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Vascular endothelial growth factor attenuates status epilepticus-induced behavioral impairments in rats

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ABSTRACT

Vascular endothelial growth factor (VEGF) is a vascular growth factor more recently recognized as a neurotrophic factor (for review, see Storkebaum E, Lambrechts D, Carmeliet P. BioEssays 2004;26:943–54). We previously reported that endogenous VEGF protein is dramatically upregulated after pilocarpine-induced status epilepticus in the rat, and that intra-hippocampal infusions of recombinant human VEGF significantly protected against the loss of hippocampal CA1 neurons in this model (Nicoletti JN, Shah SK, McCloskey DP, et al. Neuroscience 2008;151:232-41). We hypothesized that we would see a preservation of cognitive and emotional functioning with VEGF treatment accompanying the neuroprotection previously observed in this paradigm. Using the Morris water maze to evaluate learning and memory, and the light–dark task to assess anxiety, we found a selective profile of preservation. Specifically, VEGF completely preserved normal anxiety functioning and partially but significantly protected learning and memory after status epilepticus. To determine whether the ability of VEGF to attenuate behavioral deficits was accompanied by sustained preservation of hippocampal neurons, we stereologically estimated CA1 pyramidal neuron densities 4 weeks after status epilepticus. At this time point, we found no significant difference in neuronal densities between VEGF- and control-treated status epilepticus animals, suggesting that VEGF could have protected hippocampal functioning independent of its neuroprotective effect.

1. Introduction

Vascular endothelial growth factor (VEGF) is a protein growth factor originally named for its potent trophic effects on endothelial cells, but more recently recognized as a neurotrophic factor [for review, see 1]. We have previously reported that endogenous VEGF protein is dramatically upregulated after pilocarpine-induced status epilepticus in rat and that intra-hippocampal infusions of recombinant human VEGF significantly protect against the loss of hippocampal CA1 neurons in the same model [2].

Although status epilepticus results in loss of hippocampal neurons in rat models, findings regarding hippocampal damage in humans with temporal lobe epilepsy (TLE) have been inconsistent. Many studies with human patients have demonstrated that acute status epilepticus can cause extensive loss of hippocampal neurons [3,4], and that damage progression occurs in patients who continually experience seizures [5,6]. In contrast, Thom and colleagues [7] demonstrated the absence of hippocampal neuronal loss in a postmortem stereological analysis of patients with poorly controlled seizures.

In addition to the underlying neuropathology, individuals with epilepsy often experience functional impairments such as deficits in intellectual functioning, learning and memory, processing speed, attention and concentration, and executive functioning [8,9]. These impairments can lead to substantial decreases in quality of life. In fact, individuals with epilepsy are more likely to be unemployed, resulting in lower socioeconomic status, and are also less likely to marry, ultimately leading to social isolation [9]. It would therefore be important to determine whether treatment interventions that protect the epileptic brain from damage would also preserve functional integrity.

The hippocampus plays a fundamental role in learning, memory, and emotional functioning, and it is therefore reasonable to predict that loss of neurons after status epilepticus could be associated with functional deficits in these behaviors. Indeed, animal models of epilepsy have revealed seizure-associated impairments in hippocampally mediated memory tasks as well as a blunting of normal anxiety responses in anxiety tasks [10–12]. Based on our finding that VEGF decreased status epilepticus-induced cell loss in the hippocampus, we hypothesized that VEGF would also attenuate behavioral impairments.

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To investigate functional preservation, animals were continuously treated with VEGF and were given pilocarpine-induced status epilepticus. Behavioral testing was then conducted to evaluate learning, memory, and emotional functioning during the period between status epilepticus and the eventual development of chronic seizure behavior.

2. Methods

2.1. Subjects

All subjects were adult male Sprague–Dawley rats (Charles River Laboratories, Kingston, NY, USA) weighing 250–350 g. Animals were housed two to three per cage within a temperature-stabilized animal facility with food (Rat LabDiet 5001, Purina Mills, LLC, St. Louis, MO, USA) and water available ad libitum. Animals were maintained on a 12:12 light:dark cycle (lights on 07:00) and acclimated to their colony room for at least 1 week prior to any manipulations.

2.2. Proteins

The VEGF used for protein infusions was recombinant human $VEGFA_{165}$ (generously provided by Regeneron Pharmaceuticals). VEGF was stored frozen until use and then diluted in sterile phosphate-buffered saline (PBS; Sigma–Aldrich, St. Louis, MO, USA) to deliver 60 ng/day VEGF in a 12-μl volume at the rate of 0.5 μL/hour via osmotic minipump. PBS was purchased in powder form, mixed with distilled water, sterilized, and used as a vehicle control. In addition, some control animals received inactivated VEGF as a control instead of PBS to control for protein load. VEGF was inactivated by repeated freeze–thaw cycles, which has previously been shown to eliminate its bioactivity (unpublished data), rather than by heat, which results in a precipitate.

