An Examination of the Mechanism of Action of Fenfluramine in Dravet Syndrome: A Look Beyond Serotonin
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INTRODUCTION
Racemic fenfluramine (FFA) has provided unique, unexpected seizure control with a prolonged duration of effect in children and young adults with Dravet syndrome (DS), which is unusual in epilepsy

Fenfluramine (FFA) has been widely known to produce serotonergic effects via release of serotonin (5HT) by reversal of 5HT transporter (5HTT) flux and direct activation of 5HT receptors. However, it is highly unlikely that these effects are solely responsible for the unique clinical profile:

- Limited data in the preclinical and clinical literature suggest that 5HT agonism might provide some benefit against seizures
- Conversely, antidepressants have also been reported to lower seizure threshold in some instances
- The incidence of depression and anxiety is reported as high as 24% in people with epilepsy and treatment with selective serotonin reuptake inhibitors (SSRIs) for depression in epilepsy is common, suggesting that a clear anticonvulsant effect would have been reported in the literature, which is not the case
- After the first published case series using FFA, pediatric epileptologists have prescribed SSRIs for DS, but without benefit [intraclinical communication]

Recently, Martin and colleagues found that FFA alters activity at the sigma receptor at concentrations that are physiologically relevant. Based on their experiments:

- FFA did not show classic agonist or antagonist activity
- The FFA and its enantiomers potentiated the sigma 1 agonist (+)-SFK-10,047, suggesting that FFA acts as a positive allosteric modulator
- Racemic fenfluramine and its enantiomers appeared to behave as inverse agonists
- Sourbron et al. using a zebratfish model of DS, determined that FFA acts as an agonist at 5HTT1A, 5HT2A, and 5HT2C receptors and as an antagonist at the sigma 1 receptor (Sig-1R), to reduce seizure activity
- Given the above observations, we conducted additional studies to examine and better understand the effect of FFA on Sig-1R

Lorcaserin also has 5HT2C receptor agonist activity, although unlike FFA, it has shown only limited and short-term effectiveness in five patients with DS. Therefore, we also examined the effect of lorcaserin on the Sig-1R.

METHODS
Receptor binding

- Binding of FFA, norfenfluramine, and their enantiomers was evaluated using radioligand binding assays with recombinant human receptors or with rat, bovine, or rabbit receptors when human receptors were not available (Seikui Xenotech, LLC, Kansas City, KS). Data were expressed as Ki values in molar concentration
- The binding immunoglobulin protein (BiP) assay was used to measure the activity of FFA and lorcaserin in Sig-1Rs
- In the dormant state, the Sig-1R forms a complex with BiP
- Sig-1R agonists cause a dissociation of the complex so that the receptor becomes active
- Sig-1R antagonists inhibit the action of agonists by preventing the chaperone activity and mediating proper folding of membrane proteins of sigma 1 agonists
- Sig-1R modulatory molecules alter the response to an agonist or antagonist

Experimental methods: cell culture and EUSA

- CHO cells were grown in 24-well plates and treated with compounds in culture medium at 37°C for 30 min at 1 µM and 10 µM final concentrations
- Reaction was stopped by removal of 3 M Pi of 37°C
- CHO cells were harvested and suspended in phosphate-buffered saline (PBS) 7.4 followed by 5 µg/ml of diithiothreitol (dithiothreitol) and proteinase K
- Reaction was stopped by addition of 1X HCl (pH 8.3, final 0.5 mol/L)
- Fifteen minutes after incubation on ice, cells were lysed with RIPA buffer (50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 1% Triton X-100, 0.3% sodium deoxycholate, 0.1% SDS, 0.1% SDS), and the cell lysate was incubated with Sepharose protein-A
- After successive centrifugations, supernatants were analyzed by ELISA assay

Experimental methods: fenfluramine and lorcaserin

- Reference compounds: PRE084 (sigma 1 agonist) and NE 100 (sigma 1 antagonist)

