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FEED-FORWARD FACILITATION OF GLUTAMATE RELEASE BY PRESYNAPTIC $GABA_A$ RECEPTORS

I.-S. JANG,[a] Y. ITO[a] AND N. AKAIKE[b]*

[a]*Department of Pharmacology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Fukuoka 812-8582, Japan*

[b]*Research Division for Life Sciences, Kumamoto Health Science University, Izumi-machi 325, Kumamoto 861-5598, Japan*

Abstract—Disynaptic GABAergic inputs from Schaffer collateral (SC) afferents on to the soma of glutamatergic CA1 pyramidal neurons are involved in feed-forward inhibition in the hippocampal neural circuits. Here we report the functional roles of presynaptic $GABA_A$ receptors on SC afferents projecting to CA1 pyramidal neurons. Muscimol (0.5 μM), a selective $GABA_A$ receptor agonist, increased SC-evoked EPSC amplitude and decreased paired-pulse ratio in the slice preparation, in addition, it facilitated spontaneous glutamate release on to mechanically dissociated CA1 pyramidal neurons in an external Ca^{2+}-dependent manner. In field recordings, muscimol at low concentrations (≤0.5 μM) increased not only the excitability of SC afferents but glutamate release, however, it at high concentrations (≥1 μM) changed bidirectionally. These results suggest that the moderate activation of presynaptic $GABA_A$ receptors depolarizes SC afferents and enhances SC-mediated glutamatergic transmission. When endogenous GABA was disynaptically released by brief trains of stimulation of SC afferents, the axonal excitability in addition to glutamate release was increased. The effects of endogenous GABA on the excitability of SC afferents were blocked by either SR95531 or AMPA receptor blockers, which would be expected to block disynaptic feed-forward neural circuits. The present results provide a novel form of presynaptic modulation (feed-forward facilitation) of glutamatergic transmission by presynaptic $GABA_A$ receptors within the intrinsic hippocampal neural circuits. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: hippocampus, presynaptic facilitation, $GABA_A$ receptor, GABAergic interneuron, neural circuit.

The functional organization of neural circuits consists of two basic elements; excitatory principle neurons and inhibitory interneurons which can modulate the activity of the principle neuron. An interesting feature of neural circuits within the hippocampus is that distinct populations of GABAergic interneurons control the excitability of pyramidal neurons via inhibitory axon terminals that synapse on specific postsynaptic domains (Buhl et al., 1994; Halasy et al., 1996; for review, Freund and Buzsaki, 1996; McBain and Fisahn, 2001). For example, basket cells preferentially innervate CA1 neurons in the stratum pyramidale and the proximal area of strata oriens and radiatum (Peters et al., 1983; Freund et al., 1986; Cope et al., 2002). Different interneuron populations projecting to specific domains have been postulated to reflect distinct functional roles, including feedback inhibition, feed-forward inhibition, tonic inhibition and the production of intrinsic oscillations (Alger and Nicoll, 1982; Cobb et al., 1995; Chapman and Lacaille, 1999; Pouille and Scanziani, 2001; Bartos et al., 2002; Maccaferri and Dingledine, 2002). In contrast, other populations of interneurons have a more dispersed field of termination. The Schaffer collateral (SC)-associated interneurons, for example, project their terminals throughout the entire area of the strata radiatum and oriens, and their terminations overlap with the SC afferents (Hájos and Mody, 1997; Gulyás et al., 1999; Vida et al., 1998; Cope et al., 2002). The functional roles of these SC-associated interneurons within the hippocampal neural circuits are still unclear, although their location suggests they help coordinate activity between CA1 and CA3 pyramidal neurons, possibly via some feed-forward inhibition (McBain and Fisahn, 2001; Cope et al., 2002).

