Respiratory Physiology & Neurobiology 212–214 (2015) 9–19

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Activation of 5-HT1A receptors in the preBötzinger region has little impact on the respiratory pattern

Tomislav Radocaj[a,b], Sanda Mustapic[a,b], Ivana Prkic[a,b], Astrid G. Stucke[a,c], Francis A. Hopp[a,b], Eckehard A.E. Stuth[a,c], Edward J. Zuperku[a,b,*]

[a] Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI, United States
[b] Clement J. Zablocki VA Medical Center, Milwaukee, WI 53295, United States
[c] Children's Hospital of Wisconsin, Pediatric Anesthesia, Milwaukee, WI, United States

ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form 10 February 2015
Accepted 19 March 2015
Available online 5 April 2015

Keywords:
5-HT receptors
Control of breathing
Respiratory neurons
preBötzinger complex
5-HT$_{1A}$ receptors
8-OH-DPAT

ABSTRACT

The preBötzinger (preBötC) complex has been suggested as the primary site where systemically administered selective serotonin agonists have been shown to reduce or prevent opioid-induced depression of breathing. However, this hypothesis has not been tested pharmacologically in vivo. This study sought to determine whether 5-HT$_{1A}$ receptors within the preBötC and ventral respiratory column (VRC) mediate the tachypneic response induced by intravenous (IV) (±)-8-Hydroxy-2-diproplyaminotetralin hydrobromide (8-OH-DPAT) in a decerebrated dog model. IV 8-OH-DPAT (19 ± 2 μg/kg) reduced both inspiratory (I) and expiratory (E) durations by ~40%, but had no effect on peak phrenic activity (PPA). Picoejection of 1, 10, and 100 μM 8-OH-DPAT on I and E preBötC neurons produced dose-dependent decreases up to ~40% in peak discharge. Surprisingly, microinjections of 8-OH-DPAT and 5-HT within the VRC from the obex to 9 mm rostral had no effect on timing and PPA. These results suggest that the tachypneic effects of IV 8-OH-DPAT are due to receptors located outside of the areas we studied.

Published by Elsevier B.V.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) plays a key role in the modulation of the breathing pattern by acting on different subtype receptors (Hedlund et al., 2004) and especially by acting on 5-HT$_{1A}$ receptors whose stimulation during intravenous administration of 5-HT$_{1A}$ agonists increases breathing rate (Garner et al., 1989; Gillis et al., 1989; Sahibzada et al., 2000). There has been increased interest in employing subtype receptor agonists of these biogenic amines to prevent or correct the clinical problem of opioid-induced respiratory depression of breathing without diminishing their potent analgesic effect (Dutschmann et al., 2009; Guenther et al., 2009; Manzke et al., 2009, 2003; Oertel et al., 2007; Sahibzada et al., 2000). Since μ-opioids act on neurons via G-protein mediated inhibition of adenylyl cyclase and cAMP, one approach that has been used takes advantage of the convergence within the intracellular signaling pathways of messengers that are common to μ-opioid receptors and receptor subtypes for 5-HT and norepinephrine. Systemic administration 5-HT$_{4a}$ agonists (Manzke et al., 2003) and alpha-2 adrenoreceptor antagonists (Vonhof and Siren, 1991), which activate cAMP, partially reverse opiate-induced respiratory depression without antagonizing or reducing the analgesic effects of opiates. Alternatively, another approach takes advantage of the interaction among neurons within the respiratory control network, which express postsynaptic 5-HT$_{1A}$ receptors that inhibit cAMP production. The selective 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) has been shown to counteract opioid-induced respiratory depression without impairing analgesia in rats (Guenther et al., 2009; Sahibzada et al., 2000). Repinotan hydrochloride, a selective 5-HT$_{1A}$-receptor agonist already investigated in humans, has been shown to prevent and antagonize opioid-induced ventilatory depression in anesthetized rats (Guenther et al., 2012, 2010). Recently, befiradol a highly selective 5-HT$_{1A}$ receptor agonist was shown to reduce fentanyl-induced respiratory depression, however, it also reduced the analgesic effect of the opioid (Ren et al., 2015).

