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The Role of Vascular Endothelial Growth Factor in the Regulation of Development and Functioning of the Brain: New Target Molecules for Pharmacotherapy

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Abstract—There is evidence that members of the VEGF family are involved in the crucial processes in the brain: atherosclerosis, cerebral edema, neuroprotection, neurogenesis, angiogenesis, postischemic brain and vessel repair. Most of these effects are mediated by VEGF-A and the VEGFR-2 receptor. VEGF signaling pathways contain some potential targets relevant for pharmacological agents applicable for therapy of neurological diseases affecting the brain.

Keywords: vascular endothelial growth factors (VEGF), ischemic stroke, brain, neuroprotection, neurogenesis

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INTRODUCTION

Vascular endothelial growth factor (VEGF) was discovered in 1983 as a factor influencing the vascular permeability [1]. This protein plays a key role in the induction of angiogenesis in normal and pathological conditions [2]. The VEGF family includes seven members: VEGF-A, placenta growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D, and a group of viral homologs, referred to as VEGF-E and VEGF-F [3].

VEGF-A (the most common member of the family VEGF) is a homodimeric glycoprotein with a molecular mass of 36–46 kDa [4]. In the healthy organism, the highest VEGF-A mRNA levels are detected in the lungs, kidneys, heart and adrenal glands [4]. Smaller, but significant expression of VEGF-A is registered in the liver, spleen and stomach mucosa. VEGF-A exists as seven homodimeric isoforms consisting of 121, 145, 148, 165, 183, 189, or 206 amino acid residues [3]. Among these isoforms, VEGF165 is the most abundant and active [4].

VEGF-C, another well-known member of the VEGF family, is a dimeric protein with molecular mass of its pro-form of about 47 kDa [3]. This protein was discovered in 1996 as a vascular endothelial growth factor receptor-3 (VEGFR-3) ligand [5]. In the adult organism VEGF-C is predominantly expressed in the heart, placenta, ovary, small intestine and thyroid gland, while in embryos its expression is maximal in areas of lymphatic vessel sprouting from embryonic veins (e.g. in the jugular vessel area) [3]. VEGF-C is mainly involved in lymphatic vessel formation, which is carried out predominantly via VEGFR-3 activation. Besides lymphangiogenesis, VEGF-C also stimulates growth of blood vessels and regulates their permeability. In vascular endothelial cells VEGF-C interacts with VEGFR-3 or VEGFR-2 [6].

In addition to regulation of the growth and development of blood and lymphatic vessels, VEGF-C also plays an important role in the development of the nervous system during ontogenesis. Le Bras et al. have shown that VEGF-C is important for embryonic development of the brain neuroepithelial cells; acting on VEGFR-3 it influences a number of neural progenitor cells [7]. VEGFR-3 is expressed in ventricular and subventricular brain cells of mouse embryos. VEGF-C deficiency leads to rough developmental defects of mouse neuroepithelial cells expressing VEGFR-3. VEGFR-3 is expressed in oligodendrocyte progenitor cells and also in the olfactory bulb neuronal progenitor cells, which require VEGF-C for their proliferation [7].

1. VEGF RECEPTORS (VEGFR)

VEGFR along with such specific for endothelial signaling systems as angiopoetins and TIE receptors

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(tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains), VE-cadherin (vascular endothelium cadherin)/β-catenin and integrins, VEGF receptors (VEGFR) are involved at least in five processes crucial for vascular growth: vasorelaxation, stimulation of vascular permeability, endothelial cell migration, proliferation, and survival.

The VEGFR family includes VEGFR-1 (Flt-1—fms-related tyrosine kinase 1), VEGFR-2 (KDR—kinase insert domain receptor), and VEGFR-3 (Flt-4), which belong to the subfamily of receptor tyrosine kinases, constituting the group of platelet growth factor receptors. VEGFRs differ in their signaling activity and physiological functions.

The VEGFR tyrosine kinase activity is stimulated by specific ligands of the VEGF family: VEGF-A, PlGF, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F. PlGF and VEGF-B bind to VEGFR-1; VEGF-A interacts with both VEGFR-1 and VEGFR-2. VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3, while VEGF-E only activates VEGFR-2 [8].

The transmembrane protein neurophilin-1 involved in the regulation of axonal functioning, acts as a co-receptor for VEGF-B, PlGF-2, VEGF-E and the isoforms VEGF165 [9—12].

As in the case of many other tyrosine kinases, VEGFR ligand binding leads to dimerization and transphosphorylation of the receptor. This activates a complex intracellular signaling cascade, which triggers the angiogenic program of endothelial cells [13].

