

Therapeutic Intervention in Mice Deficient for Succinate Semialdehyde Dehydrogenase (γ -Hydroxybutyric Aciduria)

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ABSTRACT

Therapeutic intervention for human succinic semialdehyde dehydrogenase (SSADH) deficiency (γ -hydroxybutyric aciduria) has been limited to vigabatrin (VGB). Pharmacologically, VGB should be highly effective due to 4-aminobutyrate-transaminase (GABA-transaminase) inhibition, lowering succinic semialdehyde and, thereby, γ -hydroxybutyric acid (GHB) levels. Unfortunately, clinical efficacy has been limited. Because GHB possesses a number of potential receptor interactions, we addressed the hypothesis that antagonism of these interactions in mice with SSADH deficiency could lead to the development of novel treatment strategies for human patients. SSADH-deficient mice have significantly elevated tissue GHB levels, are neurologically impaired, and die within 4 weeks postnatally. In the current report, we compared oral versus intraperitoneal administration of VGB, CGP 35348 [3-aminopropyl(diethoxymethyl)phosphinic acid, a GABA_B receptor antagonist], and the nonprotein amino acid taurine in rescue of SSADH-deficient

mice from early death. In addition, we assessed the efficacy of the specific GHB receptor antagonist NCS-382 (6,7,8,9-tetrahydro-5-[H]benzocycloheptene-5-ol-6-ylideneacetic acid) using i.p. administration. All interventions led to significant lifespan extension (22–61%), with NCS-382 being most effective (50–61% survival). To explore the limited human clinical efficacy of VGB, we measured brain GHB and γ -aminobutyric acid (GABA) levels in SSADH-deficient mice receiving VGB. Whereas high-dose VGB led to the expected elevation of brain GABA, we found no parallel decrease in GHB levels. Our data indicate that, at a minimum, GHB and GABA_B receptors are involved in the pathophysiology of SSADH deficiency. We conclude that taurine and NCS-382 may have therapeutic relevance in human SSADH deficiency and that the poor clinical efficacy of VGB in this disease may relate to an inability to decrease brain GHB concentrations.

Murine knockout models of human inborn errors of metabolism serve as a rich resource to explore both gene and pharmacologic therapeutic strategies with potential applicability to the corresponding human disease. For example, treatment of mucopolysaccharidosis type VII mice with recombinant adeno-associated virus vectors leads to significant improvement of the disease phenotype (Daly et al., 2001). Similarly, lentiviral-directed gene therapy results in correction of neuropathology in mice with another lysosomal storage disease, metachromatic leukodystrophy (Consiglio et al., 2001). However, limitations of knockout models include genetic background effects and an inability to predict the effects of modifier genes potentially associated with the deleted gene (Gingrich and Hen, 2000).

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ABBREVIATIONS: SSADH, succinic semialdehyde dehydrogenase; GABA, γ -aminobutyric acid; GHB, γ -hydroxybutyric acid; VGB, vigabatrin (Sabril; γ -vinyl-4-aminobutyrate); CGP 35348, 3-aminopropyl(diethoxymethyl)phosphinic acid; NCS-382, 6,7,8,9-tetrahydro-5-[H]benzocycloheptene-5-ol-6-ylideneacetic acid.

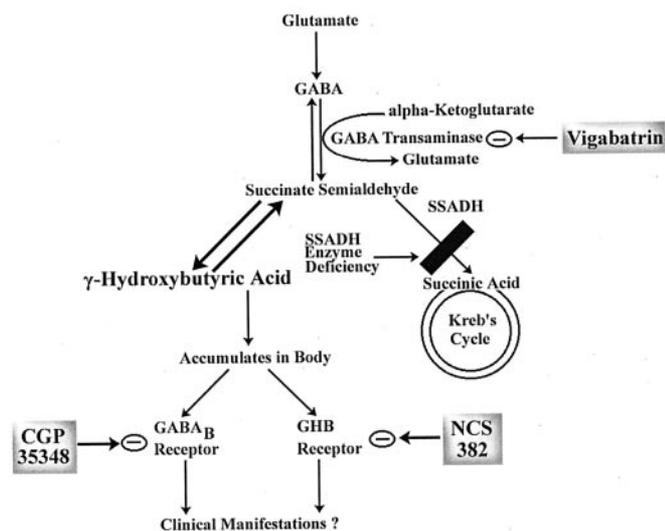


Fig. 1. The GABA catabolic pathway and pharmacologic sites of action for vigabatrin, CGP 35348, and NCS-382. The filled box depicts the site of the block in human and murine SSADH deficiency. Vigabatrin acts as an irreversible GABA-transaminase inhibitor, whereas CGP 35348 and NCS-382 function as GABA_B and GHB receptor antagonists, respectively.