While such studies demonstrate that the systemically administered 5-HT$_{1A}$ agonists are remarkably effective in increasing breathing rate, the underlying mechanisms and anatomical sites of action are unclear. Based on immunohistochemical analyses of subtype bioamine receptor expression and co-expression of

* Corresponding author at: Clement J. Zablocki Department of Veterans Affairs Medical Center & Medical Coll, Anesthesiology, Zablocki VA Medical Center, Research Service/151, 5000 W. National Ave, Milwaukee, WI 53295, United States. Tel.: +1 414 384 2000x41579; fax: +1 414 645 6550.
E-mail address: ezuperku@mcw.edu (E.J. Zuperku).

http://dx.doi.org/10.1016/j.resp.2015.03.005
1569-9048/Published by Elsevier B.V.

μ-opioid receptors (Manzke et al., 2009, 2003; Niebert et al., 2011) and on in vitro pharmacological studies (Gray et al., 1999), it has been posited that the relevant receptor actions occur within the rhythmogenic preBötzinger complex (preBötC). However, microinjection of 50 μM 8-OH-DPAT into the preBötC of anesthetized cats produced a sustained apnea (Richter et al., 1999). Moreover, neuroanatomical tracing studies show projections of raphe obscurus serotonergic neurons to multiple sites of respiratory control in medulla and pons. 5-HT-immunoreactive fibers are found in the nucleus of the solitary tract (NTS), nucleus ambiguus, retrotrapezoid nucleus (RTN), preBötC, and hypoglossal and phrenic motor nuclei (Holtman, 1988; Holtman et al., 1990a, 1984a, 1984b, 1990b; Jacobs and Azmitia, 1992; Pilowsky et al., 1990; Voss et al., 1990). A recent extensive study showed that the RTN, which consists of a group of highly chemosensitive neurons that are thought to drive the breathing network, receives direct projections from raphe serotonergic neurons. The discharge of the RTN neurons was remarkably increased by local application of 5-HT and also stimulated by substance P and thyrotropin-releasing hormone (TRH) (Mulkey et al., 2007).

In the study of Richter et al. (1999), it is noteworthy that IV 8-OH-DPAT also caused apnea, which is opposite to the tachypnea reported by others (Guenther et al., 2009; Sahibzada et al., 2000). Accordingly, these findings call into question whether the 5-HT_{1A} agonists when used systemically mediate their stimulating effects on respiration via the preBötC neurons. Thus, the current study was undertaken to gain a better understanding of the role of serotonergic 5-HT_{1A} receptors on respiratory neurons in the preBötC complex and adjacent regions of the ventral respiratory column (VRC). We studied the effects of intravenous application of 5-HT_{1A}-receptor agonist 8-OH-DPAT on the phrenic discharge pattern in an adult decerebrated dog model. In addition, local applications of selective agonists via the picoejection technique were made while recording single unit activity. Lastly, microinjections of 8-OH-DPAT were made throughout the VRC from the obex to 8 mm rostral of obex while monitoring phrenic activity. We hypothesized that local microinjection of serotonergic agonists into the VRC, which includes the preBötC, should emulate the effects of their systemic administration, if these agonists exerted their relevant effects within the VRC and preBötC.

2. Methods

This research was approved by the subcommittee on animal studies of the Zablocki VA Medical Center, Milwaukee, WI, in accordance with provisions of the Animal Welfare Act, the PHS Guide for the Care and Use of Laboratory Animals and VA policy. Experiments were performed on mongrel dogs of either sex, weighing from 8 to 16 kg. Inhalational anesthesia was induced by mask and maintained with isoflurane at 1.5–2.5% (1–2 MAC) end-tidal concentration. The animals were monitored for signs of inadequate anesthesia such as salivation, lacrimation, and increases in blood pressure and heart rate. If required, anesthetic depth was increased immediately.

