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Norepinephrine-induced calcium signaling in astrocytes in the respiratory network of the ventrolateral medulla

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ABSTRACT

The neuronal activity in the respiratory network of the ventrolateral medulla strongly depends on a variety of different neuromodulators. Since the respiratory activity generated by neurons in the pre-Bötzinger complex (preBötC) is stabilized by astrocytes, we investigated potential effects of the neuromodulator norepinephrine (NE) on the astrocytic calcium signaling in the ventral respiratory group. In acutely isolated brainstem slices from wild type mice (postnatal day 1–10) we performed calcium imaging experiments using Oregon Green 488 BAPTA-1 AM as a calcium indicator dye. Astrocytes in the preBötC, which were identified by their unique intracellular calcium rise after the reduction of the extracellular K^+ concentration, showed calcium rises in response to norepinephrine. These calcium signals persisted after blockade of neuronal activity by tetrodotoxin (TTX) indicating that they were independent of neuronal activity. Furthermore, application of the endoplasmic reticulum calcium pump blocker cyclopiazonic acid (CPA) diminished norepinephrine-induced calcium signals. This results could be confirmed using transgenic mice with astrocyte specific expression of GCaMP3. Thus, norepinephrine might, apart from acting directly on neurons, influence and modulate respiratory network activity via the modulation of astroglial calcium signaling.

1. Introduction

In the ventrolateral medulla neurons for respiratory and cardiac control are intermingled and surrounded by astrocytes that stabilize autonomous activity of the network (Hülsmann et al., 2000; Grass et al., 2004; Schnell et al., 2011; Oku et al., 2016). Not only have these cells been shown to provide supportive functions for this neuronal activity, e.g., by mediating reuptake of neurotransmitters (Gomeza et al., 2003; Szöke et al., 2006), but they also have been shown to respond to various neuromodulators by an increase of their intracellular calcium concentration (Funk et al., 2008; Härtel et al., 2009; Huxtable et al., 2010). Like other neuromodulators norepinephrine (NE) has been shown to stimulate the output of the respiratory network activity (Errchidi et al., 1991; Al-Zubaidy et al., 1996; Viemari and Ramirez, 2006; Funk et al., 2011). NE is released in the respiratory network from pontine and medullary neurons (Robertson et al., 2013) and leading to a stimulation of ventilation (Bianchi et al., 1995; Hilaire et al., 2004). Moreover, medullary NE levels are reduced in mouse models for the RETT syndrome (Viemari et al., 2005). To understand the role of astrocytes in NE-signaling we aimed to investigate NE-effects, which might involve astrocytes. Here we tested if NE exerts an effect on astrocytes and their main signaling mechanism of calcium signaling.

2. Materials and methods

2.1. Breeding of mice

Animals were hold and bred in the animal facilities of the University Hospital Göttingen in accordance with guidelines of the German Physiological Society as well as the regulations of the State of Lower Saxony Institutional registration (T19/08), and Saarland and the Federal Republic of Germany (TierSchG). Procedures were approved by the authorities of Lower Saxony State Office for Consumer Protection and Food Safety; 33.12-42502-04-14/1524 and Saarland (State Office for Consumer Protection; 72/2010).

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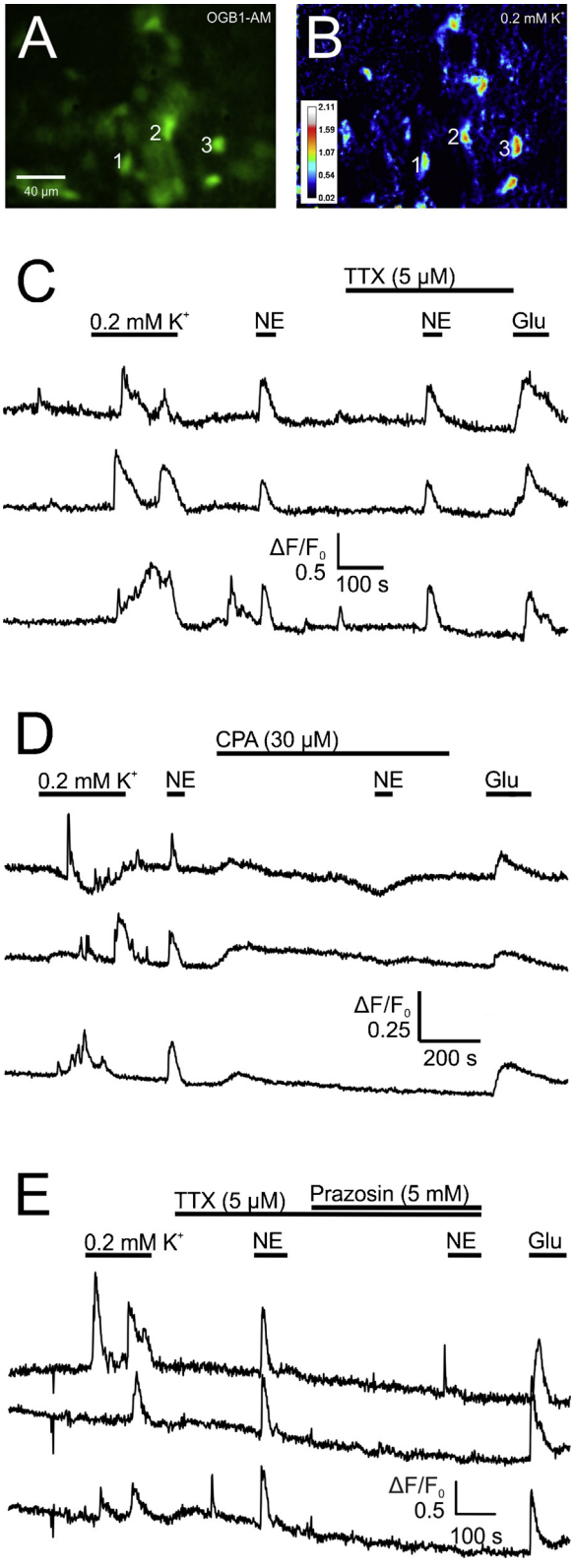

