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A 5-HT_{1D}-Receptor Agonist Protects Dravet Syndrome Mice from Seizure and Early Death

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Abstract

Mutations in the SCN1A gene encoding the Nav1.1 sodium channel cause several forms of epilepsy, the most severe is Dravet syndrome (DS). DS patients are at high risk for sudden death and seizures are often triggered by fever or hyperthermia. To improve understanding of how serotonergic ligands might influence DS in this study we tested several drugs for their effect on hyperthermia-induced seizure using a mouse model of DS consisting of a heterozygous loss-of-function of *Scn1A*. We found that a mixed 5-HT_{2A/2C} receptor agonist had no effect while a mixed 5-HT_{1B/D} receptor agonist had a modest effect reducing the severity of seizures. Hypothesizing selective agonists may be more effective, we tested selective 5-HT_{1B} and 5-HT_{1D} receptor agonists, CP-93129 and GR-46611, respectively. Of these GR-46611 significantly increased the threshold of hyperthermia-induced seizure and lowered seizure severity. Given chronically at 1/mg/kg/day, GR-46611 also significantly improved survival of DS mice. Thus 5-HT_{1D}-receptors may represent a meaningful target for pharmacotherapy for DS with potential relevance for related forms of epilepsy, particularly those with a known sensory trigger such as heat.

Keywords

seizure; epilepsy; raphe; serotonin; *Scn1A*; Nav1.1

Introduction

SCN1A encodes the pore forming subunit of Nav1.1 voltage gated sodium channels and mutations of this gene cause familial hemiplegic migraine as well as genetic forms of epilepsy. The most severe and debilitating of these disorders is Dravet syndrome (DS), which is characterized by febrile seizures beginning early in life and progresses into afebrile-general or partial seizures accompanied by moderate to severe intellectual disability and ataxia. Sudden unexpected death in epilepsy occurs at high rates in DS and appears to be linked to postictal respiratory depression (Kalume, 2013). DS is notoriously resistant to

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standard anti-epileptic treatments. However the serotonin-selective amphetamine fenfluramine has beneficial effects for DS, which raises interest in identifying potentially more specific serotonergic compounds that may have utility for this disorder (Boyd et al., 2019).

Studies using a zebrafish model of DS found that 5-HT_{1D}, 5-HT_{2A} and 5-HT_{2C} agonists have antiseizure effects (Sourbron et al., 2017). There is a long history of research into the capacity of serotonergic drugs to modify epileptic activity and indeed considerable evidence supports roles of 5-HT_{2A} and 5-HT_{2C} receptors (Bercovici et al., 2007). There is less literature linking 5-HT_{1D} receptors with epilepsy but 5-HT_{1D} receptors have been studied for their role in migraine, another disorder associated with SCN1A. It has been speculated that gain of function mutations of SCN1A are associated with migraine while loss of function with epilepsy but the situation is likely more complex and it's possible that migraine and epilepsy could share underlying mechanisms (Nye & Thadani, 2015; Mantegazza & Cestele, 2018). To determine how results in zebrafish would extend to a mammalian model here we used a mouse model of DS to understand if agonists of any of these serotonin receptors could influence the appearance of hyperthermia-induced seizure or survival.

Methods

Male and female progeny resulting from breeding male 129S-*Scn1A*^{tm1Koa}/Mmjax mice with normal female C57BL/6J mice, both purchased from Jackson Labs, were used. Controls consisted of wildtype littermates. Care and use of animals in these studies was approved by the Institutional Care and Use Committee at Boston Children's Hospital under protocol approval number 15-03-2904R.

Although phenotypic characterization of human mutations and behavioral analysis of the mouse model do not indicate substantive sensory abnormalities, seizure in this syndrome is often triggered by heat. To study hyperthermia-induced seizure susceptibility, DS mice were primed with a hyperthermia-induced seizure on postnatal day of age (P) 18 +/- 1 day. After a 5-minute acclimation to a RET-3 rectal temperature probe (Physitemp), body temperature was raised at a rate of 0.25 °C per minute using a heat lamp until the first generalized seizure resulting in a loss of posture. Subsequently the same mice were tested for seizure threshold at P21 after receiving drug pretreatments 20 minutes before repeating the hyperthermia protocol. This design was used to model treatment of affected individuals rather than modeling a prevention in seizure-naïve individuals. Seizure severity was scored using a modified Racine score of 5 for severe generalized tonic-clonic seizure involving falling and wild jumping, 4 for seizure with forelimb clonus and falling, 3 for seizure with forelimb clonus but without falling. If an individual reached the temperature of 42 degrees without seizure for one-minute they were considered seizure free.

