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Proneurotrophins, Seizures, and Neuronal Apoptosis

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Abstract

Neurons respond to numerous factors in their environment that influence their survival and function during development and in the mature brain. Among these factors, the neurotrophins have been shown to support neuronal survival and function, acting primarily through the Trk family of receptor tyrosine kinases. However, recent studies have established that the uncleaved neurotrophin precursors, the proneurotrophins, can be secreted and induce apoptosis via the p75 neurotrophin receptor, suggesting that the balance of secreted mature and proneurotrophins has a critical impact on neuronal survival or death. Epileptic seizures elicit increases in both proneurotrophin secretion and p75^{NTR} expression, shifting the balance of these factors toward signaling cell death. This review will discuss the evidence that this ligand-receptor system plays an important role in neuronal loss following seizures.

Keywords

nerve growth factor; brain-derived neurotrophic factor; p75 neurotrophin receptor; Trk; hippocampus

Cell death in the hippocampus is a well-established consequence of human epilepsy (Arzimanoglou and others 2002) and experimental animal seizure models (Represa and others 1995; Turski and others 1983). There are many potential mechanisms by which neurons may die after seizures, including excitotoxicity from excessive glutamate, release of nitric oxide, increased oxidative stress, as well as induction of apoptosis. In this review article, we will discuss the role of neurotrophins in contributing to neuronal apoptosis in the brain following seizures.

Neurotrophins are a family of neurotrophic factors that includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins (NT)-3 and -4. These neurotrophins were originally identified for their ability to support survival and differentiation of specific neuronal populations in the peripheral and central nervous systems (Barde 1994; Huang and Reichardt 2001). However, over the years these factors have been shown to have many other functions that are determined by the cellular context and suggest a greater complexity in their function than was previously appreciated. Neurotrophins are synthesized as precursors that can be cleaved intracellularly by furin and other proconvertases (Seidah and others 1996), or alternatively can be secreted in their uncleaved form as proneurotrophins (Lee and others 2001). Neurotrophins bind to two distinct types of receptors, a member of the Trk receptor tyrosine kinase family, and the p75 neurotrophin receptor (p75^{NTR}), previously referred to as the “low affinity” NGF receptor. The cleaved “mature” form of neurotrophins bind with high affinity to Trk receptors, which may be in a complex with p75^{NTR}, whereas

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pro-neurotrophins bind preferentially to p75^{NTR} in a complex with the coreceptor sortilin (Nykjaer and others 2004; Fig. 1). The Trk family of receptors has three members, TrkA, which preferentially binds NGF, TrkB, which binds BDNF and NT4, and TrkC, which binds NT3. The role of Trk receptors in mediating neuronal survival, differentiation, and synaptic function have been well defined, and the major signaling pathways activated are similar to those of other receptor tyrosine kinases, including activation of the PI₃ kinase-Akt, ras-MAP kinase, and PLC γ signaling pathways, which have been extensively studied (Friedman and Greene 1999; Kaplan and Miller 2000; Patapoutian and Reichardt 2001). In contrast, the function of the p75^{NTR} has not been as clearly defined. This receptor can form complexes with other coreceptors to mediate many diverse cellular functions such as survival, apoptosis, and axonal growth, depending on the cellular context. The most clearly defined role for p75^{NTR} thus far is in signaling apoptosis (Casaccia-Bonnel and others 1996; Coulson and others 1999; Frade and others 1996; Friedman 2000), particularly in response to proneurotrophins (Beattie and others 2002; Lee and others 2001; Volosin and others 2006). Thus, neurotrophins can regulate such opposing functions in the brain as survival or apoptosis, depending on which form of the protein is secreted and which receptor and signaling pathway is activated. There is, therefore, a finely tuned balance between neurotrophin regulation of neuronal survival or death, determined by expression and processing of the ligand and regulation of the receptors.

