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Absence seizures in succinic semialdehyde dehydrogenase deficient mice: a model of juvenile absence epilepsy

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Abstract

The succinic semialdehyde dehydrogenase (SSADH) null mouse represents a viable animal model for human SSADH deficiency and is characterized by markedly elevated levels of both γ-hydroxybutyric acid (GHB) and γ-aminobutyric acid (GABA) in brain, blood, and urine. GHB is known to induce absence-like seizures and absence seizures have been reported to occur in children with SSADH deficiency. We tested the hypothesis that the phenotype of the SSADH$^{-/-}$ mouse shows absence-like seizures because of the inordinately high levels of GHB in the brain of this mutant animal. Sequential electrocorticographic (ECoG) and prolonged video ECoG recordings from chronically implanted electrodes were done on SSADH$^{-/-}$, SSADH$^{+/-}$, and SSADH$^{+/+}$ mice from postnatal day (P) 10 to (P) 21. Spontaneous, recurrent absence-like seizures appeared in the SSADH$^{-/-}$ during the second week of life and evolved into generalized convulsive seizures late in the third week of life that were associated with an explosive onset of status epilepticus which was lethal. The seizures in SSADH null mice were consistent with typical absence seizures in rodent with 7 Hz spike-and-wave discharge (SWD) recorded from thalamocortical circuitry, the onset/offset of which was time-locked with ictal behavior characterized by facial myoclonus, vibrissal twitching and frozen immobility. The absence seizures became progressively more severe from P14 to 18 at which time they evolved into myoclonic and generalized convulsive seizures that progressed into a lethal status epilepticus. The absence seizures in SSADH$^{-/-}$ were abolished by ethosuximide (ETX) and the $GABA_BR$ antagonist CGP 35348. The seizure phenotype in the SSADH$^{-/-}$ recapitulates that observed in human SSADH deficiency. Hence, SSADH$^{-/-}$ may be used to investigate the molecular mechanisms that underpin the pathogenesis of absence and generalized tonic-clonic seizures associated with SSADH deficiency. As well, the SSADH$^{-/-}$ may represent a unique animal model of the transition from absence to myoclonic and generalized convulsive seizures that is observed in up to 80% of patients with juvenile absence epilepsy.

Keywords: SSADH deficiency; γ-Hydroxybutyric acid (GHB); γ-Aminobutyric acid (GABA); Brain; Absence-like seizures; ECoG; Generalized convulsive seizures; Status epilepticus

1. Introduction

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), is derived from L-glutamate by glutamic acid decarboxylation (Tillakaratne et al., 1995). GABA is converted to succinic semialdehyde by GABA transaminase. The succinic semialdehyde either can be converted to succinic acid by succinic semialdehyde dehydrogenase (SSADH) or to γ-hydroxybutyric acid (GHB) by the enzyme succinic semialdehyde reductase (Gold and Roth, 1977; Maitre, 1997; Kelly et al., 2002). SSADH deficiency is a rare autosomal recessive disorder that is characterized by markedly elevated levels of GHB and GABA in brain, blood, and urine (Gibson et al., 1983; Gibson and Jakobs, 2001). The clinical phenotype of children with SSADH deficiency is manifested by nonspecific psychomotor delay associated with

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hypotonia, ataxia, absence seizures, and generalized tonic-clonic seizures (Gibson et al., 1997; Pearl et al., 2003a,b). The SSADH null mouse mirrors human SSADH deficiency. The mutant animal shows markedly elevated levels of both GABA and GHB in urine and homogenates of liver and brain, and has a phenotype that is characterized by ataxia and seizures that occur in the third postnatal week of life with death, apparently from prolonged seizure activity, by postnatal day (P) 22 (Hogema et al., 2001; Gibson et al., 2002; Gupta et al., 2002).

Children with SSADH deficiency have a multiplicity of seizures, including absence seizures (Gibson et al., 1997; Pearl et al., 2003a,b). When given to animals, GHB is known to induce absence-like seizures (Snead, 1988; 2002). Therefore, we tested the hypothesis that the elevated levels of GHB in the brain of SSADH$^{-/-}$ mouse induce a phenotype that is consistent with typical absence seizures. In addition, we sought to provide electrographic and behavioral characterization of the developmental evolution of seizures to a lethal status epilepticus in these mutant animals.

