Effects of experimental cortical seizures on respiratory motor nerve activities in piglets

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Terndrup, Thomas E., Robert Darnall, Susan L. Knuth, and Donald Bartlett, Jr. Effects of experimental cortical seizures on respiratory motor nerve activities in piglets. J. Appl. Physiol. 86(6): 2052–2058, 1999.—Airway obstruction at the level of the larynx causes respiratory insufficiency during experimental seizures in spontaneously breathing, anesthetized piglets (T. E. Terndrup and W. E. Fordyce, Pediatr. Res., 38: 61–66, 1995). To investigate further the neural mechanisms of this obstruction, the activities of the phrenic nerve (PH) and the recurrent laryngeal motor branches to the thyroarytenoid (TA) and posterior cricoarytenoid (PCA) muscles were analyzed in 11 anesthetized, vagotomized, paralyzed, and ventilated piglets. After a control recording period, seizures were induced by subcutaneous penicillin G injections. Compared with baseline conditions, nerve activities became irregular during seizures. Extrinsic TA bursts during PH activation were evident in all piglets during seizures. During ictal phases of seizures, the peak integrated activities of the PH and the expiratory component of the PCA, but not TA or inspiratory PCA activities, were significantly decreased compared with interictal phases. During seizures, a significant delay in the onset of the inspiratory component of PCA activation with respect to the onset of the PH was observed. This study helps to explain respiratory impairment during cortical seizures by providing evidence of impaired timing of activation of laryngeal dilator mechanisms and coordination with those activating the diaphragm. Cyclic PH inhibition during high-intensity cortical discharges may provide a secondary mechanism producing respiratory insufficiency during seizures.

Seizures induced with subcortical penicillin G have been reported to increase ventilation and produce respiratory alkalosis in spontaneously breathing animals (13). Increased phrenic and upper airway nerve activities have also been demonstrated in anesthetized, paralyzed, and artificially ventilated cats (14). However, spontaneously breathing, anesthetized piglets with intact upper airways develop laryngeal airflow obstruction and hypoventilation during generalized seizures, whereas tracheotomized piglets do not (20). These studies suggest that laryngeal airway control and coordination with activation of the diaphragm may be unfavorably altered by experimental seizures.

We undertook the present investigation to understand better the neural mechanisms underlying laryngeal obstruction during cortically induced seizures. We chose to study the activities of a laryngeal adductor (the TA) and abductor (the PCA). In anesthetized animals, activation of the phrenic nerve (PH) is preceded by activation of the PCA branch of the recurrent laryngeal nerve (RLN), presumably preparing the larynx for inspiratory airflow. The sudden decrescendo of PH activity is normally followed nearly simultaneously by activation of the TA branch of the RLN, presumably preparing the larynx for inspiratory airflow. The sudden decrescendo of PH activity is normally followed nearly simultaneously by activation of the TA branch of the RLN, ultimately causing laryngeal constriction and presumably braking expiratory airflow and maintaining functional residual capacity. Uncoordinated activation of these two RLN motor branches may lead to laryngeal obstruction in two ways: 1) a reduced or absent late expiratory PCA activation or a delay in the onset of inspiratory PCA activation with respect to PH activation, and 2) by activation of the TA during neural inspiration.

**METHODS**

Thirteen piglets of either sex (weighing 2.2–5.8 kg, 10–13 days of age) were anesthetized with 3–4% halothane. Catheters were placed in a femoral artery and vein, and the trachea was cannulated low in the neck, taking care to avoid injury to the RLNs. Halothane was discontinued, while an induction dose of alphaxalone-alphadolone acetate was tetrated (Saffan, Pittman Moore; 9 mg/kg iv). Maintenance anesthesia was provided by an iv infusion of 1.1–3.2 mg·kg⁻¹·h⁻¹, which was continued throughout the experiment. Alphaxalone-alphadolone acetate was chosen because it reportedly interferes less with the integrative activities of the forebrain than do most general anesthetics and was utilized in the intact piglet experiments that demonstrated laryngeal obstruction (20). Animals were paralyzed with gallamine triethiodide (13.5 mg·kg⁻¹·h⁻¹) iv) and ventilated with 100% oxygen by a pump at 16–20 breaths/min. The stroke volume of the pump was adjusted so that the CO₂ concentration of end-tidal gas, sampled from the tracheal
cannula and measured by an infrared analyzer, was ~5.9–6.3%. This mild level of hypercapnia was chosen to ensure that all nerves would exhibit clear respiratory activity in the baseline state. A scalp flap was elevated, and bilateral temporal craniotomies were performed for epidural placement of silver-wire electrodes and induction of seizures by intracortical penicillin injection (see below). Body temperature was monitored with a rectal probe and servo-regulated at 38°C with a heating pad. Arterial pressure was monitored through a femoral catheter; the mean value averaged 70–90 mmHg and remained >50 mmHg during all experiments. The bladder was emptied to prevent bladder distension and reflex effects on respiratory activity.

