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Excitability of human motor and visual cortex before, during, and after hyperventilation

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Sparing R, Dafotakis M, Buelte D, Meister IG, Noth J. Excitability of human motor and visual cortex before, during, and after hyperventilation. J Appl Physiol 102: 406–411, 2007. First published September 21, 2006; doi:10.1152/japplphysiol.00770.2006.—In humans, hyperventilation (HV) has various effects on systemic physiology and, in particular, on neuronal excitability and synaptic transmission. However, it is far from clear how the effects of HV are mediated at the cortical level. In this study we investigated the effects of HV-induced hypocapnia on primary motor (M1) and visual cortex (V1) excitability. We used paired-pulse transcranial magnetic stimulation; intracortical facilitation; intracortical inhibition; partial pressure of carbon dioxide; phosphene; stimulus-response (S-R) curves; intracortical inhibition; intracortical facilitation; intracortical inhibition.

HYPERVENTILATION (HV) (or hyperpnea) is the state of breathing faster or deeper than necessary, thereby reducing the CO2 concentration of the blood below normal. What is usually referred to as HV is, in fact, hypocapnia. Since a reduction of arterial PCO2 (PaCO2) below the normal level (40 Torr) is obtained by increasing the alveolar ventilation, HV became synonymous with hypocapnia (32). HV is known to have various effects on human physiology (for a review, see Ref. 6). For instance, a reduction in PaCO2 increases the excitability of sensory and motor axons in the peripheral nervous system (21, 23, 28).

The aim of the present study was to investigate further the neural mechanisms of the HV-induced changes in cortical excitability. As measures of primary motor cortex (M1) excitability, motor threshold (MT), stimulus-response (S-R) curves (i.e., recruitment curves), intracortical inhibition (ICI), and intracortical facilitation (ICF) were determined. MT reflects primarily neuronal membrane excitability (34), while ICI and ICF relate to the excitability of separate intrinsic interneuronal circuits in the motor cortex (18, 35, 36). S-R curves may assess neurons that are intrinsically less excitable or spatially further from the center of activation by transcranial magnetic stimulation (TMS).

In the present study, we explored, furthermore, the effect of HV on the excitability of a nonmotor area, namely the primary visual cortex (V1). We measured the phosphene threshold (PT) and recorded S-R curves for the phosphene elicitation (average number or intensity of reported phosphenes at different stimulation intensities). Both measures are reliable parameters of V1 excitability (3–5, 22, 29–31). In contrast to the measurements of M1 excitability, these measures are little influenced by the concomitant effects of HV on other tissues outside the central nervous system (CNS), such as nerve fibers and muscle cells. Since it has also been proven that PT and MT do not correlate (3, 31), a decrease in PT would demonstrate that the effect of HV on human cortical excitability is not confined to the motor system alone.

MATERIALS AND METHODS

Subjects

Ten subjects (8 men, 2 women, age range 23–32 yr) participated in the study. Four subjects were unable to perceive phosphenes even at maximum stimulator output. Such a failure rate is in accordance with previous studies of phosphene elicitation (22, 29, 30). However, all subjects who were not able to perceive phosphenes had a MT in the normal range. We therefore assigned subjects to two subgroups of six subjects each to investigate motor and visual cortical excitability separately. Two subjects participated in both parts of the study. Since the measures of PT and MT do not correlate (e.g., Refs. 3, 31), it is...
 unlikely that this selection results in a potential bias. To avoid learning effects, subjects were sufficiently trained in phosphene detection before the main experiment. All subjects gave informed written consent. The study protocol was approved by the local ethics committee.