2.3. Pump implantation and protein infusion

Animals were anesthetized with 65 mg/kg sodium pentobarbital administered intraperitoneally (Henry Schein, Melville, NY, USA). Animals were placed in a stereotaxic apparatus, the skull was exposed, and three burr holes were drilled to insert anchor screws (Plastics One, Roanoke, VA, USA). A sterile 4-mm cannula (Plastics One), with an attached heat-sealed polyvinyl catheter (Plastics One) containing sterile PBS, was implanted bilaterally into the hilus of the dentate gyrus of the dorsal hippocampus (3.8 mm posterior and 2.7 mm lateral as measured from bregma). Dental acrylic was then applied to secure the cannula and anchor screws, and polyamide nylon suture thread (Henry Schein) was used to close the incision.

One week following cannula implantations, animals were briefly re-anesthetized under 2.5% isoflurane anesthesia and an incision was made at the nape of the neck. The heat-sealed tip of the catheter was snipped and an Alzet osmotic minipump (0.5 μL/hour, Durect Corp., Palo Alto, CA, USA), containing $rhVEGF_{165}$, sterile PBS, or inactivated VEGF, was attached to the catheter or catheters and glued. The pump was inserted into the subcutaneous space at the nape of the neck, and the incision was closed with nylon sutures. Pumps continuously administered VEGF protein into the hippocampus for 2 weeks.

One group of animals, the “control” group, received no surgical manipulations or protein infusions.

2.4. Acute seizure induction

Five days following pump implantations for protein infusions, animals were pretreated with 1 mg/kg atropine methyl bromide (Sigma–Aldrich) injected subcutaneously 30 minutes prior to receiving either 350 mg/kg pilocarpine hydrochloride (Sigma–Aldrich) or an equivalent volume of saline intraperitoneally (the “control” group). Seizures were scored from stages 1 to 8 based on Racine's scale [13] modified as previously described [14]. Status epilepticus was defined as seizures with no intervening return to normal behavior for longer than 5 minutes. Status epilepticus was truncated with 10 mg/kg diazepam (Henry Schein) after 60 minutes. Animals not achieving status epilepticus received diazepam 90 minutes after pilocarpine injection, and were removed from further analyses. All pilocarpine-induced animals were hydrated immediately following diazepam with 3 cc of isotonic saline and were given fresh apple slices for further hydration. Seized animals received hydration injections and fresh apple slices daily for 1 week.

2.5. Behavioral analyses

Animals underwent the behavioral tests described below 2 to 4 weeks after status epilepticus to evaluate learning, memory, and emotional functioning. Before each behavioral test, animals were acclimated to the testing room for at least 1 hour. All behavioral testing was conducted by experimenters blind to the treatment condition of the animals.

2.5.1. Morris water maze

Each animal was placed in a 130-cm-diameter water maze, made opaque with white, nontoxic paint, back end first to avoid stress. Each animal was placed in a pseudo-randomly selected start location in the pool for three trials per day with an intertrial interval of 1 minute. Each trial ended when the animal escaped onto a submerged, hidden goal platform or when the animal had been in the maze for 2 minutes. Any animal that had not located the platform within 2 minutes was guided to the platform by hand. Each animal was tested daily until acquisition of the memory was achieved. Successful acquisition was defined as the achievement of a 10 second or lower mean latency for the control rats (which had not been surgically manipulated, treated with protein, or injected with convulsant) in each cohort. This typically occurred by the fourth day (12th trial), although acquisition trials in some cohorts were extended to the fifth day. Following the acquisition trials, the goal platform was removed for a spatial probe trial in which each animal was placed in the maze for 30 seconds, and the proportion of time spent in the goal and other quadrants was recorded. Additionally, mean quadrant crossing time was calculated as a control for swim speed.

2.5.2. Grid locomotor activity

Each animal was placed in the center of an 86-cm^2 open field divided by laboratory tape into nine 29-cm squares. Animals were observed for 6 minutes to measure the number of grid crossings as a measure of exploratory locomotor activity.

2.5.3. Light–dark exploration

Each animal was placed into the open side of a 43×86-cm light–dark box in which one side was covered and painted black and the other side was open and painted white. The amount of time spent in the black chamber versus the white chamber was recorded for a total of 5 minutes as a measure of anxiety-like behaviors, with more time spent hidden in the closed side indicative of greater levels of anxiety.

2.6. Histology

After receiving an overdose of Euthasol euthanasia solution (Henry Schein, Inc.), animals were transcardially exsanguinated with heparinized isotonic (0.9%) saline, and then perfusion-fixed with 4% paraformaldehyde in acetate and then borate buffer, as previously described [15]. The brains were removed and placed in 30% sucrose borate buffer at 4 °C until sectioned.

After 3–7 days in buffered sucrose solution, brains were sectioned coronally at 40 μm using a sliding microtome (American Optical