To explore the patho-mechanisms involved in human SSADH deficiency, and to investigate the potential development of preclinical therapeutics, our laboratory developed a murine model of this disorder using standard gene targeting methodology (Hogema et al., 2001). Mutant mice are born at the expected Mendelian frequency; and show 20- to 60-fold and 2- to 3-fold accumulation of brain GHB and GABA, respectively. Animals are neurologically impaired and manifest a critical period at 16 to 22 days of life, at which time they exhibit generalized tonic-clonic seizures associated with 100% mortality (Fig. 2a). The SSADH-deficient mice fail to gain weight after about day 15 of postnatal life (Fig. 2b), associated with striking absence of body fat (Fig. 3). Seizure activity and early death are inconsistent with the human phenotype. In preliminary studies, we found that application of taurine, CGP 35348 [3-aminopropyl(diethoxymethyl)phosphinic acid, a GABA_B receptor antagonist], and VGB (vigabatrin, a GABA-transaminase inhibitor) led to significant extension of lifespan for mutant mice, although the animals ceased any demonstrable weight gain on these interventions (Hogema et al., 2001). VGB, an irreversible inhibitor of GABA-transaminase, represents an intuitively optimal treatment for both human patients and mutant mice (Fig. 1), since mechanistically it should lead to increased GABA production and decreased GHB output. However, its limited clinical use in human SSADH deficiency has shown

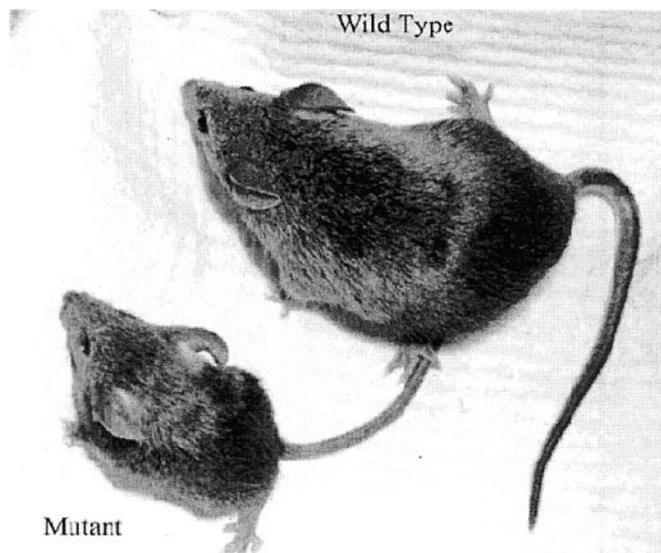


Fig. 3. Phenotypic comparison of mutant ($-/-$) and wild-type ($+/+$) mice at 33 days of age. Both animals were of mixed genetic stock (C57BL/129Sv). Note the significantly smaller size of the mutant mouse and the absence of fat mass in the hind region.

inconsistent efficacy (Gibson et al., 1995; Matern et al., 1996). Moreover, VGB intervention has been linked to visual field abnormalities, raising concerns about its chronic use (Spence and Sankar, 2001; Malmgren et al., 2001). Its therapeutic efficacy in SSADH deficiency may also be diminished due to nonspecific inactivation of other enzymes, including alanine and aspartate aminotransferases (Okumura et al., 1996; Williams et al., 1998).

Our laboratory has begun to evaluate the receptor interactions associated with high-dose GHB as targets for therapeutic intervention. In addition to association with its own high- and low-affinity binding sites, GHB has been shown (or postulated) to interact with dopamine, serotonin, *N*-methyl-D-aspartate, opiate, GABA_B, and GABA_A receptors (Gibson et al., 1998). Thus, there are a number of receptor mechanisms that could be pharmacologically antagonized, and a murine knockout represents an excellent vehicle in which to explore these treatment strategies. In the current report, we have studied the efficacy of oral versus intraperitoneal application of taurine, CGP 35348, and VGB in SSADH-deficient mice, using survival as an endpoint of treatment efficacy. In addition, we assessed the efficacy of the specific GHB receptor antagonist NCS-382 (6,7,8,9-tetrahydro-5-[H]benzocycloheptene-5-ol-6-ylideneacetic acid) using i.p. administration. Our rationale for i.p. administration was based upon the potential poor consumption of orally administered drugs due to taste

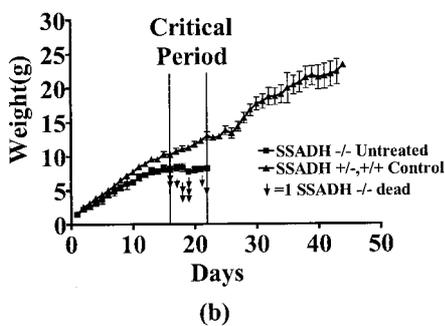
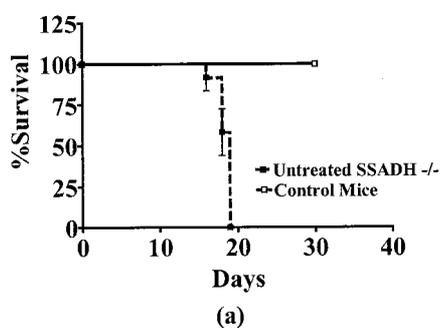


Fig. 2. Survival and weight gain of untreated SSADH $-/-$ mice. a, survival of untreated ($n = 12$) SSADH $-/-$ mice in comparison with control (heterozygous or wild-type) mice ($n = 12$). b, weight gain and critical period (16–22 days) in untreated SSADH $-/-$ ($n = 10$) and control heterozygous ($+/-$) or wild-type ($+/+$) mice; survival was inferred as lifespan beyond 30 days of life.

aversion, and the need to ascertain whether survival could be further enhanced via this mode of delivery. Finally, we explored brain neurometabolite levels in mutant mice receiving moderate- to high-dose VGB to gain insight into the poor clinical efficacy of this compound in the corresponding human disorder. The current report summarizes our findings.

