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ALZHEIMER'S THERAPEUTICS: Neuroprotection

Alzheimer's Therapeutics

Neurotrophin Domain Small Molecule Mimetics

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Abstract

Factors limiting the therapeutic application of neurotrophins to neurodegenerative diseases include poor stability and CNS penetration. Moreover, certain neurotrophin effects, such as promotion of neuronal death via interaction with the $p75^{NTR}$ receptor, might further limit their application. We have proposed that development of small molecule mimetics of neurotrophins might serve to overcome these limitations. In previous work, our laboratory established the proof-of-principle that mimetics of specific nerve growth factor (NGF) domains could prevent neuronal death. Peptidomimetics of the loop 1 domain prevent death via $p75^{NTR}$-dependent signaling and peptidomimetics of the loop 4 domain prevent death via Trk-related signaling. In current work we are designing pharmacophore queries corresponding to loop domains 1 or 4 that incorporate features of the NGF crystal structure along with features derived from peptidomimetic structure-activity-relationships. Screening of *in silico* databases containing non-peptide, small molecules has identified a number of candidate NGF domain mimetics. Preliminary assessment of these compounds using neurotrophin bioassays indicates that several are capable of preventing neuronal death. Ongoing studies will determine whether these compounds act via $p75^{NTR}$ or Trk receptors.

Index Entries: Neurotrophin; nerve growth factor; mimetic; p75; TrkA.

Introduction

The hypothesis that amyloid-β (Aβ) causes neuronal degeneration suggests that strategies inhibiting its accumulation will prevent or slow progression of AD. Although such approaches offer important promise, several scenarios raise the need for parallel therapeutic strategies. Aβ-targeted therapeutics might have to be applied during the unknown time window of the presymptomatic onset of Aβ-induced pathological cascades and might not be entirely effective in preventing neuronal damage. Moreover, Aβ-targeted mechanisms might not promote recovery or optimize function of impaired neurons. Neurotrophin-based strategies offer a number of features that might compliment Aβ-based therapies. First, neurotrophins up-regulate functions of vulnerable (e.g., cholinergic) neurons (Tuszynski, 2002). Second, evidence suggests that Aβ toxicity might be mediated, in part, via alterations in signaling

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networks shared by neurotrophins (Savage et al., 2002; Rabizadeh and Bredesen, 2003). In addition, Aβ binds to the p75 neurotrophin receptor ($p75^{NTR}$) and triggers signaling associated with $p75^{NTR}$-induced cell death (Yaar et al., 2002; Kawasumi et al., 2002). Third, neurotrophins decrease the vulnerability of neurons to a wide range of toxic mechanisms. The discoveries that a precursor form of nerve growth factor (NGF), proNGF, binds to $p75^{NTR}$ to promote neuronal death (Lee et al., 2001; Hempstead, 2002) and that levels of proNGF are increased in Alzheimer's disease (AD) brain (Fahnestock et al., 2001) raise the possibility that inhibition of this interaction might inhibit death.

There are at least two critical hurdles in the application of neurotrophins. Half-lives of neurotrophins are on the order of minutes and their ability to reach CNS targets is limited (Poduslo and Curran, 1996). A more fundamental limitation is the wide range of neurotrophin biological actions. The ability of NGF to induce sympathetic fiber sprouting and up-regulate pain mechanisms might limit its clinical application. We hypothesized that these limitations might be overcome by small molecule neurotrophin mimetics that selectively target their receptors to modulate certain but not necessarily all of the signaling cascades triggered by neurotrophins.

