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Research report

Generation and characterization of pilocarpine-sensitive C57BL/6 mice as a model of temporal lobe epilepsy

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ABSTRACT

C57BL/6 (B6) is the most widely used inbred mouse strain, but its use in epilepsy research is compromised by low sensitivity to various convulsants, including pilocarpine. We recently identified a subline of B6NCrl mice in a barrier (#8) of a German vendor (Charles River) that was much more sensitive to status epilepticus (SE) induction than B6NCrl mice from four other barriers of the same vendor and other B6 substrains. Breeding experiments indicated that the observed differences have a genetic basis, thus offering a unique opportunity to identify the genes and pathways involved and contributing to a better understanding of the underlying molecular mechanisms of seizure susceptibility. Since the pilocarpine-sensitive B6 subline (B6NCrl#8) is not further available from the breeder, we decided to generate a new highly pilocarpine-sensitive B6NCrl subline by crossing female B6NCrl#8 mice with male F1 hybrids. Further sister–brother mating of the resulting F2 generation generated a highly susceptible F3 generation. Similar to B6NCrl#8 mice, mice from the F3 generation were significantly more susceptible to SE induction than any other B6 substrain, including B6J (JAX) mice, which were particularly insensitive to seizure induction. In contrast to the marked inter-subline differences in susceptibility to induction of SE, B6 sublines did not differ in the long-term consequences of SE, i.e., development of spontaneous seizures and neurodegeneration in the hippocampus, although hippocampal damage was much less severe than previously reported for other mouse strains. We have started to search for genetic loci underlying the high seizure susceptibility of B6NCrl#8 and filial generations obtained by cross-breeding with this B6 subline. Further characterization of the genetic variations underlying high susceptibility to convulsants such as pilocarpine will facilitate our understanding of the pathomechanisms involved in the evolution of single seizures to a self-sustained SE and provide new opportunities for interventions.

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1. Introduction

The pilocarpine model of temporal lobe epilepsy (TLE) has become the most widely used rodent model of this frequent and difficult-to-treat type of epilepsy [1,2]. In this model, the cholinomimetic convulsant is used to induce a status epilepticus (SE), followed by hippocampal damage and spontaneous recurrent seizures (SRS), resembling the characteristic features of TLE [1,2]. The pilocarpine model is extensively used to study mechanisms and therapy of SE as well as mechanisms and prevention of epileptogenesis and neurodegeneration induced by SE [3–6]. This model has initially been described in rats [7], but is increasingly used in mice, including the C57BL/6 (B6) inbred strain [2]. However, induction of SE by pilocarpine in the B6 strain is difficult and associated with high mortality [8–10], which is a major drawback when using this mouse strain in studies on the molecular mechanisms underlying SE and SE-induced epilepsy.

B6 mice are one of the oldest and most widely used inbred strains in biomedical research, and are commonly used as a genetic background to create transgenic and knockout mice [11]. B6 mice are generally considered to exhibit a low sensitivity to induction of seizures by various convulsant agents, including pilocarpine [9,12–17], which hampers the use of B6 mice and B6-based transgenic mice in epilepsy research. In most of these studies, JAX® mice from the Jackson Laboratory (Bad Harbor, ME, USA) were used. There continues to be an implicit assumption that B6

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate acid; B6, C57BL/6; NMDA, N-methyl-D-aspartate; SE, status epilepticus; SNP, single-nucleotide polymorphism; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy.

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substrains can be used interchangeably [18]. However, molecular genetic studies indicate simple sequence-length polymorphisms, single-nucleotide polymorphisms (SNPs), and copy-number variants among B6 substrains that may contribute to phenotypic differences [18,19]. Multiple branches of the B6 lineage arose in the early 1950s and have been maintained as separate breeding colonies since that time; two branches in particular are denoted as C57BL/6J ("J" for The Jackson Laboratory) and C57BL/6N ("N" for National Institutes of Health) [20]. Isolation and genetic drift of these colonies has resulted in the emergence of genetically distinct substrains [18,21]. Furthermore, B6J and B6N strains obtained from different breeders and even from different barriers of the same breeder may differ in their geno- and phenotypes [18,19,21,22].

