Plasticity of postsynaptic, but not presynaptic, GABA_B receptors in SSADH deficient mice

Irina Vardya[1], **Kim R. Drasbek**[1], **K. Michael Gibson**[2], and **Kimmo Jensen**[1,3]

[1]Synaptic Physiology Laboratory, Department of Physiology and Biophysics, Aarhus University, Denmark

[2]Department of Biological Sciences, Michigan Technological University, USA

[3]Center for Psychiatric Research, Aarhus University Hospital, Risskov, Denmark

Abstract

Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal-recessively inherited disorder of γ-aminobutyrate (GABA) catabolism characterized by ataxia and epilepsy. Since SSADH is responsible for GABA break-down downstream of GABA transaminase, patients manifest high extracellular levels of GABA, as well as the GABA_B receptor (GABA_BR) agonist γ-hydroxybutyrate (GHB). SSADH knockout (KO) mice display absence seizures, which progress into lethal tonic-clonic seizures at around 3 weeks of age. It is hypothesized that desensitization of GABA_BRs plays an important role in the disease, although detailed studies of pre- and postsynaptic GABA_BRs are not available. We performed patch-clamp recordings from layer 2/3 pyramidal neurons in neocortical brain slices of wild-type (WT) and SSADH KO mice. Electrical stimulation of GABAergic fibers during wash in of the GABA_BR agonist baclofen revealed no difference in presynaptic GABA_BR mediated inhibition of GABA release between WT and SSADH KO mice. In contrast, a significant decrease in postsynaptic baclofen-induced potassium currents was seen in SSADH KO mice. This reduction was unlikely to be caused by accumulation of potassium, GABA or GHB in the brain slices, or an altered expression of regulators of G-protein signaling (RGS) proteins. Finally, adenosine-induced potassium currents were also reduced in SSADH KO mice, which could suggest heterologous desensitization of the G-protein dependent effectors, leading to a reduction in G-protein coupled inwardly rectifying potassium (GIRK) channel responses. Our findings indicate that high GABA and GHB levels desensitize postsynaptic, but not certain presynaptic, GABA_BRs, promoting a decrease in GIRK channel function. These changes could contribute to the development of seizures in SSADH KO mice and potentially also in affected patients.

List of keywords

Correspondence should be addressed to: Kimmo Jensen, M.D. Ph.D., Department of Physiology and Biophysics, Building 1160, Room 116, Aarhus University, Aarhus C, DK-8000, Denmark, kimmo@fi.au.dk, Phone: +45 8942 2792, Fax: +45 8612 9065.

Introduction

Succinic semialdehyde dehydrogenase (SSADH, *Aldh5a1*) deficiency is an autosomal-recessively inherited disorder of γ-aminobutyric acid (GABA) catabolism characterized clinically by intellectual disability, autism spectrum, sleep disturbances and epileptic seizures (Knerr et al., 2007). A mouse model of SSADH deficiency, SSADH knockout (KO) mice, has become an important tool to investigate the pathophysiology of this disease and possible treatment approaches (Gibson et al., 2005, Hogema et al., 2001, Nylen et al., 2008). SSADH is responsible for the oxidation of succinic semialdehyde (SSA) to succinic acid and participates in the break-down of GABA downstream of GABA transaminase (Turner and Whittle, 1983). Comparable to human patients, SSADH deficient mice manifest accumulation of GABA and γ-hydroxybutyrate (GHB) in the brain (Hogema et al., 2001, Jansen et al., 2008). Both GABA and GHB are agonists of metabotropic GABA$_B$Rs, which are recognized as a therapeutic target in various brain pathologies (for review see Bowery, 2006).

Dysfunction of GABA$_B$R mediated inhibition has been suggested to contribute to the pathophysiology of SSADH deficiency. Accordingly, Buzzi and colleagues found a significant decrease of [^{3}H]CGP-54626A binding in brain slices of SSADH KO mice. Moreover, they observed that electrically evoked GABA$_B$R mediated slow IPSPs (inhibitory postsynaptic potentials) in CA1 of the hippocampus were downregulated (Buzzi et al., 2006). Since GABA release does not appear to be affected in SSADH KO mice (Drasbek et al., 2008), this raises the possibility that postsynaptic GABA$_B$Rs or downstream effector systems are altered in SSADH deficiency.

GABA$_B$Rs are expressed on glutamatergic and GABAergic presynaptic terminals and on the somatodendritic region of target neurons. Presynaptic GABA$_B$Rs influence neurotransmitter release by inhibiting calcium influx, while postsynaptic GABA$_B$Rs influence excitability of neurons mainly by causing postsynaptic hyperpolarization via potassium efflux through G-protein-coupled inwardly-rectifying potassium (GIRK) channels that can shunt excitatory input (Nicoll, 2004).

GABA$_B$Rs are members of the family of G-protein coupled 7-transmembrane domain receptors (GPCRs) (Kaupmann et al., 1997) and functional GABA$_B$Rs are obligatory heterodimers composed of GABA$_B$($1_{a,b}$) and GABA$_B$(2) subunits that cross-stabilize each other (Brown et al., 2003, Kaupmann et al., 1997, Prosser et al., 2001, Schuler et al., 2001). GABA$_B$Rs are coupled to G-proteins of the G$_{i/o}$ subfamily and activation of GABA$_B$ receptors by agonists (e.g. GABA, GHB, or baclofen) results in phosphorylation of Gα (the α subunit of the G-protein complex) followed by the liberation of the Gβγ-subunits and opening of the GIRK channels (Bettler et al., 2004). Furthermore, GABA$_{B2}$ subunits are responsible for binding and activation of Gα (i/o subtypes), and for the correct trafficking of the GABA$_{B1}$ subunit to the cell surface (Calver et al., 2001, Margeta-Mitrovic, Jan and Jan, 2000, Margeta-Mitrovic, Jan and Jan, 2001, Robbins et al., 2001). GABA$_B$R heterodimers are proposed to be atypical GPCRs, as phosphorylation does not cause obligate internalization-dependent downregulation of the receptors on the neuron surface. Instead, during agonist application, GABA$_B$Rs can undergo rapid desensitization, which is explained by uncoupling of the GABA$_B$ heterodimers from Gα-GIRK complexes rather than by receptor internalization (Labouebe et al., 2007). While high levels of agonists can influence GABA$_B$ receptors and effectors, little is known about the GIRK channel responses in SSADH KO mice, which show high GABA and GHB levels in the brain.

