Neuroscience 238 (2013) 11–18

LACK OF INFLUENCE OF PRION PROTEIN GENE EXPRESSION ON KAINATE-INDUCED SEIZURES IN MICE: STUDIES USING CONGENIC, COISOGENIC AND TRANSGENIC STRAINS

J. F. STRIEBEL, B. RACE, M. PATHMAJEYAN, A. RANGEL AND B. CHESEBRO *

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA

Abstract—Prion protein (PrP) is a glycosylphosphatidylinositol (GPI) anchored cell surface protein expressed by many cells, including those of the mammalian nervous system. At present the physiologic functions of PrP remain unclear. Deletion of Prnp, the gene encoding PrP in mice, has been shown to alter normal synaptic and electrophysiologic activities, indicating a potential role in seizure susceptibility. However, published efforts to link PrP with seizures, using both *in vivo* and *in vitro* models, are conflicting and difficult to interpret due to use of various mouse backgrounds and seizure induction techniques. Here we investigated the role of PrP in kainic acid (KA)-induced seizure sensitivity, using three types of mice. In contrast to previous published results, Prnp−/− mice on the C57BL/10SnJ background had a significant *decrease* in KA-induced seizure susceptibility. In genetic complementation experiments using a PrP-expressing transgene, genes derived from strain 129/Ola, which flanked the Prnp−/− locus in C57BL/10SnJ mice, rather than Prnp itself, appeared to account for this effect. Furthermore, using coisogenic 129/Ola mice differing only at Prnp, this difference was not reproduced when comparing PrP-negative and PrP-positive mice. In contrast, substrains of PrP-expressing C57BL mice, showed large variations in KA-induced seizure sensitivity. The magnitude of these differences in susceptibility was larger than that associated with the presence of the Prnp gene, suggesting extensive influence of genes other than Prnp on seizure sensitivity in this system. Published by Elsevier Ltd. on behalf of IBRO.

Key words: prion, kainic acid; seizures; Prnp; flanking genes; knockout mice.

INTRODUCTION

Prion protein (PrP) is expressed in most cell types in all mammalian species, and is known to be essential for susceptibility to prion diseases (also known as transmissible spongiform encephalopathies or TSE diseases) (Bueler et al., 1992). The normal physiological role for PrP remains unclear, though many studies point to a role in neuronal function (Collinge et al., 1994; Aguzzi et al., 2008; Llorens and Del Rio, 2012). PrP null (Prnp−/−) mice were previously found to be deficient in hippocampal spatial memory (Criado et al., 2005) and also showed reductions in paired pulse facilitation and long-term potentiation in the dentate gyrus (Collinge et al., 1994; Colling et al., 1996, 1997; Curtis et al., 2003; Criado et al., 2005). In addition, lack of PrP has been shown to affect normal physiology of the glutamatergic synapse (Khosravani et al., 2008; Pathmajeyan et al., 2011). Furthermore, deletion of PrP using a Cre-loxP mouse system resulted in reduction of afterhyperpolarization potentials, suggesting a direct role for PrP in the modulation of neuronal excitability (Mallucci et al., 2002). In summary, lack of PrP may have an influence on synaptic electrophysiology and/or epileptiform activity.

Seizure susceptibility of Prnp−/− mice has previously been studied independently by two groups which found Prnp−/− mice to be *more* susceptible than Prnp+/+ controls to kainic acid (KA)-induced seizures (Walz et al., 1999; Rangel et al., 2007). In contrast, using *ex vivo* hippocampal slices exposed to pentylenetetrazol, bicuculline or zero-magnesium conditions, tissue from Prnp−/− mice was *less* susceptible to induction of spontaneous epileptiform activity compared to tissue from Prnp+/+ controls (Ratte et al., 2011). Variations in protocols and/or the use of mice with different genetic backgrounds might explain these results. Indeed, susceptibility to induced seizures in mice is known to be influenced by multiple genes, many of which have not been identified but still might contribute to experimental studies of specific genes such as Prnp (Schauwecker, 2011).

