Neuroscience 255 (2013) 158–176

METHYLXANTHINES DO NOT AFFECT RHYTHMOGENIC PREBÖTC INSPIRATORY NETWORK ACTIVITY BUT IMPAIR BURSTING OF PREBÖTC-DRIVEN MOTONEURONS

B. PANAITESCU, [a†] **J. KURIBAYASHI,** [a†] **A. RUANGKITTISAKUL,** [a] **V. LEUNG,** [a] **M. IIZUKA** [b] **AND K. BALLANYI** [a*]

[a] *Department of Physiology, Faculty of Medicine & Dentistry, 750 Medical Sciences Building, University of Alberta, Edmonton T6G 2H7, Canada*

[b] *Center for Medical Sciences, Ibaraki Prefectural University of Health Sciences, Ami, Ibaraki, Japan*

Abstract—Clinical stimulation of preterm infant breathing with methylxanthines like caffeine and theophylline can evoke seizures. It is unknown whether underlying neuronal hyperexcitability involves the rhythmogenic inspiratory active pre-Bötzinger complex (preBötC) in the brainstem or preBötC-driven motor networks. Inspiratory-related preBötC interneuronal plus spinal (cervical/phrenic) or cranial hypoglossal (XII) motoneuronal bursting was studied in newborn rat en bloc brainstem-spinal cords and brainstem slices, respectively. Non-respiratory bursting perturbed inspiratory cervical nerve activity in en bloc models at >0.25 mM theophylline or caffeine. Rhythm in the exposed preBötC of transected en bloc preparations was less perturbed by 10 mM theophylline than cervical root bursting which was more affected than phrenic nerve activity. In the preBötC of slices, even 10 mM methylxanthine did not evoke seizure-like bursting whereas >1 mM masked XII rhythm via large amplitude 1–10 Hz oscillations. Blocking A-type γ-aminobutyric ($GABA_A$) receptors evoked seizure-like cervical activity whereas in slices neither XII nor preBötC rhythm was disrupted. Methylxanthines (2.5–10 mM), but not blockade of adenosine receptors, phosphodiesterase-4 or the sarcoplasmatic/endoplasmatic reticulum ATPase countered inspiratory depression by muscimol-evoked $GABA_A$ receptor activation that was associated with a hyperpolarization and input resistance decrease silencing preBötC neurons in slices. The latter blockers did neither affect preBötC or cranial/spinal motor network bursting nor evoke seizure-like activity or mask corresponding methylxanthine-evoked discharges. Our findings show that methylxanthine-evoked hyperexcitability originates from motor networks, leaving preBötC activity largely unaffected, and suggest that $GABA_A$ receptors contribute to methylxanthine-evoked seizure-like perturbation of spinal motoneurons whereas non-respiratory XII motoneuron oscillations are of different origin.

Key words: apnea of prematurity, epilepsy, network oscillations, rhythm generation, seizures.

INTRODUCTION

Tonic–clonic convulsions are commonly accompanied by ictal respiratory changes (IRCs) that likely play a major role in sudden unexplained death in epilepsy patients (SUDEP) (Navelet et al., 1989; Blum, 2009). Whether IRCs manifest as accelerated or depressed breathing depends on various factors such as (cortical) subregions from which seizures originate or the animal species studied (Blum, 2009; Surges et al., 2009; Boison, 2011). Use of A-type γ-aminobutyric ($GABA_A$) receptor blockers like bicuculline or penicillin for inducing seizures in animal models revealed that potentially lethal arrest of lung ventilation ('apnea') in IRCs can include both central nervous and obstructed airway components (Paydarfar et al., 1991; Terndrup et al., 1996, 1999; Leaming et al., 1999; Budzińska, 2004). Inhibition of neural respiratory networks in the lower brainstem, the medulla oblongata, seems to be responsible for centrally depressed breathing as well as laryngospasms in IRCs (St-John et al., 2006; Tavee and Morris, 2008; Blum, 2009). Of utmost importance in that regard is the pre-Bötzinger complex (preBötC) inspiratory center whose cellular functions are studied in medullary slices or 'en bloc' brainstem-spinal cord preparations from perinatal rodents (Suzue, 1984; Smith et al., 1991; Ballanyi et al., 1999; Ballanyi and Ruangkittisakul, 2009; Feldman et al., 2013). Using the newborn rat en bloc model, we found that bicuculline-evoked seizure-like bursting in cervical nerves originates from spinal motor networks and not medullary inspiratory interneurons (Brockhaus and Ballanyi, 1998, 2000). This shows that the bicuculline seizure model evokes hyperexcitability similar to that in cortical networks in some components of the neural respiratory control system.

Convulsive seizures and associated IRCs can occur in patients that are treated with caffeine or theophylline for either countering apnea of prematurity of central origin or obstructive impairment of breathing in acute bronchial

*Corresponding author. Tel: +1-780-492-8235; fax: +1-780-492-8915.
E-mail address: klaus.ballanyi@ualberta.ca (K. Ballanyi).
† Authors contributed equally to the study.
Abbreviations: C_4, fourth cervical; CPA, cyclopiazonic acid; DMPX, 3,7-dimethyl-1-propargylxanthine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; Hepes, hydroxyethyl piperazineethanesulfonic acid; IRCs, ictal respiratory changes; P, postnatal day; PDE4, phosphodiesterase-4; pFRG, parafacial respiratory group; preBötC, pre-Bötzinger complex; RTN, retrotrapezoid nucleus; SERCA, endoplasmic/sarcoplasmic reticulum ATPase; T, thoracic; VRC, ventral respiratory column.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.09.058

asthma and chronic obstructive pulmonary disease (Barnes, 2003; Charles et al., 2008; Rottier and Duiverman, 2009; Mathew, 2011). Moreover, seizures can develop in healthy individuals upon abuse of 'energy' drinks containing these methylxanthines (Sawynok, 1995; Magkos and Kavouras, 2005; Iyadurai and Chung, 2007; Mortelmans et al., 2008). It is not known whether such IRCs are secondary to hyperexcitability in cortical circuits that subsequently influence respiratory networks or can also originate directly from the latter structures.

