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Serotonergic agents act on 5-HT_3 receptors in the brain to block seizure-induced respiratory arrest in the DBA/1 mouse model of SUDEP

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ABSTRACT

Drugs that enhance the action of serotonin (5-hydroxytrypamine, 5-HT), including several selective serotonin reuptake inhibitors (SSRIs), reduce susceptibility to seizure-induced respiratory arrest (S-IRA) that leads to death in the DBA/1 mouse model of sudden unexpected death in epilepsy (SUDEP). However, it is not clear if specific 5-HT receptors are important in the action of these drugs and whether the brain is the major site of action of these agents in this SUDEP model. The current study examined the actions of agents that affect the 5-HT_3 receptor subtype on S-IRA and whether intracerebroventricular (ICV) microinjection of an SSRI would reduce S-IRA susceptibility in DBA/1 mice. The data indicate that systemic administration of SR 57227, a 5-HT_3 agonist, was effective in blocking S-IRA in doses that did not block seizures, and the S-IRA blocking effect of the SSRI, fluoxetine, was abolished by coadministration of a 5-HT_3 antagonist, ondansetron. Intracerebroventricular administration of fluoxetine in the present study was also able to block S-IRA without blocking seizures. These findings suggest that 5-HT_3 receptors play an important role in the block of S-IRA by serotonergic agents, such as SSRIs, which is consistent with the abnormal expression of 5-HT_3 receptors in the brainstem of DBA mice observed previously. Taken together, these data indicate that systemically administered serotonergic agents act, at least, in part, in the brain, to reduce S-IRA susceptibility in DBA/1 mice and that 5-HT_3 receptors may be important to this effect.

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) is a shattering consequence of epilepsy in adult and pediatric patients [1–5] and results in a major cause of lost patient years second only to stroke among neurological disorders [6]. In the majority of witnessed cases of SUDEP, a generalized convulsive seizure and severe respiratory dysfunction were observed prior to death [3]. DBA/1 and DBA/2 mice have been shown to be relevant models of SUDEP, because they exhibit generalized convulsions that lead directly to seizure-induced respiratory arrest (S-IRA), resulting in death if resuscitation is not rapidly instituted [7–9].

Several drugs that enhance the activation of serotonin (5-hydroxytryptamine, 5-HT) receptors, including selective serotonin reuptake inhibitors (SSRIs), prevent S-IRA without blocking seizures in DBA mice, and 5-HT antagonists increase S-IRA susceptibility in nonsusceptible DBA mice [10–12]. Seizures induced by maximal electroshock or pilocarpine in Lmx1b(f/f) mice resulted in elevated seizure-induced mortality because of breathing cessation, which was also reduced by SSRI administration [13]. Since seizure susceptibility is not blocked by SSRIs in DBA mice at doses that block S-IRA, this suggests that these agents exert selective effects on respiratory arrest rather than a general anticonvulsant effect. Respiratory dysfunction after seizures is common in patients with epilepsy [14,15], and patients who exhibited partial seizures and who had been taking SSRIs exhibited less respiratory dysfunction than untreated patients in a retrospective study [16].

Serotonin is known to play a role in the normal regulation of respiration, in part, by acting on neurons in the medullary respiratory centers to enhance respiration in response to elevated CO_2 levels [17,18]. Although our previous studies demonstrated that the SSRI, fluoxetine, at a dose that suppresses S-IRA, did not enhance respiratory ventilation in the absence of seizures in DBA/1 mice, SSRIs may prevent respiratory arrest via maintaining respiratory function during generalized tonic–clonic seizures [12]. Serotonin is also involved in the arousal response via ascending projections from raphe nuclei [19,20]. Thus, deficits of serotonergic neurotransmission in both respiratory and arousal systems may contribute to S-IRA in DBA/1 mice.

Specific subtypes of 5-HT receptors are thought to be selectively relevant to control of respiration, arousal, and epilepsy [21–23]. At

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least seven subtypes of 5-HT receptors are expressed in the brainstem [24,25], and the absence of 5-HT_{2C} receptors in transgenic mice is associated with seizure susceptibility that can result in seizure-induced death [26,27], similar to that seen in DBA mice. The 5-HT_{2A} receptors are reported to contribute importantly to S-IRA induced by electroshock [13]. Our previous studies indicate that levels of several 5-HT receptor subtype proteins, including 5-HT_{2B}, 5-HT_{2C}, and 5-HT_3 receptors, were significantly reduced in DBA/1 mice compared with those in a seizure-resistant mouse strain, although a 5-$HT_{2B/2C}$ receptor agonist (mCPP) was ineffective in reducing S-IRA in DBA/1 mice [28]. Therefore, the present study examined the involvement of 5-HT_3 receptors, the only ionotropic receptor among all of the 5-HT receptor subtypes identified [29], in S-IRA in DBA/1 mice. The 5-HT receptors are also known to exist peripherally and can potentially be an important target for these S-IRA-suppressing SSRIs, particularly by exerting effects in the lung [30]. Therefore, the present study also examined the effect of administration of an SSRI directly into the brain using intracerebroventricular (ICV) route.

