ABSTRACT #1.268

PROPHYLAXIS OF SEIZURE-INDUCED RESPIRATORY ARREST (S-IRA) WITH FENFLURAMINE IN A MOUSE MODEL OF SUDEP.

Srinivasan Tupal and Carl L. Faingold

Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, Illinois 62794-9629

SIU SCHOOL of MEDICINE

ABBREVIATIONS / DRUGS

5-HT	5-hydroxytryptamine
Fenfluramine (Fen.)	5-HT release enhancer
nor-Fen.	Active metabolite of Fen.
PID	Post-ictal depression
SUDEP	Sudden Unexpected Death in Epilepsy
S-IRA	Seizure-induced respiratory arrest
AUC	Area Under the curve
AGSz	Audiogenic seizures
ED_{50}	Effective dose-50
AUC	Area under the curve
n	Number of animals

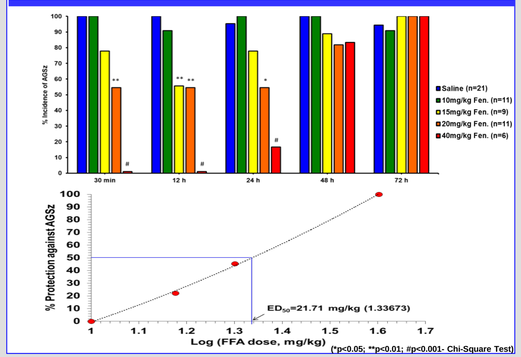
INTRODUCTION

Prevention of premature mortality due to sudden unexpected death in epilepsy (SUDEP) is a major unmet need in neurology. Most of the witnessed clinical cases of SUDEP involved generalized seizures that led to respiratory and then cardiac failure during post-ictal behavioral depression (PID). Serotonin (5-hydroxy-tryptamine, 5-HT) is an important neurotransmitter released during PID that affects respiration (Fisher and Schachter, 2000). Dravet Syndrome is an intractable form of epilepsy that shows an elevated incidence of SUDEP. A recent report indicated that patients with Dravet Syndrome have an improved clinical course when treated with fenfluramine (Ceulemans et al., 2016), a drug that enhances 5-HT release. In the DBA/1 mice model death is due to seizure-induced respiratory arrest (S-IRA) during PID following audiogenic seizures (AGSz). Fluoxetine, which blocks 5-HT re-uptake, effectively suppresses SUDEP in DBA/1 mice (Faingold et al., 2011), but not all the drugs that enhance the action of 5-HT are effective (Faingold et al., 2011, 2014. In the present study we evaluated whether fenfluramine could prevent S-IRA in the DBA/1mouse SUDEP model.

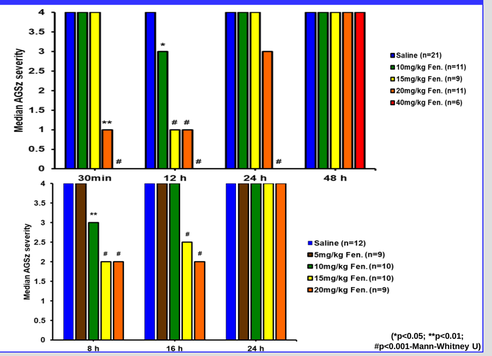
METHODS

1. DBA/1 male mice (25-26 days) were subjected to AGSz priming involving 3-4 daily tests by presenting 122 dB SPL (re: 0.0002 dyne/cm^2) broadband acoustic stimulus for ≤1 min.
2. The ordinal AGSz severity scoring used was: no seizure=0; wild running=1; clonic seizure=2; tonic seizure=3; death/S-IRA=4 (De Sarro et al., 2017).
3. Mice that showed S-IRA were resuscitated by placing the inhalation tube of a rodent respirator (200 strokes/min) over the nose.
4. At least 24 h after the final priming seizure the consistent S-IRA susceptible mice were given fenfluramine (5-40 mg/kg) or vehicle (saline) intraperitoneally.
5. The experimental paradigms used to determine the dose and time course of fenfluramine's effect on AGSz, post-ictal depression (represented by righting reflex latency) and S-IRA incidence were:
   - (A)30 min, 12 h and 24 h following fenfluramine (10-40 mg/kg) or saline (dose-response relationship).
   - (B) 8 h intervals for 24 h (selective S-IRA blocking effect).
   - The mice that didn't show a return of AGSz and S-IRA susceptibility were tested again at 24 h intervals.
6. The drug and active metabolite (nor-Fenfluramine) exposure levels (i.e. area under the curve) in brain and plasma was calculated using trapezoidal rule from the concentrations determined by liquid chromatography–mass spectrometry (Algorithme Pharma Inc.) using the samples obtained terminally (Euthasol) at 8 h intervals as in paradigm B.
7. Behaviors were recorded on videotape, quantified and analyzed offline using SPSS software.
8. The effective dose 50 (ED50) of fenfluramine against AGSz in DBA/1 mice was calculated linear regression analysis with Sigma Plot and Microsoft Excel.