The activation of postsynaptic $GABA_A$ receptors clearly contributes to the physiological functions of hippocampal interneurons, however, growing evidence has revealed that the modulation of neurotransmitter release by presynaptic $GABA_A$ receptors is much more widespread throughout the brain and occurs not just in primary afferent terminals where it was first described (Eccles et al., 1963; Segev, 1990; Cattaert and El Manira, 1999; Jang et al., 2001, 2002; Turecek and Trussell, 2002; Ruiz et al., 2003). These receptors could also be activated by endogenous GABA although the precise physiological role of this action remains unclear. In this study, we examine whether axonal and/or presynaptic $GABA_A$ receptors can also modulate excitatory transmission in the hippocampus. It is not unreasonable to further postulate that GABA spillover may activate such receptors, if present, given that the terminals of some GABAergic interneurons make inhibitory synapses in the vicinity of excitatory synapses (Megías et al., 2001). In particular the terminals of SC-associated interneurons overlap with excitatory projections of the SC afferents. In fact, GABA spillover has been shown to activate presynaptic $GABA_B$ receptors on these SC glutamatergic nerve terminals to decrease glutamate release (Isaacson

*Corresponding author. Tel: +81-96-275-2268; fax: +81-96-245-3172.
E-mail address: akaike@kumamoto-hsu.ac.jp (N. Akaike).

Abbreviations: ACSF, artificial cerebrospinal fluid; AP5, DL-2-amino-5-phosphonovaleric acid; CGP55845, (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl) ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid; CNQX, 6-cyano-7-nitroquinozaline-2,3-dione disodium; fEPSP, field EPSP; GYKI52466, 4-(8-methyl-9*H*-1,3-dioxolo[4,5-h][2,3]benzodiazepin-5-yl)-benzenamine HCl; K-S, Kolmogorov-Smirnov; NO-711, 1-[2-[[(diphenylmethylene)imino]oxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid HCl; SC, Schaffer collateral; sEPSC, spontaneous EPSC; (*S*)-MCPG, (*S*)-α-methyl-4-carboxyphenylglycine; SR95531, 6-imino-3-(4-methoxyphenyl)-1(6*H*)-pyridazinebutanoic acid HBr; TTX, tetrodotoxin; V_H, holding potential.

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et al., 1993). In the present study, therefore, we have addressed whether SC afferents express functional presynaptic $GABA_A$ receptors, and if so, whether the activation of presynaptic $GABA_A$ receptors can directly regulate SC glutamatergic transmission. Such a mechanism may represent a form of feed-forward regulation of glutamate release and could have implications for the intrinsic neuronal circuitry in the hippocampus.

EXPERIMENTAL PROCEDURES

Preparations

All experiments conformed to the guiding principles for the care and use of animals approved by the Council of the Physiological Society of Japan and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. Wistar rats (9–15 days old) were decapitated under pentobarbital anesthesia (50 mg/kg, i.p.). The brain was dissected and the hippocampus was longitudinally sliced at a thickness of 300 μm by use of a microslicer (VT1000S; Leica, Nussloch, Germany) in a cold low-Na^+ medium (in mM; 230 sucrose, 2 KCl, 1 KH_2PO_4, 1 $MgCl_2$, 0.5 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose) saturated with 95% O_2 and 5% CO_2. Slices containing the hippocampus were kept in artificial cerebrospinal fluid (ACSF; 120 NaCl, 2 KCl, 1 KH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$ and 10 glucose) saturated with 95% O_2 and 5% CO_2 at room temperature for at least 1 h. Immediately before recording, a cut was made between CA1 and CA2 area to exclude possible effects of somatodendritic $GABA_A$ receptor activation on CA3 neurons, thereafter, the slices were transferred into a recording chamber. The ACSF routinely contained 50 μM DL-2-amino-5-phosphonovaleric acid (AP5) and 3 μM (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid (CGP55845) to block NMDA and $GABA_B$ receptors, respectively, except where indicated. The bath was perfused with ACSF at 3–4 ml/min by the use of a peristaltic pump (MP-1000, EYELA, Tokyo, Japan).

In a subset of experiments, the effect of muscimol on glutamate release was directly tested using mechanically dissociated CA1 pyramidal neurons. For dissociation, hippocampal slices (350 μm in a thickness) were transferred into a 35 mm culture dish (Primaria 3801; Becton Dickinson, Rutherford, NJ, USA) containing the standard external solution (in mM; 150 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 HEPES), and the region of the hippocampus CA1 was identified under a binocular microscope (SMZ-1; Nikon, Tokyo, Japan). Details of the mechanical dissociation preparation have been described previously (Rhee et al., 1999; Akaike and Moorhouse, 2003). Briefly, mechanical dissociation was accomplished using a custom-built vibration device and a fire-polished glass pipette oscillating at about 50–60 Hz (0.3–0.5 mm). The tip of the fire-polished glass pipette was lightly placed on the surface of the CA1 region and vibrated horizontally for about 2 min. Slices were removed and the mechanically dissociated neurons allowed to settle and adhere to the bottom of the dish for 15 min. External solution containing drugs was applied to isolated cells using a 'Y-tube system,' allowing rapid solution exchange.

