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Vascular Endothelial Growth Factor Regulates Excitatory Synaptic Transmission in Hippocampal Neurons

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Abstract

Vascular endothelial growth factor (VEGF), in addition to its essential role in the processes of vascularization and angiogenesis, exerts direct effects on neural cells in the central nervous system. There is abundant evidence indicating that VEGF protects neurons against cell death induced by a variety of insults, including hypoxia/ischemia and seizures. Recent work has demonstrated the expression of VEGF and its receptors in neurons and has revealed that VEGF can act as a neurotrophic factor to regulate neurogenesis and mediate the effects of enriched environment and antidepressants on hippocampal plasticity. Current studies from our laboratory and those of others have found that VEGF can activate divergent signaling components to regulate excitatory synaptic transmission in hippocampal neurons. Here we present an overview on current understanding of cellular and molecular mechanisms by which VEGF signaling is regulated in neural cells and discuss the recent advances in the understanding of how VEGF signaling regulates excitatory synaptic transmission in hippocampal neurons. The role for VEGF in regulating synaptic plasticity will be also discussed in the article.

Keywords: VEGF; Glutamate; Synaptic plasticity; Hippocampus; Neuron

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Vascular endothelial growth factor (VEGF) is a family of basic, heparin-binding, homodimeric glycoproteins that were originally found in the endothelial cells (1) with high potency of angiogenesis (2), vascular permeability (3) and endothelial proliferation (4). The VEGF family includes VEGF-A, placental-derived growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D in mammals and two exogenous VEGF subtypes, virus genome-encoded VEGF (VEGF-E) and snake venom-derived VEGF (VEGF-F) (5). VEGF-A is the protypical member of the VEGF family. Through alternative exon splicing, human VEGF-A gene gives rise to at least six different transcripts, encoding isoforms of 121 (VEGF-A_{120} in mouse), 145, 165 (VEGF-A_{164} in mouse), 183, 189 and 206 amino acid residues (6, 7). $VEGF_{121}$ and $VEGF_{165}$ are secreted in soluble form, while $VEGF_{145}$, $VEGF_{189}$ and $VEGF_{206}$ are bound to cell-surface heparin-containing proteoglycans in the extracellular matrix. VEGF exerts its biological functions via activation of the protein tyrosine kinase receptors, VEGF receptor 1 (VEGFR-1, Flt-1) and VEGFR-2 (KDR/Flk-1), which differ considerably in signaling properties (8). VEGFR-1 and VEGFR-2 are structurally similar, consisting of an extracellular ligand-binding domain with seven immunoglobulin-like domains in the extracellular domain, a single transmembrane region and an intracellular consensus tyrosine kinase domain that is interrupted by a kinase-insert domain (9). Binding by VEGF triggers a rapid tyrosine phosphorylation of the receptors, which in turn allows the receptors to associate with various effector molecules such as the phosphatidylinositol 3-kinase (PI3K), Shc, Grb2, and the phosphatases SHP-1 and SHP-2. In addition, VEGF receptor activation can trigger

Table 1. VEGF exerts direct effects on excitatory synaptic transmission

VEGF subtype	Preparation	Effect	Signaling pathway	Reference
$VEGF_{165}$ (200 ng/ml)	Rat hippocampal slices (CA1, CA3 and DG)	(1) Decrease PS (2) Suppress spontaneous burst discharges	ND	18
$VEGF_{165}$ (200 ng/ml)	Rat brainstem slices (hypoglossal motor neuron)	(1) Decrease evoked potential	ND	19
$VEGF_{165}$ (10 ng/ml)	Rat hippocampal slices (CA1)	(1) Increase fEPSP slope (2) Enhance LTP induction	VEGFR2/ CaMKII/ERK	20
$VEGF_{164}$ (1 ng/ml)	Rat hippocampal neuron culture	(1) Increase mEPSC Frequency	VEGFR2	21

fEPSP, field excitatory postsynaptic potential; PS, population spike; DG, dentate gyrus; LTP, long-term potentiation; mEPSC, miniature excitatory postsynaptic current; CaMKII, Ca2+/calmodulin-dependent protein kinase II; ERK, extracellular signal-regulated kinase; ND, not determined.

activation of the mitogen-activated protein kinase (MAPK) signaling cascade via Raf stimulation leading to gene expression and cell proliferation, activation of PI3K leading to Akt activation and cell survival, activation of phospholipase Cγ and protein kinase C leading to cell proliferation, vasopermeability and angiogenesis (8).

