Role and Therapeutic Potential of VEGF in the Nervous System

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Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P. Role and Therapeutic Potential of VEGF in the Nervous System. Physiol Rev 89: 607–648, 2009; doi:10.1152/physrev.00031.2008.—The development of the nervous and vascular systems constitutes primary events in the evolution of the animal kingdom; the former provides electrical stimuli and coordination, while the latter supplies oxygen and nutrients. Both systems have more in common than originally anticipated. Perhaps the most striking observation is that angiogenic factors, when deregulated, contribute to various neurological disorders, such as neurodegeneration, and might be useful for the treatment of some of these pathologies. The prototypic example of this cross-talk between nerves and vessels is the vascular endothelial growth factor or VEGF. Although originally described as a key angiogenic factor, it is now well...
established that VEGF also plays a crucial role in the nervous system. We describe the molecular properties of VEGF and its receptors and review the current knowledge of its different functions and therapeutic potential in the nervous system during development, health, disease and in medicine.

I. INTRODUCTION: THE NEUROVASCULAR LINK–COMMON PRINCIPLES AND MECHANISMS

Five centuries ago, the Belgian anatomist Andreas Vesalius already documented the anatomical parallelisms between vessel and nerve patterning (Fig. 1, A and B). Despite their distinct function, the vascular and nervous systems exhibit architectural similarities and are both patterned in ramified and hierarchically ordered networks. Yet, it is only recently that the common molecular basis of neuron and vessel specification, growth, navigation, and survival has started to become elucidated. Both systems are composed of efferent and afferent networks, such as motor and sensory nerves in the nervous system, and arteries and veins in the vascular system (43) (Fig. 1, A and B). Intriguingly, nerves and vessels regularly exhibit comparable patterning, with vessels running in parallel alongside nerves (Fig. 1C). The anatomical association between both networks has raised intriguing questions as to whether there are developmental links between both networks and to what extent their development is controlled by similar (classes of) molecules. In the next paragraphs, we illustrate this neurovascular link with a few examples.

Recent studies show that common genetic pathways regulate, at least in part, the differentiation of the cellular players and development of both networks. For instance, neurogenesis and angiogenesis are closely intertwined, with endothelial cells (ECs) in vascular niches releasing cues for neural stem cells (NSCs) (see sect. IV A). Furthermore, the organization of vascular and nervous networks relies, in part, on the segregation of distinct cell populations with establishment of tissue boundaries so that cells with common functions group together, while cells with different activities are excluded. Examples include the segmentation of the hindbrain in rhombomeres (64, 187, 385) and the formation of boundaries between arterial and venous ECs in the vasculature. In both cases, the molecules, which help to establish these boundaries, belong to the Ephrin family of receptor tyrosine kinases and their membrane-bound ephrin ligands (65, 255).

More similarities between both systems have been identified when exploring the mechanisms of axon and blood vessel navigation during development. Specialized endothelial “tip” cells at the forefront of navigating vessels extend numerous filopodia that sense the surrounding tissue and respond to guidance cues, comparably to axonal growth cones that sense and navigate to their targets (107) (Fig. 2). Similar classes of cues, which control axon guidance (such as Slits, Netrins, Semaphorins, and Ephrins), also regulate vessel navigation (43), while vice versa angiogenic molecules also regulate neuronal and axon patterning. These processes will be further described in section III, A and B.
Another example of a close interaction between neuronal and vascular cells is the innervation of resistance arteries by autonomic nerves (29, 35). With the development of a pressurized circulation, certain arteries became innervated to allow proper control of the distribution of flow to vital organs (35). These autonomic nerves arise from neural crest cells that segregate from the neural tube and migrate to peripheral regions in the embryo (78, 184). Neural crest cells also differentiate to smooth muscle cells (SMCs) and pericytes (PCs), that cover some of the large thoracic arteries and vessels in the forebrain (78, 85). When deregulated, this process causes metameric appearance of vascular anomalies in the central nervous system (CNS) of patients suffering the PHACES syndrome (posterior fossa malformations, hemangiomas, arterial anomalies, cardiac defects, eye abnormalities and sternal or ventral defects). The neural crest thus contributes directly to the formation of both the nervous system and the vascular system.

The cross-talk between vessels and nerves is also important for proper functioning of neurons and blood vessels in adults. Arterioles in the nervous system consist of a single layer of ECs that are surrounded by PCs; these vessels lay in intimate contact with glial cells and nerve terminals. This neurovascular unit allows blood vessels to match oxygen and glucose delivery with neuronal metabolic demands (223). Active neurons not only induce vasodilation by releasing vasoactive substances (46, 272, 299, 312, 369, 448) but also regulate neuronal perfusion indirectly through release of glutamate, which evokes secretion of vasoactive substances from astrocytes (448).

Since VEGF is the prototypic example of the cross-talk between nerves and vessels, we focus the rest of this review primarily on VEGF. Because its role in angiogenesis has already extensively been reviewed (39, 92, 171), we here describe the molecular properties of VEGF and its receptors during angiogenesis more briefly, before highlighting its role in the nervous system in more detail.

II. BIOLOGICAL ACTIVITIES OF VASCULAR ENDOTHELIAL GROWTH FACTOR

A. VEGF Family Members

Given that excellent reviews on the molecular properties of the VEGF family and its receptors have been published previously (92, 289, 343), we restrict our discussion here to those characteristics that are relevant for understanding their role in neurobiology. VEGF-A (termed VEGF from hereon) is the founding member of a family of homodimeric glycoproteins that are structurally related to the platelet-derived growth factors (PDGF). Initially isolated as a factor increasing vascular permeability (334), the VEGF gene was thereafter cloned from pituitary cells (93). Exon 1 and part of exon 2 encode a hydrophobic signal sequence, exons 3 and 4 encode domains responsible for binding to its receptors, while exons 6 and 7 encode basic domains capable of binding to heparin (Fig. 3).

Through alternative RNA splicing, several VEGF isoforms are generated. The VEGF121 isoform (121 amino acids in humans and 120 in mice; VEGF isoforms have 1 less amino acid in mice) is freely diffusible, since it lacks the basic amino acid residues responsible for heparin binding and, therefore, does not, or only minimally, binds to the extracellular matrix (ECM) (Fig. 3). The larger
forms, consisting of 145, 165, 183, 189, or 206 amino acids, bind to heparin and heparan sulfate proteoglycans. Because VEGF165 contains some basic residues (encoded by exon 6), it is partly diffusible and partly bound to the pericellular matrix (Fig. 3). VEGF189 contains even more basic residues, explaining why it remains spatially restricted to the matrix around the VEGF-producing cell (Fig. 3). Matrix-bound VEGF isoforms are released by proteinases, such as heparinases and matrix metalloproteinases (MMPs), and thereby regulate vessel shape and lumen (41, 43, 107, 323, 364) (Fig. 4). Other isoforms have been identified, such as VEGF165b, similar in length as VEGF165, but with a different COOH-terminal tail, that may be an inhibitor of angiogenesis (21, 185, 314, 421); VEGF-D (Fig. 5). The VEGF-E gene, which is encoded by the parapoxvirus Orf virus (257) and svVEGF, which is a snake venom VEGF (155), will not be discussed. Each of these family members is characterized by the presence of eight conserved cysteine residues, which form a typical vertebral growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D (Fig. 5). The VEGF-E gene, which is encoded by the parapoxvirus Orf virus (257) and svVEGF, which is a snake venom VEGF (155), will not be discussed. Each of these family members is characterized by the presence of eight conserved cysteine residues, which form a typical
These homologs have been implicated in angiogenesis or lymphangiogenesis, although with distinct roles (Fig. 5). For instance, in addition to its key role in angiogenesis, VEGF also regulates lymphangiogenesis (122, 132, 165, 277). VEGF-C regulates lymphatic vessel growth, but also promotes angiogenesis (156); the role of endogenous VEGF-D, even though capable of stimulating lymphangiogenesis when overexpressed, in lymphatic growth remains less clear in mice (156), while it acts as a modifier of lymphangiogenesis in tadpoles (284) (Fig. 5). PlGF stimulates angiogenesis selectively in pathological conditions (41, 94, 95), while VEGF-B is a relatively weak angiogenic stimulator, with apparently restricted activity in ischemic myocardium (39, 95, 226) (Fig. 5). Of the VEGF family members, VEGF-B, VEGF-C, and PlGF also have a role in the nervous system. Their functions will be discussed in this review as well (see sect. II A for VEGF-C and sect. IX for VEGF-B and PlGF). No role for VEGF-D in the nervous system is known thus far.

**B. VEGF Receptor Tyrosine Kinases**

Three VEGF receptors (VEGFRs) have been identified: VEGFR-1 (also known as fms-like tyrosine kinase 1 or Flt1), VEGFR-2 (termed kinase insert-domain containing receptor, KDR in humans or fetal liver kinase 1, Flk1 in mice), and VEGFR-3 (Flt4). The members of the VEGF family bind to these distinct receptors, each with different affinities and selectivities: VEGF binds to VEGFR-1 and VEGFR-2, VEGF-B and PlGF bind to VEGFR-1, whereas VEGF-C and -D bind to VEGFR-3 and (depending on the species) with a lower affinity to VEGFR-2 (39, 92, 171).
In contrast to VEGF, proteolytic cleavage of VEGF-C and -D by plasmin generates mature forms, which bind VEGFR-3 and -2 with much higher affinity than their full-length forms (252). VEGFRs contain seven immunoglobulin (Ig)-like loops (except for VEGFR-3 which contains only 6) in their extracellular part and a split tyrosine-kinase domain in their intracellular region (289). The second and third Ig domain mediate ligand binding, while the fourth and seventh domain mediate receptor dimerization. VEGF receptors form homodimers, but also heterodimerize, although their function remains more enigmatic (15, 76) (Fig. 6).

VEGFR-1 and VEGFR-2 differ from each other in several aspects. VEGFR-2 is the best characterized signaling receptor, driving angiogenesis in health and disease; it stimulates EC proliferation, migration, navigation of tip cells, survival and vascular permeability (289). VEGFR-2 has a strong tyrosine kinase (TK) activity (see below) (289). In the nervous system, VEGFR-2 also stimulates migration, proliferation, and survival of various neural cell types (151, 153, 286, 358, 359, 419). Recently, a soluble form of VEGFR-2 has been discovered in human and mouse plasma (79), which traps VEGF; its levels inversely correlate with tumor progression (80). However, whether this is a splice variant or possibly a cleaved form needs further investigation.

Although VEGFR-1 was discovered before VEGFR-2 (344, 386), its role remains more enigmatic. Besides a soluble VEGFR-1 (also termed sFlt1), which traps VEGF, the transmembrane form of VEGFR-1 has weak tyrosine kinase activity but high affinity for VEGF; deletion of this domain does not affect vascular development (134). Hence, VEGFR-1 may act as a “decoy” receptor that prevents excessive VEGFR-2 activation by trapping VEGF (289). This seems to be particularly the case in development, as embryos lacking VEGFR-1 die due to disorganization of the vascular network (96).

In vitro, sFlt1 is more potent in rescuing abnormal...
vessel morphogenesis of VEGFR-1-deficient ECs than membrane-anchored VEGFR-1, presumably because it is diffusible and thereby shapes a pericellular gradient of VEGF (162, 168, 169). Other studies show, however, that VEGFR-1 stimulates postnatal angiogenesis through intracellular signaling. Indeed, mice expressing a variant VEGFR-1 without tyrosine kinase domain exhibit impaired pathological angiogenesis (133, 273, 342). Also, PIGF promotes angiogenesis directly by activating specific VEGFR-1 signaling in ECs, as well as indirectly via VEGFR-1 signaling in CD45+ vascular-modulatory inflammatory cells (15, 94, 95). Evidence is now emerging that VEGFR-1 also exerts neuroprotective effects during pathological conditions (227) (see sect. IX). VEGFR-3 is the primary receptor inducing (lymph)angiogenesis (156), but also modulates angiogenesis, especially by transmitting sprouting signals in endothelial tip cells (378); it also induces proliferation of oligodendrocyte precursors and certain other neural progenitors, as discussed below (213).

C. Neuropilin Coreceptors

Another class of receptors for VEGF are the neuropilins (NRPs) (105). Neuropilin-1 (NRP1) and neuropilin-2 (NRP2) were originally discovered as receptors for semaphorins (51, 128), the latter belonging to a large family of membrane-bound and secreted proteins. Although membrane-associated semaphorins interact directly with plexins, class 3 secreted semaphorins (Sema3A to F) bind to the NRP receptors, which form complexes with the plexins to trigger signal transduction (186, 393, 443). Genetic studies in fruit flies and mice show that semaphorins are usually repellent cues for axons and neuronal cells (393), although Sema3A can also function as a chemoattractant, depending on intracellular levels of cyclic nucleotides (361).

NRPs are single-spanning transmembrane glycoproteins, with extracellular domains A and B mediating semaphorin binding (105, 106, 118, 278) (Figs. 6 and 7A). Mutagenesis studies show that Sema3A requires an interac-
tion with the A and B domain for its binding to NRP1, while only domain B of NRP1 is necessary for VEGF binding (118). However, the additional presence of the A domain of NRP1 enhances VEGF binding considerably (237). Domain B also binds heparin and thereby enhances the interaction of VEGF165 with NRP1 more than 100-fold (237, 400). Domain C in NRP1 is important for oligomerization with VEGFR-2 (105). Initial studies showed that NRP1 binds VEGF165 and VEGF189 (297), but not VEGF121, which lacks exon 7 (encoding for the NRP1 binding site) (110, 130). A recent study shows, however, that VEGF120 is also capable of binding NRP1 in vitro (297), likely due to the fact that the COOH-terminal tail of VEGF, encoded by exon 8, may bind directly to NRP1 (13). VEGFR-2 interacts with VEGF145 and VEGF165 but not VEGF121 (110, 280). The heparin-binding form of PIGF binds NRP1 and NRP2 (110, 260), and VEGF-B binds NRP1 only (235, 260). Finally, VEGF-C and VEGF-D have also been described to bind to NRP1 and NRP2 in a heparin-independent and -dependent manner, respectively (163).

