Facilitation of the diaphragm response to transcranial magnetic stimulation by increases in human respiratory drive

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1UPRES EA 2397, Faculté de Médecine Pitié-Salpêtrière, Université Paris VI Pierre et Marie Curie; 2Service Central d’Explorations Fonctionnelles Respiratoires and 3Service de Pneumologie, Assistance Publique-Hôpitaux de Paris, Groupe Hospitalier Pitié-Salpêtrière, 75013 Paris, France

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Straus, C., C. Locher, M. Zelter, J.-P. Derenne, and T. Similowski. Facilitation of the diaphragm response to transcranial magnetic stimulation by increases in human respiratory drive. J Appl Physiol 97: 902–912, 2004. First published May 7, 2004; 10.1152/japplphysiol.00989.2003.—The human respiratory neural drive has an automatic component (bulbospinal pathway) and a volitional component (corticospinal pathway). The aim of this study was to assess the effects of a hypercapnia-induced increase in the automatic respiratory drive on the function of the diaphragmatic corticospinal pathway as independently as possible of any other influence. Thirteen healthy volunteers breathed room air and then 5 and 7% hyperoxic CO2. Cervical (cms) and transcranial (tms) magnetic stimulations were performed during early inspiration and expiration. Transdiaphragmatic pressure (Pdi) and surface electromyogram of the diaphragm (DiEMG) and of the abductor pollicis brevis (apbEMG) were recorded in response to cms and tms. During inspiration, Pdi,cms was unaffected by CO2, but Pdi,tms increased significantly with 7% CO2. During expiration, Pdi,cms was significantly reduced by CO2, whereas Pdi,tms was preserved. DiEMG,tms latencies decreased significantly during early inspiration and expiration (air vs. 5% CO2 and air vs. 7% CO2). DiEMG,tms amplitude increased significantly in response to early expiration-tms (air vs. 5% CO2 and air vs. 7% CO2) but not in response to early inspiration-tms. DiEMG, cms latencies and amplitudes were not affected by CO2 whereas 7% CO2 significantly increased the apbEMG, cms latency. The apbEMG,tms vs. apbEMG, cms latency difference was unaffected by CO2. In conclusion, increasing the automatic drive to breathe facilitates the response of the diaphragm to tms, during both inspiration and expiration. This could allow the corticospinal drive to breathe to keep the capacity to modulate respiration in conditions under which the automatic respiratory control is stimulated.

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Facilitation of the diaphragm response to transcranial magnetic stimulation by increases in human respiratory drive

THE NEURAL DRIVE TO BREATHE in humans results from the reciprocal modulation of two sources of commands. The first one, permanent, automatic, and oscillatory in nature, originates in the brain stem (61). It provides the respiratory rhythm, minute ventilation, and their homeokinetic adaptations. The respiratory rhythm depends in part on pacemaker neurons (17), but its spatiotemporal pattern eventually results from the activity of complex neuronal networks in the brain stem (see Ref. 61). Among the various afferent inputs that modulate this activity, hypercapnia is one of the most powerful. The response to a rise in CO2 is characterized by an increase in minute ventilation, respiratory rate, tidal volume, and inspiratory activity. This response is easily assessed through measures of the mean inspiratory flow, i.e., tidal volume divided by the duration of inspiration (VT/Ti), and of airway occlusion pressure (P0.1). The second source of respiratory command in humans stems from suprapontine structures allowing behavioral and volitional uses of the respiratory system. For example, through temporary overrides of the automatic ventilatory command, the cortical drive to breathe permits voluntary apnea, voluntary respiratory maneuvers, and the use of the respiratory system for nonrespiratory tasks. This suprapontine respiratory control has anatomical and functional substrates. For example, from a sensory point of view, diaphragmatic afferents project to the sensorimotor cortex (64) and to the cingulate gyrus (56). From a motor point of view, like every other striated muscle, each hemidiaphragm is represented on the contralateral primary motor cortex (9, 18, 51). The efferent motor pathway from the cortex to the spinal phrenic motoneurons is most probably direct, within the pyramidal tract (25, 51, 54). A relay in the brain stem or in the cerebellum cannot be definitely excluded, and the three possibilities may coexist (6, 12, 45).

