Focal warming in the nucleus of the solitary tract prolongs the laryngeal chemoreflex in decerebrate piglets

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Submitted 27 June 2006; accepted in final form 2 September 2006

Xia L, Damon TA, Leiter JC, Bartlett D Jr. Focal warming in the nucleus of the solitary tract prolongs the laryngeal chemoreflex in decerebrate piglets. J Appl Physiol 102: 54–62, 2007. First published September 7, 2006; doi:10.1152/japplphysiol.00720.2006.—The laryngeal chemoreflex (LCR), elicited by a drop of water in the larynx, is exaggerated by mild hyperthermia (body temperature = 40–41°C) in neonatal piglets. We tested the hypothesis that thermal prolongation of the LCR results from heating the nucleus of the solitary tract (NTS), where laryngeal afferents first form synapses in the brain stem. Three- to 13-day-old piglets were decerebrated and vagotomized and studied without anesthesia while paralyzed and ventilated. Phrenic nerve activity and rectal temperature were recorded. A thermode was placed in the medulla, and the brain tissue temperature was recorded with a thermistor ~1 mm from the tip of the thermode. When the thermode was inserted into the brain stem, respiratory activity was arrested or greatly distorted in eight animals. However, the thermode was inserted in nine animals without disrupting respiratory activity, and in these animals, warming the medullary thermode (thermistor temperature = 40–41°C) while holding rectal temperature constant reversibly exaggerated the LCR. The caudal raphe was warmed focally by ~2°C in four additional animals; this did not alter the duration of the LCR in these animals. Thermodes placed in the NTS did not disrupt respiratory activity, but they did prolong the LCR when warmed. Thermodes that were placed deep to the NTS in the region of the nucleus ambiguous disrupted respiratory activity, which precluded any analysis of the LCR. We conclude that prolongation of the laryngeal chemoreflex by whole body hyperthermia originates from the elevation of brain tissue temperature within in the NTS.

THE LARYNGEAL CHEMOREFLEX (LCR) is elicited by fluid in the larynx or hypopharynx. This inhibitory reflex consists of a variety of behavioral responses including swallowing, cough, apnea, bradycardia, and redistribution of blood flow to vital organs. The manifestations of the LCR share may features with the diving reflex, although the afferent limb of the diving reflex is in the trigeminal nerve and the afferent limb of the LCR is in the superior laryngeal nerve (SLN). The responses comprising the LCR change as animals age (3, 43). The reflex is more prominent in younger animals and consists of swallowing, cough, apnea, bradycardia, and redistribution of blood flow. In adults, the apnea and bradycardia are replaced by increased cough and swallowing, and the respiratory disruption is significantly attenuated compared with neonates. Many investigators have theorized that the chain of events leading to the sudden infant death syndrome (SIDS) is precipitated by the LCR (3, 8, 35, 36, 43–45). For example, the LCR is stimulated by gastroesophageal reflux, which occurs often in the normal course of events in all infants, and the duration of the LCR may be quite prolonged because the low pH of gastric contents may enhance the strength of the LCR (31, 37, 45). Thus anything that increases the frequency of the LCR or enhances the duration of respiratory disruption associated with it might be expected to increase the risk of SIDS.

Many biological, sociological, and environmental risk factors have been identified in epidemiological studies of SIDS. One environmental factor associated with an increased risk of SIDS is overheating. This may occur as a result of covering the baby’s head, excessive covers or increased room temperature (5, 13, 22, 25). A possible physiological basis for the relationship between overheating and SIDS was first identified by Haraguchi et al. (16). These investigators stimulated the SLN electrically and demonstrated that the latency and threshold for thyroarytenoid muscle activation decreased as body temperature was increased from 34 to 41°C in anesthetized puppies and adult dogs. The reduction in threshold as body temperature increased was much greater in younger animals, especially in neonates compared with adult dogs. Thus, overheating might increase the risk of SIDS by enhancing the respiratory inhibition associated with SLN stimulation via the LCR. To test this hypothesis explicitly, we conducted a study in decerebrate neonatal piglets in which we elicited the LCR by infusing a drop of distilled water into the laryngeal airway before and after elevating body temperature by ~2°C (6). Elevating body temperature in these animals significantly prolonged the LCR and prolonged the associated apnea. This effect cannot be attributed to an effect of elevated body temperature on the responsiveness of laryngeal water receptors, which initiate the LCR (3, 8, 27), because the receptor response to stimulation of the larynx with water is unaltered by elevating body temperature (49).

