Cell Death and Differentiation (2001) 8, 451–456

www.nature.com/cdd

Zinc inhibits p75^{NTR}-mediated apoptosis in chick neural retina

C Allington[1], IL Shamovsky[2], GM Ross[1] and RJ Riopelle*[,2]

[1] Department of Physiology, Queen's University, Kingston Ontario, Canada K7L 3N6
[2] Department of Medicine, Doran 3, Kingston General Hospital, Queen's University, Kingston Ontario, Canada K7L 2V7
* Corresponding author: RJ Riopelle, Department of Medicine, Queen's University, Kingston, Ontario, Canada K7L 2V7. Tel: (613) 548-6045; Fax: (613) 548-1369; E-mail: rriopelle@mni.mcgill.ca

Received 25.9.00; revised 24.11.00; accepted 4.12.00
Edited by C Thiele

Abstract

It has previously been documented that Zn^{2+} inhibits TrkA-mediated effects of NGF. To evaluate the ability of Zn^{2+} to attenuate the biological activities of NGF mediated by p75^{NTR}, we characterized the effects of this transition metal cation on both binding and the pro-apoptotic properties of the NGF-p75^{NTR} interaction. Binding of NGF to p75^{NTR} displayed higher affinity in embryonic chick retinal cells than in PC12 cells. NGF induced apoptosis in dissociated cultures of chick neural retina. The addition of 100 μM Zn^{2+} inhibited binding and chemical cross-linking of ^{125}I-NGF to p75^{NTR}, and also attenuated apoptosis mediated by this ligand-receptor interaction. These studies lead to the conclusion that Zn^{2+} antagonizes NGF/p75^{NTR}-mediated signaling, suggesting that the effect of this transition metal cation can be either pro- or anti-apoptotic depending on the cellular context. *Cell Death and Differentiation* (2001) **8,** 451–456.

Keywords: NGF; nerve growth factor; neurotrophins; programmed cell death

Abbreviations: NGF, nerve growth factor; Trk, Tyrosine kinase receptor; TNF, tumor necrosis factor

Introduction

Nerve growth factor (NGF) was the first identified member of the neurotrophin family. Proteins in this family are essential for growth and survival in the developing nervous system.[1–3] Neurotrophins are also vital for the maintenance of the mature nervous system, and increasing evidence suggests these growth factors act as mediators of synaptic plasticity.[4,5] However, recent data point to a paradoxical role for neurotrophins. Under specific conditions NGF can have a neurotoxic effect and mediate the elimination of certain cell populations.[6–8]

The diverse biological activities of neurotrophins may be explained by two classes of receptors with which these proteins associate. Each neurotrophin binds to a specific member of the tyrosine kinase receptor (Trk) family, and all interact with the 75-kDa common low affinity neurotrophin receptor p75^{NTR}.[9,10] The latter receptor belongs to the tumor necrosis factor (TNF) family. NGF binds specifically to TrkA, and this interaction has been well documented to support cell survival and differentiation.[10] In contrast, the role of p75^{NTR} is poorly understood, and may depend on the cellular context in which it is expressed.[11,12] When co-expressed with TrkA, p75^{NTR} can augment the trophic effects of NGF by enhancing TrkA autophosphorylation, and affinity for NGF binding.[13,14] On the other hand, in cells which express only p75^{NTR}, NGF may mediate a signaling cascade which leads to apoptotic cell death,[6–8] although this does not always occur.[15,16] When expressed in the absence of TrkA, p75^{NTR} binds NGF with a higher affinity than when expressed alone, however this parameter has not been tested in cells which undergo p75^{NTR} mediated apoptosis.[17]

The signal transduction pathways of p75^{NTR}-mediated apoptosis have yet to be fully characterized, but are thought to involve the production of ceramide,[18] activation of c-*jun* kinase,[7] the accumulation of p53,[19] and activation of various caspases.[20] NGF interaction with p75^{NTR} also leads to the translocation of nuclear factor κB (NF-κB), but this effect is thought to be anti-apoptotic.[21,22] Ligand-mediated apoptosis is a trait shared by many members of the TNF receptor family.[23]

The transition metal cation Zn^{2+} is found in abundance in the environment, and has been shown to influence many functions in the nervous system, including the regulation of apoptosis and inflammatory states.[24,25] This metal is packaged in presynaptic vesicles and released with chemical or electrical stimulation. Upon release, Zn^{2+} concentrations in the perisynaptic area can reach 100–300 μM.[26] At this concentration, it has been suggested that Zn^{2+} contributes to neuronal death in cerebral ischemia,[27] epilepsy,[28] Alzheimer's disease,[29] Parkinson's disease and amyotrophic lateral sclerosis.[24]

Zinc has previously been shown to block NGF-mediated neurite outgrowth in chick dorsal root ganglia (DRG), and rescue pheochromocytoma (PC12) cells from oxidative stress.[30,31] Both effects are mediated via the TrkA receptor. Theoretical studies have predicted that the ability of NGF to bind to p75^{NTR} can be attenuated in the presence of Zn^{2+} and other transition metal cations,[32] and Zn^{2+} blocks binding to 'low affinity' p75^{NTR}.[30] These results led to the hypothesis that Zn^{2+} may block the pro-apoptotic effects of NGF. In this study we characterize the effects of Zn^{2+} on the binding of NGF to p75^{NTR} and p75^{NTR}-

mediated apoptosis in embryonic day 5.5 (ED5.5) chick retinal cells.

