Respiratory Physiology & Neurobiology 250 (2018) 24–30

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Effect of hypercapnia on self-sustained muscle activity

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A R T I C L E I N F O

Keywords:
Hypercapnia
Central chemoreceptor
Self-sustained muscle activity
Plateau potentials

A B S T R A C T

The aim of the present study was to determine the effect of hypercapnia on motor neuromuscular activity of the human triceps surae muscle. Nine subjects participated in trials in a normal breathing condition and a CO2 rebreathing condition. In both conditions, in order to provoke self-sustained muscle activity, percutaneous electrical train stimulation was applied to the tibial nerve while each subject lay on a bed. Self-sustained muscle activity, which is an indirect observation of plateau potentials in spinal motoneurons, was measured for 30 s after the train stimulation by using surface electromyography. The sustained muscle activity was increased by CO2 rebreathing ($P < 0.05$). This finding suggests that motor neuromuscular activity may be linked to the respiratory system that is activated during hypercapnia.

1. Introduction

Carbon dioxide (CO_2) is a strong stimulus to central chemoreceptors located in the brainstem for ventilatory response, which is a homeostatic function for maintaining the internal environment of the body (Whipp and Ward 1998). Hypercapnia results in the generation of depolarizing currents known as central respiratory drive potentials in spinal motoneurons of respiratory muscles to increase ventilation through the output of the respiratory center in the medulla (Butler 2007). Additionally, it has been reported that hypercapnia facilitated plateau potentials in hindlimb motoneurons of a decerebrate anesthetized cat (Kirkwood et al., 2002, 2005). This indicates the possibility that a change in arterial blood CO_2 pressure ($PaCO_2$) is involved not only in respiratory control but also in limb motoneuronal activity in animals. Plateau potentials are sustained depolarizations of spinal motoneurons that are facilitated via activation of L-type calcium channels and persistent sodium channels of motoneurons (Bennett et al., 1998; Lee and Heckman 1999; Li et al., 2004). However, there has been no study in which the relationship between hypercapnia and plateau potentials of limb muscle motoneurons in humans was investigated.

In previous studies, it has been shown that plateau potentials can be measured by electromyography (EMG) signals in animals (Crone et al., 1988; Gorassini et al., 1999; Hounsgaard et al., 1988) and humans (Collins et al., 2001, 2002; Gorassini et al., 1998, 2002; Nozaki et al., 2003; Trajano et al., 2014; Walton et al., 2002). Nozaki et al. (2003) demonstrated that autonomous neural activity that is related to the plateau potentials of limb muscle motoneurons could be recorded as self-sustained muscle contractions, which are muscle activities that involuntarily continue after the end of percutaneous electrical train stimulation to a peripheral nerve, by using surface EMG. This indicates that sustained muscle contractions are an indirect observation of plateau potentials in spinal motoneurons. Therefore, self-sustained muscle activity, which is an enhanced sustained EMG activity, was induced in the present study by using the methods of Nozaki et al. (2003). If plateau potentials are associated with hypercapnia as indicated by Kirkwood et al. (2002, 2005), EMG activity of the sustained muscle activity in humans would be affected by hypercapnia. The purpose of the present study was to investigate whether hypercapnia induced by CO_2 rebreathing alters the self-sustained muscle activity of the human triceps surae muscle.

2. Methods

2.1. Subjects

Nine healthy males, with a mean (± standard deviation) age of 29 ± 8.4 years, who had never had a nervous or motor disorder participated in the present study. All of the subjects provided informed consent for participation in the study. This study was approved by the

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https://doi.org/10.1016/j.resp.2018.02.002
Received 30 June 2017; Received in revised form 26 December 2017; Accepted 6 February 2018
Available online 08 February 2018

Human Research Ethics Committee of the Graduate School of Education, Hokkaido University.

2.2. Experimental set-up

In the experiment, each subject lay on a bed on his left side, and the subject's right leg was placed on a cushion. The subject's right hip and knee joint were flexed approximately 60°. The subjects were instructed to remain relaxed throughout the experiment.

2.3. Recordings

Surface EMG signals were recorded from the soleus muscle (Sol) and from the medial head (MG) and lateral head (LG) of the gastrocnemius muscle of the right leg via a bipolar electrode. The ground electrode was placed over the right caput fibulae. EMG signals were amplified (Amplifier: AB-611-J, Nihon-Kohden, Japan) with band-pass filtering between 1.5 Hz and 1 kHz and converted into digital signals at a sampling rate of 2 kHz using an analog-digital converter (LabChart 8, ADInstruments, Australia). Throughout the experiment, the subjects breathed through a face mask connected to a hot-wire flow meter and a respiratory gas analyzer (AE-280S, Minato Medical Science, Japan) to measure minute ventilation (VE) and end-tidal carbon dioxide pressure ($PETCO_2$). Respiratory variables were measured with the breath-by-breath mode. A zirconium sensor and infrared absorption analyzer were used to analyze inspired and expired fractions of O_2 and CO_2, respectively. Inspired and expired flows were measured using the hot-wire flow meter. The gas analyzer was calibrated with precision reference gas (O_2, 15.17%; CO_2, 4.92%). The flow meter was calibrated with a standard 2-l syringe.

