Depression of diaphragm motor cortex excitability during mechanical ventilation

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1Respiratory Muscle Laboratory, Royal Brompton and Harefield National Health Service Trust, King’s College Hospital, London SW3 6NP; and 2Department of Respiratory Medicine, King’s College Hospital, London SE5 9PJ, United Kingdom; 2Département de Biostatistique et Informatique Médicale, Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris, 75010 Paris; and 3Service d’Exploration Fonctionnelles, Hôpital Raymond Poincaré, Assistance Publique-Hôpitaux de Paris, 92380 Garches, France

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Sharshar, Tarek, Ewen T. Ross, Nicholas S. Hopkinson, Raphael Porcher, Annabel H. Nickol, Sophie Jonville, Mark J. Dayer, Nicholas Hart, John Moxham, Frédéric Lofaso, and Michael I. Polkey. Depression of diaphragm motor cortex excitability during mechanical ventilation. J Appl Physiol 97: 3–10, 2004. First published March 12, 2004; 10.1152/japplphysiol.01099.2003.—The effect of mechanical ventilation on the diaphragm motor cortex remains unknown. We assessed the effect of mechanical ventilation on diaphragm motor cortex excitability by measuring the costal and crural diaphragm motor-evoked potential (MEP) elicited by single and paired transcranial magnetic stimulation. In six healthy subjects, MEP recruitment curves of the costal and crural diaphragms were assessed at relaxed end expiration during spontaneous breathing [baseline tidal volume (VT baseline)] and isocapnic volume cycled ventilation delivered noninvasively (NIV) at three different levels of tidal volume (VT NIV = VT baseline ± 5 ml/kg liters, and VT NIV = VT baseline ± 10 ml/kg liters). The costal and crural diaphragm response to peripheral stimulation of the right phrenic nerve was not reduced by NIV. NIV reduced the costal and crural MEP amplitude during NIV (P < 0.0001) with the maximal reduction at VT NIV = VT baseline + 5 ml/kg. Response to paired TMS showed that NIV (VT NIV = VT baseline + 5 ml/kg) significantly increased the sensitivity of the cortical motoneurons to facilitatory (>9 ms) interstimulus intervals (P = 0.002), suggesting that the diaphragm MEP amplitude depression during NIV is related to neuromechanical inhibition at the level of motor cortex. Our results demonstrate that mechanical ventilation directly inhibits central projections to the diaphragm.

transcranial magnetic stimulation; diaphragm motor-evoked potential depression

MECHANICAL VENTILATION PLAYS a key role in the treatment of acute and chronic respiratory failure. Effective mechanical ventilation reduces respiratory center output, whether measured as the diaphragm electrical activity (EMGdia) or as pressure generation [pressure-time product (PTPdia)] (10, 11). Although neuromechanical feedback mediated by thoracic mechanoreceptors on brain stem structures is thought to contribute to this inhibition (11, 28, 34), the possibility exists that it may affect motor cortical control too.

Cortical control of the diaphragm can be assessed by measuring its electrical response, the motor-evoked potential (MEP), to transcranial magnetic stimulation (TMS). TMS has previously been used to map the motor cortical area of different respiratory muscles as well as to determine excitability of their cortical neurons (7, 21, 23, 25, 33). Cortical excitability can be assessed by relating the amplitude of the MEP elicited by single stimulations to stimulus intensity (“recruitment curve”) (27, 37) and by relating the amplitude of the MEP elicited by paired TMS to the time between the stimuli (“intracortical inhibition/intracortical facilitation curves” [ICI/ICF curves]) (4, 8, 18). The latter paradigm is a reproducible method that allows assessment of both inhibitory and facilitatory intracortical circuits that modulate the excitability of cortical motoneurons (22).

The hypothesis tested in the present study was that the excitability of the motor cortex supplying the diaphragm would be reduced during mechanical ventilation. Thus we compared the recruitment and ICI/ICF curves of the diaphragm MEP in healthy subjects during spontaneous breathing and noninvasive mechanical ventilation set to produce increased tidal volumes (VT) and thus activate neuromechanical feedback.

METHODS

Subjects. Six healthy volunteers (five men), aged 25–45 yr (mean age 35 yr), participated in the study. The subjects were members of the laboratory staff, and all were free of neurological and respiratory disease. The study was approved by the ethics committee of the Royal Brompton and Harefield Hospital, and the subjects gave their informed consent. Five of the six subjects are coauthors of this paper.