Physiological and pathological examples of the interactions between the automatic and the voluntary sources of the neural respiratory drive abound, in both directions. Nevertheless, because few investigation methods are readily usable in conscious humans, the mechanisms of these interactions remain largely unknown. The neural pathway from the cerebral cortex to the respiratory muscles (including the diaphragm) can be assessed through the response of these muscles to transcranial stimulation (21, 22, 24, 25, 46). Several studies have shown that transcranial magnetic stimulation (TMS) can activate the diaphragm (e.g., Refs. 35, 41, 52, 54, 63). As for other muscles like hand muscles (29, 30), an underlying contraction of the diaphragm facilitates its response to TMS, shortening the latency and increasing the amplitude of the corresponding electromyographic signal (26, 35, 39, 41, 54, 63). Facilitation also increases the mechanical output of the contraction. The facilitation of the response of a muscle to TMS is mainly a spinal phenomenon, depending on the degree of preactivation of the corresponding spinal motoneurons (38, 57), although cortical mechanisms can be involved, as exemplified by the facilitatory effects of the mental evocation of a movement (1, 32, 33). The preactivation of the spinal motoneurons can be of ascending origin (afferent inputs conveyed by IA fibers) or from descending origin (voluntary contraction) (38, 59).

Compared with other spinal motoneurons, the respiratory ones are peculiar because they integrate descending bulbospinal...
nal inputs (automatic ventilatory command) in addition to corticospinal ones. In the cat, central respiratory drive potentials arising from medullary neurons depolarize phrenic motoneurons during inspiration whereas these motoneurons are disfacilitated during expiration (4). This disfacilitation can, however, be overcome by corticospinal inputs, as the stimulation of precise cortical areas consistently evokes a diaphragmatic response even when delivered during expiration (10, 44) (of note, in humans, a corticospinal input, as produced by TMS, can also produce a diaphragmatic response during relaxed expiration; Ref. 54). Under hypercapnic conditions, the excitatory bulbospinal input to the phrenic motoneurons during inspiration is increased. This probably suffices to explain the facilitated diaphragmatic response to a concurring corticospinal input that can be observed in humans (14, 41). During expiration, limited animal data suggest that hypercapnia could, reciprocally, strengthen the inhibition of inspiratory motoneurons (20, 47). Whether this is the case in humans and how it would affect the balance between the bulbospinal and the corticospinal input to phrenic motoneurons are not known. Yet this balance plays a crucial role in the ability to maintain activities relying on the voluntary control of breathing (and thus requiring that it preempts the automatic control, e.g., speech production) in situations of increased homeokinetic ventilatory demand (e.g., exercise).

Therefore, to gain new insights on the interactions between the automatic and the suprapontine ventilatory commands, the present study was designed to assess the effects of a hypercapnia-induced increase in the automatic drive to breathe on the response of the diaphragm to TMS during the inspiratory and the expiratory phase of the ventilatory cycle. This was achieved by studying 13 normal subjects, first in steady-state room air condition and then under hyperoxic 5 and 7% CO2, in whom the response of the diaphragm and of a hand muscle to cervical magnetic stimulation (CMS; peripheral stimulation) and TMS (supraspinal stimulation) were studied at the very beginning of inspiration and during expiration, with careful control for the degree of underlying activity of the inspiratory muscles.

MATERIALS AND METHODS

Subjects

A total of 13 healthy subjects (3 women, 10 men, 22–35 yr) participated in the study, after completion of the French legal procedure for studies in human volunteers. The study was approved by the Comité Consultatif de Protection des Personnes se prétendant à des Recherches Biomédicales, Pitié-Salpêtrière. All the subjects were naive respective to respiratory physiology experiments, did not take any drug, and did not suffer from sleep deprivation. They were informed in detail of the purpose of the study and methods used and gave written consent.

Methods

Measurement of ventilation and related pressures. The subjects were studied sitting on a chair, abdomen unbound, wearing a nose clip and breathing through a mouthpiece connected to a pneumotachometer and a one-way valve (Hans Rudolph, Kansas City, MO). The signal from the pneumotachometer gave access to the respiratory rhythm, tidal volume, minute ventilation and tidal volume-to-inspiratory time ratio (Vi/Ti) (respiratory pressure module, MedGraphics, Medical Graphic, Saint Paul, MN). End-tidal CO2 partial pressure (PETCO2) in the expiratory gas pump-sampled at the mouth was measured by an infrared gas analyzer (Medical Gas Analyzer LB-2; Beckman Instruments, Fullerton, CA). The values provided in the results section are 10-min averages of PETCO2 values obtained under steady-state conditions. Occlusion pressure at 100 ms (P0.1) was measured at the mouth during inspiration against the occluded airway (computer-driven silent expiration occlusion of the inspiratory limb of the respiratory circuit with an inflatable rubber balloon, Hans Rudolph). P0.1 was measured at random intervals, every four to seven respiratory cycles. The values hereafter provided are the average of at least 10 steady-state measurements. Esophageal (Pes) and gastric (Pga) pressures were measured by using two air-filled (1 ml) balloon catheters (length 80 cm, 1.4 mm ID; Marquat, Boissy Saint Léger, France) connected to linear differential pressure transducers (Vali-dyne MP45, ±100 cmH2O, Northridge, CA).

