Increased Expression of Erythropoietin Receptor on Blood Vessels in the Human Epileptogenic Hippocampus with Sclerosis

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Abstract. Microvascular (capillary) proliferation is a readily observed, but largely ignored phenomenon of the mesial temporal lobe epilepsy (MTLE) hippocampus. Here, we report that the proliferated capillaries in surgically resected MTLE hippocampi were strongly immunoreactive for erythropoietin receptor (EPO-r). Further, we found that these capillaries were most prominent in areas of the MTLE hippocampus with extensive neuronal loss and gliosis, i.e. the CA3, CA1, and dentate hilus. High-resolution immunogold electron microscopy revealed that the capillary EPO-r was localized to the luminal and abluminal plasma membrane of endothelial cells, to endosome-like structures of these cells, and to pericapillary astrocytic end-feet. Previous studies have shown that systemically administered EPO appears in the cerebrospinal fluid in experimental animals, and the present results are consistent with the idea that EPO enters the brain via receptor-mediated endocytosis. The enrichment of EPO-r shown here suggests a highly efficient uptake of plasma EPO into the MTLE hippocampus and a possible role for this cytokine in epileptogenesis. Moreover, the presence of EPO-r in the MTLE hippocampus may provide a new vehicle for highly efficient delivery of hitherto impermeable drugs into the epileptic brain.

Key Words: Astrocytes; Blood brain barrier; Cytokines; Electron microscopy; Immunohistochemistry; Neuroprotection; Neurotrophins.

INTRODUCTION

Mesial temporal lobe epilepsy (MTLE) is a chronic seizure disorder often refractory to medical treatment. Mesial temporal lobe structures such as the entorhinal cortex, amygdala and hippocampus are thought to be important in the causation of seizures in MTLE. Several lines of evidence support this conclusion. Firstly, depth electrode recordings of patients with MTLE indicate that the seizures originate from mesial temporal lobe structures, particularly the hippocampus (1). Secondly, magnetic resonance imaging (MRI) studies reveal increased signal intensity and atrophy of the hippocampus on T2-weighted imaging (2). Thirdly, surgical resection of the atrophic hippocampus usually leads to complete cessation of the seizures (3).

The resected MTLE hippocampus is typically fibrotic and shrunken, a condition known as hippocampal sclerosis (4). Histologically, sclerosis is recognized by a loss of neurons, particularly in the hippocampal subfields CA1, CA3, and the dentate hilus. Additionally, astroglial cells are particularly numerous in these areas. Furthermore, degeneration of peptidergic neurons in the hilus along with sprouting of dynorphin- and other peptidergic-containing axons in the molecular layer of the dentate gyrus are also features of the sclerotic hippocampus (5, 6).

Another often neglected histopathological phenomenon of the sclerotic hippocampus is a striking proliferation of blood vessels. Bratz confirmed the loss of neurons in area CA1 in the MTLE hippocampus and described a sharp boundary that separated this area of neuronal loss from the subiculum and presubiculum where the neurons were preserved (7). In addition, he noted that there was an abundance of blood vessels in the sclerotic sector; further, that these vessels were patent and lacked pathological alterations (7).

Vascular pathology in MTLE hippocampi has from time to time been suggested as a causative factor of TLE. Spielmeyer proposed that the injured areas in the hippocampus, such as CA1, had a more marginal arterial supply than resistant areas, and thus, that hippocampal sclerosis in epilepsy was an ischemic lesion brought on by vasospasm (8, 9). There is no good anatomical evidence presented in support of Spielmeyer’s theory, and certainly it is not consistent with a proliferation of blood vessels as reported by Bratz (7).

