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Long-term Suppression of GABAergic Activity by Neonatal Seizures in Rat Somatosensory Cortex

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Abstract

Here we studied the long-term effects of neonatal seizures on inhibitory synaptic transmission in somatosensory cortex. We found that recurrent flurothyl-induced seizures result in a marked reduction in amplitude of spontaneous inhibitory postsynaptic currents (IPSCs) and increases of miniature IPSCs interevent intervals. These results indicate that decreasing the inhibitory synaptic strength following neonatal seizures in neocortical neurons is not due to a postsynaptic mechanism.

Keywords

early seizures; GABA; somatosensory cortex

1. Introduction

Children are at particularly high risk for seizures during the first months of life (Hauser, 1995; Ronen et al., 1995). This high incidence of seizures is related both to birth injuries that can cause seizures and the high propensity for seizures in the developing brain (Haut et al., 2004; Ben-Ari and Holmes, 2006).

We have previously shown that neonatal seizures in rats result in a reduction of GABAergic synaptic conductivity (Isaeva et al., 2006). While these data are valuable in understanding the consequence of changes in brain function following neonatal recurrent seizures, clinical studies show that early-life seizures typically involve neocortical structures (Acharya et al., 1997; Mizrahi, 1999) and that sequelae following neonatal seizures typically involves the neocortex more than the hippocampus (Ronen et al., 2007). The goal of the present study was to determine the long-term effect of neonatal flurothyl-induced seizures on inhibitory synaptic transmission as revealed by whole-cell patch-clamp recordings of spontaneous and miniature IPSCs (sIPSCs and mIPSCs) in somatosensory cortex.

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2. Methods

Sprague-Dawley rats ($n = 16$) were used throughout the study and were treated in accordance with the guidelines set by the National Institute of Health and Dartmouth Medical School for the humane treatment of animals. Rats were subjected to 58–60 flurothyl-induced seizures from postnatal (P) day 1 to 10 (5–6 seizures per day) using previously described methods from our laboratory (Liu et al., 1999; Isaeva et al., 2006). Control rats were handled and treated in the same manner but not exposed to flurothyl.

At P20–30 rats were deeply anaesthetized with isoflurane and decapitated, brains were removed, and brain slices were sectioned (Leica 1000S vibroslicer, Leica Microsystems, Nussloch GmbH, Germany) at a thickness of 500 μm in cold (4°C) dissecting solution of the following composition (mM): sucrose 250, KCl 2, CaCl_2 0.5, MgCl_2 7, NaHCO_3 26, NaH_2PO_4 1.2 and glucose 11 (pH=7.4). Slices were then stored in warm (32°C) oxygenated (95% O_2 , 5% CO_2) artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl 126, KCl 3.5, CaCl_2 2.0, MgCl_2 1.3, NaHCO_3 25, NaH_2PO_4 1.2 and glucose 11 (pH 7.3) for at least 1.5 h before use.

sIPSCs and mIPSCs were recorded from visually identified (Olympus BX51WI, Japan layer 2/3 (L2/3) pyramidal cells of somatosensory cortex using patch-clamp technique in whole-cell configuration. For recordings of sIPSCs the slice was continuously superfused with oxygenated ACSF at 32°C . mIPSCs were recorded in the presence of tetrodotoxin (TTX, 1 μM). Patch electrodes were pulled (Narishige, PC-10, Japan) from borosilicate glass capillaries (GC150F-15, Clark Electromedical Instruments) and had an impedance of 2–3 MO when filled with a solution of the following composition (in mM): Cs-gluconate 117.5, CsCl 17.5, NaCl 8, HEPES 10, EGTA 10, Na_3GTP 0.2, and MgATP 2 (pH 7.3). Whole-cell recordings were made using an Axopatch 200B amplifier (Axon Instruments). A liquid junction potential was subtracted from the recorded membrane potentials. The series resistances were compensated on 80–90%. Recordings were digitized online with the PClamp 7 software (Axon Instruments) using a Digidata 1322A acquisition board (Axon Instruments). Spontaneous events were analyzed with the MiniAnalysis (version 6.0.7, Synaptosoft Inc., Decatur, GA), Clampfit (Axon Instruments) and Origin 7.0 (Microcal Software, Northampton, MA, USA) software. The amplitude and interevent interval of sIPSCs and mIPSCs were estimated for every single event then combined in two groups (control and flurothyl-treated) and averaged. Rise time and half-width of IPSCs were estimated and averaged for each cell and the mean values were averaged and compared for control and flurothyl-treated rats. All data are expressed as the mean $\pm$ SE. The statistical significance of differences in amplitude and kinetic parameters of IPSC were evaluated using the Student's t test. To compare the interevent interval of IPSC in groups we used the Kolmogorov–Smirnov (K-S) test. SR95531 was obtained from Tocris (Ellisville, MO, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

3. Results

In the whole-cell patch-clamp recordings sIPSCs were observed as transient outward currents from a holding potential of 0 mV (reversal potential of EPSCs). These currents were blocked by the application of SR95531 (10 μM), a GABA_A receptor antagonist, and, therefore, represent GABA_A receptor-mediated IPSCs.

