The effects of short-term hypoxia on motor cortex excitability and neuromuscular activation

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Submitted 2 June 2006; accepted in final form 3 August 2006

Suzuki, Christoph, Martin Burtscher, and Wolfgang N. Löscher. The effects of short-term hypoxia on motor cortex excitability and neuromuscular activation. J. Appl. Physiol. 101: 1673–1677, 2006. First published August 17, 2006; doi:10.1152/japplphysiol.00617.2006.—The effects of acute hypoxia on motor cortex excitability, force production, and voluntary activation were studied using single- and double-pulse transcranial magnetic stimulation techniques in 14 healthy male subjects. Electrical supramaximal stimulations of the right ulnar nerve were performed, and transcranial magnetic stimulations were delivered to the first dorsal interosseus motor cortex area during short-term hypoxic (HX) and normoxic (NX) condition. M waves, voluntary activation, F waves, resting motor threshold (rMT), recruitment curves (100–140% of rMT), and short-interval intracortical inhibition and intracortical facilitation were measured. Moreover, motor-evoked potentials (MEPs) and cortical silent periods were determined during brief isometric maximum right index finger abductions. Hypoxia was induced by breathing a fraction of inspired oxygen of 12% via a face mask. M waves, voluntary activation, and F waves did not differ between NX and HX. The rMT was significantly lower in HX (55.79 ± 10.48%) than in NX (57.50 ± 10.48%) (P < 0.01), whereas MEP recruitment curve, short-interval intracortical inhibition, intracortical facilitation, maximum right index finger abduction, and MEPs were unaffected by HX. In contrast, the cortical silent periods in HX (158.21 ± 33.96 ms) was significantly shortened compared with NX (169.42 ± 39.69 ms) (P < 0.05). These data demonstrate that acute hypoxia results in increased cortical excitability and suggest that acute hypoxia alters motor cortical ion-channel function and GABAergic transmission.

transcranial magnetic stimulation; voluntary activation; cortical silent period

STUDIES OF HYPOXIA-INDUCED CHANGES in central nervous system function have primarily focused on behavioral parameters and revealed impairments in psychomotor skills (25, 43) and cognitive performances (24, 29, 32). In addition to these deteriorations in performance, evidence of slowed visual and auditory reaction time has been demonstrated in experimental conditions corresponding to an altitude of ~6,100 m (15, 16).

Numerous in vitro studies on central neurons strongly suggested that reduced arterial oxygen saturation impairs central nervous system function (34). In particular, the cerebral neuron excitability critically depends on sufficient O2 supply, but hypoxia may not only compromise ion channels but also signaling pathways and neurotransmitter function (22, 35). So far, most of our knowledge about the processes underlying hypoxia-induced alterations of neuronal excitability and synaptic neurotransmission has been gained from in vitro patch-clamped studies.

As yet, in vivo studies addressing the functional consequences of reduced O2 supply to the neuromuscular system have primarily focused on effects on muscle force generation (13), endurance time (2), changes in muscle metabolisms (23, 42), and myoelectrical activity (41). These studies showed that hypoxia did not impair neuromuscular transmission, action potential propagation along muscle fibers, and/or the muscle membrane potential in relaxed muscles or during brief contractions (6, 21, 28, 46). The effects of hypoxia on spinal excitability were only investigated in a few studies and yielded conflicting results, indicating both decreased (46) and unaltered (28) spinal excitability levels. To date, however, little is known about the consequences of hypoxia on supraspinal mechanisms, particularly motor cortex excitability during rest and brief motoneuronal events. Olivieri et al. (40) studied the effects of chronic hypoxia on motor cortical function in patients with chronic obstructive pulmonary disease (COPD) using transcranial magnetic stimulation (TMS). This study provided the first in vivo observation of cortical dysfunction in chronic hypoxia and suggested selective GABAergic deficiency within the motor cortex.

Since no in vivo studies of the effects of acute hypoxia on motor cortex function have yet been performed, we investigated motor cortex responses to experimentally induced acute hypoxia in healthy subjects using single- and double-pulse TMS techniques.

METHODS

Fourteen right-handed, healthy male volunteers, aged 23–45 yr, participated in this study.

Cardiovascular health was additionally ascertained before the experiments by ECG and medical examination. All subjects gave written, informed consent to the experiments, and the study was approved by the Ethics Committee, Innsbruck Medical University. All experiments conformed to the Declaration of Helsinki.