Here, brain slice electrophysiology was used to study possible alterations in the function of presynaptic and postsynaptic GABA$_B$Rs of neocortical layer 2/3 pyramidal neurons in

SSADH KO mice. While presynaptic GABA$_B$R mediated inhibition of GABA release appeared to function similarly in wild-type (WT) and KO mice, pyramidal neurons of SSADH KO mice demonstrated a significant loss of postsynaptic responsiveness to baclofen and adenosine.

Materials and Methods

Mouse breeding

Wild-type and SSADH knockout mice were obtained from heterozygous breeding in a university animal facility with a 12/12-hour light/dark cycle and food and water *ad libitum*. SSADH KO mice develop absence seizures that progress into lethal status epilepticus, leading to 100% mortality at postnatal day 18-22 (P18-22). Therefore, WT and KO mice were used for experiments from P14 to P18 (Cortez et al., 2004, Hogema et al., 2001).

Brain slice electrophysiology

Mice were used in accordance with university guidelines, and European Union legislation regarding laboratory animals. Mice of either sex were anesthetized deeply with isoflurane, decapitated, and the brains were dissected out and transferred to ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM): 126 NaCl, 2.5 KCl, 2 CaCl$_2$, 2 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaCO$_3$, 10 D-glucose (osmolality 305-315 mosmol·kg^{-1}), pH 7.4 when bubbled with carbogen (5% CO$_2$, 95% O$_2$). 350 μm thick coronal slices were cut on a Vibratome 3000 Plus (Vibratome Company, St. Louis, MO). To improve brain slice quality, 3 mM kynurenic acid, 0.2 mM ascorbic acid, and 0.2 mM pyruvic acid were added during slicing and storage. Slices were allowed to rest for at least 1 hour before recording.

For recordings of IPSCs and postsynaptic GIRK currents, slices were placed in a chamber and perfused with 33-34°C bubbled ACSF at 2-3 ml·min^{-1}. Neocortical pyramidal cells were visualized by a custom-built infrared microscope (Versascope, E. Marton, CA) equipped with a 40× water immersion objective (Olympus, Ballerup, Denmark) and a CCD100 camera (DAGE-MTI, Michigan City, IN). Layer 2/3 pyramidal cells were identified under infrared video microscopy displaying large pyramidal-shaped soma with a prominent dendrite projecting to layer 1 and confirmed as regular spiking neurons in most experiments (Drasbek, Hoestgaard-Jensen and Jensen, 2007). Patch pipettes were pulled from borosilicate glass (O.D. = 1.5 mm, I.D. = 0.8 mm; Garner Glass Company, Claremont, CA) on a DMZ Universal Puller (Zeitz Instruments, Munich, Germany). For GABA$_A$ IPSC recordings, pipette resistances were 3-5 MΩ when filled with a solution containing (in mM): 140 CsCl, 2 MgCl$_2$, 0.05 EGTA, 10 HEPES, adjusted to pH 7.2 with CsOH (280-290 mosmol·kg^{-1}). For recording of postsynaptic GABA$_B$R-mediated GIRK currents, patch pipettes contained (in mM): 130 KOH, 10 KCl, 0.3 EGTA, 10 HEPES, 0.3 Na$_3$GTP, 2 MgATP and 5 disodium creatine-phoshate, adjusted to pH 7.3 with methanesulfonic acid (280–290 mosmol/kg), yielding K-methanesulfonate as the main constituent. For recording of combined slow and fast IPSCs, under conditions allowing for both GABA$_A$ and GABA$_B$ currents, internal Cl- was slightly raised and pipettes were filled with a solution containing (in mM): 120 KOH, 20 KCl, 0.3 EGTA, 10 HEPES, 0.3 Na$_3$GTP, 2 MgATP and 5 disodium creatine-phoshate, adjusted to pH 7.3 with methanesulfonic acid (280–290 mosmol/kg), yielding K-methanesulfonate as the main constituent. Whole-cell patch-clamp recordings were carried out using a MultiClamp 700B amplifier (Molecular Devices, Union City, CA). Giga seals (>1 GΩ) were always obtained before break-in. For isolated GABA$_A$ or GABA$_B$ receptor responses, neurons were voltage-clamped at a V$_{hold}$ of either -70 mV or -50 mV, and whole-cell capacitances and series resistances were noted. Resistances were compensated by 70-80% (lag 10 μs), and recordings were discontinued if series resistance changed by more than 20% or exceeded 20 MΩ (typical series resistances 10-14 MΩ). For

mixed $GABA_A$ and $GABA_B$ responses, the membrane was held between E_{Cl} (-47 mV) and E_K (-100 mV), and thus fast $GABA_A$ IPSCs are inward, but slow $GABA_B$ IPSCs are outward.

Data acquisition and analysis

All recordings were low-pass filtered (8-pole Bessel) at 3 kHz, digitized at 20 kHz, and acquired using a BNC-2110 D/A converter and a PCI-6014 board (National Instruments, Austin, TX) and custom-written LabVIEW 6.1–based software (EVAN v. 1.4, courtesy of Istvan Mody). As there was no apparent difference in cell size between pyramidal cells of WT and SSADH KO mice (21.1 ± 0.6 pF, $n = 27$, and 22.0 ± 0.5 pF, $n = 29$, respectively), the currents were presented without capacitance normalization in histograms. Unpaired two-tailed Student's t-tests were used to compare means with $P < 0.05$ as the significance level. Data are presented as means $\pm$ SEM, except for the averaged EC_{50} (means $\pm$ standard deviation), with n indicating the number of neurons. Concentration-response curves were generated by washing in different agonist concentrations, and a Hill function was used to fit current amplitudes normalized to the maximal current obtained with a saturating concentration of agonist: $y = 1/(1 + ([EC_{50}]/[x])^h)$, where EC_{50} is the concentration yielding the half-maximal response and h is the Hill coefficient. On these curves, each point represents the mean $\pm$ SEM across all experiments. Correlation of concentration-response curves was analyzed with linear regression in GraphPad Prism (Graph Pad Software, Inc., La Jolla, CA, USA).

