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STRAIN DIFFERENCES IN SEIZURE-INDUCED CELL DEATH FOLLOWING PILOCARPINE-INDUCED STATUS EPILEPTICUS

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Abstract

Mouse strains differ from one another in their susceptibility to seizure-induced excitotoxic cell death. Previously, we have demonstrated that mature inbred strains of mice show remarkable genetic differences in susceptibility to the neuropathological consequences of seizures in the kainate model of status epilepticus. At present, while the cellular mechanisms underlying strain-dependent differences in susceptibility remain unclear, some of this variation is assumed to have a genetic basis. However, it remains unclear whether strain differences in susceptibility to seizure-induced cell death observed following kainate administration are observed following systemic administration of other chemoconvulsants. In rodents, the cholinomimetic convulsant pilocarpine is widely used to induce status epilepticus (SE), followed by hippocampal damage and spontaneous recurrent seizures, resembling temporal lobe epilepsy. This model has initially been described in rats, but is increasingly used in mice. We characterized neuronal pathologies after pilocarpine-induced status epilepticus (SE) in eight inbred strains of mice focusing on the hippocampus. A ramping-up dose protocol for pilocarpine was used and behavior was monitored for 4–5 hours. While we did not observe any significant differences in seizure latency or duration to pilocarpine among the inbred strains, we did observe a significant difference in susceptibility to the neuropathological consequences of pilocarpine-induced SE. Of the eight genetically diverse mouse strains screened for pilocarpine-induced status, BALB/cJ and BALB/cByJ were the only two strains that were resistant to the neuropathological consequences of seizure-induced cell death. Additional studies of these murine strains may be useful for investigating genetic influences on pilocarpine-induced status epilepticus.

Keywords

temporal lobe epilepsy; hippocampus; inbred strains; pilocarpine; cell death; status epilepticus; seizure

Introduction

Genetic factors have been suggested to play an important role in seizure disorders. A significant genetic component has been found in predisposition to a wide variety of epilepsies (Berkovic et al., 2006; Macdonald et al., 2010; Scheffer and Berkovic, 2010).

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However, despite being the subject of enormous research efforts, genetic influences and their effects on the etiology of temporal lobe epilepsy (TLE) are not yet fully understood. This is in part due to the complexity associated with myriad genetic and environmental factors in human populations. As an alternative approach, rodents provide important model systems for studying the pathophysiology of status epilepticus (Majores et al., 2007; Müller et al., 2009b; Williams et al., 2007). Of considerable value in this regard are isogenic inbred mouse strains. Comparison of different mouse strains can provide valuable information about the importance of genetic background in behavioral and pharmacodynamic phenotypes (Jacobson and Cryan, 2005). Although there are no known inbred strains that spontaneously develop status, researchers have used induced models of status in experimental animals such as mice. In particular, selected lines and inbred strains of mice with a divergence in susceptibility represent valuable tools to dissect the genetic components of seizure-induced cell death.

A great variety of animal models of seizures and epilepsy has played and still play a major role in unraveling the pathophysiology of human epilepsies (Kharatishvili et al., 2006). In general, animal models reproduce several components of human epilepsies. They are used to study the mechanisms underlying seizures and the neuropathological consequences resulting from seizures. Typically, rodents are used to conduct these epilepsy studies and the type of rodent strain used seems crucial. Studies in inbred murine strains in particular, have yielded considerable information regarding genetic influences on both seizure susceptibility and its neuropathological consequences (Ferraro et al., 2004, 2007; Kong et al., 2008; Mazarati et al., 2004; Schauwecker, 2010; Weinshenker et al., 2001). Previously, we demonstrated that administration of the chemoconvulsant, kainic acid, results in significantly different extents of hippocampal neurodegeneration among inbred mouse strains (Schauwecker and Steward, 1997). Interestingly, the duration or severity of seizure activity in response to kainic acid (KA) is not predictive of subsequent hippocampal cell death. Resistant strains show no evidence of degeneration or cell damage until doses at or exceeding the LD₉₀ are administered (Schauwecker and Steward, 1997). Those mice susceptible to KA administration show an excitotoxic response similar to what has been described in rats (Ben-Ari, 1985; Nadler et al., 1980a,b; Sperk et al., 1983). These findings suggest that host genetic factors confer protection against hippocampal damage following seizures in resistant strains. However, whether this phenomenon exists for other chemoconvulsants remains to be determined.

