Neuroprotection and Angiogenesis: Dual Role of Erythropoietin in Brain Ischemia

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Erythropoietin, originally defined as an erythroid growth factor, is upregulated in the brain under conditions of hypoxia. So far, two functions have been identified for this locally produced cytokine: a direct protective effect on neuronal cells during cerebral ischemia and an indirect protection of brain tissue that could be provided by promoting brain vessel growth.

Erythropoietin is a cytokine that is commonly associated with its role as a hormonal stimulator of erythropoiesis. Erythropoietin is produced either in the fetal liver or the adult kidney and gets released into the blood. It then makes its way to those erythroid progenitor cells in the bone marrow that otherwise would undergo apoptosis.

The production of erythropoietin is controlled by the systemic availability of oxygen carriers, i.e., erythrocytes in a closed-loop feedback system. However, other organs, such as the brain, also produce erythropoietin under conditions of local hypoxia. This brief review will deal with this latter aspect.

Production sites of erythropoietin distinct from kidney and liver

Ratcliffe and colleagues (14) were the first to show that erythropoietin is produced in rat organs other than the kidney and the liver. In unstimulated animals, erythropoietin mRNA was detected, among other organs, in lung, testis, and brain but was not found in muscle, intestine, or bone marrow. After exposure to severe hypoxia, erythropoietin mRNA was significantly increased in the spleen and mainly in the testis and the brain (14). These findings raise several issues of great importance. First, the modulation of erythropoietin expression in many organs by hypoxia led to the hypothesis that oxygen sensing is a general phenomenon and is therefore widespread and found in other organs as well. Indeed, the subsequent discovery of a hypoxia-inducible transcription factor (HIF-1), which is expressed in all cells of the body so far investigated, proved the validity of this hypothesis. HIF-1 controls the oxygen-dependent regulation of a whole variety of genes, including erythropoietin, the glycolytic enzymes, vascular endothelial growth factor (VEGF), and many others (15). Second, because there is a functional blood-brain barrier to diffusion of substances into the blood in testis as well as in the brain, the question arose as to whether erythropoietin expression in these organs serves a local physiological function. In particular, the occurrence of erythropoietin and its receptor in the brain increased interest in the physiological role of this cytokine in the central nervous system. It is of note in this connection that blood-borne erythropoietin cannot cross the blood-brain barrier to get access to the brain. For the same reason, brain-derived erythropoietin cannot get into the circulation and contribute to hematopoiesis in the bone marrow. Accordingly, the local production of erythropoietin in the brain must account for any physiological effects within this organ.

Cellular origin of brain-derived erythropoietin and its receptor

Subsequently, erythropoietin was found in the brain of other species as well. Its mRNA was detected in biopsies from the human cortex and hippocampus and in various brain regions of rhesus monkey and mouse (6). In addition, erythropoietin protein was also measured in the supernatant of cerebrospinal fluid of human patients. Upon exposure to systemic hypoxia (8% O₂) or to 0.1% carbon monoxide, resulting in functional anemia and subsequent severe tissue hypoxia, erythropoietin mRNA in the brain of mice and monkeys increased up to 20-fold, depending on the severity of the hypoxic stimulus (Fig. 1A) (6). On a cellular level, primary cultures of both astrocytes and neurons were shown to produce erythropoietin (Fig. 1B) (1, 4, 6, 8). When incubated at a low oxygen concentration, astrocytes showed a >100-fold time-dependent erythropoietin mRNA accumulation (Fig. 1A) and also increased their erythropoietin protein production (6), whereas neuroblastoma cells only showed a twofold induction of erythropoietin mRNA during hypoxia (4), suggesting that astrocytes are the main producers of erythropoietin in the brain. Interestingly, the brain-derived erythropoietin was smaller in size (33 kDa) compared with serum erythropoietin (35 kDa), which is probably due to a lesser sialylation of the brain-derived form (8).