Electrical measurements

All electrical measurements were performed by use of a computer-controlled patch clamp amplifier (MultiClamp 700A; Axon Instruments; Union City, CA, USA). For whole-cell recording, patch pipettes were made from borosilicate capillary glass (1.5 mm outer diameter, 0.9 mm inner diameter; G-1.5; Narishige, Tokyo, Japan) in two stages on a vertical pipette puller (PB-7; Narishige). The resistance of the recording pipettes filled with internal solution (in mM; 130 CsF, 5 TEA-Cl, 5 CsCl, 2 EGTA, 5 QX-314, 2 Mg-ATP and 10 HEPES, pH 7.2 with Tris-base) was 4–6 MΩ. CsF was replaced with equimolar Cs-methanesulfonate for recording disynaptic IPSCs. Neurons were viewed under an upright microscope (DM LFSA, Leica, Bensheim, Germany) with a water-immersion objective (×63). Membrane currents were filtered at 3 kHz (MultiClamp Commander; Axon Instruments), digitized at 10 kHz (Digidata 1322A, Axon Instruments), and stored on a computer equipped with pCLAMP 8.02 (Axon Instruments). In whole-cell recordings, 10 mV hyperpolarizing step pulses (30 ms in duration) were periodically delivered to monitor the access resistance, and recordings were discontinued if access resistance changed by more than 15%. All slice experiments were performed at 32–34 °C and those using mechanically dissociated neurons at 22–25 °C. For extracellular recording, electrical measurements were performed by use of a computer-controlled patch clamp amplifier set on the current clamp mode (MultiClamp 700A; Axon Instruments). Glass electrodes (inner diameter; 3–5 μm) filled with ACSF were placed at striatum radiatum. Voltage responses, including fiber volleys and field EPSPs (fEPSPs) were directly stored on a computer equipped with pCLAMP 8.02.

To stimulate SC afferents, a glass pipette (inner diameter; 7~8 μm) filled with ACSF was placed around the center of stratum radiatum, and short voltage pulses (100 μs, 1–3 V) were applied at 0.1–0.033 Hz using a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) equipped with an isolator unit (SS-701J, Nihon Kohden). The distance between stimulating and recording electrodes was 1000–1500 μm, and this distance did not evoke any monosynaptic GABAergic transmission onto the recorded neurons (see also Fig. 1).

Data analysis

Numerical values are provided as the mean±S.E.M. using values normalized to the control. Differences in the amplitude of EPSCs, fEPSPs and fiber volleys were tested by Student's paired two-tailed *t*-test using their absolute values, rather than the normalized ones. Values of $P<0.05$ were considered significant. EPSC and fEPSP amplitudes were calculated by subtracting the baseline from the respective peak amplitude. Fiber volley amplitudes were calculated by subtracting the negative peak amplitude from the positive one. Spontaneous EPSCs (sEPSCs) were counted and analyzed using the MiniAnalysis program (Synaptosoft, Decatur, GA, USA) as described previously (Jang et al., 2001, 2002). The average values of sEPSC frequency and amplitude during the control period (5–10 min) were calculated, and the frequency and amplitude of all the events during muscimol application (2 min) were normalized to these values. The effect of muscimol was quantified as a percentage increase in sEPSC frequency compared with the control values. The inter-event intervals and amplitudes of a large number of sEPSCs obtained from the same neuron were examined by constructing cumulative probability distributions and these distributions were compared under different conditions using the Kolmogorov-Smirnov (K-S) test with Stat View software (SAS Institute, Inc.).

Drugs

The drugs and chemicals used in the present study were muscimol, AP5, tetrodotoxin (TTX), 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), 6-imino-3-(4-methoxyphenyl)-1(6*H*)-pyridazinebutanoic acid HBr (SR95531), 4-(8-methyl-9*H*-1,3-dioxolo[4,5-h][2,3]benzodiazepin-5-yl)-benzenamine HCl (GYKI52466), 1-[2-[[(diphenylmethylene)imino]oxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid HCl (NO-711) (from Sigma, St. Louis, MO, USA) and CGP55845, (*S*)-α-methyl-4-carboxyphenylglycine ((*S*)-MCPG) (from Tocris, UK).