Original articles

Respiratory rate can be modulated by long-loop muscular reflexes, a possible factor in involuntary cessation of apnea

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Key words
Musculoskeletal, metabolism, breath-hold diving, respiratory, physiology, research

Abstract
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Introduction: The main limiting factors determining apnea time are generally considered to be related to blood and cerebrospinal fluid chemistry. Several physiological (adaptive) mechanisms and some psychologic parameters, such as motivation, are also known to increase apnea time.
Aim: We wished to study the link between peripheral muscle fatigue, the concomitant alteration of long latency (transcortical) reflexes and respiratory control.
Methods: Fatigue was induced in a small hand muscle (abductor pollicis brevis) (n = 11). This muscle is sufficiently small that its fatigue and the resulting production of metabolites are unlikely to alter whole-blood biochemistry. The Hoffmann reflex, an involuntary reaction to electrical stimulation of muscle afferent sensory fibreswas studied, as was the long latency reflex (LLR) using the Dueschl method in which electrical stimulation is superimposed on a slight voluntary contraction, Different fatiguing protocols were performed, and respiratory rate continuously recorded.
Results: The 'muscular metabolites increasing protocol' (at 50% maximum voluntary contraction, MVC) showed a significant dissociation between the decreases in the H-reflex and the LLR, compared to contraction at 25% MVC. This was associated with an increase in the respiratory rate to 148.25 (SD 11.37)% of control at 3 min (the maximum time the contraction could be sustained), whereas at 25% MVC, respiratory rate did not change during the contraction.
Conclusions: This suggests a peripherally mediated, central input to the respiratory centres, triggering a powerful stimulus when metabolites accumulate in muscles. We believe this to be a possible mechanism terminating extreme breath holds.

Introduction

As part of the neural control of respiration, it is believed from animal studies that muscular afferent signals of some type may be responsible for respiratory rate modulation, especially during muscular effort.[1,2] Human studies have shown that group III and IV polymodal afferents (small diameter chemosensitive fibres located in the muscle aponeurosis and perimysia) can affect respiratory rate, but the exact mechanism has not been clarified.[3–5] During a breath-hold experiment after oxygen breathing (100% oxygen for 30 minutes), one of our subjects maintained an apnea for 13 min 54 s (in water of 1.5 metre depth). We measured alveolar gas composition at the breaking point and concluded that, since the oxygen percentage was 93.7% and carbon dioxide (CO_2) 5.9% at the end of the breath hold, these levels could not be the full trigger for its termination. In our experience, this percentage of CO_2 at end-apnea is not exceptional in arterial blood gas measurements of elite apnea divers.[6]

To understand better the reasons why our subject terminated his apnea, we considered the possible factors that might contribute to the cessation of apnea in humans: psychological factors, hypercarbia, acidosis, hypoxia and muscular afferent neurological signals. We excluded the first two factors, and considered the third and fourth as unlikely because this was a resting apnea and thus the diver was not voluntarily contracting any muscle. We hypothesised that the 'muscular afferents theory' could well fit the profile.

To investigate this hypothesis, an experiment was designed in which we studied EMG recordings from a small hand muscle whilst recording respiratory rate. The abductor pollicis brevis (APB) was chosen at two different contraction levels, 25% and 50% of maximal voluntary contraction (MVC). This muscle is small enough for its contraction not to interfere with global oxygen consumption but large enough to allow the Deuschl method for H-reflex and long latency reflexes (LLR) measurement.

Methods

SUBJECTS

Eleven healthy volunteers (8 males and 3 females; aged 22–40 years, mean 28.8 SD 5.6) took part in this investigation. All subjects were part of the Physical Education Department of

Figure 1
Pathways involved in the mechanism. During fatigue, metabolites accumulate in the contracting muscle, increasing presynaptic inhibition at the spinal level, via afferent fibres III-IV, reducing the spinal level input. In parallel, the supraspinal level will increase its input towards available synergistic neurons to overcome force reduction. This descending input will in turn down-stimulate the pneumotaxic centre, and increase respiratory rate. (HR – Hoffmann reflex, LLR – long latency reflex)

