Cerebral cortical respiratory-related evoked potentials elicited by inspiratory occlusion in lambs

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Received 21 February 2001; accepted in final form 5 July 2001

Davenport, Paul W., and Alastair A. Hutchison. Cerebral cortical respiratory-related evoked potentials elicited by inspiratory occlusion in lambs. J Appl Physiol 93: 31–36, 2002; 10.1152/japplphysiol.00177.2001.—Respiratory-related evoked potentials (RREP) elicited by inspiratory mechanical loads have been recorded in humans. Early RREP peaks were hypothesized to be generated by activation of neurons in the somatosensory cortex. An animal model was developed to test this hypothesis in chronically instrumented, awake, spontaneously breathing lambs. Electroorticogram (ECoG) was recorded bilaterally with ball electrodes on the dural surface over the somatosensory region. Inspiratory occlusions were presented through a face mask or endotracheal tube as interruptions of inspiration. Occlusion-elicited evoked potentials were obtained by computer-signal averaging the ECoG activity. A short-latency positive peak was observed bilaterally in the averaged occlusion-elicited evoked potentials in all animals breathing with the facemask and 5 of 8 lambs with the endotracheal tube. Postmortem identification of the electrode location demonstrated that the ECoG was recorded in the caudal-lateral portion of the somatosensory cortex. These results demonstrate that inspiratory occlusion elicits an evoked potential in the somatosensory cortical region of awake, spontaneously breathing lambs. The lamb cortical RREP is similar to human RREP.

inspiratory load; somatosensory; electrocorticogram

HUMAN SUBJECTS ARE CONSCIOUSLY AWARE of breathing against mechanical loads. This conscious awareness suggests that there must be activation of neurons in the cerebral cortex and that this activation is measurable. Activation of cortical neurons in humans by mechanical loads has been studied by using evoked-potential techniques similar to those routinely used in other somatosensory systems (6, 3, 15, 19, 22). Mechanical loads were applied while simultaneously recording from the scalp over the somatosensory region of the cortex in adult humans and children (3, 6, 13–15). This analysis resulted in the observation of load-elicited evoked potentials, the respiratory-related evoked potential (RREP), recorded from scalp electrodes placed over the somatosensory region of the cortex. RREP was similar to somatosensory-evoked potentials reported for the hand and leg (6). With the use of dipole model-

ing in human subjects, the first peak (P1) was a short-latency, exogenous positive voltage that was suggested to be due to the arrival of the occlusion-related afferent information at the somatosensory cortex (15). The afferents and neural mechanisms mediating this evoked cortical activity remain unknown primarily because of the lack of an adequate animal model using mechanical loads as the stimulus.

Animal studies of the neural mechanisms mediating respiratory sensations are limited. An adult animal model of resistive load detection was developed and studied in our laboratory (5). Adult dogs with a tracheal stoma were behaviorally conditioned to signal the detection of inspiratory resistive loads and occlusions. The resistive load detection threshold and Weber fraction were found to be similar to those of humans breathing through a mouthpiece, demonstrating that animals could be conditioned to signal detection of inspiratory loads. If respiratory mechanical information is processed in part by the somatosensory cortex, then somatosensory cortical neurons must be activated by respiratory mechanoreceptors. Phrenic afferents and intercostal muscle mechanoreceptors have been demonstrated to activate neurons in the somatosensory region of the cat cerebral cortex (8, 9). This projection is via the thalamus (24) similar for mechanosensation in other sensory systems. Thus animal studies have demonstrated that the neural substrate exists for respiratory mechanoreceptor and muscle afferent activation of the cerebral cortex.

The afferent pathways, cortical distribution, and neural mechanisms for somatosensory cortical processing of respiratory mechanical load information are implied by RREP studies in humans. However, specific somatosensory cortical activation during conscious, spontaneous breathing remains unknown. The present study bridges the gap between invasive, anesthetized animal studies and the scalp surface RREP techniques used with humans. Conscious, spontaneously breathing lambs chronically instrumented with cortical electrodes served as an animal model. It was hypothesized that respiratory mechanical loads applied at the mouth

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and below the larynx activate mechanoreceptors that elicit RREP in the somatosensory cortex.