Neuropilins enhance VEGF signaling by acting as coreceptors for VEGF receptors. When coexpressed with VEGFR-2, NRP1 associates with VEGFR-2 and amplifies VEGFR-2 phosphorylation and signaling (105). As the cytoplasmic domain of NRPs only contains 40 amino acids, it has been postulated that this domain does not transduce biological signals, but instead, requires an interaction with other signaling molecules, such as those interacting with VEGFR-2. Other studies have shown that NRPs contain an intracellular PDZ-binding domain (Fig. 7A), which interacts with intracellular proteins, such as synectin (105). Deletion of this PDZ-binding domain decreases complex formation between NRP1 and VEGFR-2, and diminishes the EC response to VEGF165 (304), suggesting that synectin acts as a bridge between NRPs and intracellular signaling molecules (36, 410).

During development, NRP1 expression becomes restricted to ECs in arteries, whereas NRP2 labels ECs of venous and lymphatic vessels (81). Murine embryos deficient for NRP1 exhibit defects in the vascular system, including impaired neural vascularization and abnormalities in vascular remodeling (166). In contrast, mice lacking NRP2 are viable, with no evidence of cardiovascular defects, although small lymphatic vessels and capillaries fail to form (50). Accordingly, monoclonal antibodies against NRP1 or NRP2 inhibit tumor angiogenesis and lymphangiogenesis, respectively (47, 296). Loss of synectin in mice and zebrafish also causes selective arterial defects (59).

D. Regulation of VEGF Expression

The expression of VEGF is tightly regulated; for instance, loss of even a single VEGF allele results in embryonic lethality because of defective vascular development (40, 266). Furthermore, even a reduction of VEGF levels by only 25–30% causes paralyzing motor neuron degener-
Hypoxia is a strong stimulus for angiogenesis: when cells suffer hypoxia, they release, in a feedback loop, angiogenic factors to reestablish oxygen supply through vessel formation. Hypoxia activates hypoxia-inducible transcription factors (HIFs), which function as master switches to induce expression of angiogenic factors, including VEGF. At the transcriptional level, hypoxic induction of VEGF is conferred by binding of a HIF complex to a core "hypoxia responsive element" (HRE) sequence in the VEGF promoter (264) (Fig. 3). HIF complexes are heterodimeric transcription factors, consisting of α- and β-subunits, which belong to the basic helix-loop-helix PAS family of transcription factors (408). The β-subunit, also known as the aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively expressed, while activation of the α-subunit is tightly regulated by oxygen tension. Several HIFα subunits exist, i.e., HIF-1α, HIF-2α (also known as endothelial PAS domain, EPAS) and HIF-3α, each encoded by different genes (298, 417). HIF-1α and HIF-2α share conserved structural domains, dimerize with HIF-β, and induce gene expression in a hypoxia-inducible manner (229); HIF-3α on the other hand is poorly characterized.

HIFs execute the cellular response to hypoxia, but do not sense changes in oxygen tension themselves. This is performed, in part, by HIF-prolyl hydroxylase domain (PHD) proteins, which are dioxygenases that use oxygen to hydroxylate proline residues in HIFα subunits (97, 158). Under normoxic conditions, hydroxylated HIFα interacts with the von Hippel-Lindau (pVHL) protein, a component of the E3 ubiquitin ligase complex, that triggers ubiquitination and, thereby, marks HIFα for proteasome-dependent degradation (161, 250). In hypoxia, PHDs are inactive, and HIFα subunits become stabilized and activate transcription of target genes (141, 142). The transcriptional activity of HIFs is also regulated by another type of sensor, i.e., the factor-inhibiting HIF-1 (FIH-1). HIFα subunits contain two transactivation domains, an oxygen-regulated COOH-terminal domain (CAD) and a NH₂-terminal domain (NAD) (229). The activity of CAD is regulated by hydroxylation of an asparaginyl residue by FIH-1 (209, 210, 229). In normoxia, this residue is hydroxylated and CAD activity is repressed, while in hypoxia, FIH-1 is inactive, hydroxylation of CAD does not occur, and HIFα subunits bind then, through their CAD domain, to the transcriptional coactivators CBP/p300 to activate transcription of target genes (229). Intriguingly, in conditions of low oxygen and nutrients, the transcriptional coactivator and key metabolic regulator PGC-1α also upregulates VEGF expression and angiogenesis, independently of the canonical HIF pathway (14).

Hypoxia also increases the half-life of the VEGF mRNA by increasing its stabilization (219, 434) (Fig. 3). Under low oxygen conditions, ARE elements in the 3' UTR bind mRNA hypoxia-induced stabilizing proteins, such as HuR (an Elav-like protein), poly(A)-binding protein 2 (PAIP2), and heterogeneous nuclear ribonucleoproteins L (hnRNPL) or K (hnRNPK) (90, 219, 222, 290, 345, 434). As discussed above, translation of VEGF mRNAs in hypoxic conditions occurs through IRES sequences (365) (Fig. 3), although IRES-independent mechanisms also exist (435). The relevance and relative contribution of each of these mechanisms regulating VEGF translation in hypoxic conditions require further investigation.
Although initial studies indicated that VEGF is an endothelial cell-specific factor, more recent findings revealed that VEGF also has important effects in the nervous system. We now describe the evidence on the role of VEGF in the nervous system both in development and health.

III. ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE DEVELOPING NERVOUS SYSTEM

A. VEGF Regulates Vessel and Neuronal Wiring in the CNS

In higher organisms, the neural tube (NT), from which the brain and spinal cord develop, becomes vascularized by a process involving VEGF. Indeed, current evidence indicates that vascularization of the NT occurs via the formation of a perineural vascular plexus (PNVP) surrounding the NT and by subsequent inward vessel sprouting from the PNVP (136, 195, 196). In cocultures of NT explants with presomitic mesoderm, which provides the source of ECs, VEGF inhibitors inhibit PNVP formation (136). Likewise, formation of the PNVP is blocked when presomitic mesoderm explants from VEGFR-2-deficient embryos are cocultured with wild-type NT explants. At the time of vessel sprouting, VEGF is specifically expressed in the NT by the floor plate, the neural progenitors in the ventricular zone, and the motor neurons, suggesting that VEGF also plays a role in directing vessel sprouting into the NT (136, 276, 311). One of the molecules responsible for the timely and highly specific expression of VEGF in the NT is Sonic hedgehog, an angiogenic factor which also participates in NT vascularization in part by regulating VEGF expression (211, 276, 302, 322). On the other hand, invading endothelial precursors derived from the lateral plate mesoderm and somites have also been found in the NT from quail embryos (9, 10, 58, 136, 283), suggesting that vascularization of the NT might also occur through other mechanisms apart of PNVP vessel sprouting.

The intracerebral vasculature develops through sprouting of the pial vessels located on the brain surface. The vessel sprouts grow into the brain parenchyma and converge radially towards the ventricles (195). From there, they extend branches that surround the ventricles and navigate in the reverse direction, radially towards the pial surface again (116). A recent model proposes, however, that vascular differentiation in specific brain regions may be regulated in a similar way as neuronal specification. Indeed, in the developing telencephalon, brain vascularization starts from periventricular vessels, which develop independently, according to compartment-specific expression of homeobox transcription factors that also determine neuronal specification (402).

Vascularization of other brain regions is also regulated by VEGF. For instance, VEGF is expressed by Purkinje cells and astrocytes during cerebellar development, while VEGFRs are expressed by ECs (5). Interestingly, a spatial VEGF gradient, established by various VEGF isoforms, is necessary for proper vessel patterning in the brain. Hence, in mice lacking the heparin-binding VEGF isoforms, endothelial tip cells fail to extend filopodia to the midline of the hindbrain, and vessels are disorganized and misshaped (323). In addition, in mouse embryos, in which VEGF is eliminated in neural progenitors, directed ingrowth of capillaries in the developing brain is impaired (121, 311). NRP1 also contributes to the VEGF signaling, as vascularization of the nervous system is impaired in NRP1-deficient mice (108, 166).

Blood vessels also cross-talk with neural cells during CNS vascularization. For instance, in the developing retina, astrocytes grow centrifugally from the center to the outside layers and thereby provide a template for growing vessels. Indeed, initially, the developing retina is avascular and hypoxic, which upregulates VEGF expression in astrocytes. Increased VEGF then triggers blood vessel growth (308, 366), and supply of oxygen by the nascent neovascularature will, in turn, downregulate VEGF expression in astrocytes and trigger astrocyte differentiation (418).

In addition to effects on vascularization, VEGF also regulates neuronal cell migration in the CNS. For instance, migration of facial motor neurons in the developing mouse hindbrain is regulated by VEGF and NRP1 (330) (Fig. 8A). Facial motor neurons are born in a ventral position of the hindbrain segment (rhombomere 4) and move their soma caudally to rhombomere 6. VEGF controls this migration process by interacting with NRP1. However, VEGF is not required for their axon guidance; instead, Sem3A and Sem3F cooperate to pattern facial branchiomotor neuron axons by binding to NRP1/PlexinA4 and NRP2/PlexinA3, respectively, without controlling the path-finding of their somata (330, 331) (Fig. 8). VEGF also stimulates axonal outgrowth of cortical neurons and retinal ganglion neurons (30, 54, 151, 358, 359, 445). Another ligand of the VEGF family, VEGF-C, regulates expansion of the population of oligodendrocyte precursor cells (OPCs) and neural progenitors in vitro (213). VEGF-C acts as a trophic factor for these cells in vivo, since VEGF-C-deficient mouse embryos show a selective loss of OPCs in their optic nerve (213).

The observation that VEGF is involved in wiring the developing nervous system is perhaps not surprising, given that VEGF and its receptors appeared first in the nervous system of invertebrate species, such as worms and flies, which lack a well-developed vascular network (Fig. 5). In C. elegans, a family of four tyrosine kinase receptors, structurally similar to VEGF receptors, has been identified. These receptors (vascular endothelial
growth factor receptors or ver genes) are expressed by specialized cells of neural origin, such as glial cells, chemosensorial neurons, and neurons of the dorsal ganglia (303). A PDGF/VEGF-like growth factor (pvf1) has been characterized in the worm with biochemical properties similar to vertebrate PDGF and VEGF (Fig. 5), which bind to VEGFR-1 and VEGFR-2 and induce angiogenesis in vertebrate models (384).

The fruit fly D. melanogaster expresses a receptor tyrosine kinase related to mammalian PDGF and VEGF receptors (pvr), as well as three VEGF orthologs (pvf1 to pvf3); this receptor is involved in the tubular formation of salivary glands (126) and in migration of various cell types, including hemocytes, which are the primitive blood cells in the fruit fly (60, 287). Loss of pvr also induces defects in axon patterning and positioning of glial cells (287, 333): midline neurons secrete pef ligands, which bind to pef on glial cells and regulate their survival and chemotact them to the midline (214, 287), whereas gain-of-function experiments show that pef induces supernumerary glia at the midline (214). Since glial cells are required for axon guidance, fasciculation, and axon ensheathment during development and after axon injury, VEGF may affect wiring of the developing nervous system indirectly by acting through glial cells in this invertebrate species.

B. VEGF at the Crossroad of Peripheral Innervation and Vascularization

In the peripheral nervous system, some vessels and nerves migrate along the same path and track alongside each other. For instance, in the skin of the embryonic limb, arteries are intimately associated with sensory nerves (Fig. 1C). In mouse mutants with disrupted axon patterning, arterial patterning follows that of disorganized nerves (271). Interestingly, these vessels express arterial markers once they establish close contact with nerves, indicating that nerve-vessel association regulates arterial specification, at least, in this condition (271). Coculture experiments of ECs with dorsal root ganglia (DRG) neurons and Schwann cells further demonstrate that both cell types promote arterial differentiation of ECs through release of VEGF (271). A conditional gene inactivation strategy further reveals that both nerve- and Schwann cell-derived VEGF is required to induce arterial differentiation of these vessels (270). The coalignment of vessels and nerves is, however, not dependent on the release of VEGF (270). In other cases, the opposite also occurs: vessels release guidance cues, such as VEGF, artemin, neurotrophin-3, or endothelin-3 to attract axons to track alongside the vessels (22, 138, 194, 236, 242).

VEGF also affects neural cells in the PNS. It prolongs the survival and stimulates proliferation of Schwann cells in explants of superior cervical ganglia (SCG) and DRG (328). Even though Schwann cells express VEGFR-1, VEGFR-2, and NR1, the effect of VEGF on their migration appears to be mediated predominantly by VEGFR-2 (328). Nonetheless, the precise role of VEGF in peripheral innervation remains incompletely understood.

In mice, expressing a variant NR1, that only binds VEGF but not Sema3A, neural but not vascular morphogenesis is perturbed (119) (Fig. 7, B and C). Moreover, genetic studies in zebrafish embryos show that VEGF regulates axon outgrowth via NR1; indeed, silencing of NR1 induces aberrant branching of motor axons and migration defects of motor neurons (89). Coinjection of Sema3A or VEGF morpholinos in combination with NR1 morpholinos, each at a suboptimal concentration, induces similar defects, suggesting that NR1 integrates signals from both Sema3A and VEGF during axonal outgrowth (89) (Fig. 7C). Likewise, the role of Sema3A in peripheral vascularization is not fully characterized: Sema3A deficiency did not impair the formation of the major axial
vessels, vessel branching, or vessel remodeling, neither in an inbred or outbred genetic background (404) (Fig. 7, B and C). In contrast, another study analyzing Sema3A-null mouse embryos described vascular defects (335). The reason for these contradicting findings is unclear. Silencing of PlexinD1 or Sema3A expression also induced vascular defects in zebrafish embryos (391). Indeed, Sema3A morphants displayed intersomitic vessel-patterning defects, with vessels emerging from the aorta at irregular positions, and elongating sprouts failing to track along the intersegmental boundaries and connecting with each other in an aberrant pattern (391) (Fig. 7, B and C). Further studies are thus required to resolve the controversy about the role of Sema3A in vascular development (Fig. 7B).

Since VEGF and Sema3A interact with the B domain of NRP1, both molecules have been proposed to bind to overlapping sites and functionally compete with each other (Fig. 7C). Indeed, it has been reported that VEGF antagonizes the axon-collapsing effect of Sema3A in DRG neurons (118) and inhibits its repellent effect on migrating neuroectodermal progenitors (17). In the vascular system, high concentrations of Sema3A inhibit EC migration, lamellipodia formation, and microvessel assembly in vitro, and these effects are reversed by VEGF (259, 279) (Fig. 7C). On the other hand, recent crystallographic studies, providing the first detailed picture of VEGF and Sema3A binding to NRP1, indicate that VEGF and Sema3A do not directly compete for binding to the same site in NRPs. In vitro data also reveal that VEGF fails to inhibit Sema3A-induced growth cone collapse of DRG neurons (13) (Fig. 7C), thereby favoring the latter hypothesis that VEGF and Sema3A are not competing for binding to NRP1.