Measurements. Throughout the study, subjects wore either a nasal or full-face mask. Flow was measured via a pneumotachograph head (Hans Rudolph 3700 series, Hans Rudolph, Kansas City, MO) connected to a CS5T electrospirometer (GM Instruments). Minute ventilation and VT were electronically derived. Airway pressure (Paw) was measured via a differential pressure transducer (MP-45 model, Validyne, Northridge, CA). Esophageal (Pes) and gastric pressures (Pga) were measured by using two air-filled balloon catheters (Ackrad Laboratories, Crandford, NJ) positioned in a standard manner (2, 24). Both catheters were connected to differential pressure transducers (range ±300 cmH2O, Validyne). Transdiaphragmatic pressure (Pdi) was derived electronically by subtracting Pes from Pga. End-tidal PO2 and PECO2 were determined via a nasal catheter connected to a capnograph (PK Morgan, Gillingham, Kent, UK).

Surface recordings of the right diaphragm compound motor action potential (CMAP) and MEP were obtained by using Ag-AgCl elec-

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trodes whose optimal position was determined by using transcutaneous electrical stimulation of the right phrenic nerve (40). Abdominal muscle MEP was measured by positioning surface electrodes over the rectus abdominis muscle (27).

Crural diaphragm CMAP, MEP, and electromyographic (EMG) signals were obtained by using a multipair esophageal electrode catheter, which consisted of five copper coils. The top coil was connected to Earth, and the lower four coils formed six sequential pairs of electrodes. The optimal esophageal electrode position was characterized by opposite polarity and similar amplitude of the CMAP recorded from the two pairs of electrodes. The electrode was securely taped to the subject’s nose. The crural EMG signals recorded from the two optimal electrode pairs were amplified and then passed to a personal computer running LabView 4.1 software (National Instruments). Electrocardiogram was simultaneously recorded by using three chest electrodes. Mean peak-to-peak amplitude (μV) of the MEP first deflection was calculated.

TMS and phrenic nerve stimulation. TMS was performed by using a Magstim 200 stimulator (The Magstim, Whitland, UK), and paired TMS were delivered by using two units linked via a Bistim locking device. For both types of stimulations, a 110-mm double-cone coil (The Magstim), oriented in the anterior-posterior direction, was used. TMS was delivered at the best scalp position, which was determined as previously described (31). In three subjects, this was over the vertex, in two subjects +1 cm forward, and in one subject +1 cm forward and 1 cm lateral. Throughout the study, stimuli were delivered at resting end expiration, and the interval between stimulations was at least 30 s. For TMS, surface and esophageal electrode signals were amplified with band-pass filters at 10 Hz to 10 kHz, digitized, and acquired into a five-channel EMG recorder (Medelec, Synergy, Oxford Instruments, Oxford, UK).

To assess the diaphragm recruitment curves to TMS, stimulus intensity was increased from 40% of maximal stimulator output in 10% increments up to 100% of stimulator output. The order in which stimuli from 40 to 100% were delivered was randomized. Four stimuli were delivered at each intensity level.

Motor threshold was defined as the lowest stimulator output (via the Bistim device) that produced a MEP peak-to-peak amplitude of at least 50 μV in more than 5 of 10 trials. Stimulator output was decreased from 100% by 5% increments until threshold was reached. To assess the diaphragm ICI/ICF curves, intensity of subthreshold conditioning stimulus (CS) and suprathreshold test stimulus (TS) was set at 80 and 125% of the motor threshold, respectively. The interstimulus interval (ISI), i.e., time elapsing between conditioning stimulus and TS, was set at 1, 3, 5, 7, 9, 11, 13, 15, and 20 ms, in random order (8). Five stimuli were delivered at each ISI.

Unilateral anterolateral magnetic stimulation (UAMPS) of the right phrenic nerve was performed at 100% of stimulator output by using a 45-mm figure-of-eight coil (The Magstim). In cases (n = 4) in which the CMAP elicited by UAMPS yielded unacceptable stimulation artifact, we performed supramaximal transcutaneous electrical stimulation (electrophrenic stimulation).

