Epilepsy Research (2012) **102**, 153—159

journal homepage: www.elsevier.com/locate/epilepsyres

Factors affecting outcomes of pilocarpine treatment in a mouse model of temporal lobe epilepsy

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Received 4 April 2012; received in revised form 21 May 2012; accepted 28 May 2012
Available online 19 June 2012

KEYWORDS
Pilocarpine;
Mice;
Status epilepticus;
Sex;
Age;
Body weight

Summary Pilocarpine-treated mice are an increasingly used model of temporal lobe epilepsy. However, outcomes of treatment can be disappointing, because many mice die or fail to develop status epilepticus. To improve animal welfare and outcomes of future experiments we analyzed results of previous pilocarpine treatments to identify factors that correlate with development of status epilepticus and survival. All treatments were performed by one investigator with mice of the FVB background strain. Results from 2413 mice were evaluated for effects of sex, age, body weight, and latency between administration of atropine methyl bromide and pilocarpine. All parameters correlated with effects on outcomes. Best results were obtained from male mice, 6—7 weeks old, and 21—25 g, with pilocarpine administered 18—30 min after atropine methyl bromide. In that group only 23% failed to develop status epilepticus, and 64% developed status epilepticus and survived. Those results are substantially better than that of the total sample in which 31% failed to develop status epilepticus and only 34% developed status epilepticus and survived.

Introduction

Rodents that survive status epilepticus after systemic treatment with kainic acid or pilocarpine are among the most widely used models in epilepsy research (Cavalheiro et al., 2006). Mice are increasingly popular because of the abundant availability of transgenic and knockout animals. Kainate treatment in mice can be problematic, however, because some commonly used strains are resistant to kainate's normally excitotoxic (and epileptogenic) effects (Schauwecker and Steward, 1997), which is not the case for pilocarpine (Shibley and Smith, 2002; Schauwecker, 2012). Consequently, pilocarpine-treated mice have become an important model of temporal lobe epilepsy. In its most common application the model involves intraperitoneally administering a peripherally acting muscarinic acetylcholine receptor antagonist, such as methyl scopolamine or atropine methyl bromide, minutes before a single high-dose of pilocarpine (Turski et al., 1984). Pilocarpine's actions include activation of M1 receptors, which evokes status epilepticus

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http://dx.doi.org/10.1016/j.eplepsyres.2012.05.012

(Maslanski et al., 1994; Hamilton et al., 1997). After surviving status epilepticus, mice begin displaying spontaneous seizures days later, and their epileptic condition is permanent (Turski et al., 1989; Cavalheiro et al., 1996).

Treating mice with pilocarpine is difficult, however, because many animals fail to develop status epilepticus and many of those that do die acutely. Mortality rates for mice treated with pilocarpine range from 25% to 100% depending in part on dose (Turski et al., 1984; Maslanski et al., 1994; Cavalheiro et al., 1996; Winawer et al., 2007b). Respiratory failure following convulsions is a common cause of acute death after pilocarpine treatment (Boyd and Fulford, 1961). Lower pilocarpine doses are associated with lower mortality rates, but fewer mice develop status epilepticus (Shibley and Smith, 2002; Borges et al., 2003). If status epilepticus does not occur or is too short, epilepsy will not develop (Lemos and Cavalheiro, 1995).

To improve animal welfare and outcomes of future experiments we analyzed results of previous pilocarpine treatments to identify factors that correlate with development of status epilepticus and survival. We asked whether sex, age, body weight, and timing of administration of a peripherally acting muscarinic acetylcholine receptor antagonist relative to pilocarpine affected outcome of treatment.