The induction of status epilepticus by the chemoconvulsants, pilocarpine or kainate, in rodents leads to a series of neuropathological changes and the subsequent appearance of spontaneous recurrent seizures. Many features of the rodent model, such as hippocampal sclerosis and mossy fiber sprouting, resemble human temporal lobe epilepsy (Ben-Ari, 1985; Turski et al., 1983). Similar to kainic acid, systemic administration of pilocarpine initially induces a well-characterized pattern of seizure activity that lasts for hours, followed by a latent seizure-free period of weeks preceding the development of spontaneous recurrent focal seizures that begin within 3–4 weeks (Brandt et al., 2003; Williams et al., 2007). Pilocarpine-induced status epilepticus (SE) is followed by extensive neuronal destruction in hilar, CA3 and CA1 hippocampal regions, a pattern similar to human hippocampal sclerosis (Cavalheiro et al., 1996; Curia et al., 2008; Löscher, 2002). Pilocarpine is a chemoconvulsant that initiates seizures (Turski et al., 1983) via activation of muscarinic M1 receptors (Hamilton et al., 1997). However, upon induction of seizures, seizure maintenance is dependent upon other mechanisms; mainly activation of the NMDA receptor by release of the excitatory neurotransmitter glutamate (Smolders et al., 1997).

The present study represents a systematic strain investigation of pilocarpine-induced status epilepticus, a complex trait having genetic and environmental influences. We were

interested in investigating strain-differences in pilocarpine-induced seizure severity in mice and the relation to seizure-induced cell death in a large number of inbred mouse strains. Thus, this is the first study to conduct a strain survey to compare eight commonly used inbred mouse strains for both susceptibility to pilocarpine-induced status epilepticus and its neuropathological consequences. The results of this study identify BALB/cJ and BALB/cByJ as resistant to the neuropathological consequences of pilocarpine-induced status epilepticus and C57BL/6J and FVB/NJ as significantly susceptible suggesting that these strains may be particularly useful for genetic dissection of this complex trait.

Materials and methods

Animals

All mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA), to reduce a potential source (i.e. supplier) of genetic and behavioral variation, at an age of about 6–7 weeks and housed in groups under controlled conditions (temperature: $21 \pm 0.5^\circ\text{C}$; humidity: $55 \pm 5\%$), under a 12-h light-dark cycle with food and water *ad libitum*. The animals were allowed to adapt to laboratory conditions for at least 1 week before starting the experiments. The strain survey comprised: A/J, AKR/J, BALB/cByJ, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ, SJL/J and 129T2/SvJ mice (8–10 weeks old, 25–30 g). These were selected on the basis of 1) their frequent use in studies of status and as genetic backgrounds for mouse mutant lines, 2) inclusion as “group A” priority strains in the Mouse Phenome Project, an international effort to provide the biomedical research community with phenotypic data on the most commonly used mouse strains (www.jax.org/phenome), and 3) their use as parental strains in several sets of recombinant inbred strains, including the CXB and BXD sets (see www.genenetwork.org). All experiments were performed between 8:00 a.m. and 12:00 p.m. to minimize variation because of circadian rhythms and testing was conducted in a manner counterbalanced for strain.

Pilocarpine-induced status epilepticus (Pilocarpine-induced SE)

To minimize the peripheral cholinergic side effects of pilocarpine, mice were injected with methyl scopolamine 15–30 min prior to pilocarpine (1 mg/kg each i.p. in 0.9% NaCl). We utilized a dosing protocol with repeated low-dose treatment by i.p. application of 100 mg/kg pilocarpine (in 0.9% NaCl) every 30–45 min until onset of SE was observed (Grötcke et al., 2007; Müller et al., 2009a). Compared to injection of one single high dose of pilocarpine, this ramping-up protocol allowed a more individual dosing of the convulsant, resulting in a higher percentage of mice developing SE and reduced mortality. Age-matched mice of the C57BL/6J strain were used as sham controls ($n=6$). These mice also received an injection of methyl scopolamine, similar to the pilocarpine-treated mice, but five i.p. injections of sterile saline instead of pilocarpine. All drugs were obtained from Sigma Aldrich (St. Louis, MO, USA).

