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Research Report

5-HT_{2C} and $GABA_B$ receptors influence handling-induced convulsion severity in chromosome 4 congenic and DBA/2J background strain mice

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ABSTRACT

Progress towards elucidating the underlying genetic variation for susceptibility to complex central nervous system (CNS) hyperexcitability states has just begun. Genetic mapping analyses suggest that a gene(s) on mid-chromosome 4 has pleiotropic effects on multiple CNS hyperexcitability states in mice, including alcohol and barbiturate withdrawal and convulsions elicited by chemical and audiogenic stimuli. We recently identified *Mpdz* within this chromosomal region as a gene that influences alcohol and barbiturate withdrawal convulsions. *Mpdz* encodes the multi-PDZ domain protein (MPDZ). Currently, there is limited information available about the mechanism by which MPDZ influences drug withdrawal and/or other CNS hyperexcitability states, but may involve its interaction with 5-HT_{2C} and/or $GABA_B$ receptors. One of the most useful tools we have developed thus far is a congenic strain that possesses a segment of chromosome 4 from the C57BL/6J (donor) mouse strain superimposed on a genetic background that is >99% from the DBA/2J strain. The introduced segment spans the *Mpdz* gene. Here, we demonstrate that handling-induced convulsions are less severe in congenic *vs.* background strain mice in response to either a 5-HT_{2C} receptor antagonist (SB242084) or a $GABA_B$ receptor agonist (baclofen), but not a $GABA_A$ receptor channel blocker (pentylenetetrazol). These data suggest that allelic variation in *Mpdz*, or a linked gene, influences SB242084- and baclofen-enhanced convulsions. Our results are consistent with the hypothesis that *Mpdz*'s effects on CNS hyperexcitability, including alcohol and barbiturate withdrawal, involve MPDZ interaction with 5-HT_{2C} and/or $GABA_B$ receptors. However, additional genes reside within the congenic interval and may also influence CNS hyperexcitability.

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Abbreviations: QTL, quantitative trait loci; HIC, handling-induced convulsion
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1. Introduction

Individual risk for idiopathic epilepsy and other central nervous system (CNS) hyperexcitability phenotypes including predisposition to alcohol and drug withdrawal convulsions is regarded as strongly genetic (Annegers et al., 1996; Sander et al., 1997). Complex generalized seizures characterize at least half of idiopathic epilepsies (Annegers et al., 1996), and are also the most characteristic and severe type of seizure that occurs in alcohol and drug withdrawn individuals, as well as rodent models of alcohol and drug withdrawal (Rogawski, 2005). Over the past decade, a number of genes have been associated with rare monogenic idiopathic epilepsies that have relatively simple inheritance (Mulley et al., 2005), but progress towards elucidating the genetic variation underlying susceptibility to complex CNS hyperexcitability phenotypes has just begun.

Quantitative trait loci (QTLs) are chromosome sites containing a gene(s) at which allelic variation affects a complex (quantitative) trait. Animal models and QTL analyses provide powerful tools to dissect key sites of neuronal excitability and to identify the underlying genetic factors (Flint, 2003). QTL analyses in common inbred strains of mice have identified seizure susceptibility loci on mid-chromosome 4, including QTLs for convulsions elicited by chemical, electrical and audiogenic stimuli (Neumann and Collins, 1991; Martin et al., 1995; Ferraro et al., 1997) as well as alcohol and drug withdrawal convulsions elicited by handling (Buck et al., 1997, 1999; Fehr et al., 2002; Bergeson et al., 2003; Shirley et al., 2004). Thus, a gene or genes on mid-chromosome 4 may have pleiotropic effects on multiple seizure phenotypes. For drug withdrawal convulsions, based upon positional cloning using robust behavioral models as well as sequence and expression analyses, the most compelling gene in the critical 1.8 Mb chromosomal segment is *Mpdz* (Shirley et al., 2004). This gene and its human homolog encode the multi-PDZ domain protein (MPDZ, also called MUPP1) and show widespread expression in the brain (Ullmer et al., 1998; Simpson et al., 1999; Sitek et al., 2003). The MPDZ protein is thought to affect neuronal excitability by altering the rate or fidelity of signal transduction mediated by a protein(s) with which it interacts, similar to other members of the PDZ domain protein family (Tsunoda et al., 1997; Fanning and Anderson, 1999; Sheng and Sala, 2001). Plausible mechanisms involve MPDZ's interaction with 5-HT_{2C} and/or $GABA_B$ receptors (Becamel et al., 2001; Balasubramanian et al., 2007), both of which influence neuronal excitability and are important therapeutic targets for epilepsy as well as alcohol and drug abuse (Prather et al., 1991; Semenova and Ticku 1992; Lal et al., 1993; Rezazadeh et al., 1993; Bettler et al., 1998; Gatch et al., 2000; Bowery, 2006).

