Respiratory Physiology & Neurobiology 207 (2015) 61–71

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Abrupt changes in pentobarbital sensitivity in preBötzinger complex region, hypoglossal motor nucleus, *nucleus tractus solitarius*, and cortex during rat transitional period (P10–P15)

Sara M.F. Turner, Stephen M. Johnson*

Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706, United States

ARTICLE INFO

Article history:
Accepted 22 December 2014
Available online 27 December 2014

Keywords:
Pentobarbital
preBotzinger complex
Nucleus tractus solitaries
Hypoglossal
Development

ABSTRACT

On postnatal days P10–P15 in rat medulla, neurotransmitter receptor subunit composition shifts toward a more mature phenotype. Since medullary $GABA_A$Rs regulate cardiorespiratory function, abrupt alterations in GABAergic synaptic inhibition could disrupt homeostasis. We hypothesized that $GABA_A$Rs on medullary neurons become more resistant to positive allosteric modulation during P10–P15. Medullary and cortical slices from P10 to P20 rats were used to record spontaneous action potentials in pre-Botzinger Complex (preBötC-region), hypoglossal (XII) motor nucleus, *nucleus tractus solitarius* (NTS), and cortex during exposure to pentobarbital (positive allosteric modulator of $GABA_A$Rs). On P14, pentobarbital resistance abruptly increased in preBötC-region and decreased in NTS, but these changes in pentobarbital resistance were not present on P15. Pentobarbital resistance decreased in XII motor nucleus during P11–P15 with a nadir at P14. Abrupt changes in pentobarbital resistance indicate changes in GABAergic receptor composition and function that may compensate for potential increased GABAergic inhibition and respiratory depression that occurs during this key developmental transitional period.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

GABAergic synaptic transmission plays a key role in the respiratory control network and $GABA_A$Rs are widely expressed on respiratory neurons. $GABA_A$Rs are responsible for fast, synaptic inhibition and also mediate neuronal excitability with a tonic inward inhibitory current. In preBötC neurons, $GABA_A$Rs modulate neuronal excitability and regulate respiratory rhythm generation and pattern (Bongianni et al., 2010; Janczewski et al., 2013; Shao and Feldman, 1997). Also, tonic GABAergic input can constrain the firing rate of medullary respiratory neurons by up to 50% (Zuperku and McCrimmon, 2002), and altered GABAergic signaling can significantly disrupt respiratory output (Koshiya and Guyenet, 1996; Paton and Richter, 1995).

There is a "transitional period" in respiratory motor control development during postnatal days P10–P15 in rats that is characterized by abrupt changes toward the adult phenotype in receptor subunit composition and neurotransmitter concentrations in neurons within preBötC (generates inspiratory rhythm), XII motor nucleus (regulates upper airway patency), and NTS (integrates cardiorespiratory sensory input; Liu and Wong-Riley, 2005; Wong-Riley and Liu, 2008). Although "critical period" was used by Wong-Riley and colleagues in their seminal studies of development (Gao et al., 2011; Liu et al., 2006, 2009; Liu and Wong-Riley, 2002, 2004, 2005, 2006, 2010; Wong-Riley and Liu, 2008; Wong-Riley et al., 2013), we prefer to use the term "transitional period" because "critical period" is more often used in neuroscience to define a specific developmental period whereby a perturbation (e.g., sensory deprivation) causes long-lasting changes in neural function (e.g., Hensch and Bilimoria, 2012). The rat transitional period in respiratory control development at P10–P15 represents a progression toward expressing the adult phenotype in receptor subunit composition and neurotransmitter concentrations, but it also represents a potential period of increased vulnerability for maintaining blood-gas homeostasis.

During this period, accumulating evidence suggests there is a net increase in neuronal inhibition. GABAergic inhibition is increased and excitatory signaling is decreased in the XII motor nucleus

Abbreviations: aCSF, artificial cerebrospinal fluid; cNA, compact *nucleus ambiguus*; $GABA_A$Rs, $GABA_A$ receptors; NTS, *nucleus tractus solitarius*; preBötC, preBötzinger complex; VRC, ventral respiratory column; XII, hypoglossal.