Results

Binding affinity of NGF to $p75^{NTR}$

Saturation binding was performed using dissociated ED5.5 chick neural retina and ^{125}I-NGF concentrations between 0.005 and 1 nM. Linear transformation of the steady state saturation binding data of ^{125}I-NGF to retinal cells demonstrated homogenous binding affinity parameters consistent with one binding site. Previously, it has been shown that chick retinal cells express $p75^{NTR}$ at ED4 and do not express TrkA until after ED6,[6] although one report shows that specific populations of precursor amacrine cells may express TrkA as early as ED6.[37] TrkA could not be detected in these cells with chemical cross-linking and immunoprecipitation (data not shown) thus, $p75^{NTR}$ is the only receptor available for interacting with NGF. The measured dissociation equilibrium constant (K_d) was 0.42 nM $\pm$ 0.12 (Figure 1a). To confirm this observation we also determined the IC_{50} value using various concentrations of unlabeled NGF (0.04 – 40 nM) to inhibit ^{125}I-NGF (0.15 nM) from chemically cross-linking $p75^{NTR}$ in retinal and in PC12 cells which express both $p75^{NTR}$ and TrkA.[17] Results were evaluated with SDS – PAGE and autoradiography (Figure 1b,c), followed by excision of bands and counting of radioactivity incorporated into both monomers and dimers of $p75^{NTR}$ (Figure 1d). In both cell types, the displacement of ^{125}I-NGF from monomers and dimers did not differ statistically. The IC_{50} values for the monomer of $p75^{NTR}$ in retinal cells and PC12 cells were 0.23 $\pm$ 0.03 and 0.55 $\pm$ 0.11 nM respectively, suggesting that $p75^{NTR}$ has a higher affinity in retinal cells than in PC12 cells.

Zn^{2+} inhibits binding of NGF to $p75^{NTR}$

Previously, we proposed that Zn^{2+} alters the conformation of NGF,[30,32] and inhibits binding to $p75^{NTR}$, in a low affinity state.[30] Here we demonstrate that Zn^{2+} blocks the binding of ^{125}I-NGF to $p75^{NTR}$ in dissociated ED5.5 retinal cells. As $p75^{NTR}$ is the only neurotrophin receptor which interacts with NGF in these cells, we could use both total binding and chemical cross-linking analysis to evaluate the dose response profile of Zn^{2+}. At concentrations of 100 μM, Zn^{2+} (and copper, data not shown) effectively blocks ^{125}I-NGF binding (Table 1). This finding was selective, as equal concentrations of Mg^{2+} had no effect on binding. Dose response analysis revealed that the addition of 50 and 25 μM Zn^{2+} partially blocked binding, while concentrations of 10 μM had no effect. The inhibitory effects of Zn^{2+} could be prevented by the

