HHS Public Access

Author manuscript

Epilepsia. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Epilepsia. 2018 January ; 59(1): e1–e4. doi:10.1111/epi.13960.

BAD Knockout Provides Metabolic Seizure Resistance in a Genetic Model of Epilepsy with SUDEP

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SUMMARY

Metabolic alteration, either through ketogenic diet (KD) or by genetic alteration of the BAD protein, can produce seizure protection in acute chemoconvulsant models of epilepsy. To assess the seizure-protective role of knocking out (KO) the *Bad* gene in a chronic epilepsy model, we used the *Kcna1*$^{-/-}$ model of epilepsy, which displays progressively increased seizure severity and recapitulates the early death seen in sudden unexplained death in epilepsy (SUDEP). Beginning on postnatal day 24 (P24), we continuously video monitored *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ double knockout mice to assess survival and seizure severity. We found that *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice outlived *Kcna1*$^{-/-}$ mice by approximately two weeks. *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice also spent significantly less time in seizure than *Kcna1*$^{-/-}$ mice on P24 and day of death, showing that *Bad* KO provides seizure resistance in a genetic model of chronic epilepsy.

Keywords

K_v1.1; *Kcna1*$^{-/-}$; BAD; metabolic seizure resistance

INTRODUCTION

The BCL2-associated Agonist of cell Death (BAD) protein has a well-known role in cell apoptosis, but it also acts to regulate cellular metabolism[1, 2]. Genetic knockout of *Bad* (*Bad* KO) alters cellular metabolism, reducing the ability of both neurons and astrocytes to utilize glucose, and increasing their ability to use ketone bodies (KBs) as fuel[2]. This cellular metabolic state is reminiscent of what occurs on the ketogenic diet (KD); a high fat, low carbohydrate diet used to treat pharmacoresistant epilepsy[3]. Despite the KD being used clinically for close to a hundred years, its molecular mechanism is still incompletely understood[4, 5].

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DISCLOSURE

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

We previously found that, like mice on the KD, *Bad*$^{-/-}$ mice show resistance to acute chemoconvulsant seizures[2]. This effect was not due to the loss of BAD's apoptotic function; a mutation that maintains BAD's apoptotic role but prevents activation of glycolysis by BAD also provided seizure resistance in mice. This prompted us to examine whether *Bad* KO grants seizure protection in chronic epilepsy models, such as the *Kcna1*$^{-/-}$ model of epilepsy in mice.

Loss-of-function mutations in the *KCNA1* gene, which encodes for $K_v1.1$ channels[6], can cause epilepsy in humans[7], and targeted deletion of *Kcna1* in mice results in neuronal hyperexcitability and spontaneous seizures[6, 8]. This developmental model of epilepsy causes progressively increased seizure severity that begins shortly after weaning and recapitulates the early death seen in sudden unexplained death in epilepsy (SUDEP)[9, 10]. Furthermore, it has been previously shown that the KD as well as KB administration can reduce seizures and increase longevity in *Kcna1*$^{-/-}$ mice[10–13]. For the present study, we assessed the seizure protective effect of *Bad*$^{-/-}$ in the *Kcna1*$^{-/-}$ genetic model of epilepsy by generating *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ double knockout mice. *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice survived longer and had reduced seizure severity compared to *Kcna1*$^{-/-}$ mice, showing that *Bad* KO protects from spontaneous seizures that occur in chronic epilepsy.