Proneurotrophins, p75^{NTR}, and Apoptosis

The p75^{NTR} is widely expressed in the brain during neonatal development, but its expression in the adult brain is restricted to a few neuronal populations such as the basal forebrain and striatal cholinergic neurons. However, many pathological conditions, including seizures, lesions, and traumatic injuries, have been shown to induce this receptor, suggesting an important role in mediating neuronal responses to injury (Ernfors and others 1989; Roux and others 1999). Data from a number of these injury paradigms have demonstrated that neuronal death is attenuated or absent in mice lacking p75^{NTR}, indicating that this receptor can actually mediate neuronal apoptosis (Beattie and others 2002; Troy and others 2002).

Inasmuch as mature neurotrophins can all bind p75^{NTR} with low affinity, it was unclear whether sufficient concentrations of these factors would be present in the brain to bind p75^{NTR} and activate apoptotic signaling, and if not, what the endogenous ligand for this receptor might be. Once proneurotrophins were identified as high-affinity ligands for p75^{NTR} (Lee and others 2001), it appeared that the neurotrophin precursors could serve as endogenous p75^{NTR} ligands. Thus, a key aspect of neurotrophin function is influenced by the regulation of proneurotrophin cleavage, determining whether the factor is processed intracellularly, and secreted as the cleaved “mature” neurotrophin, which would preferentially bind a Trk receptor and support neuronal survival, or whether it is secreted as the proneurotrophin, which can bind the p75^{NTR}/sortilin complex and elicit apoptosis (Fig. 1).

There has been some controversy regarding the role of proNGF in the brain, and whether it has prosurvival or proapoptotic activity (Fahnestock and others 2004). Initial studies demonstrated that proNGF did not bind to TrkA, but bound with high affinity to p75^{NTR} and was efficacious in eliciting neuronal death (Lee and others 2001). Studies confirming the proapoptotic role of endogenous proNGF have demonstrated that the proNGF extracted from brain lysates of human Alzheimer’s disease patients, or from rat brains following seizures, elicited death of cultured neurons (Pedraza and others 2005; Volosin and others 2006). Furthermore, several studies have shown that infusion of anti-proNGF antibodies, which specifically recognize the pro domain of proNGF, into the brain provided significant protection from neuronal cell death after either corticospinal lesions or seizures, indicating that endogenous proNGF has proapoptotic effects in the brain after different types of injury (Harrington and others 2004; Volosin and others 2008). However, studies from a different

group have suggested that there is some binding of proNGF to TrkA and some neurotrophic activity, although less than that of mature NGF (Fahnestock and others 2004). These studies were primarily based on overexpression of proNGF, which may alter the balance of cleavage and release of the different forms of the factor. Studies from the latter group have suggested that the relative levels of the Trk and p75^{NTR} receptors may alter the balance of signaling and determine whether proNGF induces survival or apoptosis (Masoudi and others 2009). The situations where proNGF has been demonstrated to induce neuronal apoptosis in vivo have generally been after injury or seizures, when p75^{NTR} expression is induced, which may change the relative levels of the Trk and p75^{NTR} receptors, and therefore tilt the balance of signaling toward apoptosis.

Neurotrophins, Proneurotrophins, and Seizures

Many studies have demonstrated that neurotrophin mRNAs are regulated by seizures in the brain. Different mechanisms of seizure induction, such as kainic acid, pilocarpine, and kindling, evoked elevated levels of NGF mRNA (Bengzon and others 1992; Gall and Isackson 1989) as well as BDNF mRNA (Ballarin and others 1991; Dugich-Djordjevic and others 1992). In addition, immuno-cytochemical labeling for both proNGF and proBDNF was more widespread in the hippocampus after pilocarpine-induced seizures (Volosin and others 2008). However, the induction of the mRNAs and the intracellular presence of the proneurotrophins do not provide information on which form of the factor, the pro or cleaved form, is secreted. Seizures also induce increased expression of the neurotrophin receptors, including TrkB and TrkC (Bengzon and others 1993; Merlio and others 1993) as well as p75^{NTR} (Roux and others 1999; Troy and others 2002). Therefore, it is unclear whether the increased neurotrophin expression leads to the secretion of the cleaved factor that might be neuroprotective via the Trk receptors, or the proneurotrophin that might induce neurodegeneration via p75^{NTR}. Activation of TrkB receptors in mossy fibers has been demonstrated following kindling-induced seizures, suggesting that BDNF released during seizures is at least partially processed to the mature form (He and others 2002). However, a recent publication demonstrated that prolonged status epilepticus evoked a decrease in TrkB expression with a concomitant increase in p75^{NTR}, and suggested that increased binding of proBDNF to p75^{NTR} resulted in elevated levels of neuronal death (Unsain and others 2008). Thus, the type of stimulus and duration of the seizure may have differences in the evoked response of the neurotrophins and their receptors (Xu and others 2004). These observations suggest that the seizures regulate expression of both the receptors and ligand, tipping the balance of neurotrophin signaling toward cell death.

