Prolongation in expiration evoked from ventrolateral pons of adult rats

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RESPIRATORY RHYTHM is influenced by the pons in numerous animal species and in humans (see Ref. 17 for review of references). Since the experiments of Lumsden (20), studies in cats have shown that the principal source of this influence is the dorsolateral (dl) pons (1, 3, 4, 10), although other regions in the pons may also be involved (9, 12, 13). Furthermore, in other species, pontine structures distinct from the dl pons modulate respiration (7, 16–18). These structures include the lateral and ventrolateral (vl) pons and the A5 noradrenergic cell group.

Recently, we identified a vl pontine site that influenced breathing in the adult rat (17). Bilateral injections of muscimol in the vl pons caused apneusic breathing, similar to that observed after dl pontine lesions (17, 24). In this paper, we present evidence that microinjections of the excitatory amino acid L-glutamate within the same area cause transient and consistent changes in the respiratory pattern.

METHODS

Adult rats (n = 8 male Sprague-Dawley, 312–476 g) were anesthetized with equithesin (0.3 ml/100 g ip). Atropine methyl nitrate (0.5 mg/100 g sc) was administered to reduce secretions. The trachea and left femoral artery and vein were cannulated to ventilate the animal, to measure blood pressure, and to administer drugs, respectively. Phrenic nerve activity was amplified and filtered (0.1–3 KHz; Grass P511) and integrated (Paynter filter, time constant 50 ms, CWE). The raw and moving averaged phrenic neural activity were recorded on paper (Astromed DASH 8) and tape (Hewlett-Packard). Animals were paralyzed with pancuronium bromide (0.1 mg·h⁻¹·100 g⁻¹ iv) and ventilated. The vagi were transected.

Two different surgical approaches were used to expose the central nervous system: 1) a dorsal approach, in which a craniotomy was made in the parietal plate through which the vl pons was approached; and 2) a ventral approach, in which the basioccipital cranium was opened to expose the ventral surface of the pons.

Animals (n = 4) were prepared, and a target site was identified as described previously (17). Two types of micropipettes (30- to 100-µm tip diameter) were used: 1) single-barrel micropipette (glass capillary tubes, 6020, A-M Systems) containing L-glutamate (10 mM dissolved in phosphate-buffered saline, pH 7.4) in a 2% fast green solution or 2) double-barrel micropipette (theta glass, 6070, A-M Systems) containing L-glutamate-2% fast green in one barrel and D-glutamate in the other. The tip of the micropipette was positioned at the target site, and L-glutamate (10–100 nl) was injected. In two of these animals, L-glutamate was injected every 500 µm, moving more ventrally in the same track to approach the target site. The volume of the injectate was determined by measuring displacement of the meniscus in the micropipette with the use of a reticle in an ocular lens of the microscope. Fast green was ionophoresed at the target site.

In four animals, microinjections of L-glutamate were made into the vl pons by using a single-barrel micropipette. The mapped area was just rostral and lateral to the exit of cranial nerve VI, to a depth of 2.5 mm. The dl pons was not penetrated. The most effective site was marked ionophoretically with fast green.

At the end of the experiment, the animal was perfused transcardially with heparinized saline, followed by 4% paraformaldehyde. The brain stem was removed, infiltrated with 30% sucrose-fixative solution and cut in coronal sections (50 µm) on a cryostat. The tissue section containing the injection site marked with fast green was drawn by using a camera lucida, then counterstained with 0.1% thionine.

The durations of inspiratory (Ti) and expiratory phases (Te) were measured before, during, and after injections. We used integrated phrenic nerve activity as an index of the respiratory cycle. Ti was measured from the onset of phrenic nerve activity to its offset and Te from the offset to the onset of the next phrenic burst. Measurements of Ti and Te were made for 5 consecutive cycles before the L-glutamate injections for the affected breath(s) during the L-glutamate injections and for 10 consecutive cycles after the injection.

