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Riluzole Rescues Alterations in Rapid Glutamate Transients in the Hippocampus of rTg4510 Mice

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Abstract

Those at risk for Alzheimer's disease (AD) often exhibit hippocampal hyperexcitability in the years preceding diagnosis. Our previous work with the rTg(TauP301L)4510 tau mouse model of AD suggests that this increase in hyperexcitability is likely mediated by an increase in depolarization-evoked glutamate release and a decrease in glutamate uptake, alterations of which correlate with learning and memory deficits. Treatment with riluzole restored glutamate regulation and rescued memory deficits in the TauP301L model. Here, we used enzyme-based ceramic microelectrode array technology to measure real-time phasic glutamate release and uptake events in the hippocampal subregions of TauP301L mice. For the first time, we demonstrate that perturbations in glutamate transients (rapid, spontaneous bursts of glutamate) exist in a tau mouse model of AD mouse model and that riluzole mitigates these alterations. These results help to inform our understanding of how glutamate signaling is altered in the disease process and also suggest that riluzole may serve as a clinically applicable therapeutic approach in AD.

Keywords

Alzheimer's disease; glutamate uptake; hippocampus; riluzole; tau; Tg4510

Introduction

Alzheimer's disease (AD) is characterized by accumulation of plaques consisting of the beta-amyloid protein (Aβ) (Crimins et al. 2013), tangles consisting of the tau protein (Grundke-Iqbal et al. 1986), and the slow and progressive loss of neurons, eventually resulting in memory deficits (Brier et al. 2012; Palop et al. 2003). However, those at risk for AD often exhibit a hyperexcitability of the hippocampal network in the years preceding diagnosis (Bassett et al. 2006; Bondi et al. 2005; Bookheimer et al. 2000; Sperling et al. 2010). This hyperexcitability is linked to memory impairment and later conversion to AD (Bakker et al. 2012; Kamenetz et al. 2003; Vossel et al. 2013), and recent work suggests tau may mediate hyperexcitability (DeVos et al. 2013; Holth et al. 2013; Ittner et al. 2010;

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Roberson et al. 2007). Perhaps most intriguing, the secretion and trans-synaptic spread of both Aβ and tau has been linked to synaptic activity (Cirrito et al. 2005; Liu et al. 2012), suggesting hyperexcitability could be permissive for the spread of pathology through vulnerable networks.

Our previous work suggests that glutamate dysregulation might be driving this increased hyperexcitability and represents a potential therapeutic target (Hunsberger et al. 2014a; Hunsberger et al. 2015). We have recently shown that the rTg(TauP301L)4510 tau mouse model of AD, containing the proline to leucine mutation in human tau at the 301st amino acid, exhibits increased potassium-evoked glutamate release and decreased glutamate uptake following application of exogenous glutamate. Furthermore, glutamate dysregulation correlated with learning and memory deficits in TauP301L mice. Rescuing glutamate dysregulation with riluzole treatment, which decreases glutamate release and increases glutamate uptake (Azbill et al. 2000; Frizzo et al. 2004; Gourley et al. 2012; Hunsberger et al. 2015), rectified these learning and memory deficits (Hunsberger et al. 2015).

The goal of the current study was to determine whether differences in rapid, spontaneous bursts of glutamate, termed glutamate transients, could be detected in TauP301L mice. Glutamate transients are a relatively new measure detectable only recently as a result of newer microelectrode array (MEA) technology with high temporal, low spatial, and minimal damage of the MEAs (Hascup et al. 2011). Increased transient levels have been associated with depression (Hascup et al. 2011) and seizure activity (Stephens et al. 2014), but have yet to be examined in an AD tau model. A second goal of this study was to determine whether riluzole could restore alterations in glutamate transients to control levels. Here, we implanted MEAs into anesthetized TauP301L mice and littermate controls, as well as TauP301L mice treated with riluzole, and measured fluctuations in resting glutamate in the absence of exogenous manipulators (i.e., potassium or exogenous glutamate).