EMGs. Surface recordings of the right diaphragmatic electromyogram (EMG) were obtained by using a pair of skin-taped silver cup electrodes filled with conductive paste. One electrode was placed in the eighth intercostal space, between the costochondral junction and the midclavicular line; the other electrode lay on the relief of the above rib. The distance between the two electrodes was kept to a minimum, never exceeding 2 cm. This electrode placement corresponds to a modified technique recently validated as minimizing the risk of contamination of the signal picked up by the electrical activity of extradiaphragmatic muscles coactivated by both CMS and TMS (15, 60). Surface EMG of a hand muscle, the right abductor pollicis brevis (APB), was simultaneously recorded to serve as control. EMG signals were amplified, band-pass filtered (20 Hz–5 kHz), digitized (10 kHz), and stored as computer files for subsequent analysis (Neuropack Sigma electromyograph; Nihon Kohden, Tokyo, Japan). The Pes and Pga signals (see above) were processed with the same device.

Stimulations. All magnetic stimulations were carried out with a Magstim 200 stimulator equipped with a 90-mm circular coil and providing a maximum output of 2.5 T (Magstim, Sheffield, UK). To begin with, bilateral phrenic nerve stimulation was performed using CMS to assess the integrity of the phrenic nerves, check the position of the surface electrodes aimed at recording the diaphragm EMG, and evaluate the mechanical diaphragm response. This was achieved according to the method previously described (52, 53). Briefly, the coil was centered on the spinous process of the seventh cervical vertebra, while its handle was held at a 45–90° angle to the vertebral column. The subjects kept their heads in a neutral position. In addition to the phrenic nerves themselves, CMS stimulates the spinal nerves and several cervical roots. We took advantage of this feature to assess the peripheral nervous conduction to the APB (49). The maximum output of the stimulator was always used. TMS was achieved by using the same stimulator-coil combination as for CMS. The coil was positioned over the vertex, and the maximal output intensity of the stimulator was always used. Various locations and coil positions were tried out to obtain an optimal EMG response. Because the cranial positioning of the coil can influence the relationship between the stimulating current and the stimulated neural structure and therefore can modify the characteristics of the recorded response, particular care was taken to maintain optimal coil position. This was achieved by drawing a marker on a tightly fitting rubber swimming cap that the subjects wore throughout the experiments. All the stimulations were triggered by the same value of Pes (Pes.trig), set slightly below the room air breathing end-expiratory Pes. Pes.trig thus corresponded to the Pes defined by the mechanical properties of the respiratory system under a condition of near-relaxation of the inspiratory muscles. Stimulations at Pes.trig were performed during early inspiration (I), when Pes began to fall, and expiration (E). Expiratory stimuli were delivered by firing the stimulator when Pes, ascended during its inspiratory nadir, crossed the value previously set to trigger the “early inspiration” stimulations. Of note, all stimulations, inspiratory or expiratory, were thus triggered at the same level of Pes. All the stimulations were performed without airway occlusion.
Data analysis. Responses to stimulations were observed in terms of EMG (apbEMG for the APB, DiEMG for the diaphragm; EMG, cms for CMS, EMG, tms for TMS); Pes (Pes, cms for CMS, Pes, tms for TMS); and Pga (Pga, cms for CMS and Pga, tms for TMS). The amplitudes of the EMG responses were measured between the highest and the lowest peak. The latencies of the EMG responses were measured as the time elapsed between the stimulus and the onset of the response, namely the first departure of the signal from baseline. We define the difference between the EMG, tms latency of a given muscle and the corresponding EMG, cms latency as a "central conduction time," being aware that this name is not completely correct because the EMG, cms is not the reflection of a spinal response but of a peripheral one. The values of Pes, cms, Pes, tms, Pga, cms, and Pga, tms were calculated as the difference between the value at the time of stimulation and the peak of the pressure wave induced by it. The transdiaphragmatic pressure (Pdi) in response to stimulations (Pdi, cms for CMS, Pdi, tms for TMS) was calculated offline by subtracting Pes, cms or Pes, tms from Pga, cms or Pga, tms, respectively. The values reported for each subject are the average of 5 CMS and 10 TMS.