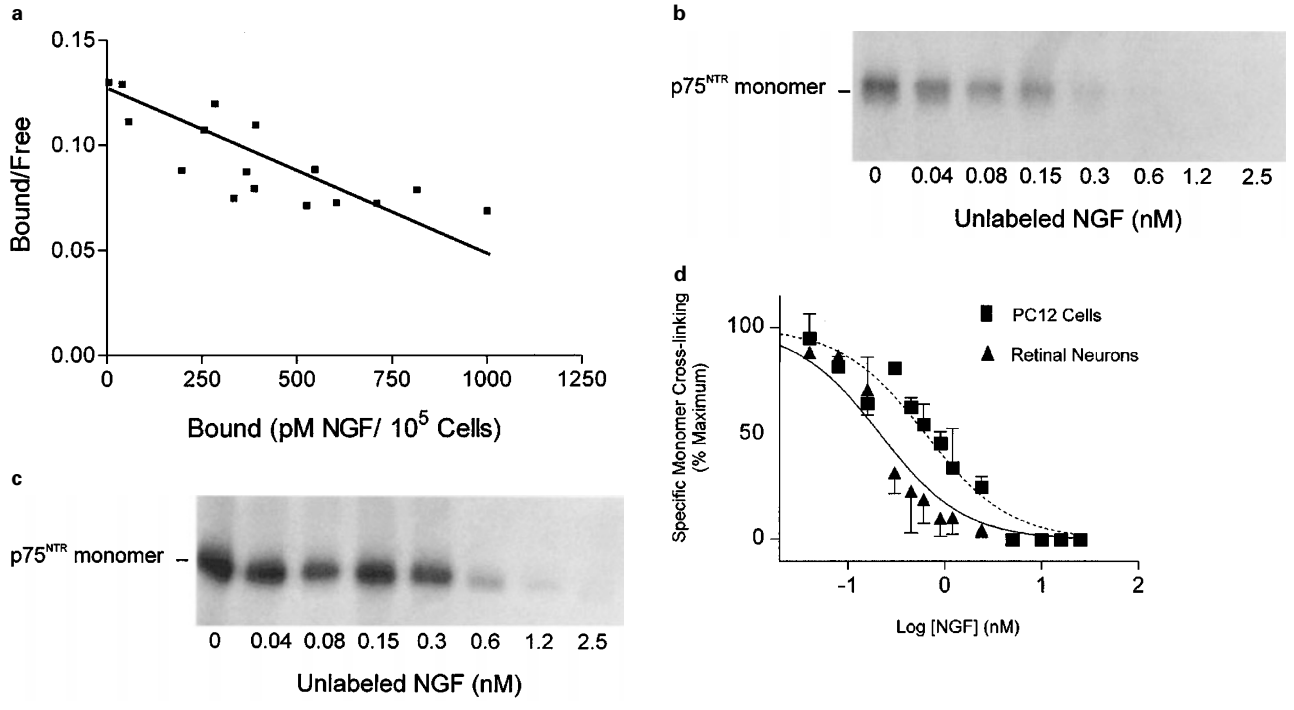

Figure 1 (**a**) Scatchard analysis of steady state binding of ^{125}I-NGF to ED5.5 chick retinal cells displays homogenous binding consistent with a single receptor binding of NGF with an equilibrium dissociation constant of 0.42 $\pm$ 0.12 nM (S.E.M. *n*=7). To compare the affinity of the $p75^{NTR}$ monomer and homodimer in retinal and PC12 cells, the ability of various concentrations of unlabeled NGF to inhibit chemical cross-linking of 0.15 nM ^{125}I-NGF to $p75^{NTR}$ was evaluated. In both cell types, the displacement of ^{125}I-NGF in the monomer and homodimer did not statistically differ. The loss of cross-linking of ^{125}I-NGF with increasing concentration of unlabeled NGF was determined by autoradiography followed by excising bands and counting incorporated radioactivity. Displacement analysis revealed that the $p75^{NTR}$ monomers had an IC_{50} value of 0.23 $\pm$ 0.03 nM in retinal cells (**b**), and 0.55 $\pm$ 11 nM in PC12 cells (**c**). The shift in monomer displacement profile between both cell types is shown in (**d**). (Results shown $\pm$ S.E.M. *n*=4)

Table 1 Effect of Zn^{2+} on binding to retinal cells. Binding of ^{125}I-NGF to $p75^{NTR}$ in chick retinal cells is blocked by 100 μM Zn^{2+}. The binding efficiency was restored with the adition of equimolar CaEDTA. Partial inhibition was achieved with the addition of 50 and 25 μM Zn^{2+}, while 10 μM had no effect. With the addition of 100 μM Mg^{2+}, no change in binding was observed

Treatment	% Control binding	S.E.M.	*n*
No treatment	100		7
Zn^{2+} 100 μM	2.3	13.6	7
Zn^{2+} 50 μM	37.1	12.0	4
Zn^{2+} 25 μM	69.0	15.3	4
Zn^{2+} 10 μM	93.3	4.5	4
Ca^{2+} 100 μM+CaEDTA 100 μM	98.2	22.7	7
Mg^{2+} 100 μM	100.0	22.4	7

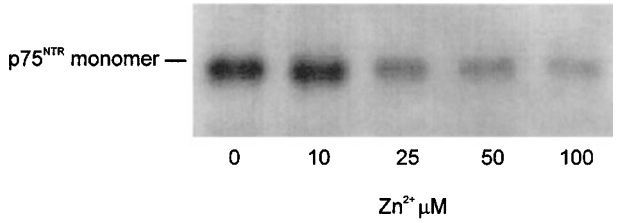

Figure 2 Zinc inhibits EDC/SNHS receptor cross-linking of ^{125}I-NGF to $p75^{NTR}$ in retinal cells at concentrations of 100 μM, partially at 50 and 25 μM and has no effect at 10 μM

addition of equimolar CaEDTA into the incubation buffer. Inhibition of EDC/SNHS cross-linking of ^{125}I-NGF to $p75^{NTR}$ with Zn^{2+} was seen with a similar dose response profile (Figure 2).

Zn^{2+} inhibits $p75^{NTR}$-mediated apoptosis

Apoptosis mediated via $p75^{NTR}$ signaling following exposure to NGF has previously been demonstrated in the normal development of chick neural retina, and in explanted cultures.[6,38] Thus, to determine the effects of Zn^{2+} on cell death, dissociated cells from ED5.5 chick embryos were plated on 16-well chamber slides. Each well was treated independently and incubated overnight at 37°C with 5% CO_2 and 95% air. To determine the number of apoptotic nuclei, nick translation TUNEL and anti-ssDNA MAb staining were performed. All slides had two control wells with either no treatment or control beads with cytochrome *c*. There was no significant difference between these two control groups in both assays. The basal percentage of apoptotic cells was taken from the cytochrome *c* treated groups which ranged between 9.5–12.7% following TUNEL staining, and 11.1–13.9% in MAb stained cultures. The control cytochrome *c* groups were normalized to 100% (Figure 3). With the addition of NGF coated glass beads, the number of TUNEL and MAb positive stained nuclei was significantly increased to 208 ± 7.5 and 258.9 ± 2.5% of control level respectively. When co-administered with NGF, Zn^{2+} (100 μM), proved to be neuroprotective as the number of TUNEL and anti-ssDNA MAb positive nuclei was decreased significantly to levels