After the pilocarpine injections, experimental animals were monitored for a minimum of 5 h to assess the severity and length of the behavioral seizures. Seizures were classified according to Racine (1972) as modified by Borges et al. (2003): normal activity (stage 0); rigid posture or immobility (stage 1); stiffened, extended and often arched (Straub) tail (stage 2); partial body clonus, including forelimb or hind limb clonus or head bobbing (stage 3); whole body continuous clonic seizures with rearing (stage 4); severe whole body continuous clonic seizures with rearing and falling (stage 5); and tonic-clonic seizures with loss of posture or jumping (stage 6). SE was defined by continuous or intermittent limbic seizure activity for at least 45 minutes without full recovery between seizures, as previously defined in humans (Lowenstein, 1999). After SE, all mice were fed moistened high-fat rodent chow and monitored daily. Mice were injected with 5% dextrose in lactate Ringer

solution as needed. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Southern California and conducted in accordance with its guidelines. Every effort was made to minimize animal suffering.

Tissue preparation and histological staining

In order to evaluate the severity of pilocarpine-induced excitotoxic brain damage in different strains of mice, brains from each strain of mice were processed for light microscopic histopathologic evaluation according to previously published methods (Schauwecker et al., 2004). Briefly, 7 days after seizure induction by pilocarpine, mice were anesthetized with Avertin and transcardially perfused with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Brains were removed and post-fixed overnight, followed by cryoprotection in 30% sucrose for at least 12–18 h. Horizontal (40 μ m) frozen sections were cut on a sliding microtome and collected as free-floating sections in 0.1M phosphate buffer (pH 7.4) until processed for light microscopic histology. Every sixth section (~240 μ m) was processed for cresyl violet staining to assess cell loss and morphology. An alternate series of sections were stained with a modified Gallyas silver stain, which stains degenerating fibers, synaptic terminals and cell bodies (Nadler and Evenson, 1983 as modified in Schauwecker 2003) and examined for the appearance of degenerative debris.

Quantitative analysis of hippocampal cell loss

Subsequently, to determine the susceptibility of individual hippocampal subfields to neurotoxic insult, we counted neurons in Nissl-stained sections. Quantitative analysis of hippocampal cell loss was performed by an observer blinded to the strain groups using unbiased stereological methods on cresyl violet-stained sections according to previously published protocols (Schauwecker and Steward, 1997; Schauwecker et al., 2000; Santos and Schauwecker, 2003). The Nissl-stained neurons in area CA3, area CA1, the dentate hilus, and the dentate gyrus were counted in both the right and left hippocampus and counting was initiated within the ventral hippocampus at the first point where hippocampal subfields could be easily identified. This level corresponded to horizontal section 54, based on the atlas of Sidman et al. (1971). Hippocampal subfields were based on Franklin and Paxinos (1997) classification and discrimination between the CA3 and the dentate hilus region was based on morphological features and locations of the cells (Sousa et al., 1998; West et al., 1991). Specifically, for dentate hilar cell counts, the hilus was operationally defined as the region bordered by the supra- and infrapyramidal granule cell layers and excluding the densely packed pyramidal neurons of area CA3.

Neuron counts were made in all subfields and the numbers for each side were averaged into single values for each animal. Surviving cells were counted only if they were contained within the pyramidal cell layer, dentate hilus or dentate gyrus, possessed a visible nucleus and characteristic neuronal morphology and had a cell body larger than 10 μ m. Six square counting frames (200 $\times$ 200 μ m) were randomly placed in the pyramidal layer of fields CA1 and CA3 or in the dentate hilus or dentate gyrus in 4–5 regularly spaced horizontal sections from each animal. Neuronal nuclei were evaluated at three different focal planes and only those in the focal plane were counted with a 40X objective and considered as a counting unit. Stereological analysis was performed with the aid of ImagePro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA) and a motorized Z-stage (Optiscan; Prior Scientific, Fairfax, VA, USA). Final cell counts were expressed as the percentage of cells as compared to intact mice. Results were assessed statistically by one-way ANOVA using the computer program, SigmaStat (Jandel Scientific, San Rafael, CA, USA), and intergroup differences were analyzed by Newman-Keuls post hoc test.

