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Research Report

Activity-mediated shift in reversal potential of GABA-ergic synaptic currents in immature neurons

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Abstract

γ-Aminobutyric acid (GABA), which is inhibitory in the adult central nervous system, can be excitatory in the developing brain. The change from excitatory to inhibitory action of GABA during development is caused by a negative shift in its reversal potential. Here, we report a presynaptic activity-mediated negative shift in the reversal potential of the GABA-mediated synaptic currents in immature deep cerebellar nuclei neurons. This shift appears to be due to an increased expression and activation of the K^+-Cl^- co-transporter type 2 (KCC-2) through the activation of protein kinase A, protein synthesis and activation of protein phosphatases. Thus, maturation of the GABA response may rely on an activity-dependent up-regulation of KCC-2.

Theme: Development and regeneration
Topic: Neurotransmitter system and channels

Keywords: GABA; Reversal potential; Deep cerebellar nucleus; Inhibitory postsynaptic current; K^+-Cl^- co-transporter

1. Introduction

γ-Aminobutyric acid (GABA), acting via $GABA_A$ receptors, induces a depolarizing (excitatory) response in immature neurons compared to the hyperpolarizing (inhibitory) one in mature neurons [1,3]. This is caused by an elevated intracellular Cl^- concentration in immature neurons and a shift in reversal potential of the GABA response towards a more depolarized level than the resting membrane potential [2,14,16]. The K^+-Cl^- co-transporters, especially the KCC-2, are thought to play a major role in rendering GABA hyperpolarizing during neuronal maturation [4,8]. KCC-2 is expressed only in neurons [21] and undergoes an up-regulation during development [13,22,23]. The cellular mechanisms involved in developmental changes in KCC-2 expression are still unclear.

Activity-mediated plasticity of GABA-ergic postsynaptic currents (PSCs) has been reported [5,19,28]. In the present study, we examined the mechanisms involved in high-frequency stimulation (HFS)-induced changes in reversal potential for the $GABA_A$ receptor-mediated PSCs in neurons of deep cerebellar nuclei (DCN) in immature rat brain slices. Part of this work has been published as an abstract [20].

2. Materials and methods

Experiments were performed on sagittal cerebellar slices (400 μm thick) prepared from 2 to 3 (P2–3) days old Wistar rats as previously described [19]. The external solution contained in mM: 120 NaCl, 3 KCl, 1.8 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 dextrose saturated with 95% O_2–5% CO_2. All experiments were carried out with (±)2-amino-5-phosphonovaleric acid (APV, 50 μM) and 6,7-Dinitroquinoxaline-2,3-dione (DNQX, 20 μM) being present

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in the perfusion medium to block *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor-mediated synaptic transients, respectively.

Whole-cell patch-clamp recordings were made using an Axopatch 200 A (Axon Instruments) on visually identified DCN neurons at room temperature. Data were acquired at 5 kHz and filtered at 2 kHz. Recordings were digitized and stored with a PC-DOS microcomputer-based data acquisition system (Digidata 1200 and pClamp6, Axon Instruments). Whole-cell recording pipettes (5–10 MΩ) contained (in mM) 135 K-gluconate, 10 HEPES, 10 KCl, 1 K_4-bis-(2-aminophenoxy)-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), 5 Mg-ATP, 0.1 $CaCl_2$, 10 Na_2-phosphocreatine, 0.4 Na_3-GTP, in addition to creatine phosphokinase 50 U/ml (pH adjusted to 7.20–7.3 with KOH). (Unless noted otherwise, all recording patch pipettes contained 1 mM BAPTA.) Recordings were considered acceptable if the series resistance (10–20 MΩ) could be properly compensated to 75% and if the holding currents were stable. If any change of series resistance more than 15% occurred during recording, the cell was discarded from analysis. The external potential was recorded at the end of the experiment and ranged from −1 to +3 mV. GABA postsynaptic currents (GABA-PSCs) were evoked by electrical stimulation of the white matter surrounding the DCN with constant current pulses (30–100 μA, 0.1–0.2 ms duration). Cells were accepted if GABA-PSCs were stable during the initial 10–15 min control period. High-frequency stimulation consisting of a train of stimuli at 10 Hz for 2–5 min was delivered under current-clamp conditions. This particular frequency train was chosen because the inferior olive neurons which innervate the cerebellar cortex as well as deep nuclei, have the propensity to fire at 10 Hz and GABA-ergic plasticity was observed when the inputs to DCN were tetanized at this physiologically relevant frequency [17]. GABA-PSC reversal potentials were calculated by recording the synaptic current at various holding membrane potentials (−90 to −35 mV, 5 mV step) produced by 500 ms voltage pulses. The GABA-PSC amplitudes were plotted against the holding membrane potential. Reversal potentials were extrapolated from a linear regression of GABA-PSC amplitude versus membrane potential.