* Corresponding author at: Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, WI 53706, United States. Tel.: +1 608 261 1104; fax: +1 608 263 3926.
E-mail address: johnsons@svm.vetmed.wisc.edu (S.M. Johnson).

http://dx.doi.org/10.1016/j.resp.2014.12.016
1569-9048/© 2014 Elsevier B.V. All rights reserved.

(Gao et al., 2011). Similarly, in all three regions, glutamate and NMDAR1 decrease, whereas GABA, $GABA_BR$, glycine receptors, and GluR2 increase at P12 (Liu and Wong-Riley, 2002, 2005, 2010). With respect to $GABA_ARs$, subunit composition switches from including predominately alpha-3 subunits (immature phenotype) to alpha-1 subunits (mature phenotype) on postnatal day 12 in preBötC and NTS neurons (Liu and Wong-Riley, 2004, 2006). Functionally, these changes may contribute to decreased neuronal excitability, and potentially cause the blunted hypoxic and hypercapnic ventilatory responses in found in P12–P15 rats (Holley et al., 2012; Liu et al., 2006, 2009; Wong-Riley and Liu, 2008). Taken together, these studies suggest that neuronal inhibition is relatively high during the second postnatal week. Despite the obvious importance of maintaining appropriate respiratory control during the transitional period, underlying mechanisms that may potentially compensate for increased inhibition are poorly understood and have received little attention.

Recently, our group identified a compensatory mechanism which likely contributes to respiratory control stability. Specifically, $GABA_ARs$ become insensitive to pentobarbital during hibernation (Hengen et al., 2009, 2011) and pregnancy (Hengen et al., 2012). This response appears to be selective for respiratory-related medullary regions, such as ventral respiratory group and NTS, since cortical neurons remain highly sensitive to pentobarbital during both conditions (Hengen et al., 2009, 2011, 2012). Since there is potentially widespread decreased excitability in the rat medulla during P10–P15, we hypothesized that respiratory-related medullary regions would increase resistance to positive allosteric modulation to maintain appropriate neuronal excitability (i.e., decrease impact of inhibition).

To address this hypothesis, spontaneous neuronal action potentials were recorded in preBötC-region, XII motor nucleus, NTS, and cortex (non-respiratory control) in acutely isolated brain slices from P10 to P20 rats using extracellular silicon multichannel electrodes. To pharmacologically assay for resistance to positive allosteric modulation, slices were exposed to pentobarbital and the subsequent decrease in spontaneous action potential firing was quantified. We found that there were complex changes in pentobarbital-resistance that were region- and age-dependent in medulla, but not cortex. Preliminary data were reported previously (Turner and Johnson, 2012).

2. Methods

2.1. Electrophysiological brain slice recordings in vitro

All experimental procedures followed NIH guidelines and this study was approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. A total of 94 rats at postnatal ages P10–P20 were used for brain slice recordings. Rats were anesthetized with 5% isoflurane (O_2 balance) until the toe-pinch response was abolished. Brains were removed, and medullary and cortical slices (400 μm thick) were cut in ice-cold artificial cerebrospinal fluid (aCSF) solution with a vibrating microtome (Campden Instruments, Layfayette, IN, USA). A series of 3–4 medullary slices used for recording contained the preBötC-region (ventrolateral to the compact *nucleus ambiguus*), XII motor nucleus, and NTS (Fig. 1A–C). Cortical slices contained primary motor and somatosensory areas (Fig. 1D). Slices were placed into an interface recording chamber (Warner Instruments, Hamden, CT, USA) and subfused with aCSF solution (8 ml/min), whose composition was (in mM): 120 NaCl, 26 $NaHCO_3$, 20 glucose, 2.0 $MgSO_4$, 1.0 $CaCl_2$, 1.25 Na_2HPO_4, 7.0 KCl. The KCl concentration was elevated to increase yield of neuronal recordings. Slices were maintained at 37 °C by an automated temperature controller (Harvard Apparatus, Holliston, MA, USA). Spontaneous neuronal activity was recorded from preBötC-region, XII motor nucleus, NTS, and cortical neurons using four 16-channel extracellular silicon electrode arrays (model a4x4-3mm100-177, Neuronexus, Ann Arbor, MI, USA). Arrays were composed of four shanks with four recording sites per shank. The distance between each shank was 125 μm, the distance between each recording site was 75 μm, and each individual recording site had a diameter of 15 μm.