2. Material and methods

2.1. Animals

The SSADH null mice with C57/129Sv background were first generated in the Oregon Health and Science University in Portland (Hogema et al., 2001). Five pairs of heterozygous (+/−) mice were transported to the Hospital for Sick Children in Toronto. The mutant mouse line was maintained by inbreeding those heterozygous (+/−) mice. The absence of SSADH in the SSADH$^{-/-}$ was confirmed by genotypic analysis of the mutants, as previously described (Hogema et al., 2001). The mice were housed in a pathogen-free facility. All animals were maintained in a controlled environment at 12 h light–12 h dark cycle with lights on at 06:00 h and given ad lib access to food and water.

2.2. Drugs

CGP 35448 was a gift from Novartis (Basel, Switzerland); γ-butyrolactone (GBL) was obtained from Sigma (St. Louis, MO). All other chemicals were obtained from standard commercial sources and were of the highest available purification.

2.3. Surgery and electrocorticography (ECoG)

A total of 96 pups were divided into three groups consisting of SSADH$^{-/-}$, heterozygotes (+/−) and (+/+) control littermates. Groups of SSADH (−/−), (+/−) and (+/+) developing mice were chronically implanted with four epidural 1 mm monopolar electrodes with their tips placed bilaterally at frontal and parietal cortices under halothane anesthesia. The electrodes were placed 1 mm deep, 2.20 mm anterior to bregma and 3 mm lateral from midline. All coordinates were measured in mm with skull surface flat and bregma 0.0 (Franklin and Paxinos, 1997). This surgical electrode placement lasted approximately for 10 min in duration in all developing mice from P10 to P20. After surgery, all animals were returned to the animal facility for 4 days of recovery with the exception of the developing animals from P10 to P14 that had acute electrocorticographic (ECoG) recordings 1 to 2 h after recovery from halothane anesthesia.

Each animal was placed in individual, warm Plexiglas chambers for a 20-min adaptation period prior to ECoG recordings in order to minimize movement artifact. For those studies in which SWD duration was measured, ECoG recordings were made on paper using a Grass Polysomnograph machine (Depaulis et al., 1989). Behavior was captured on an artifact free EEG-video recording system using a 1 to 5 contact head connector with FET preamplifiers and batteries, a signal conditioning device (5000×, 1–100 Hz filters, Axon Instruments), an A/D converter (MP100 Biopac) and video/PC–PC video computer boards for EEG and image data (Cortez et al., 2001). All baseline and test recordings were performed from 10:00 to 14:00 h to minimize circadian variations (Loscher and Fiedler, 1996).

2.4. Pharmacological characterization of seizures in SSADH null mice

The presence or absence of seizures was ascertained in all (−/−), (+/−) and (+/+) animals at baseline over 1-h ECoG recordings (see Results). Absences seizures were scored only if two channels showed bilaterally synchronous 5–7 Hz spike-and-wave discharges (SWD), associated with a frozen behavior and vibrissal twitching lasting at leas 1 s in duration. All drugs were administered intraperitoneally (i.p.) at 1 μl/g of body weight and were given only after the completion of the recovery period from surgery, and baseline ECoG recordings. The experiments were conducted from P10 to P21. All drugs were given i.p. after 1 h of baseline ECoG recordings. All experiments were performed in drug naïve mutant, heterozygous and wild-type controls. Paired vehicle controls were used in all experiments to test the effect of the anti-absence drug ethosuximide (ETX; 100 mg/kg) (Cortez et al., 2001), the $GABA_BR$ antagonist CGP 35348 (100 mg/kg), the $GABA_BR$ agonist baclofen (*p*-chlorophenyl GABA; 5 mg/kg) (Snead, 1996).