Immunostaining analysis using antibodies that recognize only the pro domains of proNGF and proBDNF revealed that, in addition to increases in proneurotrophins in hippocampal neurons following pilocarpine-induced seizures, there was also a dramatic induction of proNGF and proBDNF in hippocampal astrocytes. Analysis of cerebrospinal fluid (CSF) from control and pilocarpine-treated rats showed that secreted proNGF was undetectable under normal conditions, and was only observed following the seizures, indicating that release of proNGF occurred primarily under pathological conditions (Volosin and others 2008). Whether it is the neurons or glia, or both, that are responsible for the secretion of proNGF remains to be determined.

Although several studies have shown induction of p75^{NTR} after seizures, ischemia, oxidative stress, and injury, the mechanisms of p75^{NTR} regulation have not been clearly established. Because these situations of p75^{NTR} induction have all been associated with pathological conditions, stimuli common to those situations are likely to be key regulators. Hypo-osmolarity is one consequence of multiple types of injuries that elicits p75^{NTR} expression in brain neurons (Ramos and others 2007). Another common consequence of these different pathological situations is the induction of inflammatory cytokines, such as IL-1 and TNF α . These cytokines

are highly expressed in disease and after injury, and are up-regulated prior to p75^{NTR} induction after damage. These inflammatory cytokines have recently been shown to regulate p75^{NTR} expression in both neurons and astrocytes in vitro, and the mechanisms governing this regulation are cytokine and cell type specific (Choi and Friedman 2009). IL-1 β is also known to regulate expression of NGF mRNA in astrocytes (Friedman and others 1996); therefore, this cytokine may be a critical regulator of both the ligand and receptor under inflammatory conditions that lead to neuronal loss.

Given the proapoptotic function established for p75^{NTR}, the possibility that this receptor might be involved in mediating neuronal cell death in the hippocampus after seizures was investigated. Roux and others demonstrated that p75^{NTR} was induced in the hippocampus and cortex after status epilepticus evoked by pilocarpine (Roux and others 1999). Moreover, they found that the expression of p75^{NTR} was coincident with TUNEL labeling, suggesting a link between expression of the receptor and cell death. Subsequently, Troy and colleagues showed that pilocarpine induced apoptotic signaling, including activation of caspases and chromatin condensation, in the p75^{NTR}-positive neurons. Further, this study demonstrated that induction of seizures in p75^{-/-} mice showed an 80% reduction in the number of dying neurons compared with wild-type controls, demonstrating a significant role for this receptor in mediating neuronal loss (Fig. 2; Troy and others 2002). The increased expression and secretion of proNGF, together with the demonstrated role for p75^{NTR} in mediating seizure-induced neuronal death, suggested that this ligand-receptor system might play a critical role in regulating hippocampal cell death. Confirmation of this was obtained by infusing an antibody to proNGF into the hippocampus on one side of the brain for three days after pilocarpine treatment compared with a control antibody on the contra-lateral side. When sections through the hippocampus were examined for several death markers, including cleaved caspase-3 and fluorojade B labeling, there was a dramatic reduction in the number of dying neurons in the hippocampus that received the anti-proNGF (Volosin and others 2008), providing confirmation that endogenously secreted proNGF plays a significant role in mediating neuronal death after seizures (Fig. 3).

