ENDOCRINOLOGY

Neurosteroids and Microneurotrophins Signal Through NGF Receptors to Induce Prosurvival Signaling in Neuronal Cells

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The neurosteroid dehydroepiandrosterone (DHEA) exerts a portion of its neuroprotective effects by directly interacting with the nerve growth factor (NGF) receptors TrkA and p75^{NTR} to induce prosurvival signaling. DHEA is an intermediate in the biosynthesis of estrogens and androgens that affects the endocrine system and potentially increases the risk for developing estrogen- and androgen-dependent tumors. We have synthesized 17-spiro analogs of DHEA that lack estrogenic or androgenic properties and bind to and activate NGF receptors, thus exerting potent neuroprotective effects without the tumor risk. These synthetic DHEA derivatives may serve as lead molecules to develop small agonists of NGF receptors that can penetrate the blood-brain barrier (microneurotrophins) with potential applications in the treatment of neurodegenerative diseases. The neuroprotective properties of microneurotrophins are now being tested in various animal models of neurodegenerative diseases.

Presentation Notes

Slide 1: Science Signaling *logo*

The slideshow and notes for this Presentation are provided by *Science Signaling* (http://www.sciencesignaling.org).

Slide 2: Neurotrophin signaling

Neurotrophins control the development and maintenance of neural tissue. Neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and neurotrophins 4 and 5 (*1*). Signaling through neurotrophin receptors controls neuronal survival and axonal outgrowth, neuronal differentiation, and synaptic plasticity. The mammalian neurotrophin Trk receptors belong to the receptor tyrosine kinase (RTK) superfamily of transmembrane receptors and share a conserved architecture (*2*). TrkA binds to NGF and to NT3, TrkB recognizes BDNF, and TrkC is activated by NT3. Additionally, all neurotrophins are recognized by the pan-neurotrophin p75^{NTR} receptor, a member of the tumor necrosis factor (TNF) receptor (TNFR) superfamily, albeit with lower affinity than by the Trk receptors. Whereas Trk receptors mediate the prosurvival, neurotrophic effects of neurotrophins, p75^{NTR} is considered a proapoptotic receptor. Trk receptors are primarily activated by homodimerization followed by autophosphorylation of specific tyrosine residues in the Src homology 1 (SH1) domain, which induces a cascade of sequential phosphorylation events involving kinase Src, the SH2 domain–containing adaptor protein Shc, the Ras-Raf pathway, and mitogen-activated protein kinases (MAPKs). These phosphorylation events in turn regulate the transcription factor nuclear factor κB (NF-κB). In addition, phosphorylation of Trk receptors also results in the activation of phospholipase C–γ (PLC-γ) and protein kinase C (PKC) and subsequent activation of the transcription factor cyclic adenosine monophosphate (cAMP)–response element binding protein (CREB) (*3*). Neurotrophins, as well as their precursor proneurotrophins, bind to the p75^{NTR} receptor, inducing its homodimerization (*4*) and the recruitment of intracellular interactors, such as the TNF receptor–associated factor 6 (TRAF6), Rho guanosine diphosphate dissociation inhibitor (RhoGDI), receptor interacting protein 2 (RIP2), and neurotrophin receptor interacting factor (NRIF), to control neurite elongation and differentiation through the Jun N-terminal kinase (JNK) pathway and the activation of NF-κB (*5*). Recent experimental findings suggest that p75^{NTR} and Trk receptors may even form heterodimeric complexes that have signaling properties distinct from the homodimeric receptors (*6*). Additionally, proteins like Sortilin act as co-receptors for both TrkA and p75^{NTR} (*7*), further enhancing the complexity of neurotrophin signaling. Neurotrophins are multifaceted agents, controlling axonal growth and dendritic arborization or synapse formation, as well as neuronal proliferation, differentiation, survival, and apoptosis during development and aging.

Slide 3: NGF in neurodegeneration

A large number of experimental and clinical findings have implicated NGF in the pathophysiology of various neurodegenerative conditions, including peripheral neuropathies, Alzheimer's disease, Parkinson's disease, multiple sclerosis, glaucoma, and retinal degeneration (*8*–*15*). Decrease in NGF production is associated with neuronal loss and damage. On the basis of these data, NGF was recently identified as a therapeutic agent for the prevention of neurodegeneration. However, its clinical use has proven to be problematic because of the limited bioavailability conferred by its polypeptidic nature: It is destroyed in the gastrointestinal system and does not cross the blood-brain barrier (BBB).