Fig. 1. Analysis of norepinephrine (NE)-induced calcium signals in astrocytes of the ventrolateral medulla. A: Oregon-Green-BAPTA1-AM labeling of the tissue. B: Identification of astrocytes was performed via application of an extracellular solution with a low (0.2 mM) potassium concentration. The $\Delta F/F_0$ image identifies the position of astrocytes by the calcium rise during application of 0.2 mM K^+. C: Original traces from the cells indicated in panel A,B. NE (10 µM) is applied as indicated before and during the application of TTX. Glutamate (1 mM) was applied to prove the viability of the cells. D: Application of CPA diminished the response of NE. E: Significant reduction of calcium signals induced by NE in the presence of the α1-adrenoreceptor antagonist prazosin (50 µM).

Experiments were performed on acute brain slice preparations of NMRI (naval medical research institute), transgenic hGFAP-mRFP1 (Hirrlinger et al., 2005) and *Glast*-GCaMP3 mice (Mori et al., 2006; Paukert et al., 2014).

2.2. Induction of GCaMP3 expression in astrocytes

Glast-creERT2 mice ($Slc1a3^{tm1(cre/ERT2)Mgoe}$; Mori et al., 2006) and R26-lsl-GCaMP3 (Paukert et al., 2014) were crossbred to receive double transgenic offsprings. For induction of GCaMP3 expression in newborn offsprings, lactating mice were intraperionetally injected with tamoxifen (10 mg/ml solved in corn oil, 100 mg/kg body weight) once a day for 1–4 consecutive days on day 0–3 after birth. After the last tamoxifen injection we waited a minimum of 2 days for allowing sufficient expression of GCaMP3.

2.3. Slice preparations

Acute transversal brainstem slices were prepared as described previously (Härtel et al., 2007; Winter et al., 2009; Schnell et al., 2011). Animals were decapitated under diethyl-ether anesthesia, and brainstems were isolated and placed in ice-cooled, carbogen-saturated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF) containing 118 mM NaCl, 3 KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO4, 25 mM $NaHCO_3$, and 30 mM D-glucose; pH 7.4. The isolated brainstem was glued with cyanoacryl glue (Loctite Deutschland GmbH) to an agar block and mounted in a vibroslicer (VT 1000S, Leica). The location of the preBötC was judged based on the anatomy of inferior olive and 4th ventricle (see: Winter et al., 2009; Schnell et al., 2011).

Before staining with Oregon Green BAPTA 1-AM (OGB; see below) slices were kept in oxygenated ACSF at room temperature for at least 30 min. For imaging experiments, slices were transferred to the recording chamber and were kept submerged by a nylon fiber grid and continuously perfused with aCSF at a flow rate of 5–10 ml/min.

For OGB calcium imaging experiments 200–300 µm slices containing the pre-Bötzinger complex (preBötC) were cut from P1–P10 mice. Slices made from GCaMP3 expressing mice (P3–P11) were 650 µm thick.

2.4. Epifluorescence calcium imaging

Cells were loaded with the calcium dye Oregon Green BAPTA 1-AM by multi-cell bolus loading (Zhao et al., 2006) and fluorescence changes were recorded using upright fluorescence microscopy (Axioscope FS1, Zeiss) though a 40 × 0.8 NA water immersion objective (Zeiss) with a cooled CCD camera (SensiCam; PCO, Kelheim, Germany). A monochromator (Polychrome, T.I.L.L. Photonics, München, Germany) was used to generate excitation (494 nm). For wavelength control and image acquisition Imaging Workbench 6 (Indec Biosystems, Mt. View, USA) was used and images were captured at 1 Hz. During epifluorescence imaging, astrocytes were identified by their characteristic calcium response induced by lowering the extracellular potassium concentration from 3 mM to 0.2 mM. This procedure was also used to identify astrocytes in hGFAP-mRFP1 mice (Härtel et al., 2009; Hirrlinger et al., 2005) and has been shown to be a reliable method to identify astrocytes in brain slices (Dallwig et al., 2000; Dallwig and Deitmer, 2002; Härtel et al., 2007).

2.5. 2-photon excitation microscopy

GCaMP3 fluorescence was imaged through a 20 × 1.0 NA water immersion objective (Zeiss) with non-descanned detection by