Thus the thermally induced prolongation of the LCR is centrally mediated, but the structures within the central nervous system that might be responsible have not been identified. Afferent information from laryngeal water receptors is conducted centrally in the SLN, which projects to the nucleus of the solitary tract (NTS; Refs. 17, 40). Therefore, we tested the hypothesis that focal heating of the NTS would prolong the LCR. These studies were conducted in decerebrated neonatal piglets, and the results indicate that focally increasing the brain temperature in the region of the NTS significantly prolongs the respiratory inhibition associated with the LCR; in contrast, elevating the temperature of the caudal raphe and other medullary regions outside the NTS does not alter the duration of the LCR.

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METHODS

The Institutional Animal Care and Use Committee of Dartmouth College approved the protocols in these studies. We studied a total of 21 piglets aged 8.1 ± 0.7 days (means ± SE; range 3–13 days) with body weights ranging from 1.5 to 5.5 kg (average weight = 3.4 ± 0.2 kg). Piglets were housed with the sow in our animal care facility before they were studied.

**Surgery.** Animals were anesthetized with 2% halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Laboratories, NJ) in O2. A rectal probe was inserted, and body temperature was maintained between 37 and 38°C using a heating pad. Femoral arterial and venous catheters were inserted to measure blood pressure and administer drugs, respectively. Each animal was tracheostomized and artificially ventilated (dual-phase respirator, Harvard Apparatus, South Natick, MA) to maintain end-tidal CO2 at ~5%. The carotid sinus regions were exposed bilaterally, and the internal and external carotid arteries were ligated to facilitate decerebration. The vagus nerves were sectioned bilaterally to prevent phrenic entrainment to the mechanical ventilator rhythm. The animal was placed prone, and the head was positioned in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). The skull was opened, and the animal was decerebrated at the level of the superior colliculi. All brain tissue rostral to the section was removed by suction. Following decerebration, halothane anesthesia was discontinued, and each animal was paralyzed using pancuronium bromide (1 mg/kg iv; Elkins-Sinn, Cherry Hill, NJ). Supplemental doses of pancuronium were given as required, usually at a rate of 0.5 mg·kg⁻¹·h⁻¹. A phrenic nerve was exposed and sectioned, and the central cut end was placed on a bipolar recording electrode to monitor respiratory output. Phrenic activity was amplified (Gould Universal Amplifier, Cleveland, OH), and the moving time average (“integrated activity”) was calculated electronically (100-ms time constant; CWE, Ardmore, PA). Peak integrated phrenic nerve activity, phrenic frequency, body temperature, end-tidal CO2, and blood pressure were recorded on a computer (PowerLab, ADI, Sydney, Australia) for later analysis.

**Eliciting the LCR.** Following decerebration, each animal was left for at least 30 min to stabilize before any further interventions. A small-diameter polyethylene tube was inserted into one nostril and placed with its tip at the caudal extent of the soft palate, just rostral to the larynx. The correct position of the tube was visually checked with a laryngoscope before the outer portion of the catheter was fixed to the nose. The LCR was produced by infusing 0.1 ml of distilled water into the larynx through the nasal tube during inspiration. The water was close to body temperature, having equilibrated in the catheter within the larynx. The correct position of the tube was visually checked with a laryngoscope before injection. In previous studies, the duration of the LCR provided a single measurement that captured the multiple events that may constitute the reflex (6, 47). We did not analyze any of the cardiovascular manifestations of the LCR because the piglets in this study were vagotomized, and the heart rate and blood pressure responses, which are seen in intact animals, were markedly attenuated. We defined the duration of the LCR as the period of respiratory instability (defined as variability of phrenic amplitude and/or respiratory timing) from the beginning of the breath during which water was injected into larynx to the onset of at least five regular breaths. These five breaths did not need to have the same frequency or amplitude as the control breaths; we simply required that they be regular. The LCR measured in this way included both periods of unstable respiratory activity and apneas. The apneic periods during the LCRs were also measured and analyzed separately. Apnea was defined as a cessation of breathing greater than the duration of the two breaths preceding the breath during which the LCR was elicited. Animals were allowed to recover for a minimum of 10 min before the LCR trial was repeated. We elicited the LCR at least three times in each experimental condition and averaged responses within a treatment condition to obtain a measure of the LCR in each piglet in each treatment condition.