1.1. VEGFR-1

VEGFR-1 is a regulator of monocyte and macrophage migration [14]. Its affinity for VEGF-A is one order of magnitude higher than that for VEGFR-2 [15]. However, in contrast to VEGFR-2, it is difficult to register VEGFR-1 autophosphorylation after ligand binding. In many cases such VEGFR-2 effects as stimulation of proliferation and survival of endothelial cells can not be obtained by application of VEGFR-1 specific ligands; these effects are not detected in cells with VEGFR-1 overexpression and VEGFR-2 deficit [16]. This suggests that endothelial VEGFR-1 inhibits angiogenesis by sequestering VEGF-A and preventing VEGFR-2 activation [13].

1.2. VEGFR-2

VEGFR2 is involved in processes of normal and pathological of angiogenesis and is the main receptor mediating the VEGF action on endothelial cells [14].

Binding of VEGF-A to VEGFR-2 is accompanied by receptor dimerization and autophosphorylation. Since various intracellular proteins such as VEGF receptor-associated protein (VRAP), PLC-γ, and Shc2 (SHC-transforming protein 2) are bound to VEGFR, receptor activation causes their phosphorylation [17]. The phosphorylated VEGFR2 rapidly activates PLC-γ (phospholipase C gamma), which cleaves phosphatidylinositol 4,5-biphosphate (PIP_2) into two secondary messengers: sn-1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG directly activates certain protein kinase C isoforms, while IP3 promotes Ca^{2+} release from intracellular stores [18, 19].

VEGF-A stimulates nitric oxide release from endothelial cells; this is a consequence of increased intracellular concentration of calcium ions, which activate endothelial nitric oxide synthase (eNOS), and also Akt/PKB serine/threonine kinase (RAC-alpha serine/threonine-protein kinase/Protein kinase B alpha). Akt can phosphorylate and activate eNOS regardless of calcium ion concentrations. VEGF-A also stimulates the mitogen-activated protein kinase (MAPK) cascade [13]. Thus, VEGF-A binding to its receptor results in activation of a number of signaling pathways; this stimulates proliferation, survival, cell migration and permeability (figure).

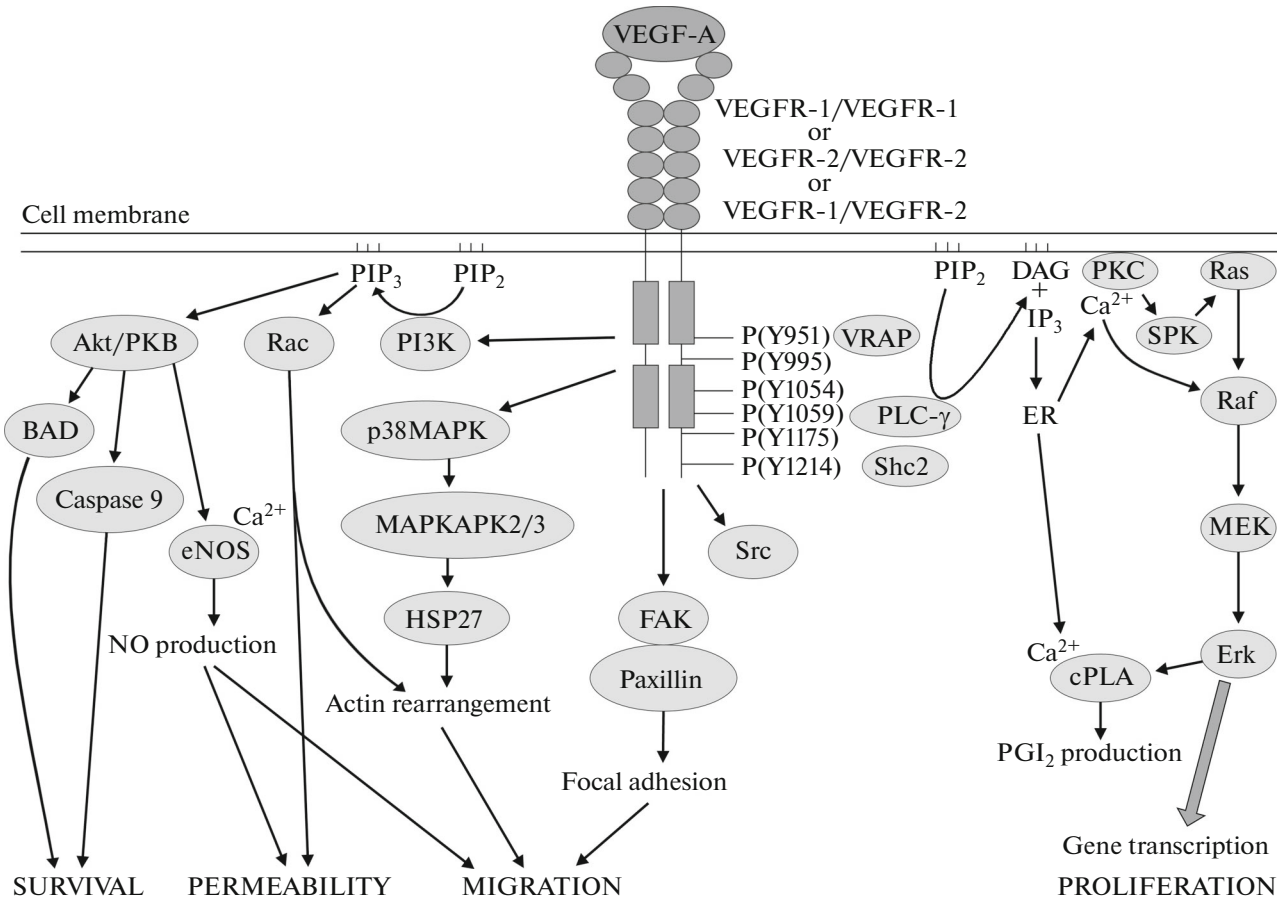
As noted above, VEGFR-3 plays an important role in the development and functioning of the endothelial cells of lymphatic vessels. Thus, VEGF-A-induced angiogenesis and neurogenesis are mainly realized via VEGFR-2 activity.