Results

Induction of status epilepticus by systemic pilocarpine

Administration of pilocarpine to all strains of mice resulted in stereotypical behavioral responses which included the following: akinesia, staring, salivation, facial automatisms, a stiffened tail, slight tremors and head bobbing. This type of behavior built up progressively into motor limbic seizures that recurred repeatedly and rapidly developed into status epilepticus (SE). Irrespective of mouse strain, SE onset was typically characterized by forelimb clonus and tonic-clonic seizures with loss of righting reflexes. SE was defined by continuous or intermittent seizures without full recovery between seizures. The latency to SE ranged from 15–29 minutes following the last injection of pilocarpine irrespective of mouse strain.

Table 1 illustrates the disposition of 11 inbred strains of mice that underwent pilocarpine-induced status epilepticus. Three inbred strains (A/J, AKR/J, and SJL/J) were not included for additional study due to high seizure mortality (A/J, AKR/J) and/or inability to develop status epilepticus (AKR/J, SJL/J). Of the eight remaining inbred strains examined, we found that 50% of the total mice from the BALB/cJ and C3H/HeJ strains administered pilocarpine were unable to develop status epilepticus. In all other strains examined, pilocarpine administration failed to induce SE in 20–30% of mice. As well, of the eight inbred strains included in the complete study, mortality following pilocarpine administration ranged from 20–50% depending on the strain. A clear relationship between mortality and cumulative dose of pilocarpine was not apparent among the inbred strains of mice.

In Table 2, data from all experiments with pilocarpine in the 8 inbred mouse strains are summarized. As illustrated, while no significant strain-dependent differences were observed with regard to the latency or duration of severe seizures, a significant strain-dependent difference in the dose of pilocarpine to SE (ED_{100}) was observed. Post hoc analysis by the Student Newman-Keuls test indicated that the CBA/J differed significantly from five inbred strains (CBA/J vs. BALB/cJ, $p=0.014$; CBA/J vs. DBA/2J, $p=0.021$; CBA/J vs. C57BL/6J, $p=0.016$; CBA/J vs. C3H/HeJ, $p=0.014$; CBA/J vs. FVB/NJ, $p=0.026$). CBA/J seemed to require the lowest dose of pilocarpine (~200 mg/kg) to induce status epilepticus. In contrast, five of the inbred strains (BALB/cJ, C3H/HeJ, C57BL/6J, DBA/2J, and FVB/NJ) had a similar dose of pilocarpine required to induce status epilepticus.

Strain differences in susceptibility to pilocarpine-induced cell death

Histological analysis was performed to determine seizure-induced neuronal damage in the hippocampal formation following systemic pilocarpine administration in the eight inbred strains of mice. Consistent with previous studies in rats (Mello et al., 1993; Olney et al., 1983; Turski et al., 1983) and mice (Cavalheiro et al., 1996; Turski et al., 1984), administration of pilocarpine induced the degeneration and reduction of hippocampal neurons in area CA3 and the dentate hilus in susceptible strains (CBA/J, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ and 129T2/SvJ). As illustrated in Fig. 1, hippocampal sections from 'susceptible' mice, such as the DBA/2J strain, showed significant loss of neurons in area CA3 and within the dentate hilus. Consistent with previous studies (Scharfman and MacLusky, 2006), neurons within the dentate granule cell layer were spared. Hippocampal sections from susceptible strains that were processed for the Gallyas silver stain for degeneration displayed degenerating neuronal cell bodies within the CA3 cell layer, as well as within the dentate hilus (Fig. 1). Furthermore, neuronal loss in susceptible strains was not simply restricted to the hippocampus, but was found in many other brain areas 7 days after pilocarpine administration. In those strains susceptible to seizure-induced neuronal damage, cell loss occurred in the thalamus, piriform cortex, striatum and amygdala. In contrast, two

representative resistant strains (BALB/cJ and BALB/cByJ) showed no detectable reduction of neurons or signs of neuronal degeneration within the hippocampus proper (Figs. 1I, i–l) and no indication of damage was noted in the septum, amygdala, pyriform cortex, neocortex or thalamic nuclei in cresyl violet-stained sections. As well, no detectable evidence of degenerative debris was observed in the two representative ‘resistant’ strains of mice.

