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Brief communication

Abnormal neuronal networks and seizure susceptibility in mice overexpressing the APP intracellular domain

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Abstract

Alterations in the processing of the amyloid precursor protein (APP) lead to familial Alzheimer's disease (AD). AD patients exhibit increased seizure susceptibility and alterations in their EEGs, which suggests that APP and its metabolites may modulate neuronal networks. Here we demonstrate that transgenic mice overexpressing APP intracellular domain (AICD) and its binding partner Fe65 exhibit abnormal spiking events and a susceptibility to induced seizures. These abnormalities are not observed in PDAPP(D664A) mice, which express high Aβ levels but harbor a mutation in the APP intracellular domain. These data suggest that alterations in the levels of AICD contribute to network dysfunction in AD.

Keywords: Alzheimer's; AICD; APP; Fe65; Electroencephalogram; Seizure

1. Introduction

Familial mutations associated with Alzheimer's disease (AD) alter amyloid precursor protein (APP) processing, resulting in early onset AD (Price and Sisodia, 1998). When these familial mutations are introduced into mice, key pathological features of AD, such as elevations in amyloid beta (Aβ) peptides and plaques, are present. These mouse models also exhibit altered neuronal activity and a susceptibility to seizure activity (Palop et al., 2007), which is consistent with the high incidence of seizures in AD patients as compared to the normal population (Menendez, 2005). However, because full-length APP is expressed in these mouse models, it is difficult to distinguish the contribution of individual peptides generated from APP processing.

Comparisons of two mouse models suggest an important contribution of the APP intracellular domain (AICD). In mice that overexpress human APP (hAPP) with familial mutations and an additional mutation in the intracellular domain, PDAPP(D664A), high levels of Aβ are present but mice exhibit no deficits in long term potentiation (LTP) or memory (Galvan et al., 2006; Saganich et al., 2006). Another transgenic line, PDAPP(J20) mice, harbors the same familial mutations in APP but lacks the mutation in the intracellular domain. PDAPP(J20) mice have similar levels of Aβ as the PDAPP(D664A) mice, as well as deficits in long term potentiation, spontaneous seizures and a susceptibility to seizure-inducing drugs (Galvan et al., 2006; Saganich et al., 2006; Palop et al., 2007) These data suggest that other APP metabolites, particularly AICD, may be involved in the electrical aberrations that are prominent in AD and AD mouse models.

AICD peptides of 59, 57 and 50 amino acids can be generated by γ-secretase, while caspase cleavage generates a fragment of 31 amino acids. AICD interacts with many cytosolic proteins, including Fe65, which may be important for the activity of AICD and AD pathology (Russo et al.,

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1998; McLoughlin and Miller, 2008). Interestingly, transgenic mice overexpressing both AICD and Fe65 have high levels of active GSK-3β (Ryan and Pimplikar, 2005) which is observed in AD patients (Hooper et al., 2008). In addition, we recently observed that these mice exhibit other AD pathological features without altering APP metabolism (Ghosal et al., in press), suggesting that imbalances in AICD may be sufficient to cause some features of AD. Here we demonstrate that mice overexpressing AICD and Fe65 exhibit abnormal EEG spiking, and these changes are correlated with a strong susceptibility to induced seizures. These data demonstrate the first in vivo biological function for AICD in regulating neuronal networks, and suggest that AICD contributes to these phenotypes in AD mouse models.

2. Materials and methods

2.1. Transgenic mice

All mice were maintained on a C57BL/6J background. Mice co-expressing AICD59 and Fe65 (FeCγ lines) or Fe65 alone (Fe27 line) under the CaMKIIα promoter were previously described (Ryan and Pimplikar, 2005). AICD protein levels in FeCγ12 are slightly elevated over wildtype (WT) while high in the FeCγ25 line, and all transgenic lines express equal amounts of Fe65. R1.40 mice express human APP containing the Swedish (K670N, M671L) familial AD mutation, and have been previously described (Lamb et al., 1997). PDAPP(D664A) mice (line B254) express hAPP with Swedish and Indiana (V717F) familial mutations and a point mutation in the intracellular caspase cleavage site (D664A), and have been previously described (Galvan et al., 2006). WT littermates were used as controls for all experiments.