2. VEGF EFFECTS IN THE BRAIN

Basic angiogenic functions of VEGF-A include endothelial cell survival [16], induction of their proliferation [20], and also stimulation of migration and invasion of these cells [21]. Besides the primary role in angiogenesis, VEGF-A is also involved in other processes in the central nervous system, such as the ontogenesis of the nervous system cells, including migration, differentiation, synaptogenesis and myelination [22]; neuroprotection [23—26]; stimulation of adult neurogenesis [27—30]; post-ischemic recovery of brain tissue [14, 31] and vessels [32—34], stimulation of the hippocampus-dependent memory formation [35]. VEGF-A is also involved in pathological processes such as atherogenesis [36, 37] and cerebral edema formation [2, 38—42].

Although most of these processes occur in response to VEGF-A activation of VEGFR-2, in some cases VEGF-B, PlGF and VEGFR-1 are also involved. The VEGF signaling pathways contain some of potential targets important for the treatment of both acute ischemic stroke and during the rehabilitation period.

All members of the VEGF family play an important role in the development and functioning of the nervous and circulatory systems and therefore it is not surprising that these proteins are involved in the pathogenesis of stroke. As mentioned above, VEGF is implicated in all stages of angiogenesis, including neuroangiogenesis: de novo blood vessel formation from mesenchymal stem cells [21]; hypoxia stimulated for-



The main pathways of VEGF-A-mediated signal tranducstion. Explanations are given in the text. Abbreviations: PIP_2—phosphatidylinositol 4,5-bisphosphate; PIP_3—phosphatidylinositol 3,4,5-trisphosphate; PI3K—phosphatidylinositol 3-kinase; Rac—Rac family protein; Akt/PKB—alpha serine/threonine kinase/protein kinase B alpha; BAD—Bcl2 antagonist of cell death; eNOS—endothelial nitric oxide synthase; NO—nitric oxide; p38MAPK—mitogen-activated protein kinase p38; MAPK 2/3—mitogen-activated protein kinase 2/3; HSP27—heat shock protein 27; FAK—focal adhesion kinase; Paxillin—structural protein paxillin; Src—Src family tyrosine kinase; VRAP—VEGFR-associated protein; PLC-γ—phospholipase C gamma; Shc2—Src homology 2 domain containing transforming protein 2; DAG—sn-1,2-diacylglycerol; IP_3—inositol-1,4,5-trisphosphate; PKC—protein kinase C; ER—endoplasmic reticulum; SPK—stress-activated protein kinase; Ras—Ras family genes; Raf—protein kinase Raf; MEK—MAP kinase kinase; Erk—extracellular signal-regulated kinase; cPLA—cytosolic phospholipase A; PGI_2—prostaglandin I_2.

mation of new capillaries from existing blood vessels [33]; expansion of arteriolar anastomoses in response to changes in the blood pressure gradient [34]. VEGF also demonstrates direct neurotrophic and neuroprotective properties [23–26, 43–47]. Thus, the role of VEGF in the pathogenesis of stroke may be attributed to a combination of their angiogenic and neurotrophic activities.