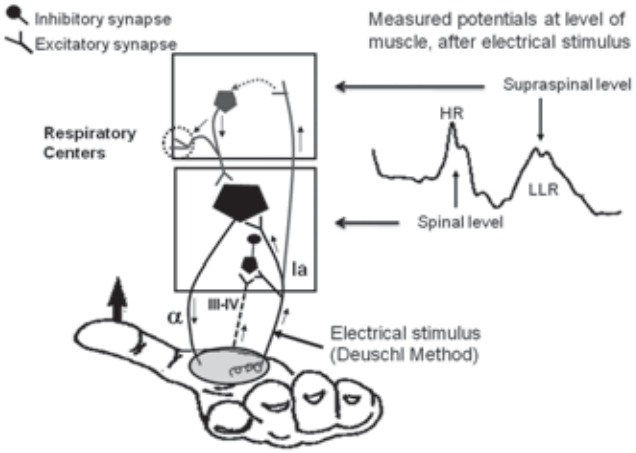
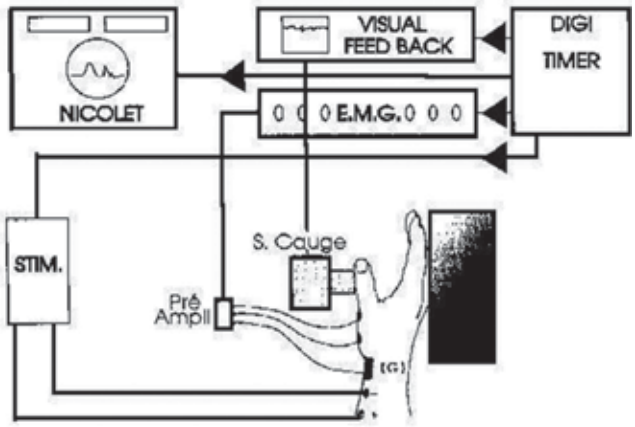


Figure 2
The experimental set-up when recording the EMG and force in the abductor pollicis brevis



the Université Libre de Bruxelles. This study was approved by the University Ethics Committee, and the subjects gave their informed consent to participate in the investigation.

STIMULATION

The Hoffmann reflex (H-reflex) is an involuntary reaction of muscles after electrical stimulation of their afferent sensory fibres (Ia afferent fibres, arising from muscle spindles). The reflex loop transits in the spinal cord. The H-reflex test is elicited using an electrical stimulator, which gives a square-wave current of short duration and small amplitude, and an electnomyelogram (EMG) set to record the muscle response. This response is called the H-wave, 28–35 ms after the stimulus (Figure 1).

In the Deuschl method, a slight voluntary contraction (10–25% of MVC) is used, which permits the elicitation of another component of the EMG during electrical stimulation superimposed on the voluntary contraction called the long latency reflex (LLR, Figure 1).[7,8] This reflex is a compound muscular reaction composed of the medium latency reflex (MLR, ±40 ms), mediated via large fibres from muscle to cortex and back to the muscle via alpha motor fibres, and the long latency reflex (LLR, ±60 ms). This signal has the same pathway as the previous one, adding some slower cutaneous afferents. These two components are better seen during rapid stretching of the muscle. Electrical stimulation does not allow separation of the two components and shows a single signal, the LLR (Figure 1). The general pathway of the LLR is described as follows: the afferent signal starts from the muscle spindle, moves toward the spinal chord via Ia large afferents, then reaches the medulla oblongata, finds a relay in the cuneatus or gracilis nuclei (gracilis for lower body afferents, cuneatus for the upper body), another relay via the lemniscus medialis with the thalamus (ventroposterolateralis nucleus), then reaches the Brodmann 3 area (somatosensory area) and finally transits to the motor cortex (Brodmann 4 area) to reach the alpha motor neuron of the anterior horn of the spinal grey matter.

Muscular contraction, when sufficiently intense to impair local microcirculatory blood flow, induces local metabolite build-up, which stimulates III-IV fibres originating from the muscle membrane. These fibres exert an inhibition at the level of the spinal cord and thus reduce the amplitude of the H-reflex, as well as the LLR at the level of the alpha motor neuron in the anterior horn.