Experimental Procedure

Effect of breathing hypercapnic gas mixtures. The subjects first breathed room air (air condition) through the pneumotachometer and the one-way valve for 15–20 min, to become familiar with the setting and to reach a ventilatory steady state. Measurements of respiratory rhythm, tidal volume, minute ventilation, VT/Ti, PETCO2, and P0.1 were performed over a 10-min period. CMS and TMS were then performed. The whole procedure was repeated while subjects breathed a 5% CO2–95% O2 gas mixture (5% CO2 condition) and then with a 7% CO2–93% O2 gas mixture (7% CO2 condition). This procedure was intended to increase the automatic respiratory neural drive through hypercapnia while inhibiting the outputs of the peripheral chemoreceptors through hyperoxia.

Effect of breathing pure O2 on the APB EMG response to CMS. The goal of this procedure was to assess the effect of a hyperoxic gas mixture on the conduction of peripheral nerves. The response of the APB to CMS performed during steady-state pure O2 breathing (100% O2 condition) was compared with the response elicited by CMS during room air breathing. In this part of the experimental protocol, which was performed in a subset of the study population including seven subjects (one in common with the first set), stimulations were triggered manually, randomly over the ventilatory cycle.

Statistical Analysis

The latencies and the amplitudes of the EMG responses as well as the values of Pes, Pga, and Pdi responses obtained in each subject were used to calculate the group mean ± SE for each condition. Differences between study conditions were tested for statistical significance (probability P of a type I error below 5%) using either the two-tail Student’s t-test or an ANOVA, followed, when appropriate (P < 0.05) by the Student-Newman-Keuls post hoc test (40). Linear regressions using the least square method were carried out, using P0.1 as the independent variable and the latencies and amplitudes of the EMG, cms as the dependent variable. The statistical analysis was performed by using the Statview 5.0 software (SAS Institute, Berkeley, CA).

RESULTS

Effects of Breathing Hypercapnic Gas Mixtures

Ventilation. Hypercapnic gas mixtures significantly increased PETCO2. As expected, this increase in PETCO2 led to an increase in minute ventilation, tidal volume, and respiratory rhythm (P < 0.0005 in all cases). Breathing hypercapnic gas mixtures also increased the respiratory neural drive expressed in terms of P0.1 (P < 0.0005) and VT/Ti (P = 0.022). VT/Ti, which was of 1.09 ± 0.43 l/s in the air breathing condition, increased significantly between the 5% CO2 condition (1.34 ± 0.10 l/s) and the 7% CO2 condition (2.46 ± 0.43 l/s). The 7% CO2 P0.1 (6.88 ± 0.82 cmH2O) was significantly greater than the 5% CO2 P0.1 (4.33 ± 0.47 cmH2O), and both were significantly higher than the air P0.1 (1.64 ± 0.23 cmH2O).

Mechanical Responses to Stimulations

A twitch-shaped Pdi response made of simultaneous negative Pes and positive Pga components was consistently associated with CMS. This was also the case for TMS, ascertaining the reality of a diaphragm response to both types of stimulation, during both I and E.

CMS. The Pdi, cms in response to I-CMS was unaffected by the increase in the inhaled fraction of CO2 (Fig. 1). Conversely, the Pdi, cms in response to E-CMS significantly fell from 11.76 ± 1.46 cmH2O during the air condition to 6.43 ± 1.15 cmH2O during the 7% CO2 condition (ANOVA, n = 7, P = 0.0001). As a result, a difference between the E-CMS Pdi, cms and the I-CMS Pdi, cms, absent during air breathing, appeared and was significant during both the 5% CO2 and the 7% CO2 conditions.

TMS. The Pdi, tms in response to I-TMS increased with increasing the inhaled fraction of CO2 (ANOVA, n = 7, P < 0.0001; Fig. 1). The difference reached significance between the air condition (7.16 ± 1.29 cmH2O) and the 7% CO2 condition (10.35 ± 1.60 cmH2O). In contrast, the Pdi, tms in response to E-TMS did not change with the inhaled fraction of CO2. During all conditions, the Pdi, tms was greater in response to I-TMS than to E-TMS.