**Focal heating of the brain stem.** We made a thermode following a design developed by Brown et al. (4). The thermode was made from two hypodermic needles. A 21-gauge needle with the end left open, was placed within a 17-gauge needle. The tip of the larger needle was sealed with solder and was water tight. The inner needle was ~33 mm long and extended to within 1–2 mm of the sealed tip of the outer needle. Warm water was circulated through the needle tip and out through the outer needle. The water was heated in a water bath and circulated through the thermode using a Millipore peristaltic pump at a flow rate of 35–40 ml/min. The temperature of the water leaving the thermode ranged from 36 to 37°C in the control condition from 40 to 41°C during the tissue heating studies. An additional thermistor was inserted adjacent to the thermode in the brain stem and recorded brain tissue temperature ~1 mm from the tip of the thermode. We did not measure the temperature at the tip of the thermode. We controlled the water temperature as it entered the thermode, and we measured the temperature of the effluent. The temperature of the water bath varied between 43 and 53°C depending on the baseline temperature of the animal. The temperature of the fluid leaving the thermode was 0.4°C cooler than the brain temperature measured 1 mm from the thermode tip. Thus, we assume that there was some heat loss in the brain tissue around the thermode, and this probably varied in proportion to the local blood flow. Before insertion of each thermode into the brain stem, the tip of the thermode was coated with a fluorescent dye, 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate (DiI; catalog no. D-3911, Invitrogen, Carlsbad, CA). This dye is sticky and was not washed off during dissection or tissue processing. It effectively marked the tip location of each thermode, which we subsequently identified in anatomic studies of the brain stem in each animal.

**Experimental protocol.** After the decerebration, the thermode, held by a micromanipulator, was slowly introduced into the brain stem tissue on the left side of the brain. Once the thermode was in position, the animal was allowed a period of stabilization for 15–30 min. Studies began with a control period during which the thermode was perfused with water at 37–38°C, and brain tissue temperature was approximately equal to body temperature. The LCR was elicited three times. Next, the brain stem tissue temperature was elevated ~2°C by circulating heated water through the thermode (water temperature 40–41°C) while rectal temperature remained at the baseline value. Once the brain stem temperature reached a stable elevated temperature, the LCR was elicited three more times. Subsequently, the thermode was perfused with water at 37–38°C, and the LCR was elicited a final three times in this follow-up control period. The baseline phrenic nerve activity, respiratory frequency and the magnitude of the reflex responses were compared before, during, and after the elevation of brain temperature.

**Neuroanatomy.** At the conclusion of each study, the brain stem was removed en bloc, placed in embedding compound for frozen specimens (Tissue-Tek, OCT compound, Sakura Finetek, Torrance, CA), and frozen with isopentane and dry ice. Fifty-micrometer sections were cut from each brain stem using a cryostat, and the sections were stained with cresyl violet. Thermode placements were expressed relative to the obex, midline and dorsal surface of the brain in millimeters (coordinates, 0, 0, 0). Serial coronal sections were cut from 2 mm caudal to the caudal pole of the obex rostrally to the caudal pole of the facial nucleus. This section of the medulla encompassed the caudal raphé and the caudal half of the NTS as far back as the commissural subnuclei. The location of the tip of the thermode was determined from the pattern of tissue damage and the location of DiI fluorescence in the brain stem tissue. Fluorescence images were obtained using a Nikon Eclipse E800 fluorescence microscope and a TricT filter cube. The location of the tip of each thermode was plotted on a set of schematic representations of coronal sections of the neonatal piglet brain stem that we developed from multiple studies over the last few years (34).
Data analysis and statistics. We analyzed each experiment using ANOVA and the average response from each animal in each condition. In experiments in which we had repeated measures, we used a one-way repeated-measures design in which body temperature or brain temperature was a within-subjects factor with three levels (control, test, and a follow-up control). Each anatomic area in which the thermode was placed was analyzed separately. In the caudal raphe studies, there were no follow-up control periods, and we made comparisons with a paired t-test. The apnea and LCR durations were not normally distributed, which we also found in our laboratory’s previous study (6). For this reason, the apnea and LCR durations were log transformed; the transformed data were normally distributed. Statistical comparisons were made on the log-transformed data, but the untransformed data are presented in all the figures. Specific preplanned comparisons were made using P values adjusted by the Bonferroni method when the ANOVA indicated that significant differences existed among treatment groups. Data are presented as means ± SE.