It was one aim of this study to investigate potentially epileptogenic methylxanthine effects on isolated inspiratory networks of newborn rats. For this, we used suction electrodes for simultaneous recording of population bursting of preBötC interneurons and preBötC-driven spinal motoneurons in anatomically 'calibrated' en bloc preparations (Ruangkittisakul et al., 2007, 2012). In calibrated slices (Ballanyi and Ruangkittisakul, 2009), we performed suction electrode recording from preBötC-driven hypoglossal (XII) cranial motoneurons and the ventral respiratory column (VRC) containing the preBötC. Moreover, we used 'blind' whole-cell patch-clamp recording (Smith et al., 1991, 1992) for analyzing membrane potential responses of inspiratory and tonically active neurons within the preBötC.

Recently, we reported that low millimolar methylxanthine is needed for countering opioid depression of the isolated newborn rat preBötC (Ruangkittisakul and Ballanyi, 2010). Based on these observations and our preliminary findings (Ruangkittisakul et al., 2010), we hypothesize that similar doses are also necessary for evoking seizure-like activity. Such methylxanthine doses block $GABA_A$ receptors, phosphodiesterase-4 (PDE4) and Ca^{2+} uptake into cellular stores whereas A_1- and A_{2A}-type adenosine receptors are already inhibited at $< 100\ \mu M$ (Fredholm et al., 1999; Chen et al., 2013). For investigating the contribution of these mechanisms to presumptive methylxanthine-evoked perturbation of inspiratory networks, we studied whether selective blockers of these various cellular processes mimic or mask methylxanthine effects.

EXPERIMENTAL PROCEDURES

Preparations and solutions

All procedures were carried out in compliance with guidelines of the Canadian Council for Animal Care and with approval of the University of Alberta Health Animal Care and Use Committee for Health Sciences. Experiments were performed on (anatomically calibrated) en bloc brainstem-spinal cords and brainstem slices from postnatal day (P) 0–4 old Sprague–Dawley or Wistar rats. Because there was no indication for differences between findings using either rat strain, the data were pooled. Animals were anesthetized with 2–3% isoflurane until disappearance of the paw withdrawal reflex. Following decerebration, the neuraxis was isolated at 18–21 °C in 'standard' solution containing (in mM) 118 NaCl, 3 KCl, 1 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 30 (in en bloc models) or 20 (in slices) D-glucose; pH was adjusted to 7.4 by gassing with 95% O_2–5% CO_2.

Three types of en bloc models were used (Fig. 1). Firstly, experiments were done on the 'Suzue' preparation (Suzue, 1984; Ballanyi et al., 1999), in which the preBötC interacts with pre/post-inspiratory active parafacial respiratory group (pFRG) neurons (Fig. 1A). pFRG neurons drive expiratory muscles in newborns, but appear to transform postnatally to a major extent into chemosensitive retrotrapezoid nucleus (RTN) neurons providing tonic excitatory drive to the preBötC (Janczewski et al., 2002; Onimaru and Homma, 2003; Guyenet and Mulkey, 2010). For generating this model, the brainstem-spinal cord preparation was transected rostrally at the caudal cerebellar artery and thus contained the facial (VII) motor nucleus ('en bloc [+VII] model') whereas the cord was cut at the last cervical (C_8) or first thoracic (T_1) level (Fig. 1A). For testing if methylxanthines evoke hyperexcitability primarily in respiratory or rather non-respiratory motoneurons, en bloc [+VII] preparations with rostral and caudal boundaries identical to the Suzue model were isolated with the cervical and brachial plexuses for recording from fourth cervical root (C_4) plus phrenic and musculocutaneous nerves (Fig. 1B). Inspiratory active cervical nerve roots (C_1–C_8) contain axons from both inspiratory and non-respiratory motoneurons. In contrast, the phrenic nerve contains solely axons from inspiratory (phrenic) motoneurons while the musculocutaneous nerve contains motor axons that innervate arm muscles (Greene, 1935; Ballanyi et al., 1999; Cho et al., 2007). Finally, calibrated en bloc preparations were used (Fig. 1C), in which brainstem transection aimed at exposing the preBötC to the rostral cut surface. In newborn rats, the preBötC extends within the VRC between −0.4 and −0.6 mm caudal to the posterior end of VII nucleus (VII_c) and spans the most anterior XII nerve root as a surface landmark (Smith et al., 1991; Ruangkittisakul et al., 2007, 2008) (Fig. 1). In seven such preparations, the mean rostral transection level was -0.22 ± 0.10 mm caudal to VII_c. In this model, the RTN/pFRG complex does not seem to be active based on the absence of pre/post-inspiratory activity in lumbar nerves that normally drives abdominal expiratory muscles (Janczewski et al., 2002; Ruangkittisakul et al., 2007; Taccola et al., 2007). Here, the cord was transected between T_2 and T_3 and suction electrodes were used for simultaneous recording of inspiratory-related ('inspiratory' for short) rhythms from ventral spinal nerves, and also in the ventrolateral aspect of the cut medullary surface containing the (partially) exposed preBötC (Fig. 1C). For studying methylxanthine effects on inspiratory XII cranial motor networks and the preBötC in the absence of major influences from rostrally and caudally adjacent (respiratory) structures, calibrated 400 µm thick transversal brainstem slices were used that contain the preBötC in the middle ('m-preBötC[400] slices') (Ruangkittisakul et al., 2006,