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THETA-BURSTS INDUCE A SHIFT IN REVERSAL POTENTIALS FOR GABA-A RECEPTOR-MEDIATED POST-SYNAPTIC CURRENTS IN RAT HIPPOCAMPAL CA1 NEURONS

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Abstract

Theta-burst stimulation of the stratum radiatum induces a negative shift in the reversal potential (RP) of γ -aminobutyric acid (GABA)-ergic postsynaptic currents (PSCs) in hippocampal CA1 neurons in brain slices from rats of age groups 3–4 day, 6–9 day and 3–4 wk. Furosemide reversed the shift in the RP. The amplitude of the evoked PSC appeared to increase following the theta-burst stimulation but this increase was secondary to the change in the RP. These results indicate that the RP for GABA-ergic PSCs undergoes an activity-dependent plasticity in not only neonatal but also adult neurons presumably through an up-regulation of a K^+ - Cl^- co-transporter. This plasticity can have significant implications for neuronal network activity in the central nervous system. Also, these results indicate that studies on GABA-ergic synaptic efficacy require a careful, parallel monitoring of the RP.

Keywords

GABA; Reversal potential; Plasticity; Hippocampus; Theta-burst; Postsynaptic currents; K^+ - Cl^- co-transporter

Tetanic stimulations of inputs induce a shift in reversal potential (RP) for γ -aminobutyric acid (GABA)-ergic postsynaptic currents (PSCs) in neurons of neonatal rat deep cerebellar nuclei (DCN) (Ouardouz and Sastry., 2000; Ouardouz and Sastry., 2005). Theta-bursts also induce a switch in the RP for GABA-A receptor-mediated PSCs in neonatal rat hippocampal CA1 neurons (Ouardouz et al., 2006). Age-related changes in the expression of neuron specific KCC-2 (Payne et al., 1996) are thought to play a major role during this plasticity (DeFazio et al., 2000; Kakazu et al., 1999). In the current study, we tested, if theta-bursts in stratum radiatum cause a change in the RP of GABA-ergic PSCs in neurons of postnatal 3–4 day, 6–9 day and 3–4 wk Wistar rat hippocampus, in a slice preparation.

Hippocampal slices were prepared according to the procedures described previously (Xu and Sastry, 2005). Slices were superfused for at least one hour before recording with artificial cerebrospinal fluid (ACSF) containing in mM: 120 NaCl, 3 KCl, 1.8 NaH_2PO_4 , 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$ and 10 D-glucose, and saturated with 95 % O_2 + 5 % CO_2 . Patch clamp

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recordings were made from pyramidal cells located in the CA1 area of hippocampal slices at room temperature (25–26 °C) with continuous oxygenated ACSF superfusion. The neurons were identified by their pyramidal shape, large soma and presence of apical dendrites. Recording electrodes were filled with a medium containing (in mM) 135 K-gluconate, 10 HEPES, 10 KCl, 1 K₄-bis-(2-aminophenoxy)-*N,N,N',N'*-tetraacetic acid (BAPTA), 5 Mg-ATP, 0.1 CaCl₂, 10 Na₂-phosphocreatine, 0.4 Na₃-GTP and creatine phosphokinase 50 U/ml (pH was adjusted to 7.20–7.30 with KOH).

Axopatch 200A and Digidata 1322 (Axon Instruments) were used for this recording. Data were acquired at 5 kHz and filtered at 2 kHz. Evoked PSCs were recorded at a holding potential of –60 mV. Dinitroquinoxaline-2,3-dione (DNQX, 20 μM) and 2-Amino-5-phosphonovaleric acid (AP-5, 50 μM) were present in the superfusing medium throughout the experiment to block *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor-induced responses (in all age groups except in slices from 3–4 day old rats in which only DNQX, but not APV, was present). The recorded PSCs were blocked by bicuculline indicating that they were GABA-A receptor mediated. This was consistent with other reports involving recordings from CA1 pyramidal neurons in hippocampal slices where, in most case, IPSCs evoked in the presence of DNQX and AP-5 were mainly mediated by GABA-A receptors (Draguhn and Heinemann, 1996; Poelchen et al., 2000). The peaks of GABA-A receptor-mediated and GABA-B receptor-mediated IPSCs are clearly separated by more than about 100 ms (Wu et al., 2005). Therefore, even if GABA-B IPSCs were present in some of our records, the amplitude measurements we made were of the GABA-A receptor mediated IPSCs.

Theta-bursts were given in the stratum radiatum (4 pulses at 100 Hz in each burst in a train consisting of 5 bursts with an inter-burst interval of 200 ms, the train repeated thrice at 30 s intervals) and their effects on the RP and the amplitude of the IPSC tested 3, 15 and 30 min following the conditioning stimulation.