Specific doses tested were selected based upon reports of other physiological effects. DOI (Sigma-Aldrich) was dissolved in normal saline and administered i.p. in a single, acute dose of 3.0 mg/kg, a dose which is sufficient to produce stereotypical behavior in mice and reduces seizures in rats (Bercovici et al., 2007). Zolmitriptan (Sigma-Aldrich) in 5% DMSO

in saline was used at a dose of 10 mg/kg (de Almeida et al., 2001). CP-93129 (Tocris Bioscience) was dissolved in saline and administered at 3 mg/kg; GR-46611 (Tocris Bioscience) was dissolved in 5% DMSO, 30% propylene glycol in saline and administered at 0.01, 0.1 and 1 mg/kg. Drug injections were given in random order with different vehicle injections interspersed throughout the experimental timeline.

In a pilot study of untreated mice we found mortality was most prevalent between P19 and P29 consistent with the literature (Hawkins et al., 2017). Therefore to study survival mice were treated from P18 to P32 with either GR-46611 at 1 mg/kg or vehicle via daily i.p. injection. At P21, mice were weaned and group housed. After P32, treatment ceased and mice were monitored to P42.

Threshold seizure temperatures were compared using ANOVA with posthoc P values < 0.05 considered statistically significant. Seizure severity was compared using independent samples Kruskal-Wallis test. Survival statistics were calculated using time-to-event analysis with P values determined with the Log-Rank test.

Results

Neither the mixed 5-HT_{2A/2C} agonist DOI at 3 mg/kg nor Zolmitriptan at 10 mg/kg had a detectable effect on hyperthermia-induced seizure threshold, but Zolmitriptan reduced seizure severity (P = .017, Fig. 1). Zolmitriptan is an agonist for both 5-HT_{1B} and 5-HT_{1D} receptors, therefore we tested CP-93129 and GR-46611, selective agonists for each of these receptors respectively. CP-93129 did not have a detectable effect on seizure severity or threshold, however, GR-46611 significantly increased the average seizure threshold (P = .001, Fig. 1B). In addition, while no mice at P18 or at P21 in any other group met the criteria for seizure free, 3 of 11 mice with GR-46611 at 1 mg/kg were seizure free (Fig. 1C). When seizures did appear in this group, the seizure severity score was lower than those of controls (P = .01, Fig. 1A). At the same time there were no detectable differences in thermoregulation between groups as body temperature after drug/vehicle treatment and rate of temperature rise did not differ between groups.

We tested GR-46611 at additional doses and found a nominally intermediate but statistically insignificant effect at 0.1 mg/kg on seizure threshold (Fig. 1B, P = 0.1) and seizure appearance (Fig. 1C, P = 0.19), while mice receiving 0.01 mg/kg were indistinguishable from vehicle-treated mice.

Next we tested if GR-46611 given chronically could improve survival by comparing DS mice receiving chronic i.p. vehicle injections and those that received GR-46611 at 1 mg/kg dose (Fig. 2). GR-46611 given for 2 or more days improved survival relative to vehicle treated controls with 89% surviving to P32 vs 36% of controls (P = 0.0029). Drug treatment was discontinued at P32 and survival was monitored for another two weeks with no spontaneous death occurring.

Discussion

Here we show in a mammalian model of DS that the 5-HT_{1D} agonist GR-46611 has protective effects by increasing the threshold for heat-induced seizure and reducing seizure severity. In addition, GR46611 given chronically reduced mortality in DS mice. These observations are consistent with the potential utility of 5-HT_{1D} agonists for DS, a finding with potential relevance to other forms of epilepsy.