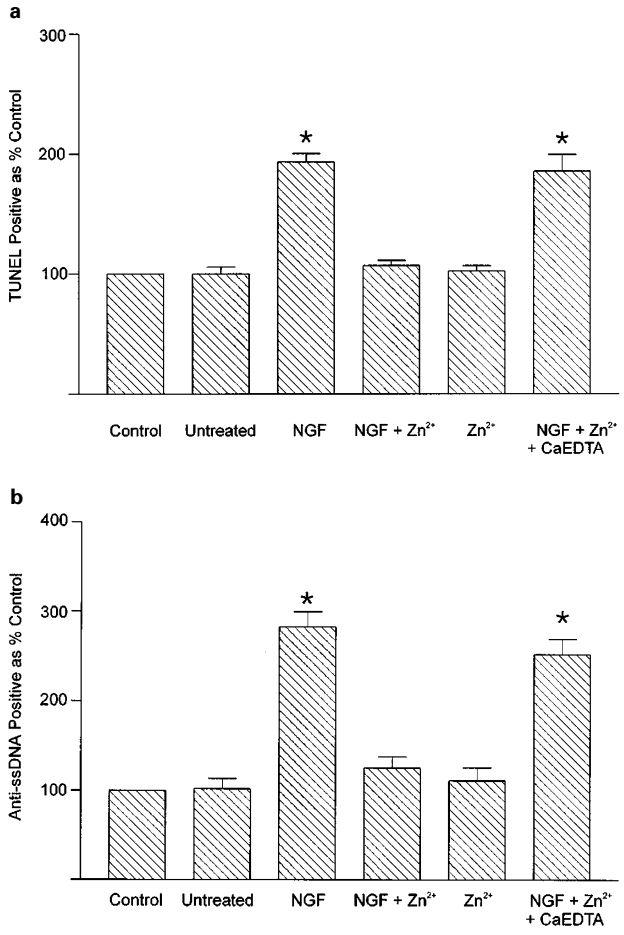

Figure 3 Exogenous NGF on glass beads induces chick retinal cells to undergo apoptosis shown using both TUNEL (**a**) and anti-ssDNA MAb (**b**) staining. The addition of 100 μM Zn^{2+} attenuates this effect, and NGF activity can be restored with the addition of equimolar CaEDTA. This effect is independent of the glass beads as the number of positively stained cells is not significantly different between the group treated with glass beads loaded with cytochrome *c* and untreated cultures. Zinc has no effect when administered without NGF. Results were analyzed using a one-way ANOVA and Dunnett's multiple comparison *post hoc* test (results shown $\pm$S.E.M.). In each culture treatment, eight fields were counted with approximately 100 cells each. (TUNEL n=5; anti-ssDNA MAb, n=4; asterisks signify $P<0.01$)

similar to control cultures. This protective effect could be blocked with equimolar CaEDTA (186 ± 14.2 and 209 ± 3.2% positive apoptotic cells). As predicted through binding analysis, this effect was specific for Zn^{2+}, as 100 μM Mg^{2+} did not alter binding and did not significantly alter NGF-mediated cell death (195 ± 9.2% TUNEL positive cells). As a positive control, we explored the anti-apoptotic effects of a known $p75^{NTR}$ antagonist, PD90780.[39] When added at 100 μM this compound had a similar effect to Zn^{2+}, and inhibited cell death in the ssDNA assay (Figure 4). As previously observed,[6] the apoptotic effect was specific for NGF, as brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) showed no significant increase in positive anti-ssDNA MAb stained cells compared to cytochrome *c* cultures when all proteins were bound to glass beads (Table 2).

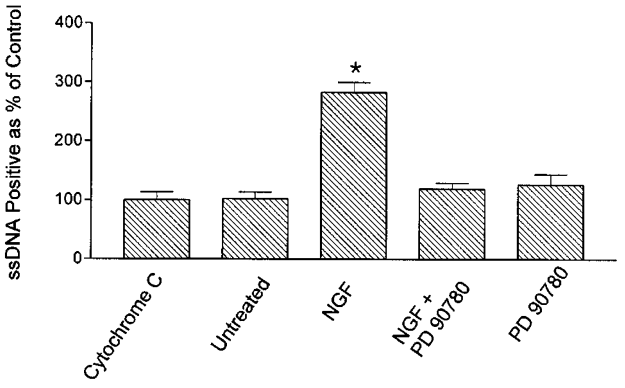

Figure 4 NGF mediated apoptosis is attenuated with the addition of the $p75^{NTR}$ antagonist PD90780 as seen when assayed for anti-ssDNA positive cells. Results were analyzed using a one-way ANOVA and Dunnett's multiple comparison *post hoc* test (results shown $\pm$S.E.M.). In each culture treatment, eight fields were counted with approximately 100 cells each