Beyond its well-established effects on vasculature, recent findings reveal that VEGF has multiple neurotrophic effects (10, 11). Studies involving the central nervous system (CNS) have demonstrated localization of VEGF and its receptors on neurons and astrocytes (12, 13). It has also been reported that VEGF induces neuronal outgrowth (14) and provides neuroprotection, particularly after ischemia or spinal injury (15, 16). Furthermore, VEGF has been shown to act as a neurotrophic factor to mediate the effect of enriched environment on neurogenesis and cognition (17). In addition, VEGF has been implicated in the regulation of excitatory synaptic transmission (Table 1). For instance, extracellular field potential recordings on rat hippocampal slices showed that application of exogenous $VEGF_{165}$ (200 ng/ml) decreased the evoked responses of hippocampal neurons to synaptic stimulation in each of the major glutamatergic pathways of the trisynaptic circuitry (18). In addition, $VEGF_{165}$ suppressed spontaneous discharges in hippocampal slices from pilocarpine-treated rats but has little effect on bicuculline-induced spontaneous discharges in hippocampal slices from control rats (18). McCloskey et al (19) also found that the expression of VEGF in hypoglossal motor neurons increased after seizure and application of exogenous $VEGF_{165}$ reduced depolarizing input to hypoglossal motor neurons in a brainstem slice preparation without an apparent influence on passive and active membrane properties. In contrast, a more recent study showed that a brief bath application of $VEGF_{165}$ (10 ng/ml) elicited a rapid and persistent enhancement of synaptic transmission in the hippocampal CA1 region through the activation of either Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) or extracellular signal-regulated protein kinase (ERK) signaling (20). In our recent work, using both pharmacological and genetic approaches, we have extended these findings by showing that hypoxia-inducible factor-1α (HIF-1α) accumulation can enhance excitatory synaptic transmission in hippocampal neuron cultures by regulating production of VEGF (21). Our results also indicate that treatment of hippocampal neuron cultures with $VEGF_{164}$ (1 ng/ ml) for 1 or 12 h elicits a significant increase in the frequency but not the amplitude of miniature excitatory postsynaptic currents (mEPSCs) and that this enhancement is blocked by the selective VEGFR-2 inhibitors or knockdown of VEGFR-2 expression by shRNA. The observed increase in mEPSC frequency by VEGF treatment is most likely attributable to an enhancement of presynaptic release probability. The lack of effect of VEGF on mEPSC kinetics also implies that its action on glutamatergic transmission is not mediated by a change in postsynaptic responsiveness to glutamate. The reasons for these discrepancies remain unclear. They may be attributable to use of different doses and time scales