While 5-HT_{2A/2C} agonist and 5-HT_{1B} agonists did not have detectable effects, we can't rule out their possible efficacy as only one dose was tested at one age. Both 5-HT_{2A} and 5-HT_{2C} agonists improve locomotor behavior and reduce epileptiform activity in the zebrafish model suggesting they have the capacity for positive therapeutic effects (Sourbron et al., 2016) and Lorcaserin, a 5-HT_{2C} agonist, may be beneficial in humans (Griffin et al., 2017). A potential explanation for this unexpected result is the age tested, because there are age-related changes in serotonin receptor function that continue to evolve over the lifespan. For example, mice lacking functional 5-HT_{2C} receptors develop epilepsy only after P90 (Sejourn et al., 2015). Thus we don't exclude the possibility that 5-HT_{2A}, 5-HT_{2C} or 5-HT_{1B} receptor ligands may have useful effects.

It was also unexpected to find positive effects of zolmitriptan and more obviously GR-46611 given the paucity of literature implicating these compounds as modifiers of epilepsy. One of the few lines of evidence for 5-HT_{1D} receptors playing a role in epilepsy is from the zebrafish model of DS (Sourbron et al., 2016). Zolmitriptan is a marketed antimigraine medication and 5-HT_{1D} receptors are also implicated in migraine. It is an interesting coincidence that SCN1A mutations are a cause of familial hemiplegic migraine, which is consistent with the possibility that migraine and seizure are mechanistically related (Mantegazza & Cestele, 2018). Currently there are no official treatment guidelines for familial hemiplegic migraine and the use of triptan class of drugs including zolmitriptan is avoided due to untested but perceived increased risk for adverse effects on cardiovascular function. However there are reports in humans that triptans can be helpful to familial hemiplegic migraine sufferers and there may not be exceptional risk in this patient population (Artto et al., 2007). Conversely these results raise the possibility that fenfluramine which appears beneficial for DS may be useful for familial hemiplegic migraine.

Our results showed that given daily GR-46611 improved overall survival. GR46611 could be acting at different sites along the neuroaxis, for example 5-HT_{1D} receptors are expressed in areas such as the thalamus where they could plausibly act to reduce the vulnerability of forebrain networks to epileptic activity (Bonaventure et al., 1998). Alternatively they may modify peripheral triggers of epileptic activity in that 5-HT_{1D} receptors are implicated in presynaptic modulation of sensory afferents (Potrebic et al., 2003). More broadly our results suggest that 5-HT_{1D} agonists as well as newer antimigraine drugs that carry less risk of cardiovascular side effects than triptans may deserve further scrutiny for their utility in the treatment of epilepsy. Speculatively these may be particularly useful when there are sensory triggers of seizure such as heat. The finding that a 5-HT_{1D} agonist had positive effects in DS

mice may also be relevant to the mechanism underlying the beneficial effects of fenfluramine in DS, although we do not rule out the contribution of other mechanisms.

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Abbreviations:

DS	Dravet Syndrome
5-HT	5-hydroxytryptamine or serotonin
P	postnatal day of age
DOI	(±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride
i.p.	intraperitoneal

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Figure (1).

A. Severity of seizures elicited by hyperthermia. DOI (n=6) had no detectable effect whereas zolmitriptan (“Zolmig” n=17) reduced severity (n = 19). Excluding individuals that were seizure-free with GR-46611 treatment (n=3), when seizures appeared after this drug-treatment they were also less severe (n=8). **B.** GR-46611 at 1 mg/kg had a significant effect on seizure threshold (also excluding individuals that were seizure free, n=8). GR-46611 at 0.1 mg/kg (n=8) was nominally intermediate but not statistically different from controls. GR-46611 at 0.01 mg/kg (n=9) and CP at 3 mg/kg (n =7) were indistinguishable from controls. **C.** Kaplan-Meier plots to visualize hyperthermia induced seizure temperature differences between vehicle, 1.0 and 0.1 mg/kg GR-46611 treatment. 1 mg/kg vs vehicle, $P < 0.0001$; 0.1 mg/kg vs vehicle, $P = 0.19$, Log-Rank test.

Figure (2). Survival with chronic treatment from P18-P32 with GR-46611 at 1 mg/kg vs. vehicle (n = 18 and 11, respectively).