RESULTS

Effect of elevating brain temperature in the NTS. In intact animals, the LCR consists of respiratory inhibition that may include apnea, coughing, swallowing, and an extended period of irregular breathing. In decerebrate animals, the reflex tends to consist of apnea and a longer period in which the respiratory frequency is slowed and integrated phrenic activity is reduced, but the irregularity of breathing and the coughing and swallowing are less prominent in decerebrated vagotomized animals. Examples of the LCR in a decerebrate piglet with and without elevated brain tissue temperature are shown in Fig. 1. The thermode was positioned in the NTS on the left side of the brain stem in this 13-day-old piglet. In Fig. 1, top, the body temperature of the animal was 38.1°C and the brain tissue temperature in the NTS was 38.0°C. When the LCR was induced, there was a ~4-s apnea and a 27-s period of respiratory instability. However, when the brain temperature was increased focally within the NTS to 40.0°C, the apnea duration was ~20 s, and respiratory slowing persisted for ~2 min after breathing was reestablished. These changes were reversible, and after the brain tissue temperature in the NTS returned to 38.3°C, the duration of apnea following elicitation of the LCR was ~6 s and the respiratory disruption was short lived. The respiratory frequency was variable throughout the protocol, but the effects of warming the NTS were consistent whether the baseline respiratory frequency increased or decreased during the protocol.

The average duration of the LCR, the length of the longest apnea, and body and brain tissue temperature adjacent to the thermode are shown in Fig. 2. Placement of the thermode disrupted respiratory activity in some animals (see below), but the data presented in Fig. 2 were taken from nine animals in which placement of the thermode within the brain stem did not disrupt the respiratory rhythm or the pattern of phrenic nerve activity. In the initial control period, the average body temperature was 38.4 ± 0.2°C, and the average brain tissue temperature adjacent to the thermode was 37.2 ± 0.1°C (slightly lower because of the exposure of the brain stem in our decerebrate preparation). The average apnea duration was 11.9 ± 2.4 s, and the average duration of the LCR was 22.9 ± 3.3 s. The apnea and LCR duration are similar to those to our laboratory found in previous studies of decerebrate neonatal piglets (6). When the brain tissue temperature was elevated to 40.0 ± 0.2°C, the apnea duration increased approximately fivefold and the LCR duration increased more than threefold. These changes were statistically significant (P < 0.001 for both comparisons). The changes in body temperature during brain tissue heating were not significant, but the elevation in brain tissue temperature was significant (P < 0.001). These changes were, on average, reversible, and when brain tissue temperature was returned to the control range, the average apnea and LCR durations also returned to the control levels. Thus elevating brain temperature in the region of the NTS was associated with marked prolongation of the LCR.

