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Brief communication

Bexarotene reduces network excitability in models of Alzheimer's disease and epilepsy

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ABSTRACT

The nuclear retinoid X receptor agonist, bexarotene, has been implicated in recovery of cognitive function in mouse models of Alzheimer's disease (AD). Since AD genetic mouse models also show abnormal neural hyperexcitability, which may play a destructive role in memory storage and retrieval, we studied whether bexarotene exerted dynamic network effects on electroencephalography cortical spike discharge rate and spectral frequency in an AD (hAPP J20 model) and non-AD (Kv1.1 null) mouse models of epilepsy. We find that oral treatment with bexarotene over 1 week acutely reduced spike discharges in both models and seizures in the Kv1.1 null mouse model without major alterations in the background frequency of brain rhythms. The effect was reversible and exhibited a similar rapid onset in hippocampal slices. While the exact mechanisms are unknown, bexarotene counteracts both amyloid-β-induced and amyloid-β-independent increases in cortical network hyperexcitability.

1. Introduction

The retinoid X receptor (RXR) agonist, bexarotene increases apolipoprotein E (ApoE) expression and exerts a striking improvement in cognitive function in an Alzheimer's disease (AD) mouse model (Cramer et al., 2012). Salient effects include reduction in amyloid-β (Aβ) plaque burden, clearance of soluble Aβ within 72 hours of treatment, and improvement in neural network activity and cognitive behaviors within 1 week. Recent studies confirmed the reduction in soluble forms of Aβ, including Aβ oligomers, in response to bexarotene treatment (Bachmeier et al., 2013; Fitz et al., 2013; Price et al., 2013; Tesseur et al., 2013; Ulrich et al., 2013; Veeraraghavalu et al., 2013) and improved cognition and memory (Fitz et al., 2013; Tesseur et al., 2013), however, plaque loss was not confirmed (Fitz et al., 2013; Laclair et al., 2013; Price et al., 2013; Tesseur et al., 2013; Veeraraghavalu et al., 2013). These data support an accumulating body of evidence linking soluble forms of Aβ, rather than plaque deposition, with memory decline.

The cellular mechanisms leading to the dynamic and potentially reversible component of dementia in Aβ-overexpression models of AD and their amelioration by bexarotene remain poorly understood. There is ample evidence at the single cell level for Aβ-linked defects in synaptic plasticity, and these are accompanied by circuit level network discharges and seizures in most experimental genetic models of AD (Blanchard et al., 2002; Minkeviciene et al., 2009; Mucke and Selkoe, 2012). Network hyperexcitability may arise from changes in intrinsic membrane properties as well as synaptic and connectivity defects and all have been described in AD models (Palop and Mucke, 2010). Clearance of Aβ from the brain is facilitated by ApoE, and ApoE expression is transcriptionally induced through the action of the nuclear peroxisome proliferator activated receptor and liver X receptors in coordination with RXRs. The primary mechanism of action of RXR receptors is to alter gene expression (Boehm et al., 1995; Hurst, 2000; Lalloyer et al., 2009), but the extent and time course of its effects on neuronal excitability mechanisms are unknown, particularly in the early stages of its exposure to central nervous system pathways.

In this pilot study, we sought evidence for early changes in brain excitability that might correlate with the improvement in cognitive function because of bexarotene, as well as to determine whether this effect was strictly dependent on Aβ pathology by comparing 2 distinct mutant mouse models of neural hyperexcitability, 1 AD and 1 non-AD model.

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2. Methods

2.1. Animals

Mice were obtained from the Baylor Developmental Neurogenetics breeding colony. The hAPP J20 mice were a generous gift from Drs L. Mucke, E. Roberson. Kv1.1 null mice (background Black Swiss N:NIHS-BC)or J20 mice (background C57BL/6+DBA/2+Swiss-Webster) were treated with a single daily oral gavage for 7 days of either 100 mg/kg/day bexarotene (Targretin capsule) dissolved in 2 mL of water (Cramer et al., 2012) or vehicle (PBS) for 5 days. Between recordings, mice were housed at 22 °C with a 12-hour light and/or dark cycle and fed ad libitum. Mouse breeding and experiments were carried out under IACUC approved protocols at Baylor College of Medicine.

2.2. Electroencephalography recordings

Silver wire electrodes (0.005″ diameter) soldered to a miniature connector were implanted bilaterally into the subdural space over frontal and parietal cortex of mice under Avertin anesthesia (250 mg/kg) several days before recording. Simultaneous electroencephalography (EEG) and behavioral video EEG monitoring was performed using a digital electroencephalograph (Harmonie 6.1, Stellate Systems, Montreal, Quebec, Canada) from 11 homozygous Kv1.1 null mice (5 vehicle, 6 bexarotene treated), aged 5–10 weeks, moving freely in the test cage. J20 mice were allowed to acclimate to the recording chamber for 1 day before acquiring baseline EEG data. A total of 23 hAPP J20 mice were recorded (aged 4–18 months, 14 received bexarotene, 9 received vehicle, ages between the groups were similar). Mice were monitored by video EEG over a 4-hour period each day. After a baseline recording period, mice were administered drug once daily and recorded at same time each day for 7 days, drug was discontinued, and mice recorded for an additional 1–7 days washout-monitoring period. Vehicle-treated (PBS) control mice were treated identically and recorded at the same time for 5 days.