What is the functional significance of the proliferated blood vessels in MTLE, and can they hold clues to the pathogenesis of epilepsy? Erythropoietin (EPO) is known to stimulate angiogenesis by binding to erythropoietin receptors (EPO-r) on the cell (10). Both EPO and EPO-r have been reported to increase in malignant tumors (11)
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<th>Case</th>
<th>Gender</th>
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as well as in brain tissue after hypoxic/ischemic insults, situations where there is angiogenesis (12, 13). In these situations, EPO-r is highly expressed on astroglial and endothelial cells (12, 14). The intracellular domain of EPO-r transmits signals that lead to the activation of transcription factor NF-kappaB (15). This transcription factor plays a key role in EPO gene regulation in response to hypoxia (16) and in regulation of other genes involved in inflammation. Increased levels of NF-kappaB are also found in reactive astrocytes and surviving pyramidal neurons in the hippocampus of patients with MTLE and in animal models of epilepsy (17, 18), suggesting the occurrence of inflammatory processes in MTLE.

In the course of a broad screening of the effects of EPO on various neurological insults we decided to examine the expression of EPO and EPO-r in patients with MTLE. In a preliminary experiment we found that EPO-r was indeed expressed in human MTLE hippocampi (19). The present report was aimed at further exploring the expression of EPO-r in MTLE; the hypothesis being that EPO-r is highly expressed on proliferated blood vessels in the MTLE hippocampus.

MATERIALS AND METHODS

Tissue

Patients with medically intractable temporal lobe epilepsy underwent presurgical evaluation at the Yale-New Haven Hospital, and those selected for surgery had their hippocampus resected according to standard procedures (20). Patients who died from nonneurological causes had their hippocampus removed at autopsy. Informed consent from each patient and institutional approval were obtained for the use of tissue in this project.

Classification and Controls

The surgically resected hippocampi could be classified into 3 main groups: 1) mesial temporal lobe epilepsy (MTLE, n = 10); 2) mass-associated temporal lobe epilepsy (MaTLE, n = 4); and 3) paradoxical temporal lobe epilepsy (PTLE, n = 4) (Table; ref. 6). In MTLE and PTLE, the seizure activity is believed to originate from the hippocampus based on noninvasive studies, depth, and/or subdural electrode recordings (21). In MaTLE, the seizures are thought to originate from a mass-lesion (usually a tumor) outside the hippocampus, but within the temporal lobe territory (21).

Neurohistological and clinical outcome studies have further defined these groups (22). MTLE is characterized by pronounced neuronal loss (>50%) in the hippocampal subfields CA1, CA3, and hilus. Also, this category shows extensive reorganization of peptidergic (dynorphin, somatostatin, neuropeptide Y, substance P) neurons in the dentate gyrus. Moreover, the clinical outcome after surgery is excellent. PTLE is recognized by a modest neuronal loss (<25%) throughout all hippocampal subfields, no reorganization in the dentate gyrus, and a poorer clinical outcome. MaTLE is similar to PTLE histologically, with a better clinical outcome than PTLE. Thus, the PTLE and MaTLE hippocampi are suitable “controls” for the MTLE hippocampi. Autopsy hippocampi (Table, n = 7) were used as additional controls.

Tissue Preparation

Immediately after surgical resection or autopsy, a 5-mm-thick slice was cut from the mid-portion of the hippocampus and immersed into a fixative containing 4% paraformaldehyde and 15% (v/v) saturated picric acid in 0.1 M phosphate buffer pH 7.4 (PB) for 1 h, followed by immersion into 5% acrolein in PB for 3 h. Thereafter the tissue was rinsed and stored in PB at 4°C. Fifty-μm coronal sections were cut on a Vibratome and processed for preembedding immunohistochemistry while 300 micrometer sections were used for freeze substitution and immunogold electron microscopy.