Noninvasive ventilation. Volume cycled noninvasive ventilation (NIV) (Brea PSV 403, Brea Medical) was delivered via a commercially available nasal mask (Comfort Classic Profile Therapeutics, Bognor Regis) in five subjects and a full-face mask (Mirage NV, Oxfordshire, UK) in one. Leaks were minimized, and subject comfort was assessed by using a visual analog score ranging from 0 (very comfortable) to 10 (very uncomfortable).

Study protocol and statistical analyses. The study protocol encompassed three studies performed on three separate occasions.

The objective of study 1 was to assess the effect of NIV on TMS recruitment curves of the diaphragm and abdominal muscles. It consisted of two periods of relaxed spontaneous breathing before and after three periods of assisted ventilation. During each of these, a VT was delivered in a random order. During the two spontaneous breathing periods [baseline (SBbaseline) and end], subjects breathed room air with the mask in position, but with the ventilator circuit disconnected. Baseline respiratory rate (RR), VT (VTbaseline, and PETCO₂, (or isocapnia)) were assessed for the last 90 s of a 5-min control period. During assisted ventilation, the ventilator was set to deliver the same RR as during SBbaseline. In each ventilator period, the volume delivered was equal to either that during spontaneous breathing or VTbaseline + 5 ml/kg or VTbaseline + 10 ml/kg.

The diaphragm and abdominal muscle recruitment curves were assessed in each condition. In addition, the costal and crural diaphragm responses to peripheral right phrenic stimulation (UAMPS or electrophrenic stimulation) were recorded during SBbaseline and the two highest levels of NIV. MEP100 referred to MEP elicited by 100% stimulator output, and MEPTS referred to MEP elicited by TS alone. For recruitment curves, MEP amplitude and latency were normalized to MEP100 response at SBbaseline. MEP100 amplitudes were compared between the two spontaneous breathing periods and the mechanical ventilation levels by using mixed-model analysis of variance with period fixed effect and random subject effects. MEP amplitude recruitment curves were analyzed by using linear mixed-effects models, with a fixed period effect, a linear intensity effect, and their interactions. Subject effects, slopes, and interactions were also added to the model. The random effects variance structure was selected by using Schwarz’ Bayesian information criterion model selection criterion (29). Post hoc tests were performed to compare SBbaseline to each of the other study periods by using least squares means for MEP100 amplitudes or estimated slopes for MEP recruitment curves and Hochberg’s procedure for multiple testing (14).

Study 2 was an abbreviated version of study 1. It included only one period of relaxed spontaneous breathing and one period of NIV with a set at VTbaseline + 5 ml/kg. The diaphragm MEP100, motor threshold, and ICI/ICF curves were consecutively assessed in the final phase of spontaneous breathing and mechanically ventilated periods. Mean peak-to-peak amplitude (μV) of the MEP first deflection was calculated. MEP100 referred to MEP elicited by TS alone. For the ICI/ICF curves, MEP amplitude was normalized to MEPTS. Diaphragm ICI/ICF curves were analyzed by using linear mixed-effects models, in which the ICI/ICF was considered to have a piecewise linear effect, with different slopes and intercepts before and after 9 ms.

In study 3, we assessed the quadriceps response to paired TMS to determine whether mechanical ventilation-related changes in ICI/ICF curves were specific to the diaphragm. During VTbaseline and VTbaseline + 5 ml/kg, quadriceps MEP amplitude was measured at 3-, 9-, and 15-ms ISIs by using the same paired TMS paradigm.

Before each period of studies 1, 2, and 3, an accommodation period of 20 min was allowed, and periods were separated by a 10-min rest, during which the mask was removed from the subject. PTECO₂ was maintained at baseline level by bleeding in a gas mixture containing 15% CO₂/85% air into the respiratory tubing proximal to the ventilator. Heart rate, Paw, Ppa, Pes, Pdi, flow, RR, respiratory time, expiratory time, and PTECO₂ signals, as well as crural EMGdia activity, were recorded over the final 5 min of each period. The peak Paw and inspiratory transdiaphragmatic PTPdia (cmH₂O·s⁻¹·min⁻¹) and, in study 1, inspiratory integrated EMG-time product (EMGdia, arbitrary units·s⁻¹·min⁻¹ and percentage of baseline EMGdia) were calculated. Throughout studies 1, 2, and 3, the subjects were seated comfortably in an armchair and were unable to view either the various PC monitors, the settings on the ventilator, or the CO₂ cylinder. Subjects were encouraged to maintain the same posture, with the upper and lower limbs and the head and neck in a relaxed position. The subjects were allowed to listen to music, but were not allowed to read or write. Subjects were checked regularly to assure that they remained awake throughout the study. Importantly, no information on the results obtained was given to the subject during the study, so as not to influence the subjects.
Table 1. Mean values of HR, comfort of breathing, RR, flow, PTPdia, and EMGdia during each period of study 1

