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Serotonin and sudden death: Differential effects of serotonergic drugs on seizure-induced respiratory arrest in DBA/1 mice

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ABSTRACT

In the DBA/1 mouse model of sudden unexpected death in epilepsy (SUDEP), administration of a selective serotonin (5-HT) reuptake inhibitor (SSRI), fluvoxamine, completely suppressed seizure-induced respiratory arrest (S-IRA) at 30 min after administration (i.p.) in a dose-related manner without blocking audiogenic seizures (AGSz), but another SSRI, paroxetine, reduced S-IRA but with a delayed (24 h) onset and significant toxicity. A serotonin–norepinephrine reuptake inhibitor, venlafaxine, reduced S-IRA incidence, but higher doses were ineffective. A selective 5-HT_7 agonist, AS-19, was totally ineffective in reducing S-IRA. In developing DBA/1 mice that had not previously experienced AGSz, administration of a nonselective 5-HT antagonist, cyproheptadine, induced a significantly greater incidence of S-IRA than that of saline. This study confirms that certain drugs that enhance the activation of 5-HT receptors are able to prevent S-IRA, but not all serotonergic drugs are equally effective, which may be relevant to the potential use of these drugs for SUDEP prevention. Serotonergic antagonists may be problematic in patients with epilepsy.

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) is an uncommon but devastating outcome in patients with epilepsy, leading to the relatively high incidence of sudden death in these patients compared with the general population [1]. Sudden unexpected death in epilepsy is the leading cause of death in patients with refractory epilepsy, with an estimated risk of 35% over a patient's lifetime [2]. Reliable animal models of SUDEP have been developed using DBA/1 and DBA/2 mice that are genetically susceptible to generalized tonic–clonic seizures induced by acoustic stimulation, which consistently lead to sudden death [3–6]. Acoustically-evoked seizures induce sudden death in 75–100% of DBA/1 mice if not rapidly resuscitated compared with ~10% when resuscitation is provided, and spontaneous sudden deaths of ~15% also occur. Observations in patients have identified several potential precipitating factors that may be responsible for SUDEP, which include respiratory, cardiac, and cerebral dysfunctions in association with generalized tonic–clonic seizures [1,7–11]. In SUDEP and near-SUDEP cases that have been witnessed, generalized seizure-induced respiratory dysfunction has occurred in the majority of these cases [1,12]. The proximate cause of death in DBA/1 and DBA/2 mice has also been established as being due to respiratory arrest, leading to cardiac arrhythmia and cardiac arrest, and prompt respiratory support will prevent death in most of these mice [4–6]. The central control of respiratory function is mediated by networks in the brainstem, which are regulated by the actions of several neurotransmitters on the neurons in these network nuclei, and prominent among these neurotransmitters is serotonin (5-hydroxytryptamine, 5-HT) [13,14]. 5-Hydroxytryptamine exerts a stimulatory effect on respiratory function, especially in response to elevated levels of CO_2 [15–18]. Even in patients who survive their generalized tonic–clonic seizures, evidence of respiratory dysfunction and elevated CO_2 levels is observed in association with their seizures [19–21]. A number of neurotransmitters are released during seizures, including 5-HT [22,23]. There are many drugs that are available clinically and others that are being developed that affect the action of 5-HT, including selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), and 5-HT receptor subtype-selective agonists, as well as 5-HT antagonists. The agents that enhance 5-HT availability are used primarily for the therapy of depression and are often used in treating patients with epilepsy who have comorbid depression [24]. Certain of these agents that lead to enhanced activation of 5-HT receptors, including SSRIs, have been successful in blocking sudden death in the DBA/1 and DBA/2 mouse models of SUDEP [4,25,26]. In addition, seizure-related respiratory depression in a retrospective study of a selected group of patients was reduced in patients with epilepsy who were taking SSRIs compared with a matched group of patients who were not taking these drugs [27].

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However, not all tested drugs that enhance the activation of 5-HT receptors in the DBA/1 mouse model of SUDEP are effective in blocking seizure-induced respiratory arrest (S-IRA) [28]. One SSRI exerts a relatively selective effect on S-IRA, while another SSRI blocks both S-IRA and the tonic seizure behaviors but not the other AGSz behaviors in a recent study [26]. Therefore, to improve our understanding of the role of 5-HT in the control of seizure-induced changes in respiration, the present study evaluated the effects of other drugs that alter the action of 5-HT to determine the effect of these agents on S-IRA in the DBA/1 mouse model of SUDEP [5]. Thus, several SSRIs and SNRIs are currently available, but it is not clear if all of these agents are effective in blocking S-IRA and, if so, whether there is a selectivity of effects on S-IRA vs. effects on the other seizure behaviors exhibited by DBA/1 mice. The present study evaluated the effects of two SSRIs, fluvoxamine and paroxetine, an SNRI, venlafaxine, as well as a selective $5HT_7$ agonist (AS-19) to determine if these agents are effective and/or selective in blocking S-IRA. The $5HT_7$ agonist was evaluated because 5-HT_7 receptors are proposed to play a role in mediating the effects of SSRIs, and these receptors are also implicated in the central control of respiration [29,30]. We also examined the effects of a nonselective 5-HT antagonist to evaluate if this agent altered the incidence of S-IRA in developing DBA/1 mice.

2. Methods

2.1. Animals

The male and female DBA/1 mice (N = 266) were obtained from Harlan Laboratories and also included offspring of these mice that were bred in our facility. These mice were screened for susceptibility to audiogenic seizure (AGSz)-induced respiratory arrest (S-IRA), beginning on postnatal day (PND) 24–30 as previously described [5]. The operational definition of S-IRA is described below. DBA/1 mice that exhibited drug effects were tested subsequently at 24-h intervals to determine if the susceptibility to S-IRA returned.

