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# Activity-mediated plasticity of GABA equilibrium potential in rat hippocampal CA1 neurons

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ABSTRACT

The equilibrium potential ($E_{GABA\text{-}PSC}$) for γ-aminobutyric acid (GABA) A receptor mediated inhibitory postsynaptic currents (PSCs) in hippocampal CA1 pyramidal neurons shifts when theta-burst stimulation (four pulses at 100 Hz in each burst in a train consisting of five bursts with an inter-burst interval of 200 ms, the train repeated thrice at 30-s intervals) is applied to the input. $E_{GABA\text{-}PSC}$ is regulated by $K^+/Cl^-$ co-transporter (KCC2). $GABA_B$ receptors are implicated in modulating KCC2 levels. In the current study, the involvement of KCC2, as well as $GABA_B$ receptors, in theta-burst-mediated shifts in $E_{GABA\text{-}PSC}$ was examined. Whole-cell patch recordings were made from hippocampal CA1 pyramidal neurons (from 9 to 12 days old rats), in a slice preparation. Glutamatergic excitatory postsynaptic currents were blocked with DL-2-amino-5-phosphonovaleric acid (50 μM) and 6,7-dinitroquinoxaline-2,3-dione (20 μM). The PSC and the $E_{GABA\text{-}PSC}$ were stable when stimulated at 0.05 Hz. However, both changed following a 30-min stimulation at 0.5 or 1 Hz. Furosemide (500 μM) and KCC2 anti-sense in the recording pipette but not bumetanide (20 or 100 μM) or KCC2 sense, blocked the changes, suggesting KCC2 involvement. Theta-burst stimulation induced a negative shift in $E_{GABA\text{-}PSC}$, which was prevented by KCC2 anti-sense; however, KCC2 sense had no effect. CGP55845 (2 μM), a $GABA_B$ antagonist, applied in the superfusing medium, or GDP-β-S in the recording pipette, blocked the shift in $E_{GABA\text{-}PSC}$. These results indicate that activity-mediated plasticity in $E_{GABA\text{-}PSC}$ occurs in hippocampal CA1 pyramidal neurons and theta-burst-induced negative shift in $E_{GABA\text{-}PSC}$ requires KCC2, $GABA_B$ receptors and G-protein activation.



## Introduction

In the adult mammalian central nervous system (CNS), γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter. Three pharmacologically and molecularly distinct GABA receptors, $GABA_A$, $GABA_B$ and $GABA_C$, are well recognized. Fast synaptic inhibition is mainly due to the actions of GABA on ionotropic ligand-gated $GABA_A$ and $GABA_C$ receptors, whereas, metabotropic $GABA_B$ receptors, which are coupled to G-proteins and second messenger systems, mediate slow inhibition via an increased $K^+$ conductance. Structurally, $GABA_A$ receptors exist as hetero-oligomeric and a pentameric assembly of various subunits ($\alpha_{1-6}$, $\beta_{1-3}$, $\gamma_{1-3}$, δ, ε and π) (Mehta and Ticku, 1999). $GABA_C$ receptors are mostly insensitive to bicuculline, allosteric modulators and other specific agonists to $GABA_A$ receptors. They exist as a homomeric assembly of two of the subunits ($\rho_{1-3}$) (Johnston, 1996). $GABA_B$ receptors are heterodimers consisting two distinct subunits, GBR1a or GBR1b and GBR2 (Jones et al., 1998).

Together, GABA receptors impart a powerful regulatory influence on the excitability of the principal cells (Freund and Buzsaki, 1996; Stelzer, 1992; Wigstrom and Gustafsson, 1983). Evidence in literature suggests that a decreased $GABA_A$ receptor activity facilitates induction of long-term potentiation (LTP) of the excitatory postsynaptic potential (EPSP). $GABA_A$ receptor antagonists also increase the range of stimulating frequencies that elicit LTP (Grover and Yan, 1999; Steele and Mauk, 1999). Similarly, in the motor cortex, theta-burst activation of the inputs induces a LTP of the EPSP only if GABA-ergic inhibition is blocked by bicuculline (Hess et al., 1996). Moreover, Costa and Grybko (2005) found that in Ts65Dn mice, in which $GABA_A$ receptor-mediated inhibition is enhanced, LTP of the EPSP is difficult to induce unless GABA-ergic inhibition is pharmacologically blocked. Therefore, any changes in GABA-ergic transmission, including those in the equilibrium potential for the GABA-ergic IPSC ($E_{GABA\text{-}PSC}$), would have significant implications.