Slide 4: Small molecules as NGF receptor agonists

Synthetic small molecules that act as NGF receptor agonists with potent neuroprotective properties may represent a new therapeutic approach for NGF-dependent neurodegenerative diseases. The idea is to synthesize small molecules with high affinity for TrkA or p75^{NTR} receptors or both, which are capable of selectively activating the neurotrophic signaling pathways without affecting those associated with hyperalgesia and pain. The development of central nervous system (CNS)–bioavailable, synthetic microneurotrophins represents a very active field in neurodegeneration therapeutics research in both academia and in industry. This presentation summarizes our experimental findings that suggest that synthetic lipophilic steroid

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derivatives may be of use as lead molecules to develop new selective agonists and antagonists of neurotrophin receptors.

Slide 5: DHEA exerts neuroprotective effects in vitro and in vivo

In the early 1980s, Etienne Baulieu described the ability of neurons and glia to produce steroids, for which the term "neurosteroids" was coined (*16*). The first neurosteroid described was dehydroepiandrosterone (DHEA). It is the most abundant steroid in humans and is a precursor for estrogen and androgen biosynthesis. It is synthesized by the enzyme cytochrome 17 (CYP17), and its concentration in the brain and in circulation gradually declines with age, in neurodegenerative conditions such as Alzheimer's disease, during chronic stress, and under conditions of neuroinflammation. In evolutionary terms, DHEA is an ancient and "sticky" molecule, because it directly binds to many neutotransmitter and steroid hormone receptors, including the *N*-methyl-D-aspartate (NMDA), γ-aminobutyric acid type A ($GABA_A$), and σ1 receptors; the estrogen receptors α and β (ERα and ERβ); and androgen receptors. DHEA increases survival signals in neural precursors in the mouse embryo forebrain, enhances neuroprotection in a rabbit spinal cord ischemia model, protects the hippocampus from excitotoxicity, and prevents MPTP-induced dopamine depletion and apoptotic loss of dopaminergic neurons in rodents, in primates, and in experimental allergic encephalomyelitis (EAE) in mice.

Slide 6: Antiapoptotic effects of neurosteroid DHEA

DHEA protects neuronal cells from apoptosis. The antiapoptotic effect of DHEA is initiated at the plasma membrane, after binding to specific membrane binding sites (mDBS) (*17,18*). DHEA binds to mDBS at nanomolar concentrations and results in the sequential activation of prosurvival kinases such as Src, protein kinase A (PKA), PKCα/β, mitogen-activated or extracellular signal–regulated protein kinases 1 and 2 (MEK1/2), and extracellular signal–regulated kinases 1 and 2 (ERK1/2). The activities of these kinases result in phosphorylation of the transcription factors CREB and NF-κB, leading to positive transcriptional control of genes encoding the antiapoptotic proteins Bcl-2 and Bcl-xL. In parallel, binding of DHEA also induces the activation of phosphoinositide 3-kinase (PI3K) and the kinase Akt, which phosphorylate and deactivate the proapoptotic protein Bad (*19*). Thus, DHEA inhibits the cellular apoptotic machinery at both the transcriptional and posttranslational levels.

Slide 7: The antiapoptotic signaling pathways activated by DHEA and NGF are strikingly similar

Both NGF and DHEA effectively protect neuronal cells against apoptosis. Their antiapoptotic effects are initiated at the plasma membrane, followed by activation of similar cascades of prosurvival kinases and the transcriptional control of antiapoptotic protein Bcl-2 through activation of transcription factors NF-κB and CREB (*20*). These similarities in the signal transduction pathways activated by DHEA and NGF suggested a role for NGF receptors in the antiapoptotic actions of DHEA.

