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REGULATION OF SYNAPTIC INPUT TO HYPOTHALAMIC PRESYMPATHETIC NEURONS BY $GABA_B$ RECEPTORS

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Abstract—The hypothalamic paraventricular (PVN) neurons projecting to the spinal cord and brainstem play an important role in the control of homeostasis and the sympathetic nervous system. Although $GABA_B$ receptors are present in the PVN, their function in the control of synaptic inputs to PVN presympathetic neurons is not clear. Using retrograde tracing and whole-cell patch-clamp recordings in rat brain slices, we determined the role of presynaptic $GABA_B$ receptors in regulation of glutamatergic and GABAergic inputs to spinally projecting PVN neurons. The $GABA_B$ receptor agonist baclofen (1–50 μM) dose-dependently decreased the frequency but not the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) and inhibitory postsynaptic currents (sIPSCs). The effect of baclofen on sEPSCs and sIPSCs was completely blocked by 10 μM CGP52432, a selective $GABA_B$ receptor antagonist. Baclofen also significantly reduced the frequency of both miniature excitatory and miniature inhibitory postsynaptic currents (mEPSCs and mIPSCs). Furthermore, uncoupling pertussis toxin-sensitive $G_{i/o}$ proteins with N-ethylmaleimide abolished baclofen-induced inhibition of mEPSCs and mIPSCs. However, the inhibitory effect of baclofen on the frequency of mIPSCs and mEPSCs persisted in the presence of either Cd^{2+}, a voltage-gated Ca^{2+} channel blocker, or 4-aminopyridine, a blocker of voltage-gated K^+ channels. Our results suggest that activation of presynaptic $GABA_B$ receptors inhibits synaptic GABA and glutamate release to PVN presympathetic neurons. This presynaptic action of $GABA_B$ receptors is mediated by the N-ethylmaleimide-sensitive $G_{i/o}$ proteins, but independent of voltage-gated Ca^{2+} and K^+ channels.

Key words: synaptic transmission, hypothalamus, presynaptic modulation, brain slices.

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Abbreviations: aCSF, artificial cerebrospinal fluid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EPSC, excitatory postsynaptic current; GDP-β-S, guanosine 5′-*O*-(2-thiodiphosphate); GIRK, G protein-coupled inwardly rectifying K^+ channel; IML, intermediolateral cell column; K_V, voltage-gated K^+ channels; mEPSCs, miniature excitatory postsynaptic currents; mIPSCs, miniature inhibitory postsynaptic currents; NEM, N-ethylmaleimide; PVN, paraventricular nucleus; QX-314, lidocaine N-ethyl bromide; sEPSCs, spontaneous excitatory postsynaptic currents; SHR, spontaneous hypertensive rat; sIPSCs, spontaneous inhibitory postsynaptic currents; SNARE, soluble N-ethylmaleimide-sensitive factor activating protein receptor; TTX, tetrodotoxin; 4-AP, 4-aminopyridine.

The hypothalamic paraventricular nucleus (PVN) plays an important role in the regulation of sympathetic vasomotor tone (Martin and Haywood, 1993; Allen, 2002), which is critical for maintenance of homeostasis and the normal function of many systems in the body. The PVN neurons project to various cardiovascular centers, such as the rostral ventrolateral medulla and the nucleus of the solitary tract, as well as the sympathetic preganglionic neurons located in the intermediolateral cell column (IML) of the spinal cord (Pyner and Coote, 2000; Hardy, 2001). The PVN presympathetic neurons may contribute to elevated sympathetic outflow in some pathophysiological conditions, such as heart failure and hypertension (Allen, 2002; Zhang et al., 2002; Li and Pan, 2006).

