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Glycine-induced currents are insensitive to the glycine receptor α_1 subunit-specific blocker, cyanotriphenylborate, in older circling mice

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ABSTRACT

The pharmacologic characteristics of glycine receptors (GlyRs) in the lateral superior olive (LSO) of circling mice, animal model for inherited deafness, were investigated using a GlyR α_1 subunit-specific receptor blocker (cyanotriphenylborate [CTB]). There was a statistically significant age-dependent increase in the antagonistic effect of CTB in heterozygous (+/*cir*) mice. In postnatal (P)0–P3 heterozygous (+/*cir*) mice, glycine currents evoked by glycine puffs were reduced to 20.4 ± 2.6, 37.1 ± 3.1, and 63.9 ± 2.5% at 0.1, 1, and 10 μM CTB ($n = 13$) compared to controls, while the glycine currents were reduced to 22.3 ± 3.5, 52.9 ± 4.1, and 78.3 ± 3.5% at 0.1, 1, and 10 μM CTB ($n = 7$) in P8–P12 heterozygous (+/*cir*) mice. In contrast, the antagonistic effect of CTB was not strong and even less than that of younger animals in older homozygous (*cir*/*cir*) mice. In P0–P3 homozygous (*cir*/*cir*) mice, the extent of inhibition was 20.2 ± 3.7, 37.8 ± 4.3, and 66.8 ± 4.2% at 0.1, 1, and 10 μM CTB ($n = 6$) compared to controls, while the extent of inhibition was 18.7 ± 2.4, 28.1 ± 3.9, and 39.1 ± 8.2% ($n = 6$) in P8–P12 homozygous (*cir*/*cir*) mice. The age-dependent decrease in the antagonistic effect of CTB indicates the abnormal development of the α_1 subunit-containing GlyRs in homozygous (*cir*/*cir*) mice.

1. Introduction

The LSO, a binaural auditory nucleus in the auditory brainstem, receives inhibitory inputs from the contralateral ear via the MNTB [1–4]. The inhibitory MNTB–LSO synapses are purely glycinergic in adults [5,6], but glutamate/GABA/glycinergic in the early developmental period [7–9].

The glycine receptor (GlyR), forming an anion-selective channel, is a strychnine-sensitive pentameric protein complex and one β and four α subunits (α_1–α_4) have been reported [10–12]. The α and β subunits are associated to form functional receptors in the following two different ways: the homomeric configuration composed of five α subunits [13]; and the heteromeric configuration comprising two α subunits and three β subunits [14]. It has been reported that neonatal α_2 subunits observed at birth are subsequently replaced by α_1 subunits in rodent spinal cord [15,16]. The age-related change of subunit compositions has also been reported in the auditory system. The α_1 subunit-specific GlyR antagonist cyanotriphenylborate (CTB) [17] blocks glycine-induced currents effectively only after the first postnatal week, but not during the first few postnatal days in the medial nucleus of the trapezoid body (MNTB) of developing rats [18]. An immunohistochemical study revealing delayed expression of α_1 subunit-containing GlyRs at P8 support the above electrophysiologic results [19].

We have demonstrated decreased glycinergic transmission at MNTB–LSO synapses and decreased immunoreactivity of GlyR in the LSO of developing circling mice [20], animal model for human hereditary deafness (DFNB6) [21–23]. Decreased immunoreactivity of GlyR opens the possibility of developmental alterations of GlyR in circling mice. However, this possibility has not been studied. Therefore, we investigated age-related modification of glycine receptor subunit composition using the α_1 subtype-specific blocker (CTB).