In addition to acute neuronal loss following status epilepticus, dendritic sprouting and synaptic reorganization lead to the formation of aberrant connections and the development of spontaneous recurrent seizures (Williams and others 2009). Several studies have demonstrated a role for mature BDNF acting via TrkB in eliciting sprouting of mossy fibers and network reorganization in the dentate gyrus (Koyama and others 2004). Thus, epileptic seizure activity in the brain results in acute neuronal cell death, as well as the possibility of chronic recurrent seizures and continued neuronal loss.

Regulation of Proneurotrophin Cleavage

Proneurotrophins can be detected in neurons under normal conditions as precursors for the cleaved neurotrophins that play a crucial role in development and maintenance of neuronal survival, differentiation, and synaptic function. Studies using virally overexpressed proNGF and proBDNF in hippocampal neurons showed that proNGF was cleaved intracellularly by furin in the trans-Golgi network and secreted by the constitutive pathway, whereas proBDNF was processed by proconvertases in secretory granules and released by the regulated secretory pathway (Mowla and others 1999). However, even under normal physiological conditions, some of these factors may be secreted as proneurotrophins (Lee and others 2001). Enzymes present in the extracellular environment also have the capacity to cleave proneurotrophins. These include enzymes such as plasmin, which is activated by cleavage of plasminogen by tissue plasminogen activator (tPA), as well as specific matrix metalloproteinases. Secretion of proNGF under physiological conditions has not been studied; however, secretion and cleavage of proBDNF has important consequences for hippocampal function. Mature BDNF is a key regulator of long-term potentiation (LTP; Korte and others 1998; Patterson and others 1996),

whereas uncleaved proBDNF promotes long-term depression (LTD; Woo and others 2005); thus, regulation of BDNF cleavage may have profound effects on hippocampal physiology. Although a recent study questioned whether proBDNF could be released from neurons under normal conditions (Matsumoto and others 2008), other studies have demonstrated that proBDNF is released from neurons in the hippocampus (Yang and others 2009), and can be cleaved extracellularly by tPA to facilitate LTP (Pang and others 2004). Thus, mature BDNF can be produced intracellularly or extracellularly to influence LTP. The mechanisms that regulate whether BDNF is cleaved intracellularly or released as proBDNF to be cleaved extracellularly remain to be defined.

The enzymes that can cleave proneurotrophins extracellularly are also regulated following seizures and have many functions other than regulation of neurotrophin cleavage. Although the activity of tPA in cleaving proneurotrophins to their mature form might be expected to be neuroprotective, tPA itself has been shown to elicit neuro-degeneration after kainic acid-induced seizures (Tsirka and others 1995). Clearly, tPA can cleave numerous substrates in addition to the proneurotrophins. Moreover, since tPA and its inhibitor, neuroserpin (Hastings and others 1997; Osterwalder and others 1998), are released in conjunction with proNGF into the extracellular environment, the regulation of proNGF cleavage is likely to depend on the balance between tPA and neuroserpin activity (Bruno and Cuello 2006; Yepes and Lawrence 2004).

In addition to plasmin, specific matrix metalloproteinases (MMPs) have the ability to cleave proneurotrophins. ProBDNF can be cleaved by MMP-3 and MMP-7, whereas proNGF can also be cleaved by MMP-7, but not MMP-2, 3, or 9 (Lee and others 2001). Thus, MMP-7 activity promotes the cleavage of proNGF and proBDNF to their cleaved forms, but tissue inhibitor of metalloproteinase (TIMP-1) would prevent cleavage and retain the uncleaved proneurotrophins in the extracellular environment. Inasmuch as proNGF has specifically been associated with neuronal death after seizures, activity of MMP-7 and its inhibitor, TIMP-1, are of particular interest. Expression of TIMP-1 is induced in both neurons and astrocytes, and is particularly strong in astrocytes three days following kainic acid-induced seizures (Rivera and others 1997), the same time that maximal neuronal death has been observed. Moreover, TIMP-1 knockout mice were resistant to neuronal degeneration in the hippocampus (Jourquin and others 2005), which is consistent with the inability to prevent MMPs from cleaving proneurotrophins. Because expression and activity of these enzymes as well as their inhibitors are regulated in the brain by seizures, the balance of their activities will ultimately determine whether secreted proneurotrophins are cleaved or not.