2.2. Seizure induction and resuscitation

All DBA/1 mice were subjected to an acoustic stimulation paradigm, consisting of a broadband acoustic stimulus generated by an electrical bell (Heath Zenith Model #172C-A) 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 a tonic seizure, which ended in tonic hindlimb extension convulsions and often resulted in S-IRA. Behaviors were recorded on videotape, and seizure-related behaviors were quantified visually off-line. After S-IRA was evoked, all DBA/1 mice received respiratory support to assist in recovery of respiration, as described below. The operational criteria for S-IRA were 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 [4,5]. Resuscitation involved the placement of the outflow polyethylene tube (4.4-mm external diameter) of a rodent respirator (Harvard Apparatus #680) over the nostrils of the supine mice. The respirator was already in operation pumping room air (180 strokes/min), and when the outflow tube was placed over the nostrils, 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 [4,5]. The mice were resubjected to the acoustic stimulation paradigm 24 h and 48 h after drug administration and at 24-h intervals thereafter, if necessary, to determine if the susceptibility to S-IRA had returned. In the case of paroxetine, the effects of semichronic 5-day, once daily, administration were also evaluated.

2.3. Drugs

The DBA/1 mice received the following drugs acutely (i.p.): the SSRIs, fluvoxamine (50–80 mg/kg in saline) and paroxetine [75–100 mg/kg in 10% dimethyl sulfoxide (DMSO)] and an SNRI, venlafaxine (25–100 mg/kg in saline). Paroxetine (20–50 mg/kg) was also administered once daily for 5 days to examine the effects of this semichronic treatment. A selective 5-HT_7 agonist (AS-19, 10–60 mg/kg in 10% DMSO) was also administered (i.p.) at 0.1-ml/10-g body weight. A nonselective 5-HT antagonist, cyproheptadine (2 mg/kg in saline), was administered to DBA/1 mice prior to the first induction of AGSz, and the incidence of S-IRA was compared with that seen with saline vehicle. The effect of drug treatment on the seizure-related behavior in DBA/1 mice that previously exhibited S-IRA following AGSz was compared statistically with the effect of the vehicle administered previously in the same mouse. All drugs were obtained from Tocris (Ballwin, MO, USA).

2.4. Statistical analysis

The behavioral study employed a paired experimental design. The videotaped seizure-induced behaviors were analyzed visually, and the incidences of AGSz behaviors (wild running and clonus, tonic extension, and S-IRA) were compared in each animal the day prior, 30, 60, and/or 120 min after drug treatment and at 24-h intervals thereafter to evaluate for the return to S-IRA susceptibility. Statistical analysis compared the effect of each predrug dose with its own control, utilizing the Wilcoxon signed-rank test or the Mann–Whitney *U* test, and differences in the incidences were considered to be statistically significant at $p < 0.05$.

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 the National Institutes of Health guidelines for the care and use of laboratory animals. Measures were included in the protocols to minimize the pain and discomfort of the animals and to minimize animal usage.

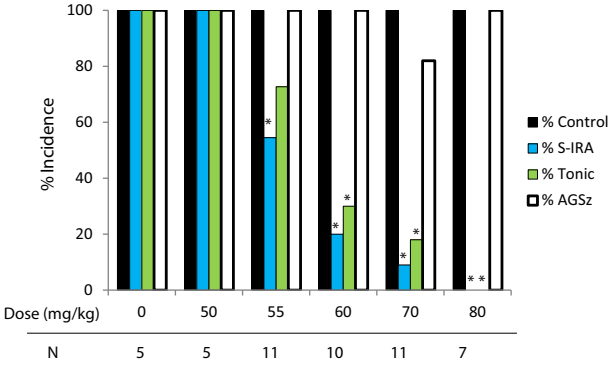

Fig. 1. Effect of administration of a selective serotonin reuptake inhibitor, fluvoxamine, on the incidence of audiogenic seizure (AGSz)-induced respiratory arrest (S-IRA), tonic (hindlimb extension) seizures, and other AGSz behaviors (wild running and/or clonus) in DBA/1 mice. Black bars indicate the % incidence of S-IRA in DBA/1 mice given (i.p.) the vehicle (in saline) injection, and blue (dark gray) bars indicate the incidence of S-IRA 30 min after fluvoxamine (50–80 mg/kg, i.p.) or vehicle (0 mg/kg) administration. Green (light gray) bars indicate incidence of tonic seizures, and white bars indicate incidence of AGSz (clonus and wild running behaviors). Fluvoxamine in doses of 55, 60, 70, or 80 mg/kg significantly reduced the incidence of S-IRA compared with the incidence 24 h before treatment. Note: All the mice remained susceptible to AGSz, and the incidence of tonic hindlimb extension was significantly reduced at the 60- to 80-mg/kg doses. Susceptibility to S-IRA and tonic seizures returned at 24–48 h in most of the mice. Those DBA/1 mice that did not return to S-IRA susceptibility exhibited health problems, including pulmonary edema, and had to be euthanized.* Significantly different from vehicle control, $p < 0.05$; (Wilcoxon signed-rank test). Note: actual p values: 55 mg/kg ($p = 0.025$ for S-IRA), 60 mg/kg ($p = 0.005$ for S-IRA and 0.008 for tonic), 70 mg ($p = 0.002$ for S-IRA and 0.003 for tonic), and 80 mg ($p = 0.008$ for S-IRA and tonic). N is the number of animals for each dose.