2. Materials and methods

2.1. Animal and slice preparation

Heterozygous females (+/*cir*) were mated with homozygous males (*cir*/*cir*) and the pups were used for this study. The heterozygous (+/*cir*) mice were used as control, because we already observed the apparent developmental change of GlyR between heterozygous (+/*cir*) and homozygous (*cir*/*cir*) mice in the previous study [20]. The data presented here were obtained from pups between P0–P3 and P8–P12. This study was conducted blind because it was hard to distinguish between homozygote and heterozygote

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animals during the period of study. Genotypes were assessed with polymerized chain reaction (PCR) analysis according to our previous report [20]. The animals were maintained in the Animal Facility of Dankook University. The Dankook University Institutional Animal Care and Use Committee (DUIAC) approved this study.

After the mice received deep anesthesia with isoflurane, the brains were removed in ice-cold artificial cerebrospinal fluid (aCSF) with 1 mM kynurenic acid, and 300 μm thick coronal slices were cut with a vibratome (LEICA VT1000s; LEICA Microsystems, Heidelberg, Germany). The slices were allowed to recover for at least 30 min in an interface chamber before recording, then transferred to a submersion-type chamber mounted on an upright microscope and perfused continuously with aCSF containing (in mM) NaCl (124), KCl (5), KH_2PO_4 (1.25), glucose (10), $NaHCO_3$ (26), $CaCl_2$ (2), $MgCl_2$ (1), and kynurenic acid (1).

2.2. Electrophysiologic recordings

Whole cell patch clamp recordings were obtained from the visualized principal type LSO neurons, as identified by the bipolar morphology. Series resistance was not compensated, but monitored by the delivery of 5 mV voltage steps before the pressure puff through experiments. The acceptable resistance was <20 MΩ. If the series resistance changed by >20% or increased over 20 MΩ, experiments were discontinued. To avoid the remnant effects of previous drug application, only one cell was tested for every slice. Because we selected the single best slice per mouse, the number of cells presented in the electrophysiologic study equaled the number of animals tested. Glycine (0.1 mM) puffs were used to elicit currents. The puffing electrode was positioned at a distance of approximately 20 μm from the cell body using a micromanipulator. Pressure pulses of 20 ms were delivered at 5–10 psi. Pressure application was controlled by a Toohey Spritzer pressure system IIe (Toohey Company, Fairfield, NJ, USA). Recording electrodes (2.5–3.5 MΩ) contained (in mM) K-gluconate (22), EGTA (10), KCl (91), HEPES (20), Na_2GTP (0.3), MgATP (1), KOH (35), and QX-314 (5) with an osmolarity of ~285 mOsm. The osmolarity was adjusted with sucrose (The Advanced™ Micro Osmometer Model 3300; Advanced Instruments, Inc., USA). All chemicals, except QX 314, CNQX, bicuculline (Tocris), and CTB were purchased from Sigma (St. Louis, MO, USA). Dr. László Fodor in the Department of Molecular Pharmacology (Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences) donated CTB.

The data were filtered at 5 kHz (EPC-8, HEKA), digitized at 10 kHz and stored in the computer via home-made program (R-Clamp 1.23) for offline analysis. The stored data were analyzed using Clampfit 9.0 (Molecular Devices) and Origin 7.0 (Origin Lab). Data are expressed as the mean ± SEM. An independent *t*-test or two-way ANOVA was used for comparison and data with a *p*-value <0.05 were considered statistically significant.

3. Results

3.1. Developmental changes of glycine currents

In the presence of CNQX (10 μM) and DL-APV (10 μM), the local pressure applications of glycine-induced inward currents at a holding potential of −60 mV in all LSO neurons of both younger (P0–P3) and older (P8–P12) mice. The mean peak amplitude of 2.88 ± 0.38 nA at P0–P3 ($n = 21$) changed to 4.76 ± 0.46 nA at P8–P12 ($n = 15$) in heterozygous (+/*cir*) mice, while the peak amplitudes were 2.30 ± 0.51 nA at P0–P3 ($n = 10$) and 4.52 ± 0.46 nA at P8–P12 ($n = 15$) in homozygous (*cir*/*cir*) mice (Fig. 1A and B). The age-dependent increases in the peak amplitude were statistically significant ($p < 0.0001$). However, there were no significant differences in the mean peak amplitudes between genotypes ($p = 0.385$) (two-way ANOVA) (Fig. 1C).