IV. ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE NERVOUS SYSTEM IN THE HEALTHY ADULT

A. Role of VEGF in Neurogenesis

Neurons and glial cells arise from NSCs; ECs influence this process. In specific areas of the nervous system ("vascular niches"), such as in the neurogenic subgranular zone of the hippocampus and the subventricular zone, NSCs proliferate in small clusters around dividing capillaries (295); ECs closely interact with NSCs and astroglial cells in these niches (143, 438) (Fig. 9). Furthermore, ECs release factors that induce differentiation of neuronal precursors (258) (Fig. 9). For example, when subventricular zone explants are cocultured with ECs, the maturation, neurite outgrowth, and migration of neurons are enhanced (218).

Emerging evidence indicates that VEGF participates in this cross-talk between ECs and neural progenitors. VEGF promotes neuronal cell proliferation indirectly by stimulating ECs (Fig. 9). In adult birds, testosterone-induced neurogenesis is preceded by an upregulation of VEGF, which stimulates expansion of vocal cord capillaries (231). ECs in turn secrete brain-derived neurotrophic factor (BDNF), which supports the survival and integration of neurons that are newly recruited from the overlying ventricular zone (231). However, VEGF also exerts a direct mitogenic effect on VEGFR-2 expressing neural progenitors (249). Genetic studies with neurospheres from mice, lacking VEGFR-2 in NSCs, show that VEGFR-2 signaling is essential for the survival of cultured NSCs (407). Furthermore, intracerebroventricular delivery of VEGF stimulates adult neurogenesis in the subventricular

**FIG. 9. VEGF at the neurovascular niche.** Neural stem cells (NSCs) reside in vascular niches in defined CNS regions, such as in the subventricular zone (or SVZ). Neurogenesis occurs in close spatiotemporal association with vessel growth in these niches. VEGF, secreted by ECs, NSCs, and astrocytes (as well as other factors, which have also been depicted), promotes astrocyte differentiation and neurogenesis, the latter by stimulating NSC proliferation, migration, and differentiation. In addition, VEGF secreted from NSCs also promotes EC proliferation and angiogenesis. [Adapted from Zacchigna et al. (430).]
and subgranular zone of the hippocampal dentate gyrus (151), and promotes subsequent neurite outgrowth (172, 317).

Several factors mediate or codetermine the angiogenic and neurogenic activity of VEGF. For instance, erythropoietin promotes angiogenesis by enhancing the secretion of VEGF from neural progenitors and upregulating VEGFR-2 expression in ECs (409). Cocultures of NSCs with brain-derived ECs elicit vascular tube formation and maintenance through the release of nitric oxide (NO) by NSCs and the subsequent NO-mediated upregulation of VEGF and BDNF expression in ECs. VEGF and BDNF then act in an autocrine manner to activate VEGFR-2 and the BDNF receptor TrkB on ECs, and in a paracrine manner to induce NSCs proliferation (225). In vitro, VEGF-mediated proliferation of NSCs relies on the presence of basic fibroblast growth factor (bFGF), which upregulates VEGFR-2 expression in NSCs (423). Granulocyte colony-stimulating factor (G-CSF) also stimulates neurogenesis in vitro by increasing the release of VEGF from NSCs and the expression of VEGFR-2 in the same NSCs. In agreement, VEGFR-2 tyrosine kinase inhibitors block neurogenesis induced by G-CSF (154), thus indicating that G-CSF exerts its proliferative effect through VEGF/VEGFR-2.

VEGF has been implicated in adult neurogenesis in various conditions. For instance, VEGFR-2 signaling is involved in the induction of neurogenesis by antidepressant treatments (414); the latter increase VEGF expression in the hippocampus (7), particularly in granule cells (414) (Fig. 10A). Conversely, chronic stress conditions, such as cold immobilization, forced cold swimming, or isolation, downregulate the expression of VEGF in hippocampal astrocytes and VEGFR-2 in hippocampal granule cells and reduce proliferation of putative NSCs near blood vessels in the hippocampus (129) (Fig. 10B). Hippocampal VEGF levels in rats decline from young to middle-age, coincident with the age-associated decrease in dentate neurogenesis (339) (Fig. 10B). Intriguingly, exercise-induced neurogenesis in part relies on circulating VEGF levels, as peripheral blockade of VEGF abolishes running-induced neurogenesis (Fig. 10A). Since VEGF does not cross the blood-brain barrier, these observations may suggest that VEGF controls neurogenesis through effects on the brain vasculature (87).

Although the exact contribution of VEGF-induced adult neurogenesis remains unclear, VEGF exerts beneficial effects in a number of assays, in which functional outcome has been associated with neurogenesis. For instance, rats raised in a physically enriched environment or trained in a Morris water maze task show increased hippocampal expression of VEGF, coincident with neurogenesis and improved learning (38) (Fig. 10A). Intracerebral administration of a viral vector encoding VEGF also improves hippocampus-dependent associative and spatial learning (38), while silencing hippocampal VEGF expression blocks neurogenesis in response to environmental enrichment (38). In behavioral models, infusion of VEGF in the brain mimics the neurogenic effect of antidepressants (414). Electroconvulsive seizures, which increase neurogenesis in adult animals, rescue defective hippocampal neurogenesis induced by low doses of irradiation. VEGF is thus a crucial mediator of neurogenesis. [Adapted from Warner-Schmidt and Duman (414), with permission from Elsevier.]

**FIG. 10.** Different environmental stimuli regulate neurogenesis through VEGF. A: in the hippocampus, VEGF becomes upregulated after different environmental stimuli such as enriched environment, exercise, or antidepressants. Uregulated VEGF in turn induces EC and NSC proliferation, resulting in neurogenesis. B: however, certain conditions, such as chronic stress or aging, downregulate VEGF and reduce proliferation of putative NSCs near blood vessels in the hippocampus. As a mediator of the cross-talk between endothelial and neuronal stem cells, VEGF is thus a crucial mediator of neurogenesis. [Adapted from Warner-Schmidt and Duman (414), with permission from Elsevier.]

**B. Role of VEGF in Synaptic Plasticity**

Emerging evidence suggests that VEGF affects neuronal plasticity in the CNS in the healthy adult animal. In cultured hippocampal neurons, it induces long-lasting increases in protein synthesis, in part by modulating the expression of calcium/calmodulin protein kinase II (CaMKII), cAMP-responsive element binding protein (CREB), and mammalian target of rapamycin (mTOR), suggesting that VEGF may participate in protracted changes of synaptic efficacy (176). Indeed, field-recording studies in hippocampal slices revealed that VEGF application prior to high-frequency stimulation of hippocampal neurons increases long-term potentiation (LTP), while a VEGFR-2 inhibitor reduces this effect (176). VEGF is also secreted
by hippocampal neurons upon activation of NMDA receptors or L-type voltage-activated channels (176). In addition, VEGF can be released from cortical neurons and astrocytes by shedding of extracellular vesicles (307, 327). Although the biological significance of VEGF secretion is not yet understood, these observations suggest that secretion of VEGF could influence the effect of neurotransmitters at the postsynaptic level.

C. Role of VEGF in Neuroprotection and Neuroregeneration

VEGF also has neuroprotective effects on many postmitotic neuronal cell types in the CNS (cortical, hippocampal, dopaminergic, cerebellar, and retinal neurons) and peripheral nervous system (sympathetic neurons) (Tables 1 and 2). It protects these cells against death induced by a wide variety of different noxious stimuli, including hypoxia, serum withdrawal, or excitotoxic stimuli; VEGFR-2 predominantly mediates this neuroprotective effect, although VEGFR-1 may also transmit some of these effects. The molecular cascades, underlying this neuroprotection, are described in the next section.

Besides effects on neuroprotection, VEGF also stimulates neuroregeneration. In a model of axotomy, regenerating motor nerves (expressing VEGFR-2) align with vasa nervorum, which release VEGF (22). A VEGF trap impairs regeneration of these axotomized nerves; however, it still needs to be determined whether VEGF acts directly on VEGFR-2 expressing nerves or indirectly through effects on the vasculature (22). In another model, VEGF enlarges the growth cone of sympathetic neurons in vitro and promotes reinnervation of denervated arteries in vivo (242). Since SMCs express VEGF and sympathetic nerve fibers express VEGFRs, SMC-derived VEGF might promote growth of sympathetic axons. VEGF also affects glial cells in the adult nervous system. For instance, VEGF has mitogenic effects on astrocytes in mesencephalic explant cultures in vitro and following intracerebral VEGF delivery in vivo (348). Most astrocytes

### TABLE 1. VEGF exerts direct effects on neural cells in the central nervous system

<table>
<thead>
<tr>
<th>Neural Cells</th>
<th>Effect</th>
<th>Receptor/Pathway</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryonic cortical neurons</td>
<td>Survival</td>
<td>VEGFR-2: MEK</td>
<td>286</td>
</tr>
<tr>
<td>Retinal ganglion cells</td>
<td>Neurite outgrowth</td>
<td>VEGFR-2</td>
<td>30</td>
</tr>
<tr>
<td>Cerebellar granule cells</td>
<td>Survival after K⁺ deprivation, glutamate, or 3-NP treatment</td>
<td>VEGFR-2: Akt/PKB</td>
<td>419</td>
</tr>
<tr>
<td>Hippocampal neurons</td>
<td>Survival after hypoxia or NMDA-induced excitotoxicity</td>
<td>Inhibition of excitotoxicity</td>
<td>374</td>
</tr>
<tr>
<td>Cortical neuron precursors</td>
<td>Proliferation</td>
<td>MEK, PLC, PKC, and PI3K</td>
<td>445</td>
</tr>
<tr>
<td>Embryonic ventral mesencephalic neurons</td>
<td>Survival and neurite outgrowth</td>
<td>ND</td>
<td>300</td>
</tr>
<tr>
<td>Cortical neurons</td>
<td>Survival after hypoxia</td>
<td>Inhibition of casp-3 activation</td>
<td>148</td>
</tr>
<tr>
<td>Cortical neurons</td>
<td>Neurite outgrowth</td>
<td>VEGFR-2: MEK, PI3K-Akt</td>
<td>317</td>
</tr>
<tr>
<td>Primary CNS neuronal cultures</td>
<td>Neuronal growth and maturation</td>
<td>VEGFR-2: MAPK</td>
<td>172</td>
</tr>
<tr>
<td>Cholinergic neurons (in vivo)</td>
<td>Survival after NMDA stimuli</td>
<td>ND</td>
<td>268</td>
</tr>
<tr>
<td>SVZ neural progenitors</td>
<td>Proliferation and differentiation</td>
<td>ND</td>
<td>276</td>
</tr>
<tr>
<td>Cortical neurons</td>
<td>Neurite outgrowth</td>
<td>VEGFR-2: Rho/ROK</td>
<td>149</td>
</tr>
<tr>
<td>Retinal explant cultures</td>
<td>Reduction of apoptosis of retinal neurons in an ischemia/reperfusion model</td>
<td>VEGFR-2</td>
<td>282</td>
</tr>
<tr>
<td>SVZ neural progenitor cells</td>
<td>Migration</td>
<td>ND</td>
<td>439</td>
</tr>
<tr>
<td>Primary motor neurons</td>
<td>Survival after hypoxia or hypoglycemia</td>
<td>ND</td>
<td>397</td>
</tr>
<tr>
<td>Primary hippocampal neurons</td>
<td>Survival after glutamate-induced toxicity</td>
<td>VEGFR-2: PI3K/Akt and MEK/ERK</td>
<td>247</td>
</tr>
<tr>
<td>Motor neurons</td>
<td>Survival after AR polyglutamine-induced toxicity</td>
<td>ND</td>
<td>362</td>
</tr>
<tr>
<td>Cerebral cortical cultures, SVZ and SCG neuronal progenitors</td>
<td>Proliferation</td>
<td>VEGFR-2</td>
<td>151</td>
</tr>
<tr>
<td>Definitive neural stem cells</td>
<td>Survival</td>
<td>VEGFR-2: NFrB</td>
<td>407</td>
</tr>
<tr>
<td>Dopaminergic neurons</td>
<td>Survival</td>
<td>ND</td>
<td>348</td>
</tr>
<tr>
<td>Dopaminergic neurons</td>
<td>Survival after 6-hydroxy-dopamine treatment</td>
<td>ND</td>
<td>431</td>
</tr>
<tr>
<td>Cortical neurons</td>
<td>Survival</td>
<td>TNFR-1 dependent</td>
<td>382</td>
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<tr>
<td>Astrogia</td>
<td>Proliferation</td>
<td>VEGFR-1: MAPK/ERK and PI3K</td>
<td>238</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>Proliferation</td>
<td>ND</td>
<td>348</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>Astrocyte activation</td>
<td>ND</td>
<td>318</td>
</tr>
<tr>
<td>Primary microglial cultures</td>
<td>Proliferation, chemotaxis</td>
<td>VEGFR-1: Akt</td>
<td>99</td>
</tr>
</tbody>
</table>

VEGF-mediated effects are summarized per cell type, receptor through which VEGF exerts its effect, as well as the described downstream pathways that are stimulated. ND, not determined.
express VEGFR-1, but little or no VEGFR-2. VEGF increases astroglial proliferation and expression of glial fibrillary acidic protein (GFAP) and nestin through VEGFR-1 signaling (238). VEGF also induces proliferation of microglia through VEGFR-1 (99).

D. Intracellular Signaling of VEGFRs in Neural and Vascular Cells

Although the intracellular signaling cascades of the VEGF receptors have been studied in more detail in ECs than in neurons, many similarities and parallelisms between both cell types exist. In general, binding of VEGF to its receptor induces receptor dimerization, upon which specific tyrosine residues become phosphorylated. Activation of VEGFR-2 results, for instance, in the phosphorylation of tyrosine residues at position 951 in the kinase insert domain, 1054 and 1059 in the kinase domain (positive regulatory sites), and 1175 and 1214 in the COOH-terminal tail (289). These phosphorylated tyrosines then act as docking sites for intracellular signaling pathways. We will briefly discuss some of the VEGFR-2 pathways according to their biological effect. It needs to be taken into account, however, that the cascades, mediating proliferation, survival, and migration are complex, interact with each other, and partially overlap. For further details, the interested reader is referred to previous reviews (289, 340).

