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Developmental stability of taurine's activation on glycine receptors in cultured neurons of rat auditory cortex

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Abstract

Taurine is an endogenous amino acid that can activate glycine and/or γ-aminobutyric acid type A ($GABA_A$) receptors in the central nervous system. During natural development, taurine's receptor target undergoes a shift from glycine receptors to $GABA_A$ receptors in cortical neurons. Here, we demonstrate that taurine's receptor target in cortical neurons remains stable during *in vitro* development. With whole-cell patch–clamp recordings, we found that taurine always activated glycine receptors, rather than $GABA_A$ receptors, in neurons of rat auditory cortex cultured for 5–22 days. Our results suggest that the functional sensitivity of glycine and $GABA_A$ receptors to taurine is critically regulated by their developmental environments.

Keywords: Taurine; Glycine receptor; γ-Aminobutyric acid type A receptor; Auditory cortex; Cell culture; Whole-cell patch–clamp; Development

Taurine is an endogenous amino acid abundantly present in the brain [10,19] and has various physiological functions including protecting against excitotoxic cell death [12,17], modulating cell excitability [22,23], inhibiting protein phosphorylation [11], modulating calcium homeostasis [5] and regulating cell osmolarity [9]. Taurine is also critically important for normal development of the nervous systems [6,7,16]. Much evidence has shown that taurine exerts its physiological functions and regulation of neural development at least through activating glycine receptors (GlyRs) and/or γ-aminobutyric acid type A receptors ($GABA_A$Rs) [7,22,23].

Not only taurine's concentration in the brain [19,21] but also its affinity to GlyRs and/or $GABA_A$Rs is developmentally regulated [24]. A recent study has shown that taurine's target on ligand-gated chloride channels undergoes a shift from GlyRs to $GABA_A$Rs in cortical neurons during natural development [24]. However, what factor determines this shift in taurine's receptor target is not fully understood. The authors of this study postulate that the shift in taurine's receptor target may be genetically programmed in the developing cerebral cortex. In the present study, we found that there was no shift in taurine's receptor target for auditory cortex neurons developing in culture, indicating that the functional sensitivity of GlyRs and $GABA_A$Rs to taurine is also critically regulated by their developmental environments.

Experimental protocols were approved by the Institutional Animal Care and Use Committee of University of Science and Technology of China. Neurons used for cell culture were dissociated from the auditory cortex of newborn Wistar rats (postnatal day 0). The neurons were isolated with a standard enzyme treatment protocol as described previously [20]. Briefly, the tissues were dissociated by trypsin (Sigma, USA) and plated (1.5×10^6 cell/ml) on poly-L-lysine (Sigma, USA) coated cover glasses. The isolated neurons were grown in Dulbecco's modified Eagle's medium with L-glutamine plus 10% fetal bovine serum, 10% F-12 nutrient mixture, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco, USA) for 24 h. Then, neuron-basal medium (1.5 ml) with 2% B27 (Gibco, USA) was replaced every 3–4 days. Treatment with 5-fluoro-5′-deoxyuridine (20 μg/ml, Sigma, USA) on the fourth day after plating was used to block cell division of non-neuronal cells, which helped to stabilize the cell population. The cultures were maintained at 37 °C in a 5% CO_2 humidified atmosphere.

The standard external solution contained: 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, and

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10 mM *N*-2-hydroxyethylpiperaxine-*N*′-2-ethanesulfonic acid (HEPES). The pH was adjusted to 7.4 with Tris–base. The osmolarity of the solutions was adjusted to 310–320 mOsm/l with sucrose and a micro-osmometer (Advanced Instruments Inc., Model 3300, USA). The patch pipette solution for whole-cell patch–clamp recordings contained: 120 mM KCl, 30 mM NaCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM ethyleneglykole-bis-(2-aminoethyl)-tetraacetic acid (EGTA), 2 mM Mg-ATP, and 10 mM HEPES. The pH of the internal solution was adjusted to 7.2 with Tris–base. When reversal potentials of I_{Tau} were measured, voltage-activated Na^+, Ca^{2+} and K^+ channels were blocked by adding 0.3 μM tetrodotoxin (TTX) and 0.2 mM