Solutions and drugs

Baclofen, CGP55845, kynurenic acid were from Tocris (Avonmouth, UK), sodium 4-hydroxybutyrate (GHB) was from Lancaster Synthesis (Eastgate, England), while pyruvic acid was from MP Biomedicals (Irvine, CA). All other drugs and reagents were from Sigma (St. Louis, MO).

Results

Similar function of presynaptic $GABA_B$Rs on inhibitory nerve terminals in WT and SSADH KO mice

Initially, the present study focused on presynaptic $GABA_B$R mediated inhibition of GABAergic transmission onto layer 2/3 pyramidal neurons by minimal stimulation of putative single GABAergic axons onto neurons clamped at -70 mV (Jensen and Mody, 2001). Using stimulating intensities of 20-40% above threshold, $GABA_A$ receptor-mediated IPSCs (eIPSCs) were elicited in layer 2/3 pyramidal neurons in the presence of the glutamate receptor antagonist kynurenic acid (3 mM) (Fig. 1). Using paired-pulse stimulation with an inter-pulse interval of 100 ms, the ratio of the second eIPSC ($eIPSC_2$) relative to the first ($eIPSC_1$) was 0.71 ± 0.03 ($n = 15$) in WT and 0.72 ± 0.06 ($n = 13$, $P > 0.05$) in SSADH KO mice (Fig. 1). Furthermore, upon activating $GABA_B$ receptors with baclofen (10 μM), the amplitude of $eIPSC_1$ was depressed identically in WT (0.54 ± 0.05, $n = 8$) and SSADH KO mice (0.55 ± 0.04, $n = 8$). Finally, the increase in the paired-pulse ratio associated with lowering of the release probability by baclofen was similar in WT and SSADH. Thus, paired-pulse ratios in baclofen were 1.20 ± 0.08 for WT ($n = 8$) versus 1.19 ± 0.12 for KO ($n = 8$) ($P > 0.05$). These results indicate that the presynaptic $GABA_B$ receptor function at GABAergic synapses is similar in WT and SSADH KO mice.

Postsynaptic $GABA_B$R mediated inhibition is reduced in SSADH KO mice

In order to investigate possible changes in the postsynaptic $GABA_B$R function in SSADH deficient mice, outward currents induced by baclofen during 10 min wash in experiments

from the layer 2/3 pyramidal neurons were recorded. A supramaximal concentration of baclofen (100 μM) led to outward currents, which was associated with a concurrent decrease of the membrane resistance (R_{in}), indicating the increase of a potassium conductance, that could be blocked by the $GABA_B$ antagonist CGP55845 (8 μM) (Fig. 2A, B). In pyramidal neurons of WT mice, baclofen (100 μM) induced a current of 110.7 ± 6.5 pA and a decrease of R_{in} of 108.6 ± 13.1 MΩ ($n = 9$) (Fig. 2A, C). The current desensitized to $66.4 \pm 5.4\%$ during 10 minutes of continuous agonist application (Fig. 2A). In pyramidal neurons of SSADH KO mice, baclofen (100 μM) induced a potassium current of 71.1 ± 7.9 pA and a decrease of R_{in} of 71.3 ± 12.0 MΩ ($n = 12$) (Fig. 2B, C). In SSADH KO mice, the baclofen-evoked current was desensitizing to $68.2 \pm 5.4\%$ during 10 minutes of agonist application (Fig. 2B). As a result, SSADH KO mice exhibited lower $GABA_BR$ mediated potassium currents in neocortical pyramidal neurons compared to WT mice, while the currents desensitized to similar extents upon acute baclofen exposure.

EC_{50} for baclofen at postsynaptic $GABA_BRs$ in WT and SSADH KO mice

Previously, it was reported that chronic administration of GHB leads to cell-specific changes in the coupling efficiency between $GABA_B$ receptors and GIRK channels and, thus, potentially a strengthening in $GABA_B$ receptor mediated inhibition (Labouebe et al., 2007, Mutneja et al., 2005). To examine if the sensitivity to $GABA_B$ receptor agonist was affected in SSADH KO mice, a concentration-response relationship for baclofen (0.1 - 300 μM) was constructed using a population of responses from different pyramidal neurons of WT and SSADH KO mice (Fig. 2D). Averaged currents were normalized to maximal responses and fitted using the Hill equation. No major change in EC_{50} for baclofen between WT (8.11 ± 4.3 μM, h = 2.0, $n = 27$) and SSADH KO mice (5.09 ± 1.4 μM, h = 1.9, $n = 28$) was found. These data suggest that the coupling between $GABA_B$ receptors and GIRK channels is not affected in SSADH KO mice.

Accumulation of GABA or GHB in the slices is unlikely to explain reduced postsynaptic baclofen responses

The partial agonist at $GABA_BRs$ GHB, which is increased in the brains of SSADH KO mice, might bind to $GABA_BRs$ in the slice during the recording and compete with the effect of baclofen (Mathivet et al., 1997). To examine if this could be mimicked in WT slices, experiments in the presence of GHB (300 μM) were performed. Ten min preincubation of WT slices with GHB (300 μM) did not decrease the response of the pyramidal neurons to baclofen (100 μM) in WT mice. Indeed, in the presence of GHB (300 μM) wash in of baclofen (100 μM) induced a potassium current of 109.0 ± 11.0 pA ($n = 5$) in WT mice, which was similar to the response without GHB (110.7 ± 6.5 pA, $P > 0.05$) (Fig. 3).

Furthermore, in SSADH KO mice it is possible that the accumulation of both GABA and GHB in the slice could lead to tonically activated $GABA_BRs$, masking the baclofen-evoked potassium currents during slice recordings. To test if a tonic $GABA_BR$ activation could be detected in SSADH KO, CGP55845 (8 μM) was applied to KO slices during recordings. CGP55845 application revealed a very small tonic potassium current of 9.0 ± 2.6 pA ($n = 6$). As a consequence, endogenous $GABA_B$ agonists are unlikely to interfere significantly with the baclofen induced $GABA_B$ response. This finding argues against the possibility that reduced postsynaptic baclofen responses are due to elevated endogenous $GABA_B$ agonists in SSADH KO mouse slices.

