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MULTI-AUTHOR REVIEW

Vascular growth factors in neuropsychiatry

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Abstract Recent advances in understanding the cellular and molecular basis of psychiatric illnesses have shed light on the important role played by trophic factors in modulating functional parameters associated with disease causality and drug action. Disease mechanisms are now thought to involve multiple cell types, including neurons and endothelial cells. These functionally distinct but interactively coupled cell types engage in cellular cross talk via shared and common signaling molecules. Dysregulation in their cellular signaling pathways influences brain function and alters behavioral performance. Multifunctional trophic factors such as VEGF and EPO that possess both neurotrophic and angiogenic actions are of particular interest due to their ability to rescue structural and plasticity deficits in neurons and vasculature. Obtaining insight into the behavioral, cellular and molecular actions of multi-functional trophic factors has the potential to open new and transformative therapeutic approaches.

Keywords Vascular endothelial growth factors · Erythropoietin · Trophic factor signaling · Neurovascular

Introduction

A substantial body of scientific evidence drawn from clinical, pre-clinical and basic research has demonstrated that neuronal atrophy and cell death are involved in psychiatric illnesses. The importance of neuronal plasticity and cellular resilience has been particularly highlighted in depression studies. Examination of the cellular response to stress has documented a reduction in BDNF levels and dysregulation of neurotrophic signaling, which could be reversed by antidepressant administration. These findings led to the formulation of the neurotrophic factor hypothesis of depression and antidepressant action [1]. Gene expression studies aimed at examining the potential involvement of other classes of trophic factors in antidepressant action revealed several additional trophic molecules and suggested that both vascular and neuronal factors are likely to be involved [2]. Subsequent studies validated the role played by multifunctional growth factors such as vascular endothelial growth factors (VEGF-A through D), erythropoietin (EPO) and basic fibroblast growth factor (bFGF) have both angiogenic and neurotrophic effects.

A clinical observation that has been known for at least 3 decades is that a bi-directional relationship exists between vascular disease and depression [3–5]. Depression is an independent risk factor for the occurrence of cerebrovascular and cardiovascular events, and conversely, vascular disease elevates rates of depression [6, 7]. In fact, depression is the predominant psychiatric disorder associated with cerebrovascular diseases [8]. A vascular hypothesis of depression that has helped guide clinical research is based primarily on the high incidence of cerebrovascular lesions observed in imaging studies of late-life depressed patients [9]. However, recent clinical imaging studies have reported impaired cerebrovascular perfusion in depressed middle-age patients, which was normalized by antidepressant treatment [10, 11]. This suggests that the association between vascular dysfunction and depression could be mechanistically coupled. Interestingly, stress-based rodent behavioral models of depression have shown a reduction in hippocampal microvessel density, providing additional support for a

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link between depression and the cerebral vasculature [12, 13]. The potential involvement of neuronal and vascular deficits in depression points toward vascular growth factors as candidate molecules worthy of investigation. This review will focus on the role of VEGF-A (the prototypic vascular growth factor) and EPO in depression and treatment response.

Role of vascular growth factors in brain function

Vascular growth factors serve key roles in the brain by influencing both neuronal and vascular function. VEGF is expressed at high levels and performs critical functions during development, guiding neuronal migration, blood vessel growth and branching [14]. Loss of VEGF in the developing brain impairs vascularization and causes neuronal apoptosis, hippocampal atrophy and microcephaly [15]. Transgenic mice with reduced levels of VEGF have reduced brain blood circulation, which could predispose them to neurodegenerative defects as a result of deficits in oxygen and metabolic support [16]. In addition to indirectly influencing neurons via its vascular actions, VEGF also has direct neuronal effects, enhancing survival and neurite outgrowth in cultured neurons and elevating neurogenesis when administered intracerebroventricularly (ICV) [17–21].

Conditional deletion of EPO in the brain decreases neurogenesis, particularly in the subventricular zone, resulting in smaller brain size [22]. These mice also exhibited atrophy of the choroid plexus, which constitutes the major blood-CSF barrier and is also the site where CSF and several neuroprotective polypeptides are produced. Lack of EPO receptor signaling also affects brain development by elevating apoptosis and limiting proliferation of neural progenitor cells [23]. In contrast, systemic delivery of EPO increases neurogenesis in the hippocampal subgranular zone [24, 25]. The mitogenic actions of EPO and VEGF on endothelial cells and neurons are indispensable for brain growth, development and function. Dysregulation in their level of expression adversely affects brain function and homeostasis.

Trophic factor-mediated neuroprotection

In addition to their mitogenic effects on neural cells, trophic factors possess neuroprotective actions that preserve neuronal function by opposing the harmful effects of cellular insults. Both VEGF and EPO are strongly induced by hypoxia because of the presence of transcription factor-binding sites for the hypoxia inducible factor (HIF) in their gene promoter regions [26, 27]. Both factors exhibit robust protection against neuronal damage associated with hypoxia and ischemia. Systemic administration of EPO has been shown to exhibit anti-apoptotic activity in protecting neurons from cell death after cerebral ischemia [28]. In vitro studies indicate that EPO is also protective against neuronal death arising from glutamate toxicity [29]. The release of excitotoxins, NMDA-induced apoptosis and free radical damage caused by proinflammatory cytokines was effectively blocked by pretreatment with EPO in cellular models of neurodegeneration [30]. The EPO receptor (EPOR) is expressed in adult rat dopaminergic neurons [31], and viral overexpression of EPO reverses the degeneration of dopamine neurons in rodent models of Parkinson's disease [32, 33].

