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Fenfluramine Has In Vivo Activity as a Positive Modulator of Sigma-1 Receptors

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INTRODUCTION

- Fenfluramine (FFA) provides unique, unexpected antiepileptic activity in Dravet syndrome (DS)[1,2]
  - High degrees of seizure reduction and freedom
  - Prolonged duration of efficacy
- FFA antiepileptic activity is thought to arise from agonist activity of neuronally-released serotonin (5-HT) or via a direct agonist effect on 5-HT receptors
- Recent data suggest that FFA's interaction with sigma-1 receptors (S1Rs) may also be responsible for its ability to reduce seizure activity
  - Studies utilizing the zebrafish model of DS have shown that:
    - FFA's antiseizure activity was due, at least in part, to agonism at several 5-HT receptors[3]
    - The S1R antagonist NE-100 also reduced seizure activity, while the S1R agonist PRE-084 reversed the efficacy of FFA, suggesting that FFA alters seizure activity by acting on S1Rs[3]
    - FFA behaves as a positive modulator at S1Rs in an in vitro S1R/HSP70 chaperone (BiP) dissociation assay[4]
- The spontaneous alternation test and the passive avoidance test are well known tests used to study the mechanism of sigma-1 activity[5]
- We used the spontaneous alternation and passive avoidance tests to investigate the possibility that FFA is a positive modulator of S1Rs in vivo to corroborate earlier in vitro data[4]

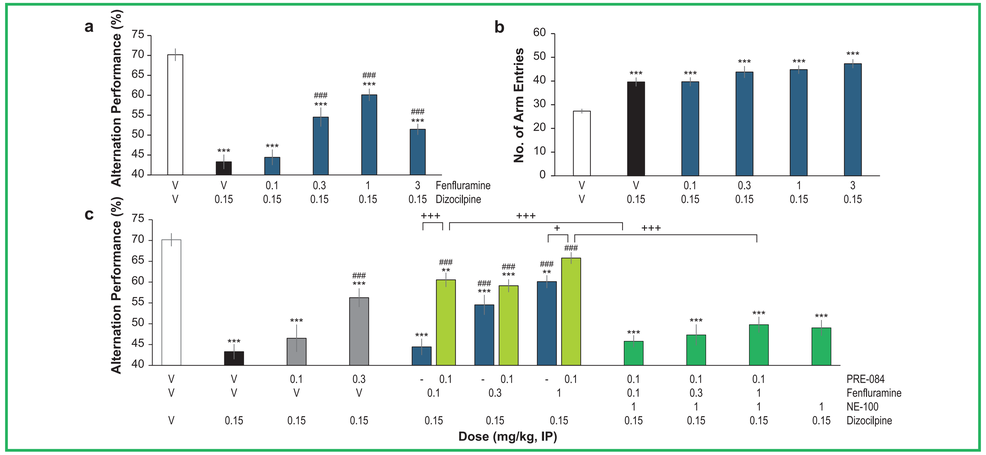
METHODS

- All studies employed male Swiss OF-1 mice, aged 7-9 weeks and weighing 32±2 g
- The dizocilpine-induced learning impairment mouse models of spontaneous alternation in Y-maze and step-through passive avoidance were used to assess any positive modulation of S1Rs by FFA (dizocilpine-induced learning impairment shown to be modulated by sigma ligands[5,6])
  - Spontaneous alternation in the Y-maze
    - An index of spatial working memory[5-9]
    - Alternation defined as successive entries into all three arms of the Y
  - Step-through passive avoidance test
    - Measures nonspatial/contextual long-term memory[8,9]
- Statistical analysis
  - Data were analyzed using a one-way analysis of variance (ANOVA, F value), followed by a post-hoc Dunnett's test or a Kruskal-Wallis nonparametric ANOVA (H value), followed by a Dunn's multiple comparison test, for passive avoidance latencies (expressed as median and interquartile range)
- Combination index (CI) to determine the interaction of two agents was calculated according to Zhao et al (2010)[10] and Maurice (2016)[9]
  - A CI <1 indicates synergy; CI=1 shows additivity; and CI >1 indicates antagonism

