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Contribution of BK_{Ca} channels of neurons in rostral ventrolateral medulla to CO-mediated central regulation of respiratory rhythm in medullary slices of neonatal rats

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ABSTRACT

We recently described that carbon monoxide (CO) participated in the regulation of rhythmic respiration in medullary slices. The present study was undertaken to further assess whether the large-conductance calcium-activated potassium channels (BK_{Ca} channels) are involved in the CO-mediated central regulation of respiratory rhythm in medullary slices. The rhythmic discharge of hypoglossal rootlets of medullary slices of neonatal rats was recorded. We observed that blocking BK_{Ca} channels could partially abolish the effects of CO on the rhythmic bursts of hypoglossal rootlets. With whole-cell patch-clamp recording technique, we further observed that CO could reversibly augment potassium current density of the neurons in the rostral ventrolateral medulla. The CO-induced increase in potassium current was entirely blocked by the pretreatment of slices with BK_{Ca} channels blocker; whereas blockade of CO generation with zinc protoporphyrin-IX produced an opposite response. Altogether, these data indicate that BK_{Ca} channels of the neurons in neonatal rostral ventrolateral medulla could be activated by CO and involved in CO-mediated central regulation of respiratory rhythm in medullary slices.

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1. Introduction

Carbon monoxide (CO) is generated in mammals during the degradation of heme by the enzyme heme oxygenase (HO) (Wu and Wang, 2005). Some metalloporphyrins, such as zinc protoporphyrin-IX (ZnPP-9), tin protoporphyrin-IX (SnPP-9) and Zn-deuteroporphyrin-bis-glycol (ZnDPBG), are selective inhibitors of HO and can reduce formation of endogenous CO (Wu and Wang, 2005). Three distinct HO isoforms encoded by different genes have been identified to date, they are inducible HO-1 (Sikorski et al., 2004), constitutive HO-2 (Shibahara et al., 1993) and constitutive HO-3 (Mccoubrey et al., 1997), among which HO-1 and HO-2 are the most studied.

CO regulates physiological functions in a wide variety of tissues as an endogenous paracrine and autocrine gaseous messenger (Wu and Wang, 2005). Endogenous CO was reported to participate in central regulation of respiration. Expression of HO-2 mRNA was seen in the rostral ventrolateral medulla (RVLM) under both control and hypoxic conditions, whereas expression of HO-1 mRNA was only seen in the RVLM by induction of hypoxia (Mazza et al., 2001). HO-2 was immunocytochemically localized in the RVLM to the pre-Bötzinger complex (preBötC) (D'Agostino et al., 2009) which has been considered to be critical for the genesis of rhythmic respiration in the medullary slice (Smith et al., 1991; Feldman and Del Negro, 2006; Peña, 2008). Electrophysiological studies on neurons dissociated from the RVLM showed that, in response to SnPP-9, many of these neurons showed increase in baseline-firing frequency that is associated with depolarization of the membrane (Mazza and Neubauer, 1998). Our preliminary results have shown that ZnPP-9 could shorten the inspiratory duration and expiratory duration, increase the respiratory frequency, and decrease the inspiratory amplitudes of respiratory-like burst activity of the hypoglossal rootlets in medullary slices of neonatal rats (Yang et al., 2007). These observations indicate that endogenous CO may be involved in central regulation of the respiratory rhythm generated in the medullary slices. The underlying mechanism, however, is not yet entirely clear, although the soluble guanylyl cyclase (sGC)–cyclic guanine monophosphate (cGMP) pathway (Zhang et al., 2007) and the nitric oxide synthase (NOS)–nitric oxide (NO) pathway (Zhang

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et al., 2011) were reported to participate in the CO-mediated regulation of respiratory rhythmic activity at the level of medulla oblongata in neonatal rats.

The stimulation of the large-conductance calcium-activated potassium channels (BK_{Ca} channels) by both exogenous and endogenous CO has been documented in many studies from different laboratories. For instance, it has been demonstrated that both exogenous and endogenous CO could activate BK_{Ca} channels in either vascular smooth muscle cells (Wang et al., 1997; Naik and Walker, 2003; Hristov et al., 2004; Xi et al., 2004; Ryan et al., 2006) or carotid body glomus cells (Prabhaker et al., 1995; Riesco-Fagundo et al., 2001; Kemp, 2005), indicating that CO could mediate vasomotion or peripheral respiratory regulation. The present study was designed to examine the hypothesis that BK_{Ca} channels of neurons in RVLM could be activated by CO and then be involved in CO-mediated regulation of respiratory rhythmic activity of neonatal brain slices.

2. Materials and methods

2.1. Agents

ZnPP-9, tetraethylammonium (TEA), dimethyl sulfoxide, glibenclamide, 4-aminopyridine (4-AP) and tetrodotoxin (TTX) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Paxilline was purchased from Biomol (Plymouth Meeting, PA, USA). CO gas was obtained in research purity from Natural Gas Company (Chengdu, China).