Responses to adenosine are reduced in SSADH KO mice

It was shown earlier that prolonged activation of $G\alpha i/o$ coupled GPCRs can lead to heterologous desensitization of responses to similar acting neurotransmitters (Cornelisse et al., 2007, Blanchet and Luscher, 2002, Wetherington and Lambert., 2002), e.g. that baclofen

exposure can decrease the response to other GPCRs, including adenosine receptors. To examine if the decreased $GABA_BR$ response in SSADH deficiency includes alterations in downstream effector proteins, the responses to adenosine were tested. Wash in of adenosine (100 μM) led to outward currents in pyramidal neurons, also indicative of the activation of a potassium conductance. In WT mice, adenosine (100 μM) induced a current of 44.3 ± 8.3 pA and a decrease of R_{in} of 52.3 ± 5.7 MΩ ($n = 5$) (Fig. 4A, C). In SSADH KO mice, adenosine (100 μM) induced a significantly smaller current of 17.2 ± 3.6 pA and a decrease of R_{in} of 24.4 ± 5.6 MΩ ($n = 6$, $P < 0.05$) (Fig. 4B, C), i.e. only 39% of the WT adenosine current.

$GABA_B$ receptor responses mediated by synaptically released GABA are decreased in SSADH KO

Synaptically released GABA can generate chloride-channel dependent fast IPSCs mediated by $GABA_ARs$, and potassium-channel dependent slow IPSCs mediated by $GABA_BRs$ (Nicoll, 1988, Mott et al., 1999). To determine if alterations in the $GABA_BR$ mediated pathway in SSADH KO mice will attenuate synaptic potassium currents in neocortex, the magnitude of evoked slow IPSCs mediated by synaptically released GABA in WT and KO mice was compared. To ensure that the stimulating electrode activated inhibitory interneurons, fast IPSCs in the absence of $GABA_ARs$ antagonists were monitored, and recordings were done under conditions allowing for combined $GABA_A$ and $GABA_B$ responses (Luscher et al., 1997). The membrane was held between E_{Cl} (-47 mV) and E_K (-100 mV), and thus fast $GABA_A$ IPSCs were inward (V_{hold} -70 mV), while slow $GABA_B$ IPSCs (V_{hold} -50 mV) were outward. In order to induce a substantial synaptic release of GABA, extracellular stimulations consisting of 7 pulses at 100 Hz were employed (Mott et al., 1999). Again, average evoked fast IPSCs mediated by $GABA_ARs$ responses were unaltered in SSADH KOs (not shown, but see Fig. 1). However, using a holding potential of -50 mV, a significant decrease to 54% of control in the synaptically evoked $GABA_BR$ mediated responses was seen in SSADH KO compared to WT. In WT mice, the amplitude of the slow IPSCs averaged 70.1 ± 5.7 pA ($n = 12$) (Fig. 5A, C) while, in SSADH KO mice, the slow IPSCs were significantly smaller and averaged 38.2 ± 4.4 pA ($n = 15$) $P < 0.001$ (Fig. 5B, C).

For further analysis, the amplitude of $GABA_BR$ mediated responses were normalized to the peak amplitude of the $GABA_AR$ response obtained from the same cell. In WT, such normalized responses were $35.8 \pm 4.0\%$ ($n = 11$) (Fig. 5D) while, in SSADH KO mice, normalized $GABA_BRs$ mediated responses averaged only $18.4 \pm 2.7\%$ ($n = 20$) (Fig. 5D) ($P < 0.01$), illustrating the significantly reduced $GABA_BR$ mediated potassium currents during comparable synaptic releases of GABA.

Discussion

In the present study, presynaptic $GABA_BR$ dependent inhibition of GABA release onto pyramidal cells was not affected in SSADH deficiency, indicating that the presynaptic receptors controlling calcium influx and the transmitter release machinery were not disturbed. On the other hand, the postsynaptic $GABA_BR$ function was significantly altered, since we found a reduction in postsynaptic $GABA_BR$ mediated currents in neurons of SSADH KO mice. GHB in relevant pathophysiological concentrations failed to mimic this effect in neurons in WT slices. Moreover, despite the increased levels of GABA there was no major basal $GABA_B$ receptor activation in neurons of SSADH KOs. Although chronic GHB exposure may lead to cell-specific changes in the coupling efficiency between $GABA_BRs$ and GIRK channels (Labouebe et al., 2007), we found no major change in the EC50 for baclofen in SSADH KO mice. On the other hand, neurons of SSADH KO mice exhibited decreased responsiveness to the similarly acting neurotransmitter adenosine,

suggesting a heterologous desensitization and pointing to alterations in proteins in the cascade downstream of GABA$_B$Rs. Finally, slow GABA$_B$ IPSCs were decreased to 54% in KOs pointing to a physiological relevance of our pharmacological findings.

Overall, our results suggest that a differential plasticity of these types of pre- and postsynaptic GABA$_B$Rs may operate in the rodent brain *in vivo*. The defective GABA$_B$Rs function could play an important role in the seizure phenotype in SSADH knockout mice, and potentially also in the human disorder as well.