Methods

All experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by a Stanford University Institutional Animal Care and Use Committee. All pilocarpine treatments were performed by the same investigator using GIN mice (FVB-Tg(GadGFP)4570Swn/J, The Jackson Laboratory, Bar Harbor, Maine) (Oliva et al., 2000). A colony of GIN mice was maintained by our laboratory for use in other experiments (Zhang et al., 2009; Halabisky et al., 2010; Buckmaster and Lew, 2011; Buckmaster and Wen, 2011; Lew and Buckmaster, 2011). The present study is a retrospective analysis of pilocarpine treatments performed for those experiments. Mice were bred in-house, lived in potentially mixed-sex sibling groups of up to 5 per cage, and had food and water available ad libitum. On the day of treatment, mice were weighed, sexed, and individually identified by tail bands made by marker pen. Female mice that appeared to be pregnant because of obvious abdominal distension were noted. Pilocarpine and atropine methyl bromide (Sigma–Aldrich, St. Louis, Missouri) were dissolved in bacteriostatic 0.9% sodium chloride solution to 50 and 1 mg/ml, respectively. Pilocarpine (300 mg/kg, i.p.) was administered 18 min or longer after atropine methyl bromide (5 mg/kg, i.p.), which began at 9:40 am ± 1 h (mean ± standard deviation, range 6:50 am till noon). Mice were behaviorally monitored continuously. Status epilepticus was recognized as persistent head nodding following stage 3 or greater seizures on the Racine (1972) scale. Some mice that failed to develop status epilepticus experienced one or more convulsive seizures but did not display continuous head nodding. Diazepam (10 mg/kg, i.p.) was administered 2 h after the onset of stage 3 or greater seizures and was repeated 1–6 h later if necessary to suppress convulsions. During recovery, mice received lactated ringers with 5% dextrose subcutaneously, and body temperature was maintained by placing cages on a heating pad. In the present study, survival/death refers only to the first 8 h following pilocarpine treatment. Results are reported as mean ± standard deviation. Statistics were performed using Sigma Stat (Systat, Chicago, Illinois) with $p < 0.05$ considered significant.

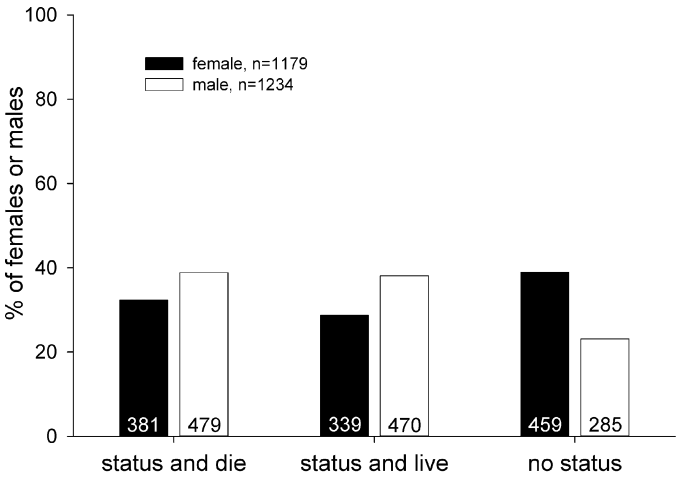

Figure 1 Percentage of male and female mice that developed status epilepticus and died or survived or failed to develop status epilepticus after pilocarpine treatment. Numbers from which percentages were calculated are indicated in bars. Male and female mice were significantly different ($p < 0.001$, Chi-square test).

Results

Results were obtained from 2413 mice that were treated in 32 batches (75 ± 23 mice/batch, range 14–129). For the entire sample, 744 (30.8%) failed to develop status epilepticus, 860 (35.6%) developed status epilepticus and died, and 809 (33.5%) developed status epilepticus and survived. Therefore, a total of 1669/2413 (69.2%) developed status epilepticus. The percentage of mice that developed status epilepticus and survived ranged widely from 11% to 72% per batch. All parameters (sex, age, body weight, and time between atropine and pilocarpine) correlated with differences in outcome ($p < 0.05$, Kruskal–Wallis one way ANOVA on ranks with Dunn's method). Therefore, each parameter was evaluated in detail.

Sex

Of 2413 mice, 1179 (49%) were female. Male mice were 1.3-times more likely to develop status epilepticus than females (Fig. 1). Of male mice, 77% (949/1234) developed status epilepticus, compared to only 61% (720/1179) of females ($p < 0.001$, Chi-square test). However, males and females that developed status epilepticus had similar probabilities of surviving (50%, 470/949 and 47%, 339/720, respectively). Consequently, male mice were 1.3-times more likely to develop status epilepticus and survive than females (38%, 470/1234 versus 29%, 339/1179, respectively, $p < 0.001$, Chi-square test).