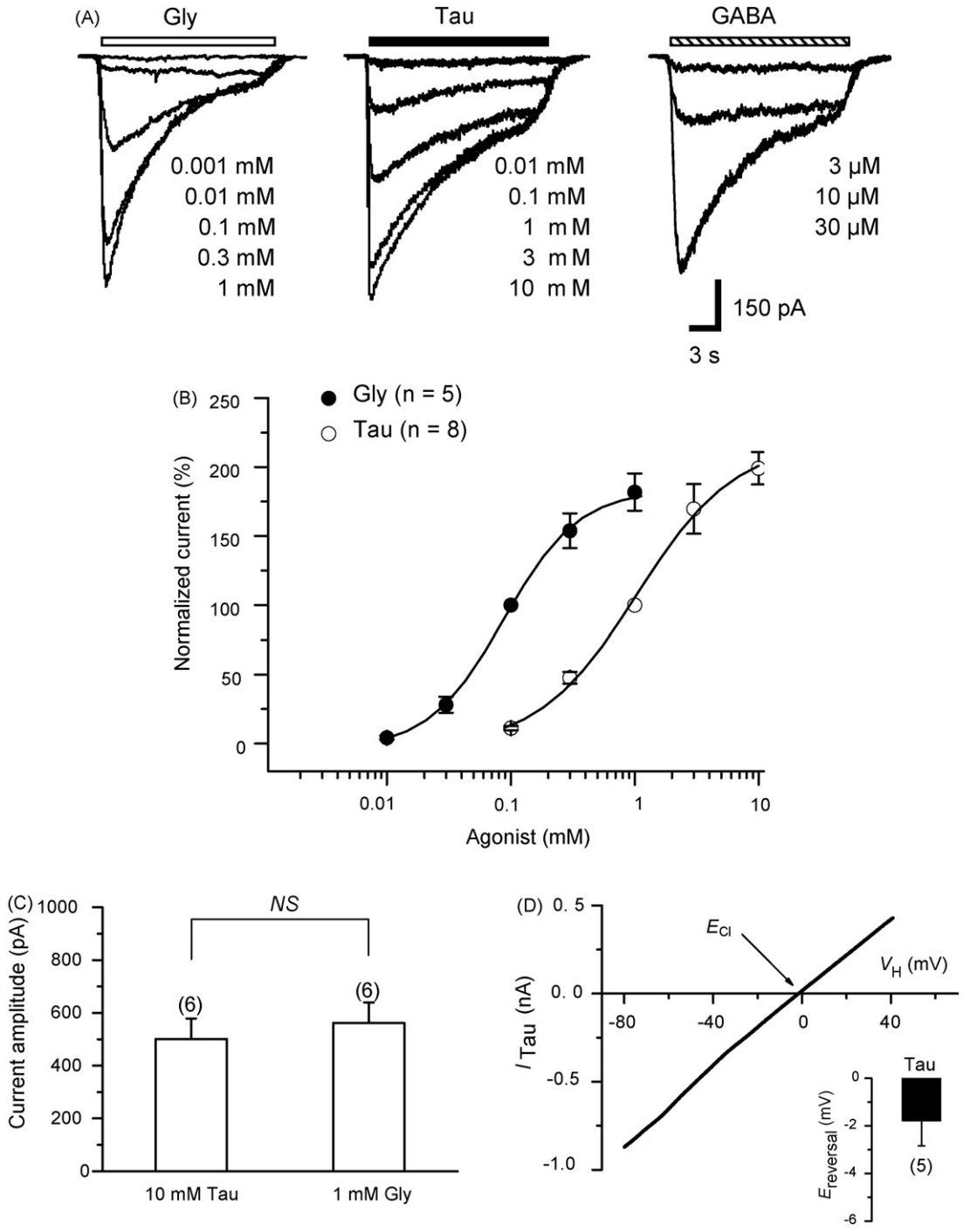

Fig. 1. Current responses of cultured auditory cortex neurons 7–14 days *in vitro* (DIV) to glycine (Gly), taurine (Tau) and GABA. (A) Sample traces of inward currents evoked by glycine, taurine and GABA at various concentrations. (B) Concentration-response relationship for glycine-induced current and taurine-induced current. Currents induced by glycine and taurine were normalized to the responses elicited with 100 μM glycine and 1 mM taurine, respectively. (C) Mean amplitudes of currents induced by 1 mM glycine and by 10 mM taurine. Bars represent standard errors. NS indicates no significant statistical difference. (D) Current-voltage relationship for current induced by 1 mM taurine (I_{Tau}) from one neuron determined with a voltage ramp protocol. Arrow indicates the theoretical chloride equilibrium potential (E_{Cl}). Inset shows the reversal potential of the I_{Gly} ($E_{reversal}$). V_H represents holding potential. Sample sizes are indicated in parentheses.