3. CELL PRODUCERS AND MECHANISMS OF REGULATION OF VEGF EXPRESSION IN THE BRAIN

In rats with transient middle cerebral artery (MCA) occlusion, the VEGF expression level significantly differed in various brain regions [4]. The most pronounced VEGF expression was observed in neurons, with a gradual decrease in the intensity from the core to the periphery of the infarction zone. A lower VEGF expression was detected in astrocytes, particularly, near the infarction zone. A significantly lower VEGF expression level was detected in non-phagocytic microglia and also in vascular endothelial cells surrounding the infarction zone [4].

Under normal physiological conditions, all cells of the adult organism are supplied with adequate amounts of oxygen needed for their metabolism. Oxygen is transported by circulating red blood cells (RBC) and RBC production is controlled by the hormone erythropoietin (EPO). In the liver and kidneys EPO-producing cells are sensitive to changes in oxygen concentrations and conditions of systemic and regional hypoxia stimulate EPO gene transcription [3].

VEGF-A plays a central role in angiogenesis and neovascularization by promoting increased delivery of oxygen and energy substrates. Hypoxia and hypoglycemia induce its expression via specific hypoxia-regulated VEGF-A gene enhancer sequences [48]. For example, hypoxia-inducible factor-1 (HIF-1) binds to the enhancer sequences of the VEGF-A gene. This induces transcription and stabilizes mRNA [49]. Ma et al. have shown [50] that hypoxia stimulates VEGF expression by neurons of the brain and physical exercises increase this effect.

Tumor necrosis factor-alpha—TNF-α is a proinflammatory cytokine that exhibits a wide range of biological effects including angiogenesis. It indirectly stimulates blood vessel formation and induces secretion of angiogenic molecules, including VEGF-A and VEGF-C [51]. Good evidence exists that TNF-α induces transcription of VEGFR-2 genes in vascular endothelial cells [52]; it appears that TNF-α is also involved in the regulation of neuorophilin-1 transcription. In addition to TNF-α, a number of tissue growth factors such as TGF (tissue growth factor), EGF (epidermal growth factor), PDGF-BB (platelet-derived growth factor BB) induce VEGF-A mRNA expression [3].

4. THE ROLE OF VEGF IN VARIOUS ASPECTS OF PATHOGENESIS OF ISCHEMIC STROKE

4.1. VEGF and Atherosclerosis

Stroke develops due to focal cerebral ischemia or (more rarely) due to cerebral hemorrhage. Causes of focal cerebral ischemia include thrombosis of large or small blood vessels or arterio-arterial/cardiogenic embolism [53]. The process of atherosclerotic vascular occlusion mainly involves VEGF-A.

Atherosclerosis is a complex degenerative inflammatory process primarily affecting large and medium-sized arteries, especially at branch points. Atherosclerotic plaques can cause a stroke either due to their destruction (leading to appearance of the thrombogenic and embologenic material in the bloodstream), or total/subtotal occlusion of the artery.

Plaques are characterized by at least two process associated with VEGF-A overexpression: inflammation and hypoxia [36]. These processes result in increased levels of HIF-1 and other transcription factors associated with the VEGF-A expression in plaque macrophages and vascular smooth muscle cells. VEGF-A acts on the vasa vasorum of arteries affected by atherosclerosis and stimulates angiogenesis, which in some cases leads to intraplaque hemorrhage and plaque destruction.

VEGF-A may also promote atherogenesis by stimulating migration of vascular smooth muscle cells; this involves activation of phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) 1/2 [37]. VEGF-B is involved in the regulation of fatty acid uptake by endothelial cells [54] and this also promotes atherogenesis.

4.2. VEGF and Angiogenesis

VEGF-A is the main mediator of cerebral angiogenesis, which is activated after stroke in rats [55] and humans [56]. Several studies have shown the spatial and temporal correlation between the expression of VEGF-A or VEGF receptors and angiogenesis after transient MCA occlusion in rats [57, 58]. Intravenous and intraventricular administration of VEGF-A also stimulated postischemic angiogenesis [2, 59]. Pretreatment of mice with intraventricular administration of VEGF-A promoted formation of mature, functioning blood vessels that provided better survival of the penumbra (the border zone of ischemia), acceleration of cerebral blood flow and stabilization of the brain energy balance after transient MCA occlusion for 90 min [60]. VEGF-A is obviously involved in the nitric oxide induced angiogenesis after ischemic stroke [61].