SSADH deficiency is associated with increased GABA and GHB

SSADH KO mice represent a genetic model of the severe case of human SSADH deficiency (Hogema et al., 2001). These mice manifest spike-and-wave discharge (SWD) and behaviors typical of absence seizures starting from age P10-14 evolving to myoclonic and generalized convulsive seizures from age P18, that progress into lethal status epilepticus (Cortez et al., 2004, Hogema et al., 2001). These changes have been electrophysiologically investigated in detail *in vivo* (Cortez et al., 2004). Comparable to human patients, SSADH deficient mice manifest accumulation of GABA (2-fold) and GHB (60-fold) in the brain (Jansen et al., 2008, Hogema et al., 2001). GHB is a weak agonist of GABA$_B$ receptors and present in low concentrations in the normal brain (2-4 μM), which is likely lower than what is necessary to activate GABA$_B$Rs (Vayer et al., 1988). Of relevance for the SSADH KO model, in pharmacological models of absence seizures, where administration of 3.5 mmol/kg of GHB induced spike-and-wave activity, rat brain concentrations of GHB reached 240 μM (Snead, 1991), comparable to the concentration found in brain of SSADH deficiency (150-240 μM) (Hogema et al., 2001). This led to the hypothesis that raised GHB is involved in the brain pathophysiology in SSADH deficiency. However, it is equally likely that accumulation of GABA might also play an important role by over-activating GABA receptors. Indeed, both GHB and GABA$_B$ antagonists partially rescue the lethal phenotype of SSADH KO mice (Hogema et al., 2001).

Presynaptic GABA$_B$Rs function on inhibitory terminals is similar in WT and SSADH KO mice

The presynaptic function of GABA$_B$Rs on GABAergic nerve terminals was not affected in SSADH KO mice upon activating GABA$_B$Rs with baclofen, since eIPSC$_1$ was depressed similarly in WT and SSADH KO mice. Also, the increase in the paired-pulse ratio associated with lowering of the release probability by baclofen was similar in WT and SSADH KOs. The synapses giving rise to these IPSCs are probably part of a perisomatic inhibitory system, since they show depressing GABA$_A$ responses. The presented data suggests that there could be a differential susceptibility for downregulation of postsynaptic G-protein-coupled GABA$_B$R function (discussed below), compared with presynaptic GABA$_B$Rs onto neocortical pyramidal neurons. Supporting this, cell-type specific mechanisms of desensitization of Gαi/o coupled pre- and postsynaptic receptors during prolonged agonist treatment have been reported. For example, postsynaptic GABA$_B$Rs and 5-HT$_{1A}$Rs of pyramidal neurons of CA1 seem to be resistant to downregulation by high levels of agonists in transporter knockout mouse models (Jensen et al., 2003, Mannoury la Cour et al., 2001). On the other hand, in dorsal raphe neurons, prolonged increased agonist levels caused by either serotonin transporter knockout (Mannoury la Cour et al., 2001) or chronic treatment with serotonin uptake inhibitor fluoxetine (Cornelisse et al., 2007) led to reduced somatodendritic 5-HT$_{1A}$R and GABA$_B$R responses. Similarly, in rat brain slices, postsynaptic, but not presynaptic, μ-opioid receptors can be acutely desensitized by the selective agonist DAMGO (Blanchet and Luscher, 2002). Finally, chronic treatment with agonists in cultured hippocampal neurons can desensitize postsynaptic, but not presynaptic, GABA$_B$Rs (Wetherington and Lambert, 2002).

The molecular background for these differences are currently unknown, although a plausible explanation can be different mechanisms of G-protein regulation in GABAergic interneuron nerve terminals versus the somatodendritic area of pyramidal cells (Cruz et al., 2004, Labouebe et al., 2007), or different subcellular expression of $GABA_B$Rs isoforms $GABA_{B1a}$ and $GABA_{B1b}$. There is also growing evidence of cell or cell-compartment specific composition of $GABA_B$R heterodimers (Bischoff et al., 1999, Huang, 2006). Indeed, it was shown that $GABA_{B1a}$ containing heterodimers are predominantly expressed at the glutamatergic presynaptic terminals (Shaban et al., 2006, Vigot et al., 2006, Waldmeier, Kaupmann and Urwyler, 2008), while $GABA_{B1b}$ containing heterodimers are mainly found at postsynaptic sites of pyramidal neurons of the neocortex (Perez-Garci et al., 2006). Interestingly, GABAergic presynaptic terminals seem to be equipped with both heterodimer isoforms (Tiao et al., 2008); they are less sensitive to low concentrations of GHB in neocortex and thalamus (Gervasi et al., 2003, Jensen and Mody, 2001, Li et al., 2007) and might be more stable with respect to agonist induced desensitization.

Postsynaptic $GABA_B$Rs mediated inhibition is reduced in SSADH KO mice

We found that postsynaptic $GABA_B$R mediated currents are significantly decreased in SSADH KO mice. This effect could potentially be explained by downregulation of the $GABA_B$R function in the SSADH KO neurons and/or by pathological extracellular conditions in slices from SSADH KO mice, such as high levels of GABA, GHB, or potassium. The latter is, however, unlikely since a similar change in membrane resistance was observed. On the other hand, we recently reported that GABA is elevated in slices of SSADH KO mice to a sufficient level for activation of extrasynaptic $GABA_A$Rs mediating a tonic current (Drasbek et al., 2008). Interestingly, in this study we found that tonic $GABA_B$ mediated currents in KO mice were absent or very small. Similarly, GABA transporter 1 deficient (GAT1 KO) mice show similar results in CA1 pyramidal neurons (Jensen et al., 2003), where no tonic $GABA_B$Rs mediated current could be revealed, despite a prominent $GABA_A$R current. This difference in basal activation levels between somatodendritic extrasynaptic $GABA_A$Rs and $GABA_B$Rs could reflect a different cell-compartment specific localization, density or sensitivity of extrasynaptic $GABA_A$Rs and $GABA_B$Rs (Brown et al., 2002, Kulik et al., 2003, Schuler et al., 2001). Finally, accumulation of GHB (a partial agonist of $GABA_B$ receptors) in the brain slices of SSADH KO mice might have reduced the effect of baclofen, by competing for the binding sites (Mathivet et al., 1997). However, exposure of WT slices to GHB in pathophysiologically relevant concentrations failed to affect the magnitude of the baclofen induced potassium currents. Overall, decreased $GABA_B$R mediated inhibition that we found in SSADH KO mice most likely reflects downregulation of $GABA_B$R function, rather then pathological conditions in the slices of KO mice during recordings.