In analogy to the erythroid tissue, in which erythropoietin exerts its function solely after binding to its receptor, one should expect expression of the erythropoietin receptor in the central nervous system. Indeed, erythropoietin binding sites were found in various areas of the brain, including cortex, hippocampus, and midbrain (2). In addition, erythropoietin receptor mRNA and protein were identified by...
During cerebral ischemia, the cellular expression pattern of erythropoietin and its receptor is specifically modulated as a function of the duration of ischemia in neurons, endothelial cells, and glial cells (1). Furthermore, erythropoietin receptor expression is upregulated in the ischemic penumbra (1, 11). These data suggest that the erythropoietin/erythropoietin receptor system might be involved in processes such as neuroprotection and tissue remodeling, e.g., angiogenesis and gliosis during the active evolution of a focal cerebral infarct. This aspect is discussed below.

The functional role of erythropoietin in the brain

Effects of erythropoietin in vivo. The above-mentioned modulation of expression of erythropoietin and its receptor during ischemia led to the idea that exogenously added erythropoietin might be effective as a therapeutic strategy for stroke. Indeed, by using various models of cerebral ischemia, independent groups of investigators came to the same conclusion: erythropoietin can act as a neuroprotective factor. In a model of focal cerebral ischemia, in which the middle cerebral artery is permanently occluded by electrocoagulation, mice were treated with recombinant erythropoietin (0.4 µg/kg) by intracerebroventricular injection 24 h before the induction of focal ischemia. The volume of the cortical infarct in the erythropoietin-pretreated group was significantly reduced by 47% compared with the vehicle-treated control group (Fig. 2) (1). The same erythropoietin dose administered at the time of occlusion was without effect, suggesting that erythropoietin might exert a preconditioning effect. In a similar model in the rat, the group of Sasaki (11) has shown that continuous infusion of erythropoietin via an osmotic minipump into the ventricles for 28 days prevented ischemia-induced place-navigation disability as well as cortical infarction and supported neuronal survival in the thalamus. The same group also used a model of global cerebral ischemia in gerbils by clamping both common carotid arteries for 3 minutes (12). This procedure is a model for cardiac arrest and typically leads to a delayed neuronal cell death in the CA1 region of the hippocampus. Infusion of erythropoietin into the lateral ventricles of the gerbils rescued hippocampal CA1 neurons from ischemic damage and increased the number of intact synapses in this area (Fig. 3). Furthermore, infusion of a soluble erythropoietin receptor into the brain of the gerbils, submitted to a mild ischemic treatment that did not produce neuronal damage by itself, induced neuronal damage, demonstrating that the endogenous brain-derived erythropoietin is crucial for neuronal survival (12). Erythropoietin, therefore, is another member of a growing number of cytokines and growth factors that have been shown to be neuroprotective.

In addition, it has been shown that erythropoietin injection into the uterine cavity stimulated angiogenesis in the endometrium in vivo and also elicited an angiogenic response in the chicken chorion allantois membrane assay, strongly suggesting that erythropoietin is indeed an angiogenic factor as well (10). Thus these findings and the expression pattern of the erythropoietin receptor, i.e., the expression in neurons, endothelial cells, and astrocytes, suggest that erythropoietin may have a direct neuroprotective action as well as an action on endothelial cells and astrocytes that might also lead indirectly to neuroprotection and tissue salvage.

Neuroprotection and angiogenesis by erythropoietin in vitro. It is commonly accepted that hypoxia/ischemia of the brain leads to a greatly enhanced release of the excitatory amino acid glutamate. In a large series of experiments, it was shown...
that the neuronal damage produced by hypoxia/ischemia is indeed mediated by the so-called glutamate neurotoxicity. An increase of glutamate has reached the range of 1 mM in the extracellular space, thus exceeding the normal concentration by a factor of ~500. The glutamate-induced neurotoxicity is mainly mediated by the so-called N-methyl-D-aspartate (NMDA) receptor. Binding of glutamate to this receptor leads to a massive entry of calcium, sodium, and water and subsequently to impairment of mitochondrial function, excessive free radical production, cell swelling, and neuronal cell death. Under the assumption that glutamate toxicity is a major player in the hypoxic/ischemic-induced damage of neuronal cells, the following in vitro findings seem of major importance.