Slide 8: siRNA against TrkA receptors blocks the antiapoptotic effects of DHEA and NGF

To determine whether NGF receptors are indeed involved in the antiapoptotic signaling cascade activated by DHEA, we used PC12 cells, which produce both the TrkA and $p75^{NTR}$ NGF receptors. Transfection of serum-deprived cells with constructs encoding small interfering RNAs (siRNAs) targeting TrkA completely blocked the antiapoptotic activity of NGF, DHEA, and a membrane-impermeable form of DHEA that is chemically linked to bovine serum albumin (BSA-DHEA, upper left panel) (*21*). Transfection of PC12 cells with a construct encoding a short hairpin RNA (shRNA) targeting $p75^{NTR}$ did not affect the antiapoptotic properties of NGF or DHEA, suggesting that TrkA receptors mediate the antiapoptotic actions of these factors. Transfection of serum-deprived PC12 cells with siRNAs directed against the *TrkA* transcript also fully prevented DHEA-induced production of Bcl-2 (lower left panel). The relative abundance of TrkA and $p75^{NTR}$ appears to determine the overall effect of DHEA and NGF on neuronal cell apoptosis: Both NGF and DHEA induced apoptosis of PC12-derived nnr5 cells, which produce only the prodeath receptor $p75^{NTR}$. Inhibiting $p75^{NTR}$ production by shRNA reversed the proapoptotic effect of both NGF and DHEA (right panel). The antiapoptotic effect of NGF and DHEA was effectively restored after transfection of nnr5 cells with a construct containing a *TrkA* cDNA. Asterisks indicate $P < 0.01$ versus control, $n = 4$.

Slide 9: DHEA binds to $HEK293^{TrkA}$ and $HEK293^{p75NTR}$ cell membranes

We previously showed that DHEA rescues neuronal cells from apoptosis by binding to specific sites on the membrane (*18*). This antiapoptotic effect of DHEA is effectively blocked by siRNA against TrkA (*21*). On the basis of these findings, we tested whether DHEA binds to sites that also contain NGF receptors. Saturation binding experiments have shown that radiolabeled DHEA ([^{3}H]-DHEA) binds to membranes isolated from human embryonic kidney 293 (HEK293) cells that have been transfected with cDNAs encoding TrkA ($HEK293^{TrkA}$) or $p75^{NTR}$ ($HEK293^{p75NTR}$), with a dissociation constant (K_D) of 7.4 ± 1.7 nM and 5.6 ± 0.5 nM, respectively [(*21*), left panels]. Transfectants were incubated with either fluorescently tagged, membrane-impermeable DHEA (DHEA-BSA-FITC) or with specific antibodies that recognize TrkA or $p75^{NTR}$. Fluorescence microscopy analysis revealed that DHEA-BSA-FITC associated with the membranes of $HEK293^{TrkA}$ and $HEK293^{p75NTR}$ transfectants (center panels). Transfection of PC12 cells, which produce both TrkA and $p75^{NTR}$, with constructs encoding shRNAs against both of these receptors resulted in a complete loss of [^{3}H]-DHEA specific membrane binding (right panel). Conversely, [^{3}H]-DHEA effectively bound to membranes from PC12 cells that had been transfected with an siRNA unrelated to NGF receptors (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) with a $K_D = 1.06 \pm 0.4$ nM. Taken together, these results support the hypothesis that DHEA binds to both the TrkA and $p75^{NTR}$ NGF receptors.

Slide 10: Immobilized DHEA pulls down recombinant TrkA and $p75^{NTR}$ proteins

Our [^{3}H]-DHEA binding experiments strongly suggested that DHEA physically interacts with NGF receptors. We tested this possibility by performing pull-down experiments using recombinant TrkA and $p75^{NTR}$ proteins and DHEA covalently linked to polyethylene glycol (PEG) amino resin beads. Western blot analysis of precipitates with specific antibodies that recognize TrkA and $p75^{NTR}$ showed that PEG bead–immobilized DHEA (DB) pulled down both recombinant TrkA and $p75^{NTR}$ (*21*). PEG beads only (no DHEA present, B), were found ineffective in precipitating TrkA and $p75^{NTR}$ proteins. Saturation of the recombinant proteins with an excess of soluble DHEA or NGF prevented the ability of DHEA-PEG to precipitate either receptor. These results suggest that DHEA binds to NGF receptors directly.