The anatomic locations of the tips of the thermodes within the brain stem are shown in Fig. 3 (solid squares). The thermode tips were in the NTS or closely adjacent to it. One animal, an 11-day-old male piglet, did not respond to brain stem heating. The apnea and LCR durations were unusually short in this animal even during control conditions (2.3 ± 0.4 and 4.1 ± 1.0 s, respectively) and unchanged by raising the brain stem temperature focally within the NTS from 36.2 ± 0.2 to 41.6 ± 0.3°C. This was not a particularly large temperature change compared with other animals in which the LCR was prolonged. The probe location was slightly deep to the NTS (the solid square enclosed with a circle in Fig. 3 indicates the location of the probe in this animal), but it was not markedly different from the other locations of thermodes that did prolong the LCR when heated.
Effect of elevating brain temperature in the NTS on respiratory rhythmicity. The respiratory rhythm was markedly disrupted in eight animals after the thermode was placed in the NTS, and we did not study the LCR response to brain tissue heating in these animals. In four of these animals, simply placing the thermode in the NTS disrupted the stability of the respiratory rhythm (see the top 2 panels in Fig. 4). We waited for as long as 2 h for the rhythm to regularize, but in no case did this occur. In the remaining four animals, the respiratory rhythm was slightly disrupted when the thermode was initially inserted into the NTS, but the regularity of the rhythm was reestablished quickly. However, when the thermode was heated, the respiratory rhythm deteriorated or ceased completely, and the LCR could not be studied (see the bottom panels in Fig. 4). We compared the thermode locations in the NTS in those animals in which the respiratory rhythm was not disrupted by placement of the thermode with those in which it was in Fig. 3. The placements of thermodes that altered respiratory activity were generally deeper (more ventral) in the medulla than those that left respiratory activity intact (open circles in Fig. 3). Moreover, those that completely suppressed breathing \((n = 5)\) were close to the ventral surface of the medulla in the region of the nucleus ambiguus. In contrast, thermode placements that made respiratory activity too irregular to permit study of the LCR, but did not completely destroy the respiratory rhythm \((n = 3)\), were more dorsally located (open circles enclosed by squares in Fig. 3). The separation of the thermode locations by the functional response to each thermode is imperfect, but in general, thermodes in the NTS did not disrupt respiratory activity and permitted study of the LCR. Thermodes placed just deep to the NTS disrupted, but did not suppress, respiratory activity, and thermodes placed close to the ventral surface completely suppressed respiratory activity.

Effect of elevating brain temperature in the caudal raphe. In four animals, we tested the anatomic specificity of the effect of elevating brain tissue temperature in the NTS, where laryngeal afferents make primary synaptic connections, by increasing the brain tissue temperature in an adjacent brain stem region with no known primary laryngeal afferents, the caudal raphe. The thermode was placed in the brain tissue as before, but we modified the experimental protocol slightly. To demonstrate that elevating brain temperature in each animal could prolong the LCR, we first elevated body temperature, which elevates the temperature of the entire brain stem. Having demonstrated the response of the LCR to whole body heating, we then tested the anatomically more specific hypothesis that elevating brain tissue temperature in the caudal raphe would prolong the LCR. The results of this study are shown in Fig. 5. Elevating body temperature from 38.6 ± 0.1°C to 42.0 ± 0.3°C (a significant change in temperature; \(P < 0.001\)) prolonged the duration of apnea and the LCR (\(P = 0.008\) and \(P < 0.02\), respectively), just as it had in our laboratory’s previous study \((6)\). Thus the animals studied were capable of demonstrating LCR prolongation during brain heating. However, when the brain tissue temperature within the caudal raphe was raised from 37.1 ± 0.5°C to 41.9 ± 0.2°C (\(P < 0.001\)), but body temperature varied insignificantly, the apnea duration was increased slightly, but not significantly, and the duration of the LCR actually decreased (also not a statistically significant change). Thus elevating brain tissue temperature focally within the caudal raphe did not prolong the LCR and did not replicate the effect of focal heating within the NTS. The anatomic locations of the thermode in the caudal raphe are also shown in Fig. 3 (solid triangles). The thermode placements were all close to the midline just above the ventral surface of the brain stem and well rostral to the nucleus ambiguous.

**Fig. 2.** The average duration of the LCR, the duration of the longest apnea and the rectal (●) and brain tissue temperature (temp) (○) are shown before, during, and after elevating the brain tissue temperature in the NTS. *Apnea duration, LCR duration, and brain tissue temperature were all significantly elevated during the treatment condition \((P < 0.001\) in all cases). None of the changes in body temperature or differences between the initial and follow-up control (cntrl-1 and cntrl-2) conditions for any of the variables was significant.

**J Appl Physiol • VOL 102 • JANUARY 2007 • www.jap.org**
DISCUSSION

There are two main findings in this study. First, elevating the brain tissue temperature by 2°C focally within the NTS prolongs the LCR and reproduces the effects that our laboratory previously found when body temperature was elevated a similar amount (6). This appears to be an anatomically specific effect because elevating the brain tissue temperature in the caudal raphe did not prolong the LCR. Second, focal heating of a region deep to the NTS near the ventral surface disrupts the rhythm and pattern of phrenic nerve activity.