Quantitative analysis of subfield group means revealed that susceptible strains lost on average 71% of dentate hilar neurons (Fig. 2) as compared to resistant strains, which showed a reduction of only 11% of dentate hilar neurons. This observation was confirmed by statistical analyses as significant effect of group for cell counts in dentate hilus ($F=38.053$; $P<0.001$). Post hoc analysis with the Student Newman-Keuls test revealed that among the susceptible strains, two inbred strains, C57BL/6J and 129T2/SvJ showed statistically significant reduced amounts of hilar cell loss (~30–40%) as compared to the other four susceptible strains (~85% loss; $p=0.003$). Within area CA3, susceptible strains showed a reduction of approximately 39% of CA3 pyramidal neurons (Fig. 3), as compared to only 6% of CA3 pyramidal neurons in resistant strains ($F=15.394$; $P<0.001$). In contrast, while we did observe a reduction in the number of cells within area CA1 in the six susceptible strains seven days following systemic pilocarpine administration (data not shown), this was statistically non-significant as compared with resistant strains of mice. Overall, resistant strains of mice (BALB/cByJ and BALB/cJ) showed no significant differences in the extent of cell death within the dentate hilus, area CA3 or area CA1 as compared to vehicle-injected sham control mice, when cell death was assessed in hippocampal subfields. As previously reported (Scharfman and MacLusky, 2006), no cell death was observed throughout the dentate granule cell layer irrespective of mouse strain (data not shown).

Discussion

Here, we conducted a strain survey to compare eight commonly used inbred mouse strains for both susceptibility to pilocarpine-induced status epilepticus and its neuropathological consequences to determine if strains reported to be ‘resistant’ to kainate-induced cell death would demonstrate a similar resistance to excitotoxic cell death induced by systemic administration of pilocarpine. Among mouse models of epilepsy, genetic background is known to affect both seizure susceptibility to chemoconvulsants (Ferraro et al., 1995; Frankel et al., 2001; Golden et al., 1995; McLin and Steward, 2006; Müller et al., 2009a), as well as susceptibility to the neuropathological consequences of seizures (McKhann et al., 2003; McLin et al., 2006; Müller et al., 2009b; Schauwecker and Steward, 1997; Schauwecker et al., 2004; Winawer et al., 2007). Previous studies in our lab as well as others have shown that the genetic background of mice significantly affects the susceptibility of hippocampal neurons to damage by systemic kainate administration (McKhann et al., 2003; McLin et al., 2006; Schauwecker and Steward, 1997; Schauwecker et al., 2004). Our results highlight three principal and novel findings from the present study: (1) genetic background plays a significant role in modulating susceptibility to seizure-induced cell death following pilocarpine administration; (2) the resistance of C57BL/6J mice to seizure-induced cell death following kainate administration does not generalize to pilocarpine administration; and (3) BALB/cJ mice are resistant to seizure-induced cell death irrespective of whether it is induced with kainate or pilocarpine.

We found significant variation in susceptibility to pilocarpine-induced cell death among eight commonly used inbred mouse strains. The results of this study identified BALB/cJ and BALB/cByJ as resistant to the neuropathological consequences of pilocarpine-induced status epilepticus and six other inbred strains as significantly susceptible, suggesting that these strains may be particularly useful for genetic dissection of this complex trait. Thus, our data confirm previous findings that the genetic background of mice significantly affects the

susceptibility of hippocampal neurons to damage by pilocarpine (Borges et al., 2003; Müller et al., 2009a; Shibley and Smith, 2002; Winawer et al., 2007), and extends these findings to a comparison of a large number of inbred strains. As prior studies have suggested that differential susceptibility to seizure-induced cell death could result from a reduction in seizure activity (Berger et al., 1986; Ferraro et al., 1995; Galanopoulou et al., 2002; Holmes, 2002; Sucholmelova et al., 2006), we examined several seizure parameters. Irrespective of mouse strain examined, the latency to onset of status epilepticus or severity of seizure activity, as defined by the percentage of mice achieving stage 4/5 severe seizures, was not different. While we, as well, found no significant differences in seizure duration following pilocarpine administration, we did observe a trend towards decreased seizure duration in C3H/HeJ and FVB/NJ strains. Furthermore, there did not appear to be a clear relationship between duration of severe seizures and susceptibility to seizure-induced cell death. While we did not observe any statistically significant differences in latency to onset of severe seizures or seizure duration, we cannot conclude that genetic background does not affect seizure susceptibility to pilocarpine administration, as we found significant differences in dose effectiveness.