4.3. VEGF and Collateral Circulation

Vascular collaterals protect tissues against ischemic damage by providing alternative bypasses for the arterial blood flow. Blood flow through existing collaterals is activated by a change in the blood pressure gradient between passable and clogged blood vessels almost immediately after occlusion. Sources of collateral blood flow in the brain include both intra- and extracranial vessels. The adequacy of the collateral flow is the determining factor that determines the stroke severity and therapeutic efficiency [62].

The new vessels, by which blood flow is redirected in response to focal hypoperfusion, are formed during the so-called arteriologenesis, when remodeled arterioles are increased in size; this results in increased volume and the rate of blood flow [34]. It is believed that VEGF-A participates in collateral vessel formation during ontogenetic development and in the recovery period after acute cerebral ischemia, rather than directly at the onset of acute ischemic stroke [63].

4.4. Ischemic Induction

Several studies have demonstrated the effect of cerebral ischemia on the expression of VEGF and VEGFR, mainly in the ischemic border zone also known as the penumbra. This area, surrounding the infarct core, remains viable pending reperfusion, and clinical outcome of stroke depends on the fate of the penumbra [53].

VEGF-A expression increased in neurons, astrocytes and macrophages, while VEGFR-1 expression increased in endothelial cells, over days to weeks following MCA occlusion in rats [4]. VEGF-A mRNA and protein also increased within several hours of

MCA occlusion in rats, with subsequent rapid decrease in neurons and more sustained expression in pial cells [64].

A comparative study of transient and permanent MCA occlusion in rats revealed increased levels of VEGF-A (in neurons and endothelial cells), VEGFR-1 (in neurons, endothelial cells and astrocytes), and VEGFR-2 (in endothelial cells and astrocytes), which were detectable at 1–3 days after occlusion. More pronounced changes were found in the ipsilateral hemisphere and after permanent MCA occlusion [65]. In the other rat MCA occlusion study, VEGF-A mRNA and protein were increased, with predominant expression in astrocytes [66], while another study revealed that microglia/macrophages were the main site of VEGF-A mRNA and protein expression [67].

In a neonatal rat model of perinatal hypoxic-ischemic injury the level of VEGF-A protein and its expression were also higher in neurons within the first 24 hours after MCA occlusion [68]. A photothrombotic ring model of stroke in rats revealed upregulation of not only VEGF-A, VEGFR-1 and VEGFR-2, but also of VEGF-C and VEGFR-3/Flt-4 proteins [69].

4.5. VEGF and Cerebral Edema

Originally, two major biological effects of VEGF-A have been discovered: the effect on angiogenesis and vascular permeability [1]. The latter effect is associated with tissue edema; in a closed compartment like the skull, it can be lethal. Cerebral edema is a frequently registered and often fatal complication of stroke [53]. For this reason, special attention is paid to the ability of VEGF-A to increase permeability of cerebral blood vessels. The main mechanisms of this process obviously include transendothelial transport of small solutes via cytoplasmic fenestrations and plasmalemmal caveolae, as well as leakage of fluid and plasma proteins and extravasation of blood cells through interendothelial tight junctions [2, 38–42].

The blood-brain barrier (BBB), also known as a neurovascular unit consists of the endothelium of brain capillaries, extracellular matrix components, and a number of cells such as astrocytes, pericytes and neurons [38]. Some effects of exogenous VEGF on endothelial cells in vitro are frequently attributed to nitric oxide (NO) generation by these cells. In vivo, NO increases the rate of cerebral blood flow. Application of exogenous VEGF increases BBB permeability via the signaling cascade NO-synthase/cGMP [61].

Experiments performed using a brain cell culture revealed that in response to oxygen and glucose deprivation neurons stimulated a cascade of biochemical reactions resulted in impaired integrity of the BBB and activation of astrocytes that increased VEGF secretion, which resulted in the damage of endothelial cell proteins, occludin and claudin-5 [42]. Inhibition of VEGF expression in astrocytes by small interfering RNA (siRNA) resulted in inhibition of these processes. Although ischemia-exposed neurons do not have direct contacts with the endothelial cells of brain capillaries, the results of this study suggest that these neurons can promote BBB damage by stimulating VEGF expression in astrocytes.