Effect on the LCR of elevating brain tissue temperatures within the NTS. The primary afferents from the larynx travel within the superior laryngeal nerve and terminate in the NTS (17, 40). Neurons within the NTS integrate sensory information from a variety of sources and begin the process of distributing sensory information throughout the central nervous system. In addition to laryngeal afferent information, there are also warm and cold sensitive neurons in and adjacent to the NTS (18). In rabbits, the warm- and cold-sensitive neurons are intermixed and concentrated in a volume of the brain stem that extends from 2 mm caudal to the obex to 4 mm rostral to the obex within ±3 mm of the midline and from the ventral surface ~6 mm deep into the brain stem, which is almost exactly the same region that our laboratory found that elevating brain tissue temperature by 2°C prolonged the LCR in neonatal piglets (6). The comparison is imperfect; adult rabbits and neonatal piglets may not have the same nuclear locations, but the concordance of locations may nonetheless be significant. The thermal effects on the LCR that we observed may be mediated by direct effects of elevated temperature to change and probably enhance the excitability of those neurons that integrate laryngeal afferent information so that the inhibitory potency of this information is enhanced and the LCR is prolonged. However, the thermode locations with the NTS that prolonged the LCR when heated were not always in the subnuclei containing neurons that receive laryngeal afferents. The thermode and volume of tissue heated may be large compared with the subnuclei, and some of the thermode locations outside the subnuclei containing laryngeal afferent terminations may still have been warmed. Alternatively, it is possible that thermal information generated within the NTS by warm and cold sensitive neurons is integrated either within the NTS or at the level of the ventral respiratory group of neurons to amplify the inhibitory effect of laryngeal stimulation and prolong the LCR. The thermosensitive neurons are not organized somatotopically but are distributed in and adjacent to the NTS (18). Thermodes outside the subnuclei in which laryngeal afferents terminate may still have altered the function of thermosensitive neurons and modified the LCR in this way. Finally, the thermodes were placed in areas that may also contain respiratory CO₂-chemosensitive neurons (10). Enhancing or blunting respiratory drive by elevating the inspired CO₂ or lesioning or inhibiting central chemosensory regions may shorten or lengthen the LCR (26, 28, 32, 47). However, if brain tissue heating mediated the thermal prolongation of the LCR by inhibiting the function of chemosensory cells, we might have expected LCR prolongation after heating the caudal raphe, which is also a putative chemosensitive site (10). How-

Fig. 3. The location of each thermode is shown on schematic representations of the piglet brain stem. The sections extend from the obex to ~6 mm rostral to the obex. ▲, Thermodes placed in the NTS; ○, thermodes that disrupted the respiratory rhythm; △, thermodes placed in the caudal raphe. Those symbols enclosed in a circle or a square had an aberrant response compared with the remainder of the piglets in this particular group; see text for a complete explanation. DMX, dorsal motor nucleus of the vagus; HG, hypoglossal nucleus; NA, nucleus ambiguus; ROB, raphe obscurus; RPa, raphe pallidus; RMa, raphe magnus; IO, inferior olive; pyr, pyramids; FN, facial nucleus.
ever, we saw no such effect, indicating that a thermal effect on non-CO$_2$-sensitive neurons restricted to the NTS or closely adjacent region probably mediated the thermal prolongation of the LCR that we observed.