Heterologous desensitization of postsynaptic $GABA_B$Rs in SSADH KO mice

Several factors could be involved in altered function of $GABA_B$Rs, including $GABA_B$R$_2$ subunit phosphorylation at several serines by Protein kinase A or 5′AMP-dependent protein kinase (AMPK) (Couve et al., 2002, Kuramoto et al., 2007). There is also evidence that constitutive turnover of $GABA_B$Rs in neurons is modulated by receptor activation or inhibition (Fairfax et al., 2004, Grampp et al., 2008, Wilkins, Li and Smart, 2008). Furthermore, the coupling between $GABA_B$R and GIRK channels are affected by phosphorylation of $GABA_B$Rs (Kuramoto et al., 2007) or changes in the expression or functioning of GIRK channel subunits (Cruz et al., 2004, Huang, Feng and Hilgemann, 1998, Labouebe et al., 2007, Logothetis et al., 2007).

$GABA_B$R signaling is also regulated by Regulators of G-protein signaling (RGS) proteins, which accelerate the rate of hydrolysis of GTP bound to the $G\alpha$ subunit (Jaen and Doupnik,

2006, Mutneja et al., 2005). Chronic administration of GHB affects the coupling efficiency between GABA$_B$Rs and GIRK channels in dopaminergic neurons of ventral tegmental area due to downregulation of RGS2 (Labouebe et al., 2007). To examine if receptor-effector coupling is affected during SSADH deficiency we compared the concentration-response relationships for baclofen in WT and SSADH KO mice. We found no major change in the EC$_{50}$ for baclofen and the rapid desensitization in pyramidal neurons of SSADH KOs showed no difference as well. Our results suggest that there is no major change in RGS activity, GIRK composition or GABA$_B$R phosphorylation in neurons associated with SSADH deficiency.

Finally, heterologous desensitization could account for our results on adenosine responses. This phenomenon is well described for opiate receptors, sharing pathways with GABA$_B$Rs (Terwilliger et al., 1991), when chronic administration of opiates to locus coeruleus neurons decreases responses to somatostatin and baclofen (Blanchet and Luscher, 2002). Similarly, the serotonin up-take inhibitor fluoxetine reduces responsiveness of dorsal raphe neurons to serotonin and baclofen (Cornelisse et al., 2007) and relevant to our study, incubation of hippocampal neurons with baclofen leads to decreased responses to adenosine (Wetherington and Lambert, 2002). Our data show that SSADH deficiency leads to decreases in both GABA$_B$ and adenosine receptor induced GIRK currents. Although a parallel downregulation of both receptor types in SSADH mice cannot be excluded, it is more likely that SSADH deficiency induces alterations in GABA$_B$R effector systems. Thus, we predict a decreased response of neocortex neurons to all neurotransmitters sharing similar pathways with GABA$_B$Rs. Overall, our data support the possibility of downregulation of GABA$_B$Rs or GIRKs, arguing against major changes in RGSs or GIRK subunit composition. However, future experiments are required to answer these questions in this mouse model of epilepsy.

Functional relevance

It is likely that the loss of slow postsynaptic inhibition could explain the progression into tonic-clonic seizures following the desensitization of postsynaptic, but not presynaptic, inhibitory GABA$_B$Rs. Accordingly, endogenous GABA and GHB accumulated in the brain could induce loss of postsynaptic inhibition on pyramidal cells, and this is likely to increase neocortical excitability and seizure prevalence that may ultimately be lethal.

Acknowledgments

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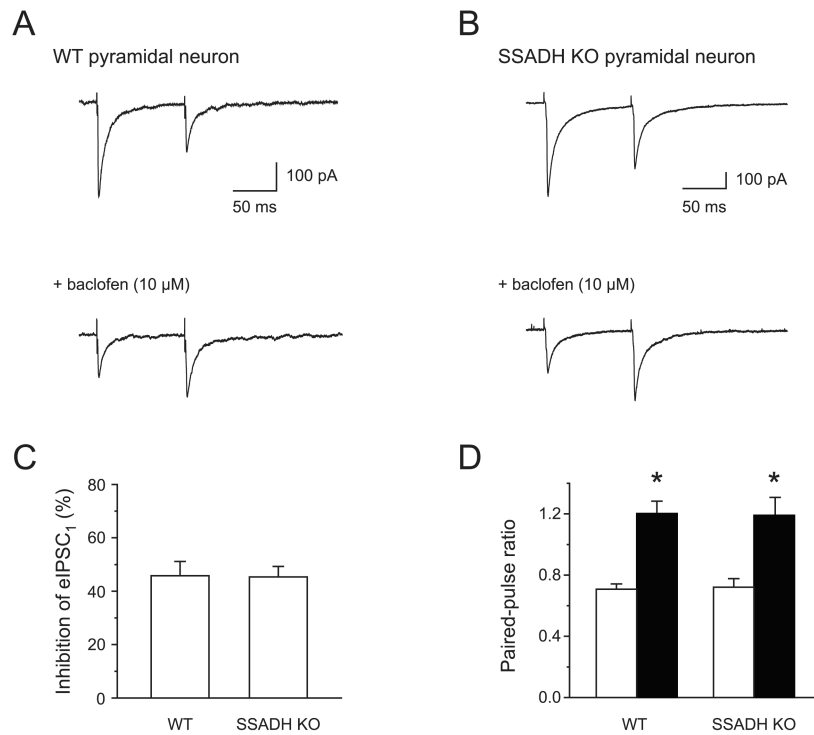
Fig. 1. Function of presynaptic GABA$_B$Rs in neocortical layer 2/3 pyramidal cells in WT and SSADH KO mice