**METHODS**

The procedures used in this study were reviewed and approved by the University of Florida, Institutional Animal Care and Use Committee. Eleven lambs, postnatal ages of 5–15 days, were studied. A lamb was initially instrumented under aseptic conditions with halothane anesthesia. After induction, the lamb was placed on an operating table. Rectal temperature probes were inserted, and the lamb’s temperature was maintained at 38 ± 1°C with a water-regulated heating pad. A femoral arterial catheter was inserted. Arterial blood gases were periodically sampled and maintained within normal values. Heart rate and blood pressure were monitored continuously. The arterial catheter was led subcutaneously to the midthoracic region, and the exposed end was capped and placed in a thoracic pouch after completion of surgery. A femoral venous catheter was similarly inserted. Fluids (dextrose 5% with normal saline) were administered intravenously at 4 ml·kg⁻¹·h⁻¹. A balloon connected to a catheter was implanted in the midcostal pleural space for recording pleural pressure (Ppl). The thoracic wound was sutured, and the exposed end of the pleural balloon catheter was capped and placed in the pouch. The rostral skull was similarly capped and placed in a thoracic pouch after completion of surgery. A femoral venous catheter was similarly inserted. Fluids (dextrose 5% with normal saline) were administered intravenously at 4 ml·kg⁻¹·h⁻¹. A balloon connected to a catheter was implanted in the midcostal pleural space for recording pleural pressure (Ppl). The thoracic wound was sutured, and the exposed end of the pleural balloon catheter was capped and placed in the pouch. The rostral skull was exposed by a midline incision. A 1-cm,ID hole was made bilaterally, 2 cm lateral to the sagittal suture in line with the rostral border of the tragus of the ear. The dura was exposed but not penetrated. Silver ball electrodes (0.75-cm diameter) were inserted through each hole and placed in contact with the brain through a minimum of 3 days postoperation. The lamb was allowed to recover from the anesthesia and was returned to its ewe. The lamb was allowed 3–4 days for postsurgical recovery before study. The animal was monitored for any signs of surgical complications. All lambs remained in good health, fed from their ewe, and had no signs of infection or postsurgical complications.

After a minimum of 3 days postoperation, the lamb was brought into the laboratory and a face mask was attached to the lamb’s nose and sealed with elastomer. Airflow (V) was recorded by using a pneumotachograph, linear between 0 and 35 l/min, attached to the face mask or endotracheal tube (Fig. 1). A differential pressure transducer, demodulator, and amplifier were used to measure V. A nonrebreathing valve was connected to the pneumotachograph. Airway pressure (Paw) was measured by using a differential pressure transducer connected to a port at the center of the nonrebreathing valve. The cortical electrodes were connected to a preamplifier. Electrocorticogram (ECoG) activity was amplified, band-pass filtered (0.3 Hz to 1 kHz), and recorded on magnetic tape. A reference electrode was placed on the scalp between the ECoG electrodes. Paw, Ppl, V, and bilateral ECoG were recorded simultaneously (Fig. 1). Arterial blood gas values, heart rate, blood pressure, and temperature were monitored periodically throughout the experiment and remained within normal values.

An occlusion device was attached to the inspiratory port of the nonrebreathing valve. The inspiratory load consisted of a total occlusion presented to the face mask or the lower trachea via an endotracheal tube. The load was presented by activating the low-resistance, rapidly closing (2-ms closure time) occlusion valve producing a total occlusion by pneumatic closure of a diaphragm. The occlusion was an interruption of inspiration presented after the visual observation of the onset of inspiration was noted in the V signal. The airway was totally occluded for ~300 ms. Each occluded breath was separated by a minimum of five unoccluded breaths. By using this method, a minimum of 100 inspiratory interruption occlusions were presented to each awake, spontaneously breathing lamb. The occlusions were initially applied with the breathing circuit attached to the face mask. A second trial, the control trial, was recorded with activation of the occlusion valve, but inspiration was allowed to continue through a bypass opening in the breathing valve. This trial controlled for any effects of noise or vibration from closure of the occlusion valve.