In mice exposed to transient MCA occlusion, the use of a protein analogue of VEGFR-1, sequestering VEGF, decreased the cerebral infraction volume and severity of edema compared with control group [39].

In another study, intravenous administration of VEGF-A to rats 1 h (but not 48 h) after MCA occlusion increased both extravasation of an intravenously administered contrast agent (as measured by magnetic resonance imaging) and the infarct size [2].

Subsequent studies have shown the ability to counteract the vascular permeability effect of VEGF-A in rats with anti-VEGF-A neutralizing antibodies [70] or the growth factor angiopoietin-1 [71].

4.6. VEGF and Neuroprotection

Despite the fact that VEGF-A has been originally investigated in the context of its effects on endothelium, results of modern studies clearly indicate that it can act on several other cell types, including neurons (table).

Neurotrophic effects of VEGF-A have been described in a variety of peripheral [43, 44] and central [45–47] neuronal preparations. VEGF-A promoted neuronal survival in cell culture models of stroke involving oxygen and glucose deprivation [72] or excitotoxicity [23–25]. It has been also implicated in some forms of hypoxic preconditioning in vitro [26]. Most of these effects are mediated by activation of VEGFR-2, PI3K, and ERK1/2.

Intranasal administration of VEGF-A to rats could improve cognitive function, synaptic plasticity and prevent hippocampal neuronal damage in a model of global cerebral ischemia [25]. VEGF-A was able to restore membrane potential, decrease the excitability of neurons and spontaneous excitatory postsynaptic potentials at an early stage of cerebral ischemia; these effects are associated with the VEGF-A action on pyramidal neurons [25]. Acting at VEGFR-2, VEGF-A, prevented death of the CA1 pyramidal neurons [25].

Topical application of VEGF-A to the cortical surface reduced [73], whereas intraventricular infusion of anti-VEGF antibodies increased [74] the infarct volume in rats, thus suggesting a neuroprotective effect of VEGF-A.

Nevertheless, reducing VEGF levels by a VEGF-sequestering analog of the VEGFR-1 protein reduced the brain infarction size; this effect is possibly determined by decreased edema zone rather than a direct adverse effect of VEGF on the ischemic brain tissue [39].

The role of VEGF in various physiological and pathophysiological processes

Process	Effect	Reference
Atherogenesis	Stimulates angiogenesis in atherosclerotic plaques	[36]
	Stimulates migration of vascular smooth muscle cells	[37]
Angiogenesis	Provides survival of endothelial cells	[16]
	Regulates endothelial cell proliferation	[20]
	Regulates migration of endothelial cells	[21]
	Regulates differentiation of vascular endothelial cells	[21, 89]
	Stimulates neovascularization in the penumbra	[57, 58, 60]
	Stimulates growth of collateral vessels in response to ischemia	[32, 33]
	Promotes expansion of existing arterial anastomosis	[34]
Vascular permeability	Promotes extravasation of plasma proteins and fluids from blood vessels	[2, 38, 39]
	Stimulates NO generation by vascular endothelial cells	[40, 41]
	Activates astrocytes and causes damage of structural proteins of vascular endothelial cells	[42]
Neuroprotection	Stimulates axonal growth in peripheral neurons	[43, 44]
	Stimulates growth of microtubules in neurons of the cerebral cortex	[45]
	Promotes growth of axons in cortical neurons	[46, 47]
	Inhibits glutamate excitotoxicity	[23–25]
	Participates in the ischemic preconditioning of CNS neurons	[26]
Neurogenesis	Stimulates maturation of neuronal progenitor cells during embryogenesis	[22]
	Participates in synaptogenesis and formation of CNS conductive pathways	[22]
	Stimulates proliferation and differentiation of neural progenitor cells during ischemic brain damage	[27–29]
	Stimulates migration of newly formed neurons to the ischemic core of the brain	[14, 31]
	Stimulates astrocytes and facilitates acquisition of stem cell properties	[30]
	Stimulates the hippocampus-dependent mechanisms of memory formation	[35]

The biphasic effect of intravenously administered VEGF-A on the infarct volume in rats should be also mentioned again: anatomic worsening was observed after administration 1 h post-ischemia, while administration at 48 h post-ischemia improved neurobehavioral functions [2]. Intraventricular administration of VEGF-A to rats started at 24 h after MCA occlusion and continuing for 3 days, reduced the infarct volume by approximately one-third at 1 month post-stroke, and also improved sensorimotor and cognitive deficits, with behavioral improvement persisting for at least 2 months [75]. Similar results have been obtained in transgenic mice overexpressing VEGF-A, as compared with the control group [76]. Delayed administration of VEGF-A to newborn pups in the neonatal stroke model accelerated recovery by stimulating angiogenesis, action on microglial cells, and modulation of the inflammatory response [77].