2.5. $GABA_AR$- vs. $GABA_BR$-mediated mechanisms of absence seizure induction in SSADH$^{-/-}$

Pharmacologically, absence seizures can be induced in rodents by the administration of either GHB, which exerts this effect by both GHB-R and $GABA_BR$-mediated mechanisms (Gervasi et al., 2003; Wong et al., 2004) or by low doses of $GABA_AR$ antagonists that induce absence seizures by antagonism of GABAergic inhibition within

thalamocortical circuitry (Snead et al., 2000). In order to determine whether either or both of these mechanisms of absence are up-regulated, and therefore may play a role in the genesis of the absence seizures observed in SSADH$^{-/-}$, the susceptibility of SSADH$^{-/-}$ and $^{+/+}$ to these two pro-absence drugs was determined. γ-Butyrolactone (GBL) was given in a dose of 100 mg/kg (Snead et al., 1999; Snead, 2002). GBL has been shown to be biologically inactive, being converted rapidly and irreversibly to its active metabolite, GHB, by a circulating lactonase (Snead, 1991). The resultant GBL-derived GHB induces absence seizures by GHB receptor- and $GABA_B$ receptor-mediated mechanisms (Gervasi et al., 2003; Wong et al., 2004). As well, the threshold of SSDAH$^{-/-}$ and SSADH$^{+/+}$ to absence seizures induced by low doses of pentylenetetrazol, a $GABA_A$ receptor antagonist (Snead, 1988; Snead et al., 2000), was determined. All animals were drug naïve at the time of the experiments.

2.6. ECoG data analyses

Absence-like seizures were scored based on behavior and ECoG correlate of SWD in the SSADH (−/−), (+/−) and (+/+) mice. SWD were quantitated by measuring duration from onset to offset of each burst and SWD duration for consecutive 20-min epochs over a 1-h recording period (N=36) (Depaulis et al., 1989). SWD was scored in each animal only if two frontal and parietal electrode derivations demonstrated the distinct 7 Hz SWD morphology that typifies rodent absence seizures (Snead et al., 1999) with an amplitude four times the baseline (Cortez et al., 2001). The generalized tonic-clonic seizures in SSADH mutants were analyzed using continuous ECoG and VEGG monitoring.

All data were expressed as an arithmetic mean± standard error of mean (S.E.M.), from eight animals for all seizure model experiments. Simultaneous comparisons of two group means were performed and were analyzed by two-tailed Student's t-test. In the ontogeny studies, timing of onset for SWD was taken as a function of age. Analysis of variance (ANOVA) for repeated measures was used to quantify the amplitude differences between ECoG baseline and SWD as a function of drug and time post injection with a probability (p) value of $p<0.05$ chosen as an index of statistical significance.

