TrkB signalling inhibits p75-mediated apoptosis induced by nerve growth factor in embryonic proprioceptive neurons

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Neurotrophins mediate their effects by binding to members of the Trk family of receptor tyrosine kinases and the neurotrophin receptor p75 [1]. Whereas Trks are essential for the trophic effects of neurotrophins [1], p75 has distinct functions in different cells. For example, it enhances the survival response of certain neurons to nerve growth factor (NGF) [2], but mediates a cytotoxic response to NGF in certain other cell types and neurons [3–6]. We investigated whether the p75-mediated responses to NGF can be modulated through the activation of different signalling pathways in the same neurons. Neurons of the embryonic trigeminal mesencephalic nucleus (TMN) are supported in culture by brain-derived neurotrophic factor (BDNF) and an unrelated neurotrophic factor, ciliary neurotrophic factor (CNTF), but not by NGF [7–9]. We found that NGF killed TMN neurons that were grown in the presence of CNTF; this effect of NGF was inhibited by anti-p75 antibodies and therefore occurred via a p75-dependent mechanism. NGF did not affect the survival of neurons grown in the presence of BDNF, and very low concentrations of BDNF inhibited NGF cytotoxicity. These results indicate that the activation of different signalling pathways in TMN neurons influences their susceptibility to p75-mediated NGF cytotoxicity.

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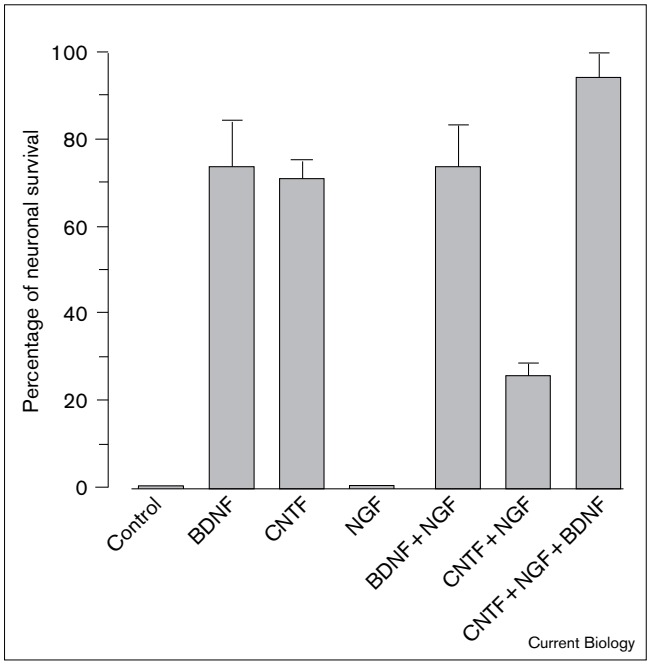
Results and discussion

We have developed a model system for investigating NGF-mediated cytotoxicity in neurons *in vitro*. Figure 1 shows that the survival of the proprioceptive neurons of the TMN was promoted by either BDNF or CNTF but not by NGF. NGF killed the majority of neurons that were grown in the presence of CNTF but had no effect on the survival of neurons grown in the presence of BDNF. Importantly, the cytotoxic effect of NGF on neurons grown in the presence of CNTF was completely prevented by the addition of BDNF. Figure 2 shows that relatively high concentrations of NGF were required to reduce the survival of TMN neurons grown with CNTF. The concentration of NGF at which this effect became saturating (125 ng/ml) was over three orders of magnitude greater than the concentration of NGF that becomes saturating for promoting the survival of embryonic sensory neurons [10], but is similar to the concentration of NGF that kills cultured oligodendrocytes [4].

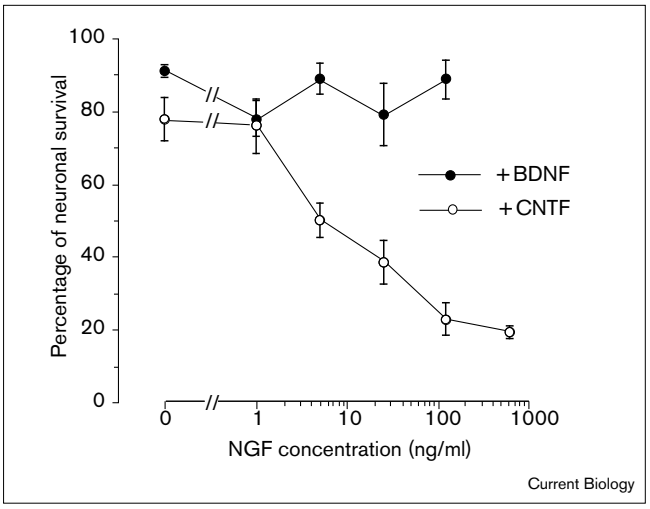
Figure 1



Bar chart of the percentage survival of E10 TMN neurons grown for 48 h without added factors (control) or in the presence of BDNF (5 ng/ml), CNTF (5 ng/ml) or NGF (125 ng/ml) alone or in the indicated combinations. The means and standard errors of the combined results of four separate experiments each set up in triplicate are shown.