After the face mask occlusion trial, an endotracheal tube was placed through the face mask port (the face mask remained in place) and mouth in eight lambs. The interruption occlusion and control trials were repeated while bypassing the upper airways in these awake, spontaneously breathing lambs. At the end of the experiment, the lambs were killed with pentobarbital sodium. The cranium was then opened at the electrode sites, and a lesion was made with a 18-gauge needle. The lambs’ brains were removed, and the electrode

**Fig. 1.** Schematic representation of the experimental preparation. L, left; R, right; ECoG, electrocorticogram; V, airflow; J/V, volume; Paw, airway pressure; Ppl, pleural pressure.
sites were identified and correlated with the surface topography of the lamb cortex (16, 20).

Data analysis. The occlusion presentation was marked on a magnetic tape recording by a transistor-transistor logic (TTL) pulse that preceded the closure of the valve. The recorded data were analyzed by digitizing Paw, Ppl, and ECoG at 2 kHz (model 1401, Cambridge Electronics Design). For each load presentation, 500 ms of ECoG activity, Paw, and Ppl (50 ms preocclusion and 450 ms postocclusion) were digitized and stored on disk for subsequent computer signal averaging (SIGAVG, Cambridge Electronic Design). The computer stored each individual load presentation. For the occlusion trial, each presentation was recalled from memory and inspected for the presence of a rapid Paw change greater than \(-1\) cmH\(_2\)O, indicative of an occlusion. If the presentation had an occlusion, it was included in the average. A minimum of 64 occlusions was included in each averaged occlusion RREP. Averaged signals were then stored on computer disk. This was repeated for the no-load control except that no Paw change was present. The TTL pulse was used to indicate occlusion-valve activation and initiate signal averaging. A minimum of 64 control breaths was included in each averaged control RREP.

The averaged RREP was then examined for ECoG voltage peaks. The P\(_1\) was the shortest latency, initially positive potential postocclusion valve closure. The point of the onset of the occlusion was indicated by a rapid change in Paw (Fig. 2) and used to set the zero time point for determination of peak latencies and amplitudes during the occlusion trials (7). P\(_1\) latency and amplitude were measured. Zero time for the control trials was the point at which the occlusion valve was activated as indicated by the TTL pulse. A paired t-test was used to test for differences between 1) the P\(_1\) latency of the RREP and the treatment (face mask or endotracheal tube), 2) the P\(_1\) latency of the RREP and the hemisphere (right or left) for face mask and endotracheal tube, 3) the P\(_1\) amplitude of the RREP and the treatment (face mask or endotracheal tube), and 4) the P\(_1\) amplitude of the RREP and the hemisphere (right or left) for face mask and endotracheal tube. The level of significance was set at \(P < 0.05\).

RESULTS

The inspiratory occlusion interruption produced a rapid negative pressure change in both Paw and Ppl (Fig. 2). An evoked potential was observed with inspiratory interruption occlusions in all lambs \((n = 11)\) breathing through the face mask (Fig. 2). No evoked potentials were noted with the face mask and endotracheal tube control trials. The P\(_1\) had a mean peak latency of 36.6 ± 3.9 ms for the left cortex and 34.1 ± 7.4 ms for the right cortex (Table 1). A similar evoked potential was observed with inspirational occlusions in five of eight lambs breathing through the endotracheal tube (Fig. 3). The P\(_1\) had a mean peak latency of 36.3 ± 9.8 ms for the left cortex and 38.8 ± 11.8 ms for the right cortex (Table 1). Again, no evoked potential was noted with the endotracheal tube control trial (Fig. 4). The P\(_1\) was observed bilaterally (Fig. 3) with both face mask and endotracheal tube ventilation. The latencies were not significantly different between right cortex and left cortex for both face mask and endotracheal tube conditions. The P\(_1\) latencies were compared in the five animals with both face mask and endotracheal tube responses, and there was no significant difference between these two conditions (Table 1).