Materials and Methods

Animals. Targeted disruption of the SSADH gene in C57BL/129Sv mice has been described (Hogema et al., 2001). Mice with mixed background were employed to assess efficacy of drug application using i.p. and oral administration. Inbreeding of heterozygous (+/−) mice was performed to derive homozygous affected (−/−) mice. Free access to food and water was provided. Mice were maintained under an artificial light/dark cycle of 12 h/12 h (6:00 AM–6:00 PM) at ambient temperature and relative humidity of 60%. All animals were drug-naïve at the beginning of all interventions. Animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (protocol A-773), Oregon Health & Science University, and were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Drugs. NCS-382 was kindly supplied by National Institute of Drug Abuse. CGP 35348 was a generous gift from Novartis Pharma Inc. (Basel, Switzerland). Taurine (2-aminoethanesulfonic acid) was obtained from Sigma-Aldrich (St. Louis, MO). Vigabatrin (VGB) was purchased from Tocris Cookson (Bristol, UK). All other reagents employed were of the highest analytical purity available.

Genotyping. Mice were genotyped using a 3-primer polymerase chain reaction method for SSADH sequences (Hogema et al., 2001). The primers employed included a common reverse primer, 5'-TTG-GTGGGCACCTTACGAAAATC-3', and two forward primers, 5'-GG-GAAGGGTTGTCTTACATCTCCTG-3' and 5'-CGTGATATTGCT-GAAGAGCTTGGC-3'.

Oral Therapy. Cohorts of mutant and wild-type/heterozygous mice ($n = 9$ – 12 each) were grouped under the following drug intervention groups: CGP 35348 (100, 200, and 2000 mg/kg body weight), vigabatrin (50–800 mg/kg body weight), and taurine (2000 and 5000 mg/kg body weight). All drugs were dissolved in drinking water, and the water was changed every 12 h. A measured quantity of water supplemented with drug (150 ml) was given in every cage. After 12 h, the water consumed was calculated based upon the remaining water, which revealed an average concentration of 4 to 6 ml/10 g body weight for all the drugs studied. This suggested that different drugs did not significantly alter water consumption. The unconsumed water was discarded, and fresh water supplemented with drug was supplied for the next 12 h. The actual doses of the drugs were calculated based upon measured water consumption. Pregnant heterozygous females were kept on regular diet and water supplemented with drug starting at 10 to 15 days' gestation. All offspring were continued on the same concentration of drug until death or until harvested after surviving beyond day of life 30.

Intraperitoneal (i.p.) Therapy. A cohort of mutant and wild-type/heterozygous mice ($n = 9$ – 12 each) was employed for each drug intervention group. Vigabatrin (15–25 mg/kg body weight), CGP 35348 (50–300 mg/kg body weight), and taurine (250–1000 mg/kg body weight) were dissolved in distilled water; NCS 382 (50–300 mg/kg body weight) was dissolved in 0.1% NaHCO₃. Aliquots of the drugs were frozen at -80°C until use. Mutants were genotyped and started on therapy at or before day 7 of life. One injection of each drug was administered daily by i.p. administration between 7:00 and 8:00 AM, with concentration adjusted according to the weight of the mouse. The injections were continued until the mice either died or survived for 30 days or more. Since the mice were to be injected over a period of 30 days, the number of injected doses was kept to a minimum of one per day, as opposed to multiple injections. Moreover, there was concern that repeated i.p. injections would irritate the

peritoneum and raise the risk of infection, affecting survival and thus interfering with interpretation of the results. Mouse weights were determined on alternate days. Survival was considered significant if mutant animals survived beyond 30 days.

Determination of GHB and GABA in Vigabatrin-Treated Mice. Because vigabatrin (VGB) is the only therapeutic analog utilized consistently in human SSADH deficiency, we investigated the concentrations of total GABA and GHB in extracts of brain, liver, and kidney from cohorts of wild-type, heterozygous, and mutant mice treated orally with vigabatrin (dose ranges approximately 50, 100, 200, 400, and 800 mg/kg body weight, respectively). Homogenates of freshly isolated organs were prepared as previously described and clarified by centrifugation (Hogema et al., 2001). GHB and total GABA were quantified using isotope dilution gas chromatography-mass spectrometry utilizing deuterium- and ¹³C-labeled internal standards, as described (Hogema et al., 2001). All metabolite concentrations were normalized for protein content in the extract.

Statistical Analyses. The GraphPad Prism program (version 3.0) (GraphPad Software, Inc., San Diego, CA) was employed to derive survival curves. Mouse survival curves were determined based upon the method of Kaplan-Meier and the standard error method developed by Greenwood (Cantor, 2001). The survival proportions were measured each time an event (death) occurred to show the percentage of surviving mutants at that time, and the log-rank test (modified Mantel-Haenszel chi square test) was used to compare the survival curves over the whole period mice were under study. Drug-dose curves were derived using linear regression analysis for optimal fit of the data points. Significance of variation of GABA and GHB levels in brain, liver, and kidney of mice on oral vigabatrin therapy, both between genotypes and various doses of vigabatrin, was determined using two-way and one-way analysis of variance, respectively.

Results

In earlier work, we found that the lifespan of mutant mice could be substantially extended by treatment with different therapeutics, including vigabatrin (VGB), CGP 35348, and taurine, all administered orally. In the present study, we extended these studies by contrasting the administration of these drugs using oral and i.p. routes, in addition to characterizing the utility of the specific GHB receptor antagonist NCS-382 using i.p. administration.