METHODS

Animals

All experiments were performed in compliance with the NIH Guide for the Care and Use of Laboratory Animals and Animal Welfare Act. Specific protocols were approved by the Harvard Medical Area Standing Committee on Animals. Animals were housed on a 12h light/dark cycle with 5058 PicoLab Mouse Diet (LabDiet) and water provided *ad libitum*. The *Kcna1*$^{-/-}$ mouse line was obtained from Bruce Tempel[6] and bred into a C57BL/6 background. *Bad*$^{-/-}$ mice have been previously described[2]. *Kcna1*$^{+/-}$ mice were bred with *Bad*$^{-/-}$ mice (and the *Kcna1*$^{+/-}$ *Bad*$^{+/-}$ progeny crossed again with *Bad*$^{-/-}$ mice) to yield *Kcna1*$^{+/-}$ *Bad*$^{-/-}$ mice. Experimental *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ double knockout mice were then generated by crossing *Kcna1*$^{+/-}$ *Bad*$^{-/-}$ mice with *Kcna1*$^{+/-}$ *Bad*$^{-/-}$ mice. This cross also yielded *Kcna1*$^{+/-}$ *Bad*$^{-/-}$ and *Kcna1*$^{+/+}$ *Bad*$^{-/-}$ mice, which were not used in this study. All mutant mice were backcrossed for at least 10 generations with an in-house long-term inbred colony of C57BL/6 mice. The genotype of the wild-type colony is estimated to be ~77% C57BL/6J and ~23% C57BL/6N, based on a C57BL/6 Substrain Characterization Panel of 150 SNPs, performed by The Jackson Laboratory.

Behavior monitoring

Male and female *Kcna1*$^{-/-}$ (n=29; 10F, 19M) and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ (n=15; 10F, 5M) mice were weaned on post-natal day 23 (P23). A two way ANOVA showed that variation in survival time was explained by genotype ($P < 0.001$) but not by sex ($P = 0.6$), and data from male and female mice within genotype were pooled for comparison. Mice were singly housed in round Plexiglas cages (Pinnacle Technologies). Continuous recordings were obtained with ACTi (Model # D21) cameras using NVR3 software (ACTi Corporation);

infrared illumination was automatically triggered at the onset of the dark phase to enable uninterrupted 24-hour recordings.

Analysis

Seizures were scored and quantified by a trained experimenter blind to genotype. Seizure behaviors were ranked on a modified Racine scale where a score of 0 indicated normal behavior. The seizure scores were assigned as follows: 1: immobility, facial seizures; 2: head bobbing; 3: unilateral and bilateral forelimb clonus with rearing but not falling; 4: forelimb clonus with rearing and falling, hindlimb clonus; 5: tonic/clonic or wild running/jumping seizures. Seizure time was assessed for five animals in each genotype (*Kcna1*$^{-/-}$: 2F, 3M; *Kcna1*$^{-/-}$ *Bad*$^{-/-}$: 3F, 2M) for 24 hours on P24 and for the 24 hours preceding death. Time spent in seizure was compared across genotypes, within each time point. The proportion of time in seizure was defined as time in seizure state/total recording time. Percent survival for *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice was plotted from P23 until death.

Statistics

The Shapiro Wilk Normality Test was used, and samples were found to be non-normally distributed, so the Mann-Whitney U Test was used to compare *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ seizure time and seizure frequency at P24 and day of death. Seizure time was calculated as a percent of total time recorded; mean ± SEM for the 5 mice in each group was plotted. Seizure frequency was plotted as events (at Racine level 2 or higher, separated by at least 1 minute) per hour over 24 hours. The log-rank (Mantel-Cox) test was used to assess significance in survival curves of *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice.

RESULTS

Bad KO increases longevity and decreases seizure severity in *Kcna1*$^{-/-}$ mice

In order to assess the *Kcna1*$^{-/-}$ seizure phenotype and potential seizure protection of *Bad* KO, male and female experimental mice were video monitored from weaning (P23) until death. The difference in mean age of death was not different between male and female mice, thus data were pooled for comparison between genotypes. *Kcna1*$^{-/-}$ mice exhibited frequent spontaneous seizures and had shortened life spans. *Kcna1*$^{-/-}$ mice also exhibited hyper-startle responses (not shown). A high proportion of *Kcna1*$^{-/-}$ mice died suddenly from seizures between 3–5 weeks of age (Fig. 1; 79%). *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice outlived *Kcna1*$^{-/-}$ mice by about two weeks (Fig. 1; *Kcna1*$^{-/-}$ survival: 30.5±1.3 days, *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ survival: 45.8±3.8 days).