| Table 1. Average P\(_1\) latencies and amplitudes for the occlusion-elicited evoked potential |
|-------------------------------|---------------------------------|---------------------------------|
|                               | Face Mask                       | Endotracheal Tube               |
|                               | Left cortex                     | Right cortex                    |
|                               | Left cortex                     | Right cortex                    |
| ECoG P\(_1\) latency, ms      | 36.6 ± 3.9                      | 34.1 ± 7.4                      |
| ECoG P\(_1\) amplitude, \(\mu V\) | 7.8 ± 5.2                      | 10.9 ± 11.1                     |
|                                | 16.1 ± 9.6                      | 12.2 ± 13.7                     |

Values are means ± SD. P\(_1\), first peak; ECoG, electrocorticogram. There were no significant differences between the latencies and amplitudes.

Fig. 2. Occlusion-elicited evoked potential with face mask breathing. Sixty-four inspiratory occlusions were averaged to obtain these waveforms. The top trace is ECoG activity, the middle trace is Paw measured at the center of the nonrebreathing valve, and the bottom trace is Ppl.

Fig. 3. Occlusion-elicited evoked potential with the lamb breathing through the endotracheal tube. Sixty-four inspiratory occlusions were averaged to obtain these waveforms. These results are from the same lamb, as presented in Fig. 2. P\(_1\), first peak.
The P1 zero-peak amplitudes were not significantly different between right and left cortex for both face mask and endotracheal tube conditions. The P1 zero-peak amplitudes were compared in the five animals with both face mask and endotracheal tube responses, and there was no significant difference between these two conditions (Table 1). Postmortem examination of the electrode location in relation to the surface topography of the lamb brain demonstrated that the electrodes were over the lateral edge of the postcruciate and suprasylvan gyri (Fig. 5A). The electrodes were over the somatosensory region of the cortex (Fig. 5B).

DISCUSSION

The P1 observed in these lambs is very similar to the initial positive primary-evoked potential recorded in cats with the use of electrical and mechanical stimulation of respiratory muscles (8, 9). This peak is also similar to the P1 of human RREP. The present study applied the method used to record the scalp RREP in humans while recording directly over the somatosensory cortex, eliminating the loss of cortical activity and spatial localization that occurs with scalp recording. These results provide the first direct evidence that inspiratory occlusion elicits a somatosensory-evoked potential in spontaneously breathing, conscious animals.

Simultaneous stimulation of a specific population of afferents produces an afferent volley that is transmitted to the somatosensory cortex. This afferent volley activates cortical columns in a specific location in the primary somatosensory cortex. The activation of a population of neurons generates a dipole that produces a positive voltage in electrodes placed on the pial surface over the activated neurons (25). Although the current from this dipole produces a voltage change recordable at a distance from the activated neurons, the amplitude falls rapidly as the electrode is moved away from the specific activated neural site. In the present study, the inspiratory occlusion elicited P1 is indicative of such a cerebral cortical dipole and is most likely produced by a dipole in close proximity to the electrodes in the somatosensory cortex.