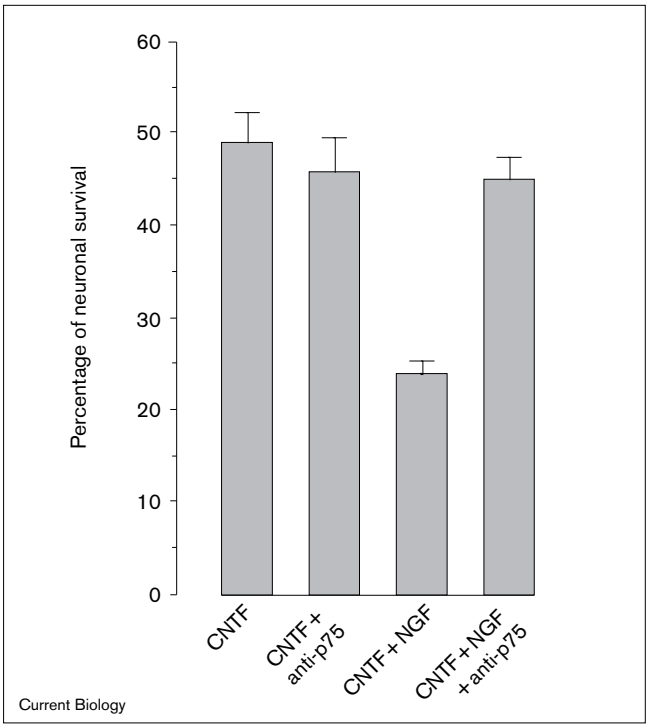
NGF mediates its effects by binding to two transmembrane glycoproteins, p75 and the TrkA receptor tyrosine kinase [1]. Previous work has shown that avian TMN neurons express relatively high levels of p75 mRNA and protein but do not express TrkA [11,12]. To demonstrate directly whether the cytotoxic effect of NGF on TMN neurons is mediated by p75, we investigated whether this effect could be inhibited by an antiserum that is directed against the extracellular domain of chicken p75 and blocks binding of NGF to p75 (ChEX antiserum [13]). Figure 3

Figure 2



Graph of the percentage survival of E10 TMN neurons grown for 48 h in the presence of BDNF (5 ng/ml) or CNTF (5 ng/ml) in combination with a range of concentrations of NGF. The means and standard errors of a representative experiment set up in triplicate are shown. Very similar results were obtained in three other experiments.

Figure 3

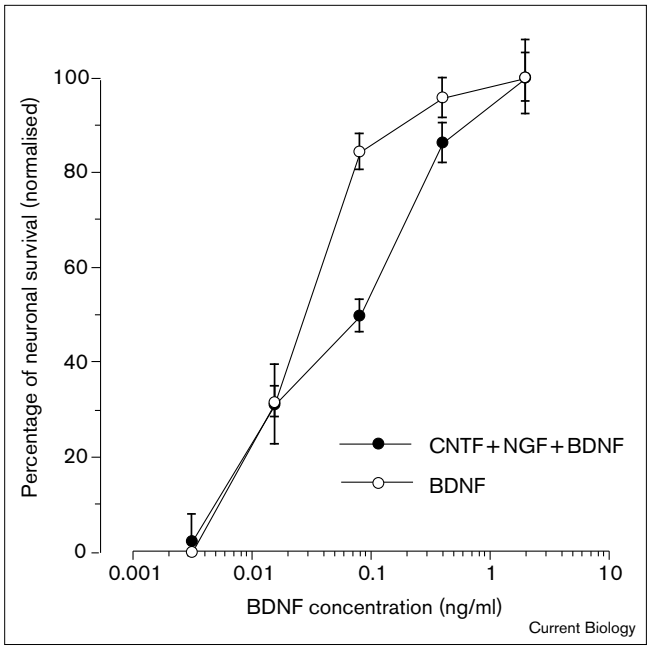


Bar chart of the percentage survival of E10 TMN neurons grown for 48 h in the presence of CNTF (5 ng/ml) alone or together with ChEX anti-p75 antiserum, NGF (125 ng/ml) or ChEX anti-p75 antiserum plus NGF. The means and standard errors of the combined results of two separate experiments each set up in duplicate in microwell dishes are shown.