It is important to note that in our study different doses of pilocarpine were administered to individual strains of mice in order to ensure that all mice achieved pilocarpine-induced SE. Thus, while we did not observe any significant differences among inbred strains with regard to latency to seizure induction or duration of severe seizures, we did observe significant strain differences in the dose of pilocarpine needed to induce status epilepticus (ED₁₀₀). For example, five of the strains examined required significantly more pilocarpine to go into SE than the others suggesting that genetic background can affect seizure susceptibility to pilocarpine administration. Therefore, it is possible that inbred strain differences in either blood-brain barrier permeability or systemically administered pilocarpine could play a role in the differential susceptibility observed.

An important difference in neuropathology between our susceptible strains was the extent of hilar neuron loss. Hilar neuron loss is one of the common neuropathological features in rodent models of temporal lobe epilepsy (Babb et al., 1991; Masukawa et al., 1995; Nadler et al., 1980b), including pilocarpine-induced status epilepticus (Lemos and Cavalheiro, 1996). Importantly, while within area CA3, susceptible strains appeared to exhibit roughly the same amount of cell loss (~30–40%), we found significant differences in the extent of cell death within the dentate hilus among susceptible strains. In particular, two strains, C57BL/6J and 129T2/SvJ, showed significantly less cell death (~40% loss) than the four remaining susceptible strains (~85% loss). Previous studies in rats have suggested few hilar neurons are truly seizure resistant, in that nearly 75% of hilar neurons are killed following pilocarpine-induced status (Jiao and Nadler, 2007; Sloviter, 2003). Our results in four of the susceptible strains are in agreement with this extensive loss of hilar neurons. However, differences in the extent of cell death across hippocampal subfields could result from differential susceptibility to particular cell populations in that approximately 60% of hilar neurons are excitatory mossy cells while nearly 40% are inhibitory GABAergic interneurons (Buckmaster and Jongen-Rêlo, 1999; Jiao and Nadler, 2007). Thus, it is possible that C57BL/6 and 129T2/Sv mice may exhibit differences in either glutamate or GABA receptors that could result in reduced susceptibility to seizure-induced damage in this particular subfield. Furthermore, pilocarpine-induced status epilepticus has also been shown, in rats, to reduce the population of GluR2⁺ hilar neurons (Jiao and Nadler, 2007). Future studies will need to assess whether mice of these two strains show a lack of a reduction in GluR2⁺ hilar neurons and/or differential regulation of GluR2 expression within the dentate hilus.

In agreement with previous studies, we found that pilocarpine-induced cell death was observed in mice of the C57BL/6J strain (Borges et al., 2003; Chen et al., 2005; Peng et al., 2004; Shibley and Smith, 2002). Thus, while previous results obtained from our laboratory have demonstrated the resistance of C57BL/6J mice to glutamate-induced excitotoxicity by kainate administration (Schauwecker and Steward, 1997; Schauwecker et al., 2000), this resistance does not extend to the current studies of pilocarpine-induced cell death. The fact that C57BL/6J mice, which are resistant to kainate-induced temporal lobe epilepsy and may carry genes that offer protection from glutamate-induced excitotoxicity, were susceptible to pilocarpine-induced TLE suggests that resistance to kainate, at least in the C57BL/6J strain, cannot be generalized to other chemoconvulsants. Furthermore, the presumed resistance of C57BL/6J mice to kainate suggests that protection against seizure-induced cell death for the kainate model may be specific for glutamate receptors, as the muscarinic M1 receptor subtype is involved in mediating pilocarpine-induced SE (Maslanski et al., 1994).