Materials and methods

Tg4510 mice expressing P301L mutant human tau linked to a hereditary tauopathy were created as previously described (Paulson et al. 2008; SantaCruz et al. 2005). Heterozygous responders (on an FVB background with tetracycline-responsive element (TRE) upstream of cDNA encoding human four-repeat tau with the P301L mutation and lacking both N-terminal inserts) and activator mice (of a 129S6 background strain with a tet-off open reading frame placed downstream of a CaMKII promoter, CKtTA) were bred together to create bigenic progeny containing both transgenes. The four genotypes of animals generated are described by the following nomenclature, TauP301L/CKtTA, and include what we refer to as TauP301L mice (+/+) and Controls (−/+, +/−, −/−). Only the resulting +/+ FVB/129S6 mice overexpress mutant P301L human tau. In the current study, we used the −/+ control because no behavioral differences among the 3 control groups have been reported, and human tau is not expressed in any of the controls (Hoover et al. 2010; Ramsden et al. 2005; SantaCruz et al. 2005), yet the −/+ allowed us to control for the expression of tTA (Mayford et al. 1996).

To prevent mutant tau expression during development, P301L tau expression was suppressed by administering 40 ppm doxycycline hyclate (DOX) in the drinking water until mice were 2.5 months of age (Hunsberger et al. 2014a; Hunsberger et al. 2014b; Hunsberger et al. 2015). Control −/+ mice also received 40 ppm during development and until 2.5 months of age. One caveat of DOX use is unintended effects. For example, 40 ppm DOX administration in adult mice has been shown to increase neurogenesis and reduce miroglia (Sultan et al. 2013). In addition, developmental exposure to 200 ppm DOX, a much higher dose than that used here, has detrimental effects on neural development (Cunningham et al. 2013).

At 2.5 months of age, DOX was removed from the drinking water, tau expression began, and three groups of mice were established: Vehicle-Controls (10 males, 7 females), Vehicle-TauP301L (11 males, 8 females), and Riluzole-TauP301L (8 males, 7 females). Riluzole (Sigma, St. Louis, MO) + 1% *w/v* saccharin (vehicle) was administered to the Rilzuole-TauP301L group via water bottles (Hunsberger et al. 2015). Water bottles were weighed daily, and riluzole water was changed every 72 hours to maintain a daily intake of 12.5 mg/kg (Gourley et al. 2012; Hunsberger et al. 2015; Ishiyama et al. 2004). At 7.5 months of age, after 5 months of P301L tau expression, mice underwent *in vivo* anesthetized microelectrode recordings to examine glutamate transients. All experimental procedures were conducted in accordance with the standards of Institutional Animal Care and Use Committee.

Fluctuations in resting glutamate, termed glutamate transients, were measured using ceramic-based MEAs (Quanteon, Nicholasville, KY) consisting of 4 platinum recording sites (Burmeister and Gerhardt 2001). Two recording surfaces were coated with glutamate oxidase (recording sites) to detect glutamate, and two sites were coated with a protein matrix to detect background noise (sentinel sites). The MEA was placed into three subregions of the hippocampus: [DG (AP: −2.3mm, ML: +/−1.5mm, DV: 2.1mm), CA3 (AP: −2.3mm, ML: +/−2.7mm, DV: 2.25 mm), CA1 (AP: −2.3mm, ML: +/−1.7mm, DV: 1.4mm)] (Paxinos and Franklin 2012). Transients were analyzed using custom Matlab software with parameters set to detect peaks with a signal to noise ratio above 2.5 with a moving baseline of 10 points (5 sec) (Hascup et al. 2011; Stephens et al. 2014). Transients were only observed on glutamate oxidase coated sites and not on the self-referencing recording sites, confirming that it was from spillover of glutamate and not noise or artifact. Five parameters were analyzed using an ANOVA (JMP, Cary, NC): 1) area under the curve (AUC), 2) amplitude (μM), 3) duration (seconds), 4) inter-transient interval (seconds), and 5) transients per minute. No differences between the males and females were observed, and thus, only the differences between the treatment groups are described.