Endogenous VEGF induced by epileptic seizures was shown to be protective against neuronal loss in the hippocampus as selective blockade of VEGF signaling by infusion of a soluble receptor abolished neuroprotection with a two-fold increase in cell death [34]. A mild preconditioning exposure to hypoxia, which induces VEGF, is neuroprotective against subsequent ischemic insults, while also elevating neurogenesis and producing antidepressant-like effects [35]. VEGF plays a central role in mediating the neuroprotective mechanism of hypoxic preconditioning via its signaling actions [36]. An acute neuroprotective effect of VEGF accompanied by improvement in neurological parameters, reduction in infarct size and elevated angiogenesis was noted after cerebral ischemia [37]. The timing of VEGF administration significantly influences its efficacy in ischemia models, as early post-ischemic (within 1 h) delivery increases BBB leakage because of its inherent vascular permeabilization property, whereas later (48 h) administration reduces neuronal deficits and improves functional recovery [38].

In examining the neuroprotective activities of VEGF and EPO in conditions of oxygen and glucose deprivation, it can be noted that there is considerable overlap in their actions. This functional similarity could be mediated by signaling crosstalk between these trophic factors and integration of signaling at tumor necrosis factor receptor I (TNFRI), which sensitizes injured neurons and enables them to efficiently utilize EPO and VEGF for survival and restoration of function [39]. The potency of these molecules to protect and rescue neuronal function could be due to their ability to simultaneously influence both neurons and endothelial cells.

Integrating the neurovascular unit in neuropsychiatric disorders

There has been a tendency in the investigation of neuropsychiatric disorders to focus primarily on neurons and pay little attention to the other cell types in the brain. It is important to recognize that neurons do not function in a network consisting only of other neurons but are intricately linked in a dynamic neurovascular network with other cell types including endothelial cells, astrocytes and perivascular cells (Fig. 1). The mammalian brain is highly vascularized,

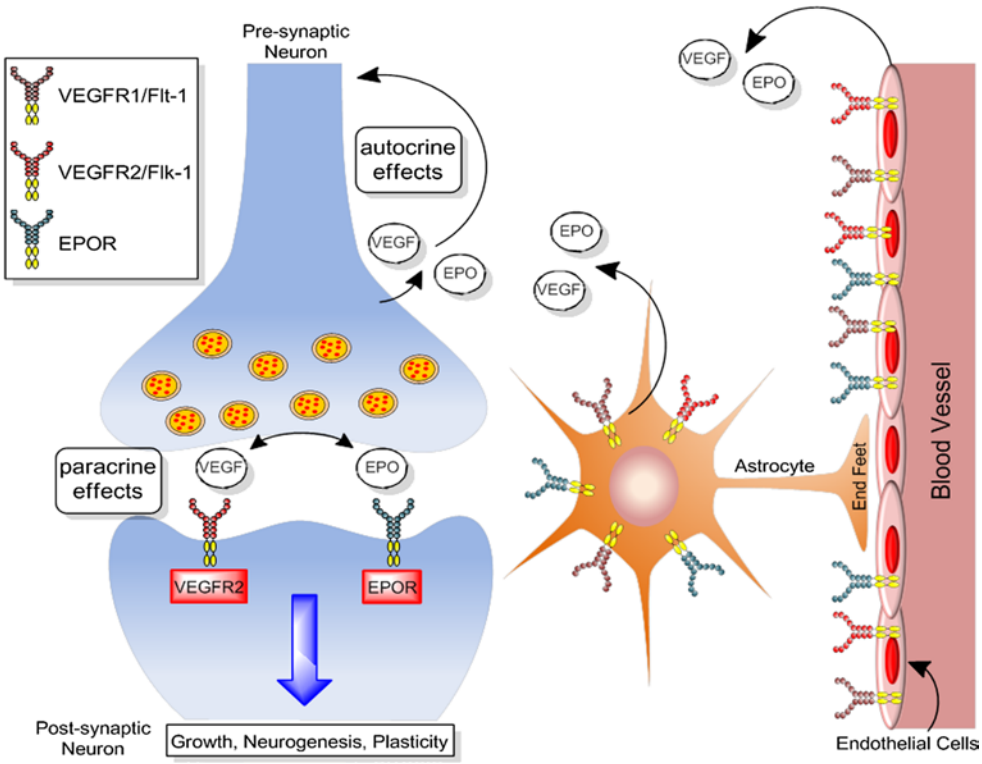

Fig. 1 Model of the neurovascular niche. Neurons, vasculature and astrocytes exist in a close anatomical and functional relationship that governs critical aspects of brain function and modulates blood flow in response to metabolic demands. Communication between these cell types occurs by the action of signaling molecules that facilitate cross talk and adaptive cellular regulation