RESULTS

In all animals (n = 8), we identified a vl pontine site at which a unilateral injection of L-glutamate prolonged expiration (Figs. 1–4). The effects on the respiratory pattern were 1) coincident with the injection, 2) transient with the greatest effect in the cycle in which the injection occurred, and 3) primarily on Te (Figs. 1A
and 2). Before the injection (first five cycles shown in Fig. 1A before the arrow), the respiratory cycle was steady. In the cycle with the L-glutamate injection (Fig. 1A, arrow), respiratory phase (TI) was shortened and subsequent TE was prolonged. Over next 5 respiratory cycles, TE returned gradually to baseline, whereas TI returned in very next cycle. Blood pressure rose after a long latency. PNA, raw phrenic nerve activity; f PNA, integrated phrenic nerve activity; AF, airflow (expiration up); BP, blood pressure. B: histological location of injection site that was approached from ventral surface of brain stem. Fast green was ionophoresed at injection site, and recovered deposit of fast green (roughly oval dark area) was ventromedial to facial nerve root. IV, fourth ventricle; 7n, nerve root of facial nerve; DT, dorsal tegmentum; g7, genu of facial nerve; NTZ, nucleus of trapezoid body; Pr5, principal sensory nucleus of trigeminal nerve; s5, sensory tract of trigeminal nerve; SO, superior olive; tz, trapezoid body.

The predominant response to L-glutamate was TE prolongation (Fig. 2). This response occurred in all animals, and TE was significantly (P < 0.05) longer in the stimulated cycle than in the preceding cycles (Fig. 2). The shortening of TI (Fig. 1A) was an inconsistent response, and no significant difference was found between the preceding control cycles and the stimulated cycle.

The prolongation of TE was independent of approach (Fig. 2). TE prolongation could be evoked by L-glutamate if a micropipette were placed from the dorsal surface of the cerebral cortex or from the ventral surface of the brain stem (Fig. 2). Thus the effect was evoked regardless of whether the shank of the micropipette penetrated the dlpons.

Histological localization of the effective site was accomplished by ionophoresing fast green contained in the glutamate solution. Sites were recovered from six of eight animals (Figs. 1B and 3). There was general agreement in the sites localized by the two approaches. The site of glutamate injection was located in the vl quadrant of the pons, lateral to the nucleus of the trapezoid body and dl to the superior olivary nucleus, in the anatomic location of the A5 area. At the rostral end, the marked sites were ventral to the trigeminal motor nucleus, and, at the caudal end, medial to the exit of the facial nerve fibers. The rostrocaudal extent of a single marked site was not >0.5 mm. However, the rostrocaudal extent of the identified sites that prolonged expiration ranged from 8.8 to 9.6 mm caudal to bregma.

Two control experiments were performed in rats by using the dorsal approach. First, comparable volumes of D- and L-glutamate (10 mM) were injected from adjoining barrels of a double-barreled micropipette at the same site (Fig. 4A). A response was only evoked by...
L-glutamate (Fig. 4A, top and bottom; similar amounts of D-glutamate did not evoke a response (Fig. 4A, middle). Second, we recorded the response to injections of L-glutamate in 500-µm steps, starting 4,500 µm below the dorsal cerebral surface (Fig. 4B). An increase in respiratory rate (decrease in TE) was evoked from 6,000 µm below the surface (Fig. 4B, top). From 6,500 to 8,000 µm, the response was negligible (Fig. 4B, middle). Then at 8,500 µm deep, L-glutamate injection evoked a prolongation in TE (Fig. 4B, bottom).

DISCUSSION

This study demonstrated that neurons with cell bodies in the vl pons can influence the breathing pattern in anesthetized vagotomized rats. The prevalent and consistent finding was a transient prolongation of expiration after unilateral L-glutamate injections into an area rostral to the facial motor nucleus, ventral to the spinal trigeminal tract and nucleus, and lateral to the lateral tegmental field of the reticular formation and overlapping with the A5 area. Furthermore, we have shown that these effects were independent of approach, i.e., regardless of whether the micropipette penetrated the dl pons.