$GABA_A$ receptor channels are selectively permeable to $Cl^-$ and $HCO_3^-$ ions. The relatively small bicarbonate permeability has only a modest influence on the $E_{GABA\text{-}PSC}$ (Bormann et al., 1987), setting the $E_{GABA\text{-}PSC}$ slightly positive to the chloride equilibrium potential $E_{Cl^-}$ (Kaila and Voipio, 1987). On the contrary, at physiological concentrations, since the curve for $E_{GABA\text{-}PSC}$ vs. $[Cl^-]_i$ is steep, even small changes in $[Cl^-]_i$ can have profound effects on the $E_{GABA\text{-}PSC}$ (Staley and Smith, 2001; Jarolimek et al., 1999). $Cl^-$ driving force across neurons is tightly regulated by a family of cation chloride co-

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transporters (Ben-Ari, 2002; Delpire, 2000). Na-K-2Cl co-transporter (NKCC1) and K-Cl co-transporters (KCCs) are co-expressed in many neurons in a spatially distinct manner aiding the maintenance of a steady-state $Cl^-$ equilibrium (Duebel et al., 2006; Gavrikov et al., 2003; Khirug et al., 2008; Price and Trussell, 2006). KCC2 is exclusively expressed in CNS neurons (Karadsheh and Delpire, 2001; Payne et al., 1996; Rivera et al., 1999). Other KCC isoforms, KCC3 and KCC4, are also known to be expressed in several brain regions (Le Rouzic et al., 2006; Li et al., 2002; Pearson et al., 2001). However, their roles are unclear since specific antagonists are currently not available (Blaesse et al., 2009). Accumulation of $Cl^-$ in neonatal neurons that occurs through NKCC1 (Achilles et al., 2007; Yamada et al., 2004) accounts for $GABA_A$ receptor mediated excitatory responses in immature neurons (Plotkin et al., 1997). In rat hippocampal neurons, expression of KCC2, a chloride extruding co-transporter, matures with age (Luhmann and Prince, 1991; Zhang et al., 1991). Increased activity of KCC2 renders GABA-ergic PSCs hyperpolarizing in adult neurons (Deisz and Lux, 1982; Jarolimek et al., 1999; Misgeld et al., 1986; Rivera et al., 1999; Thompson et al., 1988), while a knockdown of KCC2 reverses this negative shift in $E_{GABA\text{-}PSC}$ (Rivera et al., 1999).

Although the levels of KCC2 are thought to increase with age and stabilize in adult neurons (Luhmann and Prince, 1991; Zhang et al., 1991), it is possible that KCC2 and $E_{GABA\text{-}PSC}$ are both dynamically regulated at all age groups. Short- and long-term depolarizing shifts in $GABA_A$ receptor-mediated responses have been observed during neuronal trauma, epilepsy and tetanic stimulation (Avoli, 1996; Rivera et al., 2004). Interictal activity in hippocampal slices exposed to a $Mg^{2+}$-free solution, kindling-induced seizures, all lead to a downregulation of KCC2 (Jin et al., 2005; Pathak et al., 2007; Rivera et al., 2004). Downregulation of KCC2 seems to involve a postsynaptic rise in intracellular $Ca^{2+}$ levels and PKC-dependent phosphorylation (Banke and Gegelashvili, 2008; Fiumelli et al., 2005). Upregulation of KCC2, on the other hand, switches depolarizing $GABA_A$ receptor-mediated responses in neonates to hyperpolarizing (inhibitory) responses in adult neurons (Ludwig et al., 2003).

Apart from the developmental shifts in $E_{GABA\text{-}PSC}$ (Rivera et al., 1999), activity-mediated regulation has also been reported in literature (Ouardouz and Sastry, 2000; Ouardouz and Sastry, 2005; Ouardouz et al., 2006; Xu and Sastry, 2007). Theta-burst stimulation, which is extensively used to induce plasticity due to its physiological relevance (Bland, 2004; Larson et al., 1986; Oddie and Bland, 1998; Patenaude et al., 2003; Staubli and Lynch, 1987), can induce a negative shift of the $E_{GABA\text{-}PSC}$ in both neonatal and adult rats (Ouardouz et al., 2006; Xu and Sastry, 2007). In the presence of furosemide, a KCC2 antagonist (Thompson and Gahwiler, 1989), the shift in $E_{GABA\text{-}PSC}$ and associated potentiation of the GABA-ergic inhibitory postsynaptic current (PSC) are blocked (Ouardouz et al., 2006; Xu and Sastry, 2007). The trigger for, and the mechanisms involved in, upregulation of KCC2 and plasticity of $E_{GABA\text{-}PSC}$ are unknown. Modulation of $E_{GABA\text{-}PSC}$ and KCC2 through different mechanisms has been reported in literature (Fiumelli et al., 2005; Lee et al., 2005; Wang et al., 2006). It is suggested that GABA itself can set off the shift in $E_{GABA\text{-}PSC}$ and that upregulation of KCC2 requires the activation of $GABA_A$ receptors (Ganguly et al., 2001). This view is disputed by other studies (Ludwig et al., 2003). Activation of $GABA_B$ receptors can suppress neurotransmitter release and dampen neuronal excitability (Nicoll, 2004). The receptors are activated during repetitive activity such as the theta rhythm (Isaacson et al., 1993) and suggested to be involved in synaptic plasticity (Davies et al., 1990; Wagner and Alger, 1995). Moreover, baclofen, a $GABA_B$ receptor agonist, increases the expression of KCC2 in hippocampus (Kang et al., 2006). However, whether $GABA_B$ receptors are involved in activity-mediated shifts of $E_{GABA\text{-}PSC}$ is currently unknown.