I. Regulation of proliferation

By signaling through VEGFR-2, VEGF acts as a potent inducer of proliferation in ECs. In particular, through phosphorylation of tyrosine residue 1175 (i.e., Tyr1175), VEGFR-2 serves as a docking site for phospholipase C-γ (PLCγ), which activates protein kinase C (PKC) through generation of diacylglycerol and increased intracellular Ca²⁺ concentrations. PKC in turn activates mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase 1/2 (ERK1/2) via Raf-1 (375, 376) or Ras (253, 254, 347). Via a clathrin-dependent pathway, VEGFR-2 is then internalized in endosomes (28, 86, 207). The extent to which VEGFR-2 is endocytosed determines the magnitude, duration, and qualitative nature of its signaling. Indeed, in confluent quiescent ECs, which form tight contacts through adhesion with junctional VE-cadherin molecules, EC growth is inhibited. In these ECs, VEGFR-2 forms a complex with VE-cadherin at the cell surface, resulting in dephosphorylation of specific tyrosines in VEGFR-2 and the attenuation of MAPK signaling (115). However, when VE-cadherin is absent or not clustered at intercellular contacts, as is for instance the case in active ECs, that do not form intercellular contacts,

### Table 2. VEGF exerts direct effects on neural cells in the peripheral nervous system or on neural cell lines

<table>
<thead>
<tr>
<th>Neural Cells</th>
<th>Effect</th>
<th>Receptor/Pathway</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCG and DRG neurons</td>
<td>Axonal outgrowth, survival</td>
<td>VEGFR-2: MAPK</td>
<td>358, 359</td>
</tr>
<tr>
<td>DRG neurons</td>
<td>DRG growth cone formation</td>
<td>VEGFR-2: MAPK</td>
<td>54</td>
</tr>
<tr>
<td>Major pelvic ganglia</td>
<td>Fiber outgrowth, induction of NOS, and TH expression</td>
<td>VEGFR-2: MAPK</td>
<td>228</td>
</tr>
<tr>
<td>Primary Schwann cells</td>
<td>Survival, migration in hypoxia and normoxia conditions</td>
<td>VEGFR-2 and NRP1: PI3K/Akt</td>
<td>224</td>
</tr>
<tr>
<td>Schwann cells in SCGs</td>
<td>Survival, proliferation</td>
<td>VEGFR-2</td>
<td>358</td>
</tr>
<tr>
<td>Schwann cells</td>
<td>Survival, migration in hypoxia and normoxia conditions</td>
<td>VEGFR-2 and NRP1: PI3K/Akt</td>
<td>224</td>
</tr>
<tr>
<td>Schwann cells in SCGs</td>
<td>Survival, migration in hypoxia and normoxia conditions</td>
<td>VEGFR-2 and NRP1: PI3K/Akt</td>
<td>224</td>
</tr>
<tr>
<td>Cell lines</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NSC34 motor neurons</td>
<td>Survival in normal conditions and after hypoxia, H₂O₂, stimuli, serum deprivation, TNF-α or in SOD1/GSK3β</td>
<td>VEGFR-2 and NRP1: PI3K/Akt</td>
<td>224</td>
</tr>
<tr>
<td>HN33 immortalized hippocampal neurons</td>
<td>Survival after serum deprivation</td>
<td>VEGFR-2: PE3-K/Akt and NFκB</td>
<td>152</td>
</tr>
<tr>
<td>Neuroectodermal progenitor cell line (Dev)</td>
<td>Survival, proliferation, and migration after Sema3A repellent action and Sema3A-induced apoptosis</td>
<td>NRP1/VEGFR-1</td>
<td>17</td>
</tr>
<tr>
<td>Clonally derived adult rat neural stem cells</td>
<td>Survival</td>
<td>VEGFR-2</td>
<td>326</td>
</tr>
<tr>
<td>A1 human hybrid clonal neurons</td>
<td>Survival after an ischemic insult (hypoxia with glucose deprivation)</td>
<td>VEGFR-2</td>
<td>123</td>
</tr>
<tr>
<td>Glial cell line BV-2</td>
<td>Proliferation, chemotaxis</td>
<td>VEGFR-2</td>
<td>99</td>
</tr>
<tr>
<td>SK-N-SH neuroblastoma cells</td>
<td>Survival after serum deprivation</td>
<td>VEGFR-2: PKA, MEK/ERK1/2, Akt, p38 MAPK</td>
<td>111, 112</td>
</tr>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>

VEGF-mediated effects are summarized per cell type, receptor through which VEGF exerts its effect, as well as the described downstream pathways stimulated by VEGF. ND, not determined.
VEGFR-2 is more efficiently internalized and remains active as a signaling molecule for prolonged periods (207). Dynamin-2, a signal-transducing GTPase involved in receptor endocytosis, has been suggested to bind VEGFR-2 and mediate VEGFR-2 endocytosis (28). However, further studies are needed to shed more light on the molecular mechanisms regulating VEGFR-2 internalization.

Because VEGFR-1 exhibits only weak tyrosine kinase activities, its signaling pathway is less well understood. Molecular studies identified, however, several signaling molecules as possible interacting partners, including PLCγ, the p85 regulatory subunit of PI3K, growth factor receptor-bound-2 (Grb2) protein, the Src homology 2 domain-containing protein tyrosine phosphatase SHP2, and Src homology 2-containing adapter molecule Nck (289). This suggests that VEGFR-1 can trigger relevant biological effects. Deletion of VEGFR-1 also reduces EC proliferation and induces premature EC senescence due to constitutive Akt activation (281). Consistent herewith, PI GF amplifies VEGF-driven angiogenesis (41), in part because activation of VEGFR-1 by PI GF transphosphorylates VEGFR-2 and thereby amplifies its signaling (15). Intriguingly, VEGF and PI GF each induces a specific tyrosine phosphorylation pattern of VEGFR-1, i.e., Tyr1213 is phosphorylated by VEGF and Tyr1214 by PI GF. In addition, a distinct gene expression profile is triggered by both molecules in ECs (15). VEGF and PI GF also activate MAPKs differently in ECs: PI GF by acting through its receptor-bound-2 (Grb2) protein, the Src homology 2 domain-containing protein tyrosine phosphatase SHP2, and Src homology 2-containing adapter molecule Nck (289). This suggests that VEGFR-1 can trigger relevant biological effects. Deletion of VEGFR-1 also reduces EC proliferation and induces premature EC senescence due to constitutive Akt activation (281). Consistent herewith, PI GF amplifies VEGF-driven angiogenesis (41), in part because activation of VEGFR-1 by PI GF transphosphorylates VEGFR-2 and thereby amplifies its signaling (15). Intriguingly, VEGF and PI GF each induces a specific tyrosine phosphorylation pattern of VEGFR-1, i.e., Tyr1213 is phosphorylated by VEGF and Tyr1214 by PI GF. In addition, a distinct gene expression profile is triggered by both molecules in ECs (15). VEGF and PI GF also activate MAPKs differently in ECs: PI GF by acting through its cognate receptor VEGFR-1 and VEGF by acting through VEGFR-2, but not VEGFR-1 (208).

In neurons, VEGF also binds to VEGFR-2 and stimulates proliferation via similar pathways as in ECs, including the PLCγ and the MAPK pathways (99, 445) (Tables 1 and 2). Although VEGF affects neurons and Schwann cells predominantly via VEGFR-2, it also affects astrocytes and microglia by activating the MAPK/ERK and phosphatidylinositol 3-kinase (PI3K) signaling pathways through VEGFR-1 (238).

2. Regulation of survival

VEGF also promotes the survival of ECs through VEGFR-2. One of the molecules involved in triggering this effect is the adaptor molecule Shb, which binds to Tyr1175 and activates PI3K/Akt signaling pathways (102, 137); the latter also inhibits the proapoptotic proteins Bad and caspase-9 (234) and activates NFκB, which in turn will upregulate the expression of various survival factors, including Bcl-2, Bcl-xL, McI1, c-IAPs, and c-FLIP (234).

VEGF also protects neuronal cells against cell death. Indeed, VEGF promotes the survival of neural progenitors and numerous postmitotic neurons of the CNS and peripheral nervous system. The molecular mechanisms whereby VEGF regulates neuronal survival are incompletely understood, yet they seem to rely, at least partly, on the activation of the PI3K/Akt and MAPK pathways (63, 152, 224, 247, 286, 374, 419) (Tables 1 and 2). Interestingly, VEGF also promotes neuronal survival by inducing phosphorylation of the voltage-gated potassium channel Kv1.2, via a mechanism linked to the PI3K pathway (309). In addition, VEGF protects hippocampal neurons against ischemic cell death by inhibiting voltage-activated channels and reducing Ca2+ influx and overload caused by the ischemic insult (233).

3. Regulation of migration

Endothelial tip cells and axon growth cones use filopodia at the leading edge of protruding lamellipodia to sense guidance cues in their surroundings and to navigate in response to these signals (103, 107). The initiation and elongation of these filopodia require the precise regulation of polymerizing, converging, and cross-linking actin filaments (248). Once formed, lamellipodia and filopodia are then stabilized by adhering to the ECM or adjacent cells with their transmembrane receptors, which connect to the actin cytoskeleton. These newly formed adhesions then act as traction sites when the cell moves forward over them.

VEGF induces EC migration through VEGFR-2 by activating Src kinases. Activated VEGFR-2 is also capable of forming a multiprotein complex with VEGFR-associated protein (VAP), also known as T-cell specific adaptor (or TsAd), and Src kinases to trigger migration (246). Indeed, phosphorylated Tyr651 in VEGFR-2 acts as a docking site for TsAd, which forms a complex with the Src family kinases, whereas phosphorylated Tyr1214 recruits the Src kinase Fyn (82, 201, 246, 319, 320, 437). Activation of Src kinases by VEGFR-2 subsequently leads to phosphorylation of guanine nucleotide exchange factors, such as C3G and Vav2, which then activates small GTPase proteins from the Rho family, including Rap1, Rac1, and Cdc42 localized in the migratory edge of endothelial cells (44, 104, 202). While Rap1 and Rac1 promote formation of lamellipodia and membrane ruffling (354, 355), Cdc42 induces filopodia formation (203). Conversely, the PI3K catalytic subunit p110α activates the small GTPase RhoA, which is required for cell detachment during endothelial cell motility (114).

Numerous proteins bind Rho, Rap1, Rac, and Cdc42 in a GDP-dependent manner and mediate their effects on the actin cytoskeleton. For example, serine/threonine kinases, such as Rho-kinase, ROKα (RhoA-binding kinase α), and its close relative p160 ROCK (ROKβ), phosphorylate the myosin binding subunit of myosin light chain (MLC) through inactivation of MLC phosphatase and direct phosphorylation of MLC (reviewed in Ref. 315). This, in turn, enhances binding of myosin to actin filaments and promotes the formation of stress fibers, which are involved in cell contractility (8). VEGF also triggers the
activation of the focal adhesion kinase FAK in a RhoA
-ROCK-dependent manner. Phosphorylation of FAK is re-
quired to recruit paxillin and vinculin to FAK, which,
together with the activation of IQGAP1 (IQ motif contain-
ing GTPase activating protein 1), ensure formation and
turnover of focal adhesions (3, 4, 212, 425). Simulta-
neously, VEGF-induced activation of stress-activated pro-
tein kinase 2 (SAPK2/p38) leads to the phosphorylation of
the small heat shock protein HSP27, which induces the
release of phosphorylated HSP27 from capped actin fila-
ments, and thereby stimulates actin reorganization to pro-
mote EC migration (202, 319, 320). By acting through
the small GTPases of the Rho family, VEGF also activates Akt
signaling, which promotes phosphorylation of the actin-
binding protein Girdin, thereby inducing EC migration
and sprouting (183).

VEGF regulates neuronal migration, but its underly-
ing pathways remain incompletely understood. Interest-
ingly, VEGF signaling through VEGFR-2 regulates neural
progenitor migration in vitro by activation of IQGAP1,
which recruits additional partners such as Cdc42, Rac1,
and the microtubule-binding protein Lis1 (18). The PI3K/
Akt and MAPK pathways have also been implicated in
VEGF-induced neurite outgrowth (317, 358). In addition,
n neurite outgrowth after VEGFR-2 activation is blocked by
inhibitors of ROCK and Rho (149). Thus, although some
studies indicate that VEGF activates similar intracellular
pathways in neurons as in ECs, it remains to be deter-
mined how these signals and players are integrated to
mediate cytoskeleton rearrangements for neural migra-
 tion and neurite outgrowth.

V. ROLE AND THERAPEUTIC POTENTIAL OF
VASCULAR ENDOTHELIAL GROWTH FACTOR
IN NEUROLOGICAL DISEASE

A. Reduced Expression of VEGF Triggers Motor
Neuron Degeneration

Strong evidence for a role of VEGF in the nervous
system has come from studies in amyotrophic lateral
sclerosis (ALS) or Lou Gehrig’s disease, which is a pro-
gressive, adult-onset neurodegenerative disorder charac-
terized by loss of motor neurons in the spinal cord, brain
stem, and cerebral cortex, leading to muscle atrophy,
paralysis, and death, usually within 5 yr after onset of
clinical symptoms (205). A role for VEGF in motor neuron
degeneration was discovered by generating transgenic
VEGF<sup>-/-</sup> mice, in which the HRE in the VEGF promoter is
deleted (Fig. 3). Under baseline conditions, this results in
a 25–40% reduction in VEGF expression levels in neural
tissue, but not in muscle, heart, and fibroblasts, and a
reduction of 60–75% under hypoxic conditions (291) (Fig. 11,
A and B). Unexpectedly, VEGF<sup>-/-</sup> mice suffer from adult-
onset and progressive motorneuron degeneration with
neuropathological and clinical features similar to those of
ALS. Beyond 6 mo of age, VEGF<sup>-/-</sup> mice develop muscle
weakness resulting from the progressive degeneration of
lower motoneurons in the spinal cord and brain stem.
Several of the neuropathological hallmarks detected in
VEGF<sup>-/-</sup> mice are strikingly similar to those found in
patients with ALS. They include muscle atrophy in the
absence of myopathic signs, collateral nerve-terminal
sprouting, specific loss of choline acetyltransferase-
positive motor neurons, presence of axonal spheroids and
aberrant neurofilament inclusions, as well as selective
loss of large, myelinated axons. One phenotypic differ-
ence with ALS in humans is that VEGF<sup>-/-</sup> mice have a
slower progression and normal longevity, unlike patients
with ALS.