We recently compared the sensitivity to SE induction by pilocarpine in three substrains (B6JOlaHsd; B6NHsd; B6NCrl) of B6 mice and five sublines of B6NCrl mice coming from different barrier rooms of the same vendor [22]. In B6NCrl from Barrier #8 (B6NCrl#8), administration of pilocarpine resulted in a high percentage of mice developing SE, but mortality was low, whereas the opposite was found in B6NCrl mice from four other barriers of the same vendor and other B6 substrains. This observation strongly indicated that a mutation in Barrier 8 animals caused the high sensitivity to SE induction, which was thus further assessed by crossing female B6NCrl#8 with pilocarpine-resistant male B6NCrl mice from another barrier (#9). Experiments in F1 hybrids indicated X-chromosome linked genetic variation as the cause of the observed phenotypic alterations in B6NCrl#8 mice [22].

Unfortunately, the interesting pilocarpine-sensitive B6NCrl#8 subline is not further available, because the respective barrier was closed by the vendor (Charles River) before we could purchase male B6NCrl#8 for breeding. We therefore decided to perform further breeding experiments by crossing female B6NCrl#8 mice with male F1 hybrids with the aim to fix the high pilocarpine-sensitivity of B6NCrl#8 mice. In line with our aim, further sister–brother mating of the resulting F2 generation resulted in a highly susceptible F3 generation, which is characterized in the present study. In addition to studying B6 substrain and subline differences in SE induction by pilocarpine, we examined the long-term consequences of SE, i.e., development of spontaneous seizures and neurodegeneration in the hippocampus.

2. Materials and methods

2.1. Animals

Male and female C57BL/6NCrl (B6NCrl) mice were obtained from two different barriers (#8 and #9) from Charles River (Sulzfeld, Germany) and designated B6NCrl#8 and B6NCrl#9. These animals were used for cross-breeding as described below. For comparing the pilocarpine sensitivity of B6NCrl#8 and B6NCrl#9 mice and the offspring resulting from cross-breeding with that of other B6 substrains, previously published data from the following substrains and sublines were used [22]: B6NCrl mice from three additional barriers (#4, 7, and 11) of Charles River, as well as C57BL/6JOlaHsd (B6JOlaHsd) and C57BL/6NHsd (B6NHsd) from Harlan (Harlan-Winkelmann; Borchen, Germany), respectively. All three substrains (B6NCrl, B6JOlaHsd, B6NHsd) originally descended from the same C57BL/6 breeding stock and were maintained at the Jackson Laboratory (JAX; Bar Harbor, Maine, USA), but subsequent history differed, leading to three distinct substrains [23]. (1) B6JOlaHsd: sent in 1974 from the Jackson Laboratory to Laboratory Animals Centre, Carshalton; in 1983 to OLAC (now Harlan UK); in 1997 to Harlan Nederland. (2) B6NHsd: sent in 1974 from the Jackson Laboratory to the National Institutes of Health (NIH), Bethesda, Maryland; Harlan Sprague Dawley, Inc. derived the strain from this breeding nucleus. (3) B6NCrl: sent in 1951 from the Jackson Laboratory to the NIH; in 1974 to Charles River Laboratories. For the present study, additional experiments with pilocarpine were performed in the original JAX® mice, i.e., C57BL/6J (B6J) mice, which were obtained via Charles River.

All substrains were purchased from either Harlan or Charles River at an age of about 5–6 weeks. After arrival, animals were housed in groups under controlled conditions (temperature: 23 ± 0.5 °C; humidity: 55 ± 5%), and a 12 h light–dark cycle with lights on at 6.00 a.m. and food and water ad libitum. The animals were allowed to adapt to the new environment for 1–3 weeks before starting the experiments. All experiments were performed between 8:00 a.m. and 1:30 p.m. to minimize variation due to circadian rhythms. All possible steps were taken to avoid animals' suffering at each stage of the experiment. The procedures used in this study had the approval of the Institutional Animal Care and Use Committee and were carried out in accordance with the European Council Directive of November 24th, 1986 (86/609/EEC).