Antisera and Chemicals

The peptide antisera used for the classification into TLE subgroups were obtained from Peninsula Laboratories Inc, San Carlo, CA (neuropeptide Y), Diasorin Inc, Stillwater, MN (substance P and somatostatin), and Serotech Ltd., Kidlington Oxford, UK (dynorphin). An EPO-r antiserum (C 20) available from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) was used. This affinity-purified antiserum is raised against a fragment of the human receptor. Unless otherwise specified, all chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

Preembedding Immunocytochemistry

The Vibratome sections were processed free-floating for immunocytochemistry according to the avidin-biotin-complex (ABC) method (23) using a commercially available ABC-kit (Vectorstain Elite, Vector Laboratories, Burlingame, CA), as previously described (24). Immunostained sections from each patient were mounted on slides, dehydrated, coveredipped, and analyzed in the light microscope by 2 observers independently (TE and NCeL). Each observer classified the specimens as MaTLE, PTLE, or MTLE based on the pattern of peptide immunohistochemistry (25) and then rated the presence and pattern of EPO-r staining. The inter-observer reliability score was 98%.

Abbreviations: AEDs, antiepileptic drugs; cbz, carbamazepine; clo, clonazepam; F, female; flb, felbamate; gpn, gabapentin; L, left; ita, levetiracetam; ltg, lamotrigine; M, male; MaTLE, mass-associated temporal lobe epilepsy; MCA, middle cerebral artery; MRI, magnetic resonance imaging; MTLE, mesial temporal lobe epilepsy; pb, phenobarbital; ph, phenytoin; PM, postmortem; pri, primidone; PTLE, paradoxical temporal lobe epilepsy; R, right; tgb, tiagabine; tpm, topiramate; vpa, valproate. * Years since first unprovoked seizure.
Freeze Substitution

Small tissue blocks (0.3 × 0.5 × 1) mm³ of area CA1 of 2 MTLE hippocampi were dissected from the Vibratome sections and subjected to freeze substitution as described by Hjelle et al (26). Briefly, the tissue blocks were cryoprotected in glycerol and rapidly frozen in liquid propane at −190°C. The frozen tissue was immersed into anhydrous methanol containing 0.5% uranyl acetate at −90°C in an automatic freeze substitution unit (EM AFS; Leica Microsystems, Depew, NY). The blocks were infiltrated with Lowicryl HM20 Resin (Electron Microscopy Sciences, Forth Washington, PA) at −30°C, polymerized by ultraviolet light, sectioned ultrathin, transferred to 500 mesh nickel grids, and processed for immunogold electron microscopy.

Immunogold Electron Microscopy

On-grid immunolabeling was carried out according to the procedures of Laake et al (27). Briefly, the sections were incubated overnight with the EPO-r antisera diluted 1:100 followed by incubation with a 10-nm colloidal gold-conjugated secondary antiserum (EMGFAR10; BBInternational, Cardiff, UK) diluted 1:20 for 2 h. The sections were counterstained with uranyl acetate and lead citrate before being examined in a transmission electron microscope (Jeol EM300).

Specificity Controls

No staining was observed when the EPO-r C-20 antiserum was preadsorbed with purified EPO-r C-20 peptide prior to the immunohistochemical procedure (Fig. 3D). Substitution of the EPO-r antiserum with normal goat serum and normal rabbit serum also abolished the staining.

RESULTS

Light Microscopy

The EPO-r antisera stained blood vessels and neurons in all hippocampal specimens. Because blood vessels are the focus of the present study only staining associated with these structures will be emphasized here.

The staining pattern for EPO-r was similar in all MaTLE/PTLE (n = 8) and autopsy (n = 7) hippocampi. In these hippocampi, numerous capillaries were labeled in the white matter, i.e. the alveus and fimbriae (Fig. 1A), while few if any capillaries were stained in the remaining portions of the hippocampus, i.e. the subiculum (Fig. 2A), CA-fields (Fig. 2B), and dentate gyrus.

In the MLTE hippocampi (n = 10), labeling for EPO-r was also found in the alveus and fimbriae (Fig. 1B), and the labeling pattern in these regions was essentially similar to that of MaTLE/PTLE and autopsy control hippocampi. As in the MaTLE/PTLE and autopsy hippocampi, little if any labeling was observed in the gray matter of the MTLE subiculum (Figs. 1B, 2B) and area CA2, where the pyramidal neurons are typically spared. However, in areas of the MTLE hippocampi with extensive neuronal loss and astroglial proliferation, such as the CA1, CA3, and dentate hilus, strong labeling for EPO-r was evident (Figs. 1B, 2D). This was particularly noticeable in areas CA1 and CA3 where a network of EPO-r-positive capillaries infiltrated the entire width of the region, from stratum oriens all the way through stratum lacunosum moleculare (Fig. 2D). This was a consistent feature of all MTLE hippocampi.