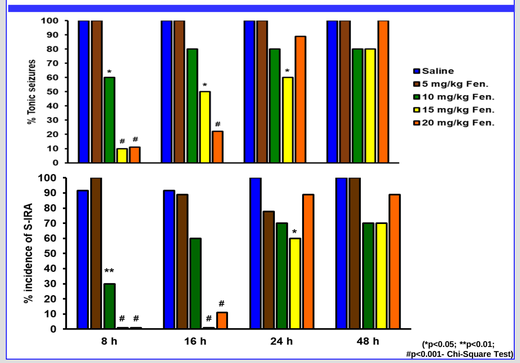
Fenfluramine significantly blocks AGSz in DBA1 mice



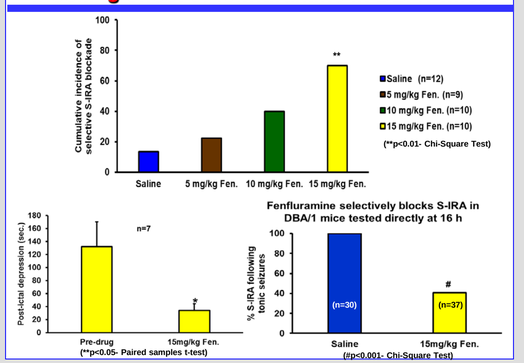
Fenfluramine dose-dependently reduces seizure severity in DBA1 mice



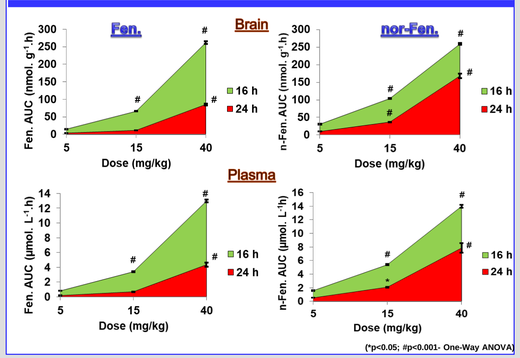
Fenfluramine exerts selective S-IRA blocking effect in DBA/1 mice



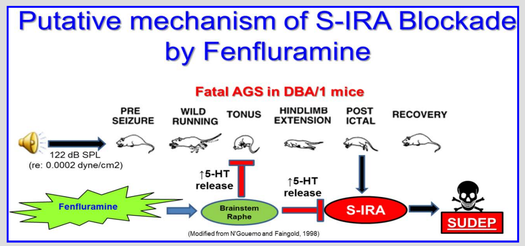
Dose and time-point for selective S-IRA blocking effect of fenfluramine in DBA1 mice



Effective doses of fenfluramine result in higher drug/ active metabolite AUC



Putative mechanism of S-IRA Blockade by Fenfluramine



SUMMARY & CONCLUSIONS

1. Fenfluramine, a drug that enhances the release of 5-HT, reduces incidence and severity of AGSz and S-IRA in DBA/1 mice.
2. Fenfluramine displayed long-lasting dose- and time-dependent anticonvulsant and S-IRA blocking effect.
3. The present study suggests a novel application of fenfluramine to prevent respiratory failure after seizure, which may further improve clinical outcomes in Dravet syndrome (Ceulemans et al., 2016).
4. These findings suggest that fenfluramine may be useful to better control seizures and is a potential therapeutic agent that deserves investigation for preventing SUDEP in epilepsy patients.

SIGNIFICANCE & FUTURE DIRECTIONS

1. Our data provide the first evidence of fenfluramine's selective S-IRA blocking effect.
2. This suggests that clinical studies should investigate seizure and SUDEP prevention with fenfluramine.
3. Further studies into fenfluramine's effect on central neurotransmitter(s) and prolonged time course of effects are needed.
4. Receptor mechanism(s) mediating the anticonvulsant and S-IRA blocking effect and sites in the brain of fenfluramine remain to be determined.
5. The effect of semi-chronic treatment is needed to further substantiate SUDEP prophylaxis potential of Fenfluramine.

REFERENCES

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