Mechanisms of p75^{NTR}-Mediated Neuronal Apoptosis

The p75^{NTR} lacks enzymatic activity and signals by recruiting intracellular binding proteins. Many proteins have been identified that can interact with the p75^{NTR}-intracellular domain (Barker 2004; Gentry and others 2004), and several of these proteins have been implicated in apoptotic signaling, such as NRIF (neurotrophin receptor-interacting factor; Casademunt and others 1999; Linggi and others 2005), NADE (Mukai and others 2000), as well as the MAGE family proteins NRAGE (Salehi and others 2002) and necdin (Kuwako and others 2004; Tcherpakov and others 2002). ProNGF treatment of cultured hippocampal neurons elicited increased interaction of p75^{NTR} with NRIF, and cultured neurons from NRIF^{-/-} mice were resistant to proNGF-induced apoptosis (Volosin and others 2008). In vivo, NRIF was required for p75^{NTR}-mediated apoptosis following seizures, as pilocarpine treatment of NRIF^{-/-} mice yielded significantly fewer dying neurons compared with wild-type mice (Volosin and others 2008). Other p75^{NTR}-interacting proteins may also be involved in p75^{NTR}-mediated apoptotic signaling after seizures. The adapter protein NADE can be co-induced with p75^{NTR} following kainate-induced seizures (Yi and others 2003), and may also play a role in cell death signaling.

In addition, NRAGE has been detected in a complex with NRIF following proNGF stimulation (Volosin and others 2008); therefore, rather than activating independent pathways, some of these p75^{NTR} adapter proteins may act together in a complex to stimulate downstream signaling.

Apoptotic signaling activated by p75^{NTR} requires phosphorylation of c-Jun N-terminal kinase (JNK; Fried-man 2000; Yoon and others 1998), phosphorylation of the proapoptotic proteins Bad (Bhakar and others 2003) and BimEL (Becker and others 2004), and activation of the intrinsic caspase pathway (Salehi and others 2002; Troy and others 2002; Wang and others 2001), requiring caspases-9, -6, and -3. This is in contrast to other death receptors that signal via the extrinsic, caspase-8-dependent pathway. Specifically how the recruitment of p75^{NTR} adapter proteins leads to activation of these downstream events is still not fully defined.

The p75^{NTR} has been implicated in many functions in different cell types; however, cell-specific differences in p75^{NTR} signaling have not been clearly characterized. For example, activation of p75^{NTR} signaling elicits activation of NFκB in Schwann cells (Carter and others 1996), where it is likely to play a role in myelination, but not in hippocampal neurons where it mediates apoptosis (Volosin and others 2008). Thus, the signaling mechanisms and functional consequences of activating this receptor depend on the cellular context.

Interaction of p75^{NTR}-Mediated Apoptotic and Trk-Mediated Survival Signaling Pathways

Although seizures induced by pilocarpine or kainic acid primarily affect hippocampal and cortical neurons, other populations can be affected as well. In the CNS, basal forebrain (BF) neurons express p75^{NTR} throughout life. Kainic acid was shown to elicit an increase in p75^{NTR} and cause cell death in this neuronal population (Oh and others 2000; Volosin and others 2006). These BF cholinergic neurons also express at least one, if not more than one, Trk receptor, and mature neurotrophins support BF neuron survival in culture and in vivo. Because neurons in the brain may be concomitantly exposed to both pro- and mature neurotrophins during development, disease, or after injury, this raises the question of how Trk and p75^{NTR} signaling pathways interact to determine the outcome of neuronal survival or death.