2.3. In vitro slice recordings

Transverse hippocampal slices were prepared from wild-type J20 mice (aged 5 to 8-week-old) as described in Holth et al. (2013). There was no spontaneous network bursting activity in normal (2.5 mM KCl) solutions, and after obtaining a baseline, artifical cerebrospinal fluid containing 7.5 mM KCl was washed on the slices. After 15–25 minutes, CA3 neurons began synchronously discharging and the burst frequency was determined using Clampfit and Origin software. Following a 20-minute period for stable acquisition of burst frequency, 10 μM bexarotene was washed on, allowed to equilibrate, and the burst frequency was measured 20 minutes after drug introduction.

2.4. Spectral analysis

Standardized samples of EEG activity (the final 30 minutes of the first hour of each recording session) were selected to ensure uniformity. Files were processed using the time frequency (Morlet wavelets) and frequency power spectrum density (Welch) transforms in Matlab (R2011b, Mathworks, Inc, Natick, MA, USA). Examples are shown (Fig. 1D and Fig. 2F) of the power spectra of Morlet wavelets for frequency ranges up to the low gamma range (40 Hz) over the 30-minute time period. Power is expressed as μV^2/Hz. For Welch transforms, 3-second epochs were analyzed with 20% overlap for grouping into major frequency bands (α, β, $\gamma 1$, $\gamma 2$, δ, θ) with parietal and temporal leads averaged. Data were compiled from 6 of the Kv1.1 null mice and 5 of the hAPP J20 mice.

2.5. Statistical analysis

All data are expressed as mean ± standard error of the mean unless otherwise stated. Graphpad PRISM version 5.04 (San Diego, CA, USA) was used to test for statistical significance. The p-values for bexarotene versus placebo treated interictal spike rate were generated by 2-way ANOVA (time and drug) with repeated measures and Bonferroni post hoc analysis. p-values for the one time treatment with bexarotene were generated by 1-way ANOVA (time) with repeated measures and Bonferroni post hoc analysis. p-values for differences in Kv1.1 null seizure rate were determined by t test. Data were tested for normality and passed unless noted and, in case of nonnormality, a Mann–Whitney test was used. A paired t test was used for the before and after drug application in the slice electrophysiology experiments. For both hAPP J20 and Kv1.1 null mice, 2-way ANOVA revealed the interaction between time and drug was significant ($p < 0.05$) as well as drug treatment ($p < 0.05$).

3. Results

3.1. Effect of bexarotene on in vivo cortical epileptiform spiking in hAPP J20 model of AD

Abnormal spontaneous epileptiform spiking is a prominent feature of the cortical and hippocampal EEG in hAPP J20 mice (Palop and Mucke, 2010; Roberson et al., 2011). We first examined the short-term effect of a daily bexarotene dose (100 mg/kg/day as used in Cramer et al., 2012) on the spontaneous discharge rate in adult hAPP J20 transgenic mice over a 1-week period (n = 14). Within the first 72 hours following administration, bexarotene reduced the spike discharge rate to 67.25% ± 16.74% of the baseline spike rate, which was significantly different from vehicle control spike rates (n = 9, $p < 0.05$, Bonferroni post hoc, Fig. 1C). The spike suppression reached a low of 53.16% ± 12.47% of the normalized spike rate after 4 days of bexarotene treatment, which was also significantly different from vehicle treated hAPP J20 mice ($p < 0.05$, Bonferroni post hoc, Fig. 1C). We next examined the background frequency spectrum of the rhythmic EEG activity before and after bexarotene treatment and found no significant difference between days of bexarotene treatment (Fig. 1D and E). This indicates that bexarotene reduces aberrant network discharge activity without major alterations in the time spent in high frequency or slow wave activity EEG patterns. Additionally, after the completion of bexarotene treatment, the spike rate in J20 mice returned to 105.47% ± 26.4% of the baseline level within 24 hours (Mann–Whitney, data not shown), indicating that the antiepileptic effects of bexarotene may be quickly reversible. While abnormal spike discharge is a hallmark of J20 EEG patterns, electrographic seizures did not occur frequently enough in this model to allow comparative analysis during the 1-week test interval.

3.2. Effect of bexarotene on in vivo cortical epileptiform spiking in Kv1.1 null model of epilepsy

We next sought to determine whether the beneficial effects of bexarotene on cortical hyperexcitability were dependent on the presence of Aβ-induced pathology in the J20 model, or whether it might be effective in other hyperexcitability models. The Kv1.1 null mouse model has been well studied due to its pronounced epileptic phenotype (Glasscock et al., 2010; Holth et al., 2013; Simeone et al., 2013; Smart et al., 1998). Somewhat surprisingly, in the absence of known Aβ pathology, bexarotene significantly reduced the spike discharge rate to 28.09% ± 3.7% (n = 6) of baseline within 3 days of daily treatment and differed significantly from the spike rate in vehicle treated (n = 5) Kv1.1 null mice