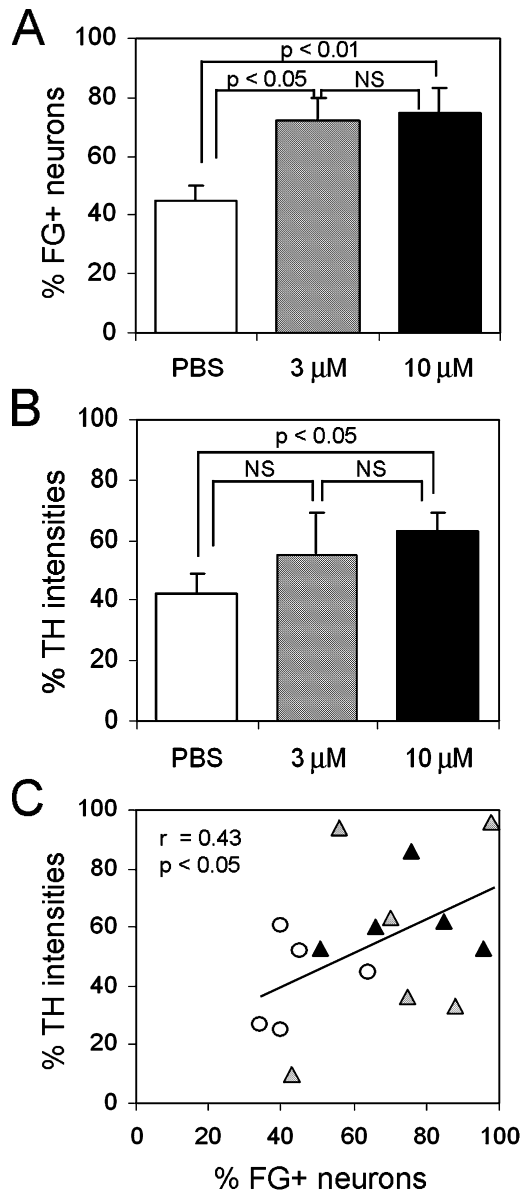

FIG. 5. Protein tyrosine phosphatase inhibition rescues nigrostriatal neurons and their projections: quantification. (A) 6-OHDA-lesioned rats infused with 3 or 10 μM peroxovanadium above the substantia nigra have significantly more fluorogold-labelled (FG) neurons in the substantia nigra pars compacta compared to PBS-treated rats. (B) Tyrosine hydroxylase immunostaining in the neostriatum was denser with 10 μM peroxovanadium infusions than with PBS, suggesting that protein tyrosine phosphatase inhibition also protects dopaminergic projections. (C) Regression analysis including all animals, irrespective of the treatment group, showed that the number of surviving neurons in individual rats correlated with the density of tyrosine hydroxylase immunoreactive processes in the neostriatum.

administered systemically remains to be determined but it is water-soluble and most likely could enter the CNS from the blood stream. Clearly, more selective tyrosine phosphatase inhibitors need to be developed to more selectively target neurotrophic signalling mechanisms, without interfering with other tyrosine phosphatase regulated mechanisms.

The neurotoxin 6-OHDA is thought to initially cause degeneration of dopaminergic terminals (Heikkila & Cohen, 1971; Sachs & Jonsson, 1975), and gradually result in the loss of dopaminergic neurons (Kostrzewa & Jacobowitz, 1974). At the highest dose,

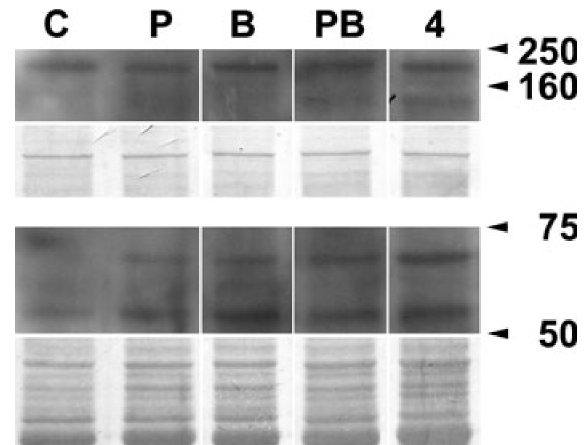

FIG. 6. Peroxovanadium treatment mimics neurotrophin signalling in the adult rat substantia nigra. Compared to control PBS (C), supranigral peroxovanadium (P) injection increases tyrosine phosphorylation in the substantia nigra 20 min later, as shown in an immunoblot of total protein extract probed for phosphotyrosine (4G10). Only molecular weight regions where changes were observed are shown, one around 145 kDa, and two between positions between 50 and 75 kDa. A similar pattern of phosphotyrosine increases is seen after injection of BDNF (B), peroxovanadium plus BDNF (PB), or NT-4 (4). The light panels are from the regions of the same membrane, restained with Coomassie Blue to ensure equal loading of protein.

