The Role of Norepinephrine in Seizure Induced Respiratory Arrest

Jared I. Rosner1,2, Kyle Dayton3, and Gordon F. Buchanan3,4

1SSTP, 2Edgmont High School, 3Neuroscience Program University of Iowa, 4Department of Neurology University of Iowa

Abstract

Background: Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with refractory epilepsy. Fluoxetine, a selective 5-HT reuptake inhibitor prevents seizure induced respiratory arrest (SIRA) and death in animal models. Fluoxetine also has activity at α1 and α2 noradrenoreceptors. Whether norepinephrine (NE) plays a role in SIRA and SUDEP is unknown. Methods: Four groups of mice underwent maximal electroshock induced seizure following treatment with vehicle, the selective NE reuptake inhibitor reboxetine, reboxetine + prazosin, an α1 antagonist prazosin, while recording cardiac, respiratory, and/or electrocerebral activity. Results: Acute injections of reboxetine (30 mg/kg) decreased mortality rate and seizure severity in the mice. Acute injections of prazosin (10 mg/kg) reversed the protective effects of reboxetine. Conclusions: Norepinephrine reduces SIRA, mortality and seizure severity in the mouse MES model.

Introduction

Epilepsy affects millions of people worldwide. A third of epilepsy patients are resistant to multiple antiepileptic drugs. Sudden unexplained death in epilepsy patients (SUDEP) is the leading cause of death in those with drug resistant epilepsy. While not much is known about SUDEP due to its difficulty to both predict and monitor, the cause of death is indicated to be SIRA by the MORTEMUS study.

Selective 5-HT reuptake inhibitors (SSRIs), fluoxetine and citalopram, reduce SIRA and seizure frequency. Fluoxetine is also active at α1 and α2 NE receptors. 5-HT and NE are both bioamines that increase in activity during a seizure. NE also modulates pacemaker and nonpacemaker respiratory neurons, suggesting that it may play a complementary role with 5-HT in recovery of respiration following a seizure. While reboxetine has been shown to increase electrocortical seizure threshold, NE’s effect on SIRA has not yet been explored. Better understanding of the roles of NE and 5-HT in respiratory recovery will contribute to improved identification of risk groups and preventative measures for respiratory issues such as SUDEP, SIRA, and apnea.

Methods

Animals: Adult male C57BL/6J mice (~20 g) from Jackson Labs were studied at 50-70 days of age. Surgical implants: Using aseptic technique under isoflurane (0.5-2%) anesthesia, all animals were implanted with EKG electrodes (Plastic One; left chest, right axilla). Some animals were also implanted with cortical screw EEG and nuchal EMG electrodes (Pinnacle). All animals receive pre, and post-operative analgesia with meloxicam (2 mg/kg).

Experimental procedure: Animals were acclimated to the recording apparatus and tether for at least one hour prior to experimentation. On the experimental trial days animals were placed into the recording chamber/plethysmograph and fitted with the EKG cable (Plastics One) and/or EEG/EMG preamplifier (Pinnacle). Signals were passed through an electrical commutator (Pinnacle), data conditioning amplifier (Brownlee), and A-D converter (National Instruments) to a computer running MATLAB. After 15 minutes baseline recording, mice received an i.p. injection (100 μL) of vehicle (1% DMSO in saline), reboxetine (10 or 30 mg/kg), or prazosin (10 mg/kg). Animals receiving prazosin received reboxetine (30 mg/kg) 15 minutes later. Thirty minutes after the final injection, a seizure was induced with maximal electroshock (50 mA, 200 msec, 60 Hz, sine wave) delivered via saline moistened ear clip electrodes with the aid of a rodent shocker (Hugo Sachs). Data and recording analysis: EKG, EEG and EMG data were captured using MATLAB. Experiments were videotaped with and Logitech quick sync. Data were analyzed with the aid of MATLAB, Origin and Excel. Seizure severity was assessed using the E/F ratio. Statistics: statistical analysis done using one-way ANOVA on Sigma Plot 13

Results

Seizure Mortality & Severity

Figure 2. (A) representative of survival (%), (n=6,5,4,5) and (B) is representative of E/F ratio (α1) which indicates seizure severity, (n=6,4,6,6).

Figure 3. Respiratory rate calculated in 15 second epoch preceding seizure induction (n=6,4,6,5).

Figure 4. Respiratory rate calculated in 15 second epochs at intervals 0-15, 15-30, 60-75, 90-105, 120-135; 0 represents the respiratory recovery for each of 4 surviving mice of the reboxetine (30 mg/mL) group.

Summary

1. Reboxetine decreased SIRA frequency and seizure severity in the MES seizure model of mice.
2. Prazosin reversed the protective effect of reboxetine on SIRA and mortality, but not severity.

Conclusions

1. The α₁ noradrenoreceptors may play a protective role in the MES mouse seizure model.
2. Prazosin plays a modulatory role on seizure severity at a location that is not the α₁ noradrenoreceptors.

Future Directions

1. Use highly specific NE agonists and antagonists to better discern the respective roles of α1 and α2 noradrenoreceptors in respiratory recovery following a seizure.
2. Study NE’s effect on hypoxia and hypercapnia.
3. Test NRIs on 5-HT deficient animals to find the varying effects of 5-HT and NE on SIRA.
4. Use combinations of fluoxetine and NE receptor blockers to better characterize the relationship between 5-HT and NE in SIRA.

References


Future Directions

1. Use highly specific NE agonists and antagonists to better discern the respective roles of α1 and α2 noradrenoreceptors in respiratory recovery following a seizure.
2. Study NE’s effect on hypoxia and hypercapnia.
3. Test NRIs on 5-HT deficient animals to find the varying effects of 5-HT and NE on SIRA.
4. Use combinations of fluoxetine and NE receptor blockers to better characterize the relationship between 5-HT and NE in SIRA.

Acknowledgements

Including Jana De Ross, Callie Ginapp and Stephen Kruse for support and assistance. This work was supported in part by the Epilepsy Foundation and the Beth and Nate Tross Epilepsy Fund.