RESULTS

Receptor Binding

Table 1. Enantiomers of FFA and Norfenfluramine: KI Values (µM) Demonstrating ≥30% Binding Based on Percent Inhibition

<table>
<thead>
<tr>
<th>Receptor</th>
<th>(-) FFA (µM)</th>
<th>(+) FFA (µM)</th>
<th>Norfenfluramine (µM)</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>µ-Agonist (non-selective)</td>
<td>1.61 x 10^-6</td>
<td>1.36 x 10^-6</td>
<td>9.76 x 10^-7</td>
<td>8.48 x 10^-7</td>
</tr>
<tr>
<td>µ-Agonist</td>
<td>8.84 x 10^-6</td>
<td>1.40 x 10^-6</td>
<td>8.60 x 10^-6</td>
<td>5.56 x 10^-6</td>
</tr>
<tr>
<td>M. Muscarinic</td>
<td>8.30 x 10^-6</td>
<td>1.15 x 10^-5</td>
<td>3.27 x 10^-6</td>
<td>4.00 x 10^-6</td>
</tr>
<tr>
<td>Sigma 1 (selective)</td>
<td>1.63 x 10^-6</td>
<td>3.51 x 10^-6</td>
<td>1.80 x 10^-6</td>
<td>2.30 x 10^-6</td>
</tr>
<tr>
<td>Sigma 2</td>
<td>1.09 x 10^-5</td>
<td>5.02 x 10^-6</td>
<td>2.61 x 10^-6</td>
<td>4.60 x 10^-6</td>
</tr>
<tr>
<td>Sigma 3</td>
<td>4.31 x 10^-6</td>
<td>8.00 x 10^-6</td>
<td>2.98 x 10^-6</td>
<td>3.21 x 10^-6</td>
</tr>
</tbody>
</table>

- 47 receptor subtypes associated with epilepsy were identified and examined for fenfluramine binding
- Receptors that showed binding ≥30% as measured by percent inhibition are shown in Table 1 (enantiomers of FFA and norfenfluramine)
- Binding and activity at the Sig-1R receptor, but not adrenergic or muscarinic receptors, occurred at physiologically relevant concentrations

Agonist and Antagonist Assays

Fenfluramine

Figure 1. Effect of FFA on BiP – Sig-1R after 30 min of incubation in culture medium

FFA potentiated the effect of the agonist PRE084 and therefore acted as a positive allosteric modulator at a concentration of 10 µM

Lorcaserin

Figure 2. Effect of lorcaserin on BiP – Sig-1R after 30 min of incubation in culture medium

Lorcaserin had no effect on the BiP – Sig-1R dissociation and therefore did not act as an agonist or an antagonist

BiP/Sig-1R Association

Statistical analysis: * p < 0.05, ** p < 0.01, *** p < 0.001

SUMMARY OF RESULTS

- Fenfluramine binds to sigma receptors
- Fenfluramine, but not lorcaserin, acts as a positive allosteric modulator of the Sig
- Lorcaserin did not show activity on the Sig-1R

CONCLUSION

- FFA is widely known as a “serotonergic” drug, yet the unexpected meaningful and prolonged reduction in seizure frequency observed after its use in DS patients distinguishes it from other serotonergic drugs such as SSRIs and lorcaserin
- Recently Martin et al. evaluated 47 receptors associated with epilepsy as possible sites for fenfluramine activity that could explain its unique effectiveness in DS and possibly other pediatric epilepsies. In this investigation, FFA demonstrated binding activity at the sigma 1 receptor
- Sig-1R effects may play a role in the anticonvulsant activity of FFA
- Activation of the Sig-1R attenuates seizures
- The Sig-1R dimerizes with other receptors, and it modulates serotonergic neurotransmission
- FFA acts as a positive allosteric modulator of the Sig-1R
- Lorcaserin did not show activity on the Sig-1R — neither agonism, antagonism, nor allosteric modulation

REFERENCES
20. Maurice F. Personal communication.

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