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Inhibition of glutamine synthetase in the central nucleus of the amygdala induces anhedonic behavior and recurrent seizures in a rat model of mesial temporal lobe epilepsy

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ABSTRACT

The prevalence of depression and suicide is increased in patients with mesial temporal lobe epilepsy (MTLE); however, the underlying mechanism remains unknown. Anhedonia, a core symptom of depression that is predictive of suicide, is common in patients with MTLE. Glutamine synthetase, an astrocytic enzyme that metabolizes glutamate and ammonia to glutamine, is reduced in the amygdala in patients with epilepsy and depression and in suicide victims. Here, we sought to develop a novel model of anhedonia in MTLE by testing the hypothesis that deficiency in glutamine synthetase in the central nucleus of the amygdala (CeA) leads to epilepsy and comorbid anhedonia. Nineteen male Sprague–Dawley rats were implanted with an osmotic pump infusing either the glutamine synthetase inhibitor methionine sulfoximine [MSO ($n = 12$)] or phosphate buffered saline [PBS ($n = 7$)] into the right CeA. Seizure activity was monitored by video-intracranial electroencephalogram (EEG) recordings for 21 days after the onset of MSO infusion. Sucrose preference, a measure of anhedonia, was assessed after 21 days. Methionine sulfoximine-infused rats exhibited recurrent seizures during the monitoring period and showed decreased sucrose preference over days when compared with PBS-infused rats ($p < 0.01$). Water consumption did not differ between the PBS-treated group and the MSO-treated group. Neurons were lost in the CeA, but not the medial amygdala, lateral amygdala, basolateral amygdala, or the hilus of the dentate gyrus, in the MSO-treated rats. The results suggest that decreased glutamine synthetase activity in the CeA is a possible common cause of anhedonia and seizures in TLE. We propose that the MSO CeA model can be used for mechanistic studies that will lead to the development and testing of novel drugs to prevent seizures, depression, and suicide in patients with TLE.

1. Introduction

Epilepsy is a common and often lifelong neurological disorder with a prevalence of approximately 1% in the general population [1]. Patients with epilepsy have a 5- to 10-fold increased risk of depression [2,3] and an 11-fold increased risk of suicide [2,4] compared with the general population. In patients with mesial temporal lobe epilepsy (MTLE), the rate of suicide is 25 times higher than in the general population [5,6]. The current treatments for MTLE, which include either the use of antiepileptic drugs or the surgical removal of the temporal lobe, can themselves increase depression and the risk of self-harm and suicide [4,7–10]. To provide more effective treatments for seizures, depression, and suicide prevention in patients with MTLE, we must achieve development of an effective animal model of MTLE with depressive comorbidity.

Commonly used animal models of MTLE include acute systemic injections of pilocarpine [11] or kainic acid [12] and electrical stimulation of the hippocampus or amygdala [13]. The depression-related behaviors that have been tested in these models have included the forced swim test and the sucrose or the saccharine preference test. Mixed results have been obtained in all these models with these tests. For the kainic acid model, studies have shown that sucrose preference is decreased and immobility in the forced swim test is increased in response to systemic administration of kainic acid in rats [14,15], indicative of depressive-like behavior; however, other studies that used the kainic acid model failed to demonstrate depressant effects [16]. Similarly, some studies using the pilocarpine model have shown a decrease in saccharin preference and an increase in immobility in the forced swim test [17–19]. Other studies, however, did not show this effect [20–22]. With respect to the stimulation models, one study showed that kindling of the ventral hippocampus produces depressant effects in the forced

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swim test and saccharine preference test [23]. Other studies have shown no such effect with amygdala or hippocampal stimulation [24,25].

We present a recently developed model of MTLE with comorbid anhedonia. While depression is a complex disorder with multiple symptoms, we have chosen to focus on the anhedonic symptom of depression because it is a key symptom of depression that is highly predictive of suicidal thoughts and behaviors [26–30] and is common in patients with MTLE [31,32]. The model we are introducing is produced by inhibiting glutamine synthetase, an astrocytic enzyme that is critical for the metabolism of glutamate and ammonia to glutamine, in the central nucleus of the amygdala (CeA). Unlike the classically used models, our approach recapitulates a possible causative mechanism of seizures and concurrent depression in humans with MTLE. This is because glutamine synthetase activity has been shown to be reduced in the amygdala in patients with MTLE [33], and glutamine synthetase levels have been shown to be significantly decreased in patients with major depressive disorder [34], in suicide victims with major depression [35], and in suicide victims with no major depression [35].

Our working hypothesis when developing the new model was that inhibition of glutamine synthetase with methionine sulfoximine (MSO) in the CeA would induce both recurrent seizures and a lack of preference for a sucrose solution in a limited access two-bottle-choice procedure. Such a model may be used to effectively investigate the anatomical and chemical mechanisms that underlie MTLE and comorbid anhedonia, thereby potentially leading to more effective ways to prevent seizures, depressive symptoms, and suicide in patients with TLE.

2. Material and methods

2.1. Chemicals and animals

All chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise noted. Male Sprague–Dawley rats were obtained from Charles River Laboratories (Wilmington, Mass). Rats were housed (2 per cage) and maintained in a temperature-controlled colony room (21 °C–23 °C) on a 12-h light–dark cycle. Rats were allowed free access to food and water and were acclimated for at least 1 week prior to surgery. All procedures were approved by the Institutional Animal Care and Use Committee at Yale University and were conducted in accordance with current guidelines.