Slide 11: DHEA induces TrkA and $p75^{NTR}$ signaling

The first step of TrkA activation by NGF is autophosphorylation of TrkA at tyrosine

residues. We tested the ability of DHEA to induce phosphorylation of TrkA in HEK293 cells transfected with a cDNA encoding TrkA (HEK293^{TrkA} cells). HEK293^{TrkA} cells were incubated for 10 or 20 min with 100 nM DHEA or with 100 ng/ml NGF, and lysates from these cells were immunoprecipitated with antibodies against tyrosine (anti-tyrosine) and then subjected to Western blotting with antibodies specific for TrkA. Both NGF and DHEA strongly increased phosphorylation of TrkA (top panel). We also tested the effects of DHEA and NGF in PC12 cells, which produce TrkA endogenously. Untreated or siRNA^{TrkA}-transfected PC12 cells were incubated for 10 min with DHEA or NGF, and cell lysates were analyzed for TrkA phosphorylation by precipitating phosphorylated tyrosine residues with phosphotyrosine antibodies and detecting in the precipitates TrkA protein with TrkA-specific antibodies. Both NGF and DHEA strongly induced phosphorylation of TrkA in control cells, and this effect was diminished in siRNA^{TrkA}-transfected PC12 cells. Next, we tested whether phosphorylation, and therefore activation, of adaptor protein Shc by DHEA is mediated by TrkA receptors by using serum-deprived PC12 cells that were either untreated or transfected with siRNA^{TrkA}. Both untreated and transfected cells were incubated for 10 min with 100 nM DHEA or 100 ng/ml NGF, and cell lysates were subjected to Western blotting with antibodies that recognized total Shc and antibodies specific for the phosphorylated form of Shc. Both DHEA and NGF induced phosphorylation of Shc in untreated PC12 cells, and this effect was abolished in siRNA^{TrkA}-transfected PC12 cells (middle panel). The low-affinity p75^{NTR} receptor modulates signaling through its association with effector proteins, such as TRAF6, which controls the nuclear translocation of the transcription factor NF-κB (*22*). We compared the ability of DHEA and NGF to facilitate the association of p75^{NTR} with TRAF6, performing cotransfection experiments in HEK293 cells transfected with cDNAs encoding FLAG-tagged p75^{NTR} and TRAF6. Transfectants were treated with 100 nM DHEA or with 100 ng/ml NGF; then lysates were immunoprecipitated with anti-FLAG and immunoblotted with p75^{NTR}-specific antibodies. Both DHEA and NGF efficiently promoted the association of p75^{NTR} with TRAF6. Taken together, these data suggest that DHEA binds to and effectively activates NGF receptors and downstream signal transduction pathways.

Slide 12: DHEA can substitute NGF in rescuing sympathetic neurons from apoptosis

We tested the ability of DHEA to substitute for NGF in various ex vivo and in vivo NGF-dependent neuronal systems. Survival of sensory neurons cultured from the superior cervical ganglia (SCG) is totally dependent on NGF and TrkA receptors (*23*). SCG neurons were cultured either in the presence of 100 ng/ml NGF or in the presence of a polyclonal NGF-neutralizing antiserum with or without 100 nM DHEA. Withdrawal of NGF resulted in increased numbers of apoptotic sympathetic neurons (Annexin-V immunostaining, top panel). DHEA effectively compensated for the loss of NGF, decreasing the numbers of apoptotic sympathetic neurons (botton panel), and this effect was blocked by a TrkA-specific inhibitor (*21*). These findings suggest that TrkA receptors mediate, at least in part, the neuroprotective effect of DHEA in NGF-dependent sympathetic neurons.

Slide 13: Does DHEA rescue NGF-dependent neurons in ngf$^{-/-}$ mouse embryos?

We have also tested the ability of DHEA to compensate for loss of NGF in vivo by using *ngf* knockout (*ngf*$^{-/-}$) mice. Heterozygotes (*ngf*$^{-/-}$) mice are normal and fertile; however, they start losing hippocampal cholinergic neurons at 4 to 6 months of age. *ngf*$^{-/-}$ mice die of starvation within the first 1 to 2 weeks of their life because of defects in the NGF-dependent peripheral sympathetic system, which includes sympathetic neurons that innervate the gastrointestinal tract (*22*). *ngf*$^{-/-}$ were crossed to generate embryos homozygous for the *ngf* disruption. Starting on the third day of gestation, pregnant animals were treated daily with a subcutaneous injection of 2 mg DHEA or vehicle only. Embryos were collected at embryonic day 14 (E14) or E18 and genotyped.