Results

P301L expression increased the net AUC in all 3 subregions of the hippocampus [DG: $F(2,43)=7.40$; $p=.002$, CA3: $F(2,47)=5.16$; $p=.009$, and CA1: $F(2,45)=5.46$; $p=.008$]. For all 3 subregions, riluzole treatment returned net AUC to control levels. Because the net AUC could be influenced by both the amount of glutamate released, as measured by amplitude, or the duration of the transient, these were measured separately. In all 3 subregions [DG:

F(2,43)=4.02; p=.025, CA3: F(2,47)=5.24; p=.009, and CA1: F(2,45)=5.11; p=.010], transient amplitudes were greater in Veh-TauP301L mice, an effect rescued by riluzole treatment, whereas the transient duration was similar among all groups in all subregions [DG: F(2,43)=21.71; p=.19, CA3: F(2,47)=.073; p=.92, and CA1: F(2,45)=.69; p=.51]. Thus, alterations in net AUC are likely mediated by changes in the amplitude of glutamate transients (Fig. 1).

We next examined the time between transients (inter-trial interval) and the number of transients per minute. With the exception of the transient per minute in the CA1 subregion, there were no differences among the groups for the inter-trial interval [DG: F(2,43)=.021; p=.98, CA3: F(2,47)=1.21; p=.31, or CA1: F(2,45)=.83; p=.44] or transients per minute [DG: F(2,43)=.38; p=.69, CA3: F(2,47)=.36; p=.70]. For the CA1 subregion, P301L tau expression increased the number of transients per minute compared to riluzole-treated P301L mice [CA1: F(2,45)=3.33; p=.04] (Fig. 2).

Discussion

The present study extends our previous finding that riluzole rescues the detrimental glutamate dysregulation observed in mice expressing P301L mutant tau (Hunsberger et al. 2014a; Hunsberger et al. 2015). Here, we have demonstrated that TauP301L mice exhibit larger glutamate transients, primarily due to differences in amplitudes. Riluzole, however, restored these transients to control levels. These larger transients could be due excessive synaptic stimulation that overwhelms astrocytic glutamate uptake transporters or a reduction in transporter expression or sensitivity. Alternatively, reduced expression or signaling of presynaptic mGluR2/3 autoreceptors could result in increased glutamate release.

Our previous work suggests that TauP301L mice exhibit an increase in potassium-evoked glutamate release, as well as a decrease in glutamate uptake (Hunsberger et al. 2014a; Hunsberger et al. 2015). These results are coupled with changes in neuronal proteins, including an increase in the vesicular glutamate transporter responsible for packaging glutamate into vesicles and shown to mediate glutamate release (Moechars et al. 2006; Wilson et al. 2005), and a decrease in glutamate uptake transporter (GLT-1) expression. Therefore, glutamate transient alterations could result from both increased release and decreased uptake. Delineating these possibilities with riluzole was not possible as both release and uptake are affected following treatment (Azbill et al. 2000; Frizzo et al. 2004; Gourley et al. 2012). While this makes riluzole noteworthy as a potential therapeutic treatment, more mechanistic studies will be required to determine the relative contribution of release versus uptake alterations in mediating changes in glutamate transients. Similarly, the use of compounds, such as tetrodotoxin (TTX), a sodium channel blocker, to examine neuronal versus non-neuronal (i.e., glial) sources of glutamate and threo-β-Benzyloxyaspartic acid (TBOA), to block glutamate transporters, would be needed to delineate the role of glutamate release and uptake in measures of amplitude and duration.

Similarly, determining how hippocampal glutamate transients affect cognition is an important next step. Now that we have demonstrated the presence of altered transients in the TauP301L mouse model, we can determine how these perturbations influence cognition by

measuring changes in transients that are time-locked to behavior (e.g., lever pressing) or stimuli (e.g., tone presentation in fear conditioning). Perhaps most critical is determining if and how alterations in glutamate transients mediate the pathophysiology of AD and how this might inform our therapeutic approaches. In addition, examining the glutamate regulation in an AD model with both Aβ and tau pathology is critical.