KCC-2 anti-sense or sense (control) phosphorothioate oligodeoxynucleotides used in this study are the same sequence as the one published by Rivera et al. [23] termed anti-sense A and sense A. Anti-sense and sense (control) oligodeoxynucleotides to the spanning translation initiation region of KCC-2 mRNA were constructed according to Rivera et al. [23], and were directed against the sequence +59 relative to the ATG starting signal. KCC-2 anti-sense sequence: 5′-TCTCCTTGGGATTGCCGTCA-3′ and sense: 5′-TGACGGCAATCCCAAGGAGA-3′. These sense and anti-sense mRNAs were constructed by Invitrogen life technologies, Burlington Ontario, CANADA. Neurons were loaded with anti-sense or sense KCC2-oligodeoxynucleotides through the recording pipette. Anti-sense and sense oligodeoxynucleotides were dissolved in the recording solution from concentrated stock solutions.

3. Results

Postsynaptic currents generated by $GABA_A$ receptor activation (GABA-PSC) from P2–3 DCN neurons were depolarizing. The GABA-PSC reversal potential ($E_{GABA\text{-}PSC}$) was more positive (−54.2 ± 0.9 mV, n = 16) (Figs. 1A, B) than the resting membrane potential of −63.2 ± 1.0 mV (t test $P < 0.05$, n = 12).

High-frequency stimulation (HFS, 10 Hz 2–5 min) of the white matter surrounding the DCN induced a significant negative shift in the $E_{GABA\text{-}PSC}$ by −10.1 ± 1.3 mV, measured 30 min after HFS (paired t test $P < 0.001$, n = 14) (Figs. 1A, B). No significant change in the PSC conductance was observed (33.6 ± 6.5 nS in controls, compared to 32.0 ± 6.6 nS, 30 min after HFS, paired t test $P > 0.05$). In control experiments, i.e., without HFS (Figs. 1C, D), the $E_{GABA\text{-}PSC}$ did not significantly change over the same period of time (the shift: −1.3 ± 0.3 mV, paired t test $P > 0.05$, n = 8). Moreover, the same HFS had no effect on $E_{GABA\text{-}PSC}$ in neurons from P7–9 animals [17]. We also examined the effect of HFS on the reversal potential in P2–3 neurons recorded with pointed tip electrodes. As in the case of patch-pipette recordings, the $E_{GABA\text{-}PSC}$ was significantly shifted by −10.3 ± 1.2 mV (paired t test $P < 0.001$; n = 9) after the HFS, suggesting that the HFS-induced shift in $E_{GABA\text{-}PSC}$ was not due to a dialysis of the intracellular contents when recordings were made with patch electrodes. Therefore, subsequent experiments were carried out using the patch-clamp recording technique.

The change in $E_{GABA\text{-}PSC}$ is probably due to a change in the concentration of intracellular Cl^-. To examine if the shift in the $E_{GABA\text{-}PSC}$ is due to an activation of Cl^- extrusion by KCC, we used furosemide, an inhibitor of this co-transporter [27]. Bath application of furosemide (0.5 mM) for 5 min did not have any significant effect on $E_{GABA\text{-}PSC}$ (−55.0 ± 1.1 mV in control versus −55.3 ± 0.9 mV in the presence of furosemide, paired t test $P > 0.05$, n = 8, Figs. 2A, B). When HFS was given in the presence of furosemide (drug application was initiated 5 min before HFS), no significant change in $E_{GABA\text{-}PSC}$ was observed (the shift was −1.2 ± 1.3 mV, paired t test $P > 0.05$, n = 5, Figs. 2A, B). The washout of furosemide, however, resulted in the unmasking of the shift in the GABA-PSC reversal potential (−69.2 ± 1.5 mV, paired t test $P < 0.001$, n = 4, data not shown). In another set of experiments, furosemide was applied 30 min after HFS was given (Figs. 2C, D). HFS induced a negative shift of $E_{GABA\text{-}PSC}$ by −9.3 ± 1.6 mV but furosemide reversibly blocked this shift to −1.0 ± 0.7 mV (paired t test $P < 0.05$, n = 4). These results raise the possibility that the HFS