2.2. Preparation of medullary slices

The experiments were performed on medullary slices of male or female neonatal (P0–3) Sprague–Dawley rats. All procedures were reviewed and approved by the Sichuan University Committee on the Use of Live Animals in Research, which is in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Medullary slices were prepared as described previously (Yang et al., 2007; Zavala-Tecuapetla et al., 2008; Zhang et al., 2010). In brief, the animals were decapitated following ether anesthesia. The brainstem was isolated in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 129 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 21 $NaHCO_3$, 1 KH_2PO_4 and 30 D-glucose, equilibrated with carbogen (95% O_2 and 5% CO_2), pH 7.4, and then glued against a cube of agar with its rostral end directed upwards, facing to the slicing blade at a 20° angle. The specimen was sectioned serially from rostral to caudal until the rostral boundary of preBötC within RVLM was reached. This region was recognized by cytoarchitectonic landmarks such as the nucleus ambiguus, hypoglossal nucleus, nucleus tractus solitarius and inferior olive nucleus, but no longer the facial nucleus (Smith et al., 1991; Koshiya and Smith, 1999; Rybak et al., 2003; Zavala-Tecuapetla et al., 2008). Then a medullary slice containing preBötC was made.

2.3. Recording and analysis of hypoglossal rootlet discharge

Thick (700–900 μm) medullary slices for recording rhythmic respiratory activity from the hypoglossal rootlets were prepared and continuously perfused with oxygenated ACSF at a rate of 4–5 ml/min at 27–28 °C, pH 7.4. To obtain and maintain consistent burst activity of the rootlets, the KCl concentration of the perfusing ACSF was raised from 3 to 7 mM. The slices were incubated for 30 min before starting the experiments. Glass suction electrode filled with ACSF was used to record the rhythmic respiratory-like discharge from the cut ends of the hypoglossal rootlets. Signals were amplified, filtered (τ = 0.001 s, F = 1 kHz) and integrated with a time constant of 50 ms by BL-420F biological signal processing system (Taimeng Biotech. Co., China). The discharge duration (DD), discharge interval (DI), discharge frequency (DF) and integrated amplitude (IA) of hypoglossal rootlets of the slices were analyzed as described previously (Yang et al., 2007; Hu et al., 2008). The slices were divided into six groups (n = 7 for each group): control, CO, paxilline, TEA, paxilline + CO and TEA + CO. In the control group, the slices were perfused with ACSF during the whole process. In the CO group, ACSF was bubbled with both pure CO gas and carbogen in an approximately 100 ml volume container at the same flow rate for 15 min immediately preceding perfusion of the slices. The estimated CO concentration in the resulting CO-ACSF was about 500 μM (Kwon et al., 2001). Slices were then perfused with CO-ACSF for 8 min. In the paxilline or TEA group, the slices were perfused with paxilline-ACSF (10 μM paxilline) or TEA-ACSF (1 mM TEA), each for 13 min. In the paxilline + CO or TEA + CO group, the slices were perfused with paxilline-ACSF or TEA-ACSF for 5 min, then simultaneously with CO, each for 8 min. The burst activity of hypoglossal rootlets before chemical application was recorded for 5 min as baseline of the activity for each group. From the end of administration of chemicals, the slices were continuously perfused with ACSF for washout. All data were compared with the baseline activity before applying chemicals. Normalized DD, DI, DF and IA of hypoglossal rootlets were reported as mean ± SEM and statistically analyzed with repeated measure ANOVA followed by LSD test. P values <0.05 were considered statistically significant.

2.4. Patch-clamp recording and analysis

Thin (about 300 μm) medullary slices containing preBötC for whole cell voltage clamp recording were prepared and continuously superfused with ACSF containing 5 μM glibenclamide, to block the ATP-sensitive potassium channels, at a flow rate of 1–2 ml/min at 20–21 °C, pH 7.4. The borosilicate glass electrode for the whole cell recording was pulled with a puller (P-97, Sutter Instrument, USA). The resistance of the pipette was 4–7 MΩ when filled with intracellular solution containing (in mM): 140 K-asp, 1 $MgCl_2$, 10 HEPES, 0.1 EGTA, 4 Na_2ATP, pH 7.2. For recording BK_{Ca} currents, membrane potential was held at −80 mV and then stepped to a 500 ms depolarizing prepulse (to 0 mV) to eliminate sodium-mediated and A-type potassium conductance, followed by application of 500 ms test potentials in 10 mV increments from −40 mV up to +60 mV with a 5 s interval. In most cases, a 500 ms prepulse to 0 mV could completely inactivate A-type potassium currents and sodium currents, if not, 0.3 μM TTX was applied to block sodium currents and/or 500 μM 4-AP was applied to block A-type potassium currents. Current signals were amplified with a patch-clamp amplifier EPC-10 (HEKA, Germany), filtered at 1 kHz and digitized at 5 kHz. The Pulse and Pulsefit software (HEKA, Germany) was used for voltage protocols as well as acquisition and analysis of data. Current densities (pA/pF) were estimated based on the membrane capacitance, presented as mean ± SEM and statistically analyzed with paired Student's t-tests or one-way ANOVA followed by LSD test. Results were considered significant if P values <0.05.

3. Results

3.1. Effects of blocking BK_{Ca} channels on CO-mediated central regulation of respiratory rhythmic activity

Paxilline, a natural non-peptide compound, exhibits a selective BK_{Ca}-blocking profile (Sanchez and Mcmanus, 1996). On the other hand, TEA in concentration of 1 mM is sufficient for blocking BK_{Ca} channels and widely used as a BK_{Ca} blocker as well

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