The H-reflex and LLR evoked by electrical stimulation were recorded during a weak sustained contraction (25% of MVC) of the APB.[8,9] Using two silver surface electrodes, the median nerve was electrically stimulated at the wrist at a frequency of 3 Hz. The stimuli were rectangular pulses of 1 ms duration, with the intensity of the stimulation set near the threshold response of the motor fibres. In order to normalise the H-reflex changes during fatigue (c.f., below), the maximal muscle compound action potential (M_{max}) (direct stimulating effect of alpha neurons on muscle, elicited by electrical stimulation) was evoked by a supramaximal stimulation of the nerve at each measuring point. Pulses were delivered from a custom-made, two-channel stimulator triggered by a digitimer (model 4030, Digitimer Ltd, Welwyn Garden City, UK). The muscle reflex responses and the M_{max} were evoked through the same electrodes.

EMG AND FORCE RECORDING

The EMG recordings (H-reflex, LLR and M_{max}) were obtained by means of a pair of silver disc electrodes (8 mm in diameter), one fixed over the muscle motor point and the other over its distal tendon (belly tendon derivation). The ground electrode was attached to the skin between the stimulating and recording electrodes. The signal was AC -amplified (1000 x), filtered (bandpass, 10 Hz to 5 kHz), and full-wave rectified. The reflex responses were averaged (64–128 sweeps).

The recordings of the APB were made with the subject's arm placed on a horizontal board in a semi-supine position, with the back of the hand fixed against a vertical restraint (Figure 2). The abduction force was recorded by pushing with the middle of the thumb against a strain gauge transducer (TC 100, Kulite, Ridgefield, *NTJ,* USA). The subjects were provided with visual feedback of the force and EMG signals in order to maintain a steady level of contraction.

The force of contraction was continuously recorded on a paper chart (Graphtech, WX2400, USA) and the EMG data were recorded and averaged by a digital oscilloscope (Nicolet 4094c, USA) then stored on disk. For each reflex component we measured the peak amplitude. The size of each reflex was defined as the distance between the peak amplitude and the mean background level computed during the 15–20 ms following the stimulus. In order to exclude fatigue-induced changes in the muscle fibre membrane response, each EMG component was normalised as a function of the peak size of the same subject's M-wave (M_{max}).

MUSCLE FATIGUE AND TESTING PROCEDURE

Muscle fatigue was induced by a sustained contraction at 25% or 50% of MVC of the APB. The experiment was terminated in both fatigue tests when the applied force fell to under the target value and the subject could not reach it again even with strong encouragements. The H-reflex and LLR responses were recorded every minute during the test. During the 50% fatigue test, the subject was instructed to reduce contraction to 25% of MVC before each measurement, and recover the 50% contraction level immediately after the measurement. To avoid any recovery from anaerobic metabolites during the recordings, a blood pressure cuff wrapped around the arm was inflated to 250 mmHg just before reducing the contraction and was maintained inflated during the entire EMG averaging time period (not more than 40 s). During this period, first a M_{max} was elicited and then the different reflex responses were averaged. In all the experiments, the temperature of the skin overlying the muscle was continuously maintained at about 35°C by means of an infrared lamp.

RESPIRATORY RATE MEASUREMENTS

Respiratory rate was measured with a spirometer (SP-304) connected to an analogue-to-digital converting board (IW-214, iWorx Systems, USA). Sampling speed was set at 100 per second and the data were stored on a personal computer for further analysis. No visual or auditory feedback was given to the subject. Measurement of respiratory rate was performed by calculating the time between respiratory peaks and averaged every 5 s.

STATISTICAL ANALYSIS

The data recorded during muscle fatigue and recovery were tested by means of an analysis of variance (ANOVA) with repeated measures on one factor and Dunnet or Tukey-Kramer post-test when appropriate (Graphpad Prism v.3.0). The level of significance was taken at $P < 0.05$.

Results

During 50% MVC, the H-reflex and the LLR amplitude, normalised to the M_{max} amplitude, decreased significantly during the fatigue tests from the first minute onwards ($P < 0.05$) (Figure 3), but the LLR decreased significantly less than the H-reflex to a mean of 71.37 (SD 6.17)% versus a mean of 54.06 (SD 5.93)% at the end of the third minute. No subject was able to keep the 50% of MVC target force beyond 3 min of contraction time. This dissociation between the two components of the reflex was significant ($P < 0.05$) from the 2-minute time point, the same time range as the increase in the respiratory rate.