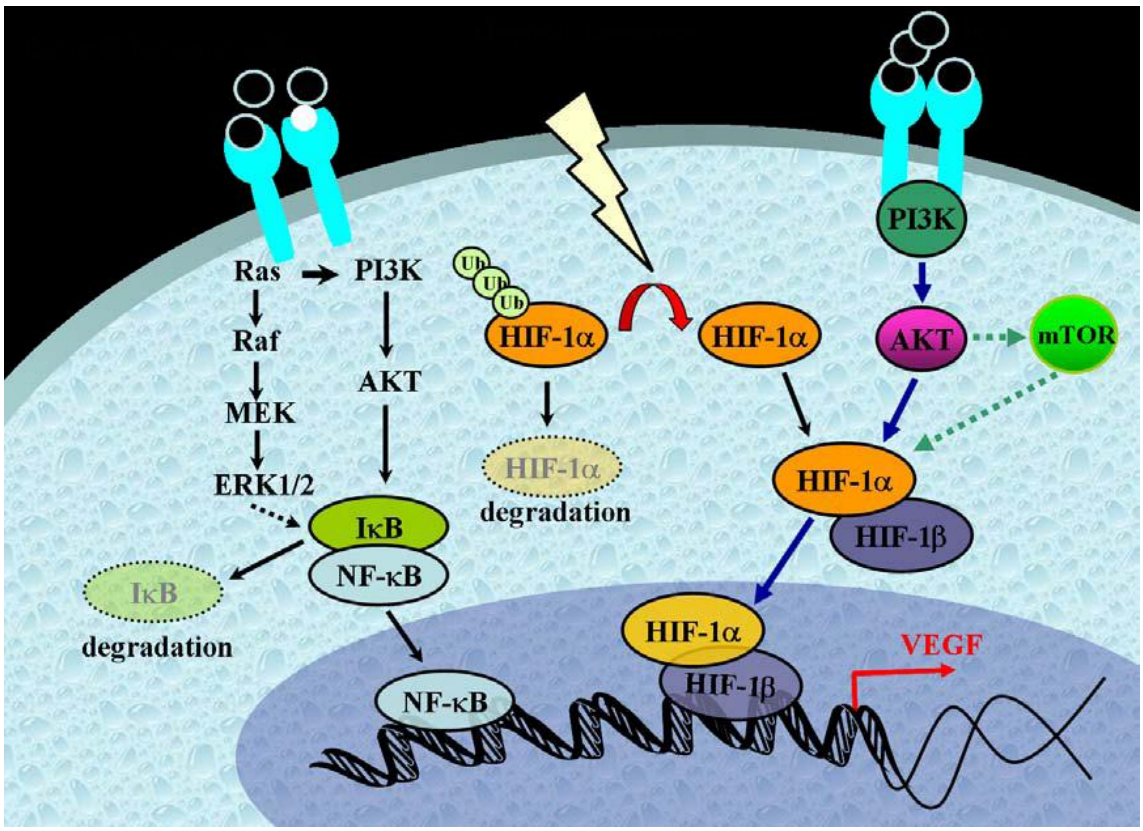

Figure 1. Proposed models for the regulation of VEGF expression in neurons. Binding of IGF-1 to extracellular IGF-1 receptors triggers activation of PI3K/Akt/mTOR signaling pathway and leads to increased HIF-1α protein expression. HIF-1α then dimerizes with HIF-1β to form the HIF-1 complex, which in turn translocates into the nucleus and binds to hypoxia response elements of the VEGF genes to activate transcription. Ubiquitin-dependent HIF-1α degradation is inhibited under hypoxic condition, leading to accumulation and translocation from cytoplasm to nucleus to induce transcriptional activation of the VEGF genes. The NF-κB activation pathway may play an adjuvant role in positive regulation of VEGF transcription. Growth factors or cytokines may activate PI3K/Akt or Ras/Raf/MEK/ERK signaling cascades, resulting in increased NF-κB activation that upregulates VEGF gene transcription. Activated Akt may increase IκB phosphorylation and promotes its degradation, which in turn increases the release of NF-κB from IκB and allows the activated NF-κB to enter the nucleus and ultimately promote VEGF gene transcription.

of VEGF challenges, leading to activation of different cellular events that vary in mode of action. Further studies would be necessary to explore this conflict issue.

VEGF expression has been localized to subpopulations of neurons in the developing and mature CNS (22-24). VEGF gene and protein expression is increased in both glial cells and neurons of the ischemic brain (25). Moreover, use of an *in vitro* culture model demonstrates that neuronal VEGF expression is hypoxia-inducible (26). Hypoxia-inducible factor-1 (HIF-1), consisting of HIF-1α and HIF-1β subunit, is a dominant transcriptional factor to regulate VEGF expression in the neural cells. HIF-1α is primarily regulated at the level of protein stability. HIF-1α protein is rapidly degraded by the ubiquitin-proteosome system under normoxic conditions (27, 28). HIF-1α degradation is inhibited under hypoxic conditions, leading to accumulation and translocation from cytoplasm to nucleus, where it dimerizes with HIF-1β to form the transcriptionally active HIF-1 complex (29). The activated HIF-1 complex binds to specific hypoxia response elements (HREs) of target genes and associates with transcriptional coactivators to induce gene expression (30). While HIF-1α is regulated mainly by oxygen tension, it is noteworthy that HIF-1α is also regulated by oxygen-independent mechanisms. For example, HIF-1α has been shown to be activated in response to insulin-like growth factor-1 (IGF-1) in cancer cells and epithelial cell lines, leading to VEGF transcription (31, 32). Previous study has also reported that activation of HIF-1α in the CNS is involved in the mechanism by which IGF-1 promotes cell survival after cerebral ischemia (33). Our recent study has indicated that IGF-1 increases expression of HIF-1α through activation of the PI3K/Akt/mammalian

target of the rapamycin (mTOR) signaling pathway and leads to increased VEGF secretion in hippocampal culture neurons (Figure 1) (21). Another transcription factor that may play a role in positive regulation of VEGF transcription is NF-κB. Many growth factors and cytokines have been shown to induce transcription of the VEGF gene through NF-κB binding to the VEGF promoter in cancer cells (34) and endothelial cells (35). However, the role of NF-κB in transcriptional regulation of VEGF in neurons remains to be elucidated.

Despite the clear significance of the role of VEGF in regulating excitatory synaptic transmission, a recent study showed that VEGF application prior to high-frequency stimulation of hippocampal neurons increases the induction of long-term potentiation (20), a putative cellular mechanism underlying learning and memory. In addition, there is strong evidence that neuronal VEGF has an additional role in linking hippocampal activity with neurogenesis, learning and memory (17). Consistent with idea that VEGF promotes synaptic plasticity and boosts memory, transgenic mice engineered to overexpress VEGF or virus-mediated VEGF gene transfer rats have been shown to perform better in associative and spatial memory tasks (17, 36). In addition, intrahippocampal administration of VEGFR-2 antagonists following spatial training impairs long-term memory (37). Moreover, pharmacological stabilization of HIF-1α expression by application of prolyl-hydroxylase inhibitors results in the elevation of VEGF concentration in the hippocampus and contributes to a long-lasting improvement of hippocampus-dependent memory performance (38). Taken together, these findings support a role for VEGF-mediated signaling in long-term memory. VEGF expression in the hippocampus is also considered important for mediating some of the behavioral effects of antidepressants (39).

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Conflicts of Interest

No potential conflicts of interest to disclose.

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