Oral administration of CGP 35348 showed 22% survival at 100 mg/kg ($n = 9$, $p < 0.01$), 25% survival at 200 mg/kg ($n = 4$, $p < 0.01$), and 22% survival at 2000 mg/kg ($n = 18$, $p < 0.01$) (data not shown). For i.p. administration, we utilized CGP 35348 at doses of 50 mg/kg, 200 mg/kg, and 300 mg/kg body weight. Under these treatment regimens, we found survival for mutant mice of 18.2% ($n = 11$, $p < 0.05$), 36.4% ($n = 11$, $p = \text{N.S.}$) and 27.3% ($n = 11$, $p < 0.05$), respectively (Fig. 4a). Mice on oral CGP 35348 were easily provoked and were hypersensitive to noise and movement, but this effect was not observed on i.p. administration of the same drug. No other adverse effects of CGP 35348 were observed. Oral administration of the sulfonic acid derivative taurine resulted in 38.5% survival at an oral concentration of 2000 mg/kg ($n = 13$, $p = \text{N.S.}$) and 30.8% at 5000 mg/kg ($n = 13$, $p < 0.01$), respectively (data not shown). Even though final survival with taurine at 2000 mg/kg was greater than that with 5000 mg/kg, it was not significant because the log-rank test employed compares survival curves over the whole range of the time period the mice are under study. For example, at 5000 mg/kg, up to 46% of the mutants were alive by day 20, whereas for mutants on 2000 mg/kg, only 38.5% were alive by

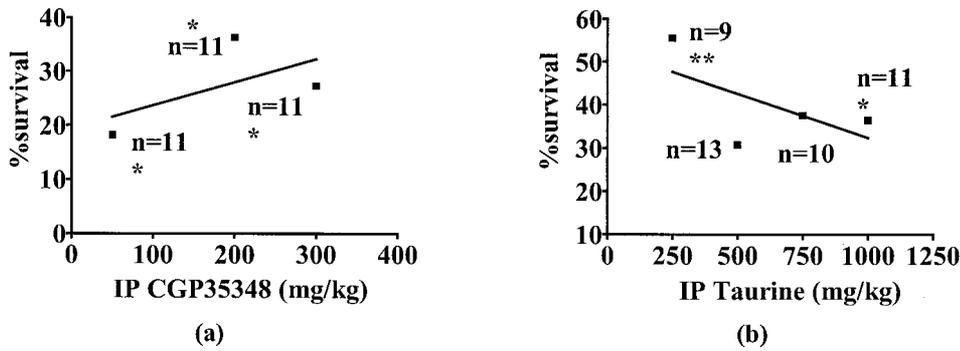


Fig. 4. Percentage survival of SSADH^{-/-} mice on CGP 35348 (a) and taurine (b); both drugs administered intraperitoneally. Statistical analysis was performed using the log-rank test, with *, $p < 0.05$; **, $p < 0.01$; or ***, $p < 0.001$ compared with control untreated SSADH^{-/-} mice (0% survival). n = number of SSADH^{-/-} mice treated; survival in SSADH^{-/-} mice was taken as lifespan beyond 30 days.

day 20. Thus, even though final survival was higher employing 2000 mg/kg taurine, the overall survival curve was statistically significant only for the 5000 mg/kg dose. We found a more robust survival for i.p. taurine administration with 55.6% survival at a daily injection level of 250 mg/kg ($n = 9$, $p < 0.01$), 30.8% survival at 500 mg/kg ($n = 13$, $p = \text{N.S.}$), 37.5% at 750 mg/kg ($n = 10$, $p = \text{N.S.}$), and 36.4% survival at 1000 mg/kg ($n = 11$, $p < 0.05$) (Fig. 4b). Higher taurine doses appeared toxic to the mutant mice as evidenced by lower survival at increasing doses.

Because GHB possesses both high- and low-affinity receptors in mammalian brain, with particularly high levels of receptors in the hippocampus, we attempted pharmacologic rescue of SSADH-deficient mice using the specific GHB receptor antagonist, NCS-382 (Maitre et al., 1990). We limited our use of NCS-382 to i.p. administration due to a limited supply of the drug. Applying dosages of 50 to 300 mg/kg body weight, we found 40% survival at 50 mg/kg ($n = 10$, $p < 0.05$), 25% survival at 100 mg/kg ($n = 12$, $p < 0.001$), 53.9% survival at 200 mg/kg body weight ($n = 13$, $p < 0.001$), and a peak survival of 61.5% at 300 mg/kg body weight ($n = 13$, $p < 0.0001$) (Fig. 5). Because the dose of NCS-382 was large, and this drug has not, to our knowledge, been previously utilized as a treatment intervention, we assessed blood chemistries on treated animals (Table 1), which revealed some significant alterations compared with wild-type littermates and untreated SSADH-deficient mice. For those mutants that survived the critical period on NCS-382, there was no mortality seen up to 55 to 60 days of life, after which time tissues were harvested for analysis. No significant adverse effects were observed in behavior or locomotion in mutant mice receiving NCS-382.

We next assessed vigabatrin intervention at dosages including 50, 100, 200, 400, and 800 mg/kg body weight. For oral application, we found 22.2% survival ($n = 9$, $p < 0.05$) at 50 mg/kg, 11.1% survival ($n = 9$, $p < 0.01$) at 100 mg/kg,

14.3% survival ($n = 7$, $p < 0.05$) at 200 mg/kg, 18.2% survival ($n = 11$, $p < 0.01$) at 400 mg/kg, and 43.8% survival ($n = 16$, $p < 0.0001$) with the maximum dosage of 800 mg/kg (Fig. 6a). In contrast, i.p. administration of similar dosages of VGB resulted in significant toxicity. Mutant mice administered 50, 100, 200, or 300 mg/kg VGB i.p. manifested significant weight loss, poor feeding, sedation, motor impairment, and death prior to day 10 of life for the majority of mice. Therefore, we lowered the i.p. VGB dose to 15 mg/kg, resulting in 12.5% survival ($n = 8$, $p < 0.01$), 20 mg/kg i.p. administration with 11.1% survival ($n = 9$, $p = \text{N.S.}$), and 25 mg/kg i.p. administration of VGB, which led to 25% survival ($n = 12$, $p < 0.0001$) (Fig. 6b). Weight gain for mutants receiving vigabatrin 25 mg/kg, i.p., is shown in Fig. 6c. A summary of maximal survival for the different interventions employed is shown in Table 2.