More detailed analysis of the EPO-r-staining in the MTLE hippocampus revealed some notable differences between the white and gray matter areas. In the white matter areas, labeling for EPO-r was confined to capillaries and astroglial cells (Fig. 3A, B). Astroglial cell bodies and processes were labeled and the processes often extended towards the capillaries (Fig. 3A). In the gray matter areas, no labeling for EPO-r was observed in astroglial cell bodies; instead the labeling was confined to the capillaries (Fig. 3C). However, it could not be determined by light microscopy which elements of the capillaries were labeled by EPO-r; therefore, high-resolution immunogold electron microscopy for EPO-r was carried out.

Immunogold Electron Microscopy

The ultrastructural analysis of EPO-r in area CA1 of the MTLE hippocampus (n = 2) confirmed the light microscopic observation that labeling for EPO-r was associated with astrocytes and blood vessels. In the white matter area (alveus) both astrocytic profiles and endothelial cells were labeled (Fig. 4A). The astrocytic labeling was found predominantly over the plasma membrane of the cell, and the gold particles appeared evenly distributed throughout the various domains of the membrane. For example, both the pericapillary end-feet and the glial processes elsewhere in the neuropil were labeled to the same extent. Few gold particles were found inside the astroglial cells, and when present, they tended to accumulate over membranous structures.

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Fig. 1. Dark-field photomicrographs of surgically resected human hippocampi stained with antibodies against EPO-r (C20). The immunostained areas appear white. A: In the MaTLE hippocampus, EPO-r-immunoreactivity is associated with blood vessels in the alveus (arrows) and fimbriae (f). B: In the MTLE hippocampus EPO-r-staining of blood vessels is also seen in the alveus (arrows) and fimbriae. However, other areas such as the hilus, CA3, and CA1 contain immunopositive blood vessels as well. Particularly strong labeling is seen in area CA1 (indicated by the asterisks within the dashed lines). Considerably fewer EPO-r-positive blood vessels are seen in the pyramidal layer of the subiculum. Scale bar = 1 mm.
Fig. 2. High-power fields of the subiculum and CA1 from the EPO-r (C20) immunostained MaTLE and MTLE hippocampi in Figure 1. In the MaTLE hippocampus only weak neuronal staining is present in the subiculum (arrow in A) and area CA1 (arrow in C). A similar staining pattern is also seen in the MTLE subiculum (B). In contrast, heavy labeling of numerous blood vessels throughout the strata oriens (or), pyramidale (py) and lacunosum moleculare (lm) is evident in CA1 of the MTLE hippocampus (arrows in D). Scale bar = 100 μm.

The endothelial cell labeling was also predominantly associated with the plasma membrane where gold particles were present on both the luminal and abluminal membrane (Fig. 4A, B). Labeling was found on vesicular structures resembling endosomes within the endothelial cells. These vesicles were particularly abundant in the abluminal compartment of the endothelial cytoplasm and they appeared to fuse with the plasma membrane (Fig.
Fig. 3. A, B: High-power fields of the alveus from the same EPO-r (C20) immunostained MaTLE (A) and MTLE (B) hippocampi as in Figure 1. In both specimens, the immunoreactivity is confined to astrocytes (arrows) and blood vessels (arrows with dot). C, D: Specificity controls. Two adjacent sections from an MTLE hippocampus were immunolabeled with EPO-r (C20) (C) and EPO-r (C20) (D) that had been preadsorbed with EPO-r (C20) peptide. The micrographs are from CA1 and demonstrate strong labeling of blood vessels with the antibody against EPO-r (C) and no labeling in (D). Scale bar = 60 μm.

