Respiratory Physiology & Neurobiology 171 (2010) 90–100

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

The role of the pontine respiratory complex in the response to intermittent hypoxia

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ARTICLE INFO

Article history:
Accepted 3 March 2010

Keywords:
Short-term potentiation
Ventilatory long term facilitation
NMDA receptors
Pontine respiratory group
Hypoxic ventilatory response
Intermittent hypoxia

ABSTRACT

These experiments were designed to determine the effects of EEG state on the response of rats to intermittent hypoxia and to test the hypotheses that short-term potentiation (STP) and ventilatory long term facilitation (vLTF) are state dependent; and that neurons with NMDA receptors in the dorso-ventral pontine respiratory group (dvPRG) modulate the development of STP and vLTF in rats. Low-doses of urethane anaesthesia (<1.3 g/kg) that do not cause significant respiratory depression or reductions in sensitivity to hypoxia result in cycling between EEG states that superficially resemble wake and slow wave sleep in rats and are accompanied by changes in breathing pattern that closely resemble those seen when unanaesthetized rats cycle between wake and SWS. When changes between these states were accounted for, intermittent, poikilocapnic hypoxia did not produce a significant vLTF. However, there was a persistent STP of tidal volume and vLTF did develop after blockade of NMDAr in the region of the PBrKF complex by microinjection of MK-801. Blockade of NMDA-type glutamate receptor-mediated processes in the dorsal pons also caused animals to cycle into State III, but did not alter the response to either continuous or intermittent hypoxia indicating that the response to hypoxia was not state dependent. This shows that neurons in the region of the PRG inhibit STP and vLTF, but no longer do so if PRG NMDA receptor activation is blocked.

1. Introduction

The hypoxic ventilatory response (HVR) is characterized by a complex interplay of physiological processes that preferentially alter the components of ventilation (frequency and tidal volume) in different ways at different times depending on whether the hypoxia is delivered continuously or intermittently (Powell et al., 1998; Mitchell and Johnson, 2003; Ling, 2008, for review). Thus, in rats during brief exposure to hypoxia (2–5 min) there is an initial acute response (AR), where frequency of breathing (f_R) and tidal volume (V_T) increase immediately (within seconds), often followed in the next few seconds to minutes by a further increase in tidal volume (short-term potentiation; STP) and/or a decline in frequency (short-term depression; STD). If the hypoxic stimulus is prolonged, there may be a secondary decrease in tidal volume (hypoxic ventilatory decline; HVD). When the hypoxic stimulus is removed, there is an acute "off" response as both f_R and V_T decline. Frequency often declines to levels below the frequency of breathing prior to the hypoxic exposure (post-hypoxia frequency decline; PHFD) (Coles and Dick, 1996), which may reflect the continuing STD. The initial decline in tidal volume and total ventilation ($\dot{V}_E$), however, are to levels above those seen prior to the hypoxic exposure, and they only then slowly decrease to initial levels, reflecting removal of the STP that developed during the HVR. Episodic hypoxia (repeated brief bouts of hypoxia) in some animals results in a progressive augmentation (PA) of the HVR, and a slowly developing increase in V_T after the hypoxic stimulus has been removed and after V_T initially returns to pre-hypoxia levels. Depending on the protocol for administering intermittent hypoxia (i.e. the level of hypoxia, the number of episodes (acute vs. chronic intermittent hypoxia) and whether the animals are poikilocapnic or isocapnic) and whether they are vagotomized or not, animals may exhibit a process termed ventilatory long term facilitation (vLTF). Of particular relevance to the present study, vLTF is smaller and of shorter duration in awake animals (where it appears primarily as an increased breathing frequency) than in anaesthetized animals (where it is expressed as an increase in tidal volume) (Powell et al., 1998; McGuire et al., 2005; Baker-Herman and Mitchell, 2008; Ling, 2008; Terada et al., 2008). It appears to be absent in awake humans (Jordan et al., 2002) unless CO_2 levels are elevated (Harris et al., 2006), but present in humans during non-rapid eye movement (NREM) sleep (Pierchala et al., 2008). In this regard, it is interesting to note that the change between sleep and wake leads to changes in total ventilation, breathing pattern and hypoxic sensitiv-

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doi:10.1016/j.resp.2010.03.005

ity (Pappenheimer, 1977), while hypoxia leads to changes in sleep state distribution (increased wakefulness) and thus, indirectly to the changes in ventilation that are associated with the changes in state (Pappenheimer, 1977; Laszy and Sarkadi, 1990). It is therefore important to account for differences in sleep state when making measurements of ventilatory time domains.

Previously we have examined the changes in EEG state that occur in animals given low-dose urethane anaesthesia, and the changes in breathing pattern that accompany these state changes (Boon et al., 2004; Boon and Milsom, 2008). It has now been shown that lightly urethane-anaesthetized rats (Boon et al., 2004; Boon and Milsom, 2008) cycle between states that, based on EEG criteria, superficially resemble the desynchronized EEG pattern of awake animals (urethane-anaesthetized State I) and the synchronized EEG pattern of animals in slow wave sleep (urethane-anaesthetized State III). It has also been shown that these species undergo changes in total ventilation, breathing pattern and hypoxic sensitivity with these changes in state that are identical to those shown when unanaesthetized animals awaken or fall asleep (Hunter and Milsom, 1998; Hunter et al., 1998; Boon et al., 2004; Boon and Milsom, 2008). Thus, while urethane-anaesthetized states may not be the same as slow wave sleep and wakefulness in other aspects, they do produce similar changes in ventilation, and offer a model for study of the control of breathing as a function of EEG state in which state can be carefully monitored and controlled. In the present study we wished to use this model to examine the effects of intermittent hypoxic exposure on state distribution, as well as the effect of changes in state distribution during intermittent hypoxia on the various components of the HVR, and in particular, vLTF.