Table 2 Per cent on anti ss-DNA Mab positive cells. Apoptosis mediated by p75 is specific for NGF as BDNF and NT-3 treated cells do not show greater number of postive cells than control cultures treated with cytochrome *c*. In each culture eight fields were counted with approximately 100 cells each (asterisk signifies $P<0.01$)

	% Anti-ssDNA MAb		
Treatment	**positive**	**S.E.M.**	***n***
NGF	25.44*	0.96	3
BDNF	9.76	1.76	3
NT-3	12.31	2.33	3
Cytochrome *c*	13.39	0.88	3

Discussion

There are three principal observations emerging from these studies. First, the pro-apoptotic effects of NGF on embryonic chick retina demonstrated initially by Frade *et al*.[6,38] have been extended to dissociated cultures. Second, in cells where NGF mediates apoptosis through $p75^{NTR}$ signaling, ^{125}I-NGF binds to $p75^{NTR}$ with high affinity. Third, Zn^{2+}, which has been shown to alter NGF conformation and influence TrkA-mediated survival functions,[30,31] also attenuates the pro-apoptotic activities mediated by the high affinity interaction of $p75^{NTR}$ and NGF.

There is an increasing body of evidence demonstrating that NGF can promote apoptosis in some cell populations which express $p75^{NTR}$ and not TrkA.[6–8] A feature of this cell death which was only reported in chick retinal cells is that exogenous NGF in solution does not promote apoptosis, while NGF bound to glass beads (solid phase) does.[38] The reason for this is unknown but it has been suggested that the way NGF is presented to developing cells *in vivo* by microglia may be critical.[38] However, whether there are other factors involved in presenting NGF to $p75^{NTR}$ expressing retinal cells *in vivo* has yet to be explored. In our cultures, solid phase NGF treated cells demonstrated a twofold increase in the number of TUNEL positive nuclei, and 2.5-fold increase in the number of anti-ssDNA MAb positive nuclei compared to control and non-treated cultures. The greater number of MAb positive cells may be attributed to the fact that this antibody is more sensitive to the early stages of apoptosis than TUNEL labeling.[36] The increased number of apoptotic cells can be attributed to the bound NGF, as groups treated with beads covered with cytochrome *c* had no significant increase in apoptotic nuclei compared to untreated control cultures.

Previous work has shown that the binding characteristics of $p75^{NTR}$ for NGF are dependent on the presence of TrkA.[17] However, binding analysis of ^{125}I-NGF to $p75^{NTR}$ has not been reported in cells that undergo cell death following this binding. In PC12 cells that express $p75^{NTR}$ and TrkA, $p75^{NTR}$ adopts a lower affinity state ($K_d \approx 4.0$ nM) than when $p75^{NTR}$ is expressed alone ($PC12^{nnr5}$ cells; $K_d \approx 0.2$ nM). When co-expressed with TrkA, $p75^{NTR}$ serves a secondary role of promoting the binding of NGF to TrkA by influencing the allosteric properties of this receptor, possibly through the formation of a heteroreceptor complex.[17] This concept is supported by studies which demonstrated that both receptors can be co-immunoprecipitated with antibodies to both $p75^{NTR}$ and TrkA when PC12 cells are incubated with EDC/SNHS prior to the addition of ^{125}I-NGF.[17] In ED5.5 chick retinal cells, we found that $p75^{NTR}$ displayed sub-nanomolar binding affinity measured by dissociation equilibrium analysis and showed that $p75^{NTR}$ binding in retinal cells displayed higher affinity than in PC12 cells by displacement studies. Therefore, apoptosis mediated by the interaction of NGF and $p75^{NTR}$ occurs when this receptor is in its high affinity conformation.

Through competition binding and receptor cross-linking studies, we have shown that 100 μM Zn^{2+} is sufficient to inhibit binding of NGF to $p75^{NTR}$ in ED5.5 chick retinal cells. Molecular modeling and ab initio calculations have been used to predict the most stable geometry of the Zn^{2+}:NGF coordination complex.[32] When Zn^{2+} interacts with NGF, it is chelated by four residues of the NGF dimer forming a 5-coordinate state with donor atoms Nε of His4, Nε of His8, Nδ of His84 and both δ-oxygens of Asp105.[30] Transition metal cations split into three groups by their intrinsic stereochemical preference in the penta-coordinated environment. The optimal geometry of the coordination site for transition metal cations within NGF, distorted square pyramidal, is consistent with intrinsic stereochemical preferences of Zn^{2+} and Cu^{2+}.[32] The conformational changes of NGF induced by Zn^{2+} results in the decreased accessibility of the $p75^{NTR}$ binding determinant within NGF, loops I and IV,[40,41] thereby inhibiting this receptor-ligand interaction.[30,42]

