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EXTRACELLULAR PH MODULATES GABAERGIC NEUROTRANSMISSION IN RAT HYPOTHALAMUS

Z.-L. CHEN AND R.-Q. HUANG *

Department of Pharmacology and Neuroscience, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107, United States

Abstract—Changes in extracellular pH have a modulatory effect on $GABA_A$ receptor function. It has been reported that pH sensitivity of the GABA receptor is dependent on subunit composition and GABA concentration. Most of previous investigations focused on GABA-evoked currents, which only reflect the postsynaptic receptors. The physiological relevance of pH modulation of GABAergic neurotransmission is not fully elucidated. In the present studies, we examined the influence of extracellular pH on the $GABA_A$ receptor-mediated inhibitory neurotransmission in rat hypothalamic neurons. The inhibitory postsynaptic currents (IPSCs), tonic currents, and the GABA-evoked currents were recorded with whole-cell patch techniques on the hypothalamic slices from Sprague–Dawley rats at 15–26 postnatal days. The amplitude and frequency of spontaneous GABA IPSCs were significantly increased while the external pH was changed from 7.3 to 8.4. In the acidic pH (6.4), the spontaneous GABA IPSCs were reduced in amplitude and frequency. The pH induced changes in miniature GABA IPSCs (mIPSCs) similar to that in spontaneous IPSCs. The pH effect on the postsynaptic GABA receptors was assessed with exogenously applied varying concentrations of GABA. The tonic currents and the currents evoked by sub-saturating concentration of GABA ([GABA]) (10 μM) were inhibited by acidic pH and potentiated by alkaline pH. In contrast, the currents evoked by saturating [GABA] (1 mM) were not affected by pH changes. We also investigated the influence of pH buffers and buffering capacity on pH sensitivity of $GABA_A$ receptors on human recombinant $\alpha1\beta2\gamma2$ $GABA_A$ receptors stably expressed in HEK 293 cells. The pH influence on $GABA_A$ receptors was similar in HEPES- and MES-buffered media, and not dependent on protonated buffers, suggesting that the observed pH effect on GABA response is a specific consequence of changes in extracellular protons. Our data suggest that the hydrogen ions suppress the GABAergic neurotransmission, which is mediated by both presynaptic and postsynaptic mechanisms.

Key words: protons, IPSC, acidification, hypothalamus, HEPES, buffer.

INTRODUCTION

GABA, the predominant fast inhibitory neurotransmitter of the adult CNS, acts principally via the $GABA_A$ receptors. Activation of the synaptic $GABA_A$ receptors generates inhibitory postsynaptic currents (IPSCs), leading to hyperpolarization of the postsynaptic neurons and preventing them from firing action potentials. In addition to the synaptic transmission, the extrasynaptic receptors are activated by low extracellular GABA concentration (0.8–2.9 μM) (Lerma et al., 1986), producing a widespread and sustained inhibition (tonic inhibition). Most brain functions employ combinations of both synaptic and tonic transmissions. Tonic inhibition plays a crucial role in regulating neuronal excitability because it sets the threshold for action potential generation and integrates excitatory signals.

$GABA_A$ receptor function is regulated by several endogenous ions, including physiological concentration of protons ($[H^+]$. Interstitial pH in mammals normally ranges between 7.36 and 7.44 (Bullock, 1990). It is known that pH in the brain changes with neural activity. Under various physiological conditions, brain pH may shift between 6.5 and 8.0 (Chesler, 1990; Chesler and Kaila, 1992). In certain pathological conditions such as stroke, seizure, hypoxia and ischemia, brain pH can shift up to 1 unit (Wang and Sonnenschein, 1955; Somjen, 1984; Siesjo et al., 1985; Carmeliet, 1999). It is well known that protons modulate neuronal excitability and this effect may be partially mediated through pH modulation of $GABA_A$ receptors.

Our previous studies and other investigations have shown that $GABA_A$ receptors are tightly regulated by extracellular pH. However, most studies focused on the pH effect on GABA-activated whole-cell currents recorded from recombinant or native preparations, which could not distinguish phasic and tonic inhibition components. A detailed elucidation of physiological relevance for pH modulation still remains insufficiently studied. While a few papers so far examined proton modulation of miniature inhibitory postsynaptic currents (mIPSCs) and GABA-evoked currents recorded from the neurons of hippocampus (Mozrzymas et al., 2003; Zhou

*Corresponding author. Address: Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, United States. Tel: +1-817-735-2095; fax: +1-817-735-0408.
E-mail address: ren-qi.huang@unthsc.edu (R.-Q. Huang).

Abbreviations: ACSF, artificial cerebral-spinal fluid; ANOVA, analysis of variance; ASICs, acid-sensing ion channels; EGTA, ethylene glycol tetraacetic acid, HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IPSC, inhibitory postsynaptic current; MES, 2-ethanesulfonic acid; mIPSC, miniature IPSC; sIPSC, spontaneous IPSC; TTX, tetrodotoxin; VDCC, voltage-dependent Ca^{2+} channel.

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et al., 2007), cerebellar granule (Dietrich and Morad, 2010) and spinal cord dorsal horn (Hugel et al., 2012), the proton effect on hypothalamic $GABA_A$ receptors has never been studied. Furthermore, how protons modulate extrasynaptic $GABA_A$ receptors is still unknown. The hypothalamus plays a vital role in endocrine, autonomic and behavioral functions (Meister, 1993). GABA suppresses the activity of hypothalamic neurons and has been suggested to be the dominant inhibitory neurotransmitter in the hypothalamus (Decavel and Van den Pol, 1990). Hypothalamic GABAergic neurotransmission through ionotroptic $GABA_A$ receptors has synaptic (phasic) and extrasynaptic (tonic) components (Park et al., 2007). In the present studies, we investigated pH modulation of GABAergic synaptic and extrasynaptic inhibition in the rat hypothalamic areas. Our results demonstrated that protons modulate GABAergic synaptic and tonic currents in the rat hypothalamus.