We have recently shown that systemic injection of MK-801, an NMDA-type glutamate receptor antagonist, delayed and reduced the hypoxic ventilatory response in rats (Boon and Milsom, 2008). However, microinjection of MK-801 into the dvPRG in the region of the parabrachial and Kölliker Fuse (PBrKF) nuclei, an area with a high proportion of NMDA receptors (NMDAr) (Alheid et al., 2004, for review) did not alter the magnitude or onset of the hypoxic ventilatory response, but amplified the effects of changes in EEG state on hypoxic sensitivity, and reduced the rate at which tidal volume returned to normal following hypoxic exposure (Boon and Milsom, 2008). The latter would suggest that NMDA sensitive neurons in the dvPRG in the region of the PBrKF region may influence short-term potentiation (STP) and/or vLTF, phenomena involving changes in tidal volume during and following the return to breathing air after exposure to hypoxia. It has been reported (McGuire et al., 2005) that injection of MK-801 into the phrenic motor nucleus of anaesthetized and vagotomized rats blocked LTF of phrenic nerve amplitude and burst frequency when isocapnia was maintained. In awake rats, the use of APV (an NMDA antagonist) eliminated vLTF when it was systemically injected either before or after episodic hypoxia, while CNQX (a non-NMDA antagonist) enhanced vLTF (McGuire et al., 2008).

We have also recently shown that MK-801 microinjections into the dvPRG in the region of the parabrachial and Kölliker Fuse nuclei in lightly urethane-anaesthetized animals promotes State III (the slow wave sleep-like EEG state) even during hypoxia (Boon and Milsom, 2008). As a consequence, MK-801 treated animals are largely in a constant state. This raises the question of the extent to which NMDA sensitive neurons in the dvPRG influence STP and/or vLTF directly or via changes in state. In the present study, we hypothesized that the HVR and state changes during the episodic bouts of hypoxia would mirror those seen in unanaesthetized animals. We also hypothesized that injection of MK-801 into the dvPRG in the region of the PBrKF, blocking activation of NMDAr, would produce a continuous State III and prevent the return of tidal volume to resting values after hypoxia, thus prolonging the effects of short-term potentiation (STP) between hypoxic episodes and enhancing the development of vLTF.

2. Methods

2.1. Animal care

All experiments were carried out on adult male Sprague–Dawley rats (390 ± 60 g) bred at the University of British Columbia (UBC) Animal Care Centre with the prior approval of the UBC Animal Care Committee and the Okanagan University College (OUC) Animal Care Committee. (Note: Okanagan University College is now the University of British Columbia Okanagan—UBCO.)

The rats were housed singly in the OUC animal care facility and allowed access *ad libitum* to food and water supplemented from time to time with sunflower seeds and fruit. The rats were kept at 25 °C, with a light/dark cycle of 12/12 h (lights on at 8 a.m.). All experiments were conducted between approximately 9 a.m. and 6 p.m. After the experiment, the rats were euthanized with an IP injection of 1 ml of Somnotol (Sodium pentobarbital, 65 mg/ml, MTC pharmaceuticals).

2.2. Experimental protocol

2.2.1. Preparation for monitoring breathing

The animals were initially anaesthetized with 2% vaporous halothane administered through a mask. They were then given an intraperitoneal (IP) injection of a 20% solution of urethane (Sigma) in saline to a final dose of 1.3 g/kg and the trachea was canulated below the larynx for the measurement of airflow. The animal was placed in a stereotaxic head frame (Kopf), adjusted such that the skull surface landmarks lambda and bregma were on the same horizontal plane. The halothane anaesthesia was slowly decreased, but was maintained until all surgery was completed and the urethane had become effective; at least 45 min. Adequacy of anaesthesia was tested repeatedly throughout the surgery and the subsequent experiment (absence of a corneal reflex, no response to a noxious toe pinch). Additional doses of 0.5 ml urethane were administered IP if there was any indication of a response. Body temperature was maintained between 36 and 37 °C by placing the rat on a servo-controlled heating pad connected to a rectal temperature probe. IP injections of 3 ml saline were given each hour to maintain fluid balance. These injections did not change the breathing pattern.

2.2.2. Placement of EEG electrodes

Once the animal was anaesthetized a dorsal-longitudinal incision was made in the skin over the crown of the cranium, extending from the orbits to the lambdoid suture. Four electroencephalographic (EEG) electrodes were implanted in the skull as described in Boon et al. (2004).

2.2.3. Recording data

A pneumotach was attached to the tracheal cannula and connected to a Validyne differential pressure transducer (Validyne, DP 103-18). The signal was amplified with a Gould amplifier and then transmitted to a two-channel data acquisition system (ATCODAS, DataQ Instruments) sampling at a frequency of 120 Hz on each channel. The breathing signal was also recorded on a chart recorder. The EEG signal was amplified and recorded on a chart recorder and on the data acquisition system on a second channel. The signal was filtered with both low and high pass filters and a 60 Hz filter to reduce noise.

2.2.4. Microinjection of vehicle and MK-801

Microinjections of vehicle (saline), MK-801 (dizocilpine maleate (+)-5 methyl-10, 11-dihydro-5H-dibenzo [a,d]cyclohepten-5,10