Piglets were vagotomized within the thorax, caudal to the RLN branch points, to abolish the influence of lung volume on the breathing pattern, while the motor activity to the RLN branches was left intact. We were not entirely successful in the first of these purposes, as 8 of the 11 animals had their baseline ventilatory activity entrained by the pump, even after vagotomy. Presumably, this reflected residual vagal afferent activity from airway receptors rostral to the section, as we have never seen such entrainment after cervical vagotomy. Bipolar wire electrodes were used to record activities of the central cut ends of a PH nerve (12 pigs) and motor branches of the RLN to the TA (13 pigs) and PCA (7 pigs) muscles. These activities and the electrocorticogram (ECoG) were amplified, filtered (0.03–50 kHz), monitored on an oscilloscope and audio system, and recorded on VHS tape. Baseline recordings were obtained when blood pressure and neural activities were stable.

After baseline records had been obtained, an aqueous solution of penicillin G (100,000 U in 0.05 ml) was injected into the left parietal cortex at the marginal or suprasylvian gyrus ~3 mm below the surface with a 27-gauge needle and microsyringe. Repeated injections of penicillin (100,000 U) were given into the same area at 20-min intervals until generalized ictal-interictal cycling occurred. Seizure activity was judged to be present when large-amplitude spiking activity was evident in the ECoG signal. Ictal-interictal cycling was judged to be present when the ECoG demonstrated periods of dense spiking alternating with periods of reduced spiking. After the 9–12 ictal-interictal cycles, phenobarbital (20 mg/kg) was given by slow iv infusion (of 5-min duration) to assess the effects of this anticonvulsant, with respect to the end of a PH burst for PCA. The mean burst timing offset was determined for each experimental condition for the inspiratory component of the PCA. The frequency of extraneous TA bursts was determined by counting the number of TA bursts occurring during 10 PH cycles for each condition. Then, to determine the peak heights of the integrated PH and PCA neurograms above their late-expiratory baselines for each condition, the respiratory neural activities were integrated with leaky integrator circuits, by using a time constant of 50 ms. The phasic expiratory peak heights of the TA were similarly determined.

Statistical comparisons were performed by using the Friedman repeated-measures analysis of variance on ranks for Ti, Te, respiratory cycle duration, the timing of the inspiratory component of the PCA burst onset with respect to PH, the proportion of breaths with extraneous TA activation, and the peak heights of integrated activities of motor nerves during baseline, interictal, and postphenobarbital conditions. When significant differences, i.e., P < 0.05, occurred between experimental conditions, pairwise comparisons were performed by using the Student-Newman-Keuls method or Bonferroni t-test for nonparametric and continuous variables, respectively.

RESULTS

After penicillin injection into the parietal cortex, gradual increases in ECoG spike frequency and amplitude were observed in 11 of 13 piglets. Data for two piglets were eliminated from analysis because no interictal-ictal cycling developed after repeated penicillin injections. In responding piglets, the amount of subcortical penicillin required before the development of ictal-interictal cycling varied from 100,000 to 300,000 U (mean ± SD; 225,000 ± 75,000 U). There was no significant change in end-tidal CO2 concentration during seizures, although transient increases of ~0.2–0.3% were observed during ictal discharges when arterial blood pressure increased by 20–30 mmHg.

There were significant reductions in Ti and Te and increases in frequency during representative ictal and interictal periods (Table 1), compared with control, in these piglets. After phenobarbital, respiratory intervals approached their baseline levels.

The peak heights of mean integrated PH neurograms were significantly increased during interictal seizure periods, compared with other conditions (Table 2). Integrated PH peak heights were also increased during ictal periods, on average, but the variance of this response was large, and it did not achieve statistical significance. The peak heights of the mean integrated expiratory component of the PCA neurograms were significantly increased during ictal, interictal, and postphenobarbital conditions, compared with baseline control. During ictal conditions, the mean peak integrated heights of the PH and expiratory component of the PCA nerves were significantly reduced, compared with interictal conditions. The mean peak integrated activities of the TA and the inspiratory phase of the PCA nerves were not significantly different during seizures, compared with baseline. After phenobarbital, nerve activities were generally reduced and approximated those under baseline conditions, except in the case of expiratory activity of the PCA, which remained increased.

