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Antidepressant and anticonvulsant effects of exercise in a rat model of epilepsy and depression comorbidity

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ABSTRACT

The bidirectional comorbidity between epilepsy and depression is associated with severe challenges for treatment efficacy and safety, often resulting in poor prognosis and outcome for the patient. We showed previously that rats selectively bred for depression-like behaviors (SwLo rats) also have increased limbic seizure susceptibility compared with their depression-resistant counterparts (SwHi rats). In this study, we examined the therapeutic efficacy of voluntary exercise in our animal model of epilepsy and depression comorbidity. We found that chronic wheel running significantly increased both struggling duration in the forced swim test and latency to pilocarpine-induced limbic motor seizure in SwLo rats but not in SwHi rats. The antidepressant and anticonvulsant effects of exercise were associated with an increase in galanin mRNA specifically in the locus coeruleus of SwLo rats. These results demonstrate the beneficial effects of exercise in a rodent model of epilepsy and depression comorbidity and suggest a potential role for galanin.

1. Introduction

People with epilepsy are at increased risk for developing depression, and individuals with active or past depression, or a family history of depression, are also at elevated risk of developing epilepsy, particularly if there is also a history of suicide attempts [1]. The bidirectional comorbidity that exists between these diseases is associated with many challenges for treatment. Patients with this comorbidity often have epilepsy that is refractory to many commonly used pharmacotherapies, require more frequent hospitalizations, and have worsened prognosis [2]. Particularly troubling is the association between many anticonvulsant medications and depressed mood, as well as the relationship between antidepressants and increased risk of seizure, as has been reviewed extensively elsewhere [3,4]. These factors make safe and effective treatment of comorbid epilepsy and depression very difficult and clearly emphasize the need for novel therapeutic approaches. Given their relative lack of negative and potentially dangerous side effects as compared to many pharmacological approaches, nonpharmacological therapies, such as aerobic exercise, are gaining more widespread interest.

Several studies have demonstrated the ability of aerobic exercise to decrease seizure frequency and/or epileptiform discharges [5–8] and improve mood in patients with depression [9–12]. Furthermore, in many epilepsy cases, exercise improves mood even in the absence of changes in seizure frequency [13]. Many of these studies emphasize the low risk and relative safety of exercise in patients with epilepsy. As an added benefit, exercise is associated with other positive outcomes, particularly in cardiovascular and overall physical health [6,14]. Combined, these studies indicate that exercise may be both safe and efficacious in treating simultaneous epilepsy and depression, although it has never been tested in a comorbidity model.

Interactions between the neuropeptide galanin and norepinephrine may underlie the antidepressant and anticonvulsant effects of exercise. Galanin mRNA is increased specifically in the noradrenergic locus coeruleus (LC) following chronic voluntary exercise [15–17]. In addition, mice overexpressing galanin in the LC are seizure-resistant [18], and the anticonvulsant effects of wheel running in a kainic acid seizure model are attenuated by the galanin antagonist, M-40 [17]. The antidepressant properties of exercise in animal models are

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well documented [19], and galanin may also have antidepressant-like properties, particularly via GalR2 receptor activation [20].

To test the antidepressant and anticonvulsant effects of exercise and the potential contribution of galanin in a comorbidity model, we compared forced swim test (FST) activity, pilocarpine-induced seizure susceptibility, and galanin mRNA in SwLo and SwHi rats that were either sedentary or given ad libitum access to a running wheel for 3–4 weeks. The SwLo rats were selectively bred for depression-like behavior in the FST and later shown to be more sensitive to limbic seizures compared to their depression-resistant counterparts, the SwHi rats [21–24]. An advantage of testing the beneficial effects of exercise in this model is that the antidepressant and anticonvulsant properties of exercise have been primarily tested independently and in "normal" animals (i.e., those without inherent risk for developing depression-like behaviors or seizures), while SwLo rats have heritable susceptibility to both of these phenotypes.

2. Methods

2.1. Animals and housing

The SwLo and SwHi rats were selectively bred based on FST phenotype, as described previously [21]. Briefly, rats were fitted with "water wings" made from rectangular rubber and placed in a tank (65 cm high, 30 cm in diameter) with 25-°C water (14 cm from the top) for 10 min. Duration of struggling (active movement of all 4 paws, forepaws breaking surface of the water) and floating (immobility) were measured by a trained researcher blinded to the line of the rat.

Twelve to fourteen male rats of each line were randomly assigned to the exercise condition, while 13–14 male rats of each line were randomly assigned to the sedentary condition. Rats were between 1.5 and 3.5 months of age at the beginning of the experiments and were from generations 56–59 of the SwHi and SwLo rat lines. All rats used were experimentally naïve to the FST and pilocarpine and were singly housed. Food and water were available ad libitum, with lights on from 0700 to 1900 h. All experiments were conducted in accordance with Emory University IACUC approval.