EMG Responses to Stimulations

Latency. The latencies of DiEMG, cms were not affected by the inhaled gas mixtures (ANOVA, n = 7, P = 0.25; Fig. 2). In contrast, the latencies of DiEMG, tms significantly decreased with increasing the inhaled fraction of CO2, in response to both I- and E-TMS (ANOVA, n = 7, P < 0.0001, Figs. 2 and 3). In response to I-TMS, the latency of DiEMG, tms decreased significantly during the 5% CO2 condition (15.78 ± 0.56 ms) and the 7% CO2 condition (15.59 ± 0.48 ms) compared with the air condition (16.87 ± 0.39 ms). A decrease DiEMG, tms latency also occurred in response to E-TMS during the 5% CO2 condition (15.50 ± 0.65 ms) and the 7% CO2 condition (14.98 ± 0.63 ms), compared with the air condition (17.49 ± 0.64 ms). Consistently, the central conduction time decreased with increasing concentration of inhaled CO2 (ANOVA, n = 7, P = 0.0012). This decrease reached significance in response to the E stimulations between the air condition (12.23 ± 0.65 ms) and the 5% CO2 condition (10.56 ± 0.63 ms), and between the air condition and the 7% CO2 condition (10.11 ± 0.65 ms).

Conversely, the latencies of apbEMG, cms increased with increasing the inhaled fraction of CO2 (ANOVA, n = 7, P = 0.0022; Fig. 4). This increase reached significance during the 7% CO2 condition (14.09 ± 0.51 ms for I-CMS and 14.20 ± 0.47 ms for E-CMS) compared with the air condition (13.38 ± 0.57 ms for I-CMS and 13.39 ± 0.59 ms for E-CMS). Similarly, the latencies of apbEMG, tms increased with the augmentation of the inhaled fraction of CO2 (ANOVA, n = 7, P =
0.0253). This increase reached significance only in response to I-TMS between the air condition (21.75 ± 0.10 ms) and the 7% CO₂ condition (22.95 ± 0.72 ms). The central conduction times measured in response to I stimulations and to E stimulations did not change significantly, whatever the inhaled gas mixture (ANOVA, n = 7, P = 0.633).

Amplitude. The amplitudes of DiEMG,ems were not affected by the inhaled gas mixtures (ANOVA, n = 7, P = 0.786; Fig. 2). In contrast, the amplitudes of DiEMG,tms increased significantly when the subjects breathed hypercapnic gas mixtures (ANOVA, n = 7, P = 0.0203; Figs. 2 and 3). In response to E-TMS, the amplitudes of DiEMG,tms increased significantly during the 5% CO₂ (157.26 ± 52.72 μV) and the 7% CO₂ breathing conditions (160.49 ± 38.96 μV) compared with the air condition (86.68 ± 23.61 μV, respectively, P < 0.05 in both cases). The amplitude of DiEMG,tms in response to I-TMS increased from 142.17 ± 38.09 to 168.08 ± 40.88 μV, but this augmentation was not statistically significant. The amplitudes of DiEMG,tms under the air condition were significantly greater in response to I-TMS than to E-TMS. Yet the DiEMG,tms amplitudes did not differ significantly between I- and E-TMS during the 5% CO₂ and the 7% CO₂ breathing conditions. The amplitudes of apbEMG,ems and of apbEMG,tms in response to both CMS and TMS were not modified by the inhalation of hypercapnic gas mixtures (ANOVA, n = 7, P = 0.633 and P = 0.566, respectively).

Relationship with P0.1. The changes in DiEMG,tms latencies induced by CO₂, expressed as a percentage of their baseline value, were linearly related to the corresponding changes in occlusion pressure, with a significant correlation (r² = 0.407, P = 0.002 for I-TMS, r² = 0.513, P = 0.0003 for E-TMS; Fig. 5). The same was true for P0.1 and DiEMG,tms amplitudes in response to I-TMS (r² = 0.420, P = 0.002) and E-TMS (r² = 0.566, P < 0.0001; Fig. 5).

Effect of Breathing Pure Oxygen on the APB EMG Response to CMS

This additional set of experiments was conducted in view of the effects of breathing 5% CO₂-95% O₂ and 7% CO₂-93% O₂ gas mixtures on the APB response to CMS (see DISCUSSION). Breathing pure O₂ instead of room air did not modify the latencies of apbEMG,ems (t-test, n = 7, P = 0.221) or their amplitude (t-test, n = 7, P = 0.145).
DISCUSSION

This study shows that increasing the ventilatory neural drive through CO₂ inhalation facilitates the response of the diaphragm to TMS applied not only during early inspiration but also during expiration. In contrast, the response of a hand muscle to the same stimulation paradigm is not facilitated by changes in the automatic respiratory drive. Before discussing our results, two methodological issues will be addressed.