The RP of the PSC was calculated by recording the synaptic current during 500 ms voltage pulses applied on the holding potential (–100 or –90 to –40 or –30 mV). Steady-state currents were measured close to the end of the 500 ms pulse and were subtracted from the peak PSC amplitude. The resulting PSC amplitude was plotted against the holding potential. The RP was extrapolated from a linear regression of the PSC amplitude vs. the membrane potential. Recordings were accepted if the series resistance could be properly compensated to 75% and if PSCs in control recordings were stable. Off-line data analysis was performed with Clampfit 9 (Axon Instruments).

Data were analyzed using a Student's *t*-test and reported as mean ± SEM. Multiple comparisons were conducted using one-way analysis of the variance (ANOVA). The level of statistical significance was taken as *P* < 0.05. *n* refers to the number of cells studied. Recordings were made from a total of 39 neurons. We used one cell per slice.

In control recordings (i.e., without the theta-burst stimulation), when RPs were monitored for 45 minutes, there was a small, but insignificant (*p* > 0.3), change (in slices from 3–4 wk old rats at 10 min: –63.56 ± 0.55 mV, at 45 min: –64.13 ± 0.41 mV, *n* = 9; in 3–4 day old rats at 10 min: –56.50 ± 1.00 mV, at 45 min: –57.90 ± 1.10 mV, *n* = 8). In neurons from slices of 3–4 wk old rats, in which theta-bursts were given to the stratum radiatum, the RP was significantly (*p* < 0.05) shifted in the negative direction when examined 3 and 30 min post-theta (before theta: –63.33 ± 2.43 mV, 30 min after theta: –67.93 ± 3.42 mV; *n* = 6) and this effect lasted for 30 min post theta-burst activation when recordings were terminated. The amplitude of the evoked PSC appeared to increase to 142.16 ± 14.20 % (*n* = 6) following the theta-burst stimulation. However, if the slices were superfused with furosemide (0.5 mM; drug was applied

after the theta-burst stimulation), the increases in the RP and the PSC amplitude induced by theta-bursts were decreased to control levels (RP: -62.10 ± 2.29 mV; $n = 5$; $p > 0.7$; PSC amplitude: 100.32 ± 3.54 %; $n = 5$; $p > 0.8$). Moreover, the slopes of I–V plots did not change after theta-burst stimulation (see Fig. 1). As in the case of 3–4 wk old slices, the RP was shifted in the negative direction in neurons from 3–4 day and 6–9 day old rats (please see Table 1 for details). Furosemide, when tested on 3 control neurons (in which no theta-bursts were given) did not significantly changed the RP of the IPSC (before furosemide application: 63.17 ± 0.59 mV & after furosemide application: 61.92 ± 0.46 mV; $P > 0.05$). This observation is consistent with those of (Banks et al., 1998; Jarolimek et al., 1996; Jarolimek et al., 1999; Ouardouz and Sastry, 2005; Pearce, 1993).

Previous studies in our laboratory indicated that tetanic stimulations of inputs induce a shift in the RP for GABA-ergic PSCs of neonatal rat DCN neurons (Ouardouz and Sastry., 2000; Ouardouz and Sastry., 2005) and neonatal rat hippocampal CA1 pyramidal neurons (Ouardouz et al., 2006). In DCN neurons, the tetanus-induced negative shift in the RP seems to be due to an increased expression and activation of KCC-2 through an activation of protein kinase A, protein synthesis and activation of protein phosphatases (Ouardouz and Sastry, 2005). Theta-burst conditioning stimulation has been extensively used to induce synaptic plasticity because of its physiological relevance (Larson et al., 1986; Staubli and Lynch, 1987). In the current study, we found that theta-burst stimulation also can introduce a shift in the GABA-ergic RP not only in neonatal but also adult rat hippocampal CA1 neurons and that any changes in the amplitude of the PSC is secondary to this shift in the RP. Furosemide, which diminishes the change in the theta-burst-induced switch in the RP, also reversed the apparent increase in the IPSC amplitude. Moreover, the IPSC conductance was not changed with theta-burst stimulation. These results are not in agreement with the conclusions of Patenaude et al. (2003) who reported that the theta-bursts induced an increase in the amplitude of the IPSC. Although, they did find that the RP switched after theta-bursts, they concluded that it was not that important for their conclusion that the amplitude of the IPSC was changed.