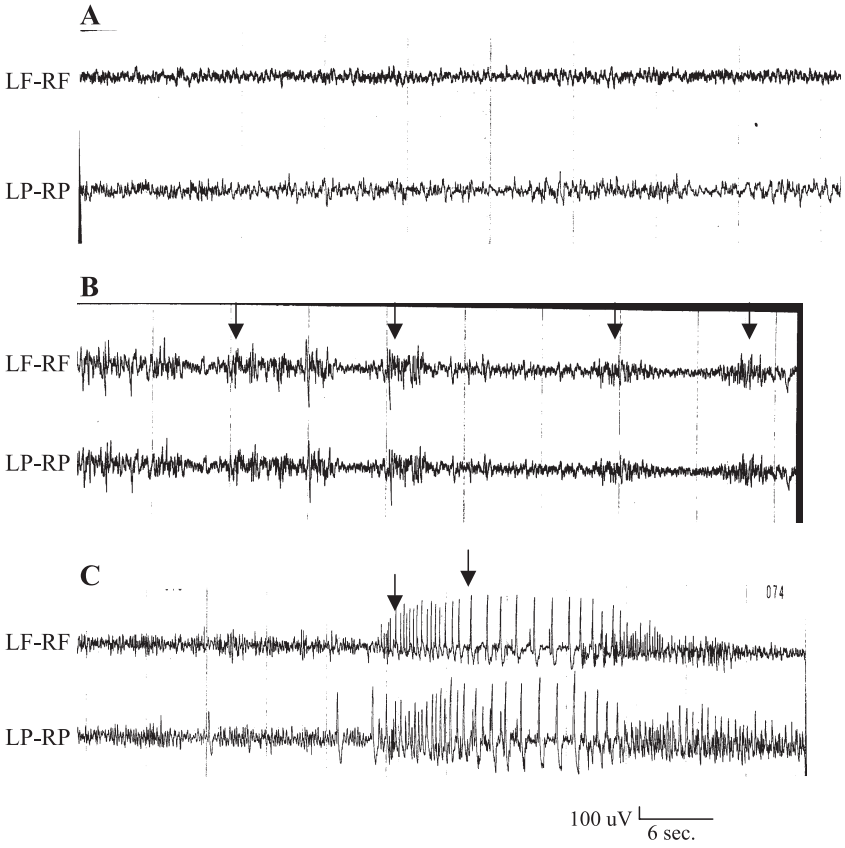

Fig. 1. (A) Baseline electrocorticogram (ECoG) recordings in a P16 wild-type (+/+) control mice (n=8) showed an uneventful baseline 35–50 μV and 5–7 Hz oscillations. (B) ECoG of P16 succinic semialdehyde dehydrogenase (SSADH) null mice (−/−) (n=8) disclosed 250–300 μV, 5–7 Hz spike-and-wave discharges (SWD) lasting 3–6 s duration, associated with frozen stare and vibrissal twitching. Spontaneous intermittent burst indicated by the arrows was recorded from P14 onwards. (C) ECoG recording in P20 SSADH null mice showed a transition from absence to a sustained rhythmic onset of generalized 600 μV at 5 Hz followed by 1.5 to 2 Hz SWD associated with tonic-clonic seizures (arrow). Generalized tonic-clonic seizures were abrupt in occurrence and lead to mortality in SSADH null mice. LF=left frontal; RF=right frontal; LP=left parietal; RP=right parietal.

3. Results

3.1. ECoG and behavioral characteristics of SSADH null mice

The baseline ECoG in wild-type mice consisted of an irregular 35 to 50 μV 4 to 7 Hz during over the developmental time period examined (Fig. 1A). The baseline ECoG in SSADH$^{+/-}$ was of similar amplitude, but with intermingled slow activity at 3 to 5 Hz (data not shown). No electrical or behavioral epileptiform events were observed in either SSADH$^{+/+}$ or SSADH$^{+/-}$ animals. The baseline ECoG in SSADH$^{-/-}$ also demonstrated excess slow activity at 3–5 Hz compared to SSADH$^{+/+}$. However, SSADH$^{-/-}$ deficient mice showed intermittent bursts of spontaneous, recurrent SWD associated with brief absence-like seizures that emerged at P14. (Fig. 1B). There was an apparent lower baseline SWD duration in the last 20-min epoch due to the appearance of high voltage slow cortical potentials consistent with sleep behavior. No alerting stimulation was performed to avoid eliciting other seizure types, such as tonic-clonic seizures. There was a three- to four-fold higher amplitude in every SWD at 7 Hz compared to baseline. The onset/offset of the spontaneous, recurrent SWD in the SSADH$^{-/-}$ was time-locked with that of the associated absence-like ictal behavior which consisted of frozen immobility, facial myoclonus, and vibrissal twitching. The absence seizures increased in frequency and duration from