and neurons are dependent on the cerebral vasculature for oxygen and metabolic and trophic support. Therefore, even transient disruptions in brain blood flow adversely impact brain function. Furthermore, recent developments in the etiology of neuropsychiatric illnesses, such as Alzheimer's disease, where mechanistic insight into vascular involvement is being obtained [40], and the failure of neuron centric approaches to treat stroke [41] indicate that it is necessary to develop a neurovascular framework to improve our understanding of neuropsychiatric disorders and develop efficacious therapies. With accumulating evidence suggesting that vascular function is impaired in depression [10, 42], it will be beneficial to consider therapeutic approaches that can promote both neuronal and vascular health.

Expression and regulation of EPO and VEGF in the neurovascular unit

Neurons, vasculature and astrocytes exist in a close anatomical and functional relationship that governs critical aspects of brain function and modulate blood flow in response to metabolic demands (Fig. 1). Communication between these cell types occurs by the action of signaling molecules that facilitate crosstalk and adaptive cellular regulation. VEGF and EPO are expressed in the three major cell types, which comprise the neurovascular unit, and it is therefore useful to examine cell-specific activity and regulation.

Neuronal

The expression of EPO and EPOR mRNA was shown in mouse brain, including specific binding sites of radiolabeled EPO [43]. EPO mRNA was detected in the hippocampus, amygdala and cortex of biopsied human brain tissue from epilepsy patients and several regions of monkey brain [44]. Neuronal expression of EPO was demonstrated using single-cell PCR in dissociated mouse cortical neurons and was elevated after systemic administration of cobalt chloride or hypoxia via a mechanism that includes transcriptional activation of hypoxia-inducible factor 1 (HIF-1) [45]. Examining EPO at the transcript level has been suggested to be more conclusive than immunohistochemical analysis because of concerns about the specificity of some commercial antibodies [45]. EPOR is expressed at higher levels in neuronal progenitors than mature neurons. Expression in neural cells, even at low levels, appears to have protective functions as cells that lack EPOR are vulnerable to hypoxia and glutamate toxicity [46]. During development, EPO facilitates the generation of neuronal progenitors from neural stem cells by functioning as an autocrine–paracrine factor [47].

Vascular endothelial growth factor is also highly expressed in developing and mature CNS tissue [48, 49]. Neuronal VEGF plays a key role in brain angiogenesis [50] and neuronal patterning during development [51]. At birth, VEGF production from neurons switches to astrocytes causing neuronal VEGF to be reduced to very low levels in the

mature neurons once angiogenesis has ceased [52]. However, VEGF expression is strongly upregulated in both neurons and glia following hypoxic and excitotoxic events [53, 54]. VEGF binds to three main subtypes of receptor tyrosine kinases—fetal liver kinase (Flk-1 or VEGFR-2), fms-like tyrosine kinase 1 (Flt-1, VEGFR-1) and fms-like tyrosine kinase 4 (Flt-4 or VEGFR-3)—as well as to a family of co-receptors called neuropilins (NRP). In adult brain, Flt-1 is predominately expressed by endothelial cells and astrocytes, whereas Flk-1 is expressed by mature neurons, neuronal progenitors and endothelial cells [55]. Interestingly, Flk-1 is more highly expressed among neuronal progenitors than mature neurons, highlighting the important role of VEGF/Flk-1 signaling in regulating neurogenesis [21, 37, 56, 57]. Of the multiple VEGF receptors found in CNS tissue, Flk-1 appears to mediate almost all of the known cellular responses to VEGF. However, expression of the VEGF receptor Flt-1, which is not normally observed in mature neurons, can be strongly upregulated after CNS ischemia, suggesting a role in brain injury [58–60].

Endothelial

It is well known that VEGF exerts potent effects on the survival and proliferation of endothelial cells, and it is the predominant cellular function that has been extensively investigated (Fig. 1). Although the endothelial actions of EPO are not as widely studied as VEGF, the angiogenic potential of EPO has been reported to be similar [61]. However, it is likely that they differ in their mechanism of action as EPO lacks the vascular permeabilization property of VEGF. Two forms of EPOR, the membrane spanning receptor and an intron-containing soluble form, were observed in rat and mouse endothelial cells, eliciting dose-dependent mitogenic actions upon treatment with EPO [62]. The endothelial and angiogenic functions of EPO can also provide an indirect neuroprotective effect by inducing the secretion of trophic factors from the vasculature [57]. EPO is known to cause vasodilation of capillaries by elevating endothelial nitric oxide synthase (eNOS) and production of nitric oxide [63]. It is interesting to note that the EPO-induced elevation of nitric oxide requires interaction between heterodimeric EPOR and beta-common receptor with the VEGFR-2 receptor, indicating interactive coupling of EPO–VEGF signal transduction in endothelial cells [64].