Our initial studies have focused on NGF mimetics. NGF interacts with the TrkA receptor to trigger signaling through the phosphatidylinositol-3-kinase (PI3K)/Akt kinase, mitogen-activated protein (MAP) kinase and phospholipase Cγ (PLCγ) pathways (Sofroniew et al., 2001). Signaling via TrkA is also likely to mediate NGF-induced upregulation of pain transmission (Bergmann et al., 1998). NGF interaction with $p75^{NTR}$ regulates its association with different intracellular effector proteins, promoting either survival or death, depending on which effectors are activated (Hempstead, 2002; Mamidipudi and Wooten, 2002; Rabizadeh and Bredesen, 2003). NGF interacts with $p75^{NTR}$ via residues in β-loop 1 and with the TrkA receptor via residues in β-loops 2 and 4 and the N terminus (McDonald and Chao, 1995). We established that NGF peptidomimetics corresponding to loop 1 act via a $p75^{NTR}$-dependent mechanism to prevent neuronal death (Longo et al., 1997) and that peptidomimetics corresponding to loop 4 act via TrkA to prevent neuronal death (Xie et al., 2000). Loop 4 mimetics activate Trk (unpublished data), as well as Erk and Akt (Xie et al., 2000). NGF peptidomimetics corresponding to loop 4 and promoting survival have also been reported by Maliartchouk et al. (2000). We are testing the hypothesis that available crystal structure of NGF, along with structure-activity relationships (SARs) of peptidomimetics, can be used to create pharmacophore queries, which in turn can be utilized to screen small molecule databases to identify nonpeptide, small molecules that act at neurotrophin receptors and modulate their signaling to prevent neuronal death. The following is an interim progress report.

Materials and Methods

Using established pharmacophore approaches with InsightII, Cerius2, and Catalyst software, we created NGF loop 1 and loop 4 pharmacophore queries based on NGF crystallographic structures and peptidomimetic SARs (Accelerys, Inc; Massa et al., 2002). Library screening was conducted on a Silicon Graphics Octane workstation. We assembled a collection of libraries containing virtual, three-dimensional conformers of ~1.2 million compounds (Massa et al., 2002). Compounds demonstrating steric and chemical features matching those of NGF loop1 or loop 4 queries were obtained for assays. Core structures of active compounds were used as secondary queries as part of an iterative approach to obtain additional active and inactive compounds with variant structures and to identify compounds with greater potency and efficacy. Structure-activity assessments were used to evaluate our starting pharmacophore hypotheses.

Compounds or peptidomimetics were assayed for neurotrophic activity in vitro using the classic NGF assay of chick embryological day 8 (ED8) dorsal root ganglion (DRG) sensory neurons (Xie et al. 2000) or mouse ED16–17 hippocampal neurons (Goslin et al., 1998). Death-preventing activity was compared to that of optimal levels of NGF (DRG neurons) or brain-derived neurotrophic factor (BDNF) (hippocampal neurons). Dependence of neurotrophic activity on Trk and on MAP kinase, PI3K/Akt, and PLCγ signaling is assessed using well-established inhibitors. Signaling is assessed using Western blot analysis for Erk, Akt, and PKC activation (Xie et al., 2000). Dependence of activity on $p75^{NTR}$ signaling is determined using DRG neurons isolated from $p75^{NTR}$ mutant mice (Longo et al., 1997). Neurotrophin binding to $p75^{NTR}$ activates sphingomyelinase, which converts sphingomyelin to ceramide (Dobrowsky and Carter, 2000). Thus, signaling via $p75^{NTR}$ is also assessed using inhibitors of sphingolipid synthesis (myriocin and fumonisin B1), as described in DeFreitas et al (2001).

Results

Results of Library Screening

A first round of studies using an initial set of queries yielded numerous "hits," as identified by fit scores. Hits were screened for steric incompatibilities visually, reducing the number by a factor >20. Compounds were favored in which chemical features were similar to those of targeted side chain features and predicted to have low conformational energies (<10 kcal). A total of 55 compounds with apparent favorable features were obtained. Out of 35 loop 1 compounds, 12 were poorly soluble in culture medium. Of the 23 soluble compounds, 4 had survival-promoting activity (Massa et al., 2002). Out of 20 loop 4 compounds, 12 were poorly soluble. Of the eight soluble compounds, three had survival-promoting activity. Overall, for the 31 loop 1 and loop 4 soluble compounds, 7, or ~23%, were active. Using DRG assays, activities occurred in the 1–100 μ*M* range, and efficacies in some cases reached approximately half of that seen with NGF at its maximum effect.