In our apoptosis assays, the addition of 100 μM Zn^{2+} to the NGF treated cells provided protection against $p75^{NTR}$-mediated apoptosis, as the percentage of TUNEL and anti-ssDNA MAb positive nuclei were attenuated to levels similar to those seen in control cultures. Previous studies have shown that chick retinal cells can be protected against NGF induced apoptosis with the addition of the peptide compound dc28–36.[38] This compound mimics loop I of NGF, a part of the binding determinant of $p75^{NTR}$, and competitively inhibits receptor

binding.[40,42] The likely mode of action of this compound is through direct competitive inhibition of $p75^{NTR}$ and not through altering ligand structure. With Zn^{2+}, we propose that protection is mediated by the ability of this cation to alter the conformation of NGF, which leads to non-competitive inhibition of binding to $p75^{NTR}$. Consistent with this hypothesis, Zn^{2+} has been shown to inhibit protomer cross-linking of the two NGF monomers suggesting that a conformational alteration within NGF has taken place.[30,32]

Zinc has been shown to affect neuronal function in many ways, including both inhibition and activation of specific receptors, by entering post-synaptic cells and interrupting cellular homeostasis.[25] The findings that Zn^{2+} can directly modulate cell functions are consistent with much of the existing experimental data indicating that Zn^{2+} contributes to the pathology of Alzheimer's disease, ischemia, amyotrophic lateral sclerosis and epilepsy.[27–29,43] Following insult, presynaptic Ca^{2+}-mediated release of vesicular Zn^{2+} results in chelatable concentrations of this transition metal between 100–300 μM.[26] Our results demonstrate that this concentration is sufficient to inhibit NGF-mediated apoptosis and suggest a novel biological function of Zn^{2+}.

The ability of $p75^{NTR}$ to contribute to neurodegeneration has been demonstrated under several conditions where Zn^{2+} may also be involved. Increased $p75^{NTR}$ expression has been observed following middle cerebral artery occlusion induced ischemia.[44] Recent experimental data also suggest that β-amyloid protein binds to $p75^{NTR}$, and this binding can lead to apoptosis.[45] However, the most compelling evidence for the direct involvement of $p75^{NTR}$ in neuronal degeneration under pathologic conditions may be the observations on cell death following both pilocarpine induced seizure activity and unilateral kainic acid administration. The effects of both insults correlate strongly with the expression of $p75^{NTR}$ in hippocampus, cortex and basal forebrain regions.[46,47] Further, the chronic administration of antibodies to rat $p75^{NTR}$ substantially decreases kainic acid induced cell death of cholinergic cells.[47] The finding that Zn^{2+} inhibits apoptosis mediated by $p75^{NTR}$ and NGF is significant, considering that vesicular Zn^{2+} release following insult occurs in many areas of the nervous system where $p75^{NTR}$ has been implicated in cell death. Therefore, this mechanism for Zn^{2+}-mediated protection offers novel insight into the potential interaction of Zn^{2+}, NGF and $p75^{NTR}$ under conditions associated with cell death in the nervous system. The characterization of $p75^{NTR}$-mediated cell death prior to the appearance of trkA (when NGF becomes trophic) will enhance further investigation of the co-modulation of these receptors.

Materials and Methods

Chick embryos

Fertilized white horn hen eggs were incubated at 38°C in 70% humidity, and staged as previously described.[33] The dissection was performed by excising both eyes from the optic cups and removing the retinal layer. Single cell suspensions of retinal cells were prepared by digesting explants in 0.05 mg/ml DNase, 0.01 mg/ml trypsin in calcium magnesium free Gey's balanced salt solution (CMF; pH 8.1) for 15 min at 37°C. Cells were then suspended in RPMI 1640 with 10% heat inactivated horse serum (Gibco), spun through fetal calf serum (Gibco) and washed three times in RPMI 1640.

^{125}I-NGF preparation

Full-length (1–118) mouse NGF was obtained from Cedarlane Laboratories. The radioination of NGF was performed as previously described,[34] with modification.[30]

Receptor binding and cross-linking

Both binding and cross-linking were used to determine the affinity of $p75^{NTR}$. PC12 cells tissue culture preparations and binding and cross-linking procedures were performed as previously described.[17]

Retinal cell culture

To culture cells for cell death determination, they were washed three times in RPMI and suspended in RPMI+2% B27 supplement (Gibco) and plated on 16-well chamber slides coated with poly-d-lysine (0.1 mg/ml overnight). Wells were individually treated and incubated at 37°C with 5% CO_2 and 95% air.

NGF adsorbed beads

Full-length mouse NGF was adsorbed on controlled pore glass beads as previously described.[35] Briefly, glass powder was incubated overnight in trifluracetic acid at room temperature, then beads were washed with distilled water and PBS six times each. Neurotrophins or cytochrome *c* were added to the activated beads at concentrations of 0.01 mg/ml and placed on ice for 1 h. Beads were washed with PBS six times and applied immediately on top of the plated cells.