The fact that C57BL/6 mice appear sensitive to injury by pilocarpine but insensitive to injury produce by kainate could be due to a number of factors. First, while the mechanism underlying the origin of this striking difference in the extent of seizure-induced cell death in C57BL/6 mice when SE is induced by kainate versus pilocarpine is not known, it is likely that complex phenotypes such as differences in the extent of seizure-induced cell death are traits that may be influenced by the interaction of multiple genes, not all of which may have strong independent effects on this phenotype. At present, the molecular regulation of seizure-induced cell death remains unclear based on difficulties in differentiation between genes that regulate seizure induction and seizure sensitivity versus those that are responsible for the subsequent neuropathological outcome. Increased expression of putative neuroprotective genes in resistant strains or the presence of those genes that predispose to excitotoxic damage in the susceptible strains, including C57BL/6, may cause the observed differences in resistance to pilocarpine-induced toxicity. Other putative mechanisms that may explain the strain discrepancies between susceptibility to seizure-induced cell death when induced by pilocarpine versus KA administration could be the result of variations in the expression of modifying genes, such as glutamate receptors. Previous studies have demonstrated that expression changes in ionotropic glutamate receptor subunits paralleled the development of susceptibility to cell death in the FVB/N strain following kainate administration (Schauwecker, 2003). At present, it remains unclear whether similar expression changes are observed among the inbred strains administered pilocarpine in the present study. Thus, C57BL/6 mice may metabolize kainate faster than pilocarpine, or the pharmacokinetics of pilocarpine delivery to C57BL/6 mice may be different than kainate delivery producing increased CNS bioavailability of pilocarpine. In addition, differences in epileptic circuitries, activation of intracellular signaling cascades, or other downstream mechanisms that might be genetically determined might contribute to the differences in the degree of neuronal injury observed. Clearly, the reason for such differences seems to be mediated, at least in part, by genetic differences, and elucidation of these factors requires further studies.

In contrast, our strain survey revealed that BALB/cByJ and BALB/cJ were resistant to the neuropathological consequences of pilocarpine-induced SE. Importantly, the resistance of BALB/cJ to pilocarpine-induced SE is similar to their resistance to KA-induced SE as reported previously (Kong et al., 2008; Schauwecker and Steward, 1997). Thus, despite the fact that the neurotoxic mechanisms of kainate and pilocarpine differ and presumably occur primarily through the actions of these excitotoxins at their respective receptors (kainate receptor and M1-muscarinic receptor), it led to the same resistance to pathological sequelae. Hence, these two strains may not be ideal to study seizure-induced cell death because they appear to be resistant to status epilepticus induced by two distinct chemoconvulsants. The higher resistance of these two strains to pilocarpine and kainate excitotoxicity clearly

indicates that genetic background is a major determinant for the susceptibility of neurons to seizure-induced neurodegeneration.

Conclusions

In summary, results of this study document substantial strain variation in susceptibility to pilocarpine-induced status epilepticus. BALB/cJ and BALB/cByJ strains were observed to be nearly resistant to the neuropathological consequences of pilocarpine-induced SE. These differences cannot be explained by a general strain-specific predisposition to seizure sensitivity. In particular, the resistance of BALB/cJ to pilocarpine-induced SE may be a more general phenomenon related to overall seizure-induced cell death resistance rather than to a specific action of pilocarpine, as BALB/cJ mice are also nearly resistant to the neuropathological consequences of kainate-induced SE (Kong et al., 2008; Schauwecker and Steward, 1997).

Another useful outcome of this study is the identification of quantitative traits that arose from the phenotypic diversity between inbred mouse strains. Future studies could center on tracking and identification of quantitative trait loci (QTL) that modify particular epilepsy processes. Successful genetic dissection of this complex trait, by finding genes and biological pathways that differentiate resistant and susceptible responders, will provide a better understanding of the mechanisms underlying susceptibility to seizure-induced cell death and represents a promising approach. Collectively, the results of this study emphasize the importance of genetic divergence between inbred mouse strains and the complex manner in which this influences susceptibility to seizure-induced cell death. Our findings could have implications for elucidating the neural and genetic basis of individual differences in susceptibility to seizure-induced cell death.

Highlights

>Eleven inbred mouse strains underwent pilocarpine-induced status epilepticus. >We compared eight of these inbred mouse strains for sensitivity to pilocarpine-induced seizures and seizure-induced cell death. >We found no significant differences in seizure sensitivity following pilocarpine administration. >Two of these strains (BALB/cJ and BALB/cByJ) are resistant to seizure-induced cell death.