In the present study, experiments were carried out to examine if low frequency (0.05, 0.5 and 1.0 Hz) and theta-burst input activity can induce plasticity in $E_{GABA\text{-}PSC}$ through changes in KCC2 activity. Whether $GABA_B$ receptor and G-proteins activation are involved in theta-burst-mediated changes of $E_{GABA\text{-}PSC}$ was also studied.

## Methods

Experiments were performed on 9- to 12-day-old male Wistar rats (Animal Care Centre, The University of British Columbia). All experimental procedures were performed in accordance to the approved guidelines of Canadian Council on Animal Care. Every effort was made to minimize the number of animals used and their suffering. Animals were anesthetized with halothane and decapitated by a guillotine. The brain was then rapidly removed and transverse sections of the hippocampus (400 μm) obtained by procedures routinely used in our laboratory (Xu and Sastry, 2007). The CA3 region was cut off from the slice to diminish the influence of spontaneous activity from CA3 neurons. Slices were then allowed to recover for 1 h in an incubating chamber, containing in mM: 120 NaCl, 3 KCl, 1.8 $NaH_2PO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$ and 10 dextrose, saturated with carbogen (95% $O_2$–5% $CO_2$); pH was adjusted to 7.35–7.4 with NaOH. Following the incubation period, slices were transferred into the recording chamber, superfused with the same solution at 1.5–2 ml/min (25–26 °C).

CA1 pyramidal neurons were visually identified by their pyramidal shape, large soma and the presence of apical dendrites, using an upright microscope (Zeiss Axioskop 4 plus, Munich, Germany) equipped with Nomarski optics and an infrared camera. Patch pipettes were pulled from thin walled, 1 mm outer diameter, borosilicate glass capillary tubing with a filament (WPI) and the resistance of the final electrode ranged between 5 and 10 MΩ. Recording pipettes were filled with (in mM) 135 K gluconate, 10 HEPES, 10 KCl, 1 BAPTA, 5 Mg-ATP, 0.1 $CaCl_2$, 10 $Na_2$-phosphocreatine, 0.4 $Na_3$-GTP and creatine phosphokinase 50 U/ml (pH adjusted to 7.20–7.30 with KOH).

Cells were accepted for further study if the evoked PSCs were stable during the initial 10–20 min control recording period. Recordings were accepted if the series resistance and capacitance were compensated to 75% and if the holding current was stable. If the series resistance changed by more than 15% during a recording, the cell was not used for analysis. PSCs were recorded using an Axopatch 200A (Molecular Devices, Sunnyvale, USA) amplifier. Records were digitized using Digidata 1322A interface and Clampex Ver. 9.0 software (Molecular Devices, Sunnyvale, USA). Low pass filtering was set at 2 kHz. Data were analyzed using Clampfit Ver. 9.2 software by (Molecular Devices, Sunnyvale, USA). Graphical data illustrating the time course of changes in the PSC amplitude was calculated by measuring the peak amplitude, following the conditioning stimulation and expressed as a percentage of the averaged controls. Data were expressed as mean ± SEM. Statistical analysis of the data was performed using a two-tailed paired Student's *t*-test. The level of significance ($p$ value) was arbitrarily chosen to be $<0.05$. Because of the pharmacological nature of the experiments and the use of conditioning tetanic stimulations, only one cell per hippocampal slice was used in these studies. Therefore, $n$ refers to number of cells. A total of 114 slices from 60 animals were used for this study.

Currents were recorded in the whole-cell voltage clamp mode. PSCs from CA1 pyramidal neurons were evoked at 0.05 Hz control frequency by stimulations in the stratum radiatum. Control recordings were made at the above frequency for 10 min before conditioning stimulations (0.5 and 1 Hz or theta-bursts) were given. We previously reported that with whole-cell patch electrodes, when stimulated at this frequency, the PSCs as well as the $E_{GABA\text{-}PSC}$ were stable for up to 45 min. In addition, stability of the recorded PSCs was not different from recordings using pointed electrodes (Ouardouz et al., 2006). We therefore used whole-cell patch recordings in the current study, both for the quality of recordings and ease of applying drugs into individual neurons.