Epilepsy Research 124 (2016) 49–54

Contents lists available at www.sciencedirect.com

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

Susceptibility to seizure-induced sudden death in DBA/2 mice is altered by adenosine

Carl L. Faingold*, Marc Randall, Srinivasa P. Kommajosyula

Departments of Pharmacology and Neurology, Southern Illinois University School of Medicine, P.O. Box 19629, Springfield, IL 62794-9629, United States

ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form 21 April 2016
Accepted 17 May 2016
Available online 19 May 2016

Keywords:
SUDEP
Adenosine
DBA/2 mice
Caffeine
Respiratory arrest

ABSTRACT

Sudden unexpected death in epilepsy (SUDEP) is rare but is an important public health burden due to the number of patient years lost. Respiratory dysfunction following generalized convulsive seizure is a common sequence of events in witnessed SUDEP cases. The DBA/2 mouse model of SUDEP exhibits generalized convulsive audiogenic seizures (AGSz), which result in seizure-induced respiratory arrest (S-IRA) in ~75% of these animals, while the remaining DBA/2 mice exhibit AGSz without S-IRA. SUDEP induction may involve actions of adenosine, which is released during generalized seizures in animals and patients and is known to depress respiration. This study examined the effects of systemic administration of agents that alter the actions of adenosine on the incidence of S-IRA in DBA/2 mice. DBA/2 mice that consistently exhibited AGSz without S-IRA showed a significantly increased incidence of S-IRA following treatment with 5-iodotubercidin, which blocks adenosine metabolism. Treatment of DBA/2 mice that consistently exhibited AGSz followed by S-IRA with a non-selective adenosine antagonist, caffeine, or an $A2_A$ adenosine receptor subtype-selective antagonist (SCH 442416) significantly reduced S-IRA incidence. By contrast, an A1 adenosine receptor antagonist (DPCPX) was not effective in reducing S-IRA incidence. These findings suggest that preventative approaches for SUDEP should consider agents that reduce the actions of adenosine.

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) is estimated to be responsible for 1–10 deaths per 1000 patient-years, and the risk of sudden death is more than 20 times higher in individuals with epilepsy than in the general population (Asadi-Pooya and Sperling, 2009; Devinsky, 2011; Ryvlin et al., 2013; Shorvon and Tomson, 2011; Tomson et al., 2016). SUDEP is second only to stroke of the major neurological disorders in the loss of patient years (Thurman et al., 2014). Respiratory difficulties associated with generalized seizures have been observed commonly in witnessed cases of SUDEP (Ryvlin et al., 2013; Surges and Sander, 2012), and a significant degree of respiratory depression is seen in association with generalized seizures in most patients examined (Bateman et al., 2008; Nadkarni et al., 2012). Cerebral oxygen saturation in patients during generalized seizures is significantly reduced, which correlated with SUDEP susceptibility (Moseley et al., 2012; Schuele et al., 2011). Other potential initiating factors for SUDEP include cardiac dysfunction and "cerebral shutdown" (Freitas et al., 2013; Glasscock et al., 2010; Moore et al., 2014).

A number of neurotransmitters and neuromodulators are released during generalized seizures in animals and humans, including adenosine (Fisher and Schachter, 2000; Lado and Moshe, 2008). Adenosine levels have been shown to greatly increase in the brains of patients and animals in association with seizures (Berman et al., 2000; During and Spencer, 1992; Pedata et al., 2001). Adenosine has been proposed to act as a key neuromodulator that acts to halt the ongoing seizure and is thought to function as an endogenous anticonvulsant (Boison, 2011, 2012; Shen et al., 2010). However, adenosine also exerts negative effects on respiration by acting, in part, on the neuronal network in the rostroventral lateral medulla that controls the rhythm and rate of both inspiration and expiration, exerting a negative effect on breathing (Panaitescu et al., 2013; Vandam et al., 2008; Zwicker et al., 2011). Other agents released during seizures, notably serotonin, can enhance respiratory parameters (Brust et al., 2014; Pilowsky, 2014). A number of animal models of SUDEP have been developed that exhibit respiratory depression as a critical precipitating factor. These models include DBA/1 and DBA/2 mice, which are inherited models (Faingold et al., 2010; Feng and Faingold, 2015; Tupal and Faingold, 2006; Venit et al., 2004), as well as models induced by genetic

* Corresponding author.
E-mail address: cfaingold@siumed.edu (C.L. Faingold).