The RP of the GABA-evoked current depends on the intracellular and extracellular Cl⁻ concentrations (Jackel et al., 1994). The expression of the KCC-2 has been correlated with the RP of GABA-ergic PSCs. The finding that the application of furosemide (which is a Cl⁻-co-transporter antagonist) reverses the effect of theta on the RP of the PSC is consistent with our earlier observations in neonatal neurons and suggests that the change in the RP is secondary to changes in the Cl⁻-co-transporter (Ouardouz and Sastry., 2005). Korpi et al. (1995) reported that furosemide but not bumetanide had effects on GABA-A receptor-mediated currents. They also felt that furosemide might not be a very selective antagonist of KCC-2. In our earlier studies (Ouardouz and Sastry, 2005), the effect of furosemide on the RP of IPSCs was mimicked by injecting KCC-2 anti-sense into the recording neurons. In the current study, it is possible that furosemide was acting on KCC-2 and further confirmation can be obtained by examining the effects of the anti-sense or checking if bumetanide had no effect on the RP. Although the levels of KCC-2 are thought to increase with age and stabilize in adult neurons, an activity-dependent up-regulation of KCC-2 function had been reported even in adult neurons (Leitch et al., 2005). The RP of GABA-ergic PSCs is also generally thought to stabilize in adult neurons making the PSC hyperpolarizing at resting membrane potentials. As can be seen in the current study, the RP for the PSC in adult neurons does shift following a theta-burst stimulation indicating that there is still plasticity in the RP of adult neuronal GABA-ergic PSCs.

In neonatal neurons, RPs for GABA-ergic PSCs are such that the amino acid is excitatory on central neurons (Chen et al., 1996; Luhmann and Prince, 1991; Misgeld et al., 1986). This excitatory action has been suggested to substitute for AMPA-receptor mediated depolarization of postsynaptic neurons in facilitating glutamatergic synaptic plasticity through the removal of the Mg²⁺ block of the NMDA receptor (Cherubini et al., 1991). Interestingly, theta-burst

stimulation of the stratum radiatum induces not only a recruitment of AMPA receptor mediated excitatory postsynaptic currents (EPSCs) in neonatal CA1 neurons but also switches the RP of GABA-ergic PSCs making them inhibitory. Depending on the RP of the inhibitory PSC (IPSC), the shunting efficacy of the IPSC in dampening excitatory transmission can vary. Since theta-bursts shifted the RP to more negative potentials, excitatory transmission can be more prone to shunting than before. Perhaps, theta activity in neuronal networks may set the level of excitation at a lower level and provide protection from hyperactivity in excitatory pathways through the plasticity of RPs for GABA-ergic PSCs.

Theta bursts were effective in shifting the RP of the IPSCs in about 70% of the cells recorded. Heterogeneity among hippocampal pyramidal cells with respect to their participation in theta generating activity was recently reported by Bland et al. (2005) and this may explain why theta is effective in switching the RP in some and not the other cells.

Theta-burst stimulations in the stratum radiatum can produce synaptic plasticity in the hippocampal CA1 area through activation of glutamatergic receptors. In the current study, we blocked the NMDA and AMPA receptors, but metabotropic receptors are still functional. Unpublished observations in our laboratory suggest that (RS)- α -ethyl-4-carboxyphenylglycine (E4CPG) dampens the theta-burst induced switch in the GABA-ergic PSCs in hippocampal CA1 neurons. Therefore, the involvement of glutamatergic metabotropic receptors is worth pursuing. Patenaude et al. (2003) reported that theta-bursts can induce a long term potentiation (LTP) of the IPSC amplitude in hippocampal CA1 neurons from 4–6 wk old rats and that a metabotropic antagonist attenuated this LTP. However, as can be seen in the current study, the change in the IPSC amplitude induced by theta-burst stimulation is secondary to changes in the RP.

In conclusion, theta-bursts can induce a shift in the RP for GABA-ergic PSCs in neonatal as well as adult hippocampal CA1 neurons. Therefore, studies examining changes in GABA-ergic synaptic potentials require a careful, parallel monitoring of the RP for the IPSC.

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Fig. 1. RP of the IPSC recorded in adult (3–4 wk) hippocampal CA1 neurons. **A:** Theta-burst stimulation induces a shift in the RP. IPSCs were evoked at different holding potentials (–100 to –40 mV in 10 mV steps). a: Control recording. b: Records taken after the theta-burst tetanus (4 pulses at 100 Hz in each burst in a train consisting of 5 bursts with an inter-burst interval of 200 ms, the train repeated thrice at 30 s intervals). c: Records taken after application of furosemide. d: I–V relationship for the same cell. **B:** Control recording for 45 min (a different cell from A, shown above). IPSCs evoked at different holding potentials (–90 to –30 mV in 10 mV steps). a: 10 min. control recording. b: 45 min. control recording. c: I–V relationships for the same cell. APV and DNQX were present (in **A** & **B**) throughout the experiment. Note

that the RP was shifted in the negative direction after theta-burst stimulation and that furosemide reversed this effect.

Table 1

Theta-burst induced shift in RP of The IPSC in 3 age groups

	RP (mV)	
	Control	After theta-burst
3–4 day (n=8)	-56.07 ± 0.91	$-63.13 \pm 1.89^*$
6–9 day (n=8)	-59.04 ± 2.33	$-64.36 \pm 2.12^*$
3–4 wk (n=6)	-63.33 ± 2.43	$-67.93 \pm 3.42^*$

Data are means $\pm$ SEM.* Indicates that the values are significantly difference from the respective controls ($p < 0.05$).