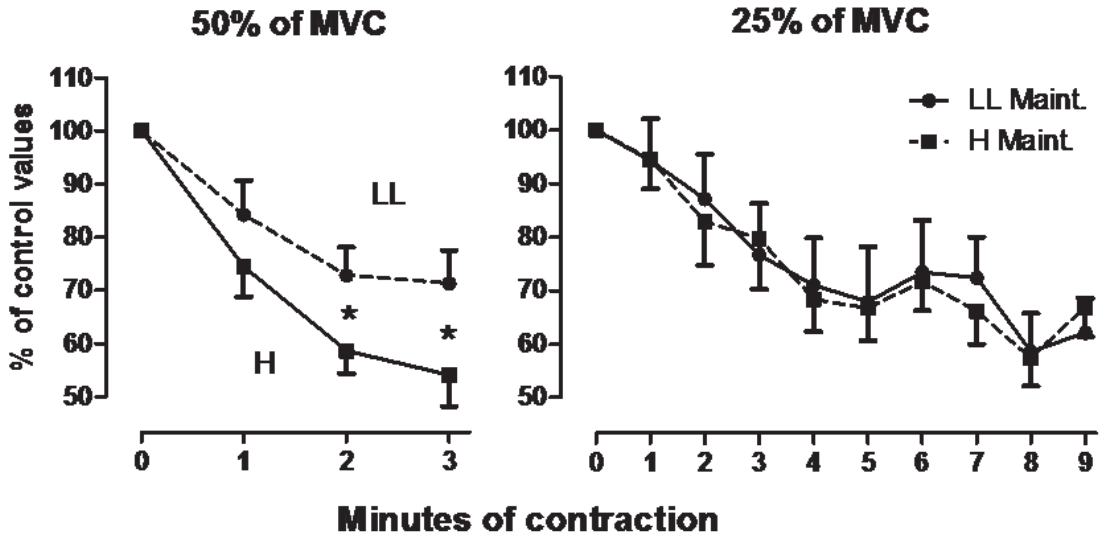
During 25% MVC, both the LLR and the H-reflex decreased concomitantly ($P = 0.05$), but there was no statistical difference between the two components throughout the test. The reduction in amplitude reached a mean of 66.9 (SD 5.48)% for the LLR and 62.14 (SD 6.43)% for the H-reflex after 9 min of contraction.

Respiratory frequency rose during the 50% MVC test only, reaching 148.25 (SD 11.37)% of control at 3 min (the maximum time the contraction could be sustained) (Figure 4). A significant ($P < 0.05$) dissociation between respiratory rates at 25% and 50% MVC appears after 110 sec of contraction and increases from then on.

Discussion

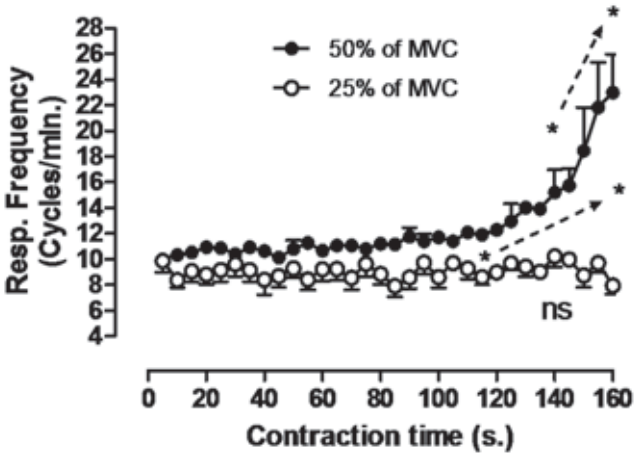
In the present investigation, the major finding is that fatigue during sustained contractions at 50% of MVC induces a decrease of the normalized H-reflex amplitude but lesser changes in the LLR, concomitant with an involuntary increase of breathing rate. This dissociation has previously been described during 100% contractions on the first dorsal interosseus muscle and the APB; however, respiratory rate was not measured.[8] During the decrease of the H-reflex, LLR was even enhanced in a neighbouring muscle which remained at rest, while the H-reflex was not changed in that muscle.[8] This suggested a central, non-specific stimulus to the LLR amplitude.