Behavioral seizures were scored using a modified Racine scale (see methods). Mice of both genotypes spent most of their time behaving normally and having stage 1 seizures (Figure 2a, b). Both genotypes also spent considerable time exhibiting moderate (stage 2–3) seizures, and limited time in severe (stage 4–5) seizures (Figure 2a, b). However, compared to *Kcna1*$^{-/-}$ mice, *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice spent less time having moderate to severe seizures (Figure 2a, b). When all Racine scores of two or above were summed and compared between groups, *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice spent less time in seizure on both P24 and day of death (Figure 2c). *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice also had lower seizure frequency on P24, compared to

Kcna1$^{-/-}$ mice (Figure 2d). In summary, *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice spent 59 ± 9% less time in moderate-severe seizures on P24 and 67 ± 12% less time in moderate-severe seizures in the 24 hours preceding death, when compared to *Kcna1*$^{-/-}$ mice ($P < 0.05$).

DISCUSSION

This study shows for the first time that genetic knockout of *Bad* provides epileptic seizure protection in *Kcna1*$^{-/-}$ mice, a genetic model of epilepsy with SUDEP. *Bad* knockout provided seizure protection in both male and female mice. Based on previous work[2], this effect is likely mediated by the non-dietary effect of BAD on cellular metabolism, rather than its effect on apoptosis. We found that *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice had increased longevity and decreased spontaneous seizure severity, characterized by decreased time spent in seizures at P24 and day of death. This is also the first time the *Kcna1* gene has been deleted in the C57BL/6 mouse strain. We found that C57BL/6 *Kcna1*$^{-/-}$ mice experienced frequent spontaneous seizures and hyper-startle responses, similar to previous studies performed in different mouse strains[14]. However, C57BL/6 *Kcna1*$^{-/-}$ mice appear to have increased seizure severity and decreased lifespans, compared to values reported for *Kcna1*$^{-/-}$ in other mouse strains[6].

We observed that nearly 80% of C57BL/6 *Kcna1*$^{-/-}$ mice died by 5 weeks of age and all died by 8 weeks of age. This is a higher mortality rate than previously reported in other *Kcna1*$^{-/-}$ mouse strains. Smart et al., 1998 reported that only 50% of *Kcna1*$^{-/-}$ 129/Sv X N:NIHS-BC hybrid mice died between 3–5 weeks of age and the rest survived to adulthood (while still experiencing spontaneous seizures)[6].

The *Kcna1*$^{-/-}$ model of epilepsy recapitulates the spontaneous seizures seen in epileptic patients[6] and early death that occurs in SUDEP[9, 10]. KD and KB treatment decrease seizures and extend the lifespan of *Kcna1*$^{-/-}$ mice[10–13]. Additionally, genetic deletion of *Bad* is associated with seizure resistance; when subjected to systemic chemoconvulsant injections of either kainic acid or pentylenetetrazol, *Bad*$^{-/-}$ mice had decreased seizure severity, compared to wild type mice[2]. This seizure resistance was not due to a lack of the apoptotic role of BAD because when BAD's ability to activate glycolysis was inhibited, but the apoptotic role of BAD was left intact, seizure protection ocurred[2]. We therefore wanted to test for seizure protection of *Bad* KO in a chronic seizure model. To do this, we tested seizures and lifespan in *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice.

We measured *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ survival and found that nearly 80% of *Kcna1*$^{-/-}$ mice died by 5 weeks of age but all *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice survived past this time point. We suspected *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice outlived *Kcna1*$^{-/-}$ mice because they had a less severe seizure phenotype. We measured time spent in seizure and found that *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice spent less time in moderate to severe seizures at an early age (P24) and during the 24 hours preceding their death. Furthermore, at P24 *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice had decreased seizure frequency, compared to *Kcna1*$^{-/-}$ mice. It is possible that a reduction in seizure frequency at a young age is what allowed for increased longevity in *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice. However, both *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice eventually succumbed to severe (stage 4/5) seizures. Thus *Bad* KO extends longevity but does not prevent eventual SUDEP

in the *Kcna1*$^{-/-}$ model of epilepsy. Nevertheless, reduction in seizure severity and extended lifespan suggests that *Bad* KO is seizure protective in a model of chronic epilepsy.