Acknowledgments

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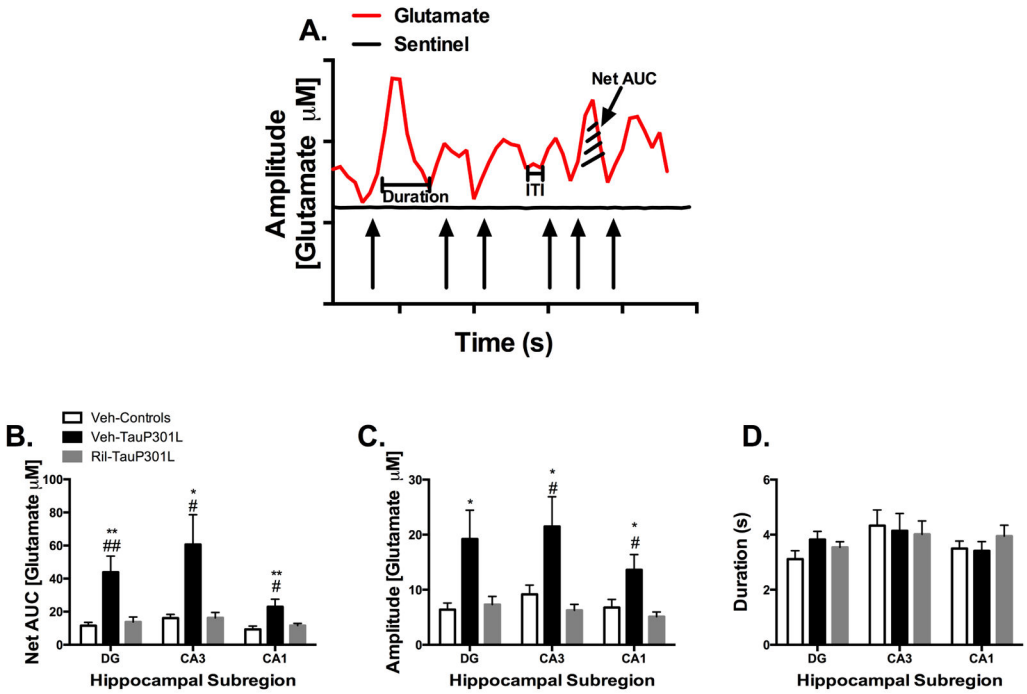

Fig. 1. Transient trace, net AUC, amplitude, and duration of glutamate transients

(A) Representative trace of spontaneous glutamate transients (arrows) in the CA1 region. Trace includes amplitude, duration, inter-transient interval (ITI), and area under the curve (AUC). Rapid fluctuations in glutamate were observed on the glutamate oxidase sites (red), but not the sentinel sites (black). (B) Net area under the curve (AUC) was increased in Vehicle-TauP301L mice in the DG, CA3, and CA1, an effect attenuated by riluzole. (C) Riluzole treatment rescued the increase in maximum transient amplitude in Vehicle-TauP301L mice in the CA3 and CA1. (D) The duration of the transient was similar among the groups. (Mean ± SEM; * p<.05, ** p<.01 Veh-Control vs. Veh-TauP301L; # p<.05, ## p<.01 Ril-Tau-301L vs. Veh-TauP301L; n = 14–19/group)

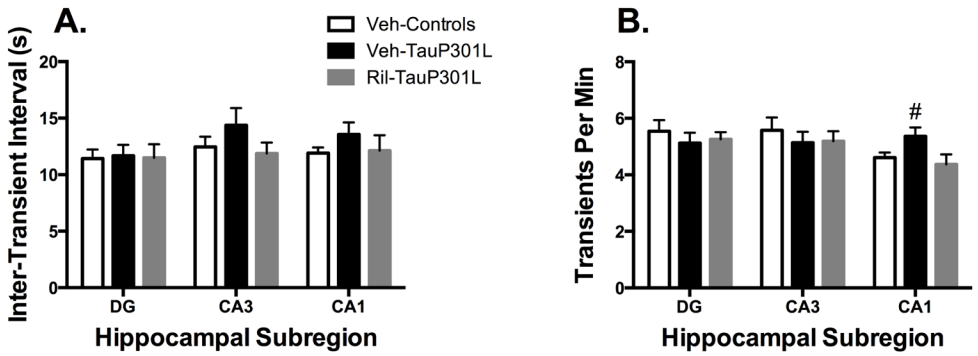

Fig. 2. Glutamate transient interval and transients per minute

(A) The time between transients (inter-transient interval) was similar among the groups in the DG, CA3, and CA1. (B) Riluzole rescued the increase in transients per minute observed in the Veh-TauP301L in the CA1, but no differences were observed in the DG or CA3. (Mean ± SEM; # p<.05 Ril-Tau-301L vs. Veh-TauP301L; n = 14–19/group)