<table>
<thead>
<tr>
<th></th>
<th>SBbaseline</th>
<th>MV1</th>
<th>MV2</th>
<th>MV3</th>
<th>SBend</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>68±10</td>
<td>68±7</td>
<td>68±8</td>
<td>74±5</td>
<td>65±8</td>
</tr>
<tr>
<td>Discomfort of breathing</td>
<td>0.1±0.2</td>
<td>2.4±1.0</td>
<td>0.6±0.2</td>
<td>1.1±0.2</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>PetCO2, kPa</td>
<td>5.45±0.34</td>
<td>5.69±0.43</td>
<td>5.48±0.33</td>
<td>5.51±0.33</td>
<td>5.55±0.36</td>
</tr>
<tr>
<td>Ti/Tr, %</td>
<td>0.41±0.01</td>
<td>0.43±0.08</td>
<td>0.42±0.07</td>
<td>0.43±0.05</td>
<td>0.40±0.05</td>
</tr>
<tr>
<td>RR, cycles/min</td>
<td>13±2</td>
<td>14±1</td>
<td>14±2</td>
<td>13±2</td>
<td>13±3</td>
</tr>
<tr>
<td>Vr, ml/s</td>
<td>560±90</td>
<td>760±150</td>
<td>1250±180</td>
<td>1830±190</td>
<td>500±190</td>
</tr>
<tr>
<td>Vt, ml/kg</td>
<td>7.3±0.9</td>
<td>10.7±2.6</td>
<td>17.1±3.5</td>
<td>23.6±3.9</td>
<td>6.2±1.9</td>
</tr>
<tr>
<td>Peak Paw, cmH2O</td>
<td>1.9±1.7</td>
<td>4.7±2.5</td>
<td>9.7±5.4</td>
<td>16.9±10.8</td>
<td>2.1±1.7</td>
</tr>
<tr>
<td>PTPdia, cmH2O µs⁻¹·min⁻¹</td>
<td>213±105</td>
<td>178±70</td>
<td>125±104</td>
<td>117±94</td>
<td>148±61</td>
</tr>
<tr>
<td>EMGdia, %</td>
<td>100.0±0.0</td>
<td>87.0±50.0</td>
<td>50.67±34.1</td>
<td>49.1±28.1</td>
<td>76.2±39.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. SB, spontaneous breathing; MV, mechanical ventilation; SBbaseline, SB before MV; MV1, first level of MV [baseline tidal volume (Vt; Vtbaseline) + 5 ml/kg]; MV2, second level of MV (Vtbaseline + 10 ml/kg); SBafter MV, SB after MV; HR, heart rate; PetCO2, end-tidal PaCO2; Ti/Tr, inspiration time-to-total respiratory time ratio; RR, respiratory rate; Vr, flow; Paw, airway pressure; PTPdia, transdiaphragmatic pressure-time product; EMGdia, normalized electromyographic activity of the crural diaphragm. PTPdia and EMGdia significantly decreased with ventilation (ANOVA, P = 0.01 and P = 0.001, respectively).

RESULTS

Effect of different levels of mechanical ventilation on the diaphragm CMAP and on the diaphragm and abdominal muscle MEP recruitment curves (study 1). There was a significant decrease in PTPdia (P = 0.01) and EMGdia (P = 0.001) with mechanical ventilation (Table 1). There was no significant difference in end-expiratory Pes, Pga, or Pdi measured at the mechanical ventilation (Table 1). There was no signifi-
cant change in mean amplitude of diaphragm CMAP between spontaneous breathing and mechanical ventilation (Table 2; see Fig. 2). Representative traces of the costal diaphragm, the crural diaphragm, and rectus abdominis during each period are shown in Fig. 1.