An important conclusion from these studies is that the timing and route of administration VEGF-A are the most crucial factors in achieving a desired therapeutic result.

4.7. VEGF and Neurogenesis

Studies performed on the human brain revealed that starting from the second trimester until the 13th month after birth VEGF-A expression was detected in progenitor cells (radial glial cells and the external granular layer) and also in migrating neurons, astrocytes, and mature oligodendrocytes [22].

These results suggest that the cerebral cortex neurons are exposed to VEGF-A during the most important developmental stages (migration, maturation, branching of axons and dendrites). High expression of VEGF-A and VEGFR-2 has been detected in most pathways of the internal capsule, in the commissures

of the telencephalon and cerebellum white matter, thus suggesting the role of these proteins in the formation of brain pathways and synaptogenesis [22].

Neurogenesis takes place in the developing as well as in the adult human brain. The most active zones of neurogenesis include the hippocampal dentate gyrus and the zone surrounding the lateral ventricles (subventricular zone). Neurogenesis in adult humans is regulated by neurotransmitters, hormones and growth factors, and also by behavior and by pathological processes [63].

Stroke-induced neurogenesis was demonstrated in various rodent models [14, 30] and in humans [31]. Activation of neural cell proliferation above physiological levels was observed in both dentate gyrus and subventricular zone, and was accompanied by migration of newborn neurons from the latter site toward the ischemic lesion [14, 29, 30].

In the brain, exposed to ischemia neurogenesis and angiogenesis represent two parallel and interrelated processes. It is suggested that the signaling pathway VEGF-A/VEGFR2 is the molecular basis that links together the processes of angiogenesis and neurogenesis. In particular, VEGF-A/VEGFR2 can play a key role in neuroblast migration from the ipsilateral subventricular zone along the blood vessels to the center of ischemic lesion [14].

Exogenous VEGF-A stimulated neurogenesis in both embryonic rat brain cultures [78] and in the brain of adult rats after MCA occlusion [59].

Astrocytes activated after cerebral ischemia express nestin, a marker of endogenous neural stem cells [79]. Such astrocytes acquire properties of stem cells and can differentiate into neurons. Nestin expression by astrocytes is also accompanied by astrocyte VEGF overexpression in the ipsilateral striatum. Moreover, increased VEGF concentration stimulates formation of new reactive astrocytes exhibiting properties of stem cells [30]. Thus, VEGF-A stimulates neurogenesis not only in healthy, but also in the ischemic brain, and can promote adaptation after stroke.

The table summarizes data on the participation of VEGF in the regulation of various physiological and pathophysiological processes.

5. TARGET MOLECULES OF THE VEGF MEDIATED SIGNAL TRANSDUCTION FOR THERAPY OF CNS DISEASES

Lack of adequate efficacy of modern neuroprotective drugs available in the market stimulates constant search for new modulators applicable for the treatment of CNS diseases. Certain attention is also paid to characterization of neuroprotective effects of existing drugs and techniques, justification of their use to improve brain functioning.

It is believed that the neuroprotective effect of the lipid-lowering drugs, statins, is mainly associated with their ability to stimulate VEGF expression. Use of atorvastatin therapy for treatment of elderly rats with experimental ischemic stroke was accompanied by VEGF mediated expression of the nerve cell differentiation genes Mash1 and TUJ1 in the subventricular zone. This effect of atorvastatin was nullified by VEGF inhibition [80]. Hyperlipidemia differentially (depending on atherogenic lipids concentrations in the blood) inhibited VEGF-induced capillary growth and pericyte coverage of the cerebrovascular endothelial layer [81]. This compromised the restoration of cerebral blood flow in ischemic stroke, thereby reducing the penumbra and increasing the core of cerebral infarction.

Warner-Schmidt et al. [82] proposed a hypothesis that VEGF is a key mediator, determining behavioral and neuroprotective effects of antidepressants. Treatment with serotonin and noradrenaline reuptake inhibitors increases VEGF levels, which stimulate proliferation of the neural progenitor cells of the subgranular zone in rats.