To further analyze the current characteristics, glycine currents were normalized to the peak amplitudes and the normalized currents were averaged to obtain a mean normalized current. Thirty current traces were normalized (7 for the younger heterozygotes, 7 for the older heterozygotes, 8 for the younger homozygotes, and 8 for the older homozygotes). The mean normalized currents recorded in older mice (P8–P12) showed faster activation than the mean normalized currents of younger mice (P0–P3) in both genotypes (Fig. 2A and B). We measured the times needed to reach a peak in the current traces induced by glycine puffs. They were 209.2 ± 17.8 ms for P0–P3 heterozygous (+/*cir*) mice ($n = 7$), 210.5 ± 14.8 ms for P0–P3 homozygous (*cir*/*cir*) mice ($n = 8$), 102.4 ± 12.7 ms for P8–P12 heterozygous (+/*cir*) mice ($n = 7$), and 122.7 ± 14.9 ms for P8–P12 homozygous (*cir*/*cir*) mice ($n = 8$). The age-dependent decreases in the time needed to reach a peak were statistically significant ($p < 0.0001$). However, there was no significant difference between genotypes ($p = 0.480$) (two-way ANOVA). Using Clampfit 9.0, we also measured the areas under the current traces from the initiation to the peak, which reflected the activation kinetics. They were 138.9 ± 8.3 pA·ms for P0–P3 heterozygous (+/*cir*) mice ($n = 7$), 141.9 ± 8.5 pA·ms for P0–P3 homozygous (*cir*/*cir*) mice ($n = 8$), 66.0 ± 11.6 pA·ms for P8–P12 heterozygous (+/*cir*) mice ($n = 7$), and 89.7 ± 11.0 pA·ms for P8–P12 homozygous (*cir*/*cir*) mice ($n = 8$). The age-dependent decreases in the area

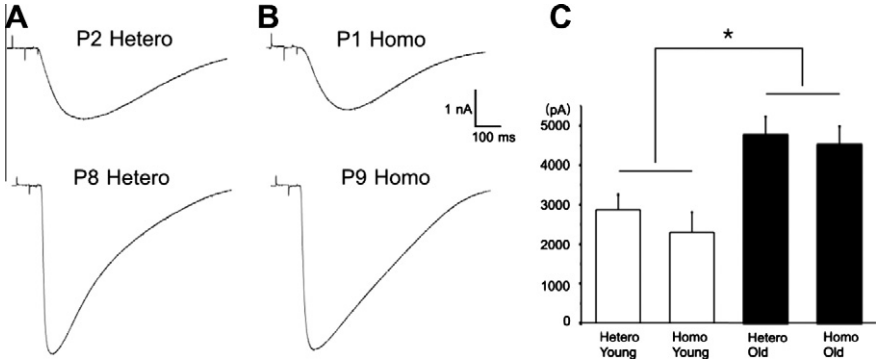

Fig. 1. Age-dependent increase in glycine-induced inward currents in LSO neurons between P0 and P12. Neurons were voltage clamped at −60 mV and glycine (0.1 mM) was applied locally via pressure puffs. A statistically significant age-dependent increase in peak amplitude of glycine-induced current was observed in heterozygous (+/*cir*) mice (total 36 neurons [21 neurons from younger and 15 neurons from older mice] were analyzed) as well as in homozygous (*cir*/*cir*) mice (total 25 neurons [10 from younger and 15 from older mice] were analyzed) (two-way ANOVA, $p < 0.001$) (C). Examples of the maximal peak current are shown in (A) and (B). (Homo: homozygous (*cir*/*cir*) mice, Hetero: heterozygous (+/*cir*) mice. Young: P0–P3, Old: P8–P12). '*' indicates the statistical significance.