4B). Scattered gold particles were also observed in the basal lamina between the endothelial cell and astrocytic end-foot (Fig. 5).

The ultrastructural distribution of EPO-r in the gray matter of area CA1 (i.e. oriens to lacunosum moleculare) of the MTLE hippocampus was essentially similar to that of white matter with one notable exception. While the endothelial cell labeling in gray matter was similar to white matter, the glial labeling in gray matter was predominantly associated with pericapillary end-feet surrounding EPO-r-positive endothelial cells, and not with glial processes elsewhere in the neuropil (Fig. 5).

DISCUSSION

Since the observation made by Bratz (7) on the proliferation of the microvasculature in the sclerotic hippocampus in MTLE, relatively little attention has been paid to the role of blood vessels in epilepsy. Comparisons of spiking and nonspiking cortices have revealed differences in capillary morphology. In spiking cortex, the capillaries show increased vessel wall thickness (particularly of the basal lamina), increased pericyte cytoplasmic density, and even pericyte degeneration (28). There is also evidence of changes in membrane protein expression. For instance, the glucose transporter GLUT1 is upregulated in microvessels in epileptogenic tissue (29).

In the present study we provide data that another membrane-associated protein, EPO-r, is highly expressed on blood vessels in the MTLE hippocampus, particularly in areas of peak neuronal loss and astroglial proliferation, i.e. the dentate hilus and areas CA3 and CA1. The fact that EPO-r is found in the luminal as well as abluminal domain of the endothelial cell membrane, and also in endosome-like vesicles, suggests that EPO-receptors and their ligand EPO may be shuttled through the endothelial cell. Recent studies have indeed shown that EPO can cross the blood brain barrier under normal and pathological conditions. For example, when rats are given a single dose of recombinant human (r-hu) EPO (5,000 U/kg) intraperitoneally (i.p.), the levels of EPO increase in the cerebrospinal fluid (30). Moreover, if biotinylated r-huEPO is given i.p. to rats and the brains are studied histologically for the presence of biotinylated r-huEPO, the biotin reaction product is found in vesicles of endothelial cells, strongly suggesting a transport of EPO across the blood-brain barrier (19). However, the mechanism underlying this transport is not known. The present results provide a possible explanation as they suggest that EPO may be transported through the endothelial cell via receptor-mediated endocytosis. If so, this transport would be very efficient in the MTLE hippocampus if judged from the dense expression of EPO-r particularly in the hilus and areas CA3 and CA1. Thus the possibility needs to be considered that the endogenous levels of plasma EPO influence hippocampal function in patients with MTLE.

What are the actions of EPO in the brain? One of the main effects of EPO in the periphery is to promote growth and differentiation of red blood cell precursors into mature erythrocytes (31). A similar trophic role has also been suggested for EPO in the central nervous system (CNS). In 1993, Konishi et al discovered that local
administration of EPO rescues septal neurons from degeneration after fimbria-fornix lesions (32). In a series of immunohistochemical studies on human fetal brains, Juul et al demonstrated time- and location-specific increases in EPO- and EPO-r immunoreactivities (33, 34). Both EPO and EPO-r were highly expressed in neural progenitor cells of the subventricular zone. Studies in rats have shown that r-huEPO induces the production of neural
progenitor cells in embryonic and adult animals (35) and that infusion of EPO-blocking antibodies into the brain of these animals slows the production of such cells (35). Finally, transgenic animals with reduced levels of EPO-r display severe neurodevelopmental abnormalities (36). In summary, EPO appears to play a critical role for growth and development of neural elements in the CNS.