GABAergic and glutamatergic synaptic inputs play an important role in tonic control of PVN presympathetic neurons (Martin and Haywood, 1993; Allen, 2002; Li et al., 2004; Li and Pan, 2006). The majority of the local synaptic inputs to PVN neurons are GABAergic (Tasker and Dudek, 1993; Boudaba et al., 1996). The $GABA_A$ receptor antagonist, bicuculline, significantly increases the firing of PVN presympathetic neurons in the brain slice (Li and Pan, 2005, 2006), and microinjection of bicuculline into the PVN increases the sympathetic nerve activity and blood pressure in anesthetized and conscious rats (Martin et al., 1991; Martin and Haywood, 1993). Furthermore, many important neuromodulators, including nitric oxide and angiotensin II, regulate the firing activity of PVN presympathetic neurons through their effect on synaptic GABA release (Li et al., 2002, 2003). Synaptically released GABA not only activates the ionotropic $GABA_A$ receptors to produce postsynaptic inhibition, but it also stimulates the metabotropic $GABA_B$ receptors located at the presynaptic and postsynaptic sites (Misgeld et al., 1995). $GABA_B$ receptors are widely distributed in the brain including the PVN (Margeta-Mitrovic et al., 1999). Both $GABA_A$ and $GABA_B$ receptors contribute to the regulation of neuronal activity and sympathetic outflow. In this regard, microinjection of baclofen, a specific $GABA_B$ receptor agonist, into one of the hypothalamic pressor areas, the ventromedial hypothalamus, decreases sympathetic nerve activity, blood pressure, and heart rate in normotensive rats and spontaneous hypertensive rats (SHR) (Takenaka et al., 1996). However, it remains uncertain how $GABA_B$ receptors regulate different synaptic inputs to PVN presympathetic neurons.

Therefore, in this study, using retrograde labeling and whole-cell recordings in the brain slice, we determined the role of presynaptic $GABA_B$ receptors in the control of synaptic GABA and glutamate release to spinally projecting

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PVN neurons. We also attempted to determine the possible signaling mechanisms involved in the presynaptic effect of $GABA_B$ receptors in the PVN.

EXPERIMENTAL PROCEDURES

Retrograde labeling of spinally projecting PVN neurons

Male Sprague–Dawley rats (3–5 weeks old, Harlan, Indianapolis, IN, USA) were used in this study. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine and University of Texas M. D. Anderson Cancer Center and conformed to the National Institutes of Health guidelines on the ethical use of animals. All efforts were made to minimize both the suffering and number of animals used. Under halothane (2% in O_2) anesthesia, the rat spinal cord at the T_1–T_4 level was exposed through dorsal laminectomy. Then a glass micropipette (20–30 μm tip diam) was filled with rhodamine-labeled fluorescence microsphere suspension (FluoSpheres, 0.04 μm, Molecular Probes, Eugene, OR, USA) and placed at about 500 μm from the midline and 500 μm below the dorsal surface with a micromanipulator (Li et al., 2002, 2003). The dye was pressure-ejected (Nanojector II, Drummond Scientific Co., Broomall, PA, USA) bilaterally into the IML region of the spinal cord in three or four separate 50-nl injections. After dye injection, animals were allowed to recover for 3–10 days to permit retrograde tracer being transported to the PVN.

Slice preparations

Three to 10 days following tracer injection, the rats were rapidly decapitated under halothane anesthesia. The brain was quickly removed and transferred into ice-cold artificial cerebrospinal fluid (aCSF) solution saturated with 95% O_2–5% CO_2 for 1–2 min. A brain block containing the hypothalamus was trimmed and the coronal slices (300 μm in thickness) containing the PVN were cut using a vibratome (Technical Product International, St. Louis, MO, USA), as we described previously (Li et al., 2002, 2003). The slices were then incubated in the aCSF continuously gassed with 95% O_2–5% CO_2 at 34 °C for at least 1 h before recording. The aCSF perfusion solution contained (in mM) 124.0 NaCl, 3.0 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1.4 NaH_2PO_4, 10.0 glucose, and 26.0 $NaHCO_3$.

Recordings of postsynaptic currents of PVN neurons

The slice was placed in a glass-bottomed chamber (Warner Instrument, Hamden, CT, USA) and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steel weight. The labeled neurons were briefly identified with the aid of epifluorescence illumination and then visualized with differential interference contrast optics on an upright microscope (BX51WI, Olympus, Tokyo, Japan). The slice was continuously perfused with aCSF at 3.0 ml/min at 34 °C maintained by an in-line solution heater and a temperature controller (model TC-324, Warner Instruments). At this perfusion rate, the final concentration of the drug in the bath can be attained in about 1 min.