Adaptations of contractile properties, neuromuscular transmission, spinal and motor cortical excitability, and voluntary activation in response to short-term hypoxia (HX) were studied in the first dorsal interosseus muscle (FDI) and compared with normoxia (NX). Electrical stimulation of the ulnar nerve was performed to assess contractile properties and neuromuscular transmission, and F waves were recorded as a measure of spinal excitability. To study motor cortex excitability, resting motor threshold (rMT), cortical recruitment curve, short-term intracortical inhibition (SICI), and intracortical facilitation (ICF) were measured at rest. Moreover, motor-evoked potentials

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Hyoxia was induced by breathing a HX air mixture containing a fraction of inspired O2 of 12% in nitrogen via a face mask (Hypoxicci). The inspiratory tube was connected to a 20-liter bag that contained a constant 12% O2 mixture. Hyoxia was induced by gradual reduction of fraction of inspired O2 to ensure appropriate adaptation. Arterial O2 saturation (SaO2) and pulse rate were continuously monitored throughout the experiment by an oxymeter placed at the left index finger (Onyx, Nonin Medical). After a stable reduction of SaO2 was achieved, after 20–30 min of HX air inhalation (4), the experiments were performed. Total time spent under stable hyoxia was ~45 min. In NX, subjects also wore a mask, which was detached from the air bag, and waited 20–30 min before the experiment started. Although the subjects were not informed about the choice of condition, most subjects experienced a transient period of light-headedness during the induction of HX, and thus complete blinding was probably not achieved.

Data analysis. Peak-to-peak MEP amplitudes were measured. To determine the input-output curve, MEP amplitudes were averaged for each stimulation intensity and expressed as percentage of the mean peak-to-peak M wave for each subject. For SICI and ICF, amplitudes were calculated separately as a mean size of evoked 10 MEP values for TS, SICI and ICF for each subject. The inhibited and facilitated responses were presented as percentage of the mean TS MEP. The highest 10 of 20 F-wave peak-to-peak amplitudes were collected, and expressed as percentage of the mean M wave to assess the spinal excitability. Twitch force peak amplitudes and M-wave peak-to-peak amplitudes were calculated and expressed as the mean of three stimulations.

The maximal force of MVCs was calculated as the highest value before TMS. Three maximal force values were averaged. To measure voluntary activation, the force increment evoked by TMS was measured and expressed as percentage of the mean voluntary force during 100 ms preceding the stimulation (voluntary activation (%)) = (1 – (superimposed twitch/background force)) × 100). MEP amplitudes during MVCs were also measured peak to peak. The duration of the CSP was determined manually and was measured from the stimulation artifact to the return of continuous voluntary EMG.

Statistical analysis. Data distribution was ascertained using the Shapiro Wilk’s W test. The effects of condition, stimulus intensity, and inter-stimulus interval on MEP amplitude in input-output curves, SICI, and ICF were tested with two-way repeated-measures ANOVA (main effect: condition; levels: stimulus intensity and inter-stimu- lus interval). Further comparisons were made using the Student’s t-test, and Bonferroni correction was applied when necessary. Two-tailed paired sample t-tests were performed to compare motor threshold, F wave, twitch amplitude, MVCs, M wave, MEP, CSP, and voluntary activation in NX and HX. Linear correlation analyses between changes in rMT and CSP with the reduction of SaO2 were also calculated.

Group data are presented as means ± SD within the text and displayed as means ± SE in the figures. Statistical analyses were performed using STATISTICA (6.1 StatSoft, Tulsa, UK), and statistical significance was set to P < 0.05.

RESULTS

Expectedly, SaO2 decreased significantly from 97.14 ± 0.77% in NX to 75.07 ± 2.40% in HX (P < 0.001), and heart rate was significantly increased in HX (80.79 ± 12.34 beats/min) compared with NX (69.21 ± 11.59 beats/min) (P < 0.001).

Electrical supramaximal stimulations in relaxed muscle condition. The maximal M-wave amplitude in the resting FDI remained unchanged in HX (17.41 ± 2.71 mV) compared with NX (17.47 ± 3.85 mV). Likewise, the amplitude of the resting twitch was similar in the two conditions (NX, 8.35 ± 2.45 N).
vs. HX, 7.85 ± 3.02 N). Also, HX did not significantly change F-wave amplitudes (NX, 2.34 ± 1.33% vs. HX, 2.10 ± 1.10%).

**TMS in relaxed muscle condition.** rMT was significantly lower (55.79 ± 9.40%) in HX compared with NX (57.50 ± 10.48%) \((P < 0.01)\) (Fig. 1). The reduction of rMT did not correlate significantly with the reduction of \(\text{SaO}_2\) \((r = 0.20)\). The input-output curve of the motor cortex showed a gradual increase of normalized MEP amplitude in NX \((P < 0.001)\) and HX \((P < 0.001)\). However, no significant differences were observed between the both conditions at any of the five stimulation intensities (Fig. 2).

During the SICI and ICF test protocol, the MEP amplitudes of the TS did not differ between the two conditions (NX, 0.52 ± 0.36 mV vs. HX, 0.62 ± 0.34 mV). During NX and HX, the MEP was significantly reduced at 2 ms ISI \((P < 0.001)\) and significantly increased at 12 ms \((P < 0.001)\), and both SICI and ICF did not differ between HX and NX (Fig. 3).

**Corticomotoneuronal excitability and voluntary activation during MVCs.** MEP amplitude during the brief MVCs in HX (6.14 ± 2.71 mV) did not differ significantly from the MEPs in NX (5.26 ± 1.85 mV). In contrast, the SP duration was shortened in HX compared with NX \((158.21 ± 33.96 \text{ ms} \text{ vs. } 169.42 ± 39.69 \text{ ms}, P < 0.05; \text{Fig. 4})\). The decrease in CSP duration did not correlate significantly with the reduction of \(\text{SaO}_2\) \((r = 0.09)\).