In endothelial cells, binding of VEGF triggers rapid phosphorylation of VEGFR-2, which in turn allows the receptor to be associated with various effector molecules, including phosphatidylinositol 3-kinase (PI3K)–Akt, Raf–MAPK and phospholipase Cγ-protein kinase C (PLCγ–PKC). Important endothelial functions, including proliferation (via activation of the Raf–MAPK signaling cascade), survival (via activation of PI3K–Akt) and vasopermeability and angiogenesis (via PLCγ–PKC), have been shown to be regulated through VEGF/Flk-1 signaling [65]. Although the effects of EPO stimulation in endothelial cells have not been well described, in neurons, EPOR has been shown to transduce signaling via the Jak 2 and NF-κB pathways [23, 30]. However, the induction of other neurotrophic factors, such as BDNF, GDNF and neuritin by EPOR [66, 67], is likely to involve the MEK–Erk and PI3K–Akt signaling cascades [66].

Astrocytic

The strongest induction of EPO and VEGF has been observed in astrocytes following exposure to hypoxic conditions [68]. Cultured astrocytes exposed to low oxygen levels (1 %) exhibit a 100-fold elevation in EPO [44]. Breathing a gas mixture that contained 8 % oxygen (20 % is normoxic) elevated EPO levels in monkey brain threefold, and this increased to 20-fold when exposed to 0.1 % carbon monoxide, demonstrating that the degree of EPO induction in astrocytes is dependent on the severity of hypoxia [44, 69]. This is similar to the hypoxia-induced elevation of VEGF that is acutely dependent on oxygen levels. VEGF contributes to new blood vessel formation at 10 % oxygen, but at levels below 8 %, blood vessels become leaky, suggesting thresholds for beneficial and detrimental effects [70]. The high levels of inducible EPO expression in astrocytes have been suggested to serve paracrine functions by acting on neurons and protecting against neuronal damage [69, 71]. VEGF and EPO can also act on astrocytes in an autocrine manner to enhance astrocyte proliferation and also promote maturation of immature oligodendrocytes (Fig. 1) [51, 72]. Moreover, expression of VEGF receptors Flt-1 and Flk-1 is strongly expressed in the astroglial endfeet that closely surround the nearby endothelium. Astroglia treated with EPO were protected against apoptotic cell death caused by exposure to cellular stressors [73]. EPO prevents astrocytes from swelling-induced injury during conditions of brain edema by regulating water permeability via the aquaporin 4 water channel [74].

Role of VEGF and EPO in depression and stress responses

Increasing evidence suggests that vascular dysfunction plays a critical role in the etiology of depression [75–78]. For example, decreased cerebral blood flow and metabolism in the hippocampus and prefrontal cortices are frequently observed in patients with depression [79–83]. Moreover, decreased levels of circulating bone-marrow derived endothelial progenitor cells have been reported in patients with depression compared to healthy controls [84, 85]. Treatment with antidepressants can influence

endothelial function [86], and several drugs used to manage vascular disease (e.g., calcium-channel blockers, statins, angiotensin-converting enzyme inhibitors) were able to reduce depressive symptoms in preclinical studies [87–91]. Because VEGF and EPO act as key signaling molecules in the CNS, being involved in neuroprotection, neuronal survival and synaptic plasticity, altered expression and/or function of these vascular factors could contribute to the cellular and morphological changes observed in animal models of depression and in patients with depression. Below we will summarize the clinical literature highlighting the relationship between VEGF and EPO-signaling in depressive illness and antidepressant action.

VEGF in depression

Over the last few years, the relationship between VEGF and stress-related disorders, including depression, in clinical populations, has been extensively examined. For example, VEGF mRNA expression in peripheral leukocytes was found to be elevated in patients with depression compared to healthy controls, and this difference was normalized after 8-weeks of antidepressant treatment and was associated with clinical improvement [92]. In addition, increased serum levels of VEGF have been reported in depressed patients with comorbid borderline personality disorder [93] and bipolar disorder [94]. On the contrary, other groups have noted significant decreases in peripheral VEGF/Flk1 levels with depression [85, 95–97], and a recent study showed higher plasma VEGF levels in 16 major depressive disorder patients who were in partial or full remission compared to controls [98]. Moreover, preclinical studies (described below) have typically observed decreases in brain VEGF and Flk-1 expression with exposure to chronic stress.

Although it is not clear what might account for the divergence across these studies, clinical factors such as such as age, gender, treatment history, depressive episodes (recurrent vs. acute), comorbidity with other health conditions (e.g., heart disease) and small patient group sizes could be important contributors. Another possibility is that there may be differences between blood and brain levels of VEGF in patients with depression. Indeed, recent data indicate that while serum VEGF levels remain unchanged, VEGF levels in the hippocampus and frontal cortex were significantly lower in a genetic rat model of depression [99]. Nonetheless, further work is necessary to develop the diagnostic and prognostic value of peripheral VEGF as a biomarker for clinical depression and antidepressant efficacy.