2.2. Voluntary exercise

Rats in the exercise condition were given free 24-h access to a stainless steel rodent activity wheel (Mini Mitter, Bend, OR). Each wheel was connected to an electromagnetic counter that measured the number of wheel revolutions, which were recorded daily between approximately 0900 and 1000. The daily distance run was calculated for each rat by multiplying the number of wheel rotations by the wheel circumference (107.75 cm) and converting the result to km.

2.3. Forced swim test

Animals were exposed to a 15-min FST, as described above, following 3 weeks of exercise or sedentary conditions, and the data for each 5-min time bin were analyzed for struggling and floating behaviors. Following the test, rats were returned to their exercise or sedentary conditions for one additional week.

2.4. Pilocarpine-induced seizures

One week following the FST (during which the exercise group still had access to running wheels), rats were assessed for seizure susceptibility by measuring latency to first pilocarpine-induced behavioral seizure, limbic motor seizure, and status epilepticus (SE), as described previously [23]. Briefly, rats were injected with atropine methyl bromide (2 mg/kg, s.c., Sigma-Aldrich, St. Louis, MO) 30 min prior to pilocarpine hydrochloride administration (380 mg/kg, i.p., Sigma-Aldrich, St. Louis, MO), and latencies were measured during continuous video monitoring for a maximum of 2 h following pilocarpine administration or until SE was achieved. First behavioral seizure was defined as the initial incidence of facial automatism and/or head shaking (i.e., stage 1 or 2 on a modified Racine scale) [25]. Limbic motor seizure was defined as rearing and falling with bilateral forelimb clonus (i.e., stage 3 or higher on a modified Racine scale) [25]. Status epilepticus was defined as 30 min of continuous seizure. A booster dose of pilocarpine (190 mg/kg) was administered to any rat that did not demonstrate a limbic motor seizure within the first hour following pilocarpine administration. Significantly more SwLo rats in the exercise condition (10/14) required a pilocarpine booster compared with sedentary SwLo rats (2/14) (Fisher's exact test, $p < 0.01$), indicative of an anticonvulsant effect of exercise in these rats. By contrast, there was no significant difference between the number of exercising (5/12) and sedentary SwHi rats (10/13) that required a booster (Fisher's exact test, $p = 0.11$). Animals were euthanized 2 h after pilocarpine administration or following 30 min of continuous seizure, whichever came first.

2.5. Galanin mRNA quantification

Galanin mRNA was measured in the LC and hippocampus by quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR). Following pilocarpine experiments, animals were anesthetized with isoflurane and decapitated. The LC and hippocampus were isolated on ice, flash-frozen, and stored at −80 °C until further use. Tissue samples were homogenized using a motorized Kontes Pellet Pestle and passed through a QiaShredder (Qiagen), and RNA was isolated using the RNAqueous-Micro Kit (Invitrogen). Ribonucleic Acid (RNA) was eluted using RNAse-free water, and purity and concentration were verified using a Nanodrop spectrophotometer to measure A260/280. Values between 1.8 and 2.0 were considered acceptable, and all groups fell within this range (1.86 ± 0.02 for the LC, 1.94 ± 0.01 for the hippocampus). Furthermore, there were no significant differences between groups for LC ($F_{3,\ 25} = 1.203$, $p = 0.33$) or hippocampal samples ($F_{3,\ 29} = 0.11$, $p = 0.33$).

The RNA samples were then used to synthesize cDNA for qRT-PCR using the SuperScript III First-Strand Synthesis System (Invitrogen). Primers for the reference gene β-actin and the target gene galanin were purchased from SABiosciences (Qiagen) and were tested on an agarose gel to verify single band products of expected size.

A Bio-Rad CFX96 was programmed to perform qRT-PCR amplification and melting curve analysis using SYBR® Green SuperMix for iQ™ (Quanta). Ten-fold serial dilutions of cDNA from a nonselectively bred rat line were used to calculate the efficiency for both the β-actin and galanin primers. The mean efficiency value for each primer was then used for analysis with the Pfaffl method. All samples were run in triplicate, with galanin as the target gene and β-actin as the reference gene. A relative expression value was calculated for each sample as described by Pfaffl [26] using the Pfaffl spreadsheet found at http://pathmicro.med.sc.edu/pcr/realtime-home.htm, such that:

$$\text{Relative Expression Ratio} = (E_{Gal})^{\Delta Ct\,Gal(control-sample)} / (E_{ActB})^{\Delta Ct\,ActB(control-sample)}.$$

2.6. Statistical analysis

Wheel running data were analyzed by repeated measures two-way ANOVA. Forced swim test and pilocarpine data were analyzed using Student's *t*-tests. Galanin mRNA data were analyzed using a Student's *t*-test or Mann–Whitney *U* test, depending on variance. An F-test was used to compare variances between groups. If $p < 0.05$, variances were considered unequal, and a Mann–Whitney *U* test