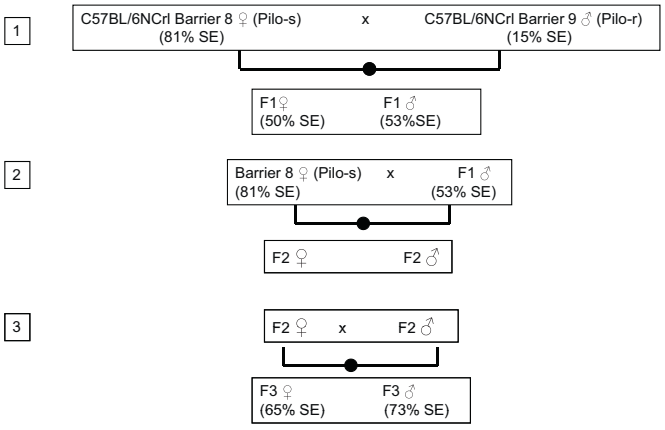

Fig. 1. Breeding scheme used for generation of the pilocarpine-sensitive C57BL/6NCrl F3 mice presented and characterized in this study. The female and male parental mice of barrier #8 and #9 are designated as pilocarpine-sensitive (pilo-s) and -resistant (pilo-r), respectively. The percentage of mice developing SE with the ramping-up dosing protocol used for pilocarpine administration (see Section 2) is shown in brackets for the parental and filial generations. Note that the F2 generation could not be tested with pilocarpine, because only one litter was available, which was needed for further breeding. For further explanation see text.

2.2. Mouse breeding

Based on the results of our experiments in different B6 strains (see Müller et al. [22], and Section 3), showing that B6NCrl mice from Barrier 8 were exceptionally sensitive to SE induction by pilocarpine, we assumed that a private mutation must have arisen in these mice. We therefore cross-bred female Barrier 8 and male Barrier 9 mice to produce F1 hybrids (Fig. 1), which were then tested at an age of 6–8 weeks with pilocarpine, using the same protocol as in the parental generation (see below). Our previous breeding studies with B6NCrl#8 and B6NCrl#9 mice had indicated X-chromosome linked genetic variation as the cause of the observed phenotypic differences between B6NCrl#8 and B6NCrl#9 mice [22]. Therefore, we performed further breeding studies as outlined in Fig. 1. The aim of these breeding studies was to generate pilocarpine-sensitive B6 mice with high susceptibility in both genders to the convulsant activity of pilocarpine, but low mortality, i.e., mice comparable to the pilocarpine-sensitive B6NCrl#8 subline. This aim became particularly important after Charles River closed down Barrier 8, and later Barrier 9, during the course of our experiments, so that pilocarpine-sensitive B6NCrl#8 mice are not further available from this vendor.

2.3. Induction of seizures by pilocarpine

For comparing the sensitivity of the different B6 substrains and sublines to the convulsant pilocarpine, we used a ramping-up dosing protocol that allows a more individual dosing of pilocarpine compared to injection of the same high dose in each animal per group, thereby reducing interindividual variability in SE induction and mortality [24]. In order to avoid peripheral cholinergic effects, methylscopolamine (1 mg/kg i.p.) was administered 30 min before the application of pilocarpine. For induction of seizures, 100 mg/kg pilocarpine were injected i.p. every 20 min until onset of SE. SE was defined as continuous limbic seizure activity, which was occasionally interrupted by clonic forelimb seizures, generalized clonic-tonic seizures or running and jumping seizures (see Section 3 for more detailed description), which typically lasted for several hours if not terminated earlier by diazepam (10 mg/kg i.p.). In B6 mice it usually took three injections to a first seizure (typically a score 4 or 5 seizure), but for development of SE it was important to continue the injections of pilocarpine after the occurrence of this first seizure, until SE started. Typically, 1–2 additional injections were needed after the first seizure to induce SE. If SE was not induced after 7–8 injections in an individual animal, usually additional injections failed to induce SE, but mice died in individual convulsive (tonic-clonic) seizures with running and jumping, due to respiratory arrest. Thus, the maximum number of repeated pilocarpine injections was restricted to about 10. In addition to recording the dose and latency to onset of SE, the dose and latency to the first seizure occurring before onset of SE were noted. Furthermore, all seizures occurring in mice that did not develop SE were recorded. Seizure severity was rated by the Racine scale [25]. All mice that developed SE received diazepam (10 mg/kg i.p.) after 90 min of SE to decrease mortality. Diazepam blocked any generalized seizure activity, but some mice still exhibited focal seizures. In case of mortality, mice usually

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