The Action of Fenfluramine to Prevent Seizure-Induced Death in the DBA/1 Mouse SUDEP Model is Selectively Blocked by an Antagonist or Enhanced by an Agonist for the Serotonin 5-HT4 Receptor

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**ABBRVIATIONS / DRUGS**

5-HT: 5-hydroxytryptamine  
AEDs: Antiepileptic drugs  
S: Selective  
Sz: Sodium  
FFA: Fenfluramine  
GTC: Generalized tonic-clonic seizures  
S-IRA: Seizure-induced respiratory arrest  
SSRI: Selective serotonin reuptake inhibitor  
SUDEP: Sudden Unexpected Death in Epilepsy

**INTRODUCTION**

SUDEP cases exhibit generalized tonic-clonic seizures (GTCS) leading to post-ictal apnea and subsequent asystole. Proposed approaches to reducing SUDEP include methods to mitigate respiratory compromise4,5, SUDEP is known to be associated with sub-therapeutic levels of AEDs, and addition of AEDs may reduce SUDEP incidence in drug resistant epilepsy1,2. Therefore, there is a need for add-on AEDs with an indication for SUDEP prophylaxis. A recent study proposed that post convulsive central apnea is a biomarker for SUDEP1, and treatments that can block this apnea may be therapeutically useful in SUDEP prevention.  

DBA/1 mice are a valuable SUDEP model that consistently exhibits post convulsive central apnea following by cardiac failure, mimicking the sequence seen in most witnessed cases of human SUDEP. DBA/1 mice exhibit increased susceptibility to seizure-induced respiratory arrest (S-IRA) and death in response to several seizure induction methods, including intense acoustic stimulation (audionic seizures, Sz)6, the Sz in DBA/1 mice consist of GTC followed by post-ictal S-IRA. Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter released post-ictally in epilepsy models and patients, which is known to enhance respiration3,10. The SSRI, fluoxetine, enhances 5-HT availability, and prevents S-IRA in DBA/1 mice by acting via 5-HT3 receptors12. Fenfluramine (FFA), which enhances 5-HT release in the brain, is an effective add-on agent in Dravet syndrome11,12 and is able to block Sz and selectively prevent S-IRA in DBA/1 mice13. S-HT4 receptors, are expressed in the brainstem respiratory network, where 5-HT enhances respiration14. The present study examined the role of specific 5-HT receptor subtypes in mediating the ability of FFA to prevent S-IRA by treating DBA/1 mice with specific 5-HT antagonists. We observed that a 5-HT4 antagonist was most effective in reversing the selective S-IRA blockade by FFA and examined the effect of a 5-HT4 agonist on S-IRA in DBA/1 mice. The present experiments also further evaluated the serotonin hypothesis of SUDEP, which proposes that altering the action of serotonin can control SUDEP susceptibility15,16.

**METHODS**

1. DBA/1 mice (age: 25-26 days) were subjected to Sz priming.  
2. Mice that showed S-IRA were resuscitated using a rodent respirator (200 strokes/min).  
3. At least 24 h after priming, the consistently S-IRA susceptible mice were given FFA or vehicle (saline) by i.p. injection.  
4. The following experimental paradigms were used to determine the 5-HT receptors that mediate the protective effect of FFA:  
   a. Determination of dose and time at which FFA-treated mice showed the lowest incidence of S-IRA following tonic seizures: Mice were tested at 8 h intervals after receiving FFA (5-20 mg/kg, i.p.) for 24 h (to determine the selective S-IRA blocking effect). The mice that did not show a return of S-IRA susceptibility were reassessed at 24 h intervals.  
   b. Determination of 5-HT receptors that mediate S-IRA prophylaxis and anticonvulsant effects of FFA: Selective 5-HT receptor antagonists were administered (i.p.) 30 min prior to seizure testing, which was done at a dose- and time-point at which significant selective S-IRA blockade by FFA was seen.  
   c. Based on the results of the previous experiments, the effect on S-IRA incidence of a 5-HT4 agonist (BIMU-8), alone or with a sub-effective dose of FFA was evaluated  
5. Behaviors were recorded on video and quantified, and changes in the incidence of tonic seizures and S-IRA were analyzed off-line using the Chi-square test with a significance level set at p<0.05.

**SUMMARY & CONCLUSIONS**

1. A significant reversal of the FFA-mediated reduction in S-IRA incidence in DBA/1 mice was elicited by the 5-HT4 receptor agonist (GR12247).
2. Pretreatment with the 5-HT4 receptor agonist, BIMU-6 (30-40 mg/kg) selectively reduced the incidence of S-IRA as well as tonic seizures.
3. Co-administration of ineffective doses of BIMU-6 and fenfluramine also selectively reduced the incidence of S-IRA and tonic seizures.
4. These findings strongly implicate a crucial role of 5-HT4 receptors in mediating the selective block of S-IRA by FFA.
5. The 5-HT4 and SHT receptors may also play a role in modulating the anticonvulsant effect of FFA in reducing S-IRA susceptibility in these mice, but the antagonists at these receptors do not selectively block S-IRA.

**REFERENCES**


**SIGNIFICANCE & FUTURE DIRECTIONS**

1. We show for the first time that 5-HT4 receptors mediate the seizure-induced sudden death prevention effect of FFA in DBA/1 mice.  
2. This is also the first report that the selective 5-HT4 agonist is effective in blocking seizure-induced sudden death in DBA/1 mice.  
3. The relevance of selective 5-HT4 blockade to the hypothesis that a cardiac action is exerted on the respiratory network in the brainstem.  
4. Studies involving intracerebrovascular injection of 5-HT receptor antagonists, along with the i.p. injection of FFA, are needed to confirm its mechanism of action.  
5. These findings further support the serotonin hypothesis of SUDEP/4.

**SUPPORT**

Zogenix International Limited  
Southern Illinois University School of Medicine