Of the four therapeutic interventions studied in the current report, only vigabatrin should have a demonstrable effect on brain neurochemistry. Whereas CGP 35348 and NCS-382 are receptor antagonists, VGB is an inhibitor of GABA-transaminase; theoretically, application of this compound should raise brain GABA levels with concomitant decrease of brain GHB levels (via reduction of the substrate for the formation of GHB, succinic semialdehyde; see Fig. 1). We measured both total GABA and GHB in brain extracts from mutant mice on a variety of VGB dosages, comparing both brain and peripheral organ levels (Fig. 7a–c). We found that control mice (wild-type and heterozygous genotypes) were relatively resistant to the GABA-elevating capacity of VGB at concentrations up to 400 mg/kg, but that higher concentrations (800 mg/kg) did result in significant increases in GABA (Fig. 7). Similar results were observed in mutant mice treated at the same dosages, although there was a significant elevation of GABA in kidney homogenates at lower VGB concentrations. Of note, concentrations of VGB up to 400 mg/kg actually resulted in an increase in GHB concentra-

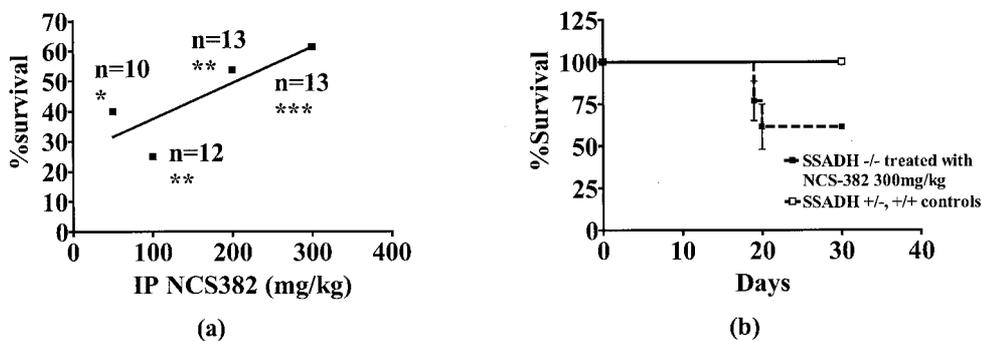


Fig. 5. Survival of SSADH^{-/-} mice on various doses of NCS-382 administered i.p. a, percentage of survival seen on different doses of NCS-382; statistical significance was derived using the log-rank test, with *, $p < 0.05$; **, $p < 0.01$; or ***, $p < 0.001$ compared with control untreated SSADH^{-/-} mice (0% survival). n = number of SSADH^{-/-} mice treated with NCS-382. b, curve showing survival of SSADH^{-/-} mice on 300 mg/kg NCS-382 (i.p.) ($n = 13$) compared with control +/-, +/+ mice ($n = 12$). Survival in SSADH^{-/-} mice was taken as lifespan beyond 30 days.

TABLE 1

Blood chemistries for wild-type (+/+), untreated mutant (-/-), and NCS-382-treated mutant (-/-) mice

Control (+/+) mice and untreated (-/-) mice = 17 days old; NCS-382-treated (-/-) mice = 80 to 111 days old. It was not possible to age-match untreated (-/-) mice since these animals uniformly died at age 16 to 22 days in the absence of therapeutic intervention. NCS-382-treated mice were on i.p. therapy for the first 30 to 32 days of life, after which the injection of drug was stopped. All values are means \pm S.E.M.

Panel	SSADH +/+ (<i>n</i> = 7)	SSADH -/- (untreated, <i>n</i> = 7)	Mutant (treated, <i>n</i> = 5) ^e
Bilirubin ^b	0.13 \pm 0.03	0.14 \pm 0.03	not done
Alk Phos ^c	394.6 \pm 23.9	419.3 \pm 20.6	127.5 \pm 42***
Protein ^d	4.37 \pm 0.09	4.46 \pm 0.12	4.8 \pm 0.25
BUN ^b	20.4 \pm 2.1	16.7 \pm 0.52	21 \pm 1.2**
Creatinine ^b	0.06 \pm 0.02	0.07 \pm 0.02	not done
Phosphorus ^b	11.0 \pm 0.77	10.1 \pm 0.30	7.05 \pm 0.45***
Calcium ^b	9.2 \pm 0.29	9.74 \pm 0.24	7.58 \pm 0.14***
Amylase ^c	2809 \pm 217.7	1985 \pm 63.8	1817 \pm 59.8
Ala Trans ^c	217.3 \pm 71.9	42.0 \pm 6.73	81.0 \pm 21.9*
Glucose ^b	165.1 \pm 20.0	157.6 \pm 8.30	159.8 \pm 18.4
Cholesterol ^b	121.6 \pm 7.35	156.9 \pm 7.76	121.5 \pm 10.71*

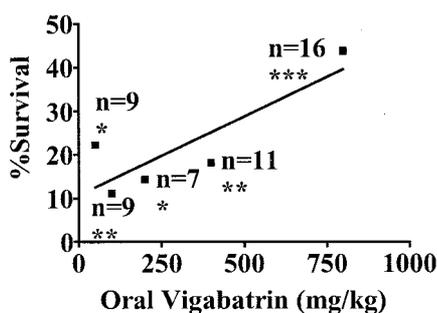
Alk Phos, alkaline phosphatase; BUN, blood urea nitrogen; Ala Trans, alanine transaminase.