2.2. Kainic acid injections

3–5-Month-old female mice were injected i.p. at a dose of 25 mg/kg body weight with kainic acid (Sigma). Mice were video monitored for 65 min, and maximum seizure severity was scored every 5 min. Mortality was determined as the time when mice ceased breathing and did not respond to a noxious stimulus. A modified version of Racine's scale (Racine et al., 1972), previously reported for other AD mouse models (Palop et al., 2007), was used to score seizure severity, where (0) normal activity, (1) freezing/immobility, (2) mild twitch, (3) tail extension, (4) forelimb clonus/repeated forelimb extensions, (5) consistent loss of balance/tonic-clonal seizures, (6) hyperactivity with jumping, (7) full body extension, and (8) death.

2.3. EEG surgeries and analysis

EEG surgeries were performed according to the manufacturer's protocol (Pinnacle Technology, Lawrence, KS). Briefly, mice were anesthetized with Avertin (0.02 ml/g body weight) and mounted in a stereotaxic device (Kopf). A mouse EEG head implant (Pinnacle technology) was affixed to the skull with four intracortical screws. EEG screws were positioned in the frontal and parietal cortices, using the headmount frame as a guide to ensure proper placement and spacing. The headmount was sealed with orthodontic resin and mice were allowed to recover for at least 3 days before chronic EEG monitoring.

EEGs were sampled at 200 Hz over 24 h intervals using Sirenia (v8.1.2), accompanied by video monitoring in a cylindrical plexiglass chamber with access to food and water ad libitum. Data were screened with video recordings and only those episodes not exhibiting environmental artifacts were exported for offline processing using Origin (v7.5). Abnormal EEG episodes were defined as amplitudes that were at least three times greater than the baseline and lasted at least 3 s. Events that occurred within 5 s were considered to be part of the same episode.

2.4. Statistical analysis

Data were processed using Prism (v4.0), and considered significant if $p < 0.05$.

3. Results

We previously generated mice that overexpress AICD and Fe65 or Fe65 alone and found that they exhibit some features observed in AD, including hyperactivation of GSK-3β (Ryan and Pimplikar, 2005). As these mice aged (>18 months) we noticed frequent behavioral seizures characterized by forelimb clonus and tonic–clonic seizures (11 of 32 mice, data not shown). However, these phenotypes were never observed in Fe27 mice that overexpress only Fe65, or wildtype (WT) littermates. To determine if AICD expression contributes to neuronal circuit disruptions at an earlier age, transgenic mice co-expressing Fe65 and high (line FeCγ25) or low (line FeCγ12) levels of AICD were implanted with intracortical EEG electrodes at 3–4 months of age and video monitored for several days. WT mice displayed consistent EEG readings without any abnormal spiking episodes (12 of 12) (Fig. 1A). The majority of mice (6 of 7) expressing Fe65 alone (line Fe27) resembled WTs, suggesting that elevated Fe65 is not sufficient to induce abnormal EEGs (Fig. 1B). Interestingly, both lines of FeCγ mice displayed abnormal spiking events detectable by EEG, (FeCγ12: 7 of 8) and (FeCγ25: 7 of 7) (Fig. 1C and D), with episodes lasting from a few seconds up to several minutes. However, we did not observe the same severe behavioral alterations seen in aged mice during these episodes. These data suggest AICD overexpression contributes to alterations of neuronal networks that may give rise to spontaneous spiking episodes.

Since AD mouse models have abnormal EEGs (Wang et al., 2002; Palop et al., 2007) we compared EEGs from FeCγ mice to those obtained from the R1.40 AD mouse model.