Figure 3
Normalised reflex amplitude (long latency reflex – LL, Hoffmann reflex – H) during 50% of maximum voluntary contraction (MVC) (left) and 25% MVC (right) (mean and SEM , $n = 11$; * $P < 0.05$). Subjects were instructed to maintain the contraction as long as possible; at 50% MVC they were unable to do so beyond 3 min.



In our experiment, the H-reflex decreased during fatigue-inducing contractions at both 25% and 50% MVC. Mechanisms for this may be related to (1) motor neuron adaptation processes, (2) reduced muscle spindle output or (3) increased presynaptic inhibition of Ia terminals and/or inhibition of interneurons in the oligosynaptic pathway. Adaptation of motor neuron properties to constant excitatory drive as observed in anesthetised cats,[9] does not appear to be preponderant with respect to the H-reflex decrease recorded in our experiments because the time course of the phenomenon that we observed was slower and there was no recovery if the pressure cuff was maintained after the fatiguing contraction. The adaptation of motor neuron properties is not present during voluntary contractions.[10]

Figure 4
Respiratory frequency (breaths per min) after the two fatigue tests (mean and SEM, $n = 11$, * $P < 0.05$)



The mono- and oligosynaptic pathway contributions to the short latency reflex evoked by stretch or electrical stimulation (H-reflex) differ slightly, but the main difference is that the H-reflex bypasses the spindle.[11,12] The comparison of the normalised H-reflex with previously reported reductions of stretch latency shows a very similar decrease of both signals under comparable conditions.[13,14] This suggests that the decrease in muscle spindle sensitivity should not play a key role in the mechanism.

In our experiment, the significant reduction (see Figure 2) of the H-reflex could be explained by a decline in transmission along neural elements between the nerve stimulation point and the H-responding motor neurons as a result of the activation of the muscle afferents (III-IV fibres), causing presynaptic inhibition of the Ia terminals and/or inhibition of interneurons of the oligosynaptic pathways at the level of the spinal cord. Because of the slow time course of the H-reflex decrease during fatigue, it is suggested that this is induced by chemical processes or metabolite accumulation in the muscle that trigger the small afferents from the fatigued muscle.[14,15] This conclusion is supported by the fact that no recovery was seen if ischaemia was maintained.[16,17]

The H-reflex and LLR evoked by electrical stimulation of the median nerve at the wrist have the same origin and are both transmitted by Ia fibres, but the LLR is routed transcortically.[7,18] The different behaviour of the LLR compared to the H-reflex in the sustained contraction at 50%

of MVC can thus be understood by the fact that metabolite-induced afferent input from type III or IV polymodal fibres will increase presynaptic inhibition, which in turn will decrease the segmental reflex (H-reflex) without influencing the transcortical pathways (LLR). In order to detect this central 'drive', a sufficient level of metabolites needs to be generated, explaining why the two signals show a parallel behaviour during weaker contraction (25% of MVC).

Thus, it seems that, in static contracting muscles (approaching 50% of MVC), fatigue induces afferent feedback, which provides the motor neurons with less excitation and, as a reflex compensation, a stronger descending supraspinal drive. This conclusion is strongly supported by the significant increase in respiratory rate, which is by definition a central mechanism, in subjects contracting the APB at 50% of MVC and no significant change at 25% of MVC. Our results are consistent with the presence of a long-loop mechanism underlying respiratory rate control in humans, with an accumulation of metabolites in contracting muscles as the trigger. Even small muscles can build up sufficient metabolites to induce this neural response. In respiratory physiology, such reflexes have long been proposed for respiratory control but until now the mechanisms involved remained unexplained. Previously published reports on phrenic afferents agree with our findings.[19] Whereas most of the studies on muscle afferents and respiratory drive have been performed on anesthetised dogs, our findings in awake, healthy humans confirm that these can also play a role in real-life situations. Recent studies utilising other approaches confirm this phenomenon in humans.[20–22]

Our results could offer an explanation for the termination of prolonged apneas, where hypercapnia and/or hypoxia are an insufficient explanation, even if these apneas are performed in a totally relaxed state. In this situation, diaphragmatic and/or intercostal muscle spasms can, with time, build up enough metabolites to trigger the system and induce the cessation of apnea.[20] This mechanism is probably relevant and deserves to be considered when discussing the limitations of extreme apnea.[23,24]

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