(A) Whole-cell recordings of evoked IPSCs (eIPSCs) in neocortical layer 2/3 pyramidal cells using extracellular stimulation. In the WT (wild type) slice, the averaged eIPSC showed paired-pulse depression of 0.50 (amplitude of eIPSC$_2$ relative to eIPSC$_1$) at a 100 ms interval. The GABA$_B$R agonist baclofen (10 μM) depressed eIPSC$_1$ by 59%, and converted the paired-pulse depression into facilitation. **(B)** In the SSADH KO slice, paired-pulse depression of eIPSCs was observed. Baclofen depressed eIPSC$_1$ by 66%, again turning depression to facilitation. **(C)** Baclofen reduced the amplitude of eIPSC$_1$ in pyramidal cells equally in WT and SSADH KO mice. eIPSC$_1$ was depressed by 45.6 $\pm$ 7% in WT slices ($n = 8$), and by 45.9 $\pm$ 5% in SSADH KO slices ($n = 6$). **(D)** The histogram shows paired-pulse depression of eIPSC2, expressed as a ratio of eIPSC$_1$ (100 ms inter-pulse interval) for WT (left, $n = 15$) and SSADH KO (right, $n = 13$). Baclofen (10 μM) (filled bars) equally converted the paired-pulse ratio to facilitation in WT (left, $n = 8$) and SSADH KO (right, $n = 6$) mice, indicating similar properties of presynaptic GABA$_B$Rs.
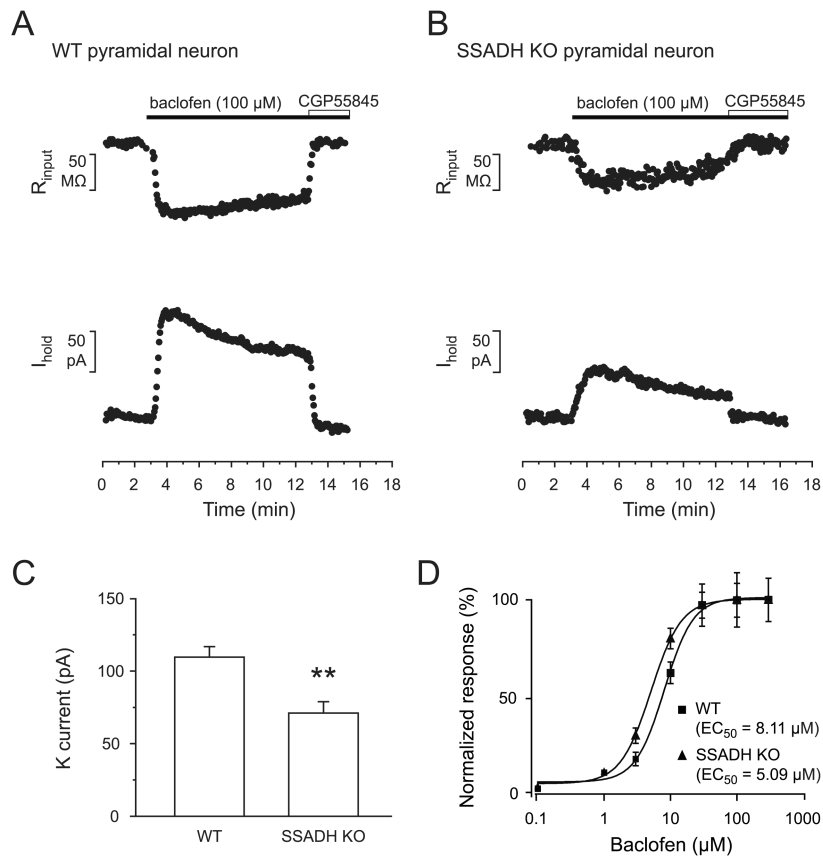
Fig. 2. Postsynaptic GABA$_B$R-mediated currents are reduced in layer 2/3 pyramidal cells in SSADH KO mice

(A-B) The GABA$_B$R agonist baclofen (100 µM) induced outward currents in pyramidal neurons in WT **(A)** and SSADH KO mice **(B)**, which were fully blocked by GABA$_B$ antagonist CGP55845. Note the concomitant reduction in input resistance (upper traces). The neurons were voltage-clamped at -50 mV. **(C)** Bar graphs representing the peak amplitude of the baclofen-induced outward currents in pyramidal cells of WT ($n = 9$) and SSADH KO ($n = 12$) mice (***: $P < 0.001$). **(D)** Concentration-response curves for baclofen are shown for pyramidal neurons of WT (squares; total $n = 27$) and SSADH KO (triangles; total $n = 28$). The mean EC$_{50}$ of baclofen-evoked currents were 8.11 µM and 5.09 µM in pyramidal neurons of WT and SSADH KO mice respectively.
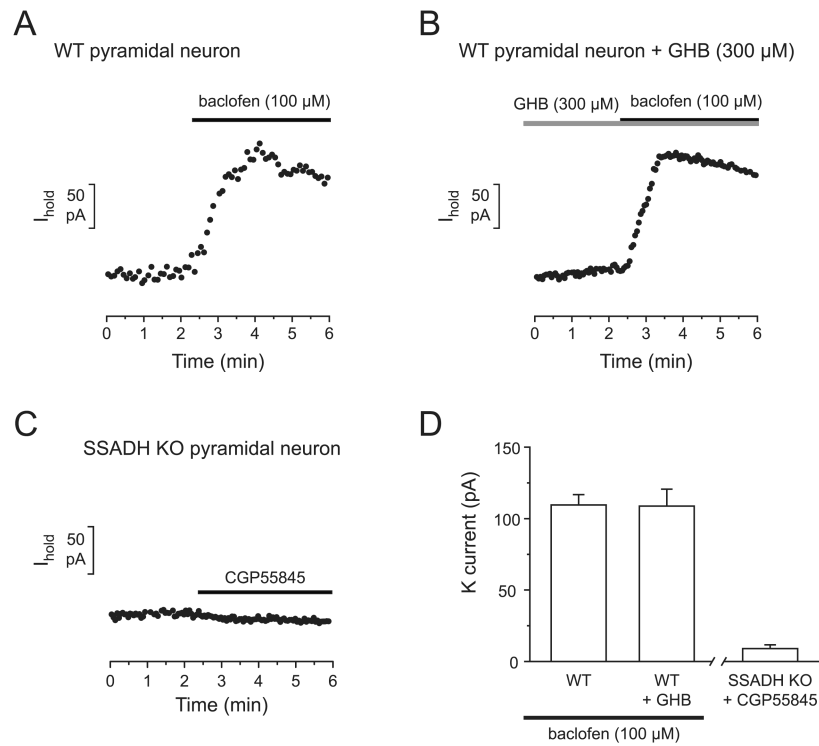
Fig. 3. Reduced baclofen currents in SSADH KO mice are not explained by accumulation of endogenous GHB or GABA in the slice
**(A-B)** Representative recordings of currents induced by baclofen (100 μM) in the absence **(A)** or presence **(B)** of GHB (300 μM) in pyramidal neurons of WT mice. GHB (300 μM) did not affect the peak response to baclofen. **(C)** Recording of the effect of CGP55845 in pyramidal neurons of SSADH KO mice. Application of CGP55845 to a SSADH KO slice revealed little tonic activation of GABA$_B$Rs by endogenous agonists. **(D)** Bar graphs represent the peak amplitude of the outward current evoked by baclofen (100 μM) in control ($n = 9$) and in the presence of GHB (300 μM) ($n = 5$) in WT slices. The rightmost bar shows the amplitude of tonic GABA$_B$ currents ($n = 5$) in the absence of exogenous agonists in SSADH KO mice.

