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Research paper

Activation of synaptic and extrasynaptic glycine receptors by taurine in preoptic hypothalamic neurons

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HIGHLIGHTS

- Taurine activated glycine receptors on preoptic hypothalamic area neurons and responses were action potential independent.
- Taurine activated homo- and hetro-meric glycine receptors on preoptic hypothalamic area neurons.
- At higher concentration taurine activated $GABA_A$ receptors on preoptic hypothalamic area neurons.

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ABSTRACT

Taurine is an essential amino-sulfonic acid having a fundamental function in the brain, participating in both cell volume regulation and neurotransmission. Using a whole cell voltage patch clamp technique, the taurine-activated neurotransmitter receptors in the preoptic hypothalamic area (PHA) neurons were investigated. In the first set of experiments, different concentrations of taurine were applied on PHA neurons. Taurine-induced responses were concentration-dependent. Taurine-induced currents were action potential-independent and sensitive to strychnine, suggesting the involvement of glycine receptors. In addition, taurine activated not only α-homomeric, but also αβ-heteromeric glycine receptors in PHA neurons. Interestingly, a low concentration of taurine (0.5 mM) activated glycine receptors, whereas a higher concentration (3 mM) activated both glycine and gamma-aminobutyric acid A ($GABA_A$) receptors in PHA neurons. These results suggest that PHA neurons are influenced by taurine and respond *via* glycine and $GABA_A$ receptors.

1. Introduction

Taurine (2-aminoethane sulfonic acid) is one of the most abundant amino acids in mammals [12], second in abundance only to glutamate [11]. Taurine has been considered one of the most important amino acids and is required for normal development of the nervous system [16,18]. Additionally, it accumulates in the synaptosomal population [33] and in synaptic vesicles [31]. Reportedly, depletion of taurine causes a wide range of pathological conditions such as cardiomyopathy [41], loss of retinal photoreceptors [32], renal dysfunction [40], and retarded cell differentiation and migration in the cerebellum, visual cortex, and pyramidal cells, as has been demonstrated in monkeys and cats [17,21,35]. In addition, taurine is an important factor for adult neurogenesis [7].

In the hypothalamus, taurine is present throughout various nuclei [6]. Specifically, taurine is released by astrocytes in hypothalamic nuclei such as the supraoptic nucleus (SON) and paraventricular nucleus (PVN) [6] to regulate osmoregulation, providing tonic regulation of neuronal excitability [10]. In addition, taurine reportedly helps in the increase of vasopressin and oxytocin from the neural lobe of the magnocellular hypothalamo-neurohypophysial system [34]. Although several studies have

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analyzed the influence of taurine on the hypothalamic area and hormone release from the anterior hypothalamus, the present study elucidated the influence of taurine on preoptic hypothalamic area (PHA) neurons by examining the role of taurine on various neurotransmitter receptors using whole cell patch clamp electrophysiology.

2. Materials and Methods

2.1. Animals

All experiments were approved by the Chonbuk National University Animal Welfare and Ethics Committee. Mice were housed under 12-h light, 12-h dark cycles (lights on at 07:00 h) with access to food and water *ad libitum*.

2.2. Brain slice preparation

Brain slices were prepared as described previously [2]. Briefly, male and female juvenile mice (5–20-day-old) were decapitated, and their brains removed rapidly and placed in ice-cold bicarbonate-buffered artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 126 NaCl, 2.5 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 11 D-glucose, 1.4 NaH_2PO_4, and 25 $NaHCO_3$ (pH 7.3–7.4 when bubbled with 95% O_2 and 5% CO_2). Brains were glued with cyanoacrylate to the chilled stage of a vibratome (Microme, Germany), where 150–250-μm-thick coronal slices containing the PHA were cut. The slices were allowed to recover in oxygenated ACSF for at least one hour at room temperature.