First, cell death, induced by exposure to 1 mM glutamate, of hippocampal and cortical primary neurons is prevented by pretreatment of the cells with erythropoietin before glutamate challenge. This protective effect is dose-dependent, starting at 3 µM erythropoietin (10 µU/ml) and reaching a maximum between 30 and 300 µM (1 U/ml). Simultaneous addition of a soluble erythropoietin receptor completely abolishes the protective effect of erythropoietin (9). For manifestation of neuroprotection, erythropoietin pretreatment has to start at least 8 h before exposure to glutamate to be effective. However, a brief incubation with 30 µM erythropoietin for as little as 5 min, 24 h before exposure to glutamate, is as effective as 24 h pretreatment with the same dose of erythropoietin. Second, in similar experiments, 24 h pretreatment with 300 µM erythropoietin protects primary mouse neocortical neurons from injury induced by subsequent exposure to 15 µM NMDA (1). When erythropoietin and NMDA are added at the same time, the cortical neurons are not protected from cell damage and die, pointing to a preconditioning effect of erythropoietin also in vitro. Third, moreover, erythropoietin at higher doses (3 mM) rescues neuroblastoma cells from apoptosis induced by exposure to hypoxia (4) and protects neuronal cultures from nitric oxide (NO)-induced cell death (12). Finally, erythropoietin was shown to be involved in angiogenesis by inducing mitogenesis in brain endothelial cells. Below we will consider some possible mechanisms underlying neuroprotection and angiogenesis by which erythropoietin may protect the brain against hypoxia/ischemia.

Possible mechanisms of neuroprotection by erythropoietin

Brain-derived erythropoietin could protect neurons by direct and indirect mechanisms. The direct pathway very likely involves inhibition of hypoxia/ischemia-induced apoptosis, whereas the indirect one probably has to do with vessel growth and neovascularization induced by erythropoietin. Let us first consider the direct erythropoietin effect.

Direct neuroprotective effect of erythropoietin. In analogy with the well-known antiapoptotic action that erythropoietin exerts on erythroid precursor cells in the bone marrow, we can advance the hypothesis that erythropoietin represses apoptosis in neurons by either maintaining expression of Bcl-2 and Bcl-xL, as is the case in erythroid precursor cells, or by inactivating polyadenosine ribose polymerase (PARP). Interestingly, mice that were homozygous null for the PARP gene were significantly better protected against hypoxic/ischemic insults (5). Erythropoietin could also act protectively by upregulating enzymes that scavenge oxygen radicals as well as by down-regulating enzymes that consume large amounts of ATP, e.g., polyadenosine ribose polymerase (PARP). Interestingly, mice that were homozygous null for the PARP gene were significantly better protected against hypoxic/ischemic insults (5).

Indirect neuronal protection by affecting endothelial cell growth. Hypoxia/ischemia-induced erythropoietin might stimulate angiogenesis in the brain. Newly formed vessels would transport more red blood cells, thereby increasing the amount of oxygen delivered to the hypoxic tissue and thus counteracting
the detrimental effects of stroke on neurons (indirect protective effect). Endothelial cells and hematopoietic cells are believed to be derived from the same mesenchymal precursor, the so-called hemangioblast. This may explain why endothelial cells carry the erythropoietin receptor and can be stimulated by erythropoietin. Activation of the Janus family protein kinase (JAK-2) and the transcription factor signal transducer and activator of transcription 5 are essential for erythropoiesis and may also be involved in the signaling pathway in endothelial cells and lead to angiogenesis. Erythropoietin might also be a survival factor for endothelial cells and could prevent apoptosis, as it does in erythroid precursors. Alternatively, it has been suggested that erythropoietin acts indirectly on endothelial cells via activation of the VEGF/VEGF receptor system. VEGF is the most important specific regulator of endothelial cell growth and differentiation and the major angiogenesis factor not only during embryonic development but also in many pathological conditions, such as tumor growth and ischemic disease. In addition, VEGF is also a survival factor for endothelial cells. VEGF is expressed in the normal adult brain, and its expression is induced during cerebral ischemia. Furthermore, VEGF is able to reduce ischemic damage after transient ischemia when applied topically on the surface of the reperfused brain (3), thereby indicating that promoting angiogenesis is indeed a strategy to protect brain tissue after stroke.