Reduced levels of VEGF also increase the severity of
motor neuron degeneration in the standard model of ALS,
i.e., in mice overexpressing the G93A mutant SOD1 pro-
tein (i.e., SOD1<sup>G93A</sup> mice). Indeed, VEGF<sup>-/-</sup>/SOD1<sup>G93A</sup>
double-transgenic mice exhibit earlier onset of muscle
weakness due to motor neuron loss and reduced life span
(206). Although reduced VEGF levels in VEGF<sup>-/-</sup> mice do
not result in an abnormal number or size of blood vessels
in the spinal cord, laser-Doppler and microsphere mea-
surements in the brain and spinal cord reveal that base-
line neural blood flow is reduced by 50% in paralyzed
VEGF<sup>-/-</sup> mice (291). It is not entirely clear, however,
whether reduced neural perfusion is present before the
onset of motor neuron degeneration or is the conse-
quence of neuronal loss, and whether reduced neural
blood flow leads to suboptimal delivery of oxygen to
neuronal tissue, thereby triggering the selective degener-
ation of motor neurons in VEGF<sup>-/-</sup> mice.

A second mechanism whereby reduced VEGF lev-
els in VEGF<sup>-/-</sup> mice might contribute to motoneuron
degeneration is by reducing the direct neuroprotective
effects of VEGF. Indeed, VEGF exerts direct neuropro-
ective effects on many different types of neurons in
vitro, including primary motor neuron cultures and cell
lines (291, 397). When transgenic Thy:VEGFR-2 mice,
overexpressing VEGFR-2 under control of the Thy1.2
promoter which drives expression in postnatal neu-
rons, were intercrossed with SOD1<sup>G93A</sup> mice, these
double-transgenic Thy:VEGFR-2 × SOD1<sup>G93A</sup> mice ex-
hibit improved motor performance with deterioration
at a later age compared with single transgenic SOD1<sup>G93A</sup>
mice (367). Transgenic mice overexpressing VEGFR-2 also
live longer, confirming that VEGF-2 in neurons delays
the degeneration of spinal motor neurons in SOD1<sup>G93A</sup>
mice by transmitting survival signals of endogenous
VEGF (367). Intriguingly, intrathecal infusion of VEGF-2
antisense oligonucleotides in rats, followed by a daily
hypoxic challenge, also results in loss of half of the motor
neurons (346). The activation of Akt and ERK under
hypoxic conditions is markedly inhibited in motor neurons of these rats (346), indicating that interference with the direct neuroprotective activities of VEGFR-2 renders motor neurons more susceptible to degeneration.

Expression of mutant SOD1 also destabilizes VEGF mRNA and downregulates VEGF protein levels in the spinal cord of SOD1<sup>G93A</sup> mice before the onset of weakness (60 days of age). This dysregulation is mediated through binding of a complex containing mutant SOD1 to the AREs in the VEGF 3'/H11032-UTR (232), indicating that posttranscriptional processing of VEGF mRNA is blocked by mutant SOD1 and results in lower VEGF expression levels (Fig. 11C). Other studies confirm that VEGF expression in SOD1<sup>G93A</sup> motor neurons is minimally upregulated by hypoxia, unlike in wild-type mice (275). Exposing mutant SOD1<sup>G93A</sup> mice for prolonged periods to hypoxia does not affect their life span (397). Moreover, VEGF levels are reduced before disease onset and become progressively lower with disease progression in SOD1<sup>G93A</sup> rats (424).

In summary, these data suggest that mutant SOD1 dysregulates posttranscriptional processing of VEGF, thereby reducing VEGF expression and accelerating neurodegeneration in ALS.

B. VEGF Is a Modifier of Sporadic ALS in Humans

The connection between VEGF and ALS in rodents was confirmed in humans, as VEGF plasma levels in Swedish ALS patients are reduced by ~50% compared with healthy spouses (206). High levels of VEGF and erythropoietin are also observed in the cerebrospinal fluid (CSF) from hypoxemic neurological controls, whereas only erythropoietin, but not VEGF, is increased in the CSF from hypoxemic ALS patients (157). Likewise, VEGF levels in the CSF from hypoxemic ALS patients are lower than in normoxemic ALS patients (275).
patients, whereas hypoxemic neurological controls displayed higher levels than normoxemic controls (267). VEGF baseline levels in the CSF of ALS patients are lower than in controls during the first year of the disease (73). Each of these studies thus supports a causative role for reduced VEGF expression in ALS patients. Other studies report, however, that VEGF is not reduced in the CSF or serum from ALS patients (68, 144), or is even increased in serum from ALS patients (285). These contradictory results could be due to the small groups of patients and controls studied. Moreover, as platelets release VEGF quite abundantly, serum levels of VEGF are difficult to interpret. Finally, impaired respiratory function leading to hypoxia in ALS patients could also affect VEGF expression levels, warranting caution to correctly interpret these data. Stratification according to the disease stage of ALS patients in which VEGF levels are measured might be advisable.

In ALS patients, fewer motor neurons express VEGF or its receptor VEGFR-2 (33). As VEGF<sup>−/−</sup> mice lacking the HRE in the VEGF promoter developed an ALS-like phenotype, ALS patients were screened for the presence of mutations in the HRE of the VEGF gene, without, however, revealing any mutations (206). Intriguingly, however, haplotypes of three genetic variations, consisting of the at risk −2578A, −1154A, and −634G alleles, in the VEGF gene increased the risk of sporadic ALS in four distinct European populations from Sweden, England, and Belgium (206). When assessing the influence of these haplotypes in vitro, they reduced VEGF expression by ~40%. Individuals with these genotypes also exhibit significantly lower VEGF in plasma. In a smaller follow-up association study, these at-risk haplotypes exhibit a threefold increased risk for ALS (387). On the contrary, studies performed in British (34), Dutch (399), American (52), and Italian populations (72) reported that there was no association between VEGF haplotypes and sporadic ALS.

Yet another study reported that the role of VEGF in ALS in the German population might be dependent on the gender of the patients; the results of this study, however, did also not entirely confirm findings of the initial study, as the common −1154G allele, rather than the −1154A risk allele, is associated with female ALS in this study (91). Thus, although genetic studies in mice firmly establish a link between VEGF and ALS, the identification of a genetic link in human ALS has remained more challenging.

ALS is, however, a heterogeneous disease, in which multiple different pathways contribute to motor neuron death (205). A wide variety of genetic variations might therefore increase the risk for ALS, but the size of each risk effect would be expected to be modest. This implies that genetic studies in ALS would require the inclusion of several thousands of cases and controls to reliably identify real associations. Given the modest effect of risk factors and the typically small sample sizes employed, it is not surprising that several genetic reports in relatively small populations, originally claiming a positive association of candidate genes with ALS, were subsequently challenged by reports lacking any association or even contradicting it (336). One approach to determine whether a gene contributes to ALS susceptibility is to combine all published data in meta-analyses (146, 147). The strength of this approach lies in its ability to identify subtle genetic effects that none of the single studies would have the power to detect. In addition, a meta-analysis is considered to give a more robust estimate of the overall relevance and risk in humans. A recent meta-analysis of all the association studies on VEGF in ALS, combining all 8 European and 3 American populations and analyzing over 7,000 patients in total, revealed, however, that homozygous carriers of the −2578A risk allele exhibit a significantly increased risk for ALS (204) (Fig. 11D). This effect is specific for male ALS patients. The increased susceptibility to ALS in male patients thus reappraises the link between reduced VEGF levels and ALS, as originally revealed by mouse genetic studies.

C. Therapeutic Potential of VEGF in Motor Neuron Degeneration

Insights on the significance of reduced VEGF in the pathogenesis of ALS also raised the question whether VEGF could have therapeutic effects in ALS rodent models. To evaluate this possibility, a rabies-G pseudotyped equine infectious anemia virus (EIAV) lentiviral vector encoding the human VEGF gene, EIAV-VEGF, was constructed. Intramuscular administration of virus revealed that it is retrogradely transported to the neuronal cell body and efficiently transduces motor neurons (16). Indeed, 4 wk after the injection of an EIAV vector, carrying the LacZ reporter gene into muscles of SOD1<sup>G93A</sup> mice, reporter gene expression is observed in up to 60% of the motor neurons. When EIAV-VEGF treatment is started before onset of symptoms in SOD1<sup>G93A</sup> mice, at the age of 3 wk, VEGF remarkably improves motor performance and prolongs survival by, respectively, 28 and 38 days, thereby resembling the survival effect of gene therapy with insullin-like growth factor I, as one of the most effective therapeutic strategies in the field (164). When treatment is started at disease onset, a smaller but still significant effect is also observed.

Although clinical trials with viral vectors are being considered, the clinical applicability of gene therapy for ALS still remains to be established. Delivery of recombinant neurotrophic growth factors, on the other hand, is clinically attractive, as it offers flexible control of the dose and duration of the administered protein. Although systemic delivery of recombinant VEGF to SOD1<sup>G93A</sup> mice prolonged the survival of SOD1<sup>G93A</sup> mice in one study (441), this effect was not replicated in another study.
Previous clinical trials with systemic delivery of a neurotrophic factor have also failed to show therapeutic effects, because these recombinant factors are insufficiently active as they are not capable of crossing the blood-brain barrier, trigger an immunogenic reaction, or are rapidly cleared from the circulation. As an alternative method to deliver recombinant proteins to motor neurons, recombinant VEGF was therefore delivered into the CSF from SOD1<sup>G93A</sup> rats (367). Distribution studies with radiolabeled <sup>125</sup>I-VEGF revealed that intracerebroventricularly administered <sup>125</sup>I-VEGF diffused from the CSF into the parenchyme of the brain and spinal cord, where it reached motor neurons and remained intact for several hours. Subsequent studies revealed that intracerebroventricular delivery of 0.2 μg VEGF·kg<sup>-1</sup>·day<sup>-1</sup> delays onset and prolongs life expectancy in SOD1<sup>G93A</sup> rats. VEGF treatment also changed the disease subtype from a severe to a much milder form. Indeed, fewer SOD1<sup>G93A</sup> rats suffered from severe forelimb onset type of disease after VEGF than after artificial CSF delivery. Compared with CSF-treated rats with hindlimb or forelimb onset, VEGF-treated rats, respectively, survived 17 or 27 days longer. The more pronounced therapeutic effect of VEGF on forelimb than hindlimb muscles is likely attributable to the higher VEGF levels in the bulbar/cervical than in the lumbar spinal cord upon intracerebroventricular delivery. This is a relevant finding, as involvement of brain stem and cervical disease results in a worse prognosis in both rats and humans. Intriguingly, the levels of phospho-Akt in motor neurons were also increased after intracerebroventricular administration of VEGF to mutant SOD1 rats (74). Overall, these findings revive interest in delivering recombinant neurotrophic factors to ALS patients.

SOD1<sup>G93A</sup> mice were also crossed with mice overexpressing VEGF in neurons and shown to exhibit delayed motor neuron loss, motor impairment, and prolonged survival (413). Expression of the mutant human SOD1 protein in zebrafish embryos also induced a dose-dependent motor axonopathy. Lowering VEGF in SOD1<sup>G93A</sup>-overexpressing embryos induced a more severe phenotype, whereas upregulating VEGF rescued the mutant SOD1<sup>G93A</sup> axonopathy (217). The effect of VEGF on motor neurons is thus also replicated in a small animal model for ALS. Overall, these findings have primed interest in the use of VEGF as a novel candidate for the possible treatment of ALS, and clinical trials to deliver recombinant VEGF intracerebroventricularly to ALS patients are underway.

A recent study also assessed the potential of delivering VEGF directly into the CNS following intranasal administration (426). The highest CNS tissue concentration following intranasal delivery was found in the trigeminal nerve, followed by the optic nerve, olfactory bulbs and tubercle, striatum, medulla, frontal cortex, midbrain, etc. Although this delivery method may not be attractive to target VEGF to the motor neurons, the intranasally delivered VEGF approach may be attractive for other neurons in CNS diseases (see below).

**D. Therapeutic Window of VEGF Delivery in the Brain**

The angiogenic activity of VEGF may, however, limit its therapeutic usefulness. Indeed, when different doses of VEGF are infused into the right lateral ventricle in rats, the vessels density increases dose-dependently in animals receiving daily doses of 1 and 5 μg VEGF/kg body wt (125). Significant enlargement of the lateral ventricles and capillary permeability are also observed in the highest-dose group (125). VEGF also induces an angiogenic response when infused directly into the cortex of the adult rat brain, or when applied to the cerebellum by way of injecting an adenoviral vector (306). Likewise, when administered to the brain surface or parenchyme, VEGF increases vascular permeability (77). In another model, delivery of VEGF in the frontoparietal cortex induces extravasation of serum proteins and fluorescent microspheres (67). Remarkably, even a low dose of VEGF, which produces no appreciable changes in vascular morphology, leads to extravasation of leukocytes, as determined with the pan-leukocyte marker OX1 (67). As another response to VEGF infusion, VEGFR-1 is upregulated in reactive astroglia, while VEGFR-2 expression is increased in vascular endothelium and neuronal somata (188).

These studies indicate that delivery of (high doses of) VEGF directly into the brain parenchyma induces inflammatory and angiogenic responses with potential detrimental effects. However, when delivered intracerebroventricularly, 0.2 μg VEGF·kg<sup>-1</sup>·day<sup>-1</sup> is safe, does not induce inflammation or angiogenesis, but exerts neuroprotective effects in ALS rats (367), thus confirming a therapeutic window for the intracerebroventricular delivery of VEGF in the brain, in which VEGF is neuroprotective but not angiogenic.