Cortical surface-evoked potentials have been used to demonstrate phrenic and intercostal afferent activation of neurons in area 3a and 3b of the cat somatosensory cortex (8–10). In the present study, it was not possible to place the electrodes on the pial surface of the cortex. It was, therefore, necessary to predict the location of the somatosensory cortex for electrode placement from cranial surface topography. The somatosensory region of the sheep cortex has been shown to be caudal to the cruciate gyrus (20). It is presumed that the sheep homunculus is similar to other mammal body structures, i.e., the face, head, neck, represented laterally, and the caudal body structures such as the hindlimb located medially. This means that the trunk would be represented in an intermediate position similar to the location reported for intercostal muscle representation (8). The electrode location in these animals was placed on the surface of the dura over the lateral edge of the somatosensory region, presumably near the head and face region. The observation of the P1 in all face mask experiments supports this lateral placement of the cortical electrodes.

The endotracheal tube, however, bypasses the mechanoreceptors in the face, nose, pharynx, and larynx, leaving only lower airway, lung, and respiratory pump mechanoreceptors exposed to the occlusion stimulus. The cortical activation with the face mask occlusion and the absence of this P1 with endotracheal tube occlusions in some animals suggests a specific face,
nose, and/or upper airway mechanoreceptor activation of this region of the somatosensory cortex in three of eight animals. The presence of the P1 in five of eight animals with occlusion presented through the endotracheal tube demonstrates a cortical activation selectively from lower airway, lung and/or respiratory pump mechanoreceptors. The upper airway, nose, and face cannot be contributing to this response because of the bypass of these structures by the endotracheal tube. These results demonstrate that inspiratory occlusion produces ventilatory mechanical changes that activate mechanoreceptors projecting to somatosensory cortical neurons by an unknown pathway. These results further demonstrate inspiratory occlusion-activated mechanoreceptors in the face, nose, pharynx, and/or larynx that activate cortical neurons and that mechanoreceptors in the lower airways, lung, and/or respiratory pump also activate somatosensory cortical neurons. Thus somatosensory cortical representation elicited by inspiratory occlusion probably occurs from multiple respiratory mechanoreceptor populations.

The specific location of the neurons mediating this evoked potential remains unknown. The electrodes were on the dural surface of these lambs. Specific localization of cortical columns requires electrodes placed on the pial surface of the cortex (8–10) or microelectrodes inserted into the cortex (17). In the present study, the electrodes were separated from the pial surface by the arachnoid, dura, and cerebral fluid. This separation is much better than scalp recordings but may have resulted in recording activity conducted at a distance from the neurons generating the dipole. Although this resulted in a less specific localization of the neural generator than pial surface recordings, the recordings are much more specific to the somatosensory cortex than scalp recordings. Electro cortical potentials recorded with this type of electrode can be generated by neuronal activity in the cerebral cortex or subcortical structures (1, 11, 18, 21). If the P1 of the lamb evoked potential was of subcortical origin, then the activity recorded would be similar for both the face mask and endotracheal tube conditions (both of these conditions elicit a P1). This similar peak would be because the deep neural generator would produce an electrical current from the subcortical nucleus, and the electrodes would be nearly the same distance from the neural generator over the entire somatosensory cortical area. The electrodes would record the current of similar amplitude for both face mask and endotracheal tube conditions from this generator with little effect of the medial-lateral placement of the electrodes over the somatosensory region. However, if the evoked neural activity is of cortical origin, then the probability that the electrode would record the activated cortical column dipole would depend on the proximity of the electrode to the activated cortical column. If the electrode was placed too far away from an activated cortical column, then no evoked potential would be observed with this placement. The fact that an evoked potential was observed in some animals when the electrode was placed on the lateral edge of the somatosensory cortex (closest to the head and neck region) with face mask occlusions, and not with endotracheal tube occlusions, suggests that the P1 is most likely of cortical origin. However, future studies with pial surface electrodes and microelectrodes will be necessary to specifically determine the site of the neural generator of the lamb RREP P1. These results do, however, support a somatosensory origin of the RREP in lambs and humans.