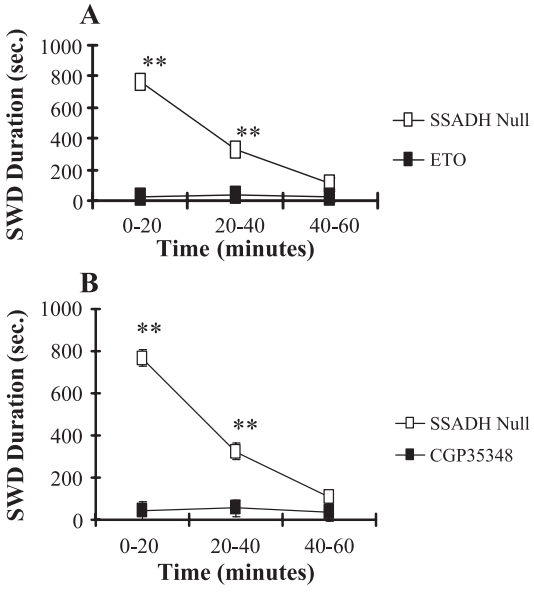

Fig. 2. (A) SWD duration measured in seconds/20-min epochs for 1-h period of time for SSADH null (−/−) prior (upper panel) and after ethosuximide (ETX), 100 mg/kg (lower panel). ETX suppressed the SWD in SSADH null mice (**p<0.005), Student's t-test. (B) SWD duration measured in seconds/20-min epochs for 1-h period of time for SSADH null (−/−) prior (upper panel) and after CGP35348 (CGP), 100 mg/kg (lower panel). CGP suppressed the SWD in SSADH null mice (**p<0.005), Student's t-test. Each data point represents means+S.E.M., N=8 for both treatment groups.

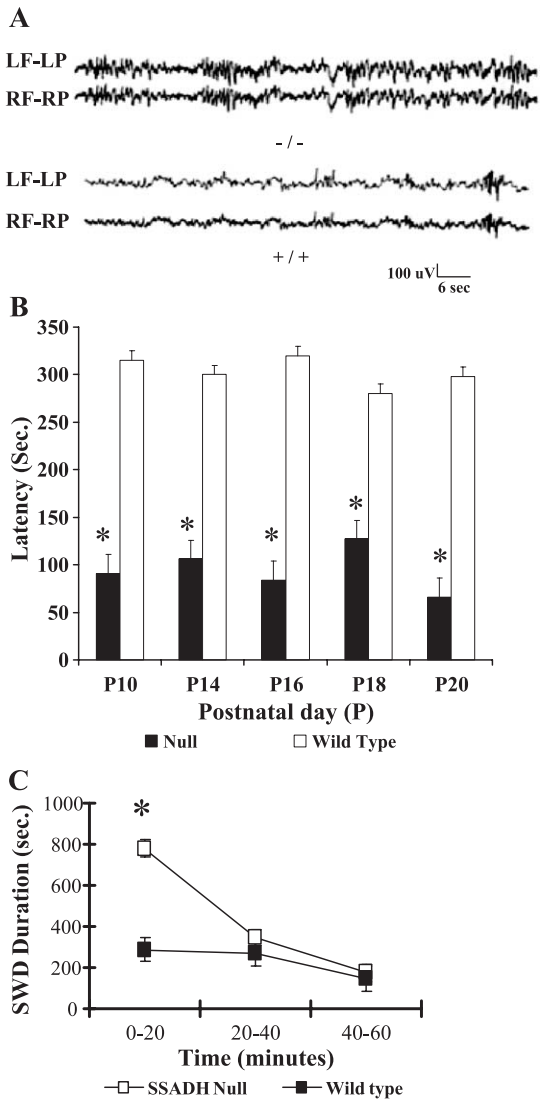

Fig. 3. (A) Gamma-hydroxybutyrate (GHB) induced spike-and-wave discharges (SWD) in succinic semialdehyde dehydrogenase (SSADH) null mice (−/−) (n=8) (upper panel) following administration of 100 mg/kg γ-butyrolactone (GBL) were characterized by a rapid onset and evolution from intermittent medium amplitude (SWD) at 7 to 9 Hz to a burst suppression pattern, associated with frozen stare, vibrissal twitching and facial myoclonus, compared to the regular GHB induced SWD progression in wild-type (+/+) mice (n=8) (lower panel). (B) The latency to onset of absence seizures in succinic semialdehyde dehydrogenase (SSADH) null mice and their wild-type controls, after systemic administration of GBL (100 mg/kg; i.p.) from postnatal day (P) 10 to 20. SSADH null mice were significantly more susceptible than controls (*p<0.05). Data are expressed as mean± S.E.M., N=96, n(−/−)=48, n(+/+)=48. The comparison between SSADH null mice and wild-type ECoG recordings starting at P10 [n(−/−)=8] [n(+/+)=8]; P12 [n(−/−)=8] [n(+/+)=8]; P14 [n(−/−)=8] [n(+/+)=8]; P16 [n(−/−)=8] [n(+/+)=8]; P18 [n(−/−)=8] [n(+/+)=8]; P20 [n(−/−)=8] [n(+/+)=8]. Latency to GHB seizure onset was shorter in SSADH null mice at each age point during development. (C) Total SWD duration in succinic semialdehyde dehydrogenase (SSADH) null mice and wild-type controls, after systemic administration of GBL (100 mg/kg; i.p.) at postnatal day (P) 18. SSADH null mice were more susceptible than (+/+) group at postnatal day (P) 18 of maturation, (*p<0.05), Student's t-test. Data are expressed as mean±S.E.M.; n(−/−)=8, n(+/+)=8, for each postnatal including P18 (n=8) and 20 (n=8), both groups with high mortality rate. (Abbreviations: LF=left frontal; RF=right frontal; LP=left parietal; RP=right parietal.)