**E. VEGF in Other Motor Neuron Disorders**

X-linked spinal and bulbar muscular atrophy (SBMA; Kennedy’s disease) is a rare lower motor neuron disease (170) caused by an expansion of a polymorphic CAG repeat in the first exon of the androgen receptor gene (197). Transgenic mice expressing an expanded human androgen receptor develop a progressive disorder, characterized by muscle weakness, atrophy, and early death, and manifested histologically by the loss of motor neurons from the anterior horns of the spinal cord and neurogenic muscle fiber atrophy (362). Motor neurons from these mice show reduced viability in cell culture, which can be rescued by VEGF.
(362). Intriguingly, in this form of muscular atrophy, the overexpressed levels of the androgen receptor also interfere with transcription of the VEGF gene by squelching the transcriptional coactivator CREB-binding protein (CBP), which together with its coactivator p300 is necessary to allow the HIF-1α complex to induce VEGF gene transcription (362). As a result, a reduction in VEGF expression, approximating that observed in the HRE-deleted VEGF−/− mice (291), is noted long before androgen receptor mice develop a neurogenic muscle atrophy (Fig. 11E). Thus a disturbance in the neuroprotective effects of VEGF is also observed in an animal model of SBMA.

F. Role of VEGF in Excitotoxicity of Motor Neurons

VEGF also protects motor neurons in a nontransgenic paradigm of motor neuron degeneration: in rat spinal cord organotypic cultures challenged with a model of chronic glutamate excitotoxicity, in which glutamate transporters are inhibited by threo-hydroxy-aspartate leading to sustained elevation of glutamate levels, VEGF exerts neuroprotective effects (389). This effect is mediated through the PI3K/Akt signal transduction pathway as it is blocked by the specific PI3K inhibitor LY294002 (389). VEGF is also effective in an in vivo model of excitotoxic motor neuron degeneration, triggered by infusing the glutamate receptor agonist α-amino-3-OH-5-methyl-4-isoxazole propionate (AMPA) in the lumbar spinal cord. AMPA infusion produced dose-dependent progressive hindlimb motor deficits, reaching complete bilateral paralysis in ~10 days, which was correlated with the loss of spinal motoneurons. VEGF administration together with AMPA completely prevented the motor deficits in this model and reduced motor neuron death by 75% (392).

VI. VASCULAR ENDOTHELIAL GROWTH FACTOR IN OTHER NEURODEGENERATIVE DISEASES

A. VEGF in Alzheimer’s Disease

Alzheimer’s disease (AD) is a chronic neurodegenerative disease characterized by a progressive impairment of cognitive functions and memory loss. Neurofibrillary tangles, β-amyloid plaques, neuron loss, and astrogliosis are major hallmarks of the AD brain (124, 381). In contrast to the traditional neurocentric view of AD, recent findings indicate that neurovascular dysfunction contributes to cognitive decline and neurodegeneration in AD. According to this hypothesis, faulty clearance of amyloid β-peptide (Aβ) across the blood-brain barrier (BBB), aberrant angiogenesis, and senescence of the cerebrovascular system could initiate neurovascular uncoupling, vessel regression, brain hypoperfusion, and neurovascular inflammation (109, 446, 447). As a factor exerting important effects on ECs and neurons, VEGF is a likely candidate to be involved in AD.

Indeed, in AD, increased VEGF levels in the CSF (383) and plasma (57) have been reported. VEGF immunoreactivity is also enhanced in clusters of reactive astrocytes in the neocortex, in the walls of many large intraparenchymal vessels, and in different perivascular deposits (160). It has been proposed that the increase in VEGF expression levels occur as a secondary response to hypoperfusion and hypoxia in the AD brain. On the other hand, a reduction in VEGF production has also been demonstrated in peripheral immune cells of AD subjects (356) and in serum from AD patients (244). This observation has been explained by the toxic effects of Aβ on VEGF expression. In the brains of patients with AD, VEGF is colocalized with Aβ plaques. VEGF also coaggregates with Aβ and is only slowly released from the coaggregated complex (427). Continuous deposition of VEGF in the amyloid plaques could thus result in VEGF depletion and may contribute to neurodegeneration and vascular dysfunction in the progression of AD (Fig. 11F). Moreover, VEGF inhibits Aβ-induced cytotoxicity on PC12 cells, possibly by exerting direct neuroprotective effects on these cells (428).

A functional polymorphism within the promoter region (~2578C/A) of the VEGF gene that lowers VEGF expression has also been associated with increased risk of AD in an Italian population (71). However, in subsequent studies, this polymorphism did not confer a greater risk for AD, nor did it modulate the extent of brain vascular lesions in AD patients from French or Spanish populations (48, 245). In a fourth study, the ~2578AA genotypes were associated with an increased risk of developing AD and with an accelerated cognitive decline in APOEε4-positive patients with AD (57). Additional studies are therefore warranted to either confirm or refute the association of this low-VEGF allele with AD. Intriguingly, low VEGF –2578AA genotypes are also significantly over-represented in long-lived subjects compared with a population of young and elderly subjects, suggesting that VEGF variability can be considered as a genetic factor influencing life span (70).

B. VEGF in Parkinson’s Disease

VEGF has a neuroprotective effect on dopaminergic neurons, which are the main target cells of neurodegeneration in Parkinson’s disease (PD) (Table 1). Indeed, VEGF protects cultured mesencephalic dopaminergic
neurons against 6-hydroxydopamine (6-OHDA)-induced cell death. Immunohistochemical analysis of midbrain tissues from PD patients further reveal prominent VEGF immunoreactivity in the substantia nigra of patients, in particular in reactive astrocytes (406). VEGFR-1, but not VEGFR-2, expression is also upregulated in ECs, astrocytes, and neurons (406), but VEGF serum levels are not associated with PD (145). In vivo, transplantation of VEGF-secreting baby hamster kidney (BHK) cells in the striatum of adult rats also protects these animals against 6-OHDA, both at the behavioral and pathological level. Interestingly, a protective effect is still evident when transplantation is performed after induction of the 6-OHDA lesion (430, 432, 433). Transplantation of human neural progenitor cells into the subthalamic nucleus also induces significant functional recovery following amphetamine-induced rotations, possibly through VEGF, as the transplanted neural progenitor cells secrete VEGF for up to 5 mo after implantation in the host brain (11). VEGF may thus have a therapeutic potential in PD. VEGF may also be involved by acting through the vasculature, since nonexercised rats display age-dependent decreases in the density of nigral microvessels and VEGF mRNA expression, which were reversed by physical exercise (405).

When assessing the effect of VEGF on human embryonic stem cells, VEGF upregulates the expression of the neuroectodermal genes Sox1 and Nestin during germ layer formation in embryoid bodies and efficiently increases the number of neural rosettes expressing both Pax6 and Nestin (175). The neural progenitors, generated by VEGF treatment of embryonic bodies, further differentiate into cells that show a similar pattern of gene expression as observed during dopaminergic neuronal development (175). Intriguingly, conditional inactivation of HIF-1α in murine neural progenitor cells, which reduces the expression of VEGF, also reduces expression of these dopaminergic markers, including tyrosine hydroxylase and aldehyde dehydrogenase (263). The number of tyrosine hydroxylase positive neurons in these mice is also reduced by 31% compared with wild-type mice (263), without, however, an effect on dopamine concentrations or locomotor behavior in these mice. Overall this indicates that HIF-1α, at least partially by acting through VEGF, is a key transcription factor during development and survival of substantia nigra dopaminergic neurons.

VII. VASCULAR ENDOTHELIAL GROWTH FACTOR IN PERIPHERAL NEUROPATHIES

Various studies provide evidence that administration of VEGF might also be promising for the treatment of peripheral neuropathies, such that clinical trials testing this concept have either been proposed or are underway. Here, we discuss findings that VEGF is involved in neuropathies, associated with diabetes, chemotherapy, ischemia, and nerve injury. An intriguing question is whether the beneficial properties of VEGF in these conditions are linked to its ability to promote vascular remodeling in the compromised tissue, or whether VEGF also confers direct neuroprotection when the blood supply is intact.

A. VEGF in Diabetic and Chemotherapy-Induced Neuropathies

Although the exact mechanisms of diabetic microvascular complications, such as diabetic neuropathy, are largely unknown, reduced vascular perfusion has been causally linked with diabetic neuropathies. Indeed, the number of vessels and the extent of nerve blood flow are markedly attenuated in rats with streptozotocin (STZ)-induced diabetes (329), suggesting that diabetic neuropathies may actually improve when angiogenesis is stimulated through increased expression of VEGF (394). In two different animal models of diabetes, i.e., in STZ and alkloxan-induced diabetic models, respectively, in rats and rabbits, VEGF gene transfer reverses the deficits in nerve conduction velocities (329). Since VEGF also improves the nerve blood flow in these models, the vessel perfusion-promoting effects of VEGF seem to be responsible for these effects. Similar observations were obtained in chemotherapy-induced neuropathies: in animal models of cisplatin-, taxol-, and thalidomide-induced neuropathies, nerve blood flow is attenuated and the number of vessels in the vasa nervorum is reduced, suggesting that these neuropathies might benefit from angiogenic cytokine therapies as well. Indeed, intramuscular gene transfer of plasmid DNA encoding VEGF results in recovery of vascular density and improved nerve electrophysiology (179, 180).

Several other studies have confirmed the therapeutic potential of VEGF in diabetic neuropathies: subcutaneous inoculation of herpes simplex virus (HSV) vector-mediated gene transfer, resulting in the expression of VEGF in nerves and DRGs, prevents sensory nerve amplitudes, preserves autonomic function measured by pilocarpine-induced sweating, reduces nerve fiber loss in the skin, and improves neuropeptide calcitonin gene-related peptide and substance P expression in DRG neurons in a mouse model of diabetic neuropathy (49). Gene transfer of an engineered zinc finger protein transcription factor, designed to upregulate expression of VEGF, also protects against diabetic neuropathy in rats (305). In addition, in a rat model of diabetes, VEGF gene transfer in the corpus cavernosum also improves erectile function, a cause of decreased quality of life in more than 70% of diabetic men (69). Progressive endothelial dysfunction, a reduction in VEGF expression and loss of intraepidermal nerve fibers have also been observed in the foot skin of diabetic patients with increasing neuropathic severity (310). Fre-
quency-modulated electrical stimulation, which improves pain control and nerve conduction velocity in diabetic patients, also results in an increase in circulating VEGF levels (26). Preliminary results from clinical studies further demonstrate that the sensory neuropathy in diabetic patients improves after intramuscular injection of a plasmid DNA encoding VEGF (351).

One study reports that intramuscular VEGF gene transfer promotes the recovery of sensory deficits without inducing angiogenesis in the sciatic nerve, thereby suggesting that other mechanisms than angiogenesis in the endoneurium of the peripheral nerve may be responsible for the observed effects (274). A possible mechanism is that VEGF exerts direct neuroprotective effects on sensory DRG neurons. An intense signal for VEGF is detectable in DRG cell bodies and sciatic nerve fibers of normal and STZ-induced diabetic animals (325, 357, 358). In vitro, supplementation of VEGF has also been shown to have neuronal growth-promoting effects on sensory neurons (359), indicating that VEGF is present on DRG neurons and may indeed exert neuroprotective effects in neuropathies. It is, however, difficult to assess whether neural repair or neuroprotection is the result of a direct neurotrophic effect in vivo, or, rather, are secondary to VEGF-driven angiogenesis and improved perfusion of the ischemic territory. Another mechanism whereby VEGF may exert its therapeutic effects is by directly affecting Schwann cells. Although a direct in vivo effect of VEGF on Schwann cells during diabetic neuropathy has not yet been reported, VEGF stimulates chemotaxis and proliferation of Schwann cells in vitro (328). VEGF stimulation of Schwann cells also leads to phosphorylation of VEGFR-2, indicating that VEGFR-2 binding sites are functionally active in these cells. VEGF may thus also act directly on Schwann cells during nerve recovery (328). High levels of VEGF have also been implicated in the pathogenesis of the POEMS (peripheral neuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) syndrome, suggesting that VEGF is also involved in the pathogenesis of this disorder (416).

**B. VEGF and Nerve Repair**

VEGF is also protective in various nerve injury models. When 2-cm gaps in rat peroneal nerves are repaired with VEGF-treated acellular peripheral nerve isografts, there is a significant increase in the total number of axons and in the percentage of neural tissue in the VEGF-treated group (321). Also, intracavernosal VEGF injection facilitates nerve regeneration and recovery of erectile function after cavernosal nerve injury (140). Another study demonstrated that VEGF application via Matrigel in a silicone sciatic nerve chamber enhances myelin axon counts by 78% and significantly improves motor performance (135). In these experiments, VEGF increases angiogenesis in the chamber, but also enhances Schwann cell proliferation and migration. Schwann cells also upregulate VEGF expression secondary to chronic nerve compression injury and can thus also be involved in mediating the effects of VEGF after a nerve injury (120).

**C. VEGF in Retinal Diseases**

Diabetic retinopathy has traditionally been regarded as a disease of the retinal microvasculature. Histologically, vascular lesions in the early stages of diabetic retinopathy are characterized by capillary basement membrane thickening and microaneurysms, pericyte-deficient capillaries, blood-retinal barrier breakdown, and other vascular cell changes (100). Damage to nonvascular cells of the retina has also been documented: loss of retinal ganglion cells (RGCs) has been detected in diabetic rats and humans, whereas glial cells change from a quiescent to an injury-associated phenotype, with high levels of expressed GFAP (19, 230, 324). VEGF is highly expressed during early stages of diabetic retinopathy (37, 360). Intravitreal injection of VEGF protein and delivery of slow-release implants also cause vascular abnormalities similar to those observed in diabetic retinopathy patients, including hemorrhage, retinal detachment, microaneurysms, venous tortuosity, and capillary nonperfusion (200, 338, 398). Mice overexpressing modest to high levels of VEGF under control of a truncated rhodopsin promoter are characterized by similar abnormalities (Fig. 12A). The degree of vascular abnormalities in these models depends on the extent to which VEGF is overexpressed, with more severe changes documented in mice overexpressing the highest VEGF levels (200, 338, 398). This illustrates that increased VEGF expression contributes to diabetic retinopathy by acting on the vasculature.