Experimental Setup and Stimulation Procedure

Subjects were seated in a comfortable reclining chair. The end-tidal PCO2 (PETCO2) was measured with a capnometer (Siemens, Munich, Germany). This measure is a good approximation of alveolar PCO2 in healthy subjects (12). The PETCO2 is usually 2–6 Torr lower than the PacCO2 (33). The capnometer was placed in front of the subject to supply direct visual feedback. Since it has to be considered that changes in the PacCO2 and changes in the brain tissue and cerebrospinal fluid do not occur simultaneously, the level of the PETCO2 may not play the only role; rather, the duration of HV and the time point at which the motor-evoked potential (MEP) or phosphene testing is conducted may also play a role. We therefore designed our study protocol according to previous TMS studies [i.e., 15 Torr/10 min compared with 22.5 Torr/3 min in Kong et al. (16); <15 Torr/10 min in Priori et al. (25); 15 Torr/3 min in Seyal et al. (27)] to most likely detect significant effects of HV. Subjects were instructed to hyperventilate briskly until a PETCO2 of 15 Torr was reached. This was the case after approximately 2–3 min, which is in accordance with previous observations (27). Subjects could then reduce their efforts but were still required to maintain a mean PETCO2 of 15 Torr until a period of 10 min of HV was over. All measurements of cortical excitability during HV were performed in the second half of this period. Figure 1 illustrates the experimental procedures.

Measurements of motor excitability. Surface EMG recordings were made from the first dorsal interosseous (FDI) muscle. A tightly fitting white Lycra swimming cap was placed over the subject’s head to mark the site for stimulation and to facilitate an exact repositioning of the coil during the entire experiment. Focal TMS was applied to the optimal point for stimulation of the FDI using a BiStim-module (Magenti, Whitland, Dyfed, UK) equipped with a 9.0-cm figure-of-eight coil. The optimal point was defined as the point from which stimuli at the minimal excitability threshold of TMS triggered MEPS of maximal amplitude and minimal latency in the target hand muscle. Resting MT was defined as the minimum stimulation intensity necessary to induce a response of at least 50 μV in 5 of 10 consecutive trials (26). The level of muscle relaxation was monitored continuously by means of audiovisual feedback. MEPS were amplified and digitized by using a PowerLab 3T module (ADInstruments, Colorado Springs, CO) with a bandpass of 20–1,000 Hz at a sampling rate of 4 kHz and stored for offline analysis. Subjects were tested in two separate sessions with a minimum of 2 days between them.

S-R curves. MT was determined as described above. Single-pulse TMS was started 10% below the resting MT and increased in 10 steps of 2% and 2 steps of 5% at the end (referring to maximum stimulator output). For each stimulation intensity, four TMS pulses were given with an ISI of at least 5 s. The mean MEP at each intensity was normalized to the mean MEP at 100% stimulation intensity before HV in each subject. This normalization to the preintervention MEP amplitude was performed to optimize the evaluation of intraindividual HV-induced changes on S-R curves. We recorded the S-R curves before HV, during HV (starting 7 min after the onset of HV), and 10 min after stopping HV (Fig. 1).

Paired-pulse stimulation. We used paired-pulse TMS with short interstimulus intervals (ISIs). Such protocols have been proven useful to separately investigate ICI and ICF in the M1 (18, 35, 36). Its effect is inhibitory at ISIs of 2–5 ms and facilitatory at ISIs of 7–20 ms. We modified the paired-pulse protocol of Kujirai et al. (18): a subthreshold conditioning stimulus (CS, at 80% MT) and a suprathreshold test stimulus (TS) were delivered at four different ISIs of 2, 4, 10, and 15 ms, respectively. Before each experimental block, we adjusted the TS intensity to produce a MEP of ~1-mV amplitude to account for any changes in test MEP size due to HV-related modulations of MT. The first block was recorded before HV, the second during HV (starting 7 min after the onset of HV), and the third and fourth block 1 and 10 min, respectively, after stopping HV (Fig. 1).

Measurements of visual excitability. Subjects were blindfolded during the experimental trials. However, to prevent dark adaptation and light deprivation-induced changes in V1 excitability, they were repeatedly reexposed to light (5). During the 10 min of HV, one of the experimenters informed the subject continuously of the PETCO2 concentration. For each subject the optimal point for phosphene elicitation was chosen within a grid of 9 points centered over Oz, according to the International 10–20 EEG electrode system, each point 2 cm apart. The coil was placed with its handle pointing upward. The site where a given TMS intensity evoked the brightest phosphenes in the contralateral hemisphere was determined as the optimal point for eliciting phosphenes. PT was then determined as the minimum intensity over the optimal point capable of eliciting phosphenes in three of five trials. TMS was started at a subthreshold intensity (around 25–30% of maximum stimulator output) and was increased in 3% steps (10 steps) and 5% (last 2 steps). To keep the subject blind to the level of the stimulation, the starting intensity was randomly varied by ±2–5% across the different stimulation conditions. At each intensity level, five stimuli were given with a minimum ISI of 5 s. The subjects were instructed to report the presence or absence of a phosphene and, if present, the intensity of the phosphene on an arbitrary scale of 1–5, with 5 being the brightest phosphene. The S-R curves were deter-