Apoptosis determination

To determine the number of apoptotic cells, TUNEL and anti-ssDNA Mab staining were performed. For TUNEL staining, cells were fixed in PBS with 4% formaldehyde and 4% sucrose, then solubilized in 0.25% Triton X-100 for 5 min, then suspended in 3% H_2O_2 for 5 min. PBS with 2% BSA was used as a blocker (30 min). DNA nick translations were marked with biotin-16-dUTP as described in manufacturers instructions (Boehringer Mannheim). Adding 300 mM sodium chloride and 30 mM sodium citrate for 45 min stopped the reaction. Biotin labeled DNA was marked using ABC kit as per manufacturers instruction (Vectastain). Anti-ssDNA MAb staining was performed as described.[36] Briefly, cells were fixed with methanol/PBS (6:1) at −20°C, then resuspended with 4.5 mM MgCl in PBS and heated to 100°C for 5 min. Following a wash with PBS, plated cells were incubated with 3% H_2O_2 for 5 min and 0.1% BSA for 30 min. Then washed and exposed to MAb F-27 (Alexis) for 30 min, biotin conjugated goat anti mouse IgM (Sigma) for 15 min, and to ExtraAvidin peroxidase (Sigma) for 15 min with washings between each step. In TUNEL and MAb assays, cells were stained with DAB (Vectastain) solution as described by manufacturer. Slides were then washed with water and dehydrated in ethanol (75–100%) and xylens for 5 min each. Permount was used to fix the coverslips for microscopy.

Acknowledgements

These studies were supported by the Amyotrophic Lateral Sclerosis Society of Canada (GMR), the Heart and Stroke Foundation of Ontario (4468), and the Canadian Institutes for Health Research. GM Ross is a Queen's National Scholar.