Mean values were determined for each experimental condition for inspiratory duration (Ti, the time from the onset of PH activity until its sudden decrescendo), expiratory duration (Te, the time from the end of Ti until the onset of the next PH burst), and respiratory cycle duration by using nonintegrated nerve activities. The timing of the activation of the TA and the inspiratory component of the PCA with respect to PH activity was examined by analyzing the onset of these bursts with respect to the end of a PH burst for TA and the onset of a

PH burst for PCA. The mean burst timing offset was determined for each experimental condition for the inspiratory component of the PCA. The frequency of extraneous TA bursts was determined by counting the number of TA bursts occurring during 10 PH cycles for each condition. Then, to determine the peak heights of the integrated PH and PCA neurograms above their late-expiratory baselines for each condition, the respiratory neural activities were integrated with leaky integrator circuits, by using a time constant of 50 ms. The phasic expiratory peak heights of the TA were similarly determined.

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Respiratory intervals, timing relationships, heart rate, and arterial blood pressure in 11 pigs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Baseline</th>
<th>Interictal</th>
<th>Ictal</th>
<th>Postphenobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>ti, s</td>
<td>11</td>
<td>0.90 (0.75–1.0)</td>
<td>0.63 (0.55–0.96)</td>
<td>0.59 (0.40–0.90)</td>
<td>0.94 (0.71–1.3)</td>
</tr>
<tr>
<td>Te, s</td>
<td>11</td>
<td>1.6 (1.4–1.7)</td>
<td>0.99 (0.90–1.2)</td>
<td>0.89 (0.79–1.3)</td>
<td>1.81 (1.5–2.2)</td>
</tr>
<tr>
<td>Frequency, min⁻¹</td>
<td>11</td>
<td>25 (±1.8)</td>
<td>36 (±2.8)</td>
<td>42 (±3.8)</td>
<td>23 (±2.1)</td>
</tr>
<tr>
<td>PH-PCA, ms</td>
<td>6</td>
<td>16.8 (9.0–30)</td>
<td>-12.5 (–17.5–16.3)</td>
<td>-9.7 (–21.1–3.8)</td>
<td>12.6 (3.0–21.1)</td>
</tr>
<tr>
<td>TA-PH</td>
<td>11</td>
<td>14.7 (11.3–15.5)</td>
<td>37.9 (18.0–64.8)</td>
<td>19.9 (16.0–27.0)</td>
<td>22.9 (21.5–24.8)</td>
</tr>
<tr>
<td>Heart rate, min⁻¹</td>
<td>11</td>
<td>285 (268–294)</td>
<td>301 (292–308)</td>
<td>304 (273–315)</td>
<td>268 (246–281)</td>
</tr>
<tr>
<td>Arterial pressure, mmHg</td>
<td>11</td>
<td>85 (72–89)</td>
<td>93 (77–109)</td>
<td>88 (70–128)</td>
<td>66 (50–82)</td>
</tr>
</tbody>
</table>

Table 2. Peak integrated nerve activities in 11 pigs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Interictal</th>
<th>Ictal</th>
<th>Postphenobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>11</td>
<td>158± (89–151)</td>
<td>144 (65–160)</td>
<td>97± (52–146)</td>
</tr>
<tr>
<td>Expiratory</td>
<td>6</td>
<td>307± (89–151)</td>
<td>231± (65–160)</td>
<td>265± (52–146)</td>
</tr>
<tr>
<td>PCA, Inspiratory</td>
<td>6</td>
<td>155 (87–184)</td>
<td>120 (54–184)</td>
<td>138 (93–151)</td>
</tr>
<tr>
<td>TA</td>
<td>11</td>
<td>81 (53–104)</td>
<td>93 (62–113)</td>
<td>62 (49–66)</td>
</tr>
</tbody>
</table>

The increased ECoG spiking intensity during seizures was associated with irregular nerve activity (Fig. 1). During ictal seizures, the PH and PCA had an intermittent or stuttering pattern. Extraneous TA bursts during neural inspiration were often evident during seizures. Increased tonic activity was often observed in the PCA neurogram. After phenobarbital, ECoG spiking was reduced, and nerve activities were similar to those under baseline conditions.