P14 to P20. At P18–20, the bilaterally synchronous SWD and associated absence seizure activity began to evolve into sporadic and abrupt myoclonic jerking movements that quickly evolved into generalized tonic-clonic seizures (Fig. 1C). Soon after the appearance of the generalized convulsive seizures, the SSADH$^{-/-}$ went into a status epilepticus from which they did not recover. The mean age of death for the SSADH$^{-/-}$ that developed status epilepticus was 23±3 days (N=8). During the status epilepticus in the SSADH$^{-/-}$, the ECoG was characterized by generalized fast waves that evolved into 550–600 μV generalized SWD (tonic phase) that gradually decreased in amplitude within 1 s and were followed by intermittent high amplitude spikes followed by slow waves and generalized suppression of cortical activity (clonic phase) that persisted following the seizure.

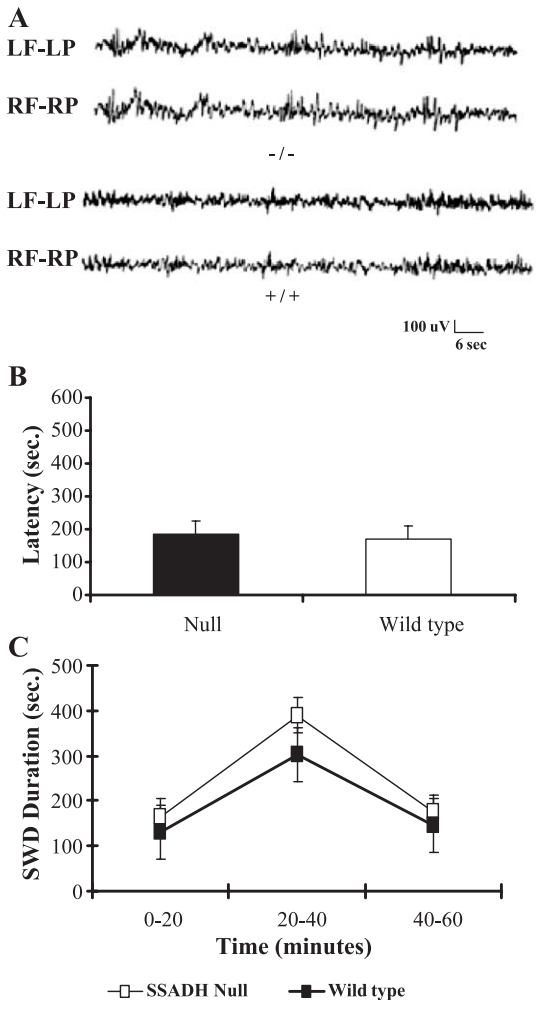

Fig. 4. (A) Pentylenetetrazol (PTZ) induced spike-and-wave discharges (SWD) in succinic semialdehyde dehydrogenase (SSADH) null mice (−/−) (n=4) (upper panel) were characterized by intermittent medium amplitude (SWD) at 7 to 9 Hz, associated with frozen stare, vibrissal twitching and facial myoclonus in both wild-type (+/+) and SSADH knockout (−/−) mice (n=4) (lower panel). (B) The latency to onset of absence seizures in succinic semialdehyde dehydrogenase (SSADH) null mice and their wild-type controls, after systemic administration of PTZ (30 mg/kg; i.p.). The SSADH null mice were not more susceptible than controls (p<0.40). (C) SWD duration measured in seconds/20-min epochs for 1-h period of time for both SSADH null (−/−) and wild-type (+/+). SWD was not different in SSADH null mice compared to wild-type controls (p<0.50). Each data point represents means±S.E.M., N=4. (Abbreviations: LF=left frontal; RF=right frontal; LP=left parietal; RP=right parietal.)

3.2. Pharmacology of seizures in SSADH null mice

The absence seizures in the SSDAH$^{-/-}$ mice were abolished by pretreatment with either the anti-absence drug, ethosuximide (Fig. 2A), or the GABA$_B$R antagonist CGP 35348 (Fig. 2B). The seizure control achieved within the first 20 min with ETX and CGP 35348 lasted during the respective half-life of these drugs, but spontaneous SWD and ictal absence behavior recurred in the SSADH null mice as the drugs were eliminated. The absence seizures in the SSDAH$^{-/-}$ were exacerbated by the GABA$_B$R agonist, (−)-baclofen, another pharmacological characteristic of experimental absence seizures (Snead, 1996).

3.3. GABA$_A$R- vs. GABA$_B$R-mediated mechanisms of absence seizure induction in SSADH$^{-/-}$