Slide 14: DHEA rescues the DRG phenotype in ngf$^{-/-}$ mouse embryos

At E14, *ngf*$^{-/-}$ embryos from *ngf*$^{+/-}$ mothers that were not treated with DHEA showed a decrease in the density of neurofilament 200Kd network in dorsal root ganglia (DRG), the anterior gray horn, and the collaterals that extend from the dorsomedial region of the dorsal funiculus into the dorsal spinal cord (SC). The neurofilament 200Kd network was restored in *ngf*$^{-/-}$ embryos from *ngf*$^{-/-}$ mothers that were treated with DHEA and indistinguishable from *ngf*$^{-/-}$ embryos. We also stained embryonic sections for tyrosine hydroxylase (TH), specifically expressed in peripheral neuronal cells of DRG to effectively demarcate them from SC neurons, which are TH-negative.

Slide 15: DHEA decreases the apoptotic loss of sensory neurons in ngf$^{-/-}$ DRG

DRG from *ngf*$^{-/-}$ embryos showed lower numbers of sensory neurons than DRG from *ngf*$^{-/-}$ embryos because of apoptosis (*23*). Sections of embryos were stained for caspase-3 and subjected to terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) to indicate apoptotic cells and stained for Fluoro jade C to mark all degenerating neurons. *ngf*$^{-/-}$ embryos at E14 showed a dramatic increase in the number of apoptotic and degenerating neurons in the DRG compared with *ngf*$^{-/-}$ embryos. Maternal DHEA treatment decreased the numbers of dying neurons in the *ngf*$^{-/-}$ DRG to levels comparable to those observed in *ngf*$^{-/-}$ embryos (*21*). DHEA treatment significantly decreased the numbers of TUNEL-positive, apoptotic neurons in *ngf*$^{-/-}$ embryos to levels observed in *ngf*$^{-/-}$ embryos.

Slide 16: Neurosteroid DHEA signaling through NGF receptors

Our findings suggest that the neurosteroid DHEA protects neuronal cells from apoptosis, mimicking some of the neuroprotective effects of NGF. DHEA binds with high affinity (K_D at nanomolar levels) to the TrkA and p75^{NTR} NGF receptors. Binding of DHEA to TrkA results in TrkA autophosphorylation and the initiation of a downstream cascade of prosurvival kinases, such as the Shc-PI3K-Akt and Src-MEK-ERK signaling axes. Binding of DHEA to the prodeath receptor p75^{NTR} affects the association of p75^{NTR} with its effectors TRAF6, RIP2, and RhoGDI. The relative abundance of prosurvival TrkA and prodeath p75^{NTR} receptors present in neuronal cells is thought to determine whether their fate is apoptosis or survival.

Slide 17: Neurosteroid DHEA—A "prehistoric" neurotrophin?

DHEA and the enzyme CYP17, which is required for DHEA biosynthesis, appeared early in evolutionary history. Both CYP17 and a TrkA homolog (AmphiTrk) are present in the invertebrate cephalochordate amphioxus (*24*), where AmphiTrk can mediate NGF signaling (*25*). It is of note that neurotrophins emerged with the appearance of vertebrates 530 to 550 million years ago, coinciding with the development of a complex neural system (*26*). Invertebrate cephalochordata like amphioxus appeared around

600 million years ago, before the emergence of vertebrates. On the basis of these observations, we hypothesize that DHEA could represent the "ancestral" neurotrophic factor, directing the development of anatomically simpler nervous systems prior to the emergence of the vertebrates. Later in evolution, when the complexity of nervous system increased, peptidic neurotrophins would have evolved to more effectively support and more precisely control brain development.