2.2. Surgery

Rats were anesthetized with 0.25–3% isoflurane (Baxter, Deerfield, Ill.) in O_2 and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif.). A 30-gauge stainless steel cannula, with a length of 8.1 mm, attached to a plastic pedestal (Plastics One, Roanoke, Va.) was stereotaxically lowered into the CeA using the following coordinates, with bregma marking zero for the mediolateral (ML) and anteroposterior (AP) directions, and the top of the skull marking zero for the dorsoventral (DV) direction: AP = −2.6 mm, ML = +4.6 mm, DV = −8.1 mm.

The cannula was lowered into the brain until the pedestal touched the skull. The pedestal was then glued to the skull with medical grade cyanoacrylate (Vetbond Tissue Adhesive, Butler Animal Health Company, Chicago, IL). The cannula and pedestal were connected via plastic tubing to a subcutaneously implanted Alzet osmotic pump (Model 2004, Durect Corp., Cupertino, Calif.) which delivers a continuous flow of 0.25 μL/h for ~28 days. Treatment pumps were filled with MSO (2.5 mg/mL; dissolved in Dulbecco's phosphate buffered saline (PBS)) to achieve a delivery of 0.625 μg of MSO per hour. Control pumps were filled with PBS. Following placement of the cannula and pedestal, four stainless steel epidural screw electrodes (Plastics One, Roanoke, Va.) were implanted to record cortical EEG activity. Two electrodes (one in each hemisphere) were positioned in the epidural space over the cortex. On the left side (contralateral to the injection), the recording screw electrode was placed in the skull above the dorsal anterior hippocampal formation (AP = −2.0 mm, ML = −2.5 mm). On the right side, the recording screw electrode was placed in the skull above the temporal lobe (AP = −6.25 mm, ML = 5.4 mm). One screw electrode was positioned in the epidural space (AP = −8.5 mm, ML = −2.2 mm) to serve as the reference. A fourth electrode, which was positioned above the cerebellum (AP = −10.0 mm, ML = 1.5 mm), served as the ground. Three additional stainless steel mounting screws (Plastics One) were inserted into the skull to reduce the risk of headcap detachment. Two screws were positioned in AP = 2.5 mm, ML = ±2.5 mm, and one was positioned in AP = −4.5 mm, ML = 4.0 mm.

The female socket contacts on the ends of each electrode were inserted into a plastic pedestal (Plastics One), and the entire implant was secured by UV light cured acrylated urethane adhesive (Loctite 3106 Light Cure Adhesive, Henkel Corp., Rocky Hill, Conn.) to form a headcap.

2.3. Video-intracranial EEG monitoring and seizure quantitation

Video-EEG recording was conducted over the first 21 days following MSO pump placement. Sucrose preference testing was performed after the completion of the EEG recordings. We chose to carry out EEG recording for the first 21 days because in other models we have developed, for example, where MSO is infused into the molecular subiculum, there is an increase in the percent severity of seizures over time [36]. We wanted to test if this was also the case with MSO infusion into the CeA. The experimental setup for recording video-EEG was adapted from Bertram et al. [37]. The rats were placed individually in custom-made Plexiglas cages. A spring-covered, 6-channel cable was connected to the electrode pedestal on one end and to a commutator (Plastics One) on the other. A second cable connected the commutator to the digital EEG recording unit (CEEGraph Vision LTM, Natus/Bio-Logic Systems Corp., San Carlos, Calif.). Digital cameras with infrared light detection capability were used to record animal behavior (two cages per camera). The digital video signal was encoded and synchronized with the digital EEG signals. Seizures were identified by visual inspection of the EEG record. As detailed in Avoli and Gloor [38], seizures were defined by EEG characteristics and not by the duration of the discharge. Specifically, seizures displayed distinct signal changes from background (interictal) activity. Such signal changes included sustained rhythmic or spiking EEG patterns and a clear evolution of signal characteristics from onset to termination. Subclinical seizures were distinguished from clinical seizures by examination of the video record. The start and stop points of seizures were identified by the following commonly used method. By visual inspection of the EEG, we determined a point that was unequivocally within the seizure. Next, we moved backward in time to determine the seizure start time as the first point where the EEG was different from background activity and forward in time to establish the seizure end time. The video record was examined to stage the seizures, using a modification of Racine's criteria [39] as follows: subclinical — no remarkable behavior; stage 1 — immobilization, eye blinking, twitching of vibrissae, and mouth movements; stage 2 — head nodding, often accompanied by facial clonus; stage 3 — forelimb clonus; stage 4 — rearing; and stage 5 — rearing, falling, and generalized convulsions.

2.4. Sucrose preference testing

After the completion of video-EEG monitoring, the animals proceeded to sucrose preference testing. For the sucrose preference test, rats were disconnected from the EEG recording equipment, taken out of the Plexiglas EEG recording cages, and housed individually in standard rat cages. Following placement, rats were given one day to acclimate to their new cage. During this time, they were provided with food and water ad libitum. Following this 24-hour period, the water bottle was removed and replaced with a bottle containing a 1% sucrose solution, which was the only source of fluid for 48 h. Sucrose preference testing occurred following the 48-hour period of acclimation to the