References

1. Levi-Montalcini R (1997) The nerve growth factor: thirty five years later. EMBO J. 6: 1145 – 1154
2. Oppenheim RW (1991) Cell death during development of the nervous system. Annu. Rev. Neurosci. 14: 453 – 501
3. Segal RA and Greenberg ME (1996) Intracellular signaling pathways activated by neurotrophic factors. Ann. Rev. Neurosci. 19: 463 – 489
4. Cowen T and Gavazzi I (1998) Plasticity in adult and ageing sympathetic neurons. Prog. Neurobiol. 54: 249 – 288
5. Schuman EM (1999) Neurotrophin regulation of synaptic transmission. Curr. Opin. Neurobiol. 9: 105 – 109
6. Frade JM, Rodriguez-Tebar A and Barde YA (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature 383: 166 – 168
7. Casaccia-Bonnefil P, Carter BD, Dobrowsky RT and Chao MV (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature 383: 716 – 719
8. Sedel F, Bechade C and Triller A. (1999) Nerve growth factor (NGF) induces motoneuron apoptosis in rat embryonic spinal cord in vitro. Eur. J. Neurosci. 11: 3904 – 3912
9. Chao MV (1994) The p75 neurotrophin receptor. J. Neurobiol. 25: 1373 – 1385
10. Lewin GR and Barde YA (1996) Physiology of the neurotrophins. Annu. Rev. Neurosci. 19: 289 – 317
11. Barker PA (1998) $p75^{NTR}$: A study in contrasts. Cell Death Differ. 5: 346 – 356
12. Barrett GL (2000) The p75 neurotrophin receptor and neuronal apoptosis. Prog. Neurobiol. 61: 205 – 229
13. Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF and Chao MV (1991) High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature 350: 678 – 683
14. Verdi JM, Birren SJ, Ibáñez CF, Persson H, Kaplan DR, Benedetti M, Chao MV and Anderson DJ (1994) p75LNGFR regulates Trk signal transduction and NGF-induced neuronal differentiation in MAH cells. Neuron 12: 733 – 745
15. Ladiwala U, Lachance C, Simoneau SJ, Bhakar A, Barker PA and Antel JP (1998) p75 neurotrophin receptor expression on adult human oligodendrocytes: signaling without cell death in response to NGF. J. Neurosci. 18: 1297 – 1304
16. Susen K, Heumann R and Blochl A (1999) Nerve growth factor stimulates MAPK via the low affinity receptor p75(LNTR). FEBS Lett. 463: 231 – 234
17. Ross GM, Shamovsky IL, Lawrance G, Solc M, Dostaler SM, Weaver DF and Riopelle RJ (1998) Reciprocal modulation of TrkA and p75NTR affinity states is mediated by direct receptor interactions. Eur. J. Neurosci. 10: 890 – 898
18. Dobrowsky RT, Werner MH, Castellino AM, Chao MV and Hannun YA (1994) Activation of the sphingomyelin cycle through the low-affinity neurotrophin receptor. Science 265: 1596 – 1599
19. Aloyz RS, Bamji SX, Pozniak CD, Toma JG, Atwal J, Kaplan DR and Miller FD (1998) p53 is essential for developmental neuron death as regulated by the TrkA and p75 neurotrophin receptors. J. Cell. Biol. 143: 1691 – 1703
20. Gu C, Casaccia-Bonnefil P, Srinivasan A and Chao MV (1999) Oligodendrocyte apoptosis mediated by caspase activation. J. Neurosci. 19: 3043 – 3049
21. Gentry JJ, Casaccia-Bonnefil P and Carter BD (2000) Nerve growth factor activation of nuclear factor kappaB through its p75 receptor is an anti-apoptotic signal in RN22 schwannoma cells. J. Biol. Chem. 275: 7558 – 7565
22. Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhauser N, Bohm-Matthaei R, Baeuerle PA and Barde YA (1996) Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. Science 272: 542 – 545
23. Ashkenazi A and Dixit VM (1998) Death receptors: signaling and modulation. Science 281: 1305 – 1308
24. Cuajungco MP and Lees GJ (1997) Zinc metabolism in the brain: relevance to human neurodegenerative disorders. Neurobiol. Dis. 4: 137 – 169
25. Choi DW and Koh JY (1998) Zinc and brain injury. Annnu. Rev. Neurosci. 21: 347 – 375
26. Xie XM and Smart TG (1991) A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. Nature 349: 521 – 524
27. Koh JY, Suh SW, Gwag BJ, He YY, Hsu CY and Choi DW (1996) The role of zinc in selective neuronal death after transient global cerebral ischemia. Science 272: 1013 – 1016
28. Harrison NL and Gibbons SJ (1994) Zn^{2+}: an endogenous modulator of ligand- and voltage-gated ion channels. Neuropharmacology 33: 935 – 952
29. Bush AI, Pettingell WH, Multhaup G, Paradis M, Vonsattel JP, Gusella JF, Beyreuther K, Masters CL and Tanzi RE (1994) Rapid induction of Alzheimer A beta amyloid formation by zinc. Science 265: 1464 – 1467
30. Ross GM, Shamovsky IL, Lawrance G, Solc M, Dostaler SM, Jimmo SL, Weaver DF and Riopelle RJ (1997) Zinc alters conformation and inhibits biological activities of nerve growth factor and related neurotrophins. Nat. Med. 3: 872 – 878
31. Wang W, Post JI, Dow KE, Shin SH, Riopelle RJ and Ross GM (1999) Zinc and copper inhibit nerve growth factor-mediated protection from oxidative stress-induced apoptosis. Neurosci. Lett. 259: 115 – 118
32. Shamovsky IL, Ross GM, Riopelle RJ and Weaver DF (1999) Theoretical and biochemical studies on the selectivity of nerve growth factor for transition metal cations. J. Am. Chem Soc. 121: 9797 – 9806
33. Hamburger V and Hamilton H (1951) A series of normal stages in the development of chick embryo. J. Morph. 88: 49 – 92
34. Sutter A, Riopelle RJ, Harris-Warrick RM and Shooter EM (1979) Nerve growth factor receptors. Characterization of two distinct classes of binding sites on chick embryo sensory ganglia cells. J. Biol. Chem. 254: 5972 – 5982
35. Gotz R, Kolbeck R, Lottspeich F and Barde YA (1992) Production and characterization of recombinant mouse neurotrophin-3. Eur. J. Biochem. 204: 745 – 749
36. Frankfurt OS, Robb JA, Sugarbaker EV and Villa L (1996) Monoclonal antibody to single-stranded DNA is a specific and sensitive cellular marker of apoptosis. Exp Cell. Res. 226: 387 – 397
37. Karlsson M, Clary DO, Lefcort FB, Reichardt LF, Karten HJ and Hallbook F (1998) Nerve growth factor receptor TrkA is expressed by horizontal and amacrine cells during chicken retinal development. J. Comp. Neurol. 400: 408 – 416
38. Frade JM and Barde YA (1998) Microglia-derived nerve growth factor causes cell death in the developing retina. Neuron 20: 35 – 41
39. Spiegel K, Agrafiotis D, Caprathe B, Davis RE, Dickerson MR, Fergus JH, Hepburn TW, Marks JS, Van Dorf M and Wieland DM (1995) PD90780, a non peptide inhibitor of nerve growth factor's binding to the p75 NGF receptor. Biochem. Biophys. Res. Commun. 217: 488 – 494
40. Ibáñez CF, Ebendal T, Barbany G, Murray-Rust J, Blundell TL and Persson H (1992) Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product. Cell 69: 329 – 341
41. Ibáñez CF (1994) Structure-function relationships in the neurotrophin family. J Neurobiol. 25: 1349 – 1361
42. Shamovsky IL, Ross GM, Riopelle RJ and Weaver DF (1999) The interaction of neurotrophins with the p75NTR common neurotrophin receptor: a comprehensive molecular modeling study. Protein Sci. 8: 2223 – 2233
43. Johansen FF, Tonder N, Berg M, Zimmer J and Diemer NH (1993) Hypothermia protects somatostatinergic neurons in rat dentate hilus from zinc accumulation and cell death after cerebral ischemia. Mol. Chem. Neuropathol. 18: 161 – 172
44. Kokaia Z, Andsberg G, Martinez-Serrrano A and Lindvall O (1998) Focal cerebral ishemia in rats induces expression of P75 neurotrophin receptor in resistant striatal cholinergic neurons. Neuroscience 84: 1113 – 1125
45. Yaar M, Zhai S, Pilch PF, Doyle SM, Eisenhauer PB, Fine RE and Gilchrest BA (1997) Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. J. Clin. Invest. 100: 2333 – 2340
46. Roux PP, Colicos MA, Barker PA and Kennedy TE (1999) p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. J. Neurosci. 19: 6887 – 6896
47. Oh JD, Chartisathian K, Chase TN and Butcher LL (2000) Overexpression of neurotrophin receptor p75 contributes to the excitotoxin-induced cholinergic neuronal death in rat basal forebrain. Brain Res. 853: 174 – 185