The MVC force in NX (54.34 ± 12.90 N) was similar to that in HX (53.79 ± 11.59 N). Also, voluntary activation during brief maximal efforts remained unchanged by HX (NX, 96.95 ± 1.82 vs. HX; 96.56 ± 1.77%).

**DISCUSSION**

The present experiments showed that acute hypoxia did not affect SICI, ICF, and cortical input-output relationship but led to a decrease in rMT and shortening of the CSP. However,
these motor cortical responses to hypoxia had no impact on force production and voluntary activation during brief MVCs. Hypoxia resulted in a small but significant reduction of rMT, indicating increased cortical excitability. Since spinal excitability, assessed by F waves, and neuromuscular transmission and action potential propagation along muscle fibers, assessed by M waves, remained unchanged during hypoxia, the reduction of rMT must have resulted from adaptations within the motor cortex. Although F-wave measurements may not be sufficiently sensitive to rule out changes in spinal excitability, which might have contributed to the reduced rMT, previous studies also failed to demonstrate changes in spinal excitability using more sensitive H-reflex measurements (28, 46).

Threshold TMS over the target motor cortical area preferentially activates the corticospinal neurons transsynaptically through excitatory interneurons and corticocortical axons (1, 11). Several studies demonstrate that rMT critically depends on the membrane excitability of motor cortical neurons and thus ion-channel function (3, 8, 50). In vitro studies demonstrated that channel function in isolated cerebral neurons is directly affected by the amount of delivered oxygen (17, 33, 37) and that hypoxia results in neuronal hyperexcitability (12). It therefore appears that acute hypoxia also modifies motor cortical ion-channel function in humans, resulting in cortical hyperexcitability evidenced by a reduction of rMT.

The duration of the CSP has been shortened during acute hypoxia. The stimulation intensity used to elicit CSP was related to the rMT during each condition. This might have resulted in an artificial shortening of the CSP as slightly lower stimulation intensities have been used in HX due to the reduced rMT. However, as the MEP recruitment curve was not altered by HX, it appears unlikely that the CSP recruitment curve was affected by HX. However, to convincingly show that the shortened CSP was caused by HX, further studies should also investigate the CSP threshold and stimulus-response curves (30).

Although the initial part of CSP depends on spinal mechanisms, its later part originates in a complex circuitry of intracortical interneurons within motor cortex (5, 7, 18, 26). It has been suggested that the duration of the CSP depends on the function of inhibitory GABAergic synapses (27, 45, 48). HX in mammalian cortical neurons led to decreased presynaptic GABA release (39, 44) or reduced postsynaptic GABAergic mechanisms (36), both of which reduce the synaptic efficacy of inhibitory transmission. The shortened CSP in our HX condition, therefore, also indicates a deterioration of GABAergic intracortical inhibition in vivo.

The recruitment curve represents the corticospinal excitability and is assumed to be influenced by ion-channel properties and GABAergic mechanisms (3, 10). Despite the reduction of rMT and shortened CSP in our protocol and the assumption of hypoxia-induced alterations in ion-channel function and GABAergic mechanisms, hypoxia did not change the MEP recruitment curve in our experiment. Similarly, short-interval intracortical inhibition and facilitation, which are also to be under GABAergic control, remained unchanged during acute hypoxia. Different mechanisms underlying SICI, ICF, and CSP might explain this difference. It has been suggested that the CSP is mediated by GABA_B receptors, whereas SICI and ICF are thought to be GABA_A receptor-dependent (45, 48, 49). The present results therefore suggest that hypoxia impairs GABA_B receptor-mediated inhibition, whereas it has no impact on GABA_A receptor-dependent inhibition.

Despite these changes in cortical excitability, acute hypoxia had no effect on MVC force, confirming previous results on finger abduction (19), hand grip (6), elbow flexion (14, 41) and knee extension (9, 14, 20, 47) force. Also, voluntary activation was not altered during acute hypoxia.

Motor cortex physiology in HX conditions has so far only been studied in patients with COPD who served as a model for chronic hypoxia (40). In contrast to the present results during acute hypoxia, rMT in these patients was not different from controls, but SICI and CSP duration were reduced. This suggests that the reduction of rMT found in the present study represents an acute response that normalizes during chronic hypoxia. On the other hand, SICI seems to change only after chronic hypoxia, whereas the duration of the CSP is altered in acute as well as in chronic hypoxia. However, factors other than chronic hypoxia might also influence cortical excitability in COPD patients.

In conclusion, the present results show that acute hypoxia results in increased cortical excitability, as evidenced by a decreased rMT and a shortened CSP, and indicate alterations in cortical ion-channel properties and GABAergic transmission, similar to the results obtained in in vitro studies. However, these changes had no impact on force production and voluntary activation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the experimental assistance of M. Liebensteiner.

GRANTS

This work was supported by the Austrian Society for Alpine and High Altitude Medicine (ÖGAHM) and the University of Innsbruck.

REFERENCES