Methodological Issues

Putative interferences from peripheral conduction velocity. Interpreting a muscle response to TMS without carefully taking the peripheral component of this response into account carries the risk of drawing false conclusions. In this study, in which the effects of TMS on the cortical commands to the diaphragm and to the APB were studied, it was of particular importance to verify whether changes in the characteristics of the EMG, tms did or did not include a peripheral factor, hence our careful assessment of the effects of CO₂ on the responses to CMS. Incidentally, this study seems to provide the first assessment of peripheral nerve velocity during acute hypercapnia in humans. The latencies of apbEMG, cms increased under 7% CO₂-93% O₂. Because they were unaffected by pure O₂, it can safely be deduced that acute hypercapnia decreased the velocity of the conduction in the corresponding nerve. This is consistent with in vitro studies that showed that hypercapnia decreases the excitability and the velocity of conduction in peripheral amphibian nerves (36) and in the rat phrenic nerve (16). Conversely, the latencies of DiEMG, cms, i.e., the velocity of conduction along the phrenic nerve, were unaffected by acute hypercapnia. This is at variance with the results reported by Ellis (16) in the phrenic nerve of the rat and showing a correlation between the percentage of increase in conduction time and the concentration of CO₂ in the surrounding milieu. One very speculative hypothesis to reconcile our findings, obtained in vivo, and those of Ellis, obtained in vitro, could be that the phasic inspiratory activity of the phrenic motoneurons provides a conditioning stimulus to the phrenic nerve. Conditioning a nerve by a first stimulus is known, in the frog, to facilitate and accelerate the response of this nerve to a subse-

Fig. 2. Example of the effect of breathing hypercapnic-hyperoxic gas mixtures on the electromyographic (EMG) responses of the diaphragm and the abductor pollicis brevis to magnetic stimulations, in one subject. Breathing hypercapnic-hyperoxic gas mixtures facilitates the diaphragmatic response to TMS, as shown by the increase in amplitude and the decrease in latency of the response. The responses of the diaphragm to CMS and of the abductor pollicis brevis to TMS are not facilitated. The lower tip of the arrows shows the latency of the motor potentials evoked by TMS.
quent stimulus, under hypercapnic conditions (36) (but to our knowledge, this phenomenon was never demonstrated in mammalian nerves). According to our observations, the function of the human phrenic nerve in vivo seems “protected” against the deleterious effects of acute hypercapnia by an unidentified mechanism that could possibly be the phasic inspiratory activity. The reality of this hypothetical mechanism and its clinical relevance will have to be investigated.

The absence of effects of hypercapnia on phrenic nerve conduction allows us to reason directly in terms of DiEMG,tms latency. Regarding the APB, it appears more relevant to consider central conduction times as we define it, rather than the whole conduction time.

**Reality of the diaphragm facilitation-Pdi response to TMS.** First of all, the consistent Pdi response to I-TMS and to E-TMS indicates that the diaphragm was truly activated by TMS in both conditions. The Pdi measured in response to CMS applied at the beginning of inspiration was not influenced by CO₂. The significant CO₂-related increase in Pdi in response to I-TMS can thus reasonably be taken as the mechanical correlate of a neural facilitation. In contrast, hypercapnia induced a decrease in the Pdi response to CMS applied during expiration. In the absence of facilitation, we should have observed a concomitant decrease in the Pdi response to TMS. Therefore, the lack of effect of hypercapnia on the Pdi response to TMS during expiration (see Fig. 1) implies a certain degree of facilitation. This was supported by the CO₂-related DiEMG,tms latency decrease and amplitude increase (see Fig. 3).

The CO₂-related decrease in the Pdi response to CMS during expiration (E-CMS) can be accounted for by several factors. Hypercapnia itself could have impaired the intrinsic contractile properties of the diaphragm (31). However, this was likely not the case because, in line with observations reported by Mador et al. (37), the Pdi response elicited by I-CMS was identical under normo- and hypercapnic conditions. An increase in lung volume (28, 55) or a change in thoracoabdominal configuration (8) is a more likely explanation. Because of the very design of the study, the value of Pes that triggered E stimulations should have corresponded to a higher lung volume than the value that triggered I stimulations, because of CO₂-related hyperventilation. This would reduce the ability of the diaphragm to produce force and pressure (28, 55).