We do not know what neurotransmitter mechanisms are involved in the LCR, but GABAergic mechanisms modulate laryngeal apnea induced by electrical stimulation of the superior laryngeal nerve (1). Intravenous or intracisternal administration of bicuculline, a GABA$_A$-receptor antagonist, reduced the duration of apnea after superior laryngeal nerve stimulation in neonatal piglets without affecting baseline respiratory activity (1). Moreover, we found in a preliminary study that systemically administered bicuculline completely prevented the thermal prolongation of the LCR (L. Xia, unpublished observations). In addition, expiratory respiratory neurons within the ventral respiratory group were hyperpolarized during electrical stimulation of the superior laryngeal nerve, and this hyperpolarization was reversed by chloride injection, suggesting that it was mediated by a GABAergic mechanism (7). Finally, hyperthermia generally elevates GABA levels within the brain extracellular fluid (14), which may have some neuroprotective benefits. Thus we imagine that increasing the temperature of neurons in the NTS that receive afferent stimulation from the larynx may increase GABAergic inhibition of respiratory neurons and prolong apnea duration and the LCR. Thermally sensitive neurons in the NTS may amplify the inhibitory activity of neurons within the NTS that are excited by laryngeal afferents or may interact directly with neurons within the ventral respiratory group that also integrate information derived from the larynx through the NTS. In either case, we suspect that GABAergic neurotransmission plays a major role, and this hypothesis is consistent with our laboratory’s previous work in which dialysis of muscimol, a GABA$_A$-receptor agonist, into the ventral medulla prolonged the LCR in intact neonatal piglets (47). Moreover, this proposed circuit is quite similar to the putative organization of another respiratory inhibitory reflex, the Hering-Breuer reflex, in which afferent pulmonary stretch receptor information is also integrated within the NTS. The central processing of the Hering-Breuer reflex relies on second-order relay neurons within the NTS, called pump cells, which are largely GABAergic and to a lesser extent, 

Fig. 4. Two examples of the changes in integrated phrenic nerve activity, which occurred when the thermode was introduced into an area ventral to the NTS, are shown. The unraveling of the respiratory rhythm following thermode placement and heating was a slow process, and the tracings are not continuous. The delay between records is indicated on each segment of the strip-chart recording. In the first example, respiratory activity was compared before and after the thermode was placed in the NTS (first and second panels), and in the second example, respiratory activity was compared before and after the thermode was placed in the NTS and before and after the brain tissue was heated (third through fifth panels).

Fig. 5. The average duration of the LCR and apnea are shown before, during, and after elevating the brain tissue temperature in the caudal raphé. Initially, the effect of elevating body temperature on apnea and LCR duration was tested (○), and when body temperature was elevated, the apnea and LCR durations were significantly prolonged (*P < 0.02 in both cases). The body and brain tissue temperatures are given in parentheses beside each point. When only brain tissue temperature was elevated (●), there was no significant change in the apnea or LCR duration or in body temperature, even though brain tissue temperature was significantly elevated (P < 0.01).
ELEVATED NTS TEMPERATURE PROLONGS THE LCR

extent glycineric (9) and some of which also receive laryngeal afferent information. These inhibitory pump cells project to a variety of neurons within the ventral respiratory group that control the duration and depth of each breath (24).

Effect of elevating brain tissue temperatures within the NTS on respiratory function. Elevating the brain tissue temperature within or adjacent to the nucleus ambiguus disrupted or inhibited regular respiratory phrenic activity. We attribute this to disruption of the network properties of the neurons that generate the respiratory pattern. It may be that elevated temperature alters the coordination among the inspiratory, expiratory, and phase-spanning neurons that are known to exist within the ventral respiratory group (2). It is also possible that simple tissue destruction associated with placing the relatively bulky thermode contributed to the disruption of the respiratory pattern, but there should have been similar tissue damage in the caudal raphe, where thermode placement did not disrupt the respiratory pattern or alter the response to laryngeal stimulation. The heterogeneity of responses to brain tissue heating among the NTS, caudal raphe, and nucleus ambiguus argues strongly that the prolongation of the LCR during NTS heating and the respiratory disruption during nucleus ambiguous heating are anatomically specific and related to neuronal activities and functions unique to each site.

Thermal stresses and the LCR: implications for SIDS. We have been pursuing the hypothesis that SIDS occurs in infants when an underlying vulnerability is revealed by an exogenous stressor during a critical period of development (11). We believe that elevated body temperature may act as an important exogenous stressor. The Back to Sleep campaign significantly reduced the incidence of SIDS (29), but the mechanism(s) whereby supine sleeping reduces the risk of SIDS remains a subject of debate. Prone sleeping enhances rebreathing of CO2, but it also reduces heat loss. Epidemiological evidence derived from death scene investigations and physiological evidence derived from studies of neonatal animals and humans strongly support the hypothesis that prone sleeping impairs thermoregulation (15). Thermal stress has been identified as a risk factor for SIDS in a number of studies. Infants who died of SIDS often had a preceding upper respiratory tract infection and may have had a fever (42). Babies may become overheated when they are covered or swaddled excessively or sleep prone (41). Heavy wrapping and excessive room heating independently increased the risk of SIDS, especially in infants greater than 70 days of age (13), and more of the infants that died of SIDS were found with covers over their heads than control infants (12, 25). Babies lose much of their heat through the head and face, particularly when the rest of the body is covered (33). Therefore, prone sleeping significantly reduces the capacity for heat loss (46). Prone sleeping and excessive covering may also increase rebreathing, but it is worth noting that rebreathing itself may impair heat loss. Rebreathing water saturated air at body temperature impairs heat loss just as inhaling gas with an elevated CO2 concentration impairs removal of CO2. Thus the prone sleeping position reduces the effective surface for heat loss in infants and causes rebreathing, which impairs respiratory heat loss in addition to increasing the risk of hypercapnia and hypoxia (20).