A second round of screening was conducted using the core structures of active compounds as queries to find available variants. Relatives of active compounds tended to fall into groups containing common core structures, with variations formed by attachment of a variety of functional groups at a restricted set of locations. Core structure searches yielded 1052 relatives of two active loop 1 compounds and 260 relatives of one active loop 4 compound. These lists were further reduced by consideration of chemical diversity and coverage of substitution points, as well as overall size and shape. For loop 1, 12 compounds were ordered, 5 obtained, and 3 found to be active. For loop 4, 12 compounds were ordered, 11 obtained, and 8 found to be active. Interestingly, efficacies tended to be lower that those of the first set of identified compounds, suggesting that the original pharmacophore queries contain features important for identifying active compounds.

Preliminary studies indicate that the activity of at least two loop 1 compounds is inhibited by the sphingomyelin synthesis inhibitors myriocin and fumonisin B1, providing initial support for the possibility that these compounds might prevent death via $p75^{NTR}$-related signaling. The activity of at least one loop 4 compound was inhibited by K252a, suggesting that this activity might be mediated via Trk signaling.

Four of the loop 1 compounds demonstrating activity in DRG assays were tested using ED16–17 hippocampus assays. ED16–17 hippocampal neurons are primarily pyramidal neurons expressing $p75^{NTR}$ and TrkB. Neurotrophin-induced 75^{NTR} signaling has been shown to promote either survival or death of embryonic hippocampal neurons, possibly depending on the degree of induced ceramide production (Brann et al., 2002). In preliminary studies, one of these loop 1 compounds, which was shown to be inhibited by myriocin and fumonisin B1, was found to prevent death of ED16–17 hippocampal neurons at low nanomolar concentrations with 100% of the efficacy of BDNF.

Discussion

NGF loop 1 and loop 4 peptidomimetic studies provided the first proof of principle that small molecules mimicking specific NGF domains might be found that modify or mimic neurotrophic activity (Longo et al., 1990, 1997; Maliartchouk et al., 2000; Xie et al., 2000). The present preliminary findings support the hypothesis that it may be possible to use pharmacophore queries based on NGF structure and peptidomimetic SARs to identify nonpeptide compounds that act via neurotrophin receptors to prevent death at nanomolar concentrations.

The identification of a small molecule preventing death via $p75^{NTR}$ signaling is of particular interest. NGF interaction with $p75^{NTR}$ was initially shown to trigger cell death (Casaccia-Bonnefil et al., 1996; Frade et al., 1996). From the perspective of these studies, it seemed unlikely that an NGF mimetic acting via $p75^{NTR}$ would prevent, rather than cause, death. More recently, however, studies have demonstrated that NGF interaction with $p75^{NTR}$ in a variety of settings prevents cell death (Defreitas et al., 2001; Khursigara et al., 2001; Roux et al., 2001; Mamidipudi and Wooten, 2002; Rabizadeh and Bredesen, 2003). The identification of a number of intracellular adaptor proteins that interact with $p75^{NTR}$ indicates that the survival vs death function is determined by the specific set of interacting adaptor and signaling proteins (Hempstead, 2002; Mamidipudi and Wooten, 2000). Moreover, the selection of adaptors interacting with $p75^{NTR}$ might be regulated by the nature of the $p75^{NTR}$ ligand (Dobrowsky and Carter, 2000). These findings, along with our peptidomimetic and small molecule studies, raise the intriguing possibility that loop 1 small molecule mimetics might be identified or designed that trigger $p75^{NTR}$ signaling in a way that favors prevention rather than promotion of cell death. Whereas the domains used by

proNGF to bind to $p75^{NTR}$ are not yet known, it is also possible that $p75^{NTR}$ binding of small molecules might also be used to interfere with proNGF activation of $p75^{NTR}$.

Current work is focused on further iterations of query modification and compound identification, formal dose-response studies of compounds using DRG and hippocampal assays, further verification of whether compounds act via TrkA or $p75^{NTR}$ signaling mechanisms, and structure-activity analysis to form a basis for lead compound design.

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