2. Materials and methods

2.1. Animals

The male and female DBA/1 mice were obtained from Harlan Laboratories (Indianapolis, IN) and were housed and/or bred in the animal facility with food pellets and water available ad libitum. Multiple groups of DBA/1 mice were screened for susceptibility to S-IRA induced by audiogenic seizures (AGSz), beginning on postnatal day (PND) 24–30, and acoustic stimulation was presented daily for 3–4 days at which time 90 to 100% of DBA/1 mice in each group developed S-IRA susceptibility, as previously described [8]. This "priming" process at a young age is important to developing the chronic consistent S-IRA susceptibility that allows the animals to model SUDEP and the testing of SUDEP prevention treatments [8,31]. A total of 97 mice were used in the current study. The operational definition of S-IRA is described below. Only those mice that consistently exhibited S-IRA were used in these experiments. The experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Southern Illinois University School of Medicine and Massachusetts General Hospital, which are in accordance with the National Institute of Health guidelines for 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.

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 or FOS 4771L, Tecumseh, MI) at an intensity of 96–110 dB SPL. Mice were individually placed in a plastic cylinder within a sound-isolation room. 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 consistently resulted in S-IRA. Behaviors were recorded on videotape, and seizure-related behaviors were quantified visually offline. 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 by the appearance of a deep respiratory gasp and relaxation of the pinnae determined visually, which were invariant indicators in previous studies that S-IRA had begun and death was imminent [8]. Although S-IRA was behaviorally determined by qualitative (visual) breathing assessment, our previous quantitative studies using plethysmography indicated that S-IRA leads to terminal asystole and death in DBA/1 mice [32]. Resuscitation was accomplished by placing the outflow polyethylene tube (4.4 mm external diam.) of a rodent respirator (Harvard Apparatus 680, Holliston, MA) over the nostrils of the supine mice. The respirator was previously in operation pumping room air (180 strokes/min), and when the outflow tube was placed over the nostrils, the 1-cm^3 volume induced visible displacement of the chest. Initiation of resuscitation began within 5 s after the final deep respiratory gasp to effectively revive the mice [7,8]. 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.

2.3. Intracerebroventricular (ICV) injection

The ICV cannulation was carried out as described in earlier studies [33]. In brief, mice were anesthetized with ketamine/xylazine (100/10 mg/kg, i.p.). A guide cannula (26G, Plastics One, Roanoke, VA) was stereotaxically implanted based on coordinates from Paxinos and Franklin [34] (AP − 0.4 mm; ML − 1.0 mm; and V − 2.0 mm). The guide cannula was fixed to the skull using mounting screws (BASi, West Lafayette, IN) and dental cement (A-M Systems, Sequim, WA). A stainless steel stylet was used to occlude the guide cannula when not in use. The animals were then allowed to recover for a week, during which tetracycline (1 g/l) was administered in the drinking water to reduce infection. One week after implant surgery, each mouse was tested to verify that it remained susceptible to seizures and S-IRA and then resuscitated. The following day, microinjections were made using a Hamilton syringe and a pump (11 Elite Nanomite, Harvard Apparatus), which was connected to the internal cannula (33G, Plastics One) by a polyethylene tubing, and 1.0 μl of fluoxetine or the vehicle, dimethyl sulfoxide (DMSO), was administered at a rate of 0.5 μl/min into the left lateral ventricle. The injection cannula was left in place for a further 1 min before being slowly withdrawn to avoid back flow.

2.4. Histology

Verification of the placement of the ICV guide cannula was done at the end of experiments. Fast green was injected ICV to mark the ventricular space. Each mouse was euthanized with an overdose of ketamine/xylazine and transcardially perfused with 30 ml PBS (pH 7.4), followed by 30 ml 4% paraformaldehyde. The brains were removed and stored in 4% paraformaldehyde at 4 °C. Each brain was sectioned into 50-μm thickness of coronal slices using a freezing microtome (CM 1850 UV, Leica, Buffalo Grove, IL). The placement of the guide cannula was observed using a light microscope. Only data from animals with correct cannula placement were used for statistical analysis.

2.5. Drugs

Because of the very small volume used for ICV injection, the dose of fluoxetine (120 nmol) was not soluble in saline, and dimethyl sulfoxide (DMSO) was used as the vehicle for these experiments. The DBA/1 mice received the following drugs acutely: the SSRI, fluoxetine, administered i.p. (40 mg/kg in saline) 30 min prior to AGSz induction or ICV (60, 90, or 120 nmol in DMSO) 15 min prior to AGSz. The selective 5-HT_3 agonist SR 57227 (20–40 mg/kg in saline) or the 5-HT_3 antagonist ondansetron (0.5–1 mg/kg in distilled water) was administered 30 and 35 min prior to AGSz, respectively. All drugs were obtained from Sigma-Aldrich (St. Louis, MO).

2.6. Statistics

The incidence of S-IRA between drug and vehicle groups was compared using Mann–Whitney U test. Statistical significance was inferred if $p < 0.05$.