The phasic timing among PH, TA, and PCA neurograms was influenced by experimentally induced seizures, especially during ictal phases. During seizures, extraneous TA bursts often occurred during neural mid-inspiration, frequently accompanied by a reciprocal inhibition of PH and PCA activity (Fig. 2). The percentage of abnormal, extraneous TA bursts during seizures was significantly greater than during baseline conditions (Table 1). Under baseline conditions, the onset of activation of the inspiratory component of the PCA always preceded that of the PH. However, in animals in which PCA activity was recorded, there was a significant delay in the onset of activation of the inspiratory component of the PCA during seizures (Fig. 3).

During prolonged ictal discharges, periods of reduced integrated peak phasic neural activities were observed to some extent in all 11 pigs. A representative response (Fig. 4) from one piglet demonstrates the sudden degradation in integrated activities, especially in the PH and PCA neurograms, associated with a high-intensity cortical discharge. The peak integrated activities of the PH, TA, and PCA return to their pre-high-intensity cortical discharge levels when the cortical discharge intensity lessens.

**DISCUSSION**

These results confirm previous findings of increased ventilation or PH activity in cats during experimental, cortical seizures (13, 14). Progressive increases in minute ventilation were observed during the development of generalized seizures induced with subcortical penicillin in spontaneously breathing, tracheotomized cats (13). In a follow-up investigation, vagotomized, glomectomized cats demonstrated progressive increases in peak phrenic activity and respiratory frequency during seizures (14). The present study demonstrates that, in addition to respiratory nerve stimulation, seizures produce irregular activation and altered timing in the activation of the TA and the inspiratory component of the PCA with respect to PH activation.

Increased peak integrated nerve activity, respiratory frequency, and expiratory activation of nasolabial and hypoglossal nerves have also been demonstrated in cats during cortical seizures (21). Furthermore, cortical seizures entrained laryngeal respiratory nerve activities, especially those of the TA branch, during prolonged ictal discharges (21). The findings of the present study suggest a mechanism by which upper airway obstruction may occur during seizures by demonstrating impaired timing of the activation of nerves, which results in laryngeal constriction and dilation with respect to the PH.

Our findings also indicate that, in young pigs, cyclical phrenic inhibition during high-intensity phases of ictal discharges reduces the peak integrated phrenic activity significantly below the activation observed during other phases of seizures. This cyclical inhibition of phrenic activity was observed in all piglets during ictal discharges and resulted in a significant reduction in the peak integrated activity of phrenic discharges during the ictal phases of seizures. This was not observed in adult cats under otherwise identical experimental seizure conditions, a finding suggesting either species or developmental differences. Synchronization of inspiratory motoneurons in the medium-frequency bands is more apparent in the early development of piglets studied during the first month of life (18). In these piglets, phrenic and RLN discharges contained peaks in the medium-frequency band, which are indicative of...
common inputs. Although phrenic spectra demonstrated peaks in the high-frequency band, such peaks were uncommon in RLN spectra. We speculate that the high-intensity cortical discharges during seizures may lead to more frequent or profound entrainment of PH activities or that Ti could be sufficiently shortened to result in decreased peak PH activity.

During seizures, there was a significant delay in the onset of activation of the inspiratory component of the PCA, compared with control conditions. RLN activity normally precedes phrenic activation (5), and PCA muscle activity precedes inspiratory airflow (2). During systemically induced seizures, anesthetized, spontaneously breathing piglets demonstrated reduced glottal area at the onset of inspiratory pressure changes during the ictal and interictal phases of seizures, compared with hypoxic rebreathing, and after anticonvulsants reduced seizure activity (20). These data demonstrate the functional importance of alteration in the timing or magnitude of PCA activation during seizures in producing a laryngeal airway with limited flow resistance.

How might the spiking activities of cortical discharges during seizures influence respiratory nerve activity? Evidence of entrainment of respiratory nerve activities during seizures has been reported. Paydarfar...
et al. (14) reported entrainment in the relationship between ECoG spikes and PH activity in cats. An acute intercollicular decerebration in these cats resulted in normalization of the respiratory rhythm. The authors concluded that an important cause of the respiratory response is by a feed-forward mechanism, whereby activation of subcortical suprapontine structures results in stimulation of breathing. We have also previously reported entrainment of PH and TA nerve activities by large-amplitude ECoG spikes in anesthetized, ventilated cats during seizures (21). The present study expands our understanding of how seizures may affect the respiratory control system, by providing evidence of altered timing of respiratory nerve activities controlling laryngeal responses. The alterations in timing favor laryngeal constriction and would be expected to increase resistance to inspiratory airflow.