http://dx.doi.org/10.1016/j.eplepsyres.2016.05.007
0920-1211/

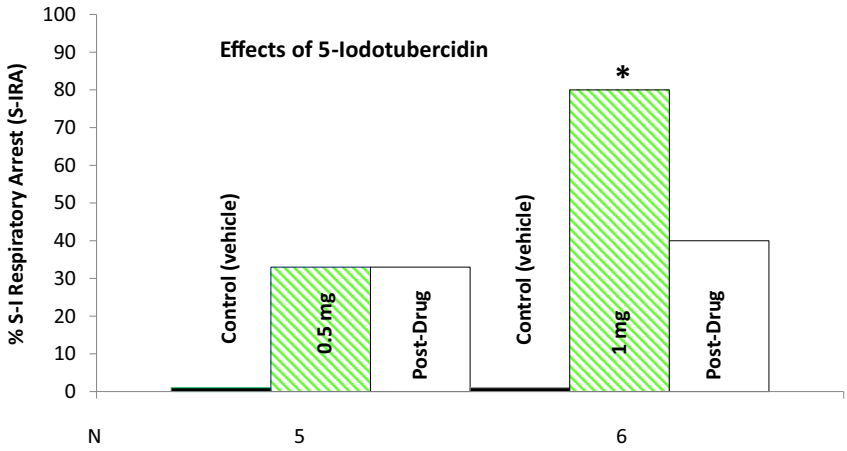

Fig. 1. 5-Iodotubercidin Enhances Susceptibility to Seizure-Induced Respiratory Arrest in DBA/2 Mice. Effect on DBA/2 mice that did not initially exhibit S-IRA of administration of an adenosine kinase inhibitor (5-iodotubercidin in 10% DMSO), 0.5 (in 5 mice) or 1 mg/kg i.p. (in 6 mice). Gray (green) hatched bars (drug) or black bars (vehicle) indicate the incidence of audiogenic seizure (AGSz)-induced respiratory arrest (S-IRA). Thirty min after 5-ITU administration S-IRA susceptibility was increased which reached statistical significance with the 1 mg/kg dose ($p < 0.05$, Wilcoxon signed rank test) as compared to the effect of vehicle alone in a second group of DBA/2 mice. Higher doses of 5-ITU reduced the incidence of AGSz in these mice. The susceptibility to S-IRA persisted in many DBA/2 mice 24 h after 5-ITU administration (white bars) which subsequently return to baseline susceptibility (data not shown). Note: actual p value: ($p = 0.02$ for S-IRA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

manipulation and/or drug or electroshock treatments (Buchanan et al., 2014; Richerson et al., 2016; Shen et al., 2010). DBA mice are subject to sound-induced (audiogenic) seizures (AGSz) that are often lethal due to seizure-induced respiratory arrest (S-IRA) but which can be reversed in most animals if prompt mechanical resuscitation is instituted (Faingold et al., 2010; Tupal and Faingold, 2006). In DBA/2 mice this S-IRA is induced in ~75% of mice, and the remainder of these animals exhibit AGSz without S-IRA (Tupal and Faingold, 2006). These seizure patterns of susceptibility to S-IRA remain consistent over the ~10 day period prior to postnatal day 30 if resuscitation is provided.

In one SUDEP model involving administration of kainate, the incidence of seizure-induced death is significantly elevated by drug treatments that block the breakdown of adenosine (Shen et al., 2010), and blockade of adenosine catabolism in another seizure model, the genetically epilepsy-prone rats (GEPR-9s), also results in a significant elevation of seizure-associated mortality in a recent study (Kommajosyula et al., 2016). The kainate-induced lethality seen following administration of blockers of adenosine catabolism can be significantly reduced by treatment with a non-selective adenosine antagonist, supporting an adenosine theory of SUDEP (Massey et al., 2014; Richerson et al., 2016; Shen et al., 2010, 2014). Agents are available that are antagonists that act selectively on the major subtypes of adenosine receptors (Burnstock, 2013). Therefore, the present study examined the effects of agents that alter the action of adenosine on the seizure-induced respiratory arrest in the DBA/2 model of SUDEP.

2. Methods

2.1. Animals

Male (N = 70) and female (N = 42) DBA/2 mice were obtained from Jackson Laboratories. These mice were screened for susceptibility to S-IRA on postnatal day (PND) 21-23 as previously described (Tupal and Faingold, 2006). Initial screening of DBA/2 mice in this age range that were subjected to AGSz indicated that the incidence of S-IRA in male and female DBA/2 mice was not statistically different, mirroring the lack of gender difference in the incidence of S-IRA observed in DBA/1 mice (Faingold and Randall, 2013). The operational definition of S-IRA is described below. DBA/2 mice that exhibited drug effects were tested subsequently at 24 h intervals to determine if changes in susceptibility to S-IRA returned to pre-drug patterns. The experimental protocols used in this study were approved by the Laboratory Animal Care and Use Committee of Southern Illinois University School of Medicine, which are in accordance with National Institutes of Health guidelines for the care and use of laboratory animals. Measures were included in the protocols to minimize pain and discomfort to the animals and minimize animal usage.

2.2. Seizure induction and resuscitation

All DBA/2 mice were subjected to an acoustic stimulation paradigm, consisting of a broad-band acoustic stimulus generated by an electrical bell (FOS 4771L, Tecumseh, MI) at an intensity of 110 dB SPL (re: 0.0002 dyn/cm^2). Mice were individually placed in a plastic cylinder (43 cm diameter) within a sound-attenuating chamber. The stimulus was given for a maximum duration of 60 s or until the mouse exhibited tonic hindlimb extension, which was followed by S-IRA in most of these mice. Behavior patterns were recorded on videotape, and seizure-related behaviors were analyzed visually off-line. After S-IRA was evoked, all DBA/2 mice received respiratory support to assist in recovery of respiration, as described below. The operational criteria for S-IRA was defined visually by the appearance of a deep respiratory gasp and relaxation of the pinnae, which were invariant indicators in previous studies that S-IRA had begun and death was imminent (Tupal and Faingold, 2006). When S-IRA occurred, resuscitation was initiated, which involved placement of the outflow polyethylene tube (4.4 mm external diam.) of a rodent respirator (Harvard Apparatus #680) over the nostrils of the recumbent mice. The respirator was already pumping room air (180 strokes/min), the outflow tube was placed over the nostrils, and the one cc volume induced visible displacement of the chest. Initiation of resuscitation began 2–5 s after the final deep respiratory gasp to effectively revive the mice and was successful in all of the mice in which it was used (Tupal and Faingold, 2006). The mice were re-subjected to the acoustic stimulation paradigm 24 h and 48 h after drug administration to determine if the pre-drug susceptibility had returned.

One group of DBA/2 mice exhibited AGSz but not S-IRA, which is a consistent pattern for ~25% of DBA/2 mice (Tupal and Faingold,