EPO in depression

Erythropoietin has been tested in several human imaging studies for its ability to modulate brain function and behavior. A single high dose of EPO reduced neuronal response to fear 1 week after administration in healthy volunteers, without evoking any erythropoietic alterations [100]. A specific reduction in blood oxygen level-dependent (BOLD) signal change in response to fearful stimuli was noted in the cortex of the EPO group in comparison to placebo treatment. A short-term effect of improved mood was reported in the first 3 days following EPO administration along with increased neural and cognitive processing of facial expressions [101]. Interestingly, the acute effects of EPO included heightened recognition of happiness and fear in a manner comparable to that of serotonin-reuptake inhibitors and similarly reversed after 1 week [101–103]. In a double-blind study comprising 19 acutely depressed patients, EPO was found to reduce left amygdala-hippocampal response to fearful stimuli [104]. This could reflect an ability to reverse negative emotional bias in this group of patients. Additional studies in larger clinical groups and later testing time points with conventional rating scales are awaited as phase II trials are ongoing.

Regulation of VEGF and EPO by stress and antidepressant treatments

Vascular endothelial growth factors and Flk-1 expression in the hippocampus and frontal cortex are lower with exposure to chronic stress or stress hormones [105–107]. In one study, Bergstrom and colleagues [107] showed a significant reduction of VEGF mRNA in the ventral hippocampus—a region with strong input to the amygdala and prefrontal cortex—following exposure to chronic mild stress. Interestingly, this decrease in VEGF expression only occurred in stress-sensitive rats; a subpopulation of stress-resistant rats did not show a similar reduction in VEGF after chronic mild stress. These findings suggest that VEGF signaling may play an important role in stress adaptability. Consistent with this view, corticosteroids are well-known inhibitors of angiogenesis, and previous work has shown that chronically stressed mice have decreased capillary density in the hippocampus [12]. Given the importance of VEGF in regulating neurogenesis and angiogenesis in the adult brain [21, 108], there has been interest in determining whether stress-induced changes in VEGF contribute to the decreases in hippocampal neurogenesis seen in depression. Indeed, stress-induced reductions in adult hippocampal cell proliferation are more pronounced near blood vessels than in areas not covered by blood vessels [105], indicating that VEGF is a key factor for promoting neurogenesis that is secreted from the vascular niche [108]. For further discussion on the effects of stress on VEGF and neurogenesis, the reader is directed to [109].

Early microarray experiments from our laboratory identified VEGF as a possible target of antidepressant action. We showed that electroconvulsive seizure (ECS), which is one the most effective options for the treatment of refractory

depression, resulted in a rapid and robust upregulation of VEGF expression in the rat dentate gyrus [2]. Extending this early work, multiple antidepressants, including selective serotonin reuptake inhibitors, noradrenalin-serotonin reuptake inhibitors and tricyclic antidepressants, have now been shown to increase VEGF mRNA and protein expression in rat hippocampus [2, 110–112]. The induction of VEGF by chemical antidepressants requires chronic administration (~2 weeks), whereas ECS produces rapid changes in VEGF expression along a much shorter time frame (~48 h). This differential profile of VEGF induction closely overlaps with the onset of clinical effects associated with these treatments. Interestingly, chronic treatment with fluoxetine upregulated VEGF mRNA expression in neuronal and endothelial cells, but not in astrocytes, suggesting that antidepressant treatment might preferentially stimulate VEGF production and possibly release from different cell sources [105]. Other treatments known to produce an antidepressant response, such as exercise and sleep deprivation, also increase VEGF expression [113, 114]. Interestingly, a recent genetic study examining a functional polymorphism of the VEGF gene (2578 C/A) revealed that this allele was more frequent in patients with treatment-resistant depression than in healthy controls or a population of treatment-responsive patients [115]. These findings underscore the importance of VEGF as a common downstream target of antidepressant action and highlight this growth factor as an important mediator in the therapeutic response of antidepressant treatment.

While EPO has been shown to possess antidepressant-like activity in rodent models and clinical studies, regulation by chemical antidepressants is yet to be examined. However, robust induction of EPO gene expression was observed specifically in the rat dentate gyrus after ECS [67]. This is similar to the induction pattern of VEGF after ECS [2]. Clearly, further work is necessary to identify the full role of EPO in the therapeutic action of antidepressant treatments.

Vascular growth factors in pre-clinical behavioral models of depression and cognition

Studies of VEGF in models of depression and cognition

Vascular endothelial growth factor induces antidepressant and anxiolytic effects in various animal models. For example, mice overexpressing VEGF in forebrain neurons show reduced immobility in the forced swim test and make a greater number of open arm entries in the elevated plus maze [116]. In addition, ICV infusions of VEGF also mimic the action of antidepressant drugs in several behavioral tests, such as the forced swim test, learned helplessness and novelty-suppressed feeding tests [110]. In the forced swim test, VEGF increased swimming, but not climbing behavior. This is noteworthy given that swimming behavior in this test is influenced by SSRI antidepressants [117]. Interestingly, 5-HT_{1a} receptor antagonists block both the increase in VEGF and the behavioral effects induced by the SSRI fluoxetine in chronically stressed rats, indicating that VEGF may exert its antidepressant actions through modulation of the serotonergic system [112]. Finally, central administration of VEGFR-2 (Flk-1) inhibitors (SU5416, SU1498) has revealed that the behavioral and neurogenic effect of chemical antidepressants and ECS appears to require VEGF/Flk-1 signaling [110, 112, 118]. However, there is some question regarding the specificity of the applied Flk-1 inhibitors since these compounds can exert effects on other growth factor such as FGF-2 and BDNF.