EXPERIMENTAL PROCEDURES

Brain slice preparation

Hypothalamic slices were acutely prepared from postnatal day (P) 15–26 (day of birth = P1) Sprague–Dawley rats (either sex, Indianapolis, IN, USA). The rat was anesthetized with isoflurane and rapidly decapitated. All procedures were conducted in accordance with the *National Institutes of Health Guide for Care and Use of Laboratory Animals*. All stages of brain dissection and tissue slicing were conducted in ice-cold (~4 °C) artificial cerebral-spinal fluid (ACSF) of the following composition (in mM): 124 NaCl, 5.0 KCl, 1.3 $MgSO_4$, 26 $NaHCO_3$, 1.24 KH_2PO_4, 2.4 $CaCl_2$ and 10 glucose; 300 mOsm and pH ~7.4 after equilibration with a 95%O_2/5%CO_2 gas mixture (carbogen). Thin hypothalamic slices (200 μm) were cut with a vibratome (VSL, World Precision Inst., Inc); slices were submerged in ACSF (22–25 °C) aerated with the carbogen gas mixture. Slices were stored at room temperature in carbogen-bubbled ACSF until used.

Cloned receptors

Human embryonic kidney cell lines (HEK293) stably expressing recombinant human α1β2γ2 were used to investigate the effect of buffers on pH modulation of $GABA_A$ receptors. Cells transiently expressing human glycine α1 receptors were also studied. HEK293 cell line was transiently transfected with recombinant receptor subunit using PolyJet DNA *In Vitro* tranfection reagent (SignaGen Laboratories, Rockville, MD, USA). Briefly, HEK293 cells were washed and placed in fresh Dulbecco's modified eagle medium containing 10% FBS and antibiotics (penicillin 100 U/mL). 0.5 μg of human glycine α1 subunit cDNA was added to cells growing exponentially on poly-L-lysine coated coverslips placed in a 35-mm culture dish. Transfected cells were used for electrophysiological analysis 24–48 h after the transfection.

Electrophysiology

Whole-cell patch recordings were made at room temperature (22–25 °C) at a holding potential of −70 mV for brain slice or −60 mV for recombinant receptors. Patch pipettes of borosilicate glass (M1B150F, World Precision Instruments, Inc., Sarasota, FL, USA) were pulled (Flaming/Brown, P-87/PC, Sutter Instrument Co., Novato, CA, USA) to a tip resistance of 7–8 MΩ. The pipette solution contained (in mM): 140 CsCl, 10 EGTA, 10 HEPES, 4 Mg-ATP; pH 7.2. For brain slice studies, a single slice was transferred to a recording chamber (~2 ml) and superfused continuously (7–10 ml/min, 22–25 °C) with external solution consisting of the following (in mM): 140 NaCl, 3.0 KCl, 2 $MgCl_2$, 10 HEPES, 2.4 $CaCl_2$, 10 glucose, 330 mOsm and pH 7.3. Individual hypothalamic neurons within the slice were visualized using an upright, fixed stage microscope (Nikon Optiphot-2UD) equipped with standard Hoffman modulation contrast (HMC) optics and a video camera system (Sony model XC-75 CCD video camera module, DOT-X monitor). Location of hypothalamic neurons studied in this investigation was identified to be in the posterior, dorsomedial, lateral, ventomedial and arcuate nuclei according to a stereotaxic atlas for adult rats (Paxinos and Watson, 1986) as previously described (Huang and Dillon, 2002). Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in the whole-cell configuration in the presence of glutamate receptor antagonist kynurenic acid (1 mM, K3375, Sigma, St Louis, MO, USA). mIPSCs were isolated with additional tetrodotoxin (TTX, 0.5 μM, T8024, Sigma, St Louis, MO). For studies on cloned receptors, a coverslip containing cultured cells was placed in a small chamber (~1.5 ml) on the stage of an inverted light microscope (Olympus IMT-2) and superfused continuously (5–8 ml/min, 22–25 °C) with the following external solution containing (in mM): 125 NaCl, 5.5 KCl, 0.8 $MgCl_2$, 3.0 $CaCl_2$, 10 HEPES, 10 glucose and pH 7.3. The currents (GABAergic IPSCs, tonic currents and GABA- or glycine-induced Cl^- currents) from the whole-cell configuration were obtained using a patch-clamp amplifier (PC-501A, Warner Instruments, Hamden, CT, USA) equipped with a 5101-01G headstage. Signals were filtered at 5 kHz, monitored on an oscilloscope and a chart recorder (Gould TA240), and sampled at 30 kHz using a Digidata 1200 and pClamp software (pClamp 6.0, Axon Instruments, Foster City, CA, USA) and stored on a computer for offline analysis. The series resistance (R_s) was compensated online by 60–70% in voltage-clamp mode to reduce voltage errors. To monitor the possibility that access resistance changed over time or during different experimental conditions, at the initiation of each recording we measured and stored the current response to a 5-mV voltage pulse on our digital oscilloscope. This stored trace was continually referenced throughout the recording. The cells in which access resistance or baseline showed instability during recording period were not included in the analysis.

Experimental protocol

For the studies on hypothalamic slices, pH of external solutions was altered by addition of NaOH or HCl, and routinely checked before and during experiments. The osmolarity of the control external medium (pH 7.3) was 330 ± 9.1 mOsm and was not changed in basic or