There was a significant reduction with mechanical ventilation in MEP100 amplitude of the costal and crural diaphragm but not of the rectus abdominis (Fig. 2). Changes with mechanical ventilation in MEP amplitude recruitment curves were also statistically significant for both the costal and crural diaphragm (fixed group by intensity interaction effect, P = 0.005 and P = 0.03, Figs. 3 and 4) but not for the rectus abdominis (P = 0.57, Fig. 5). Post hoc tests showed that the MEP amplitude recruitment curves were significantly steeper during SBbaseline than during the second and third periods of mechanical ventilation, for both the costal and crural diaphragm. There was no significant change with mechanical ventilation in MEP100 latency of the costal diaphragm, crural diaphragm, and rectus abdominis (P = 0.17, P = 0.71, and P = 0.95, respectively).

Effect of mechanical ventilation on the diaphragm ICI/ICF curves (study 2). Study 2 was conducted in five healthy subjects. The crural electrophysiological signals were interpretable in four of them. The effect of mechanical ventilation on breathing pattern and PTPdia was similar to that observed in study 1 (Table 3). There was also a reduction with mechanical ventilation in the costal and crural diaphragm MEP100 amplitu-

Values are means ± SD. Pes, esophageal pressure; Pga, gastric pressure; Pdi, transdiaphragmatic pressure. There was no significant change in Pes, Pga, and Pdi with ventilation (ANOVA, P = 0.31, P = 0.08, and P = 0.19, respectively).
The motor cortex and mechanical ventilation (Table 4). The motor threshold did not significantly change with mechanical ventilation (Table 4). For both the costal and crural diaphragms, there was a significant inhibition by 40% of MEP at ISI of 3 ms ($P = 0.04$) and facilitation by 10.220.33.6 on November 5, 2016 http://jap.physiology.org/ Downloaded from

Fig. 2. Change with MV in supramaximal compound motor action potential (CMAP; open symbols) and MEP$_{100}$ (solid symbols) amplitude of the costal diaphragm (diamond), crural diaphragm (square), and rectus abdominis (triangle). Each point corresponds to mean size ($\pm$SE) of CMAP or MEP in 6 subjects. There was an increase in costal and crural CMAP amplitude, but this difference did not reach the level of significance ($P = 0.47$ and $P = 0.58$). ANOVA showed a significant change in MEP$_{100}$ amplitude of the costal diaphragm ($P = 0.007$), the crural diaphragm ($P = 0.0005$), but not the rectus abdominis ($P = 0.10$). SBbaseline, SB before MV; MV1, first level of MV ($V_T_{baseline}$); MV2, second level of MV ($V_T_{baseline} + 5$ ml/kg); MV3, third level of MV ($V_T_{baseline} + 10$ ml/kg); SBend, SB after MV.

Fig. 3. Relationships between transcranial magnetic stimulation (TMS) intensity and MEP amplitude of the costal diaphragm during the 5 different periods: baseline (•) and SBbaseline (■), MV with 0 ml/kg (●), MV with 5 ml/kg (▲), and MV with 10 ml/kg (○) above $V_T_{baseline}$. The different levels of $V_T$ were randomly applied. In each test, TMS intensity was randomly delivered. Each point corresponds to mean size ($\pm$SE) of MEP in 6 subjects. There was a significant change in the recruitment curve ($P = 0.005$), with its slope being significantly steeper during SBbaseline than during MV2 and MV3 ($V_T + 5$ ml/kg and $V_T + 10$ ml/kg, respectively).

Fig. 4. Change with MV in supramaximal compound motor action potential (CMAP; open symbols) and MEP$_{100}$ (solid symbols) amplitude of the costal diaphragm (diamond), crural diaphragm (square), and rectus abdominis (triangle). Each point corresponds to mean size ($\pm$SE) of CMAP or MEP in 6 subjects. There was an increase in costal and crural CMAP amplitude, but this difference did not reach the level of significance ($P = 0.47$ and $P = 0.58$). ANOVA showed a significant change in MEP$_{100}$ amplitude of the costal diaphragm ($P = 0.007$), the crural diaphragm ($P = 0.0005$), but not the rectus abdominis ($P = 0.10$). SBbaseline, SB before MV; MV1, first level of MV ($V_T_{baseline}$); MV2, second level of MV ($V_T_{baseline} + 5$ ml/kg); MV3, third level of MV ($V_T_{baseline} + 10$ ml/kg); SBend, SB after MV.

Fig. 5. Relationships between transcranial magnetic stimulation (TMS) intensity and MEP amplitude of the crural diaphragm during the 5 different periods: baseline (○) and SBbaseline (■), MV with 0 ml/kg (●), MV with 5 ml/kg (▲), and MV with 10 ml/kg (○) above $V_T_{baseline}$. The different levels of $V_T$ were randomly applied. In each test, TMS intensity was randomly delivered. Each point corresponds to mean size ($\pm$SE) of MEP in 6 subjects. There was a significant change in the recruitment curve ($P = 0.03$), with its slope being significantly steeper during SBbaseline than during MV2 and MV3 ($V_T + 5$ ml/kg and $V_T + 10$ ml/kg, respectively).

Fig. 5. Relationships between transcranial magnetic stimulation (TMS) intensity and MEP amplitude of the rectus abdominis during the 5 different periods: baseline (○) and SBbaseline (■), MV with 0 ml/kg (●), MV with 5 ml/kg (▲), and MV with 10 ml/kg (○) above $V_T_{baseline}$. The different levels of $V_T$ were randomly applied. In each test, TMS intensity was randomly delivered. Each point corresponds to mean size ($\pm$SE) of MEP in 6 subjects. There was no significant effect of MV on the rectus abdominis MEP amplitude recruitment curves ($P = 0.57$).
200% at 9 ms (P < 0.04), during spontaneous breathing. The costal MEP amplitude was higher during mechanical ventilation than during spontaneous breathing (Fig. 6), for ISIs longer than 9 ms (P = 0.007) rather than for ISIs shorter than 7 ms (P = 0.06). The crural MEP amplitude was higher during mechanical ventilation than during spontaneous breathing at both short and long ISI (P = 0.02 and P = 0.0005, respectively). The greatest facilitation was at an ISI of 9 ms (Table 4).

**Table 3. Mean values of the costal and crural diaphragm MEP amplitude elicited at 100% of stimulator output and second level of isocapnic noninvasive mechanical ventilation (study 2)**

<table>
<thead>
<tr>
<th></th>
<th>SB baseline</th>
<th>Vt + 5 ml/kg</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor threshold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragm, %</td>
<td>92±7</td>
<td>91±8</td>
<td>0.65</td>
</tr>
<tr>
<td>MEP_{100} amplitude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costal MEP_{100}, %</td>
<td>100±0.0</td>
<td>62.8±12.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Crural MEP_{100}, %</td>
<td>100±0.0</td>
<td>74.6±8.1</td>
<td></td>
</tr>
<tr>
<td>Costal MEP_{100}*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISI 3 ms, %TS</td>
<td>62.0±25.9</td>
<td>91.1±51.5</td>
<td>0.08</td>
</tr>
<tr>
<td>ISI 9 ms, %TS</td>
<td>193.5±58.4</td>
<td>378.0±139.8</td>
<td>0.04</td>
</tr>
<tr>
<td>ISI 15 ms, %TS</td>
<td>530.9±333.9</td>
<td>848.2±456.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Crural MEP_{100}*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISI 3 ms, %TS</td>
<td>60.3±20.4</td>
<td>87.7±34.2</td>
<td>0.05</td>
</tr>
<tr>
<td>ISI 9 ms, %TS</td>
<td>165.4±82.1</td>
<td>244.9±95.0</td>
<td>0.05</td>
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<tr>
<td>ISI 15 ms, %TS</td>
<td>415.7±211.0</td>
<td>548.9±213.2</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Values are means ± SD. MEP_{100}, motor-evoked potential (MEP) elicited by 100% stimulator output; TS, test stimulus; ISI, interstimulus interval. Crural electrophysiological signals were available in 4 subjects. *Comparisons were tested by Wilcoxon’s signed rank test.

**Table 4. Mean values of discomfort of breathing, P_{ETCO2}, RR, Vt, and PTPdia during spontaneous breathing and second level of isocapnic noninvasive mechanical ventilation (study 2)**

<table>
<thead>
<tr>
<th></th>
<th>SB baseline</th>
<th>Vt + 5 ml/kg</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discomfort of breathing</td>
<td>0.2±0.3</td>
<td>0.2±0.3</td>
<td></td>
</tr>
<tr>
<td>P_{ETCO2}, kPa</td>
<td>5.04±0.34</td>
<td>4.97±0.76</td>
<td></td>
</tr>
<tr>
<td>T/Tr, %</td>
<td>0.37±0.1</td>
<td>0.37±0.1</td>
<td></td>
</tr>
<tr>
<td>RR, cycles/min</td>
<td>13±1</td>
<td>14±3</td>
<td></td>
</tr>
<tr>
<td>Vt, ml</td>
<td>524±76</td>
<td>1170±217</td>
<td></td>
</tr>
<tr>
<td>PTPdia, cmH2O s^{-1}min^{-1}</td>
<td>150±50</td>
<td>64±13</td>
<td></td>
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</tbody>
</table>

Values are means ± SD. PTPdia significantly decreased with ventilation (ANOVA, P = 0.04).