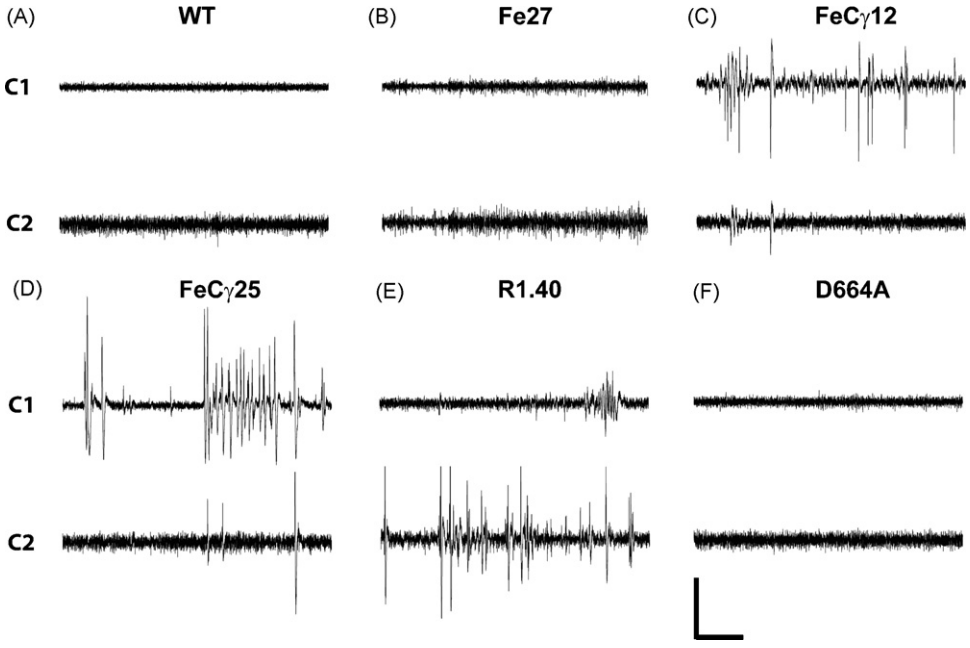

Fig. 1. Abnormal EEG episodes in AICD/Fe65 and R1.40 mice. Representative EEG rhythms in WT mice (12 of 12) (A) and Fe27 mice (6 of 7) (B). Representative EEGs from abnormal spiking periods in FeCγ12 (7 of 8) (C), FeCγ25 (7 of 7) (D), and R1.40 mice (6 of 6) (E). Representative EEG trace from PDAPP(D664A) mice (4 of 5) (F). Scale bar in (F): vertical 100 μA, horizontal 20 s. C1: EEG channel one: parietal cortex and C2: EEG channel two: frontal-parietal cortices.

This mouse has elevated Aβ (Lamb et al., 1997) but also high AICD levels (Ryan and Pimplikar, 2005). As expected, 3–4-month R1.40 mice showed abnormal spiking in the EEG (6 of 6) (Fig. 1E), and the majority of these episodes resembled those from FeCγ mice. Rarely, a more complex pattern was observed, first growing in intensity then followed by a sudden depression in the amplitude (data not shown), which resembles an epileptic EEG pattern. However, neither type of spiking episode was correlated with abnormal behavior. Interestingly, 3–4-month PDAPP(D664A) mice, which have high levels of Aβ but mutated AICD, exhibited relatively normal EEGs (4 of 5) (Fig. 1F).

When we quantified the number of abnormal spiking events among WT and transgenic lines, there was a significant increase in the number of spiking episodes occurring per hour in FeCγ25 (5.40, SEM ± 3.07) and R1.40 mice (3.59, SEM ± 1.35) compared to WT mice ($p = 0.0016$), as well as a strong trend in FeCγ12 mice (1.34, SEM ± 0.45). Thus, there may be a dose-dependent, positive correlation between the level of AICD and the occurrence of spiking events. The importance of AICD in relation to spiking events is further underscored by the observation that the number of episodes in Fe27 (0.34, SEM ± 0.31) and PDAPP(D664A) (0.16, SEM ± 0.16) mice is not significantly different from WT and again suggest that elevated Aβ alone is not sufficient for this phenotype.

Mice overexpressing AICD and Fe65 may be susceptible to stress that challenges the balance of excitation/inhibition.

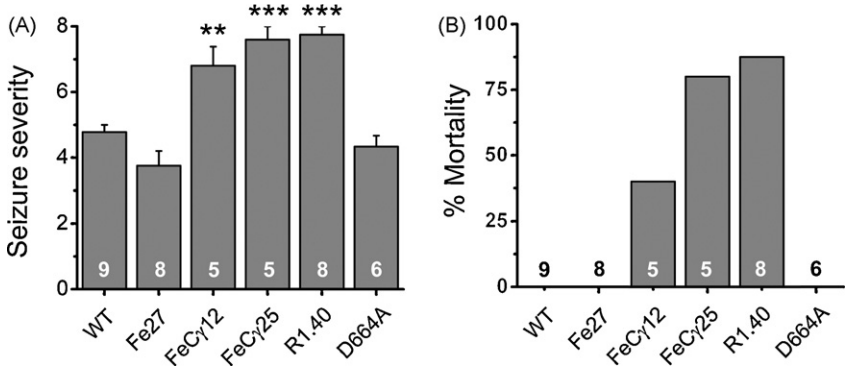

Fig. 2. Increased seizure susceptibility to kainic acid in FeCγ and R1.40 mice. (A) Quantification of maximum seizure severity exhibited over a 65 min monitoring period in mice of the indicated lines injected i.p. with kainic acid. (B) Percent mortality during the monitoring period after kainic acid injection. Data expressed as ±SEM, $^{**}p < 0.01$, $^{***}p < 0.001$ (one way ANOVA, Bonferroni's post-test).