The implication of our recent studies is that overheating may amplify the respiratory inhibition associated with the LCR (6), and the present study indicates that this thermal response arises, at least in part, from elevation of the brain temperature in the region of the NTS. The effects of bicuculline on laryngeal apnea and muscimol on the LCR (1, 47) suggest to us that the prolongation of the LCR reflects enhanced GABAergic inhibition of respiratory activity. The neurotransmitter receptor defects described in babies who died of SIDS are restricted to acetylcholine, glutamate, and serotonin receptors (21, 38, 39). These neurotransmitters are generally, but not exclusively, excitatory within the brain stem. Therefore, a deficiency of receptors for “excitatory” neurotransmitters will make infants more susceptible to enhanced inhibitory neurotransmitter activity evoked by elevated temperatures and inhibitory respiratory reflex responses. It is our hypothesis, therefore, that those infants with neurotransmitter receptor defects typical of infants who died of SIDS will be particularly susceptible to the thermal enhancement of respiratory inhibition associated with the LCR, which has already been identified as a likely precipitating factor leading to sudden infant death (8, 19, 30, 45).

Limitations of the methods. The nature of the decerebrate preparation is a major limitation of our study. Descending influences from the hypothalamus, which regulate body temperature, are absent from the decerebrate animal. Effective thermoregulation might limit the effects of brain heating and reduce the prolongation of the LCR that we observed. Moreover, inputs from the midbrain and cortex may provide an excitatory drive that supports regular respiratory activity and might also blunt the prolongation of the LCR that we observed. The “waking stimulus,” for example, provides an excitatory drive that enhances respiratory responses to a variety of stimuli such as hypoxia and hypercapnia (23). The LCR may also be prolonged in the decerebrate animals because the usual clearance mechanisms that remove the offending stimulus from the larynx and airway, swallowing and coughing, are limited or absent in the decerebrate animal, in part, because the animal is tracheostomized, and the stimulus may remain in the larynx longer than in intact animals. In addition, hypoxia and hypercapnia develop as the duration of the respiratory disruption associated with the LCR persists, and both of these stimuli may shorten the duration of the LCR in intact animals (26, 47, 48). Hypoxia and hypercapnia never developed in the decerebrate animals because they were ventilated continuously throughout these studies. Nonetheless, the direction of change in the LCR and the central mechanisms within the brain stem responsible for the thermal prolongation of the LCR are intact in the decerebrate animals. Although the details of the LCR responses may differ in intact animals, the responses in decerebrate piglets still accurately represent the basic mechanisms of the LCR.

We did not conduct time control studies in this protocol. However, when our laboratory conducted time control studies in the past (6, 47), we found no evidence that apnea duration or the LCR duration changed over the time course of these experiments. The LCR is stable within each animal unless some intervention is made to modify the reflex, such as elevating brain temperature.

Finally, we have assumed that focal warming of the NTS excited or enhanced the excitability of either neurons within the NTS that integrate laryngeal afferent information or sense brain tissue temperature in and adjacent to the NTS. We do not have any single unit recordings to justify this assumption, and
It is equally possible that the thermal effects that we observed are mediated by inhibition of neuronal activity within the NTS. In summary, we found that the thermal prolongation of the LCR, which our laboratory described previously in decerebrate piglets (6) and which cannot be ascribed to thermal effects on laryngeal receptors (49), can be attributed to elevation of the thermosensitivity affects respiratory muscle responses to laryngeal stimulation in the supine position. Clin Exp Pharm Physiol 28: 533–539, 2001.