Because of possible problems with control mice and Prnp−/− flanking genes in the above-mentioned *in vivo* studies, as well as the conflicts in the conclusions of the *in vivo* and *ex vivo* experiments, we decided to re-examine sensitivity to KA-induced seizures using more appropriate controls for possible variations between Prnp+/+ versus Prnp−/−mice. In the present work we used both congenic and coisogenic Prnp−/− mice versus Prnp+/+ control mice. In our initial experiments using congenic mice on the C57BL/10SnJ background, by three criteria Prnp−/− mice were less susceptible to KA-induced seizures, which conflicted

*Corresponding author. Tel: +1-406-363-9354; fax: +1-406-363-9286.
E-mail address: bchesebro@nih.gov (B. Chesebro).
Abbreviations: KA, kainic acid; i.p., intraperitoneally; PrP, prion protein; RML, Rocky Mountain Laboratories.

0306-4522/13 $36.00 Published by Elsevier Ltd. on behalf of IBRO.
http://dx.doi.org/10.1016/j.neuroscience.2013.02.004

with the published literature. To eliminate the effects of possible variations in genes flanking the Prnp−/− locus, we tested KA-induced seizure sensitivity in Prnp−/− and Prnp+/+ mice using coisogenic 129/Ola mice, but found minimal significant differences related to Prnp expression. We also used genetic complementation in a transgenic mouse line expressing the Prnp−/−genotype with or without a transgene (tga20) expressing PrP. Failure to rescue the Prnp+/+ phenotype by the PrP transgene demonstrated that the altered phenotype in C57BL/10SnJ Prnp−/−mice was not due to the lack of PrP expression, but rather was explained by the influence of non-Prnp genes flanking the Prnp−/− locus.

EXPERIMENTAL PROCEDURES

Animals

All mice were housed at the Rocky Mountain Laboratories (RML) in an AAALAC-accredited facility and experimentation followed NIH RML Animal Care and Use Committee approved protocols (NIH/RML Protocol #2010-10). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council. Mice were housed individually for at least 24 h prior to experimentation in a temperature-controlled environment (22 °C) under a 12-h light/dark cycle and allowed free access to food and water.

C57BL/10SnJ mice were originally obtained from Jackson Laboratories (Bar Harbor, Maine) and have been maintained at RML for several years as an inbred colony. C57BL/10J and C57BL/6J mice were obtained from Jackson Laboratories. C57BL/10Hsd mice were obtained from Harlan Sprague Dawley, Madison, WI.

C57BL10/SnJ-Prnp−/− mice were created at RML by serially backcrossing 129/Ola-Prnp−/− mice (Manson et al., 1994) to C57BL10/SnJ mice for nine generations (Chesebro et al., 2010). At each generation mice carrying the Prnp null allele were selected by PCR analysis (Chesebro et al., 2005) for further backcrossing. Following the ninth backcross mice hemizygous for the Prnp gene were interbred and homozygous Prnp null offspring were selected and bred to establish the current C57BL/10SnJ Prnp−/− line. Analysis of 1449 SNPs (Taconic Laboratories) showed that a 47.4-Mb region flanking the Prnp gene on chromosome 2 was still of the original 129/Ola strain.

Transgenic tga20 mice homozygous for the ‘‘half-genomic’’ PrP DNA transgene and for the Zürich version of the Prnp−/− gene (Fischer et al., 1996) were obtained from EMMA (Munich, Germany) on a mixed background of C57BL/6 and 129/S7. At RML these mice were backcrossed for five generations to C57BL/10SnJ-Prnp−/− mice (Chesebro et al., 2010), which were homozygous for the Prnp−/− gene developed in Edinburgh (Manson et al., 1994). At each backcross PCR was used to identify and select mice with the tga20 transgene and the Edinburgh-Prnp− allele. At the fourth and fifth backcrosses tga20 transgene-hemizygous (TgPrP+/−) and tga20 transgene-negative (TgPrP−/−) littermates were identified by PCR and used for seizure experiments at the appropriate age. These mice were homozygous for the Edinburgh Prnp−/− genotype and for the adjoining flanking genes from 129/Ola mice. Their background genes were 93.8–97.0% C57BL/10SnJ as determined by SNP analysis.