VEGF also affects other cell types in the retina. Transgenic Nse:VEGF mice, which express VEGF under control of the neuronal Nse promoter and only modestly overexpress VEGF, do not exhibit vascular changes in their retina (174). When Nse:VEGF mice are challenged in a model of RGC axotomy, which normally induces a stereotypic pattern of apoptotic death in 80% of ganglion cells, RGC neurons are protected (174) (Fig. 12B). Western blots also revealed increased phosphorylated ERK1/2 and Akt, reduced phosphorylated p38, and reduced activated caspase-3 levels in axotomized VEGF-transgenic retinas (173). In a model of ischemia/reperfusion injury, VEGF also induces a dose-dependent reduction in retinal neuron apoptosis. While mechanistic studies show that VEGF elevates blood flow to the retina, which is partially responsible for the neuroprotection, ex vivo studies on retinal cultures also demonstrate a direct neuroprotective effect for VEGF (282). Ischemic preconditioning 1 day...
before ischemia reperfusion injury also increases VEGF levels in RGC neurons, and substantially decreases the number of apoptotic retinal cells. The protective effect of ischemic preconditioning is reversed after inhibition of VEGF. Overall, these data indicate that modest increases in VEGF expression seem to exert beneficial neuroprotective effects in ischemic and injury-induced retinopathies without affecting the vasculature. Higher levels of VEGF, however, rather contribute to diabetic retinopathy-like abnormalities.

Many pathological conditions in the eye are known to result from the formation of abnormal neovessels, including age-related macular degeneration, which is the most common cause of blindness in the elderly. Intriguingly, these conditions are all characterized by overexpression of VEGF (37). Recently, antagonists directed against VEGF have successfully been developed for the treatment of age-related macular degeneration (12, 159). Based on the common pathological grounds of these ocular diseases, therapeutic strategies involving intravitreal injections of anti-VEGF agents are also being applied as new therapies for progressive diabetic retinopathy and diabetic macular degeneration (350). However, the use of intravitreous anti-VEGF treatment in diabetes patients has yet to be evaluated in phase III trials, and some caution is warranted. Indeed, one study reported that chronic inhibition of VEGF in the adult mouse eye could lead to a significant loss of neuronal RGCs (282) (Fig. 12C). Another concern is that prolonged VEGF inhibition may damage fenestrated vascular beds, which are more dependent on VEGF, such as for instance the choriocapillaris in the retina. Although a recent study failed to report regression of established choroidal neovascularization as well as damage to RGC axons in the optic nerve after transgenic overexpression of a VEGF antagonist (395), caution is warranted. Anti-VEGF drugs delivered within the vitreous can also pass into the systemic circulation, where neutralization of circulating VEGF levels could produce systemic adverse effects, ranging from teratogenicity, thrombosis, hypertension, bleeding, disrupted wound healing, hypothyroidism, fatigue, glomerular thrombotic microangiopathy, proteinuria and edema, posterior leukoencephalopathy, leucopenia, and skin toxicity (like rash and hand-foot-syndrome), increased incidence of stroke, and myocardial infarction (84, 98, 403).

VIII. VASCULAR ENDOTHELIAL GROWTH FACTOR IN ACUTE NEUROLOGICAL DISORDERS

VEGF has also been implicated in several acute neurological disorders, in which it can have both positive and negative effects, depending on the context. Indeed, in addition to its protective effects on neurons and ECs, VEGF has also negatively been implicated in BBB breakdown after ischemia and in mediating inflammatory responses (238, 348). These pleiotropic activities of VEGF make it challenging to foresee whether VEGF will aggravate or improve clinical outcome of experimental paradigms, and whether these effects are due to neuro-, glio-, or endotheliotrophic activities. Here, we first discuss the role of VEGF in ischemic brain diseases, followed by a description of the functions of VEGF in spinal cord injury, status epilepticus, and multiple sclerosis.
A. VEGF in Ischemic Brain Disease

VEGF has been implicated in brain ischemia, where it can mediate recovery according to three different mechanisms: VEGF can stimulate angiogenesis and modulate vascular permeability, exert direct neuroprotective effects, or promote neurogenesis. We will now discuss the evidence supporting a role for VEGF in each of these processes. In patients who have suffered a stroke, neuronal loss is proportional to the reduction in blood flow, and early reperfusion significantly correlates with survival (193, 241). Autopsy studies show that brain ischemia stimulates angiogenesis, especially in the ischemic penumbra, where blood flow is reduced and small increases in perfusion can determine the difference between cell death or survival (192). Histological studies have also demonstrated that HIF-1α triggers many downstream molecules in the ischemic border, including the expression of VEGF and its receptors, which can drive angiogenesis in the ischemic border (243). Overall, these observations suggest that enhancing the VEGF/VEGFR signaling pathway to stimulate angiogenesis after cerebral ischemia may exert beneficial effects in the brain.

Numerous studies investigating the therapeutic potential of VEGF have been performed. The first studies focusing on the role of VEGF in stroke did, however, not indicate that VEGF has a beneficial effect in models of brain ischemia. Indeed, intravenous delivery of VEGF early after the insult (within 1 h) worsens stroke outcome by increasing BBB leakage and hemorrhagic transformation of the ischemic lesions (396, 440). These detrimental effects of VEGF are due to its potent vessel permeability effects, which enhance acute brain edema immediately after ischemic stroke. In addition, VEGF administration enhances BBB disruption specifically in the ischemic penumbra through activation of the NO synthase pathway (55, 56). Consistently, intravenous delivery of VEGF antagonists immediately after the ischemic insult also reduces brain edema and infarct size (178). However, when systemically administered at later time points, i.e., after 48 h of the ischemic insult, VEGF exerts beneficial effects (440). Topical or intracerebral delivery of VEGF early after a stroke also exerts acute beneficial effects in various models of stroke (127, 167, 380). The continuous intracerebral administration of VEGF through encapsulated grafts (215, 261, 430) and viral-mediated hypoxia-inducible VEGF expression cassette has also been demonstrated to be beneficial (337). A study focusing on the functional outcome of intracerebroventricular delivery of VEGF further demonstrates that the sensorimotor and cognitive deficiencies following focal cerebral ischemia are improved after VEGF delivery (411). The delivery route (systemic versus local) and the timing of VEGF delivery (early versus late) thus seem to determine the outcome of VEGF therapy after an ischemic insult.

The beneficial effects of VEGF in these studies are at least partly related to its positive effects on the vasculature, as a denser vasculature in the ischemic penumbra correlated with improved neurological recovery (373, 440). Moreover, exercise-induced expression of VEGF increased microvessel densities in the brain and reduced neurological deficits and infarct volume after stroke (75). In addition, various studies report that VEGF also protects neurons from ischemic death. Although these neuroprotective effects might occur indirectly, as a result of improved perfusion of the ischemic penumbra, there is evidence that VEGF also directly affects the neurons. For instance, low doses of systemic VEGF promote neuroprotection of ischemic brains without inducing angiogenesis, whereas a high dose of VEGF induces angiogenesis but does not protect ischemic brains (239). VEGF overexpression, by the neuronal Nse promoter, in mice also significantly alleviates neurological deficits, infarct volume, and reduces disseminated neuronal injury and caspase-3 activity, thereby confirming earlier observations that VEGF has neuroprotective properties (440). The increased VEGF levels in these mice also result in a faster and more pronounced angiogenic response in areas exhibiting neuronal injury. However, cerebral blood flow studies suggest that VEGF overexpression induces a hemodynamic steal phenomenon, in which the higher vessel density in transgenic animals promotes a pronounced steal of blood flow away from ischemic to nonischemic areas which becomes more apparent towards the core of the ischemic area (440). Caution is thus warranted to prevent that inappropriate VEGF administration evokes such unfavorable hemodynamic effects. Intracerebroventricular infusion of VEGF also decreases infarct volume and brain edema without affecting cerebral blood flow (125). A study focusing on the association of VEGF expression to remote cortical areas after an infarct in the primary motor cortex also detects increased expression of VEGF in neurons of cortical areas functionally or behaviorally related to the area of infarct, suggesting that enhanced expression of VEGF in related remote cortical areas may be neuroprotective and stimulate recovery of function after stroke (368). VEGF thus seems to confer direct neuroprotection in the context of cerebral ischemia. These neuroprotective effects of VEGF are mediated, at least partly, by signaling through VEGFR-2 and its downstream PI3K/Akt signaling pathways (173).

A third mechanism by which VEGF might exert beneficial effects after brain ischemia is by stimulating neurogenesis. Intracerebroventricular delivery of VEGF stimulates neurogenesis, as bromodeoxyuridine (BrdU) incorporation in the two principal neuroproliferative zones of the mammalian brain, i.e., the subgranular zone (SGZ) of the hippocampal dentate gyrus and the rostral subventricular zone (SVZ), colocalizes with the neuronal lineage marker Dcx (151). It is thus likely that VEGF also con-
tributes to neurogenesis in a paradigm of cerebral ischemia. However, as an ischemic insult also increases BrdU labeling in the dentate gyrus and SVZ (150), the stimulatory effect of VEGF on neurogenesis can be masked by the ischemic insult. VEGF also exerts a survival effect on neuronal progenitor cells in the ischemic brain, as significantly more BrdU-labeled cell from the neuronal lineage are present in the ischemic zone after 4 wk in the VEGF-treated group, suggesting that VEGF not only stimulates the proliferation but also enhances the survival of neural progenitor cells. In addition, neuronal overexpression of VEGF enhances migration of newborn neurons toward sites of ischemic injury to replace neurons that undergo ischemic death (412). All these findings thus suggest that VEGF enhances postischemic neurogenesis. This also offers VEGF an additional therapeutic advantage for chronic brain repair.

Intriguingly, the therapeutic effects of VEGF in ischemic brain injury are also observed in models of spinal cord injury: in a model of severe spinal cord ischemia induced by clamping the thoracic vessels, most of the ventral horn neurons degenerate leading to complete paralysis in mice (206). VEGF treatment rescues some of the motor neurons from death in this model and results in improved neurological outcome (206). In another study, intubation of spinal subarachnoid space with particles releasing VEGF, also improves rehabilitation after a spinal cord ischemia, as evidenced by improved spinal-evoked and motor-evoked potentials after the VEGF treatment (53).

However, as mentioned previously, VEGF can also exert toxic side effects when delivered in the nervous system. This is further illustrated by the observation that ischemic brains treated with low doses of VEGF, which are insufficient to induce angiogenesis, exhibited reduced numbers of macrophages, whereas ischemic brains treated with an angiogenic dose of VEGF showed high macrophage density (240). Thus, although VEGF is a therapeutic candidate for stroke and other ischemic disorders, therapeutic use of this molecule warrants caution and careful consideration.

B. VEGF in Status Epilepticus

One day after a status epilepticus, VEGF expression is highly upregulated both in neurons and glial cells of the hippocampus and limbic cortex (66). VEGF also protects cultured hippocampal neurons against glutamate excitotoxicity (247). Intrahippocampal infusion of VEGF appears to protect hippocampal neurons from seizure-induced damage, as there is less neuronal loss with fewer pyknotic cells, whereas a VEGF trap significantly worsens cell loss relative to animals treated with the control (66). Seizure scores are, however, not significantly different from controls in these experiments. When VEGF is administered to adult rat hippocampal slices, it reduces the amplitude of excitatory responses elicited after stimulation and is able to suppress epileptiform activity in epileptic rats, but not in normal rat slices (251). VEGF may thus also play a role to decrease epileptiform activity in the epileptic brain.

C. VEGF in Multiple Sclerosis

There is considerable evidence that VEGF is also involved in various autoimmune disorders, as serum VEGF levels correlate with disease activity in a large number of autoimmune diseases (45). Very little is known, however, about the role of VEGF in multiple sclerosis (MS), the most common autoimmune disorder of the nervous system. Already over 130 years ago, MS lesions were found to be associated with abnormal blood vessels, and fingerlike projections (Dawson’s fingers) of demyelination were shown to extend into the white matter alongside the course of blood vessels (379). Increased formation, permeability, and perfusion of vessels have also been documented in MS lesions, whereas perfusion of grey matter is reduced, possibly reflecting the decreased metabolism secondary to neuronal and axonal loss (181, 182). Evidence for the occurrence of neovascularization in MS is observed with contrast-enhanced MRI in the appearance of “ring enhancement” at the periphery, but not at the center of chronic lesions (131). Another MRI study shows a positive correlation between VEGF serum levels and the length of spinal cord lesions, suggesting that VEGF might be involved in the formation of spinal cord lesions of MS (370). Increased levels of VEGF and its receptor VEGFR-1 are found in astrocytes in MS plaques during the inflammatory phase (113, 182, 306). In addition, intrastralatal infusion of VEGF aggravates plaque inflammation at the site of VEGF injection (306). Moreover, as VEGF expression is highly influenced by inflammatory cytokines and ischemia (see further below), the accumulation of VEGF may be the result, not the cause, of MS. When given intracerebrally to experimental autoimmune encephalomyelitis (EAE) rats, recombinant sVEGFR-1 reduces disease severity compared with untreated rats and decreases ED1 immunoreactivity, as a marker of inflammatory cells, in the CNS (444). While all these findings may suggest that VEGF, as a factor affecting vessels or inflammatory cells, aggravates MS, VEGF, as a neuroprotective factor, can also protect against axonal damage in MS. Thus the precise role of VEGF in MS remains enigmatic. Possibly, VEGF exerts a dual role in MS lesions: increased levels of VEGF can amplify vascular permeability in vessels and inflammation in glial cells during the acute phase of the disease, but can also stimulate the proliferation of neurons and their axons during the chronic phases of the disease.
D. VEGF in Spinal Cord Injury

VEGF has also been investigated for its ability to promote nerve repair following spinal cord injury. Expression of VEGF and its receptors is increased in traumatic injury lesions within the spinal cord or brain (352, 401). In the injured brain, VEGFR-2 expression is mainly upregulated in ECs (188), whereas the upregulation of VEGF and VEGFR-1 is predominantly associated with glial cells in the vicinity of the lesion (190). After a contusion injury, VEGF and VEGFR-1 expression is visible within 1 day of the injury in microglia, macrophages, and reactive astrocytes and persists for at least 14 days (61). Expression of VEGF stimulates axonal regeneration in preparations of sciatic nerves in vitro (135), suggesting that VEGF could exert beneficial effects in nervous system injury models. Indeed, adenoviral VEGF gene transfer promotes regeneration of corticospinal tract axons in rats following transection of the spinal cord (88). After a traumatic spinal cord injury, local delivery of VEGF also improves the recovery, which appears to be associated with increased vessel density and reduced neuronal apoptosis in the lesion area (420). Inhibition of VEGF-2 immediately after brain injury enlarges the hemorrhagic area, increases serum levels of the neural injury marker neuron-specific enolase and the glial injury marker S100, and leads to increased neuronal apoptosis (353), whereas delivery of a hypoxia-inducible VEGF plasmid improves the outcome in a rat spinal cord injury model (62). Although it is attractive to postulate a direct neuroregenerative role of VEGF in these studies, it is difficult to assess whether neural repair or neuroprotection are the result of a direct neural effect or are secondary to VEGF-driven angiogenesis and hence improved perfusion of the injured region. On the other hand, caution is warranted, since VEGF therapy can also worsen spinal cord injury, possibly secondary to its effect on vascular permeability (25).