2.2. Experimental protocol

Slices were allowed to equilibrate in aCSF solution with electrodes inserted for 60–90 min before recording baseline activity for 30 min. Afterwards, 200 and 300 μM sodium pentobarbital (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) were sequentially bath-applied to the slices for 45 min each to determine neuronal sensitivity to pentobarbital. The rationale for these concentrations is based on extensive previous work (Hengen et al., 2009; 2012). The pentobarbital concentration sufficient to decrease neuronal firing rates in vitro is dependent on overall neuronal excitability in the slice, which is modulated by bath $[K^+]$. The goal was to find a balance between the need to increase neuronal yield (by increasing $[K^+]$) and demonstrate positive allosteric modulation by pentobarbital. Our experience demonstrates that larger pentobarbital concentrations are required to decrease neuronal firing when bath $[K^+]$ is increased (Hengen et al., 2009, 2012). For this study, bath $[K^+]$ was increased slightly above normal ($[K^+]$ = 3–5 mM) to 7.0 mM and sequential increasing pentobarbital concentrations (200 μM and 300 μM) were tested. This strategy allowed us to: (1) test pentobarbital concentrations that were sufficient to decrease neuronal firing rates in control rats; (2) compare our present results as much as possible with previous results; and (3) minimize animal use by giving the two concentrations sequentially in the same experiment. During the last 30 min of many experiments, muscimol (20 μM, $GABA_A$ receptor agonist, R&D Systems, Minneapolis, MN, USA) was bath-applied to the slices to directly activate $GABA_ARs$ and confirm their presence, especially in neurons that were pentobarbital-resistant.

2.3. Electrophysiological data analysis

Raw extracellular recordings of spontaneous action potentials were processed as described previously (Hengen et al., 2009, 2012). Individual neurons were identified and separated using Principal Component Analysis (Adamos et al., 2008). To group waveforms associated with an individual neuron, all waveforms were projected into multi-dimensional space spanned by the three eigenvectors with the largest associated eigenvalues. The Klustakwik unsupervised clustering algorithm (Harris et al., 2000) was used to identify waveform clusters, assumed to correspond to individual neurons. Neuronal activity was averaged in 1.0-min bins throughout each experiment and normalized to the mean firing rate during a 60-min baseline recording prior to drug application. Individual neurons that were recorded on multiple, adjacent channels were counted only once. Neuronal activity was averaged in 5-min bins and normalized to the mean firing rate during the 30-min baseline recording prior to drug application. Neuronal waveforms were discarded from analysis if any one of the following criteria were met: mean baseline firing rate was <0.01 Hz, absence of action potentials for >10 consecutive min during the baseline period, or there was a consistently decreasing firing rate during baseline to <50% of the normalized value. Individual bins were discarded if the absolute firing rate was >500 Hz, or if traces exhibited evidence of mechanical disturbances (i.e. normalized firing rate increased and then decreased more than 50 standard deviations from the baseline mean in <3 min). Based on these criteria, 9.3% of waveforms and 0.1% of data bins were discarded.

Download English Version:

https://daneshyari.com/en/article/2846884

Download Persian Version:

https://daneshyari.com/article/2846884

Daneshyari.com