These findings provide important information regarding the role of VEGF in the behavioral and cellular actions of antidepressants. However, one major challenge of pharmacological studies is that they are complicated by issues of specificity, short ligand half-life, solubility, accessibility of the ligand to target tissues and side effects; problems that are compounded when chronic, rather than acute effects, need to be investigated. One strategy to circumvent many of the problems inherent in behavioral pharmacology is to use transgenic or knockout approaches. This powerful approach permits the study of single gene function in the nervous system and offers a high degree of molecular specificity over pharmacological blocking agents to probe important brain-behavior relationships. Through utilizing a conditional gene knockout strategy to achieve inactivation of the VEGF gene in neurons of the murine forebrain, we are currently examining the contribution of neuronal VEGF in depressive behavior and antidepressant response.

The effects of VEGF are not limited to depressive behavior. For example, hippocampal VEGF levels are increased with water maze training [119], and administration of VEGFR-2 inhibitors directly into the hippocampus following spatial training impairs long-term memory [120]. These findings raise the possibility that VEGF may play an important role in processes related to neuronal plasticity and behavior. Indeed, field recording studies in hippocampal slices revealed that direct application of recombinant human $VEGF_{165}$ prior to high-frequency stimulation increases long-term potentiation [121]. Consistent with the idea that VEGF promotes synaptic plasticity and boosts memory performance, previous work has shown that overexpression of VEGF through either AAV-mediated gene transfer in the rodent hippocampus or globally in the forebrain of transgenic mice increases associative learning and spatial memory tasks [122, 123]. Finally, an elegant study by Licht and colleagues [124] recently showed that VEGF can modulate neuronal plasticity in the hippocampus and improve learning through a process that was independent of changes in either vascular perfusion or neurogenesis. These

findings underscore the diverse pleiotropic actions of VEGF on brain function, although the exact receptors mediating these diverse effects have not been characterized.

Studies of EPO in models of depression and cognition

The antidepressant-like effects of EPO and non-erythropoietic variants have been tested in rodent behavioral models. Three days of EPO administration yielded a positive effect in the widely used forced swim model of antidepressant activity [67]. The reduction in immobility time was mainly due to increased swimming rather than climbing behavior, similar to the actions of VEGF in this test (see above), and to SSRI antidepressants [117]. Administration of EPO for 4 days decreased novelty-induced hypophagia in mice, measured as a reduction in the latency to approach the food source in the novel cage environment [67]. The effect was comparable to that obtained by chronic treatment (3 weeks) with SSRI antidepressants [125]. A biochemically modified (carbamoylated) form of EPO (CEPO) that is devoid of erythropoietic activity [126] reduced despair behavior in the tail suspension test, indicating that the antidepressant effects of EPO are independent of hematopoietic properties [25].

In addition to antidepressant-like efficacy, EPO has been successfully tested in models of neurocognition. Both EPO and CEPO improved performance in the spatial and object recognition tasks [25]. These effects are likely to be mediated by modulation of hippocampal plasticity and long-term potentiation [127]. The memory effects are selective and not accompanied by increases in spontaneous activity, exploratory behavior or motor performance [127]. Although EPO was dosed for 3 weeks and elevated hematocrit in the Adamcio study, the cognitive effects are not likely to be associated with erythropoiesis as the behavioral improvement persisted for an additional 3 weeks after the last EPO dose, a time point when the elevated hematocrit would have normalized. Conclusive evidence that the cognitive-enhancing effects are unrelated to hematopoiesis was provided by the use of a short 11-amino acid peptide that was derived from a portion of the three-dimensional structure of EPO. This non-erythropoietic peptide increased performance in the novel object recognition task with equivalent efficacy to the drug galantamine despite a plasma half-life of only a few minutes [128]. The behavioral actions of EPO and other EPO-like molecules and derivatives suggest that vascular trophic factors are worthy of further investigation as candidate molecules for treatment of psychiatric disorders.

Therapeutic potential

The ability to alter signaling in multiple brain cell types and produce trophic effects that lead to modulation of behavior in the setting of neuropsychiatric disorders qualifies vascular growth factors as potentially unique therapeutic agents with a novel mechanism of action. However, an important parameter that has to be addressed for CNS use is the issue of transport across the blood-brain barrier (BBB). While biologics are advancing as attractive candidates for drug development because of their specificity of action, resulting in higher rates of FDA approval than small molecules, their utility in treating CNS diseases can be challenging. Systemically administered EPO has been shown to traverse the BBB via a potential receptor-mediated translocation [129]. The efficacy of systemic administration in eliciting cellular and behavior effects is reflected by substantial literature of EPO use (over 350 papers) for CNS cytoprotective and neurotrophic activity. Nevertheless, the efficiency of CNS transport is far below that of small molecules, and hence large doses are needed to produce appreciable efficacy. This results in higher cost of treatment and can eventually limit applicability in the field. Strategies to address this challenge include producing recombinant molecules in alternate hosts such as plants, bacteria [130, 131] and chemical synthesis of peptides [128] with similar activity.