2.4. Experimental protocol

Each subject participated in trials in two respiratory conditions, a CO_2 rebreathing condition and a normal breathing condition, which were conducted on the same day (Fig. 1). The subjects remained relaxed for 152 s (−90 to 62 s) in each trial. In the CO_2 rebreathing condition, respiratory dead space (1500 ml) was added to the respiratory mask from 30 s after the initiation of the trial to the end of the trial (−60 to 62 s) in order to make the subjects rebreathe expired CO_2. In the normal condition, each subject remained rested and spontaneously breathed room air through the respiratory mask. At 90 s after the start of a trial in the both conditions, 2-s train stimulation was delivered to the right tibial nerve. Then each subject remained relaxed for 60 s after the 2-s train stimulation in both conditions. This trial was repeated 6 times with intervals of 5–10 min. During each interval, maximal M-wave (M-max) and Hoffmann's reflex (H-reflex) of the soleus muscle were measured three times and five times respectively. Three of the 6 trials were performed in the CO_2 rebreathing condition. Three trials in each condition were sequentially performed and the order of the two conditions was random among subjects. It has been shown that $PaCO_2$ is increased by rebreathing expired air through the increased respiratory dead space (Koppers et al., 2006; Smolka et al., 2014).

Electrical stimulation. While each subject remained reclined on the bed, the tibial nerve was percutaneously stimulated by applying a rectangular electrical pulse of 1 msec in duration to the popliteal fossa using a constant current stimulator (DS7AH, Digitimer Ltd, UK). Sustained muscle activity was elicited by 50-Hz electrical train stimulation for 2 s (Nozaki et al., 2003). In this study, activity of the soleus muscle was mainly assessed sample. Thus, the stimulation intensity was 120% of the H-reflex threshold of the soleus muscle. The cathode of the stimulation electrodes was secured to the right leg popliteal fossa, and the anode was attached to the patella. The subjects were asked to ignore the electrical train stimulation as much as possible. In order to measure M-max during intervals between trials, the electrical stimulus delivered by the stimulator was increased gradually until the M-wave of the soleus muscle reached a plateau while the subject was at rest. The level of stimulation was then set 20% above this point to ensure maximal activation of these muscles. Additionally, the stimulus intensity of H-reflex was 120% of the H-reflex threshold of the soleus muscle during an interval.

Safety of subjects. In both respiratory conditions, the subjects were asked about their sensation of dyspnea after recording self-sustained muscle activity using the modified Borg scale (Borg 1982) in order to determine whether the experiment could be continued. Using a pulse oximeter (PULSOX-300i, Konica Minolta, Japan), arterial oxygen saturation of pulse oximetry (SpO_2) was measured noninvasively to check whether SpO_2 was within safety level (over 90%).

2.5. Data analysis

The root mean squares (RMSs) of EMG of the Sol, MG, and LG muscles were calculated from 10-s and 30-s windows immediately before and after the train stimulation. The RMSs in each respiratory condition were averaged by the 3 trials. The magnitudes of M-max and H-reflex were measured as peak-to-peak amplitude and averaged over 3 times and 5 times, respectively, for use in the following analyses. The H-reflex amplitude was evaluated as percentage of M-max amplitude.

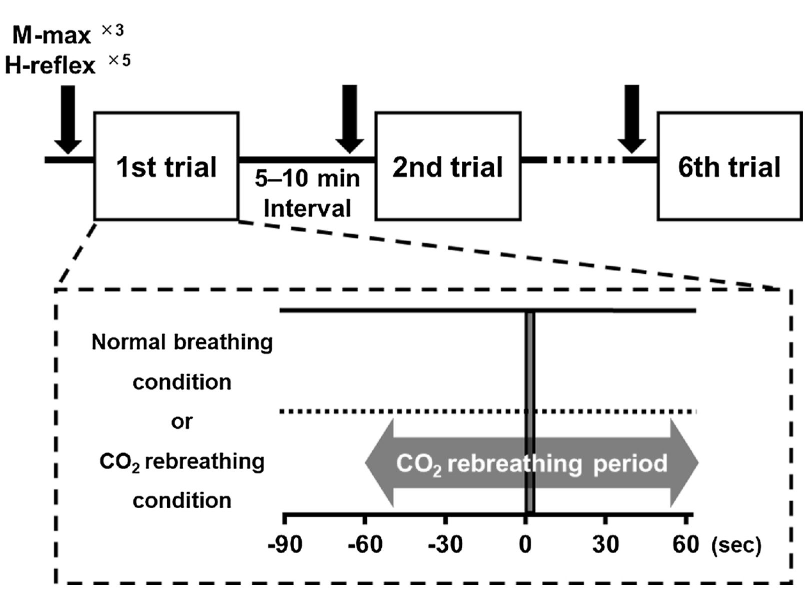

Fig. 1. Schematic representation of the experimental protocol of the normal breathing condition and/or CO_2 rebreathing condition. The sustained muscle activity trial was repeated 6 times with intervals of 5–10 min. M-max and H-reflex of the soleus muscle were measured three times and five times, respectively, before the trial (*vertical arrows*). CO_2 rebreathing was performed from 30 s after the initiation of the trial to the end of the trial (*horizontal arrow*). The vertical gray bar indicates the electrical train stimulation period.