Outlook

Brain-derived erythropoietin is upregulated by hypoxia, and expression of both erythropoietin and its receptor are specifically modulated during ischemia. Furthermore, erythropoietin is able to reduce infarct size in mice after stroke when administered intracerebroventricularly. It could serve this important function by direct neuroprotection and by inducing angiogenesis. Even if there are no immediate therapeutic actions in sight, it must be clearly stated that hypoxically upregulated erythropoietin is a naturally self-regulated physiological protection mechanism in the mammalian brain, especially under conditions of chronically reduced blood flow (chronic ischemia). Under clinical conditions, the possibility could be tested that, in patients suffering from acute stroke, intravenous injection of erythropoietin may represent a realistic therapeutic approach. In such an infarcted area, the blood-brain barrier becomes leaky, so that the brain cells may become accessible to blood-borne erythropoietin.

References

A vascular blood pressure control system consists of afferent and efferent signals in the heart and the vasculature by the following mechanism (Fig. 1): changes in arterial blood pressure lead to corresponding changes in vascular shear stress. This mechanical stimulus causes an increase in cytosolic Ca\(^2\)+ content in the endothelial cells. The resulting Ca\(^2\)+-calmodulin complex activates endothelial nitric oxide synthase (eNOS). The subsequently formed nitric oxide (NO) diffuses into the adjacent vascular smooth muscle cells, where it binds to guanylyl cyclase and stimulates the production of cGMP. cGMP acts by reducing the intracellular free Ca\(^2\)+ concentration in the smooth muscle cells. This vasodilation antagonizes the initial increase in arterial blood pressure.

Blood pressure instability may promote cardiovascular morbidity. Recent data suggest a role of nitric oxide in stabilizing arterial blood pressure. A rise in blood pressure enhances endothelial shear stress and nitric oxide release. The resulting vasodilation antagonizes the initial increase in arterial blood pressure. This response antagonizes the initial changes in cardiac output.

How does the human body protect itself against these deleterious blood pressure fluctuations? The most intensively studied mechanism to buffer blood pressure fluctuations is the arterial baroreceptor reflex. Changes in arterial pressure are sensed by baroreceptors in the carotid sinuses and aortic arch, where sympathetic and parasympathetic efferent signals are generated. This response antagonizes the initial changes in cardiac output.

A considerable amount of evidence suggests that a second mechanism that can respond within less than a minute. This short-term blood pressure control mechanism exists besides the arterial baroreceptor reflex function. For example, the sensitivity of the reflex is markedly reduced in atherosclerosis, heart failure, autonomic neuropathy, or pure autonomic failure. Because the requirement to stabilize arterial blood pressure is such a fundamental necessity for cardiovascular function, it is surprising that there should be only one short-term blood pressure control mechanism to buffer blood pressure fluctuations in the absence of hypertension, has been identified as an independent cardiovascular risk factor. For example, the risk for coronary heart disease was found to be increased in patients with high blood pressure variability compared with subjects with low blood pressure variability. Moreover, the severity of Goldblatt hypertension may rely on variations in systolic blood pressure variability (5). Enhanced blood pressure variability is an indicator of increased cardiovascular risk and mortality. This suggests that interventions to reduce blood pressure variability may be beneficial in preventing cardiovascular disease.

References: