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Influence of acute inspiratory loading upon diaphragm motor-evoked potentials in healthy humans

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Ross EZ, Nowicky AV, McConnell AK. Influence of acute inspiratory loading upon diaphragm motor-evoked potentials in healthy humans. *J Appl Physiol* 102: 1883–1890, 2007. First published January 18, 2007; doi:10.1152/japplphysiol.00694.2006.—Acute prior activity of the inspiratory muscles can enhance inspiratory muscle strength and reduce effort perception during subsequent inspiratory efforts. However, the mechanisms subserving these changes are poorly understood. Responses to magnetic stimulation in 10 subjects were studied after an acute bout of nonfatiguing inspiratory muscle loading (IML), corresponding to 40% of subjects' initial maximal inspiratory pressure (MIP), and after an acute bout of nonloaded, forced inspiration (NLF). Motor-evoked potentials elicited by cortical stimulation (MEP_c) and by phrenic nerve stimulation (MEP_p) were recorded transcutaneously from the diaphragm before, immediately after, and 15 min after two sets of 30 inspiratory efforts, at rest and during an MIP effort. After IML, MIP increased to 113 ± 3% (SE) of baseline and diaphragm MEP_p (during MIP) significantly increased (129 ± 10% of baseline). Diaphragmatic MEP_c (during MIP), expressed as a percentage of maximal MEP_p, decreased after IML (from 29 ± 9% to 20 ± 6%; $P = 0.017$) and after NLF (from 43 ± 5% to 31 ± 5%; $P = 0.032$). Observations from the biceps brachi demonstrated that changes after IML and NLF were specific to the inspiratory muscle, since no significant changes were observed in biceps force generation or in MEP_p or MEP_c amplitudes. These data indicate that after IML increased global inspiratory strength is accompanied by increased peripheral excitability and by a dampening of corticospinal excitability of the diaphragm.

transcranial magnetic stimulation; inspiratory muscles; end-tidal carbon dioxide

THE CONTRACTILE RESPONSE OF muscle is influenced by its activation history. A short bout of submaximal muscle activation can result in an enhanced contractile response (postactivation potentiation), whereas a prolonged period of activation results in an impaired or attenuated contractile response (fatigue) (31). Although fatigue and potentiation have opposing effects, the performance of a muscle after contractile activity reflects the net balance between the processes that serve to enhance force development and those that diminish it (15).

It is generally agreed that fatigue causes depression of motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation [cortical motor-evoked potential (MEP_c)], termed postexercise depression (1, 3, 11, 27, 43). However, after shorter periods of nonfatiguing effort, facilitation of the MEP_c response has been observed (14, 21). The facilitating effect of a sustained voluntary contraction has been found to last beyond the cessation of contraction (35) and has been termed postexercise facilitation. The cause and origin of these activity-dependent phenomena are still widely debated (19, 21, 30, 35).

Acute prior activity of the inspiratory muscles has been shown to enhance inspiratory muscle strength (46) and athletic performance (45, 47) and to reduce the effort perception of exercise hyperpnea (45) and magnitude estimation of inspiratory loads (33). The mechanisms subserving these changes are poorly understood. It is clear that activity-dependent changes occur in the excitability of human motoneurons after exercise or repeated muscular contraction; accordingly, the purpose of the present study was to examine central and peripheral changes that occur in response to acute, nonfatiguing inspiratory muscle loading (IML) with the use of evoked responses to magnetic stimulation of the phrenic nerve and the motor cortex.

The present study was designed to test the hypothesis that an acute bout of submaximal, nonfatiguing loaded inspirations would induce changes in peripheral and central excitability of the diaphragm. We also examined whether these changes occurred during voluntary activation, as well as during relaxation. Furthermore, we assessed whether any changes were due to the imposition of a load or to the act of deep, voluntary breathing by repeating the protocol without an added load (10, 18, 20).

METHODS

Participants

Ten healthy participants (6 men), free from any disease or relevant medical history, volunteered to take part in the study [age 24 ± 3 yr, stature 173 ± 10 cm, body mass 74 ± 18 kg; maximal inspiratory mouth pressure (MIP) 124 ± 22 cmH_2O]. The four female participants used oral contraceptives, which attempted to minimize the previously observed effects of changing ovarian steroid levels during various stages of the menstrual cycle on cortical excitability (37, 38, 48). Written, informed consent was obtained from each subject, and the Institutional Ethics Board of Brunel University approved all procedures. The study conformed to the standards set by the Declaration of Helsinki.

Procedures

Maximal inspiratory mouth pressure. Inspiratory muscle strength was assessed by a surrogate measure, MIP, during a quasi-static effort commencing at residual volume (RV). Initial MIP was measured with a mouth pressure meter (Precision Medical, Pickering, North Yorks, UK).

RV was verified at every MIP measurement by asking subjects to exhale from total lung capacity via a Microloop spirometer (Micro

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Medical, Kent, UK). This ensured consistency of achieving RV over the course of the whole experiment. Subjects were then encouraged verbally to make a maximal inspiratory effort for 2–3 s. Because all subsequent data were collected from the subjects in a seated upright position, this initial MIP measurement was also performed in this position. A minimum of 5 and a maximum of 10 technically satisfactory measurements were conducted, and the highest of three measurements with <5% variability or within 5 cmH_2O difference was defined as maximum (49). This initial MIP value was used to calculate the magnitude of the inspiratory load to be used in the acute inspiratory loading bout.

Throughout the subsequent experimental protocol, MIP was measured using a system that incorporated a manually operated three-way stopcock with a flanged mouthpiece (Hans Rudolph, Kansas City, MO). The mouthpiece also contained a small 1-mm leak to avoid glottis closure and was attached via 1 m of 2-mm internal diameter polyethylene tubing to a Validyne DP15 differential pressure transducer (±230 cmH_2O) (Validyne, Northridge, CA) and a Validyne CD15 carrier demodulator. This signal was digitized with an analog-to-digital converter (micro1401; Cambridge Electronic Design, Cambridge, UK) and acquired by commercially available software (Spike 2, Cambridge Electronic Design) on a personal computer (Packard Bell, NEC Computers, Angers, France). Real-time mouth pressure traces were displayed to the subject.

Evoked potential responses to magnetic stimulation. Diaphragm EMG activity in response to magnetic stimulation was recorded transcutaneously from pairs of bipolar differential electrodes (Goldy Karaya gel electrodes, 28 mm diameter, silver/silver chloride, Arbo; Henley Medical, Stevenage, UK) placed on cleaned, abraded skin. The electrodes were placed in the lowest intercostal spaces on the right side of the body, at the midclavicular line. A ground electrode was placed on the sternum. For the biceps brachii, electrodes were placed on the right arm over the belly of the muscle. To ensure that electrodes were placed in exactly the same position on repeat visits to the laboratory, the positions of the electrodes in relation to prominent anatomic landmarks, e.g., the umbilicus and inferior border of the sternum, were traced onto clear acetate for each individual.

The evoked potentials have been termed MEP_p when stimulated via the phrenic nerve or, in the case of the biceps at Erb's point and MEP_c when stimulated transcranially. To eliminate the influence of ECG, only those responses with a constant shape and a stable baseline before and after stimulation were accepted to be included in the averaging procedure (24).

The EMG responses were amplified (gain × 3,000) (1902; Cambridge Electronic Design), band-pass filtered between 20 Hz and 5 kHz, digitized at a sampling rate of 4 kHz using an analog-to-digital converter (micro1401; Cambridge Electronic Design), and finally acquired and later analyzed with commercially available software (Spike 2 v4.11; Cambridge Electronic Design). Peak-to-peak amplitude of the averaged evoked response was measured.

Peripheral magnetic stimulation. To achieve peripheral stimulation of the diaphragm, unilateral, magnetic stimulation of the phrenic nerve was performed with a Magstim 200 stimulator equipped with a 50-mm figure of eight coil (Magstim, Whitland, Wales, UK) capable of a maximum output of 2.2 Tesla. The coil was positioned at the posterior border of the sternomastoid muscle at the level of the cricoid cartilage (29).

The optimal stimulation position was determined during relaxation by delivering a number of single stimuli at a submaximal, constant stimulation intensity. The optimal coil position was determined as the site that elicited the largest and "clearest" MEP_p in the diaphragm. When the optimal position of the coil was located, the position was marked with indelible ink to ensure consistent coil placement, and this location was used for all subsequent stimulations.

Supramaximal diaphragm and biceps activation (demonstrated by recruitment curves comparing the amplitude of the MEP_p obtained with stimulations at 50, 60, 70, 80, 90, and 100% of the maximal power output of the stimulator) was achieved in all subjects. All stimulations were subsequently performed with the output of the stimulator at 100% of its maximal possible intensity.

Single stimuli were delivered with the neck and head in a neutral position. Stimuli were delivered during relaxed sitting at functional residual capacity and during a maximal inspiratory effort from RV against an occluded airway. An average of six stimuli were delivered during the relaxed condition, and a single stimulus was delivered during each of the two maximal inspiratory efforts.

Peripheral stimulation of the biceps brachii was achieved by delivering stimuli at Erb's point. Six stimuli were delivered with the biceps relaxed, and two stimuli were delivered with the subject performing an isometric maximal voluntary contraction (MVC). All stimuli were delivered at 100% of the stimulator's power output.

Transcranial magnetic stimulation. Transcranial magnetic stimulation (TMS) of the diaphragm and biceps brachii was carried out with a Magstim 200 monopulse magnetic stimulator and a 70-mm figure of eight coil (Magstim), with a maximum output of 2 Tesla. Before the experimental protocol, a mapping procedure was carried out to establish the optimal cortical site for activation of the inspiratory muscles. The optimal coil position was determined as the site that elicited the largest and clearest MEP_c in the diaphragm in response to TMS at 100% stimulator output. This position was marked with indelible ink to ensure reproducibility of the stimulation conditions for that individual during the experimental protocol. The mapping procedure revealed a mean diaphragmatic "hot spot" at coordinates 3.5 cm lateral to and 1.8 cm anterior to the vertex.

Before the experimental protocol, motor threshold for the diaphragm was identified by constructing a recruitment curve for each individual. The method used to establish threshold was that the stimulator output was increased from 40% by 5% steps until a diaphragmatic response was visible, the criteria for this being that the evoked response was present in less than one-half of eight stimuli (36). To assess the diaphragm MEP_c recruitment curve, stimulator output was then increased by 10% of threshold up to 100% of stimulator output. Eight successive stimuli were delivered at each level of stimulation intensity. To standardize the intensity of TMS between subjects, during the experimental protocol, stimulations were delivered at 1.2 × motor threshold for the diaphragm.

The same mapping procedure used for the diaphragmatic response was also carried out to find the hot spot for the biceps brachii. Because in all subjects this hot spot was located very near the diaphragm site, the diaphragm site was used to elicit MEPs from all target muscles during the experiment. This reduced the number of stimuli delivered via TMS compared with using two separate sites for the inspiratory muscle and biceps and thus reduced the time from the end of the breathing efforts to complete data collection.

IML

A pressure threshold inspiratory loading device (POWERbreathe; Gaiam, Warwickshire, UK) was used to provide the acute bout of IML. The intensity and duration of the acute bout were based on the protocol used by Volianitis et al. (46, 47), in which two sets of 30 breaths at an intensity of 40% of the MIP measured before the experimental procedure are performed. The subjects were instructed to perform dynamic inspiratory maneuvers starting near RV and terminating toward total lung capacity for each breath. Subjects were given no further specific instructions related to breathing pattern during the IML; however, because of the increased tidal volume, breathing frequency was lower than that at rest, a pattern spontaneously adopted by the subjects in an attempt to offset the effects of hypocapnia. To this end, a low duty cycle resulted, which also helped to ensure the acute bout was nonfatiguing.

Assessment of a Nonrespiratory Muscle

To assess the responses of a nonrespiratory muscle to the acute bout of IML, data were also collected from the biceps of the right arm with the subject seated relaxed and during an isometric MVC. This MVC involved the subjects seated with their elbows flexed at 115° and forearms supported on a flat surface. Subjects held a T-bar in their hand and pulled upward against a fixed platform. Isometric elbow flexor force was measured with a force transducer (load cell) (Globus Italia, Codogne, Italy) connected to an external unit composed of a microprocessor and LCD feedback display. TMS and phrenic nerve magnetic stimulation were also delivered at the same time points as outlined for the diaphragm to the relaxed and maximally contracted biceps brachii muscle.

Experimental Procedure

Before the main experimental procedure, subjects performed initial tests of MIP. Subjects also attended at least one further session to individually calibrate the pressure threshold device according to their MIP and to perform the TMS mapping procedure.

The main experimental procedure is shown in Fig. 1. Subjects attended the laboratory on two occasions. The main experimental procedure was the same over the two sessions, but the method of stimulation was changed (phrenic nerve magnetic stimulation or TMS). Before the breathing sets (baseline), immediately after *set 1*, immediately after *set 2*, and 15 min after the breathing task, experimental data were collected. Data collection immediately after the breathing sets was initiated within 20 s of completion of the set, and, during the 15-min recovery, subjects sat relaxed and refrained from talking. During the breathing task, end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) was measured breath by breath with an automated on-line metabolic system (Oxycon Pro; Jaeger, Hoechberg, Germany), which was connected to the expiratory port of the pressure threshold device. The CO_2 analyzer is a high-speed device based on the infrared absorption principle (range, 0–15%; accuracy, 0.05%; resolution, 0.01%; stability, 0.02%/h; minimum rise time $T_{10-90\%}$ 40 ms). At each of the data collection points, maximal inspiratory efforts of 2- to 3-s duration were performed two times, with a 30-s break between each. MIP data represent the highest values recorded in these efforts and are presented as a mean of the highest measurement from each of the two times that the protocol was repeated. At least 24 h separated each experimental testing session.

In a subgroup of eight subjects, the experimental paradigm was repeated, but the acute bout of loaded inspirations was replaced by an acute bout of nonloaded but forced inspirations. Subjects breathed through a flanged mouthpiece attached to plastic casing with no internal structures to induce a resistance. Subjects performed two sets of 30 forced, dynamic maneuvers starting at RV and terminating at total lung capacity. The purpose of this additional study was to address whether our main observations were due to the imposition of a load or to the act of deep, voluntary breathing.

In addition, in six subjects from the original group, a "sham" protocol was performed. The purpose of this was to provide confirmation that the observations of the main study were not simply a learning effect of test maneuvers but were effects of the acute bout of inspiratory loading. For this protocol, the experimental paradigm remained the same; however, instead of performing loaded inspiratory efforts, subjects sat relaxed and breathed through a flanged mouthpiece with no resistance. Data were collected at precisely the same time points as in the first protocol.

Data Analyses

For stimuli delivered to the relaxed muscle, the average MEP response of eight transcranial stimuli or six phrenic nerve stimuli was analyzed. For stimuli delivered during maximal maneuvers, the average MEP of two magnetic stimuli, delivered when subjects reached peak inspiratory pressure, or peak force, was used for analyses of both centrally and peripherally evoked responses.

The MEP_c amplitude is expressed as a percentage of the maximal MEP_p (MEP_pmax). To account for activity-dependent changes in muscle fiber action potentials, the peak-to-peak amplitude of each MEP_c elicited at rest or during a maximal inspiratory effort was normalized to the mean amplitude of the MEP_pmax elicited at rest or during maximal inspiratory efforts, respectively (e.g., Refs. 12, 40, 42).

MIP and evoked response amplitude results were analyzed by one-way repeated-measures ANOVA and Bonferonni post hoc test to assess the differences between the baseline and those after *set 1*, *set 2*, and 15-min values in both the loaded and nonloaded breathing tasks. A one-way ANOVA with a within-group comparison was used to

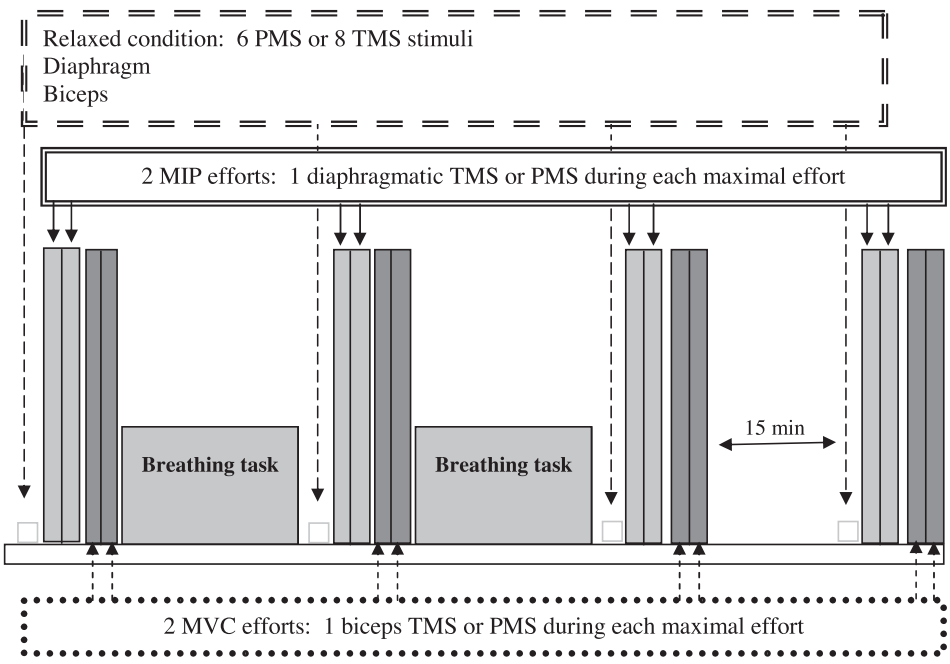

Fig. 1. Protocol design. Subjects attended the laboratory on 2 occasions. The main experimental procedure was the same over the 2 sessions, but the method of stimulation was changed [phrenic nerve magnetic stimulation (PMS) and transcranial magnetic stimulation (TMS)]. The breathing task comprised 2 × 30 loaded inspirations. MIP, maximal inspiratory mouth pressure; MVC, maximal voluntary contraction.

compare the percent change from baseline for subjects who participated in both the experimental and sham trials. Values of $P < 0.05$ were considered statistically significant. To prevent strong and important effects from being reported as nonsignificant (22), effect size was also calculated. The effect size is the degree of association between an effect and the dependant variable and is expressed here as partial eta squared (η_p^2) (8). Values of η_p^2 over 0.138 are considered a large effect size (6).

Data points are expressed as percentage of baseline, with baseline being 100%, and are means ± SE. All statistical analyses were performed with SPSS v11.5 for Windows (SPSS, Chicago, IL).

RESULTS

Acute Inspiratory Efforts

For the breathing tasks, mean time taken to perform 30 breaths was 168 ± 28 s for the loaded inspirations and 161 ± 34 s for the forced, nonloaded inspirations. Respiratory frequency was reduced during the IML compared with resting tidal breathing (9.3 ± 1.6 compared with 13.4 cycles/min; $P < 0.001$) but was increased during the nonloaded, forced breathing protocol (19.2 ± 1.3 cycles/min; $P < 0.001$). Minute ventilation was elevated during both the loaded and nonloaded acute bouts of breathing efforts compared with resting tidal breathing (43.1 ± 5 and 56.2 ± 9 l/min compared with 11.6 ± 8 l/min, respectively; $P < 0.001$).

P_{ETCO_2}

P_{ETCO_2} fell significantly from baseline values during the loaded inspiratory task (from 38.6 ± 5.8 to 22.4 ± 1.3 Torr; $P < 0.01$) and during the nonloaded forced inspiratory task (from 39.3 ± 6.1 to 18.9 ± 1.3 Torr; $P = 0.001$). There was a significant difference in the fall in P_{ETCO_2} between the breathing tasks ($P = 0.03$).

MIP

After the acute bout of IML, MIP was 112 ± 4% ($P = 0.007$) of baseline after the first set of loaded breathing and 113 ± 3% ($P = 0.0001$) of baseline after the second set. Values returned to near baseline levels after 15 min of relaxed recovery (105 ± 3%) (Fig. 2). MIP also increased significantly above baseline after the first set of nonloaded, forced inspiration (NLF; 109 ± 2% of baseline; $P = 0.007$), but there were no further changes after the second set or after 15-min recovery (107 ± 3%, $P = 0.442$ and 107 ± 3%, $P = 0.242$, respectively). In the sham trial, no significant changes were observed in MIP throughout the protocol.

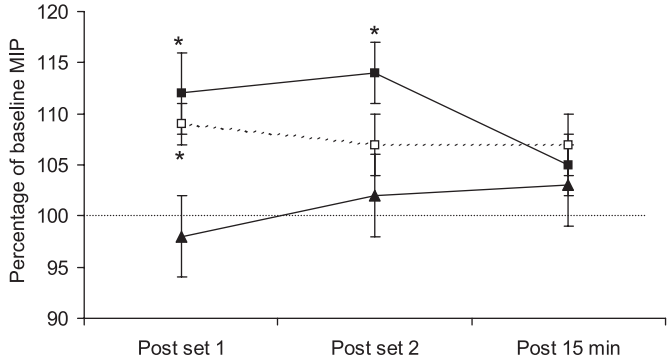

Fig. 2. MIP, expressed as a percentage of baseline values, after an acute bout of inspiratory muscle loading (■), nonloaded forced inspirations (□), or quiet breathing (▲). The horizontal line marks baseline at 100%. *$P < 0.01$.

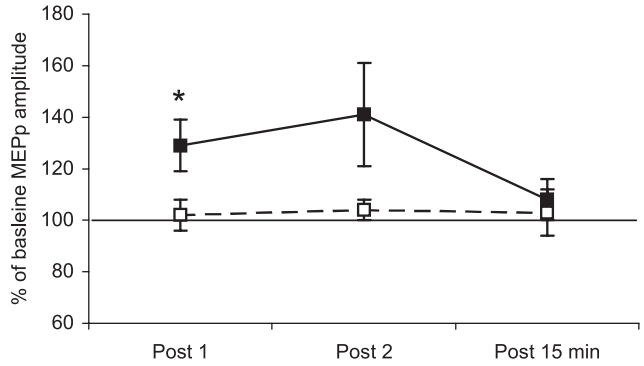

Fig. 3. Changes in amplitude of diaphragm peripherally evoked motor-evoked potential (MEP_P), at rest (□) and during maximal inspiratory efforts (■), following acute bout of inspiratory loading. Data are presented as a percentage of baseline values. The horizontal line represents baseline at 100%.

Responses to Phrenic Nerve Stimulation

After IML, no significant changes were observed in peak-to-peak amplitude of relaxed diaphragm MEP_P (1.94 ± 0.82 m at baseline). MEP_P amplitudes did increase significantly during the maximal inspiratory effort after the first set of loaded breathing in the diaphragm (from 1.19 ± 0.47 mV at baseline to 1.48 ± 0.56 mV after *set 1*; $P = 0.016$). This increase was maintained after the second set of loaded inhalation, and, although these results did not reach significance, the η_p^2 measures for effect size were large for this change (0.734). Figure 3 depicts changes in the MEP_P amplitude data for the main inspiratory loading protocol, and Fig. 4 shows a representative MEP_P trace elicited during a maximal inspiratory effort pre- and post-IML.

After the NLF breathing task, a significant increase in MEP_P amplitude of the relaxed diaphragm was observed at 15 min after cessation of the breathing task (122 ± 17%; $P = 0.0001$; effect size of 0.944) (Table 1). No significant changes were observed in MEP_P amplitude of the diaphragm during the MIP effort after NLF. Furthermore, no significant changes were observed in the peak-to-peak amplitude of the diaphragmatic MEP_P after the sham trial, both in the relaxed condition and during MIP efforts.

Responses to TMS

A diaphragm and biceps brachi response to TMS was observed in all subjects. Diaphragmatic MEP_C amplitude (expressed at $\%MEP_P max$) significantly decreased during maximal inspiration after the first set of breaths in the IML protocol [from 29 ± 9% (1.22 ± 0.52 mV) at baseline to 20 ± 6% (1.14 ± 0.55 mV) after breathing *set 1*; $P = 0.017$] (Table 1). Figure 4 shows a representative MEP_C trace elicited during a maximal inspiratory effort pre- and post-IML.

A significantly decreased MEP_C amplitude was observed in the relaxed diaphragm (from 26 ± 9% at baseline to 16 ± 6% after breathing *set 1*; $P = 0.006$) and during MIP efforts (from 43 ± 5% at baseline to 31 ± 5% after breathing *set 1*; $P = 0.032$) after the first set of NLF (Table 1). No significant changes in MEP_C amplitude were observed throughout the sham protocol in the relaxed diaphragm or during MIP efforts.

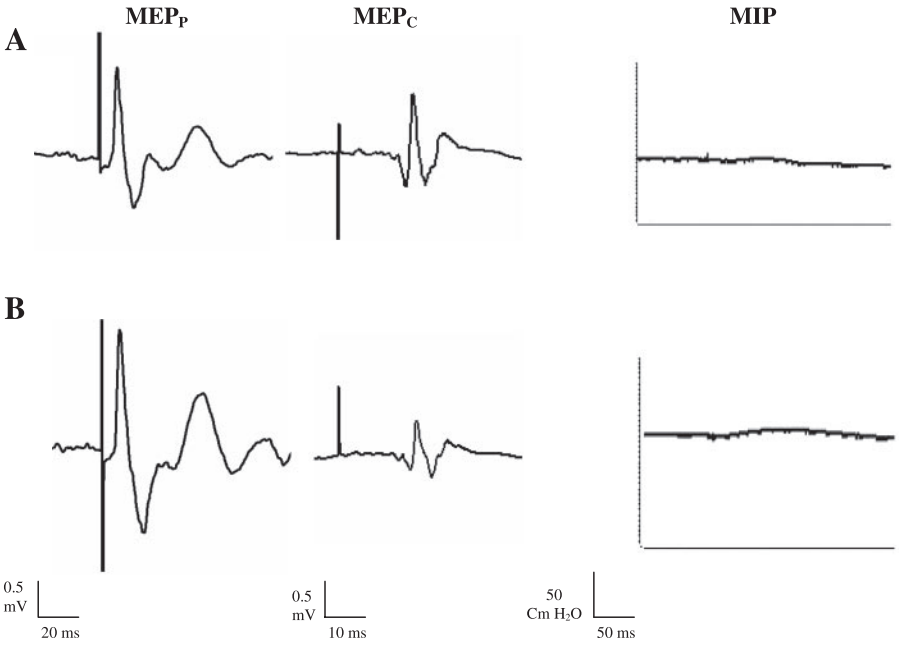

Fig. 4. MEP_p and cortical motor-evoked potential (MEP_c) diaphragmatic responses and MIP generation at baseline (*A*) and after inspiratory muscle loading protocol (*B*) in 1 subject.

Assessment of a Nonrespiratory Muscle (the Biceps Brachi)

No significant changes were observed in the relaxed or maximally contracted bicep MEP peak-to-peak amplitude after the IML (Table 2).

DISCUSSION

Main Findings

Our data suggest that the increase in the pressure-generating capacity of the inspiratory muscles that occurs after an acute bout of nonfatiguing, submaximal inspiratory loading is attributable to both central and peripheral neurophysiological processes. The return to baseline values of the variables after 15-min recovery suggests that the changes exhibited are transient in nature. Although MEP_p responses were increased, some centrally evoked responses showed a depression in amplitude.

Methodological Issues

When interpreting changes in electromyographic and evoked responses, it is important that no significant methodological changes have occurred. In the present study, maintaining a constant relationship between the stimulating coil and the stimulating site throughout the experiments was achieved by the use of indelible ink marks on the scalp, neck, and coil. Furthermore, measures were taken to ensure consistency of surface electrode placement for each subject throughout the experiments (see METHODS). Because both peripheral magnetic stimulation and TMS are incapable of selectively activating a given muscle individually, coactivation of several muscles is inevitable with this technique. To limit signal contamination, electrodes were placed in a way that has been shown to minimize recording muscle cross talk (7, 13, 25, 44). By taking these precautions, we are confident that variations in stimulation or recording technique did not play a significant role in the changes that we observed.

Although the central and peripheral stimulation sites allow investigation of the entire brain-muscle pathway, encompassing cortical and spinal motoneurons, the comparison is limited in its ability to clearly define spinal vs. cortical changes. It is therefore difficult to speculate regarding distinct changes in the spinal circuitry and the supraspinal pathways, since the two stimulation sites do not distinguish between these. However, by examining the differences in MEP_p and MEP_c responses

Table 1. *Changes in MEP_p amplitude, expressed as a percentage of baseline (with baseline being 100%), and MEP_c amplitude, expressed as a percentage of maximal MEP_p, after the first (post-set 1) and second (post-set 2) set of inspiratory efforts and after 15 min of recovery*

	Inspiratory Muscle Loading				Nonloaded Forced			
Variable	Baseline	*Post-set 1*	*Post-set 2*	After 15-min Recovery	Baseline	*Post-set 1*	*Post-set 2*	After 15-min Recovery
Di MEP_p RLX		102±6	104±4	101±9		103±9	108±10	122±17*
Di MEP_p MIP		129±10*	141±20	108±8		118±23	121±14	126±23
Di MEP_c RLX	17±4	20±7	14±3	24±10	26±9	16±6*	20±8	27±9
Di MEP_c MIP	29±9	20±6*	25±9	27±8	43±5	31±5†	39±6	44±5

Values are means ± SE. Di, diaphragm; MEP_p, motor-evoked potential in response to phrenic nerve magnetic stimulation; MEP_c, motor-evoked potential in response to transcranial magnetic stimulation; RLX, relaxed condition; MIP; maximal inspiratory mouth pressure effort. Significantly different from baseline: *$P < 0.05$ and †$P < 0.001$.

Table 2. *Changes in bicep MVC and amplitude of evoked responses after 2 sets of inspiratory muscle loading*

	Baseline	*Post-set 1*	*Post-set 2*	After 15-min Recovery
MVC force		101±3	96±4	102±6
MEP_p RLX		104±10	111±11	92±14
MEP_p MVC		111±13	101±12	90±14
MEP_c RLX	42±10	38±6	53±12	58±13
MEP_c MVC	55±11	53±12	59±14	68±14

Values are means ± SE. MEP_p are expressed as percentage of baseline (with baseline as 100%), and MEP_c are expressed as a percentage of maximal MEP_p elicited during contraction of the same strength. MVC, maximal voluntary contraction.

observed in the present study, we are able to distinguish between changes manifested at the peripheral level and those occurring "upstream" at a central level after the acute bout of inspiratory efforts.

The MEP_c elicited at rest or during maximal inspiratory efforts was normalized to the MEP_pmax elicited at baseline with the muscle in the same state, to account for activity-dependent changes in muscle fiber action potentials (12, 40, 42). However, normalizing MEP_c to baseline MEP_pmax does not account for the peripheral changes that occur because of the respiratory intervention. Only central or peripheral stimulation was used throughout the experimental protocol on any one day. Therefore, the inherent day-to-day variability of EMG (which has been found to be higher than within-day variability) (50) meant that normalization of MEP_c amplitude after the intervention to MEP_pmax measured after the same intervention on another day was not performed. As a consequence, the observed reduction in MEP_c after IML may have been underestimated, since the MEP_p amplitude was seen to increase after the breathing task.

Although the submaximal load and volume of the acute bout used in the present study was selected to avoid fatigue, fatigue has been reported to occur after submaximal voluntary contractions (32) and can persist in the absence of gross metabolic or electrical disturbance to the muscle (16). It is therefore recognized that without measures of the effects of phrenic stimulation on transdiaphragmatic pressure it cannot be assumed that inspiratory muscle fatigue did not occur. It is recognized that, after an acute bout of muscle and motor unit activation, potentiation and fatigue of the neuromuscular apparatus may simultaneously occur (5, 31). Thus in vivo performance is a measurement of the interaction between these two variables at any one time (5).

We chose to control for the effects of hypocapnia by assessing a nonrespiratory muscle, rather than by trying to maintain isocapnia. Maintaining true isocapnia is very difficult because what is measured at the mouth, as $P_{ET_{CO_2}}$, is not representative of what is happening at the tissue milieu. Because we observed no significant changes in amplitude of either MEP_p or MEP_c of the biceps brachi after the breathing tasks (IML or NLF), we can assume that changes that we observed in the inspiratory muscles were due to specific neuromuscular mechanisms acting on the target muscle, i.e., the diaphragm, and were not an artifact of increased global excitability due to the resultant hypocapnia. However, it should be noted that the neural pathways to the diaphragm and to the biceps brachii may be differentially affected by changes in arterial P_{CO_2}. Straus et al. (39) showed that acute hypercapnia depressed the peripheral conduction to a hand muscle, whereas it had no effects on phrenic nerve conduction. Similarly, Mador et al. (26) found that acute moderate hypercapnia mildly depressed limb contractility, whereas it did not produce any significant changes in diaphragm contractility.

Although hypocapnia was induced in all subjects during the loaded breathing tasks, the greatest fall in $P_{ET_{CO_2}}$ was experienced during the NLF experiment. The subsequent depression of MEP_c amplitude observed in the relaxed diaphragm after this task, but not in the relaxed diaphragm after the loaded breathing protocol, may represent motor cortical excitation being integrated at, and relayed by, brain stem respiratory neurons in response to the greater hypocapnia elicited by the NLF.

Finally, because no significant changes were observed in MIP during the sham trial, the suggested learning effect that improves the performance of voluntary maneuvers (23, 27) was apparently overcome by an adequate familiarization procedure.

Peripheral Excitability Changes After an Acute Bout of Inspiratory Effort

No significant changes were seen in the MEP_p elicited in the relaxed diaphragm, but there were significant changes induced by the inspiratory efforts when the MEP_p was evoked during maximal activation. This suggests that the effectiveness of electrical propagation across the neuromuscular junction and along the muscle surface membrane during voluntary contraction, as assessed by the amplitude of the superimposed MEP_p during MIP (2), is enhanced by the IML.

The increased pressure generation of the inspiratory muscles may reflect an increase in the number of motor units that were activated during a maximal effort after IML. The concept that initial efforts of maximal inspiratory activation may be submaximal has been confirmed by McKenzie et al. (28). These authors found that, during a 20-min inspiratory resistive loading task, voluntary activation of the diaphragm was submaximal (94.7%) at the beginning of the task, as assessed by phrenic nerve stimulation and twitch interpolation, but then became maximal after the first minute of loaded breathing and remained near maximal throughout the trial. MIP measurements were also found to increase significantly from the beginning to the end of the loaded task. The acute bout of inspiratory efforts performed in the present study may have allowed more full activation of the diaphragm during subsequent maximal efforts.

Corticospinal Excitability Changes After an Acute Bout of Inspiratory Effort

Subsequent to both the IML and NLF inspirations, a depression in the amplitude of the MEP_c was observed in the diaphragm during a maximal inspiratory effort. Normalization of the MEP_c to MEP_pmax also recorded during a maximal inspiratory effort accounts for any activity-dependent changes in the muscle fiber action potential (40). Decreases in MEP_c, when normalized to MEP_pmax, have previously been observed with increased force production (42) . The decreased amplitude of MEP_c in the present study may represent a decreased probability of activating additional motoneurons with an added synchronized synaptic input, thus supporting the aforemen-

tioned suggestion that there is a fuller voluntary activation of the diaphragm after IML. This mechanism may further explain why the depression in MEP_c after IML is only observed when stimuli are delivered during the MIP effort but not at rest.

Similar to the results of the present study, Carroll et al. (4) also found a depression in cortically evoked MEPs during voluntary contraction of the first dorsal interosseous muscle but not in MEPs evoked in the relaxed muscle. Although these changes were seen after a chronic period of exercise training, rather than an acute bout of activity, it is known that isolated exercise sessions elicit acute, transient responses, which if repeated frequently cause these adaptations to become more permanent (41). Carroll et al. (4) suggested that the mechanism responsible for the reduction in MEP_c amplitude that they observed is either an increase in firing rate or an increase in the duration or amplitude of afterhyperpolarization potential trajectory, which would reduce the response probability of individual motor units and thus reduce the resulting MEP_c amplitude.

Acute Bout of NLF Inspirations

The purpose of performing the NLF inspirations was to examine whether loading per se is responsible for the observed changes after an acute bout of inspiratory effort. The nature of the two breathing tasks differs considerably. During NLF, inspiratory effort would be equivalent to the duration of inspiratory flow, whereas flow and effort are uncoupled in threshold loading (as used in IML) and can be separated by as much as 367 ms while enough pressure is generated to open the valve (51). Furthermore, IML is characterized by high-pressure, low-flow and NLF by low-pressure, high-flow conditions. However, despite this, there were no significant differences in the changes occurring after IML compared with NLF. This suggests that it is not the loading but more the act of deep voluntary inspirations that may be responsible for eliciting the observed changes in MIP and evoked responses in the diaphragm.

The one noticeable difference between the two conditions is that, after NLF, the amplitude of the relaxed diaphragmatic MEP_c was significantly reduced compared with baseline. The greater degree of hypocapnia experienced during NLF may have contributed to the depression of diaphragm MEP_c amplitude, which was not observed after IML where $P_{ET}CO_2$ remained significantly higher. A small drop in $P_{ET}CO_2$ in sleeping humans elicits apnea (8), which would reflect a decreased respiratory drive to reestablish normocapnia. Our depression of MEP_c amplitude after NLF could represent motor cortical excitation being integrated at, and relayed by, brain stem respiratory neurons.

In conclusion, the present study has shown that an acute bout of inspiratory loading elicits an augmentation of MIP, an increase in the peripherally evoked diaphragm response during maximal inspiratory efforts, but a reduction in the amplitude of the centrally evoked diaphragm response during MIP. The acute bout of IML may act as a "conditioning" activity for fuller voluntary activation of the diaphragm during maximal inspiratory maneuvers.

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