^a Significantly different from untreated SSADH -/-, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

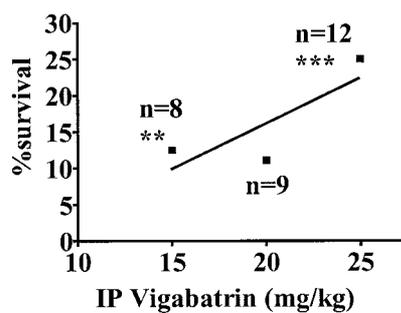
^b Values in mg/dl.

^c Values in IU/liter.

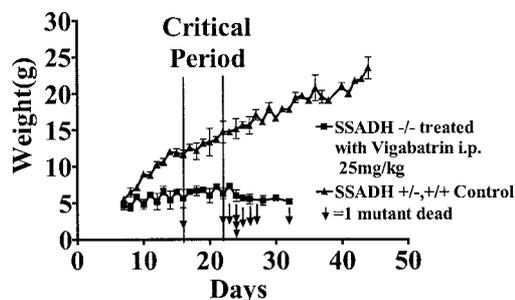
^d Values in g/dl.



(a)



(b)



(c)

Fig. 6. Effect of vigabatrin on survival and weight gain by SSADH -/- mice. Percentage survival of SSADH -/- mice treated with various doses of orally administered vigabatrin (a) and intraperitoneally (IP) administered vigabatrin (b). Statistical analysis was performed using the log-rank test, with *, *p* < 0.05; **, *p* < 0.01; or ***, *p* < 0.001 compared with control untreated SSADH -/- mouse survival (0% survival). *n* = number of SSADH -/- mice treated; survival in SSADH -/- mice was taken as lifespan beyond 30 days. c, weight gain and survival beyond the critical period in SSADH -/- mice (*n* = 12) treated intraperitoneally (IP) with vigabatrin (25 mg/kg) compared with control +/-, +/+ mice (*n* = 12).

TABLE 2

Maximum survival with different drug applications in mutant mice

All dosage units are in terms of mg/kg body weight (for oral administration, assuming an average mutant mouse weight of 10 g with consumption of 4–6 ml of drinking water/day). Mutant mice living beyond 30 days were considered to have survived.

Drug	i.p. Administration			Oral Administration		
	Dose	<i>p</i> value	Survival %	Dose	<i>p</i> value	Survival %
NCS-382	300	<0.0001	61.5			
Taurine	250	0.0022	55.6	5000	0.0013	30.8
CGP 35348	300	0.0447	27.3	2000	0.0016	22.2
VGB	25	<0.0001	25	800	<0.0001	50

tions in all tissues, including brain (Fig. 7d, mutant only shown; values for wild-type and heterozygous mice were at or near the limit of accurate detection). At the highest dose of VGB studied, however, there was a significant decrease of

GHB in kidney and liver in comparison with untreated mutants. In mutant kidney, the GHB concentration (micro-moles/gram protein) was 4.56 ± 1.05 (*n* = 7, mean \pm S.E.M., untreated) compared with 2.54 ± 1.03 (*n* = 4, treated with