Abbreviations

SE	status epilepticus
KA	kainic acid
TLE	temporal lobe epilepsy
QTL	quantitative trait locus

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Fig. 1. Strain differences in susceptibility to pilocarpine-induced cell loss and degeneration among two representative inbred strains of mice, as compared to a sham control mouse of the C57BL/6J strain. Low magnification cresyl violet staining of horizontal sections through the hippocampus and high-magnification cresyl violet and Gallyas silver staining of the dentate hilus and area CA3, seven days following systemic administration of pilocarpine. Note that significant pilocarpine-induced cell loss and degeneration is observed in the DBA/2J strain within the dentate hilus and area CA3. In contrast, no significant cell loss or degeneration was observed after systemic pilocarpine administration to BALB/cByJ mice. CA1 and CA3 denote the hippocampal subfields; H, dentate hilus. Scale bars = 750 μ m (A, E, I); 100 μ m (a-l).

Fig. 2.
Quantitative analysis of neuronal density in the dentate hilus following systemic administration of pilocarpine to eight inbred strains of young adult mice. Viable surviving neurons were estimated by cresyl violet staining. Bars denote the percentage of surviving neurons (as compared with saline-injected sham control C57BL/6J mice) in the dentate hilus. Differences in the extent of cell loss were observed 7 days following pilocarpine administration and were dependent on the mouse strains examined. Data represent the mean $\pm$ SEM of at least 5 mice/strain. Asterisk denotes a significant difference as compared to vehicle-injected sham control mice ($P < 0.05$).

Fig. 3. Quantitative analysis of neuronal density in area CA3 following pilocarpine administration to eight inbred mouse strains. Viable surviving neurons were estimated by cresyl violet staining. Bars denote the percentage of surviving neurons (as compared with saline-injected sham control C57BL/6J mice) in area CA3. A strain-dependent difference in cell loss in area CA3 was observed at 7 days following pilocarpine administration. Data represent the mean $\pm$ SEM of at least 5 mice/strain. Asterisk denotes a significant difference as compared to vehicle-injected sham control mice ($P < 0.05$).

Table 1

Disposition of mice following seizures induced by systemic administration of pilocarpine

Mouse strain	Initial # of mice	Seizure deaths	Final # mice	Mean dose (mg/kg)	Excluded due to inadequate seizure duration	Seizure mortality (%)
A/J	15	13	2	222 ± 20.9	0	87
AKR/J	17	13	1	392 ± 13.7	3	76
BALB/cByJ	20	10	5	273 ± 10.6	5	50
BALB/cJ	20	5	5	410 ± 14.8	10	25
CBA/J	21	9	7	210 ± 27.5	5	43
C3H/HeJ	10	0	5	375 ± 23.9	5	50
C57BL/6J	35	17	5	429 ± 7.1	13	49
DBA/2J	10	2	5	410 ± 16.3	3	20
FVB/NJ	20	9	6	393 ± 9.1	5	45
SLI/J	25	4	0	502 ± 19.6	21	16
129T2/SvJ	9	2	4	264 ± 22.1	3	33

Table 2

Seizure parameters

Mouse strain		Latency to onset of severe seizures (minutes)	Duration of severe seizures (minutes)	ED ₁₀₀ ^a (mg/kg) induction of S.E.
BALB/cByJ	(n=5)	27.40 ± 8.38	82.40 ± 21.43	250.00 ± 15.81 ^b
BALB/cJ	(n=5)	14.75 ± 4.09	68.25 ± 13.55	387.50 ± 23.94 ^c
CBA/J	(n=7)	27.86 ± 7.84	79.29 ± 14.43	207.14 ± 42.86 ^b
C3H/HeJ	(n=5)	23.00 ± 9.33	38.20 ± 12.41	360.00 ± 43.01 ^c
C57BL/6J	(n=5)	26.25 ± 3.75	59.00 ± 10.65	375.00 ± 43.30 ^c
DBA/2J	(n=5)	17.50 ± 3.79	53.25 ± 15.43	375.00 ± 14.43 ^c
FVB/NJ	(n=6)	15.00 ± 2.55	47.25 ± 6.85	350.00 ± 0.00 ^c
129T2/SvJ	(n=4)	29.00 ± 6.52	89.00 ± 9.25	256.25 ± 35.90 ^b

^aThe estimated dose necessary to induce status epilepticus in 100% (ED₁₀₀) of mice. No significant strain-dependent differences were noted with regard to latency or duration of severe (stage 4/5) seizures. Statistically significant strain-dependent differences in the ED₁₀₀ were found (F=4.488, P=0.002). Bars with the same letter superscripts are not significantly different from each other.