**Fig. 6. Relationships between interstimulus interval and MEP amplitude of the costal diaphragm during SB baseline (■) and MV with 5 ml/kg (▲) above V_{baseline}.** In each test, interstimulus intervals were varied in random order. Each point corresponds to mean size (+SE) of normalized MEP (%) in 5 subjects. MV induced a significant change of the interstimulus interval curves, notably for interstimulus interval longer than 9 ms (P = 0.007).

**Discussion**

The main finding of this study is that mechanical ventilation is associated with both a reduction in the diaphragm MEP and an increase in the response to paired TMS at facilitatory ISIs. By contrast, a similar trend was not observed for the quadriceps, suggesting a specific effect on the diaphragm motor cortex. These phenomena demonstrate that, first, mechanical ventilation specifically downregulates the cortical excitability and output of the corticospinal projections to the respiratory muscles, and, second, that mechanical ventilation induces a compensatory increase in the excitability of facilitatory intracortical neurons that modulate the excitability of the respiratory cortical motoneurons.

Critique of the method. Our results raise several methodological issues. First, we used chest wall surface electrodes to record the costal diaphragm MEP, rather than a needle electrode, because of the risk of pneumothorax during mechanical ventilation. The risk of contamination of action potentials recorded by using surface electrodes was minimized as their position was consistent with that previously validated (40) and was optimized with phrenic stimulation. In addition, a diaphragm origin of the MEPs was supported by the use of esophageal electrode, which provided similar data as surface ones.

We acknowledge the concern that behavior might have contributed to the depression of the MEP as the subjects were coached to be relaxed and synchronized to the ventilator and blinding to the level of mechanical ventilation was not possible. In addition, subjects were members of laboratory staff, and most of them are coauthors of the paper. This is potentially a major concern as they might have exerted a voluntary control, influencing the response to single and paired TMS. We are not able to rule out this hypothesis but attempted to prevent biasing of the results by performing data analyses only after data
collection had been concluded. Therefore, each subject was not aware of the result found in the preceding subject and his or her own response. There are several additional arguments against the possibility of a behavior effect. First, one may argue that decrease in the diaphragm MEP amplitude to single TMS was foreseeable as mechanical ventilation reduces the EMGdia activity. Our subjects knew of this effect. However, we did not find a correlation between reduction in the diaphragm MEP amplitude and EMG activity (data not presented). Moreover, we believe that the diaphragm responses to paired TMS were not foreseeable, and various scenarios could have been preconceived with an equal plausibility. This study was the sequel of a previous one in which our laboratory confirmed the existence of a neuromechanical feedback inhibiting the respiratory center output (30). Thus we hypothesized that this neuromechanical feedback might influence the motor cortex supplying the diaphragm, but we did not know how. Second, focusing attention on breathing should have induced an increase in amplitude of the diaphragm response to single TMS, as it has been shown that thinking about a movement alone facilitates MEP amplitude (16). We found the opposite result. Third, it has also been shown that volitional inhibition of movements enhances the intracortical inhibition rather than facilitation (35). We found the opposite results. Therefore, we doubt that voluntary suppression of diaphragm activity would result in the observed data.