Fig. 1. Time line of the experimental paradigms performed in this study. Experimental part I (motor cortex): in session A, stimulus-response (S-R) curves were recorded before hyperventilation (HV; S-R curve I), during HV (starting 7 min after the onset of HV; S-R curve II), and 10 min after stopping HV (S-R curve III). In separate session B, paired-pulse transcranial magnetic stimulation (PP-TMS) of primary motor cortex (M1) was performed before HV (PP-TMS I), during HV (starting 7 min after the onset of HV; PP-TMS II), and after HV (1 min (PP-TMS III) and 10 min (PP-TMS IV), respectively, after stopping HV). Experimental part II (visual cortex): S-R curves were determined before HV (S-R curve I), during HV starting 5 min after the onset of HV (S-R curve II), and 10 min after stopping HV (S-R curve III).
mined before HV, during HV starting 5 min after the onset of HV, and 10 min after stopping HV (Fig. 1).

_data analysis_

The collected MEPs were rectified, and the peak amplitude was measured. MEPs were averaged for each condition. For statistical evaluation of S-R curves, we collapsed the data to six different TMS intensity levels in 5% steps, e.g., <50%, <55%, <60%, <65%, <70%, and <75% of maximum stimulator output. We performed ANOVA with condition (time point of measurement (before, during, and after HV)) and intensity (level of TMS intensity (<50%, <55%, <60%, <65%, <70%, and <75%)) as main factors. Data of the paired-pulse stimulation were submitted to a repeated-measures ANOVA with condition (time point of measurement (before, during, 1 min and 10 min)) and ISI (2, 4, 10, and 15 ms) as factors. Duncan’s test was used for post hoc analyses. Differences were considered significant at a level of \( P < 0.05 \).

RESULTS

All subjects were able to achieve a constant mean \( \text{PETCO}_{2} \) of 15 Torr. Serious adverse events were not observed. However, some of the subjects reported paresthesias, dizziness, slurred speech, and tingling in the lips and lower limbs when they had reached the targeted value of 15 Torr. Thus a spread of excitability in the motor cortex from the respiratory muscle region to the hand region is unlikely to account for the present findings.

MT and PT

The resting MT showed a significant decrease during hyperventilation (decrease from 40% to 35%, \( P = 0.02 \)). PT also dropped during hyperventilation from 42% to 37% \( (P < 0.01) \) (Fig. 2). Both PTs and MTs returned to normal values 10 min after the end of HV.

S-R Curves

At the motor cortex, ANOVA considering together MEP amplitudes recorded during the three different experimental conditions (before, during, and after HV) showed a significant difference \( [F(2,10) = 6.69, P = 0.02] \). Moreover, we found a significant main effect for TMS intensity \( [F(5,25) = 28.92, P = 0.001] \) and a significant interaction between both factors \( [F(10,50) = 2.49, P = 0.03] \). The analysis of single contrasts revealed that S-R curves were significantly enhanced at lower stimulation intensities during HV (<50%, <55% and <60% stimulator output; \( P < 0.04 \)). S-R curve onset was slightly steeper than that obtained before and after HV. This increase in excitability resolved at 10 min after the end of HV (Fig. 3).

A similar pattern was found at the visual cortex: ANOVA showed main effects of condition and intensity but no significant interaction \( [F(2,10) = 6.59, P = 0.02; \text{intensity, } F(5,25) = 47.74, P < 0.001] \). S-R curves were significantly higher at stimulation intensities of <50% and <55% stimulator output \( (P < 0.04) \) (Fig. 4). At higher stimulation intensities, it seemed that a plateau level was reached.