A summary histogram of different characteristics of sIPSC recorded from neocortical neurons in slices from control rats and rats with neonatal seizures is presented in Figure 1. We found that recurrent flurothyl-induced seizures resulted in a marked reduction in GABA_A ergic inhibition in neocortical neurons, as shown by a highly significant decrease in amplitude of sIPSC (Fig. 1A). As in the previous work on hippocampal CA3 pyramidal neurons, there was

no alteration of frequency of sIPSC (shown as interevent interval in this study) (Fig. 1B). The 10–90% rise time and half-width of averaged sIPSCs were not significantly different between controls and flurothyl-treated rats, indicating largely unchanged kinetic properties of sIPSCs (Fig. 1C,D).

To evaluate the effect of neonatal seizure on action potential-independent synaptic release, mIPSCs were recorded in the presence TTX, a voltage-dependent sodium channels blocker. The amplitudes of mIPSCs were not modified by neonatal seizures (Fig. 2A). However we found significant increases in the interevent intervals of mIPSC recorded in slices from flurothyl-treated rats comparatively to controls (KS test: $p = 0.033$; KS = 0.062) (Fig. 2B). There was no difference in the kinetic parameters of mIPSC recorded from neocortical pyramidal cells in control and flurothyl-treated rats (Fig. 2C,D).

4. Discussion

The present study indicates that early-life seizures cause down-regulation of GABAergic synaptic transmission in the neocortex. These data is in agreement with our previous study from the hippocampus where flurothyl-induced seizures evoked in rats at P1–P5 significantly decreased amplitude of sIPSCs in CA3 pyramidal neurons of P15–P17 rats (Isaeva et al., 2006). It has been shown that seizures evoked by flurothyl in neonatal rats do not cause cell loss in the neocortex (Riviello et al., 2002). In our study flurothyl-induced seizures did not alter the interevent interval and kinetic properties of sIPSCs but significantly reduced the amplitude of sIPSC. These results suggest that in flurothyl-treated rats there is an alteration in inhibitory synaptic strength. The strength of synaptic connections between two neurons is characterized by: i) the number of release sites on the presynaptic cell; ii) the probability of transmitter release at those sites; and iii) the average size of the postsynaptic response, which is dependent on number and sensitivity of postsynaptic receptors (Walmsley et al., 2006). In the experiments with TTX we did not find a difference in amplitude of action-potential independent IPSCs recorded in control and flurothyl-treated rats, indicating that decreases of sIPSC amplitude in rats experiencing neonatal seizures is not due to postsynaptic alterations. The increased interevent interval of mIPSCs in the flurothyl-treated rats along with a decrease in amplitude of sIPSCs and unchanged interevent interval and kinetic parameters of sIPSCs could be explained by a change in the probability of GABA release in neocortical neurons. Another possible explanation of the down-regulation of GABAergic transmission in the flurothyl-treated group could be a decrease in the number of release sites between the two connected neurons. In other words, interneurons in the flurothyl-treated rats could have less synaptic connections with innervated neurons than interneurons from the control group.

Down-regulation of GABAergic transmission in neocortical neurons reported here could explain, at least in part, the increased susceptibility to seizures later in life in flurothyl-treated animals (Huang et al., 1999) and add further information concerning the adverse neurodevelopmental impact of recurrent seizures on neocortical function.

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Figure 1.

Effect of neonatal flurothyl-induced seizures on sIPSCs in L2/3 pyramidal cell of somatosensory cortex. Summary histograms of amplitude (A), interevent interval (B), rise time (C) and half-width (D) of sIPSCs recorded from L2/3 pyramidal cells in control rats (black) and flurothyl-treated (white) rats. Within the parenthesis for A, B is number of events from 19 control and 22 flurothyl-treated cells and for C, D number of cells.

Figure 2. Effect of neonatal flurothyl-induced seizures on mIPSCs. Summary histograms of amplitude (A), interevent interval (B), rise time (C) and half-width (D) of mIPSCs recorded from L2/3 pyramidal cells in control rats (black) and flurothyl-treated (white) rats. Within parenthesis of A, B is number of events from 16 control and 17 flurothyl-treated cells and for C, D number of cells.