peroxovandium showed neuroprotective effects on the dopaminergic projections and terminals in the neostriatum. Peroxovanadium also appeared to rescue the tyrosine hydroxylase-positive dendrites in the substantia nigra itself. However, the significant correlation between the number of surviving neurons and the neostriatal projections, when analysing the numbers irrespective of the treatment (Fig. 5C), suggests that as long as the neurons survive, their terminals remain intact. This raises the possibility that peroxovanadium does not directly affect the neostriatal terminals, consistent with the treatment being applied in the region of the cell bodies. In other words, peroxovanadium might enhance neuroprotective mechanisms in the cell body, which in turn contribute to the protection of the terminals. The potential advantage of this type of ‘cell body-mediated’ neuroprotective treatment is the smaller region into which the protective compound needs to diffuse.

The neuroprotective effects of peroxovanadium most likely include the inhibition of protein tyrosine phosphatases, which dephosphorylate tyrosine kinases associated with the dopaminergic survival pathways, thus prolonging and/or increasing kinase activity. It is conceivable that the survival-promoting kinases would include neurotrophic factor receptors, as the dopaminergic neurons can be rescued by treatment with a variety of neurotrophic factors (Pezzoli *et al*., 1991; Hagg & Varon, 1993; Frim *et al*., 1994; Beck & Hefti, 1995; Tomac *et al*., 1995; Lu & Hagg, 1997; Hagg, 1998; Kordower *et al*., 2000; Lu *et al*., 2002; Quesada & Micevych, 2004). In other words, the neuroprotective effects of peroxovanadium might result from enhancing endogenous neurotrophic factor-induced survival pathways. Endogenous BDNF seems to be important for the survival of the dopaminergic neurons as inhibition of BDNF gene expression results in their degeneration (Porritt *et al*., 2005). Here, peroxovanadium injection over the substantia nigra increased tyrosine phosphorylation in three protein regions in a Western blot, one around 145 (the expected size of TrkB) and two between 50 and 70 kDa. The same protein bands showed increased tyrosine phosphorylation after injection of BDNF or NT-4, both TrkB ligands. This suggests that peroxovanadium enhances endogenous TrkB and downstream

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intracellular signalling, consistent with our previous pharmacological data that suggested that peroxovanadium enhances the effectiveness of BDNF in the substantia nigra of adult rats (Lu *et al*., 2002). The identity of the peroxovanadium-induced tyrosine-phosphorylated proteins remains to be determined. Using the UniProtKB database (http://www.pir.uniprot.org) we identified 57 phosphotyrosine proteins in the rat between molecular weight of 50–70 kDa. Several of these are known to be involved in cell survival pathways, including activin receptor type 1B, protein kinase B, erythropoietin receptor, mitogen-activated protein kinase 10, extracellular signal-regulated kinase 7, and SHP-2. Peroxovanadium is known to induce ERK1/2 phosphorylation (Ruff *et al*., 1997; Cerovac *et al*., 1999; Rumora *et al*., 2004), but we did not observe changes in the expected 40–50 kDa range.

The identities of tyrosine phosphatases whose inhibition leads to improved neuronal survival remain to be identified. Phosphatases such as SHP-1 are candidates, as it negatively regulates nerve growth factor (NGF)-dependent neuronal survival *in vitro* through dephosphorylating TrkA (Marsh *et al*., 2003) and conversely, ischemia-induced neuronal death is decreased in SHP-1 deficient mice *in vivo* (Beamer *et al*., 2006). Several other protein tyrosine phosphatases undergo increased expression in polyglutamine expansion-induced neurodegeneration and their inhibition can prevent neuronal cell death *in vitro* (Wu *et al*., 2002). This also raises the possibility that tyrosine phosphatases contribute to the degeneration of the dopaminergic neurons after 6-OHDA and axotomy lesions in the adult rats. Whatever the mechanisms of neuroprotection are, our current results show that tyrosine phosphatase inhibition with a small molecule drug is a viable strategy *in vivo*. Identification of subsets of tyrosine phosphatases involved in regulation of survival pathways and the subsequent development of more selective inhibitors is expected to provide much more refined treatment options for specific neurological disorders, including Parkinson's disease.

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Abbreviations

6-OHDA, 6-hydroxydopamine; BDNF; brain-derived neurotrophic factor; bpV(phen), potassium bisperoxo(1,10-phenanthroline)oxovanadate (V); GDNF, glial cell line derived neurotrophic factor; PBS, phosphate buffered saline; TH, tyrosine hydroxylase.

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