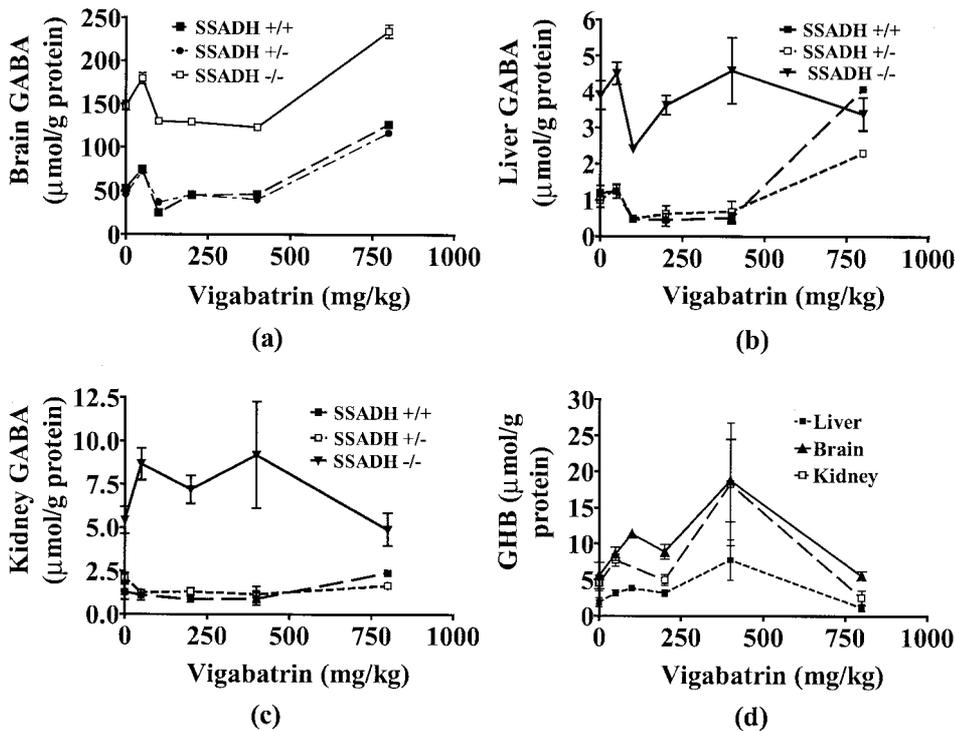


Fig. 7. GABA and GHB concentrations (micromoles per gram protein) in different tissue extracts for mice treated orally with vigabatrin. In a to c, GABA concentrations of SSADH $-/-$, SSADH $+/-$, and control $+/+$ tissues are shown for brain (a), liver (b), and kidney (c), under increasing doses of VGB. $p < 0.0001$ for SSADH $-/-$ mice in comparison with heterozygous ($+/-$) and control ($+/+$) mice as assessed by two-way analysis of variance ($n = 4-17$); in the case of SSADH $-/-$ kidney, there was no significant difference among the different VGB doses for GABA. d, GHB as a function of increasing oral vigabatrin application, showing levels in SSADH $-/-$ mice only; values for heterozygous ($+/-$) and control ($+/+$) mice were at or near the limit of accurate detection and are not shown. Significant variation in GHB levels was found in each tissue on different vigabatrin doses with $p < 0.01$ in brain and $p < 0.05$ in liver and kidney ($n = 4-17$).

VGB at 800 mg/kg body weight, $p < 0.05$); similarly, in mutant liver the values were 1.93 ± 0.56 ($n = 8$, untreated) compared with 1.19 ± 0.46 ($n = 4$, treated; $p < 0.05$). Conversely, the brain level of GHB was not significantly lowered (5.56 ± 1.75 ; $n = 8$, untreated versus 5.60 ± 0.64 ; $n = 4$, treated with 800 mg/kg body weight vigabatrin; $p = \text{N.S.}$).

Discussion

A mouse model of human SSADH deficiency represents a useful vehicle in which to explore patho-mechanisms associated with the human disorder, investigate new preclinical treatment strategies, and explore gene therapeutic approaches. Treatment strategies for human SSADH deficiency may focus on two approaches: 1) decreasing the production of GHB and 2) antagonizing receptor interactions known to exist for GHB (Gibson et al., 1998). With regard to the former, it is clear that VGB should represent an excellent treatment intervention, with the capacity to decrease the production of succinic semialdehyde (the hypothetical precursor of GHB) while concomitantly increasing brain levels of GABA. VGB is a widely prescribed antiepileptic that has nevertheless had very mixed efficacy in human SSADH deficiency, which has been puzzling. One potential drawback to the utility of VGB is the possibility that peripheral and neural GABA-transaminases share different kinetic properties. This could result in differential levels of inactivation leading to resupply of GHB from the periphery to brain, since GHB can easily cross the blood-brain barrier (Howells et al., 1992; Gibson et al., 2002). This hypothesis remains unsubstantiated. Moreover, there is concern with the use of VGB related to constriction of the visual field. Thus, we have begun to explore alternate treatment strategies, starting with preclinical treatment paradigms in the mutant mouse model, and focusing specifically on GHB-receptor interactions.

GHB is known to interact with GABA_B receptors and its own high- and low-affinity specific GHB receptors (Snead and Liu, 1984; Mathivet et al., 1997; Maitre, 1997; Lingenhoehl et al., 1999; Snead, 2000; Mehta et al., 2001). Similarly, there is a well established association between GHB and the dopaminergic system (Maitre, 1997; Snead, 2000; Kish et al., 2001). In addition, it is likely that there are interactions between GHB and the *N*-methyl-D-aspartate and opioid receptor pathways, with the latter potentially mediating the euphoric effects of GHB consumed illicitly (Nicholson and Balster, 2001). At the high GHB concentrations found in the brain tissue of our mice (150–250 μM), it is likely that GHB functions as an agonist at the GABA_B receptor, so that it is not surprising to observe therapeutic success with a GABA_B receptor antagonist, CGP 35348 (Olpe et al., 1990). There are, however, opposing effects for GABA_B receptor antagonists that have been observed in absence and convulsive-type seizures. Although the GABA_B receptor antagonist CGP 35348 suppresses absence seizures, it exacerbates convulsive seizures at the same doses that offset absence seizures (Vergnes et al., 1997; Motalli et al., 1999). For our mouse model, this would imply that although the absence seizures may be treatable, CGP 35348 would actually exacerbate tonic-clonic seizures that occur during the critical period. Because of this dual action, CGP 35348 efficacy for long-term treatment may be limited, suggesting little likelihood of exploring GABA_B receptor antagonists in human patients (Maitre et al., 1990; Hechler et al., 1993).

We found that treatment with NCS-382 led to the most significant extension of lifespan for any therapy attempted to date, suggesting a role for the metabotropic GHB receptor in pathophysiology. However, our results are at odds with recently published findings on the sedative/hypnotic effects of GHB (Carai et al., 2001). Work from the laboratory of Carai and colleagues (Carai et al., 2001) revealed that coadminis-

tration of GHB and NCS-382 to DBA mice exacerbated the loss of righting reflex in comparison with GHB administration alone; conversely, pretreatment with GABA_B receptor antagonists prior to GHB administration in the same animals abolished the sedative/hypnotic effects of GHB. One hypothesis for this discrepancy is potential overlapping activities for CGP 35348 and NCS-382, with both compounds having antagonistic capacity at GHB and GABA_B receptors (Olpe et al., 1990). Another possibility is that the GHB receptor is a GABA_B receptor subtype, which will be clarified when the specific GHB receptor is cloned and sequenced (Snead, 2000). The significant alterations in blood chemistries in NCS-382-treated mice (Table 1) suggests that more extensive safety analyses will need to be performed prior to consideration of intervention with this drug in humans.