RESULTS

- Spontaneous alternation and passive avoidance tests demonstrated positive modulation of FFA on S1Rs
  - Dizocilpine (learning impairment agent) produced substantial reductions in spontaneous alternation (**Figure 1**) and passive avoidance learning (**Figure 2**)
    - FFA significantly attenuated both deficits; the most effective doses were 0.3 and 1 mg/kg IP
  - PRE-084 (selective S1R agonist) attenuated dizocilpine-induced deficits at 0.3 but not 0.1 mg/kg

Figure 1. Dose-response protection by FFA of dizocilpine-induced learning impairment in mice in spontaneous alternation (Y-maze) test: (a) spontaneous alternation performances and (b) total number of arm entries in the Y-maze test. (c) Combination of FFA with the S1R agonist PRE-084. Fenfluramine, PRE-084, and/or NE-100 (S1R antagonist) were administered 10 min before dizocilpine, injected 20 min before the session. Bars are mean±SEM.

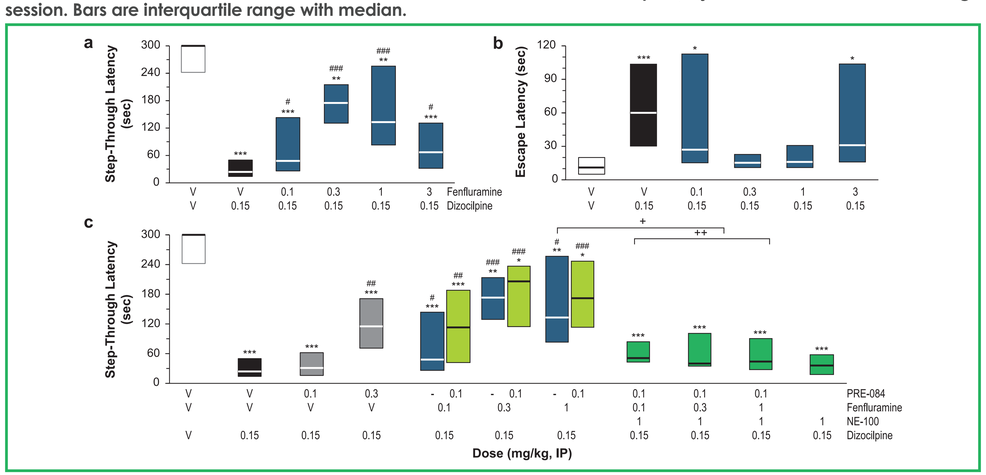


**p<0.01, ***p<0.001 vs vehicle-treated group and ###p<0.001 vs dizocilpine-treated group, ANOVA and Dunnett's test; +p<0.05, +++p<0.001, Student's t-test.

FFA, fenfluramine; IP, intraperitoneal; SEM, standard error of the mean; V, vehicle.

- FFA prevented the inhibition in alternation performance (AP) induced by dizocilpine but had little effect on the number of arm entries (panels a and b)
- The selective S1R agonist PRE-084 also partially prevented the inhibition in AP induced by dizocilpine (panel c, gray bars)
- FFA prevention of AP induced by dizocilpine was enhanced by a nonactive dose of PRE-084 (panel c, blue and light green bars)
- FFA prevention of AP and enhancement of FFA by PRE-084 was blocked by the selective S1R antagonist NE-100 (panel c, dark green bars)

Figure 2. Dose-response prevention by FFA of dizocilpine-induced learning impairments in mice: (a) step-through latency and (b) escape latency in the passive avoidance test. (c) Combination with the S1R agonist PRE-084. Fenfluramine, PRE-084, and/or NE-100 were administered 10 min before dizocilpine, injected 20 min before the training session. Bars are interquartile range with median.