**Reality of the diaphragm facilitation-EMG response to TMS.** DiEMG,cms and DiEMG,tms were obtained from surface electrodes. This approach theoretically carries the risk of signal contamination from the electrical activity of extradiaphrag-
matic muscles coactivated by the stimulation. Therefore, even though DiEMG,tms amplitude increased with hypercapnia, it is impossible to definitely rule out a possible contribution of other muscles to the EMG,tms amplitude recorded with surface electrodes. However, recent studies of our group (15, 60) showed that the location of our surface electrodes permits a reliable recording of the latency of DiEMG,tms. The at least partial diaphragmatic origin of EMG,tms in this study was further supported by the concomitant twitch-shaped rise in Pdi, ascertaining a concomitant diaphragm contraction, and by the parallelism that we found between the facilitation of the mechanical response to TMS and the facilitation of the EMG,tms even during expiration.

The hypercapnia-related facilitation of the diaphragmatic response to TMS was hallmarked by a decreased DiEMG,tms latency (I-TMS and E-TMS), a decreased central conduction time (E-TMS), and an increased DiEMG,tms amplitude (E-TMS). The absence of such changes in response to I-CMS and E-CMS rules out confounding factors such as lung volume-related changes in the electrode-muscle relationship (23). We are therefore confident about the reality of the facilitation of the diaphragmatic response to TMS at least in terms of latency. This facilitation can thus be discussed in terms of the corresponding neural phenomenon.

**Putative Source of the Hypercapnia-Induced Facilitation of the Diaphragm Response to TMS**

The preactivation of the spinal motoneurons is the most commonly accepted mechanism to explain the facilitation of a given muscle response to TMS (38, 57). Peripheral afferents are involved in this preactivation as soon as muscle contraction starts (34). Accordingly, an underlying contraction facilitates the response to TMS in general, and this is also true for the diaphragm (54). In our study, the intensity of the contraction of the inspiratory muscles including the diaphragm was controlled for at the time of the stimulation and identical under the three conditions studied. Thus we feel safe to assume that the role of peripheral afferents was not a dominant factor of the facilitation that we observed during hypercapnia. Spinal motoneurons can also be preactivated by central inputs from the motor cortex. This explains why the diaphragmatic response to TMS is facilitated by a voluntary inspiration (14, 54) and why hypercapnia is not likely to increase the corticospinal input to phrenic motoneurons (41). Indeed, Corfield et al. (11), using positron emission tomography, emphasized that “no neuronal activation was seen within the primary motor cortex” during CO2-induced hyperventilation, which was later confirmed by others (5). A cortical site for the CO2-related facilitation that
we observed is thus unlikely, all the more so because cortical facilitation, as observed in response to imagined movements, increases the amplitude of EMG,tms but does not decrease its latency (32). This is in contrast with our study. Postexercise facilitation is another mechanism that can enhance the response of relaxed muscles to TMS (42). As cortical facilitation, it increases EMG,tms amplitude without change in latency, a pattern that we did not observe. Furthermore, because the diaphragm contracts phasically and continuously, facilitation due to a postexercise phenomenon should have been constant throughout the experiment, whereas we observed that it increased with the inhaled fraction of CO₂. Finally, our results could be explained by a direct effect of hypercapnia on the excitability of the phrenic motoneurons. However, hypercapnia tends to hyperpolarize phrenic motoneurons (27). In addition, hypocapnia, not hypercapnia, increases the excitability of the human corticospinal pathway (50).

All in all, we feel entitled to submit that the most likely origin of the facilitation of the diaphragm response to TMS that we observed is the CO₂-related increase in the automatic breathing command. The statistical relationship between occlusion pressure and the facilitation of the EMG response of the diaphragm to I-TMS lends support to this hypothesis (Fig. 5). Additional arguments can also be derived from the lack of effects of hypercapnia on the response of the APB (see below).

**Differential Effects of Hypercapnia on the Diaphragm and APB Responses to TMS**

To our knowledge, our study provides the first description of the response of a hand muscle to TMS during hypercapnia. It shows the lack of facilitatory or inhibitory effect. Yet other studies have investigated the influences of the respiratory control system on locomotor function. In line with our results, Corfield et al. (13) showed that ventilatory tracking performances were significantly worsened by hypercapnia whereas manual tracking performances were not. Importantly, the response of the diaphragm and of a hand muscle to TMS are concomitantly facilitated during voluntary inspiration (12). The absence of facilitation of the APB response to I-TMS under hypercapnic conditions in our study thus provides an additional argument against a cortical source for the diaphragmatic facilitation.