One question to be resolved in our animal model is the appearance of seizures at day of life 16 to 22. It is well known that at even the highest doses, GHB itself does not induce tonic-clonic seizures (Snead, 1996). However, it is of interest that convulsive activity correlates, in our mutant mice, with the probable appearance of GHB binding in brain. In the rat, there is concordance between GHB-induced absence seizures and the developmental appearance of [³H]GHB binding in the frontal cortex at day 18 postnatally (Snead, 1994). The appearance of functional GHB receptors at this time may explain the clinical efficacy of NCS-382, which has been shown to block the electroencephalographic disturbances, cGMP level modifications, and alteration of inositol phosphate levels in the rat hippocampus following GHB administration (Maitre et al., 1990).

Our earlier limited success in rescuing SSADH-deficient mice with taurine was corroborated in the present study. The extensive literature on taurine suggests roles as neuromodulator and osmoregulator (McBride and Frederickson, 1980; Wade et al., 1988; Kontro and Oja, 1990; Huxtable, 1992; del Olmo et al., 2000). Taurine ameliorates epileptic symptomatology in experimental animals and human patients, is known to interact with both GABA_A and GABA_B receptors in different brain subsections, and may play a role in protection against free radical damage in neural tissue (Saransaari and Oja, 2000). Similar protective properties have been suggested for GHB, but at much lower concentrations than those seen in our mutant mice (Artru et al., 1980; Mamelak, 1989). The latter observation is in contrast with our preliminary work that suggests hippocampal damage associated with GHB accumulation in the knockout mouse (Hogema et al., 2001). Taurine plays a prominent role in the developing hippocampus (Clements et al., 1989), and the possibility exists that taurine exhibits neuroprotective features in the presence of high concentrations of GHB. In the current study, higher doses of taurine led to decreased survival, suggesting potential toxicity. Conversely, lower doses showed significant survival. The exact mechanism through which taurine is effectively extending the lifespan of mutant mice remains to be determined, but our data suggest the potential for clinical intervention with taurine in human SSADH deficiency.

Using i.p. administration, we found high-dose VGB toxic in SSADH-deficient mice. Increased levels of GABA may be responsible for decreased spontaneous locomotion, altered feeding, and increased sedation seen at higher doses of vigabatrin (Hout and Palfreyman, 1982; Grant and Heel, 1991). Although significant survival was observed with vigabatrin

treatment, and mutants were able to cross the critical period, most of them died on average between 22 and 30 days (Fig. 6c). This suggests a lack of long-term efficacy for VGB intervention and, perhaps, tolerance to its anticonvulsant effect when administered on a chronic basis. Rundfeldt and Löscher (1992) hypothesized that drug experience was not sufficient for maximal development of tolerance. These authors further suggested that functional tolerance developed fully only when the subject actually experienced the anticonvulsant effect of vigabatrin during seizure activity (Rundfeldt and Löscher, 1992). This suggests that the SSADH mutant mice experience the anticonvulsant effect of vigabatrin during the critical period, for the first time, when they begin manifesting seizures. Therefore, VGB is effective during this time, but eventually the efficacy of drug decreases. On the other hand, treatment with NCS-382 resulted in an extended survival of the mutants up to 55 to 60 days or more.

A particularly important aspect of our work was the ability to study neurochemical markers in mutant animals treated with VGB at different concentrations (Fig. 7). In particular, oral consumption of VGB approximating 50 to 400 mg/kg body weight in mutants did not lead to a substantial increase of total GABA in brain or liver, but did raise GABA concentrations in kidney significantly (Fig. 7). These high levels in kidney may also reflect efficient transport to the nephron for eventual excretion. Another potential explanation was poor consumption of the drug, potentially associated with limited bioavailability. Higher oral VGB administration (800 mg/kg) did result in increased total GABA levels in all tissues derived from control mice and in the brains of mutant mice, although there was not a corresponding elevation of GABA levels in peripheral mutant tissues. Paradoxically, intermediate dosages of oral VGB (50–400 mg/kg) led to increased GHB levels for mutants in all tissues, yet at high-dose VGB, the GHB levels dropped significantly below pretreatment levels in liver and kidney, but not in brain. There remains the unexplored possibility that other sources of GHB exist beyond the GABA pathway, or potentially other enzymes act on GABA to produce GHB, but data to support this speculation are lacking. At least at high-dose administration, our data support the concept that VGB may show variable efficacy in human SSADH deficiency due to an inability to lower GHB concentrations in neural tissue, potentially associated with resupply of GHB from the periphery.

In view of the number of receptor interactions for GHB, it is likely that combinatorial therapy may benefit our human patients. Our results suggest that pathophysiology in mouse SSADH deficiency involves at least the GHB and GABA_B receptors, and that taurine and NCS-382 may have therapeutic relevance in the human disease. Our preliminary neurochemical findings suggest that VGB is a suboptimal treatment for human SSADH deficiency, based upon its inability to lower brain GHB concentrations. This observation, which requires further investigation, in conjunction with increasing reports of visual field alterations associated with the use of VGB, strongly suggests that other treatment modalities should be considered in human SSADH deficiency. To this end, combinatorial treatment strategies are currently under investigation in the SSADH-deficient mouse model.

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