Certain features of the hippocampal pathology in MTLE are associated with neurotrophic changes. Synaptic plasticity with formation of new and more powerful excitatory circuits appears to take place in the MTLE hippocampus. In vitro electrophysiological recordings of surgically resected human TLE hippocampi have demonstrated an increased excitability in MTLE compared to PTLE and MaTLE hippocampi (37). This excitability is partly mediated by glutamate and is particularly pronounced in the dentate gyrus. Furthermore, immunohistochemical and ultrastructural studies have revealed the presence of new and strengthened excitatory circuits in the hyperexcitable areas of the MTLE hippocampus. In the dentate hilus of the human MTLE hippocampus we recently discovered a large population of surviving mossy cells, despite the widely held notion that such cells degenerate in TLE (24, 38). The mossy cells in the MTLE hippocampi were studded with complex, presynaptic structures (“thorny excrescences”) that expressed high levels of the AMPA-receptor subunit GluR1. Moreover, the excrescences were heavily innervated by putative glutamatergic terminals from granule cell axons. These novel synaptic connections, which were not observed in MaTLE or PTLE hippocampi, could explain the hyperexcitability of the MTLE hippocampus and thus the occurrence of recurrent seizures in MTLE. Our finding that EPO-r are heavily expressed on blood vessels in the MTLE dentate hilus opens up the possibility that EPO may play a role as a neurotrophic factor in this region and contribute to the formation of epileptogenic circuits.

Clinical trials and studies on animals have indicated that r-huEPO modulates the occurrence and severity of seizures. In patients undergoing hemodialysis for chronic kidney failure, long-term treatment with r-huEPO increases the incidence of seizures (39). Because most other patient categories receiving r-huEPO do not have a higher incidence of seizures, mechanisms other than a direct central nervous system effect of EPO may be responsible for the pro-convulsant effects in chronic kidney failure. Such mechanisms probably involve r-huEPO-induced...
hypertension and uremia-associated electrolyte derangements. When EPO is administered to animals with experimentally induced seizures, EPO has clear anticonvulsive effects. For instance, in a mouse model of kainic-acid induced seizures, pretreatment with r-huEPO significantly increases the latency of seizure onset and reduces the mortality by 45% compared with controls (19). No effect of r-huEPO on seizures or mortality is seen if administered 30 min before kainic acid or after the development of motor seizures. It has been suggested that the delayed response of r-huEPO on seizures is associated with the expression of genes that afford continued protection in the absence of r-huEPO (19).

EPO has direct effects on excitability and synaptic activity in the hippocampus. A study by Kawakami et al. demonstrated that EPO reduces hippocampal excitability in slices by inhibiting glutamate release via EPO-r mediated cell signaling (40). Weber et al. reported that EPO maintains synaptic activity during hypoxia (41). However, both these effects of EPO are neuronal and thus, may not be relevant to the EPO-r-enriched areas of the severely neuron-depleted, end-stage MTLE hippocampus. Nevertheless, it is possible that EPO modulates excitability in the early stages of epileptogenesis when a higher number of neurons presumably is present. Due to the obvious limitations of end-stage human tissue, animal models are more suitable to resolve this issue.

EPO is also neuroprotective in various paradigms of CNS injury. Systemic administration of r-huEPO ameliorates neuronal injury and improves clinical outcome in animal models of stroke (19), blunt head trauma (19), and spinal cord injury (42). The possibility should be considered that the upregulation of EPO-r on blood vessels in the MTLE hippocampus serves to protect against seizure-induced neuronal damage. While a similar mechanism may be in effect in the MTLE hippocampus, the concentration of EPO in the hippocampus may not be sufficient to maintain neuroprotective levels during seizures. Nevertheless, because the blood vessels in the MTLE hippocampus are highly “primed” for the transfer of plasma EPO into the brain parenchyma, it is feasible that a booster dose of systemically administered r-huEPO could raise the hippocampal EPO to neuroprotective levels. The use of r-huEPO in the treatment of neuronal damage caused by status epilepticus therefore warrants further investigation. Another intriguing option generated from the data in the present study is the potential to produce a new type of highly specific antiepileptic drugs by linking an antiepileptic compound to a moiety that recognizes EPO-r. By doing so, it may be possible to deliver a range of hitherto impermeable drugs to the brain and target them to the hippocampus, which is the focus of the seizures in MTLE.

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REFERENCES


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