Interesting results on the role of VEGF-mediated signal transduction in the treatment of depression were obtained by Huang et al. [83]. The authors placed adult mice for 7 days in cages under so-called conditions of enriched environment. After 7 days these animals had marked positive changes in behavior, as well as the increase in the number of dendrites of pyramidal neurons in the hippocampal CA1 area. These effects were apparently determined by stimulation of VEGF expression through a transcriptional mechanism involving HIF-1α, which blocks endogenous miR-107.

In mice with transient MCA occlusion, the use of the VEGFR-1 protein analog, mFlt(1-3)-IgG, sequestering VEGF, decreased the cerebral infraction volume and severity of edema compared to control group [39]. Subsequent studies have demonstrated the ability to suppress the effect of vasogenic VEGF-A in rats by means of anti-VEGF-A neutralizing antibodies [70] and by applying the growth factor angiopoietin-1, which is a ligand for the endothelium-specific receptor tyrosine kinase TIE2. This receptor protects adult human peripheral vasculature against plasma extravasation in the extracellular space [71].

Recently it has been demonstrated that activation of integrin-associated CD47protein regulates mechanisms responsible for BBB damage and formation of cerebral edema in cerebral ischemia. Application of 4N1 K, a specific CD47-activating peptide, stimulated expression of VEGF and matrix metalloproteinase-9 [84]. This suggests that CD47 may serve as a potential molecular target for the treatment of ischemic stroke.

Another well-known modulator of VEGF activity is EPO, which improves functional recovery after traumatic brain injury through VEGF expression and VEGFR2 phosphorylation. The study by Xiong et al. [85] demonstrated that EPO significantly stimulated

cell proliferation, angiogenesis, and neurogenesis in the hippocampal dentate gyrus.

The signaling pathway caveolin-1/VEGF plays an important role in the regulation of signal transduction, endocytosis, transcytosis, molecular transport, development of the embryonic vasculature, angiogenesis in adulthood, normal growth and tissue development, wound healing, and in some pathologic processes (ischemia, tumor growth) [86]. The study of the impact of treadmill training exercise on cerebral angiogenesis in rats after MCA occlusion has shown that the signaling pathway caveolin-1/VEGF may be a potential target for therapeutic intervention in ischemic stroke [87].

The other study performed on rats with transient MCA occlusion followed by physical rehabilitation in the form of treadmill exercises also demonstrated that VEGF played an important role in hypoxic initiation of proliferation and migration of endothelial cells within posthypoxic angiogenesis, and migration of newly formed neurons [50]. Migration of proliferating cells requires controlled degradation of the basement membrane and the surrounding extracellular matrix. This degradation involves proteinases. Matrix metalloproteinases are a large family of zinc-dependent proteases that can degrade components of the extracellular matrix. It is known that VEGF stimulates the formation of the active form of the matrix metalloproteinase 2 (MMP-2) by inhibiting the activity of tissue inhibitor of metalloproteinases-2 [88]. After MCA occlusion expression of MMP-2 and VEGF was stably increased and treadmill exercises caused further stimulation of expression of these proteins [50]. After 16 days postischemia, animals exposed to treadmill exercises were characterized by a significant increase in the rate of cerebral blood flow, as compared to rats that did not perform such physical exercises. Running also significantly improved the results of neurobehavioral test compared with the control group [50]. The effect of treadmill exercises on MMP-2 expression, regional cerebral blood flow, and neurological deficit was blocked by bevacizumab, the anti-VEGF monoclonal antibody. Methods of physical rehabilitation favorably influence the outcome of ischemic stroke. These effects are largely due to the activation of signaling cascades involving VEGF [50].

CONCLUSIONS

Members of the VEGF family play an important role in various aspects of pathogenesis of nervous system diseases, including ischemic stroke. They are involved in the pathogenesis of atherosclerosis, which is a frequent cause of cerebrovascular diseases. VEGFs are potent stimulators of angiogenesis, allowing the formation of new vascular collaterals, which supply oxygen and nutrients to the brain infarct zone. VEGF-A expression is elevated in the ischemic penumbra and remote areas of the brain involved in postischemic recovery of lost functions. VEGF-A stimulates neuroprotection, neurogenesis, and angiogenesis in the acute and rehabilitation periods of ischemic stroke and traumatic brain injury, it plays the well-known beneficial role in stress and depression. In animal models of various diseases of the nervous system it has been shown that the use of both the VEGF-A, and pharmacological agents stimulating its expression, as well as methods of physical therapy, has a positive therapeutic effect. However, taking into consideration such pathological effects of VEGF-A, as the ability to increase vascular permeability and enhance cerebral edema, further research is needed to assess the prognostic significance of VEGF-A, and the possibilities of its therapeutic use.

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