2.1. Surgical procedures

Dogs were intubated with a cuffed endotracheal tube and their lungs mechanically ventilated with a ~60% O_2-2.5% isoflurane and balance of air mixture. After cannulating the femoral artery for blood pressure recording and blood gas sampling and femoral vein for continuous infusion of maintenance fluids and administration of drugs, a bilateral pneumothorax was performed to reduce motion artifacts. The animal was then decerebrated by midcollicular transection (Tonkovic-Capin et al., 1998) and isoflurane was discontinued. After decerebration the animal was ventilated with an air–O_2 mixture (FiO_2 ≥0.6) and maintained in hyperoxic normocapnia. The dorsal surface of the medulla oblongata was exposed by an occipital craniotomy for neuronal recording and microinjections. Phrenic nerve activity was recorded from the desheathed right C5 rootlet. The phrenic neurogram (PNG) was obtained from the moving-time average (100 ms) of the amplified phrenic nerve activity and was used to produce timing pulses corresponding to the beginning and end of the inspiratory phase for the measurement of inspiratory duration (T_I), expiratory duration (T_E) and fictive breathing frequency (f_B) in breaths per minute (BPM). Peak phrenic activity (PPA) was also obtained from the PNG and expressed as % of control. Continuous neuromuscular block was achieved with pancuronium (0.1 mg/kg/h) to reduce motion artifacts during neuronal recordings.

2.2. Data recording and microinjection technique

A minimum of 1 h was allowed for preparation stabilization before data collection. Extracellular neuronal recordings were obtained using multibarrel micropipettes (10–30 μm composite tip diameter) consisting of a recording barrel containing a 7-μm thick carbon filament and three drug barrels. Neuronal action potentials with appropriate amplification and band-pass filtering (0.1–3 kHz), phrenic activity, and picoejection marker pulses, were recorded with the CED Power 1401 mk II and Spike2 data acquisition system (Cambridge Electronic Design Limited, Cambridge, UK). The phrenic neurogram (PNG), spike discharge rate, cyclic T_I and T_E, airway CO_2 concentration and systemic blood pressure were continuously displayed/recorded on a digital polygraph (PowerLab/16SP, ADInstruments). All neuron locations were documented by their coordinates with respect to obex, midline and dorsal surface of the exposed brainstem (i.e., rostral to obex, lateral from the midline, and ventral to the dorsal surface). The tilt of the medulla relative to the horizontal plane was measured in each preparation and used to correct the coordinates for the subsequent analyses. CED Spike2 software was used to extract spikes from background signals via template matching and generate cycle-triggered histograms (CTHs; 10–30 respiratory cycles/CTH) using timing signals from the PNG. Peak and time-averaged discharge frequency (Fn) values were obtained from the CTHs, where the time range for calculating the average Fn was taken as the time over which the neuron was active during the pre-ejection control period. This time period was also used for the data obtained during picoejections. The micropipettes were used to locate VRC- and preBötC neurons and to either picoeject agonists onto single recorded neurons or microinject agonists into a group of VRC neurons. All drugs (see below) were dissolved in artificial cerebrospinal fluid (aCSF) consisting of (in mM) 124 NaCl, 2 KCl, 2 MgCl, 1.3 KH_2PO_4, 0.9 $CaCL_2$, 26 $NaHCO_3$, and 11 glucose. The microinjected drug volumes were measured via height changes of the meniscus in the pipette barrel with a 100× magnification microscope equipped with a reticule (resolution ~2 nl). We used three criteria to locate the preBötC region in dogs: (1) predetermined stereotaxic coordinates based on previous studies (Krolo et al., 2005), (2) ability to extracellularly record a mixture of respiratory neuron subtypes within these predetermined coordinates of the VRC, and (3) tachypneic response of the phrenic nerve (PNG) to DLH microinjections (30–40 nl; 20 mM) within the predetermined region of the VRC. All methods are described in more detail by Mustapic et al. (2010).

2.3. Protocols

2.3.1. Protocol 1: Effects of IV administration of 8-OH-DPAT on respiratory phase timing and peak phrenic nerve activity

The phrenic nerve responses to systemic (IV) administration of 8-OH-DPAT were studied. Doses of agonist were increased incrementally until a maximum effect was observed.