Recordings of postsynaptic currents were performed using whole-cell voltage-clamp techniques (Li et al., 2002, 2003). The recording electrode was pulled from borosilicate capillaries (1.2 mm OD, 0.86 mm ID; World Precision Instruments, Sarasota, FL, USA). The resistance of the electrode filled with the pipette solution was 3–5 MΩ. Recordings of postsynaptic currents usually began 5–7 min after forming the whole-cell configuration and the current reached a steady state. Signals were processed using an Axopatch 700B amplifier (Axon Instruments, Foster City, CA, USA), filtered at 1–2 kHz, digitized at 20 kHz using Digidata 1322 (Axon Instruments), and saved to a hard drive of a computer.

Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded at the holding potential of 0 mV using internal solution containing (in mM): 110.0 mM Cs_2SO_4, 2.0 $MgCl_2$, 0.1 $CaCl_2$, 1.1 EGTA, 10.0 Hepes, 2.0 Na_2ATP, 0.3 Na_2GTP, adjusted to pH 7.25 with 1 M CsOH (280–300 mOsm). A glutamate non-*N*-methyl-D-aspartate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) was bath perfused to block excitatory postsynaptic currents (EPSCs). To record sEPSCs, we used a different electrode internal solution containing (in mM): 130.0 potassium gluconate, 2.0 $MgCl_2$, 0.1 $CaCl_2$, 1.1 EGTA, 10.0 Hepes, 2.0 Na_2ATP, 0.3 Na_2GTP, adjusted to pH 7.25 with 1 M KOH (280–300 mOsm). Bicuculline (20 μM) was applied continuously to block the IPSCs at the holding potential of −70 mV. The miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of 1 μM tetrodotoxin (TTX). A sodium channel blocker, lidocaine N-ethyl bromide (QX-314, 10 mM), was included in the pipette solution to suppress action potential generation. To block the postsynaptic action mediated by $GABA_B$ receptors, a general G protein inhibitor, guanosine 5′-*O*-(2-thiodiphosphate) (GDP-β-s, 1 mM), was added into the pipette solution during the whole-cell recording. In the preliminary study, inclusion of 1 mM GDP-β-s in the intracellular solution abolished the postsynaptic $GABA_B$ current evoked by puff application of 100 μM baclofen (data not shown).

Baclofen, 4-aminopyridine (4-AP), $CdCl_2$, N-ethylmaleimide (NEM), CNQX, bicuculline and GDP-β-s were purchased from Sigma (St. Louis, MO, USA), TTX and QX-314 from Alomone Laboratories (Jerusalem, Israel), and CGP52432 from Tocris (Ellisville, MO, USA). All the drugs and solutions were freshly prepared before the experiments. Drugs were delivered at the final concentration using syringe pumps.

Data analysis

Data are presented as means±S.E.M. The amplitude and frequency of EPSCs and IPSCs were analyzed off-line using a peak detection program (Minianalysis; Synaptosoft, Leonia, NJ, USA). The IPSCs and EPSCs during a period of 3 min during the control, drug application and washout were analyzed. IPSCs or EPSCs were detected by the fast rise time and slow decay of the signal over an amplitude threshold set above the background noise. The amplitude detection threshold was typically 6–10 pA. The background noise level was constant throughout the recording period of the same neuron before and during drug application. We manually excluded the event when the noise was erroneously identified as the IPSCs/EPSCs by the computer program. The cumulative probability of the amplitude and inter-event interval of EPSCs and IPSCs was compared using the Komogorov-Smirnov test, which estimates the probability that two cumulative distributions are similar. At least 100 EPSCs and IPSCs were used in each analysis. All the decay phases of mIPSCs and mEPSCs were analyzed with one or two exponential functions based on the curve fitting R^2 values. The effect of drugs on the amplitude and frequency of EPSCs and IPSCs was determined by repeated measures ANOVA with Dunnett's post hoc test. $P<0.05$ was considered to be statistically significant.

RESULTS

Whole-cell voltage-clamp recordings were performed on a total of 126 labeled PVN cells. The spinal cord around the level of T_1–T_4 was taken out after killing the rat and sectioned into slices in 50 μm. The spinal cord slices were viewed under the fluorescence microscope to verify the injection and diffusion sites of the tracer as described