There was a marked exacerbation of GHB-induced absence seizures in SSADH$^{-/-}$ vs. wild-type control mice. This potentiation of GHB-induced absence seizures was observed at P10, 14, 16, 18 and 20 (Fig. 3A). The latency to GHB-induced absence seizure onset was significantly shorter in SSADH null mice from P10 to 20 (*p<0.05, One-way ANOVA) (Fig. 3B) and this was most significant at P18 (p<0.005, Student's t-test). The total GHB-induced SWD duration was significantly greater in SSADH null mice than in wild-type controls (Fig. 3C).

In contrast to the GBL experiments, there was no significant difference observed between wild-type control animals and SSADH$^{-/-}$ in the experimental absence seizures induced by low doses (30 mg/kg) of the GABA$_A$R antagonist PTZ (Snead, 1988; Snead et al., 2000) (Fig. 4A). There was no significant difference between any of the PTZ-treated groups in latency (Fig. 4B) or total SWD duration (Fig. 4C).

4. Discussion

These data demonstrate that SSADH null mice manifest typical rodent absence epilepsy (Snead et al., 1999) with spontaneous, recurrent SWD at a frequency of 5–7 Hz that are time-locked in onset and offset with ictal behaviors that included sudden immobility, vibrissal twitching, and facial myoclonus. The spontaneous, recurrent absence seizures in the mutant mice had the classical pharmacological profile of experimental and human absence seizures, being abolished by ETX and GABA$_B$R antagonist and exacerbated by a GABA$_B$R agonist. The spontaneous, recurrent absence seizures, i.e. absence epilepsy, appeared in the SSDAH$^{-/-}$

mutant mice at P14 and were persistent until around P18–20 when the absence seizures were superceded by the occurrence of myoclonic seizures that evolved rapidly into generalized convulsive seizures that culminated in status epilepticus that often was lethal by P23.

The SSADH$^{-/-}$ manifests absence seizures in the second and third week of life, likely due to the markedly elevated levels of GHB and GABA, both of which are sufficient to induce absence-like seizures (Snead et al., 1999; Snead, 2002; Wong et al., 2003). The marked excess of GABA in the SSDH$^{-/-}$ brain would be predicted to act both via $GABA_AR$- (Wong and Snead, 2001), and $GABA_BR$-mediated (Snead et al., 1999; Crunelli and Leresche, 2002) mechanisms to induce absence seizures. Similarly, the elevated GHB in brain of SSADH$^{-/-}$ would be predicted to have $GABA_BR$ agonist effects (Bernasconi et al., 2002; Gervasi et al., 2003) as well as stimulate GHB-mediated activity in the brain (Maitre, 1997; Snead, 2000). GHB had little or no effect on the $GABA_AR$ (Benavides et al., 1982; Snead and Liu, 1993); however recently, it has been shown that GHB has a relevant effect on GABAA receptors via GABAB receptors (Gervasi et al., 2003).