129/Ola Prnp−/− and 129/Ola Prnp+/+ mice (Manson et al., 1994) were obtained from Dr. Jean Manson at the Roslyn Institute, Edinburgh, UK and bred and maintained at RML. These mice are co-isogenic as they are genetically identical to the parental strain with the exception of a genetic change at a single locus (Prnp). Due to breeding difficulties the 129/Ola-Prnp−/− mice were bred to 129/Ola-Prnp+/+ mice to improve fertility and litter size. Hemizygous 129/Ola-Prnp+/− mice from this breeding scheme were then bred to each other and weanlings were genotyped by PCR (Chesebro et al., 2005) to select Prnp−/− and Prnp+/+ mice to be used for experimentation.

In order to assess the potential influence of gender, data were analyzed and presented separately for males and females. Because age is known to influence KA susceptibility in mice (McCord et al., 2008), Prnp+/+ and Prnp−/− adult mice of similar ages were compared.

Administration of KA and analysis of behavioral response

Naturally derived KA monohydrate, extracted from *Digenea simplex* (Sigma–Aldrich, K2389, Missouri) was dissolved in phosphate-buffered saline (pH 7.4) and administered intraperitoneally (i.p.) for induction of seizures. KA was stored at 4 °C protected from light, and KA solutions were prepared fresh on the day of each experiment. In the course of these studies, five different lots of KA were used. Each lot of KA was assessed by comparing seizure induction in mice of the same wild-type strain. No differences in seizure scores or doses required were noted, and results were pooled for the analyses presented.

Previous papers studying the effect of Prnp expression on sensitivity to KA-induced seizures used two different KA administration protocols. One group used a single i.p. injection protocol at a dose of 10 mg/kg (Walz et al., 1999), and the other group used an injection protocol with 4 i.p. doses of 8 mg/kg at 30-min intervals (Rangel et al., 2007). In our initial experiments with the single dose protocol at various doses, we had difficulties reproducibly inducing stage 5 seizures in mice without inducing a high percent of death. However, the multiple injection protocol consistently induced a high incidence of stage 5 seizures with a minimum number of fatalities. Importantly, the use of this multiple injection protocol allowed for a direct comparison of our results with those of the previous study where Prnp expression and genetic backgrounds appeared to be accurately controlled (Rangel et al., 2007). Thus all strains of mice received four doses of KA (1 mg/ml) at 8 mg/kg at times 0, 30, 60 and 90 min.

After the first KA injection at time = 0 min, mice were placed back in their original cages and observed for changes in behavior and/or seizures over a 240 min total observation period. Mice were removed for re-injection of KA at 30, 60, and 90 min, respectively. Mice were scored using a modified Racine scale behavior score (Racine, 1972; Walz et al., 1999; McLin and Steward, 2006). Stage 1: immobility, facial clonus, staring and panting; stage 2: head nodding, tail rigidity; stage 3: myoclonic jerks, forelimb clonus, hunchback posture; stage 4: discrete rearing and falling seizures, continuous forelimb clonus; stage 5: repetitive rearing/falling or running/bouncing seizures. For each 5-min interval for 48 intervals over 240 min of observation the highest seizure stage reached during the interval was recorded. Mice were euthanized if stage 5 was recorded for five consecutive time intervals. These guidelines were established in consultation with our Institutional Animal Care and Use Committee (IACUC). Observers were blind to mouse genotypes in all experiments. PBS control solution administered intraperitoneally to mice resulted in no detectable behavioral changes.

Data analysis

The analysis of the seizures induced by KA focused on seizure stages 3, 4 and 5, as these were the most clinically severe stages. Data were analyzed and presented in three ways: (1)

Download English Version:

https://daneshyari.com/en/article/6274970

Download Persian Version:

https://daneshyari.com/article/6274970

Daneshyari.com