To test this hypothesis we challenged mice with the seizure-inducing drug kainic acid (KA) and assessed seizure severity. We could induce convulsive seizures (levels 4–5) in WT mice at a dose of 25 mg KA/kg body weight (Fig. 2A). Fe27 mice were comparable to WTs, suggesting that elevated Fe65 alone does not change seizure susceptibility. However, seizure severity was significantly increased in FeCγ12 and FeCγ25 mice (Fig. 2A, $p = 0.0001$), suggesting that both lines expressing AICD were more susceptible to excitatory stimuli. AD mouse models are more susceptible to seizures (Del Vecchio et al., 2004; Palop et al., 2007) and we confirmed that R1.40 mice also reacted strongly to KA (Fig. 2A). The severity of seizures positively correlated with the levels of previously described AICD levels: the FeCγ12 line has moderately increased expression of AICD (Ryan and Pimplikar, 2005) and a moderate but significant increase in seizure severity, while the FeCγ25 and R1.40 mice have the highest levels of AICD (Ryan and Pimplikar, 2005) and the most severe seizures. Mortality after KA injection was also greatly increased in mice with elevated AICD levels (Fig. 2B). Importantly, PDAPP(D664A) mice reacted similarly to WTs despite their high levels of Aβ (Fig. 2A and B), suggesting that elevated Aβ alone does not increase susceptibility to KA. In contrast, mice with elevated AICD levels are more susceptible to a seizure-causing stimulus, suggesting elevated AICD levels contribute to this phenotype in AD mouse models.

4. Discussion

While Aβ may induce neuronal circuit disruptions in AD, these interpretations are confounded by the issue that AD mouse models also overexpress other APP metabolites. Here we examined the in vivo function of AICD, and demonstrate that elevated AICD and Fe65 can alter neuronal circuit stability. By 3–4 months of age, we found a high incidence of abnormal spiking in the EEGs of both FeCγ lines as well as a susceptibility of induced seizures that correlates with elevated AICD levels. It is unclear whether Fe65 in conjunction with AICD is essential for this phenotype, but two lines of evidence suggest altered AICD levels are sufficient to make neurons more sensitive to excitability. First, overexpression of Fe65 alone is clearly insufficient to cause abnormal EEGs or seizure susceptibility. Second, cultured neurons from an independent transgenic mouse line that only overexpresses AICD are also highly susceptible to KA (Giliberto et al., 2008). While it is possible that overexpression of AICD could interfere with endogenous APP signaling, we have detected no changes in the processing of endogenous APP in FeCγ mice, nor any alterations in Aβ generation when FeCγ mice are crossed to the R1.40 AD mouse model (Ghosal et al., in press). However, we cannot rule out that AICD may interfere with signaling events downstream of APP.

Studies of additional mouse models suggest alterations in AICD are also linked to increased seizure susceptibility. Here we confirmed that another AD mouse model, R1.40 (which has elevated Aβ and AICD levels), shows an increased response to KA and spontaneous seizure-like events in the EEG. In contrast, PDAPP(D664A) mice, which have high levels of Aβ but a mutation in the AICD, more closely resemble WT mice. While these findings support a role for AICD in seizure susceptibility, the role of the AICD in PDAPP(D664A) mice is still uncertain. It is possible that loss of the caspase cleavage site (and therefore loss of the C31 peptide), is important for neuronal excitability. It is also possible that this mutation disrupts a potential binding site for important signaling proteins, rendering AICD inactive. Nevertheless, the D664A mutation does not interfere with Aβ production (Galvan et al., 2006; Saganich et al., 2006), further indicating that Aβ alone is not sufficient to cause these alterations. However, alterations to APP metabolites may not be fully responsible for seizures in AD patients. Familial presenilin 1 (PSEN1) mutations are also linked to seizure susceptibility, despite their ability to decrease the levels of cytosolic AICD (Wiley et al., 2005). PSEN1 also cleaves a voltage gated sodium channel subunit (Wong et al., 2005) and its activity regulates calcium homeostasis (Shideman et al., 2009). Therefore, PSEN1 mutations may cause seizures independently of APP processing. Future studies will need to examine how each metabolite of APP can work independently or in conjunction with Aβ species or familial AD mutations to cause the pathological features of AD.

Conflict of interest

All authors state they have no potential or actual conflict of interests.

Disclosure statement

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