Our results that *Bad* KO confers seizure protection and extends longevity in the *Kcna1*$^{-/-}$ model of epilepsy shows that genetic alteration of cellular metabolism can alleviate epileptic seizures and prolong life in a model of SUDEP.

Acknowledgments

The authors thank Dr. Carolina Lahmann for supervising the maintenance of the animal colony and for management of the IACUC protocol. We also thank Nidhi Nathwani for her assistance in maintaining the mouse lines used for experiments performed in this paper.

Funding:

This study was supported by a Louis Perry Jones Postdoctoral fellowship (to J.F.), by the NIH Director's Pioneer Award (DP1 EB016985-03; to G.Y.) and by an NIH/NINDS research (R01 NS083844 to N.N.D. and G.Y.).

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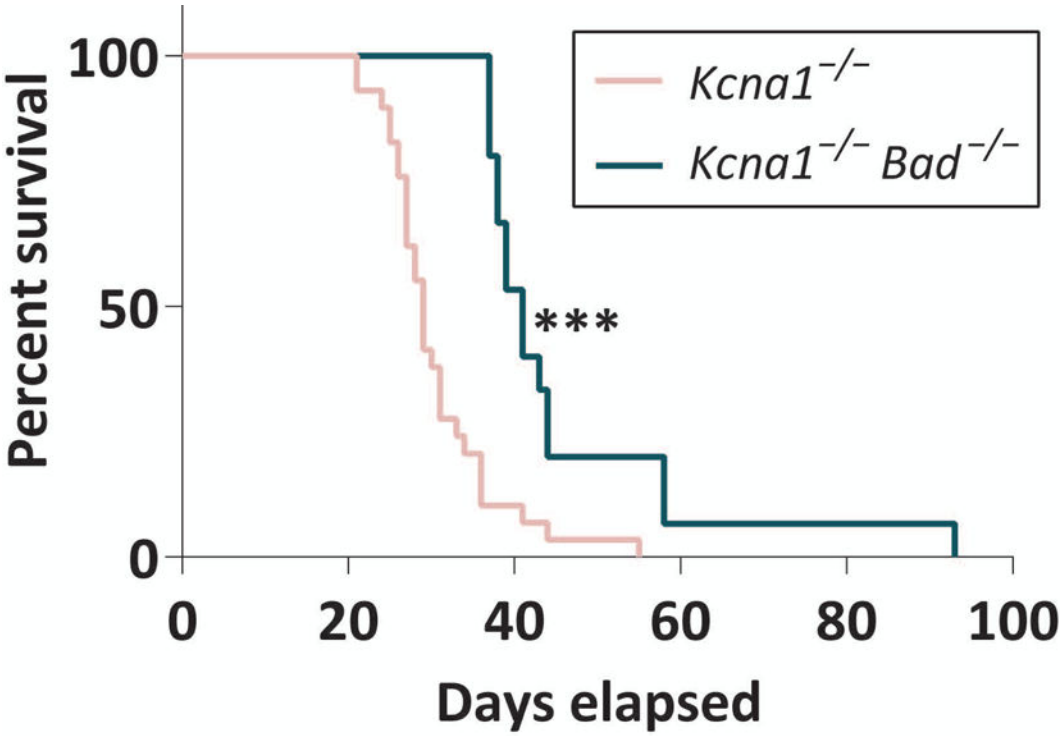

Figure 1. *Bad* KO increases longevity in *Kcna1*$^{-/-}$ mice

(**A**) *Kcna1*$^{-/-}$ (n=29) and *Kcna1*$^{-/-}$ Bad$^{-/-}$ (n=15) mouse survival. ***P<0.001, log-rank (Mantel-Cox) test.