shows that the ChEX antiserum completely prevented the cytotoxic effect of NGF; there was no significant difference between the number of neurons grown in the presence of CNTF alone, or neurons grown in medium containing CNTF, NGF and the ChEX antiserum. This result indicates that NGF exerts its cytotoxic effect on proprioceptive neurons by binding to p75. Likewise, anti-p75 antibodies have been used to demonstrate that NGF kills cultured neonatal rat oligodendrocytes through a p75-dependent mechanism [4], and administration of anti-p75 and anti-NGF antibodies has shown that endogenous NGF kills a subset of embryonic chicken retinal cells by acting via p75 [3]. The p75 protein also appears to mediate the death of a subset of basal forebrain cholinergic neurons during development because the death of these neurons is prevented by systemic treatment with a p75-inhibiting peptide, and these neurons fail to die in *p75*$^{-/-}$ mice [5].

We have found that the cytotoxic effect of NGF can be prevented by BDNF (Figure 1). Because BDNF binds to both p75 and the TrkB receptor tyrosine kinase [1], it is possible that BDNF antagonises the cytotoxic effects of NGF by acting via either of these receptors or by competing with NGF for binding to p75. Although it is clear that TrkB mediates the trophic actions of BDNF — because the distinctive neuronal deficiencies in *trkB*$^{-/-}$ mice [14] are similar to those observed in *BDNF*$^{-/-}$ mice [15,16], and ectopic expression of TrkB in neurons confers a BDNF survival response [17] — the functional significance of BDNF binding to p75 is unclear. Whereas neurons from *p75*$^{-/-}$ mice have distinctive changes in their dose responses to NGF and NT3, p75-deficient sensory neurons respond normally to BDNF [2,18]. To exclude the possibility that BDNF antagonises the cytotoxic effect of NGF by binding to p75, we studied the effectiveness of very low concentrations of BDNF in this experimental paradigm. BDNF at concentrations as low as 1 ng/ml completely prevented the cytotoxic effects of 125 ng/ml NGF (Figure 4). Because NGF and BDNF bind p75 with equal affinity [19], the binding of BDNF would be negligible compared with NGF under these experimental conditions (that is, more than two orders of magnitude lower than NGF). Thus, BDNF appears to antagonise the cytotoxic actions of NGF by binding to and activating TrkB. Comparison of the dose response of TMN neurons to a range of concentrations of BDNF alone with the dose response of these neurons to the same range of BDNF concentrations plus CNTF and NGF (Figure 4) shows that BDNF promoted neuronal survival and antagonised the cytotoxic effects of NGF over the same concentration range. At the lowest concentration of BDNF used (3.2 pg/ml), BDNF did not promote the survival of any neurons and did not antagonise the cytotoxic effect of NGF. At a five-fold higher concentration, BDNF promoted the survival of approximately 30% of TMN neurons and rescued a similar proportion of neurons from

Figure 4



Graph of the dose responses of E10 TMN neurons grown for 48 h with BDNF over a range of concentrations either alone or together with CNTF (5 ng/ml) plus NGF (125 ng/ml). To facilitate comparison of the BDNF dose responses with and without CNTF plus NGF, the level of survival at the highest concentration of BDNF used (2 ng/ml) has been normalised in both dose-response graphs to 100%, and the level of survival in medium containing CNTF and NGF alone (approximately 22%) has been normalised to 0% for the CNTF + NGF + BDNF dose-response graph. The means and standard errors of the combined results of two separate experiments each set up in duplicate are shown.

NGF cytotoxicity. At higher BDNF concentrations, the number of neurons that survived with BDNF alone, and the proportion that were rescued from NGF cytotoxicity, increased to similar extents.