Animal studies of the neural mechanisms mediating respiratory mechanical load sensations are limited. An adult animal model of resistive load detection was developed and studied in our laboratory. Adult dogs with a tracheal stoma were behaviorally conditioned to signal the detection of inspiratory resistive loads and occlusions (5). The resistive load detection threshold and Weber fraction was found to be similar to those of humans breathing through a mouthpiece. The application of the loads through the dog stoma, similar to the endotracheal tube condition in the present study, excluded afferent systems in the mouth, nose, pharynx, larynx, and upper trachea from mediating this sensation. The remaining afferents would be lower airway, lung and respiratory muscle mechanoreceptors. Thus respiratory load detection can occur without activation of upper respiratory and facial mechanoreceptors. The present study extends these observations by the demonstration that inspiratory mechanical loads specifically elicit somatosensory cortical neural activity in awake, spontaneously breathing lambs with the face and upper airways excluded. These results further suggest that activation of the somatosensory cortex may be one component of the neural mechanism mediating respiratory load sensation.

The activation of cortical neurons by mechanical loads has been studied by using evoked-potential techniques similar to those routinely used in other somatosensory systems. An inspiratory occlusion was applied while simultaneously recording from the scalp over the somatosensory region of the cortex in adult humans (3, 6, 15, 19, 22). Signal averaging of occlusion-elicited evoked potentials was similar to the present study, and an analogous P1 appears to be generated in the somatosensory region of the cortex (3, 15, 23). Also similar to the present study, the P1 of the RREP was found bilaterally (3, 15, 19, 23). The P1 of the human RREP was a positive voltage suggested to be due to the dipole that occurs when a cerebral cortical column was depolarized by the arrival of activity from a population of afferents activated by the occlusion stimulus (14). This P1 in humans is similar to the P1 recorded from the cortex in this study’s awake lambs, supporting the hypothesis that inspiratory mechanical loads elicit cortical neural activity in the somatosensory cortex. The observation of an inspiratory occlusion-related somatosensory evoked potential with an endotracheal tube bypassing the mouth, pharynx, and larynx differs from the absence of a negative pressure-elicited evoked response in humans with a laryngeal mask (2). An evoked response was elicited by large negative pressures (−10 cmH2O) applied to the mouth of humans, but this cortical activity was attenuated when the
mouth and pharynx were bypassed with a laryngeal mask. It is not surprising that a respiratory motor-driven stimulus (inspiratory occlusion) differs from an externally applied pressure stimulus. The application of a negative pressure to the breathing circuit will stimulate pressure-related mechanoreceptors with or without active respiration. An inspiratory occlusion requires an active inspiratory effort for a mechanical stimulus to occur. The afferent populations activated by the negative pressure stimulus would be primarily airway mechanoreceptors, which, on the basis of the evoked response results, are in the upper airways. Inspiratory occlusion produces much smaller negative evoked response results, are in the upper airways. Thus the use of negative pressure would elicit an evoked response with mouth or face mask breathing but not with the laryngeal mask, whereas the inspiratory occlusion-evoked potential would be present with and without intact upper airways.

The observation of the occlusion-related activation of the cerebral cortex in the awake, spontaneously breathing lamb demonstrates that 1) this mechanical load stimulates respiratory tract and/or muscle mechanoreceptors; 2) face, nose, upper airway, lower airway, lung, and/or respiratory pump muscle mechanoreceptors may activate cortical neurons; and 3) these mechanoreceptor-activated neurons are in the somatosensory region of the cerebral cortex. This lamb inspiratory interruption occlusion-elicited evoked potential is analogous to the P1 of the RREP recorded in human studies (15). The afferent pathways, cortical distribution, and neural mechanisms for respiratory sensation remain unknown. The chronically instrumented, awake, spontaneously breathing lamb appears to be a useful animal model for mechanical load-elicited RREP studies. Future studies are needed to determine the specific neural pathways and mechanoreceptors mediating the somatosensory cortical activation by ventilatory changes induced with mechanical loads.

The technical assistance of Sondi Mohrman is appreciated. This study was supported by a grant from the American Lung Association of Florida.

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