2.3. Electrophysiology and data analysis

Slices transferred to the recording chamber were submerged and continuously superfused with carboxygenated ACSF at a rate of 4–5 ml/min. The slices were viewed using an upright microscope (BX51WI; Olympus, Tokyo, Japan). Neurons were identified at 10× and 40× objective magnification and patched under Nomarski differential interference contrast optics. Patch pipettes were pulled from thin-wall borosilicate glass-capillary tubing (PG52151-4, WPI, Sarasota, FL, USA) on a Flaming/Brown puller (P-97; Sutter Instruments Co., Novato, CA, USA). The tip resistance of the electrode was 4–6 MΩ. The pipette solution was passed through a disposable 0.22-μm filter and contained the following (in mM): 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 MgATP, and 10 EGTA (pH 7.3 with KOH). Whole cell patch clamp recordings were performed using an Axopatch 200B (Axon Instruments, Union City, CA, USA). The cells were clamped at −60 mV after nullifying the junction potential between the patch pipette and bath solution. Membrane current

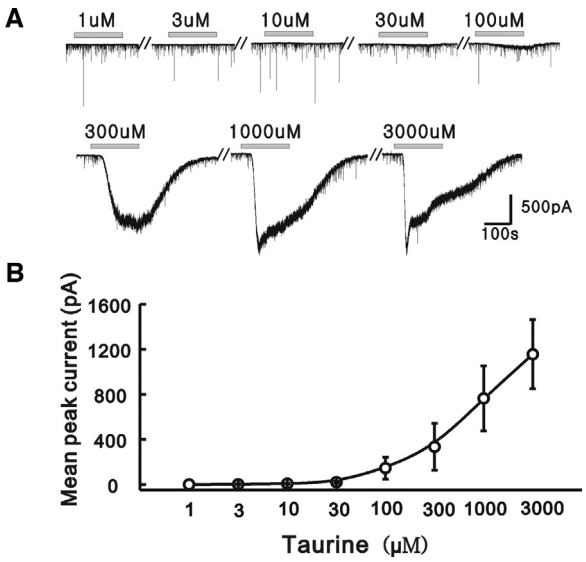

Fig. 1. Taurine-induced concentration-dependent activation of PHA neurons. (A) Taurine application at different concentrations induced inward currents in a concentration-dependent manner in PHA neurons. (B) Concentration-dependent curve of peak current induced by different concentrations of taurine in PHA neurons ($n = 5$).

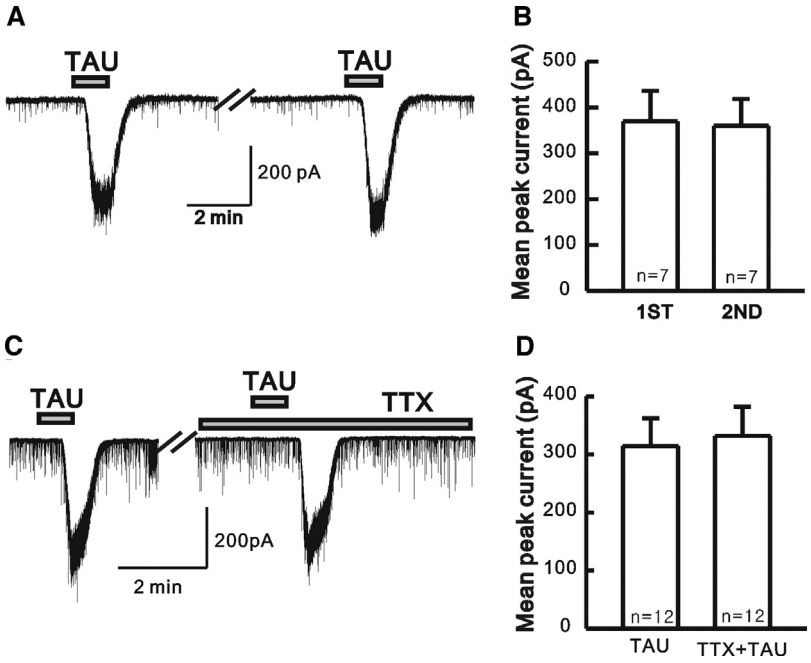

Fig. 2. Direct postsynaptic action of taurine in PHA neurons. (A) A representative trace showing inward current induced by taurine applications at two different time intervals. (B) Bar graph showing the mean peak current induced by taurine in repeated applications. (C) A representative trace showing existence of taurine-mediated inward current in the presence of TTX (0.5 μM), suggesting the postsynaptic action of taurine in PHA neurons. (D) Bar graph showing the mean peak current in the presence of TTX. "*n*" represents the number of neurons recorded in each case.