Although the seizure model data would suggest that $GABA_AR$-mediated mechanisms may not be operative in the absence seizures observed from P14 to 18 in the SSADH$^{-/-}$, $GABA_AR$-mediated activity could be involved in the transition from absence seizures to myoclonic and generalized convulsive seizures in SSDH$^{-/-}$ from P18 to 20. It is conceivable that the mechanism by which the absence seizures evolve into generalized convulsive seizures might involve a breakdown in GABA-mediated inhibition in the SSDH$^{-/-}$ mice due to a use-dependent down-regulation of $GABA_BR$ and $GABA_AR$ secondary to very high levels of GHB and GABA (Buzzi et al., 2003).

The SWD and behavior observed in the SSADH$^{-/-}$ from age P14 to 18 were identical for that reported for typical absence seizures in other rodent models of absence (Snead et al., 1999). The pharmacology of the absence epilepsy in the SSADH$^{-/-}$ also comported with the pharmacology of typical absence seizures in both human absence epilepsy and experimental absence seizures. ETX treatment was particularly efficacious to suppress SWD in the SSADH null mice which indicated that the Ca^{2+} channel modulating mechanism involved in absence seizures was intact in the SSADH null mice.

The EEG-behavioral data in the SSDAH$^{-/-}$ mice are consistent with clinical observations in human SSADH deficiency. There is a 50% incidence of seizures in children with SSADH deficiency. The epilepsy that occurs in this disorder is characterized by absence, myoclonic, and convulsive seizures, as well as convulsive status epilepticus (Gibson et al., 1997; Pearl et al., 2003a,b), a seizure phenotype similar to that documented in the SSADH$^{-/-}$ in the current experiments. The EEG findings in human SSADH deficiency include diffuse and frontal background slowing, generalized spike–wave discharges with significant activation during sleep, and focal spike discharges in central/temporal areas (Gibson et al., 1997). These EEG changes in the clinical condition also are quite similar to the abnormal EEG background rhythms and epileptiform discharges observed in the SSADH$^{-/-}$ in the current experiments. Photosensitive epilepsy with myoclonic seizures have been reported in a patient heterozygous for SSADH deficiency (Dervent et al., 2004); however, we observed no epileptiform activity, either electrographically or behaviorally in the SSADH$^{+/-}$ although the background activity in the SSADH heterozygotes was slower than that of wild-type control animals. The EEG behavioral data in the current experiments suggest that SSADH$^{-/-}$ may be used to investigate the molecular mechanisms that underpin the pathogenesis of absence and generalized tonic-clonic seizures associated with SSADH deficiency.

In addition to mirroring the epilepsy that occurs in human SSADH deficiency, the progression of EEG and behavior changes from typical absence epilepsy to myoclonic jerks and generalized convulsive seizures in SSADH$^{-/-}$ also is reminiscent of the transition of absence seizures to myoclonic and generalized convulsive seizures in juvenile absence epilepsy in humans. Unlike childhood absence epilepsy which is fairly benign, juvenile absence seizures progress to generalized convulsive and myoclonic seizures in 80% of patients and the seizures may be life long (Panayiotopoulos, 1997; Loiseau et al., 1995; Bartolomei et al., 1997; Obeid, 1994; Wirrell, 2003). The mechanism by which absence seizures in juvenile absence epilepsy transition to generalized convulsive seizures in children is completely unknown, because until now, there has been no animal model of generalized absence seizures that reflects this transition. SSADH$^{-/-}$ mice manifest absence seizures early in life that evolve into myoclonic and generalized convulsive seizures in the 3rd–4th postnatal week. Although these seizures evolve in the context of a complex metabolic disease in these mutant animals, the P14–18 SSADH$^{-/-}$ could represent a model of the transition from absence to generalized convulsive seizures in children with juvenile absence epilepsy.

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