Slide 18: Synthetic DHEA 17-spiro analogs as NGF receptor agonists

The lack of effective treatments for devastating neurodegenerative diseases has stimulated great interest in the development of neuroprotective agents that can prevent or reverse progressive loss of neural function. There is a large unmet need for the discovery of new compounds that target pathways controlling apoptosis, survival, or neurogenesis to protect or repair damaged neurons. Several international research groups are actively working on the development of BBB-permeable small-molecule agonists for neurotrophin receptors for potential therapeutic applications in neurodegeneration and brain trauma. We have shown that the neurosteroid DHEA protects various neuronal cell types by binding to neurotrophin receptors and repressing the apoptotic machinery. This property of DHEA makes it a candidate for potential use in the treatment of neurodegeneration. However, DHEA is metabolized in vivo to estrogens, androgens, and related metabolites that affect the endocrine system, thus altering the hormonal microenvironment in the brain. Therefore, the long-term use of DHEA as a potential treatment or prophylactic in neurodegeneration is problematic, particularly in patients with genetic predisposition to hormone-dependent tumors (breast, endometrium, ovaries, prostate). Additionally, DHEA is a "sticky" molecule that interacts with many neurotransmitter and steroid hormone receptors (*16*). To overcome these effects of DHEA, we synthesized DHEA analogs with modifications at positions C3 and C17 and evaluated these for neuroprotective activity (*27*). The most potent compounds were the spiro-epoxy derivatives 17-β-spiro[5-androstene-17,2'-oxiran]-3β-ol, (20S)-3β,21-dihydroxy-17β, 20-epoxy-5-pregnene, and (20R)-3β,21-dihydroxy-17α,20-epoxy-5-pregnene with median inhibitory concentration (IC_{50}) values of 0.19 ± 0.01, 99.0 ± 4.6, and 6.4 ± 0.3 nM, respectively. These synthetic 17-spiro derivatives of DHEA bind to TrkA and $p75^{NTR}$ receptors to induce TrkA phosphorylation and $p75^{NTR}$ dissociation from its effector protein, RhoGDI. These compounds are not readily metabolized to yield products with estrogenic or androgenic activity. On the basis of these properties, we named these synthetic compounds "neurosteroidal microneurotrophins." We propose that these novel, nontoxic, synthetic 17-spiro neurosteroid analogs (WO2008155534A2 and WO2011/030116A1, which are owned by the University of Crete–related Bionature E.A. Ltd.) may serve as lead molecules for developing CNS-bioavailable small-molecule agonists or antagonists of neurotrophin receptors for the treatment of neurodegenerative conditions.

Slide 19: Microneurotrophin selectivity

In contrast to DHEA, our synthetic 17-spiro derivatives showed no affinity for ERα, ERβ, androgen receptors, TrkB, or TrkC (*27*). Additionally, these synthetic microneurotrophins lack estrogenic or androgenic activity, in contrast to DHEA, which is converted into estrogens and androgens in vivo (*27*).

Slide 20: Neuroprotective and neurogenic effects of microneurotrophins in vivo

We are testing the efficacy of these synthetic microneurotrophins to protect various NGF receptor–bearing and NGF-responsive neurons in vivo by using various animal models of neurodegenerative conditions. Our unpublished preliminary data suggest the following: Microneurotrophins interact with NGF receptors and induce the phosphorylation of TrkA and the dissociation of RhoGDI from $p75^{NTR}$ receptors. They also mimic DHEA in rescuing loss of NGF-dependent embryonic sensory neurons of $ngf^{-/-}$ mice. Synthetic microneurotrophins prevent and suppress the development of EAE in mice by acting on regulatory T lymphocytes and inhibiting the neurotoxic Th17 response. They protect the retina in an ex vivo model of chemical ischemia, in the in vivo model of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) excitotoxicity (*28*), and in the streptozotocin (STZ)-induced model of diabetic retinopathy. Lastly, synthetic microneurotrophins exert neurogenic effects in vivo and in vitro. They increase the number of BrdU-positive neurons in the hippocampus of adult mice and induce self-renewal but no differentiation of embryonic neural stem cells.

Slide 21: Conclusions

Many experimental and clinical findings both in animals and in humans strongly support the hypothesis that the hormonal microenvironment within the brain affects neuronal cell survival and function. We have shown that the neurosteroid DHEA protects various neuronal cell types by binding to neurotrophin and NGF receptors to activate prosurvival signaling. Recently, we have synthesized 17-spiro derivatives of DHEA (microneurotrophins), which exert potent neuroprotective actions both in vitro and in vivo, through their interaction with and activation of NGF receptors. These DHEA derivatives lack estrogenic or androgenic actions and may serve as lead molecules for the development of CNS-bioavailable small molecules with neurotrophin receptor agonist or antagonist activities with potential applications in the treatment of neurodegenerative diseases. These studies were funded by Bionature E.A. Ltd., which was cofounded by A. Gravanis. Use of the synthetic neurosteroids we have developed requires a materials transfer agreement (MTA). Bionature E.A. Ltd. has patent applications pending for the synthetic neurosteroids (WO 2008/155534A2 and WO 2011/030116A1) described in this Presentation. C. Neophytou is the managing director of Emergo (Cyprus) Ltd., which is an indirect shareholder in Bionature E.A. Ltd.

Editor's Note: This contribution is not intended to be equivalent to an original research paper. Note, in particular, that the text and associated slides have not been peer-reviewed.

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