From another point of view, there are known connections between respiratory muscles and locomotor muscles. Inspiratory resistive loading, which preferentially activates inspiratory muscle afferents, increases motor unit recruitment in muscles such as the adductor pollicis (19) but not in muscles such as the biceps brachialis or the vastus lateralis. Reciprocally, expiratory resistive loading, which activates vagal afferents and expiratory muscle afferents, decreases motor unit recruitment.
in extensor muscles such as the vastus lateralis (19) and the triceps brachialis (58). The response of limb muscles to tonic vibrations suggests that resistive loading can influence their gamma motor drive (3). Regarding the APB, it participates in the abduction movement of the thumb. However, when this muscle contracts alone, the thumb moves inward and forward (48). The function of the APB itself is thus adductive, like the adductor pollicis. Because the motor unit recruitment of the latter muscle increases when inspiratory muscle afferents are stimulated, the cofacilitation of the APB with that of the diaphragm in response to TMS could have been expected. The absence of such a cofacilitation is an additional argument against a significant role of respiratory afferents and of suprapontine structures in our observations. Indeed, the modifications of the motor control of limb muscles induced by respiratory loading can stem from interactions either at the level of spinal alpha or gamma motoneurons (3), at the cortical level (because of the behavioral nature of the ventilatory response to resistive loading, Ref. 62), or both.

Interactions Between the Bulbospinal and Corticospinal Pathways to the Phrenic Motoneurons

During inspiration, the activity of the phrenic spinal motoneurons is facilitated by excitatory inputs (central respiratory drive potentials) arising from the brain stem central pattern generators that govern the automatic respiratory neural drive, often referred to as the “respiratory centers.” The intensity of these inputs is augmented by hypercapnia. Therefore, the facilitation of the diaphragm response to I-TMS that we observed is not a surprise and probably corresponds to a preactivation of the phrenic motoneurons by the medullary and pontine inputs. In humans, a rapidly conducting oligosynaptic corticospinal pathway (25) traveling through the pyramidal tract (54) can bypass the respiratory centers (12). The inputs that this pathway conveys are integrated with other inputs at the spinal motoneurons level (2). Their effects will thus depend on the state of the motoneurons at the time of their arrival. In this view, our results can be interpreted as follows: the CO2-driven increase in the bulbospinal drive preactivates the phrenic motoneurons, hence facilitating a corticospinal input independently generated by TMS.

During expiration, and as opposed to inspiration, the activity of phrenic motoneurons is disfacilitated by inhibitory inputs (4) in the cat. However, in the same animal, Colle and Massion (10) and Planche (44) described diaphragm responses to cortical stimulation applied during expiration. This indicates that a corticospinal input occurring during expiration can overcome the prevailing inhibition both under resting breathing conditions (54) and during CO2-stimulated breathing. The exact underlying mechanisms remain to be elucidated. Of note, the parallel evolution of occlusion pressure and of the degree of expiratory facilitation (Fig. 5) suggests that the effectiveness of the corticospinal input to the diaphragm tends to increase as the automatic drive to breathe augments.

Perspectives

From a teleological point of view, our results could be interpreted as a substrate of the possibility of voluntary respiratory commands to override the automatic ventilatory drive even after it has been powerfully increased to meet homeokinetic requirements. In that, our observations are in line with the ability of humans to disrupt ventilation to the benefit of speech during exercise, however heavy, or during CO2 rebreathing (7, 43). Taking into account the facts that 1) speech is possible and generally preserved in the presence of an increased automatic drive to breathe (7, 43); 2) hypercapnia decreases the precision of the voluntary control of breathing (13), and 3) hypercapnia facilitates corticospinal inputs (this study), one can speculate that an increased drive to breathe “opens the gate” for corticospinal inputs to the phrenic motoneurons, allowing speech to appear quantitatively normal but decreasing the precision of a voluntary command through a decrease in the “selectivity” of this command. In other words, a large number of motoneurons being in a hyperexcitable state, a corticospinal command is more likely to “diffuse,” hence less precision. This is highly speculative and needs to be verified. However, this hypothesis may be easily testable experimentally by simultaneously studying speech, voluntary control, and responses to magnetic stimulation during CO2 breathing. From a practical point of view, TMS could provide a means to clinically study the respiratory neural drive and its variations over time.

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