Recent studies of postnatal sympathetic neurons have provided some evidence that BDNF can exert a cytotoxic effect by a p75-dependent mechanism [20]. Whereas high concentrations of BDNF kill sympathetic neurons grown in the presence of depolarising levels of KCl, and anti-p75 antibodies partly prevent this loss, *BDNF*$^{-/-}$ mice have increased numbers of sympathetic neurons [20]. BDNF does not affect the survival of sympathetic neurons grown in the presence of saturating levels of NGF, indicating that TrkA signalling is able to abrogate the cytotoxic effects of BDNF. High concentrations of BDNF, however, reduce the number of neurons surviving in the presence of suboptimal concentrations of NGF. Although this latter result has been interpreted to indicate that p75-mediated apoptotic signalling can override survival signals mediated by low levels of TrkA signalling, it is possible that this result could be due at least in part to competition between BDNF and NGF for binding to p75 as NGF binding to p75 enhances the survival response of postnatal sympathetic neurons to suboptimal concentrations of NGF [18,21,22]. In our study of TMN neurons, we found no evidence for p75-mediated apoptotic signalling overriding survival signals mediated by low levels of TrkB signalling; low levels of BDNF that promote the survival of only a small proportion of TMN neurons abrogated the cytotoxic effect of NGF to a similar extent (Figure 4).

Like TMN neurons, oligodendrocytes that are susceptible to NGF-induced apoptosis in culture, and cells that are killed by NGF in the developing retina and forebrain, express p75 but not TrkA [3–5]. NGF has not been reported to have a cytotoxic effect on cells that co-express p75 and TrkA; indeed in neurons that express similar levels of p75 and TrkA, binding of NGF to p75 enhances the survival response of the neurons to NGF [21,22]. Our demonstration that activation of signalling pathways downstream of TrkB in TMN neurons abrogates p75-mediated NGF cytotoxicity implies a selective interaction between Trk and p75 signalling pathways, and this in turn provides a possible explanation for the differing effects of p75-mediated responses to NGF in some cell types. In TMN neurons, activation of signalling pathways downstream of the CNTF receptor complex do not abrogate the cytotoxic effects of NGF. Binding of neurotrophins to Trks results in phosphorylation of cytoplasmic tyrosine residues that become docking sites for adaptor proteins which include Shc and phospholipase Cγ-1 (PLCγ-1). Shc activates the Ras and phosphoinositide (PI) 3-kinase signalling pathways, and PLCγ-1 stimulates release of intracellular Ca^{2+} and activation of cAMP response element binding protein (CREB) [23]. In contrast, binding of CNTF to its receptor complex leads to phosphorylation of members of the Jak family of protein tyrosine kinases and the activation of the STAT family of transcription factors [24]. Jaks can, however, also activate the Ras pathway by interactions with the Shc adaptor protein [25]. In further work it will be important to establish which of the signalling events downstream of Trks antagonise the p75-mediated cytotoxic effects of NGF.

NGF does not invariably promote the apoptosis of cells expressing p75 without TrkA. For example, whereas both neonatal rat oligodendrocytes and adult human oligodendrocytes express p75 without TrkA in culture, only neonatal oligodendrocytes are killed by NGF [4,26]. Schwann cells also express p75 without TrkA, yet NGF does not kill these cells but stimulates their migration [27]. Like TMN neurons, the parasympathetic neurons of the ciliary ganglion express p75 without TrkA and are supported by CNTF in culture [12], but unlike TMN neurons, NGF does not kill ciliary neurons grown in the presence of CNTF (data not shown). It remains to be determined whether this difference in the response of TMN and

ciliary neurons is due to the absence of the necessary p75-activated death-effector machinery in ciliary neurons or to the relatively low level of p75 in these neurons [12] or because cytokine signalling pathways inhibit p75-mediated killing in these neurons.

In summary, we have shown that TMN neurons are susceptible to NGF-induced cell death by a p75-mediated mechanism when signalling pathways downstream of CNTF are activated, and that low levels of TrkB signalling abrogate this cytotoxic response to NGF. These results imply selective interactions between different signalling pathways in the cell that regulate cell survival and death.

Materials and methods

The median part of the TMN was dissected from E10 chicken embryos, and following trypsinisation and dissociation, the neurons were purified by differential sedimentation [28]. The neurons were plated in poly-ornithine/laminin-coated 35 mm diameter tissue culture petri dishes containing F14 medium supplemented with 10% heat-inactivated horse serum. The number of neurons within a 12×12 mm grid in the centre of each dish was counted 3 h after plating and the number of neurons surviving in the same 12×12 mm grid was counted 48 h later and expressed as a percentage of the number plated. For studies with anti-p75 antiserum, the neurons were grown in the 10 mm diameter wells of 4-well dishes (Greiner) in a volume of 100 µl. For these experiments, all neurons in each well were counted. In all experiments, neurotrophic factors were added to the culture medium prior to plating the neurons.

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