DISCUSSION

The main findings of this study were that HV of 10 min with a \( \text{PETCO}_{2} \) of \( \sim 15 \) Torr increased the excitability of the motor and visual cortex, which was indicated by the measurement of threshold as well as S-R curves. A similar response pattern was observed at both cortical sites, with a greater effect of HV at low stimulation intensities.

Effects of HV on MT and S-R Curves

In an early experiment on HV, Foerster (13) already investigated the effects of direct electric stimulation of the exposed motor cortex in two patients undergoing neurosurgery. However, there has been controversy concerning the extent to which HV influences the amplitude of the MEP evoked by TMS. Kong et al. (16) were unable to demonstrate increased excitability of corticospinal neurons. This negative finding was probably due to the study protocol that resulted in a low change of the mean \( \text{PETCO}_{2} \) (22.5 Torr). Seyal et al. (27) demonstrated a direct relationship between \( \text{PETCO}_{2} \) levels and the MEP amplitude. They found a negligible change in the MEP amplitude to 30 or 20 Torr, whereas a \( \text{PETCO}_{2} \) of 15 Torr significantly enhanced the MEP amplitude and shortened the MEP.
onset latency. In contrast, Priori et al. (25) did not observe any effect of HV on the amplitude or latency of the MEP although HV reduced the PETCO2 significantly below 15 Torr. Seyal et al. (27) argued plausibly that the use of very high TMS intensities in the study of Priori et al. (25) most likely accounted for the lack of HV-induced change in MEP amplitude. This explanation is supported by our findings that HV decreased MT and that the absolute increase of mean MEP amplitudes was more prominent at lower TMS intensities. Seyal et al. (27) demonstrated furthermore a direct relationship between PETCO2 levels and changes in MEP amplitudes and latency onset, which makes it unlikely that not HV but effects of effort, attention, or any other nonspecific factor influence visual or motor cortex excitability. While MT predominantly probes excitability of corticocortical axons directly excited by TMS, it has been assumed that S-R curves (especially at clear suprathreshold intensities) test excitability of the corticospinal system responsible for the generation of late I waves (11). The measure of recruitment may involve neurons other than those in the core region that is activated at threshold. These neurons may have higher threshold for activation, either because they are intrinsically less excitable or because they are further from the center of activation by the TMS pulse. Such intensity-dependent effects, e.g., the growth of MEP size as a function of stimulus intensity, are less well understood compared with the measure of MT. However, it has recently been argued that MEP amplitude and ICF may behave in similar ways (14). This hypothesis would be supported by our observations that HV did not influence ICF and exert larger effects on MEP amplitudes at lower TMS intensities.

Effects of HV on Visual Cortex Excitability

In contrast to the motor cortex, the excitability of the visual cortex can be determined by TMS without any possibly confounding effects of HV on other tissues, such as the spinal neurons, peripheral axons, or muscle cells. Our data show that low PCO2 levels significantly enhance the excitability of V1.
Interestingly, the S-R curves of both cortices, V1 and M1, showed a similar pattern with more prominent effects of HV at lower stimulation intensities. However, we cannot exclude the possibility that HV produces changes of V1 excitability by acting on subcortical pathways since the exact anatomic origin of phosphene generation is still unknown (22). Previous results, which have found that HV shortened the latency of visual-evoked potentials in demyelinating disease (2, 9), support the view that axonal effects underlie the effect reported here.

ICI and ICF

The results of the paired-pulse stimulation of M1 suggest that HV affects predominantly inhibitory intrinsic interneuronal circuits (ICI). An influence on ICF was not depicted with the method used in the current experiment. Our findings are in good agreement with Priori et al. (25), who reported that HV resulted in a shortening of the corticol silent period (CSP) during active isometric contraction of the FDI. The evaluation of the CSP and ICI seen with paired-pulse stimulation reflects complementary approaches for the evaluation of inhibitory mechanisms on the cortical level (10, 25). It has been argued that HV increases excitability of spinal motoneurons (26) and motor axons (21, 23, 28). However, the HV-induced changes in CSP and ICI, seen in the present study, provide good evidence that HV has also distinct effects on neuronal excitability at the cortical level. Although paired-pulse techniques have revealed important insights into the physiology of intracortical excitability, a potential problem with this protocol is that data interpretation may be hampered if the presence of task-related threshold changes cannot be excluded. In particular, MT changes may influence the size of the CS, which in turn has an effect on the magnitude of ICI and ICF (18, 34). Large variations of CS intensity are also known to cause “floor” and “ceiling” effects. In our study, we attempted to compensate potential conflicting effects of a floating baseline by adjustments in TS intensity. However, we cannot exclude that changes in threshold account for the observed decrease of ICI. Two findings may argue against such an explanation: we did not observe any significant ICF changes, and the decrease of ICI during HV is consistent with the findings of a reduced CSP duration (25). Nevertheless, we suggest that the results of the paired-pulse paradigm should be interpreted with care.