Structure-based design of biomimetics

The availability of high-resolution crystallographic structural information [132–134] and receptor affinity data [135]

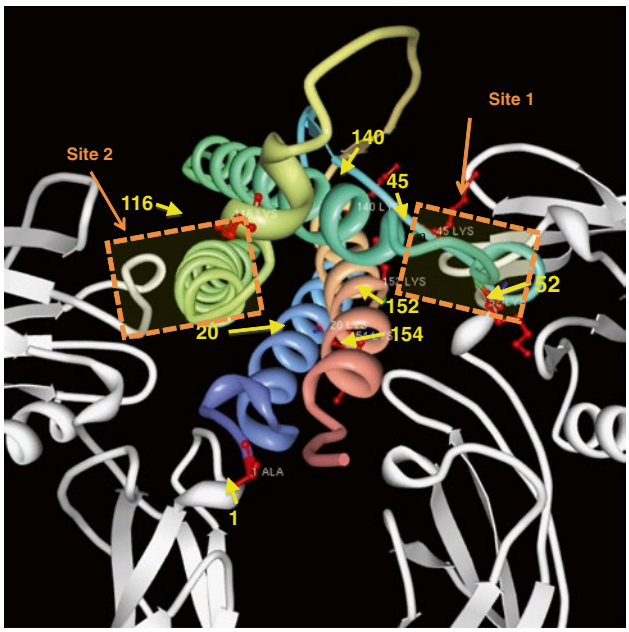

Fig. 2 Crystal structure of EPO and EPOR (1EER, protein data bank). EPOR dimer is colored *gray*, and helices of EPO are shown *multi-colored* and bound to EPOR. The two active sites, site 1 and site 2, are indicated by *orange boxes*. Potential sites of carbamylation are indicated by *red atoms*, *yellow arrows* and residue number of the amino acid sequence

has enabled modeling approaches to design molecules that activate receptor-mediated cell signaling (Fig. 2). Initial studies were performed using phage display methodology to identify peptides that exhibited strong EPO mimetic actions [136]. Interestingly, these peptides did not correspond to the primary sequence of EPO and yet produced the entire spectrum of biological activity with regard to erythropoiesis. A short peptide derived from surface-simulation analysis and composed of adjacent amino acids that represent the aqueous face of helix B, one of the four (A–D) helices of EPO, reproduced the neuroprotective actions of EPO both in vitro and in vivo, without any effect on erythropoiesis [128].

The success of this approach paved the way for the synthesis of additional biomimetic peptides. A non-erythropoietic tetrameric peptide corresponding to the C helix of EPO and the low-affinity site of EPOR produced neurite outgrowth in cultured neurons, effectively crossed the BBB and reduced kainic acid-induced toxicity in the brain [137]. Precisely how short peptides are able to activate signaling via receptor binding is currently unclear. The authors tested monomer, dimer and tetramer versions of the peptide and found that only the tetramer acquired an aqueous solution conformation that resembled EPO's C-helix. It is useful to note that although the neurotrophic effects were comparable to EPO and required the EPO receptor, they were obtained only at doses that were 10^3 higher than full-length EPO [137]. The same group also generated another tetrameric, non-erythropoietic peptide toward the high-affinity receptor site using publicly available x-ray crytallography structural information [138]. This peptide had twofold lower affinity for EPOR than recombinant EPO, which could be due to partial coverage (428 Å) of the total intermolecular contact area (920 Å) of the high-affinity binding site. The fact that peptide agonists of the EPOR remain a useful avenue for CNS drug development is most likely due to their short plasma residence time, which precludes hematological consequences.

Downstream signaling molecules as drug candidates

Cellular signal transduction modulated by EPO starts with binding to the membrane-bound receptor, dimerization and activation of the Janus protein tyrosine kinase 2 (Jak2) (Fig. 3). Jak2 then phosphorylates multiple tyrosine residues in the cytoplasmic region of EPOR [139]. This causes phosphorylation and activation of the transcription factor, signal transducer and activator of transcription 5 (STAT5), which subsequently translocates into the nucleus and binds to specific promoter elements to initiate transcription of target genes. The Jak-STAT pathway is considered the canonical EPO signaling cascade; however, it is not