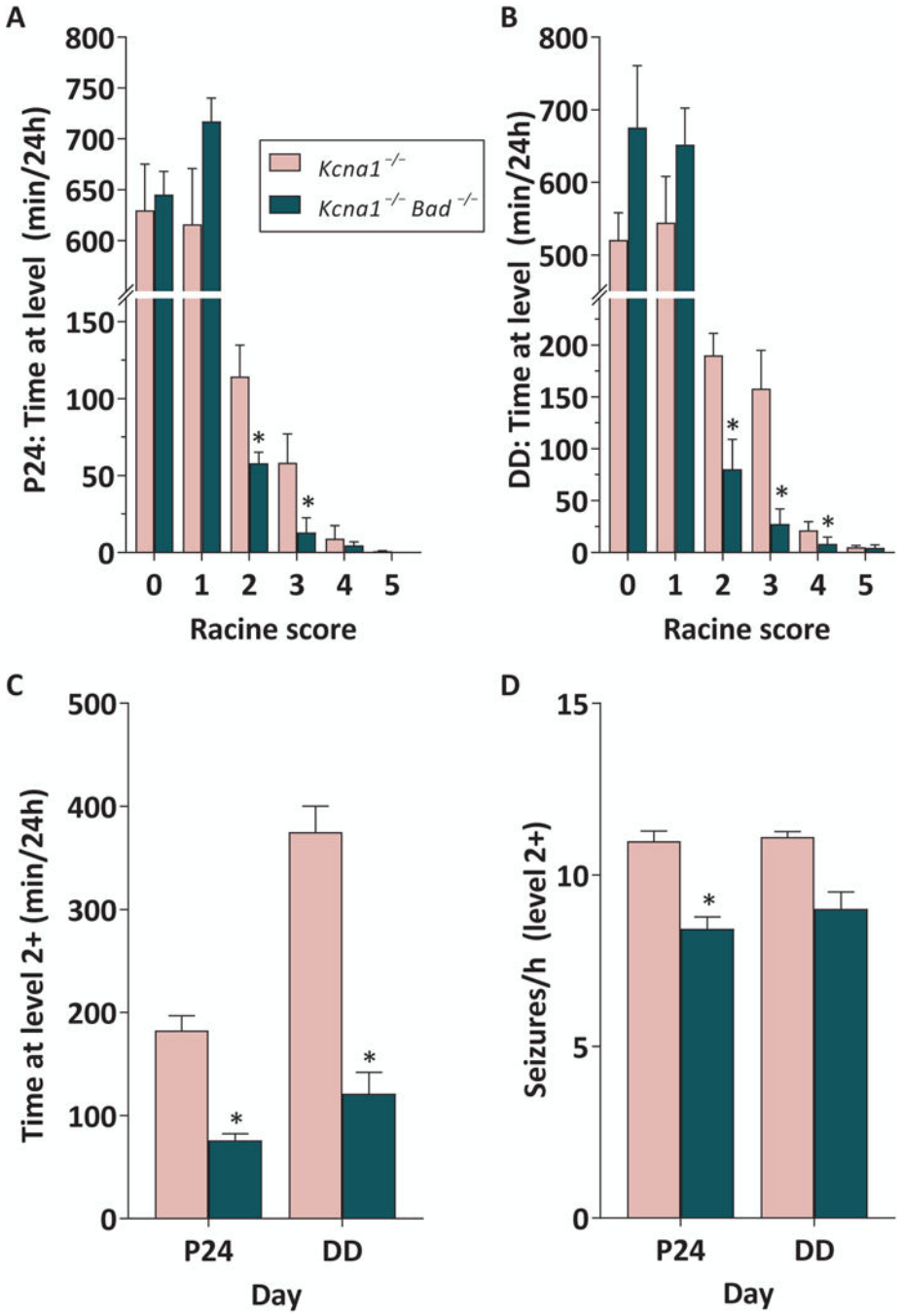

Figure 2. *Bad* KO decreases time spent in moderate to severe seizures

(**A**) Time spent in seizure at each seizure level (0–5) in *Kcna1*$^{-/-}$ (n=5) and *Kcna1*$^{-/-}$ Bad$^{-/-}$ (n=5) mice on P24. (**B**) Time spent in seizure at each level during the 24 hours preceding death (DD). (**C**) Time spent in seizure (stage 2+) on each day (P24 and day of death [DD]) for *Kcna1*$^{-/-}$ and *Kcna1*$^{-/-}$ *Bad*$^{-/-}$ mice. (**D**) Seizures (stage 2+) per hour on each day. Data are presented as mean ± SEM, *P<0.05, Mann-Whitney U Test.