Possible Pathophysiological Mechanisms of HV on Neuronal Excitability

Ziemann et al. (35), who tested the effects of CNS-active drugs on motor cortical excitability by means of TMS, suggested that changes in MT are dependent on conductivity of voltage-gated ion channels and may reflect membrane excitability, while changes in intracortical excitability are caused by GABA-controlled interneuronal circuits in M1 (35). In the peripheral nervous system, studies of excitability of motor and sensory axons demonstrated that HV has a rather selective action on persistent Na⁺ channels (24), while conventional Na⁺ and K⁺ channels seem to be relatively unaffected (21, 23, 24). Assuming that such mechanisms are also critical for central excitability, the observed decrease of MT, induced by low PaCO₂ levels, results presumably from changes in Na⁺ channel conductances, which in turn could be mediated by HV-induced pH alterations (1, 8, 24).

HV is well known to induce alkalosis (8) in conjunction with a reduced plasma concentration of Ca²⁺ (6, 9). A reduction of extracellular Ca²⁺ and/or changes in pH could potentially interact with the synaptic transmission of GABA (19), the major inhibitory neurotransmitter in the human CNS, and with excitatory glutaminergic transmission mediated by N-methyl-D-aspartate (NMDA) receptors (8). A large body of work on intracortical excitability has demonstrated that ICI probes inhibitory interneuronal circuits in M1 dependent on neurotransmission through the GABA_A receptor (14, 17, 34, 36). The mechanisms of ICF are less well understood. ICF probably reflects an overlap of inhibition through GABA_A receptor and facilitation through the NMDA receptor (17, 36). In our study, we did not detect an HV-induced change in ICF intensity. However, our findings suggest that HV decreases ICI by possibly interfering with GABA transmission. This assumption is supported by the previous demonstration of an HV-induced shortening of the CSP (27), which is most likely mediated through the GABA_A receptor. Both ICI seen with paired-pulse stimulation and CSP are thought to represent measures of intracortical inhibition. It has been hypothesized that they correspond to the excitability of different subtypes of inhibitory interneurons (17).

In contrast to motor cortex physiology, little is known about the mechanism underlying phosphenes and PT, respectively. However, it has recently been suggested that analogous mechanisms exist in the visual system as well. Boroojerdi and coworkers (5) found involvement of GABAergic inhibition and NMDA receptor activation in rapid processes of human visual cortex plasticity by using PT as a measurement of V1 excitability. On the contrary, it has been shown that the mechanisms underlying phosphene induction in V1 are probably different from those underlying intracortical inhibition and facilitation in M1 (29).

Conclusion

Our findings extend data from previous studies by providing evidence of HV-induced increases in both motor and visual cortex excitability. Low PaCO₂ levels seem to affect the human brain as a whole, which has also been suggested by the observation that HV causes a generalized slowing of the EEG (19). Aside from EEG studies, further evidence has recently provided by magnetoencephalographic studies, which demonstrated that HV enhances cortical excitability in widely distributed networks (7, 15). Overall, HV (i.e., hypocapnia) exhibits multiple differential effects both on systemic human physiolo-gy and, in particular, on the physiology of the neuronal tissue. Even vascular effects, which are not possible to address with the techniques used in the present experiments, may play a role for changes in cortical excitability. For instance, HV lowers intracranial pressure by the induction of cerebral vasoconstric-tion, with a subsequent decrease in cerebral blood volume (32). Further exploration of these complex pathophysiological mechanisms may contribute to the understanding of the various HV-related clinical phenomenona in humans.

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