One of the most useful tools we have developed thus far is a congenic strain that possesses a segment of chromosome 4 from the C57BL/6J (B6) donor strain superimposed on a genetic background estimated to be >99% from the DBA/2J (D2) mouse strain (Fehr et al., 2002). Through the elimination of genetic "noise" from additional QTLs on other chromosomes, comparisons of congenic and background strain mice are invaluable to investigate the gene(s) and mechanism(s) underlying a QTL's behavioral effects in a relatively isolated context. Previously, we reported that this congenic strain exhibits significantly less severe alcohol withdrawal CNS hyperexcitability than background strain mice (Fehr et al., 2002). Here, we compare the chromosome 4 congenic and background strains for their susceptibilities to CNS hyperexcitability in response to three chemiconvulsants with well-defined sights of action: SB242084, a selective 5-HT_{2C} receptor antagonist (Kennett et al., 1997); baclofen, a selective $GABA_B$ receptor agonist (Blake et al., 1993); and pentylenetetrazol (PTZ), which exerts its convulsant effects by impairment of $GABA_A$-mediated neurotransmission (Corda et al., 1991; Kulkarni and George, 1995). As in previous work from our laboratory, we use the handling-induced convulsion (HIC) as a sensitive measure of CNS hyperexcitability because it is a well-established index to assess genetic differences in chemiconvulsant sensitivity and severity of alcohol and drug withdrawal (Goldstein, 1973; Crabbe et al., 1980, 1991a, 1993). Although HICs can be exhibited by naïve mice, and their severity differs among genotypes (Crabbe et al., 1980), they are also increased and decreased in severity by convulsant and anticonvulsant drugs, respectively (Crabbe et al., 1991a). D2 and B6 strain mice do not differ in brain PTZ levels (Kosobud et al., 1992), but the impact of potential strain differences in pharmacokinetics for many other chemiconvulsants is not known. For this reason, we measured plasma baclofen levels in both chromosome 4 congenic and D2 background strain mice in order to assess whether potential strain differences in drug metabolism may influence the results observed.

The present studies implicate a potential role for allelic variation in *Mpdz*, or a linked gene, on SB242084 and baclofen-enhanced HICs, but not PTZ-enhanced HICs. We discuss potential roles of 5-HT_{2C} and/or $GABA_B$ receptors in mediating *Mpdz*'s influence on alcohol and drug withdrawal and other CNS hyperexcitability phenotypes influenced by a QTL on chromosome 4.

2. Results

2.1. *Baseline handling-induced convulsions (HICs)*

Individual mice and different strains can vary in baseline (predrug) HIC scores. No significant main effect of sex ($F_{[1,190]}=0.4$, $p=0.9$) or sex by strain interaction ($F_{[1,190]}=1.8$, $p=0.2$) was observed in baseline scores. However, after collapsing the data across both sexes for all of the mice tested, we observed a small but significant difference between chromosome 4 congenic and background strain mice in average baseline HIC scores (mean ± SEM = 0.18 ± 0.06 and 0.51 ± 0.08, $n=97$ per genotype, respectively; $p<0.05$), with both strains showing extremely low baseline HIC scores. This is consistent with previous data on baseline HIC scores in chromosome 4 congenic and background strain mice (Shirley et al., 2004). Although small, this difference is significant. In order to create an index of chemiconvulsant response that is independent of individual differences in baseline HIC scores and reflects the genotype-dependent difference in convulsion severity, all post-chemiconvulsant HIC scores were corrected for the individual's baseline score.

2.2. *Chemiconvulsant-enhanced HICs*

We compared chromosome 4 congenic and D2 background strain mice for severity of HICs enhanced by three chemiconvulsants