There are few other states or diseases that are characterized by poorly coordinated respiratory neural activities in laryngeal motor nerves. Suppression of upper airway stabilizing muscle activity has been reported in non-rapid-eye-movement sleep and with certain anesthetics (1, 8, 9, 11). Hiccups have been characterized as abrupt, inspiratory muscle contractions resulting in brief inspiratory flows terminated by upper airway or laryngeal closure (3). Hiccuping spells are characterized by hyperventilation and respiratory alkalosis in intubated human infants, whereas infants with intact upper airways developed an increase in obstructed breaths, decreased respiratory frequency, decreased minute ventilation, and apnea. Glottal or upper airway closure briefly persists in adults after the onset of a hiccuping spell (6, 15). Paradoxical movement of the vocal folds has also been described with...
functional problems producing stridor or increased translaryngeal resistance when voluntary relaxation produces excessive vocal-fold closure (16). However, hiccups are thought to activate both adductor and abductor muscles simultaneously, whereas we often found reciprocal inhibition of abductor (PCA) activity when adductor (TA) nerve activities occurred during seizure conditions.

Phenobarbital reduced cortical ECoG spiking and generally returned nerve activities toward those observed under baseline conditions in our piglets. Benzodiazepines and barbiturates are widely used in the clinical treatment of generalized seizure states (4, 17). These anticonvulsant agents are well known to have respiratory effects, and some evidence points to their selective depressant effects on upper airway motor nerve activities (1, 19). The results of this study are largely in agreement with the expected effects of phenobarbital, with the exception that, after administration, the nerve activities returned to approximately baseline levels rather than to more depressed levels. These responses probably reflect a balance of the seizure-related excitation and drug-related depression.

Generalized seizure activity is associated with loss of coordinated muscular activity, resulting clinically in falls and tongue biting. The inability of the central nervous system to modulate respiratory responses properly may be responsible for some of the respiratory complications observed during seizures. It is well known that patients with generalized seizures become cyanotic and may develop respiratory acidosis if seizures are prolonged. The acute treatment of human seizures with anticonvulsants may depress respiratory function and often requires endotracheal intubation (4) and artificial ventilation (19). Other respiratory complications, such as apnea and aspiration pneumonitis, are also reported (12). Once the upper airway recovers or is controlled with a tracheal tube and the effects of anticonvulsants have dissipated, adequate spontaneous ventilation usually resumes (23).

Tracheotomized piglets develop hyperpnea and hypocarbia during experimental seizures, whereas piglets breathing through intact upper airways develop subglottic obstruction and respiratory acidosis (20). Paydarfar et al. (13) studied a single, spontaneously breathing cat in which the upper airway was intact; no evidence of upper airway obstruction was observed in this animal. Thus experimental seizures appear to produce a neurological response that favors upper airway obstruction in species with susceptible airways, but obstruction is not a universal response.

What neural pathways may be involved in altering the coordination of inspiratory and expiratory motoneurons during seizures? The excitation of respiratory nerves and the entrainment of nerve activities to cortical spikes during seizures suggest that the propagation of cortical seizure discharges includes activation of brain stem structures. There is some evidence of an overlap in the activation of a population of respiratory neurons in the ventral, lateral medulla by hypoxia and seizures (10). This convergence of neural pathways of activation by hypoxia and seizures may help us to understand how these challenges are handled by brain stem structures.

There are several limitations to the present investigation into the respiratory consequences of cortical seizures. Although general anesthesia was required for these studies, its presence reduced ventilatory activity to some extent. Our model eliminated the important influences of secondary reflexes from respiratory movements and the muscular exercise of actual seizure activity. This choice simplified the interpretation of the results but at the expense of deviating greatly from the conditions of naturally occurring seizures. As noted in METHODS, the intrathoracic vagotomies may not have been complete (Fig. 4), allowing some contribution from stretch-receptor activation to the nerve activities. Finally, our piglets were maintained in a steady state of hyperoxia and mild hypercapnia, whereas changes in blood gases during true seizures occur as a consequence of alterations in both ventilation and metabolism. Such changes may then modify secondarily the ventilatory responses to seizure activity.

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