VEGFR-2 and VEGFR-1 seem to have a differential role during the response to brain injury (189). Infusion of anti-VEGFR-2 antibodies decreases vascular proliferation and causes endothelial cell degeneration, however, without an effect on astrogliosis. In contrast, infusion of neutralizing antibodies to VEGFR-1 inhibits astrogial proliferation and increases endothelial degeneration slightly (189). Neutralization of VEGFR-1, but not of VEGFR-2, also reduces astrogial expression of the growth factors CNTF and FGF-2. This suggests that after injury, VEGF acts through VEGFR-1 on reactive astrocytes to increase their proliferation and to facilitate expression of trophic factors (189). Endogenous VEGF may thus also play an important role in the formation and maintenance of the astrogial scar, which is important to provide structural support and isolate the injury by restricting the migration of inflammatory cells into healthy tissue.

IX. VASCULAR ENDOTHELIAL GROWTH FACTOR-B: AN ANGIogenic OR NEUROTROPHIC FACTOR?

A. Angiogenic Activities of VEGF-B

VEGF-B is a secreted growth factor with sequence homology to VEGF-A. The VEGF-B gene, located on human chromosome 11q13, yields two polypeptide forms, i.e., VEGF-B167 and VEGF-B186 through alternative splicing (117, 288, 293). The amino acid sequence of VEGF-B167 is 44% identical to that of VEGF165, and the intermolecular disulfide bridging pattern of both molecules is similar. VEGF-B is therefore predicted to form a cysteine-knotted, disulfide-linked homodimeric protein, similar to VEGF (332). Unlike VEGF-B186, VEGF-B167 has a COOH-terminal heparin binding domain, allowing it to associate with pericellular heparin-like glycansaminoglycans, that anchor this growth factor in the extracellular matrix (288). The predominant isoform in mice is VEGF-B167, which is expressed at approximately four times the level of VEGF-B186. Interestingly, the VEGF-B promoter lacks putative binding sites for hypoxia-regulated factors, and consequently, VEGF-B is not regulated by hypoxia (83). VEGF-B is abundantly expressed in most tissues: during fetal development, it is expressed in cardiomyocytes, skeletal muscle, and SMCs of large vessels, while in adult mice, VEGF-B is abundant in heart, kidney, and brain in addition to other sites (1, 24, 198, 199). Mice lacking VEGF-B are healthy and fertile, with apparently normal developmental and physiological angiogenesis, indicating that this molecule is redundant in healthy conditions.

Studies using VEGF-B knockout mice have yielded conflicting results regarding the role of VEGF-B in angiogenesis and the development of the cardiovascular system. One study reported that VEGF-B knockout mice have smaller hearts, dysfunctional coronary arteries, and impaired recovery from experimentally induced myocardial ischemia (24), while another study documented that these mice show a subtle cardiac phenotype and that VEGF-B is not required for proper development of the cardiovascular system either during development or for angiogenesis in adults (2). Some reports also documented an angiogenic effect of VEGF-B (269, 349, 422), whereas others did not (2, 27, 313, 316, 377). More recently, reevaluation of the activity of VEGF-B in knockout mice on a congenic background proposed that the angiogenic activities of VEGF-B are restricted to the infarcted area of the myocardial heart, where it promotes angiogenesis and myocardial function (226, 388). Overall, VEGF-B seems to have a rather weak and restricted angiogenic activity, raising the question whether it might have arisen in evolution as a molecule with other activities, perhaps in the nervous system.

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B. Novel Role for VEGF-B in the Nervous System

In contrast to the minor effects of VEGF-B in the vascular system, recent studies established a more convincing role for VEGF-B in the nervous system. First of all, studies focusing on the role of VEGF-B in cortical neurons, spinal motor neurons, and RGCs revealed that VEGF-B is expressed in these cell types (227, 301, 372). Unlike VEGF, VEGF-B is not essential for development or maintenance of these neurons under normal conditions, suggesting that VEGF-B is less important than VEGF in the nervous system (227, 301, 372). However, upon ligation of the middle cerebral artery, the infarct volume in mice lacking VEGF-B increases by as much as 40% and results in a more severe neurological impairment (371). Since the cortical vasculature seemed unaffected in VEGF-B-deficient mice undergoing a ligation and VEGF-B protects cortical neurons in vitro against hypoxia, the protective activity of VEGF-B in these mice is most likely mediated through a direct neuroprotective effect of VEGF-B on neurons. This hypothesis was indeed confirmed in another study, which demonstrated that intracerebroventricular delivery of VEGF-B increases BrdU incorporation in cells of neuronal lineage both in vitro and in vivo (372). VEGF-B-deficient mice also display impaired neurogenesis, whereas intraventricular delivery of VEGF-B in VEGF-B-deficient mice restores neurogenesis to wild-type levels (372), thus indicating that VEGF-B is capable of regulating adult neurogenesis. Moreover, VEGF-B treatment rescues neurons from apoptosis in the retina and brain in mouse models of ocular neurodegenerative disorders and stroke, respectively, without promoting neovascularization, thus supporting the role of VEGF-B in the nervous system (227).

C. VEGFR-1 Mediates Neuroprotection and Glial Activation

In the nervous system, the VEGF-B receptor VEGFR-1 is also detectable in cortical and hippocampal neurons of neonatal and adult rat brain (429), as well as in motor neurons from adult mice (301). In situ hybridization studies further reveal that VEGFR-1 mRNA is detectable in the proliferating stem cells of the ventricular and subventricular zones, as well as in postmitotic differentiating cells of the embryonic cortex and hippocampus (177). In addition, VEGFR-1 is expressed in proliferating progenitor cells of the anterior horn (SVZa) of the cerebral lateral ventricle of adult macaques, as well as in cortical neurons from humans (390). Intriguingly, VEGFR-1 expression is strongly upregulated in reactive astrocytes in response to injury, e.g., following permanent and transient occlusion of the middle cerebral artery, neural grafting, tumor cell implantation, and after mechanical spinal injury (190, 191). Likewise, VEGFR-1 expression is strongly upregulated in activated astrocytes surrounding degenerating motor neurons from paralyzed SOD1 mice (301). Application of VEGF to cortical explants also increases astroglial proliferation, presumably by acting though VEGFR-1 expressed on reactive astrocytes, as silencing of VEGFR-1 further decreases astrogliosis (238). In focal cortical dysplasia patients, VEGFR-1 is expressed in reactive astrocytes, as well as in cells from the microglial/macrophage lineage (31). Some studies also found that endothelial cells upregulate VEGFR-1 (352, 420), although this was not confirmed by others (189).

A recent study confirmed that VEGF-B, through VEGFR-1 signaling, is critical for cell survival by down-regulating genes involved in apoptosis pathways in models of optic nerve crush injury, NMDA-induced retinal neuron apoptosis, or ischemic neuronal death induced by middle cerebral artery occlusion (227). VEGF-B also increases BrdU incorporation into cells of neuronal lineage (372) and protects motor neurons against degeneration. Indeed, when primary motor neuron cultures are isolated from normal mice, VEGF-B is neuroprotective (Table 3). In contrast, when motor neurons are isolated from tyrosine kinase-deficient VEGFR-1 mice, VEGF-B fails to exert any effect, thus confirming the role of VEGFR-1 as a neuroprotective receptor. In vivo, mice lacking VEGF-B or expressing a tyrosine kinase-deficient VEGFR-1, also develop a more severe form of motor neuron degeneration when intercrossed with mutant SOD1 mice (301). Intracerebroventricular delivery of VEGF-B186 also prolongs the survival of mutant SOD1 rats. Notably, at a dose effective for neuron survival, VEGF-B treatment does not seem to affect normal or pathological angiogenesis or influence blood vessel permeability. Compared with a similar dose of VEGF, VEGF-B is thus safer and does not cause vessel growth or BBB leakiness. Rather than acting as a molecule with combined angiogenic and neuropro-

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>VEGF-A</th>
<th>VEGF-B</th>
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<tbody>
<tr>
<td>Neurons</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Schwann cells</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Neuronal progenitor cells</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>Microglia</td>
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<td>ND</td>
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<tr>
<td>Astrocytes</td>
<td>+</td>
<td>ND</td>
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<td>Endothelial cells</td>
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VEGF, originally considered as an endothelial specific growth factor, has recently been shown to have direct effects on different neural cell types. These include the following: neuronal cells, on which VEGF stimulates survival and axonal outgrowth; neural stem cells, where VEGF activates proliferation, differentiation, and migration; Schwann cells, astrocytes, and microglia, on which VEGF triggers proliferation, survival, and migration. In contrast to VEGF, the effect of VEGF-B has little or no effect on the vascular system. However, VEGF-B also exerts potent effects on neural progenitor cells and neurons.
tective activities (such as VEGF), VEGF-B thus seems to have a more restricted neuroprotective effect in the nervous system.

Upregulation of PlGF, which also binds selectively to VEGFR-1, in the CNS has also been observed after focal cerebral ischemia (23). Although the precise role of PlGF in the CNS is not known, PlGF significantly reduces astrocyte cell death induced by oxygen and glucose deprivation (101), suggesting that it may also be protective under pathological conditions. In addition, PIGF-2, a PIGF isoform that binds to NRP1, was shown to chemoattract DRG axons in vitro (54).

X. CONCLUDING REMARKS

Since the discovery of the neuroprotective effects of VEGF on DRGs in 1999 and the fortuitous discovery in 2001 that a reduction in VEGF causes motor neuron degeneration in mice, a formidable amount of research has been performed to unravel the role of VEGF in the nervous system. By now, it is well-established that VEGF exerts direct in vitro effects on a variety of neural cells (Fig. 13). However, the molecular mechanisms activated by VEGF in neurons are mostly unknown. The unexpected effect of VEGF on neurons raises numerous exciting questions. For instance, is VEGF also acting as an axon guidance cue? Does VEGF regulate cytoskeleton rearrangements in neurons? What are the intracellular pathways activated by VEGF that lead to VEGF-induced neuronal proliferation survival, migration, or differentiation? How is VEGF regulating synaptic plasticity?

VEGF also has in vivo effects in acute or chronic models of neurodegeneration, including nerve and brain injuries. VEGF can thus be considered as a modifier capable of aggravating or reducing neurodegeneration, depending on whether its expression is reduced or increased. Many other growth factors have been reported to exert similar effects in these models. The effects of VEGF are, however, of particular interest, since they may result from stimulating both the nervous and vascular systems (Fig. 15). The protective effects of VEGF may indeed result from the combined effect on vessels, neuronal and nonneuronal cells; in nerve injury models, for instance, VEGF stimulates axonal outgrowth, Schwann cell proliferation, and nerve perfusion. In fact, these pleiotropic “multitasking” effects make VEGF a particularly suitable and promising candidate for therapeutic studies, especially since the treatment of neurodegeneration has so far been largely neurocentric.

There is also clear evidence that reduced VEGF levels cause neurodegenerative diseases. A causative role for VEGF has been documented in various motor neuron degenerative diseases. Motor neurons thus seem to be particularly dependent on sufficient VEGF for their survival. The observation that reduced levels of VEGF are causally involved in neurodegeneration might have implications for antiangiogenic therapies targeting VEGF in cancer or ocular diseases. Despite potential side effects, the evidence provided in this review illustrates that VEGF can be considered as an attractive therapeutic target. Indeed, gene-based therapy using a VEGF-expressing lentiviral vector, MoNuDin, is being developed for human use, and clinical studies delivering VEGF intracerebroventricularly for ALS patients have been initiated. Deciphering the therapeutic value of VEGF thus promises to be an exciting journey, which will hopefully lead to the discovery of an effective therapy for the neurodegenerative diseases.
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VEGF IN THE NERVOUS SYSTEM


Vascular endothelial growth factor (VEGF) has been extensively studied for its role in numerous physiological and pathological processes, including angiogenesis, neuroprotection, and neurodegeneration. Research has revealed the multifaceted effects of VEGF in the nervous system, with implications for diseases such as stroke, Alzheimer's disease, and multiple sclerosis.

1. **Angiogenesis**
   - VEGF plays a crucial role in the development and remodeling of blood vessels. In the central nervous system, VEGF is involved in the formation of new blood vessels during brain development and in response to injury.

2. **Neuroprotection**
   - VEGF can protect nerve cells from damage, especially under conditions of oxygen and glucose deprivation, by promoting survival and survival pathways.

3. **Neurodegeneration**
   - VEGF signaling is activated in response to injury and disease, contributing to neurodegeneration by promoting survival of neuroprotective mechanisms.

4. **Stroke and Alzheimer's Disease**
   - VEGF has been implicated in the response to oxygen and glucose deprivation in stroke models, as well as in the pathology of Alzheimer's disease, where it may contribute to neurodegeneration.

5. **Multiple Sclerosis**
   - VEGF affects immune cell trafficking and the development of demyelinating lesions. It also influences the formation of new blood vessels in multiple sclerosis, potentially contributing to disease progression.

6. **Brain Pathology**
   - VEGF can be upregulated in response to injury, contributing to both beneficial and detrimental outcomes, depending on the context and cell type.

7. **Genetic and Environmental Factors**
   - The role of VEGF in neurological diseases is modulated by genetic and environmental factors, influencing the severity and progression of these conditions.

8. **VEGF Receptor Signaling**
   - The VEGF receptor family is critical for VEGF signaling, with distinct receptors mediating different biological effects.

9. **VEGF Signaling in the Central Nervous System**
   - The complex interaction between VEGF and its receptors in the central nervous system highlights the need for further research to fully understand the role of VEGF in neuroprotection and neurodegeneration.

10. **Future Directions**
    - Continued research into the molecular mechanisms of VEGF signaling in the nervous system is essential for developing targeted therapeutic strategies for neurological diseases.

This comprehensive understanding of VEGF's role in the nervous system underscores the importance of targeted interventions in neurological diseases, potentially leading to improved patient outcomes.

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