More importantly, MEP facilitation is known to occur during and after muscular contraction (12). Thus, if at end-expiratory time the diaphragm was more active and, accordingly, the diaphragm MEP more facilitated during spontaneous breathing than during mechanical ventilation, then this might explain the MEP amplitude reduction during mechanical ventilation. However, there are some arguments against this possibility. The end-expiratory Pes, Pga, and Pdi tended to be greater during the second and third levels of mechanical ventilation than during spontaneous breathing, indicating that, in term of pressure, the diaphragm was not more facilitated at baseline. In addition, the MEP latency and motor threshold remained constant throughout the present study, whereas they are both known to be reduced by voluntary facilitation (31, 33). In addition, from our laboratory’s previous study (31), we can infer that, to achieve the 50% “reduction” from spontaneous breathing to the second level of mechanical ventilation, there would need to be an end-expiratory Pdi >20% maximum Pdi during spontaneous breathing, which we should certainly have detected. Finally, it has previously been shown in various skeletal muscles that a voluntary contraction (of ~5–10% of maximum) induces a marked reduction in ICI and abolition of ICF (1). We found that, during spontaneous breathing, the diaphragm MEP was significantly inhibited and facilitated at short and long ISIs, respectively. This supports the premise that the diaphragm was not facilitated at end expiration. An alternative hypothesis, therefore, is that the amplitude of MEP elicited at end-expiratory time was decreased because mechanical ventilation had reduced diaphragm activation during the inspiratory phase. The neurophysiological mechanisms linking reduction in inspiratory effort and expiratory MEP amplitude are now addressed.

Significance of findings. The reduction in the diaphragm MEP amplitude during mechanical ventilation could be related to either peripheral or central mechanisms. A peripheral mecha

We are not able to rule out inhibitory mechanisms at the spinal level. In contrast to other nerves, it is impossible to assess motoneurone excitability by analyzing H-reflex and F-waves for the phrenic nerve. Although a diaphragm response to magnetic stimulation at the brain stem level has previously been demonstrated (39), we and others (T. Similowski, personal communication) have been unable to elicit this response; nevertheless, this perhaps represents a future direction for the study of the effects of mechanical ventilation.

The respiratory muscles are controlled via bulbospinal projections from the pontomedullary respiratory centers and also by motor cortical areas via the corticospinal pathways (13). Whether stimulation of the motor cortex acts via the brain stem respiratory centers remains unresolved, and our results do not allow us to draw a clear conclusion. Nevertheless the short latencies observed would be consistent with an oligosynaptic corticospinal pathway, bypassing medullary centers. This hypothesis is supported by data from Corfield et al. (7), who found that diaphragm MEP amplitude is not altered during hypocapnia, when the respiratory “oscillator” is presumed to be silent.

Our finding of a mechanical ventilation-related increase in MEP amplitude at facilitatory ISIs supports a cortical origin of the diaphragm MEP depression. It is well established that the paradigm of paired TMS that we used enables investigation of intracortical inhibitory and facilitatory circuits that modulate excitability of cortical motoneurons. Our findings indicate that mechanical ventilation made the motoneurons of the primary motor cortex more sensitive to facilitatory ISIs. Additional arguments support our hypothesis of a cortical mechanism. First, in addition to the motor cortex, imaging studies using positron emission tomography and functional magnetic resonance have demonstrated that various supratentorial brain regions are involved in breathing control, including the supplementary motor area, premotor area, sensorimotor cortex, striatum, and limbic system (6, 9, 26). Interestingly, it has been reported that these accessory regions are less active during passive mechanical ventilation with high V̇t (1.3 liters) than during active breathing (9). We can speculate that MEP amplitude depression was possibly related to the inactivation of these accessory areas. Second, the effect of sensory input on motor cortex excitability has been investigated in various skeletal muscle limbs. Muscle vibration augments MEP amplitude in the vibrated muscle (5, 17) but depresses it in ipsilateral and contralateral antagonist muscle (17, 32). Sensory electrical stimulation of the median nerve results in MEP inhibition, as does electrical cutaneous stimulation (36, 38). Sensory deafferentation is also associated with an increase of motor cortex excitability (3). It is believed that MEP amplitude modulation by sensory afferents takes place at the cortical levels and involves GABAAergic intracortical inhibitory circuits. Similarly, it has previously been shown that, independent of arterial Pco2 levels, mechanical hyperventilation inhibits the neural drive to the diaphragm, whether measured by PTPdia or EMGdia (11, 34). Such a phenomenon was observed in the present study. This inhibition is mediated by the thoracic mechanoreceptors via the vagal and other proprioceptive af-
Mechanical ventilation has modulated the amplitude of MEPs. This modulation is likely mediated by a common mechanism. In fact, these phenomena related depression of the response to single TMS and enhanced facilitation of the response of the diaphragm to transcranial magnetic stimulation. Our data show that mechanical ventilation can modulate the excitability of intracortical inhibitory and facilitatory neurons, suggesting that the diaphragm may also have clinical implications for the understanding of patient-ventilator interaction.

REFERENCES