*p<0.05, **p<0.01, ***p<0.001 vs vehicle-treated group and #p<0.05, ##p<0.01, ###p<0.001 vs dizocilpine-treated group, Kruskal-Wallis ANOVA and Dunn's test. +p<0.05, ++p<0.01, pairwise Mann-Whitney's test.

FFA, fenfluramine; IP, intraperitoneal; V, vehicle.

- FFA prevented the inhibition in step-through latency (STL) and reduced the escape latency induced by dizocilpine (panels a and b)
- The selective S1R agonist PRE-084 also partially prevented the inhibition in STL induced by dizocilpine (panel c, gray bars)
- FFA prevention of STL induced by dizocilpine was mildly enhanced by a non-active dose of PRE-084 (panel c, blue and light green bars)
- FFA prevention of STL and enhancement of FFA by PRE-084 was blocked by the selective S1R antagonist, NE-100 (panel c, dark green bars)
- Examination of CI values in both tests showed that a noneffective dose of PRE-084 (0.1 mg/kg) resulted in either a synergistic or additive effect when administered in combination with different doses of FFA (CI values ranging from 0.36 to 0.99); no evidence of antagonism was observed

DISCUSSION

- Recent experimental data[3,4,11] suggest that FFA may act not only on 5-HT receptors, but also on S1Rs to reduce seizures
  - Positive modulators of S1Rs were recently shown to have antiepileptic activity in pentylenetetrazol and bicuculline mouse seizure models[12]
- FFA interaction with S1Rs was investigated using the spontaneous alternation test and the passive avoidance test
  - FFA acted synergistically with S1Rs to prevent/reduce the inhibition in alternation performance induced by dizocilpine; this combined effect was blocked by S1R antagonism with NE-100
  - FFA acted synergistically with S1Rs to prevent/reduce the inhibition in STL induced by dizocilpine; this combined effect was blocked by S1R antagonism with NE-100
- Thus our data suggest that, following administration of FFA, there is a positive interaction between FFA and S1Rs
  - Although an S1R antagonist reduced seizure activity in the zebrafish model,[3] while an S1R agonist reduced seizure activity in mice[12] and prevented dizocilpine-induced deficits in the spontaneous alternation and passive avoidance tests, this apparent discrepancy may be due to dose since S1R ligands may show a biphasic dose-response curve, and can have different and opposite effects depending on the dose of the ligand[13]

CONCLUSIONS

- These data provide in vivo confirmation of earlier in vitro results suggesting that FFA's antiepileptic activity may not be solely due to serotoninergic effects, but could involve a synergistic effect on the sigma-1 receptor

REFERENCES

1. Ceulemans B, et al. *Epilepsia*. 2016;57:e129-34.
2. Schoonjans A, et al. *Eur J Neurol*. 2017;24:309-14.
3. Sourbron J, et al. *ACS Chem Neurosci*. 2016;7:588-98.
4. Martin P, et al. SOBP 2017 Annual Meeting, May 18-20, 2017, San Diego, CA. Poster 663.
5. Maurice T, et al. *Brain Res*. 1994;647:44-56.
6. Maurice T, et al. *Pharmacol Biochem Behav*. 1994;49:859-69.
7. Maurice T, et al. *Neuroscience*. 1998;83:413-28.
8. Meunier J, et al. *Br J Pharmacol*. 2006;149:998-1012.
9. Maurice T. *Behav Brain Res*. 2016;296:270-8.
10. Zhao L, et al. *Front Biosci*. 2010;2:241-9.
11. Sourbron J, et al. *Front Pharmacol*. 2017;8:191.
12. Vavers E, et al. *Behav Brain Res*. 2017;328:13-8.
13. Rousseaux C, et al. *J Recept Sig Transduct*. 2015;36:327-88.

DISCLOSURE

PM, AG, GMF, BB, BSG: Employee, Zogenix; Stock ownership, Zogenix.
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