In sympathetic neurons, activation of p75^{NTR} can signal neurons to die via activation of JNK and the p53 tumor suppressor (Aloyz and others 1998). However, TrkA activation can suppress p75^{NTR}-mediated signaling, tipping the balance toward survival (Miller and Kaplan 2001). In these neurons, TrkA signaling silences the p75^{NTR}-JNK-p53 pathway via activation of ras and the PI₃ kinase-Akt pathway (Mazzoni and others 1999).

In the CNS, BF neurons express p75^{NTR} together with TrkA, TrkB, or TrkC. In contrast to sympathetic neurons, activation of any of these Trk receptors was not sufficient to prevent apoptotic signaling via p75^{NTR}. In cultured BF neurons apoptosis could still be induced by proNGF, even in neurons with a phosphorylated (activated) Trk receptor. However, if Akt was phosphorylated, the neurons were resistant to p75^{NTR}-mediated cell death (Volosin and others 2006). Thus, unlike sympathetic neurons where Trk phosphorylation was sufficient to prevent p75^{NTR}-mediated apoptosis, in CNS neurons phosphorylation of the receptor was not sufficient for neuroprotection. However, once the downstream Akt pathway was activated, the neurons were resistant to proNGF-induced cell death, indicating that the Trk and p75^{NTR} signaling pathways interact downstream of the receptors, but upstream of Akt activation, revealing a novel critical checkpoint in survival versus apoptotic signaling. When ligands for both receptors were present, p75^{NTR}-mediated apoptotic signaling suppressed the ability of BDNF to elicit Akt activation via TrkB (Fig. 4). In contrast, treatment with BDNF did not suppress the ability of proNGF to elicit caspase cleavage and apoptosis via p75^{NTR} (Volosin and others

2006). Similar to the basal forebrain, in the hippocampus many neurons express TrkB or TrkC, yet may still die when p75^{NTR} is induced in these neurons, because of the ability of proNGF-activated p75^{NTR} signaling to suppress Trk-mediated signaling and induce apoptosis.

Conclusions

The effects of neurotrophins on neuronal survival depend on which form of the factors is secreted, which receptor complex is engaged, and which signaling pathways are activated. Proneurotrophins can be cleaved intracellularly or extracellularly, and the mechanisms that regulate cleavage are not yet fully understood, yet are clearly of great importance. Secreted proNGF was only detectable following injury or seizures, suggesting that these conditions alter the balance of mature and proneurotrophins present in the extracellular environment. Moreover, these conditions also lead to induction of the p75^{NTR}, shifting the balance of neurotrophin signaling toward apoptosis.

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Figure 1.
Schematic diagram showing the receptor interactions of mature and proneurotrophins.

Figure 2.

p75 is required for neuronal death following pilocarpine-induced seizures. Sections through the hippocampus from wild-type (*a*) or p75^{-/-} (*b*) mice were stained with fluoro-jade B to label dying neurons. In the p75^{-/-} mice, there was an 80% reduction in the number of dying neurons in the hippocampus compared with wild type. Size bar = 100 μ m. Reprinted with permission from Troy and others. 2002. J Biol Chem 277(37):34295–302.

Figure 3.

Anti-proNGF prevents neuronal loss after seizures in vivo. Rats were cannulated bilaterally in the dorsal hippocampus 1 week prior to seizure. Following seizures anti-proNGF (1.5 μ g/0.5 μ L) was injected on one side and control rabbit IgG on the other side. Cleaved caspase-3 (*a*, *b*) and fluorojade B labeling (*c*, *d*) combined with immunostaining for p75^{NTR} (*e-h*) demonstrated a decrease in the number of dying neurons with the anti-proNGF relative to the control IgG in the same brains. Size bar in *a* = 100 μ m and is the same for *b*; size bar in *h* represents 50 μ m and is the same for *c-g*. Reprinted with permission from Volosin and others. 2008. *J Neurosci* 28(39):9870–9.

Figure 4. Schematic diagram showing the interaction of the p75^{NTR} and TrkB signaling pathways. Activation of p75^{NTR} blocks signaling from TrkB downstream of the receptor, allowing the apoptotic pathway to proceed.