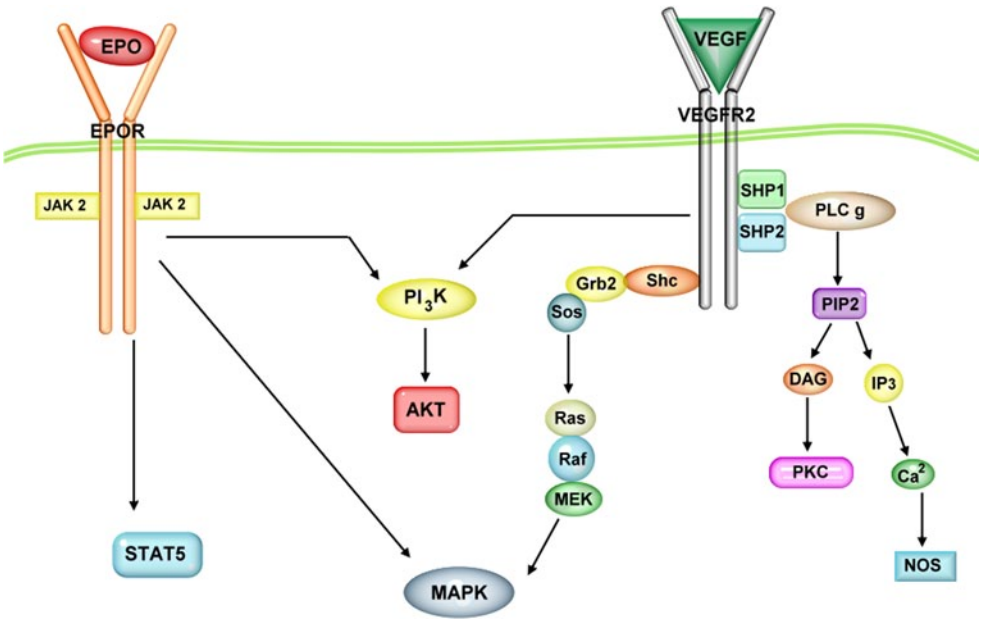

Fig. 3 Schematic of EPO and VEGF receptor signaling pathways. Intracellular cascades are shown activated by EPO and VEGF binding to EPO receptor (EPOR) and VEGF receptor 2 (VEGFR-2), respectively. Janus kinase 2 (JAK 2), signal transducer and activator of transcription 5 (STAT 5), phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT/PKB), mitogen activated protein kinase (MAPK), Src homology 2 domain containing transforming protein (Shc), growth factor receptor- bound protein 2 (Grb2), sons of sevenless (Sos), rat sarcoma GTPase (Ras), rapidly accelerated fibrosarcoma kinase (Raf), mitogen-activated protein kinase kinase (MEK), Src homology region 2 domain containing phosphatase-1, 2 (SHP1, SHP 2), phosphoinositide phospholipase C (PLC), phosphatidylinositol 4,5-biphosphate (PIP2), inositol triphosphate (IP3), diacylglycerol (DAG), protein kinase C (PKC) and nitric oxide synthase (NOS)

activated by carbamylated EPO, which lacks erythropoietic activity [126] but retains the neurogenic and angiogenic properties of EPO [140]. In addition to the Jak-STAT pathway, EPO can also signal via the PI3kinase–Akt and MAPK–ERK pathways [141]. In a similar manner, VEGF-signaling also promotes activation of the PI3-kinase–Akt and MAPK–ERK pathways [65]. Activation of the MAPK and Akt pathways is well known to mediate important trophic effects related to synaptic plasticity, neuronal survival/protection and neurogenesis [20, 21, 37, 121, 142, 143]. However, the precise involvement of these signaling pathways in the behavioral actions of EPO and VEGF is yet to be elucidated and is an interesting and important field of investigation. Dissecting these pathways and identifying the particular signaling molecules that contribute to functional output in cellular and behavioral assays can provide key targets for drug development and also help reduce undesirable side effects.

Summary and conclusions

The neuronal and vascular actions of growth factors such as VEGF and EPO are intricately intertwined to the extent that a new term "angioneurin" was coined to highlight their dual functionality [144]. The robust induction of these molecules in the brain in response to insults such as stroke or hypoxia and their ability to provide robust protective effects against cellular damage reveal that they are key endogenous components of homeostasis and survival strategies employed by the mammalian brain. The fact that these growth factors act on multiple cell types suggests that simultaneously exerting trophic actions on neuronal and vascular cells could provide superior efficacy in producing regenerative effects. Substantial evidence accruing from clinical and pre-clinical studies indicates that cellular atrophy is an important element in the pathophysiology of neuropsychiatric illnesses. The ability to reverse cellular and behavioral deficits by trophic factor administration reinforces support for testing this class of growth factors, their derivatives and biomimetics as novel therapeutic compounds for the treatment of psychiatric and neurodegenerative diseases. However, it will be critical to address the erythopoietic activity of EPO and the vascular permeability effect of VEGF as they can have detrimental hematological and BBB weakening consequences. Interestingly, another member of the VEGF family, VEGF-B, also possesses neurogenic and neuroprotective properties but does appear to exert vasopermeability effects [145]. However, whether VEGF-B expression and/or signaling are adversely affected in neuropsychiatric illness or upregulated by treatments will require further investigation. In conclusion, advancing our understanding of vascular, glial and neuronal mechanisms of action can enable us to develop safe angioneurin-based treatment strategies that maximize the clinical benefit of vascular trophic factors and preclude adverse effects when applied for the treatment of neuropsychiatric or neurological disorders.

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