A WT pyramidal neuron

B SSADH KO pyramidal neuron

C
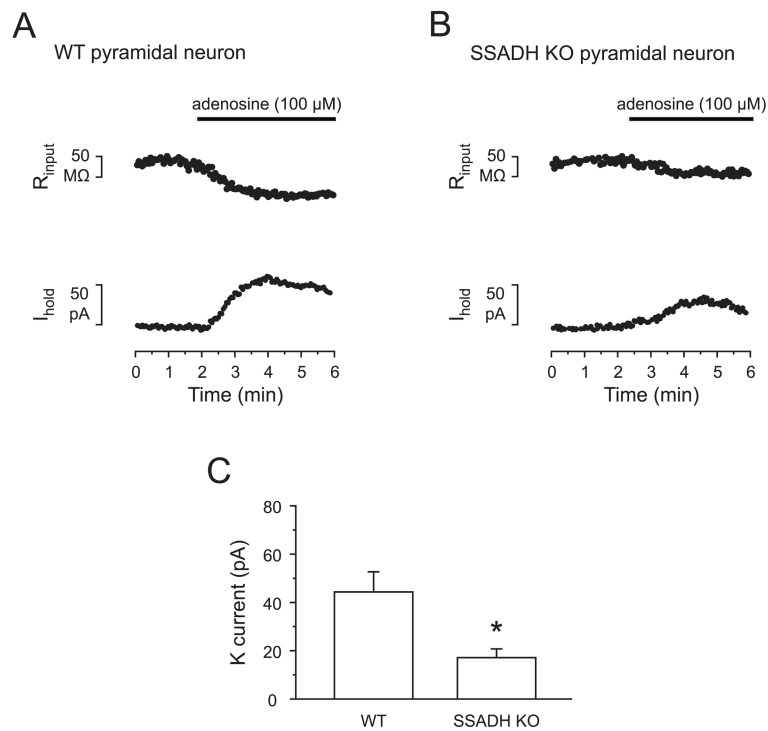
Fig. 4. Decreased responsiveness to adenosine in SSADH KO mice
(A-B) Adenosine (100 μM) induced outward currents in pyramidal neurons in WT **(A)** and SSADH KO mice **(B)**. Responses to adenosine (100 μM) were smaller in SSADH KO than in WT. Note the concomitant reduction in the input resistance (upper traces). The neurons were voltage-clamped at -50 mV. **(C)** Bar graphs representing the average peak amplitude of adenosine-induced outward currents in pyramidal cells of WT ($n = 5$) and SSADH KO ($n = 6$) mice (*: $P < 0.05$). In SSADH KO, the adenosine current was 39% of WT.
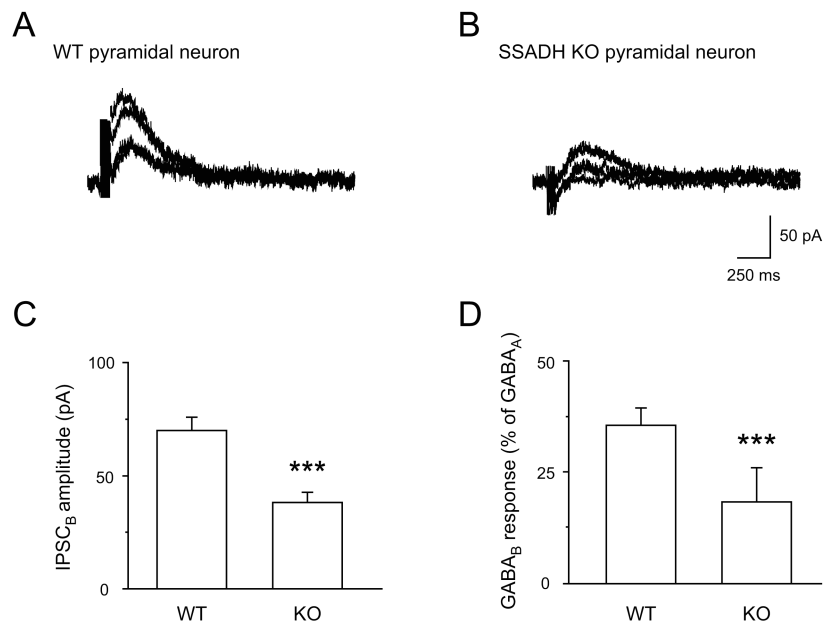
Fig. 5. Reduced synaptically evoked slow GABA_B receptor IPSCs in SSADH KO mice
(A-B) Slow GABA$_B$R-mediated IPSCs in pyramidal neurons of WT **(A)** and SSADH KO **(B)** mice. Currents were obtained in whole-cell configuration, by increasing the extracellular stimulus intensity above threshold evoking increasing GABA$_B$R IPSCs. Stimuli consisted of 7 pulses at 100 Hz, and the membrane was held at -50 mV (E_K = -103 mV) to minimize a GABA$_A$R component (E_{Cl} -47 mV) **(C)** Bar graphs representing the amplitude of the slow IPSCs in pyramidal cells of WT (n = 12) and SSADH KO (n = 15) mice (***: $P< 0.001$). **(D)** Bar graphs representing the amplitude of the GABA$_B$ IPSC normalized to the amplitude of the GABA$_A$ IPSC obtained from pyramidal cells of WT (n = 11) and SSADH KO (n = 20) mice (***: $P< 0.001$).