Lesions in the vl pons caused an apneustic type of breathing with prolongation of Ti and the reversal of the ratio of Ti to Te (17). The present findings that chemical excitation of the neurons in the same anatomical loci results in prolongation of TE complement the results of the lesion experiments.

The predominant effect of L-glutamate microinjections in the vl pons was TE prolongation. TE was prolonged, independent of the time that the injection was made during the cycle. Furthermore, TE was prolonged in subsequent cycles before returning to prestimulus duration. These data suggest an “expiratory-promoting” role for neurons located in this area.

Fig. 3. Histological identification of injection sites. Representative coronal section displaying 5 of 6 recovered injection sites. Hatched circles, fast green deposits. Sixth injection site is displayed in Fig. 1B. Injection sites were located dorsolateral to nucleus of trapezoid body and ventral to principal sensory nucleus of trigeminal nerve. Abbreviations are defined as in Fig. 1 except for following: Mo5, trigeminal mononucleus; Py, pyramidal tract; BC, brachium conjunctivum.

Fig. 4. Specificity of stimulus. A: we compared response to chemical stimulus to that of mechanical stimulus of a bolus injection at same injection site. Top: at 8,000 µm below dorsal cerebral surface, an injection (arrowhead) of L-glutamate (10 nl, 10 mM) prolonged TE. Middle: expiration was not prolonged after injection of biologically inactive stereoisomer D-glutamate (30 nl, 10 mM) from an adjoining barrel at same site. Bottom: subsequent injection of L-glutamate at same site prolonged expiration again. B: we compared respiratory response to L-glutamate at progressively more ventral depths, starting 4,500 µm below cerebral surface and testing every 500 µm. Top: at a depth of 6,000 µm, L-glutamate injections (a total of 30 nl in 3 pulses, 10 mM) prolonged inspiration, shortened expiration, and increased respiratory rate. Middle at a depth of 8,000 µm, an injection of L-glutamate (20 nl, 10 mM) had a negligible effect on breathing pattern. Bottom: at 8,500 µm deep, 500 µm deeper, L-glutamate injection (20 nl, 10 mM) prolonged expiration, decreasing respiratory frequency. Expiratory prolongation elicited at most ventral site was distinctly different from pattern elicited at most dorsal site, and these sites were separated by a site from which no response was evoked. Abbreviations are defined as in Fig. 1A.
In contrast, Ti was not significantly affected in the stimulated cycle nor in the subsequent cycles. The absence of a consistent effect on Ti may be due to the time that the microinjections occurred in the respiratory cycle; most occurred during expiration. However, Ti was not affected in subsequent cycles, whereas Té was. Therefore, the effect of vl pontine neuronal activity was primarily on Té.

The response to L-glutamate injection was immediate, independent of approach, and distinct from that evoked by direct dl pontine stimulation. Therefore, we conclude that the prolongation of Té arises from activation of a population of neurons located in the vl pons.

The evoked response was due to chemical rather than mechanical activation of the neurons. Injections of similar volumes of the biologically inactive D-glutamate isomer had no effect on respiration. Furthermore, repeated L-glutamate injections at the same site elicited consistent effects, indicating the absence of depolarization blockade affecting the respiratory response (19).

A role for pontine structures in respiratory control was identified by Lumsden’s (20) description of the “pneumotaxic centre” in 1923. Specifically, although rhythmic respiration does not depend on the pons, the dl pons, especially the medial parabrachial and Kölliker-Fuse nuclei, was involved in phase switching because breathing became apneustic when this area was lesioned bilaterally in vagotomized animals (3, 4, 6, 11, 22, 24). More recent physiological and anatomical data suggest that ventral areas of the pons may influence respiration also (13, 17, 24). Data presented here and previously (17) indicate that neurons, not just fibers of passage, in the vl pons may be components of a pontine respiratory neuronal network.

A wide variety of responses have been evoked by L-glutamate injection in the dl pons (2, 10). Furthermore, sites in the dl pons where L-glutamate injection prolonged inspiration were near, and when mapped, even overlapped those that prolonged expiration (2, 10). In contrast to the heterogeneity of responses evoked by dl pontine L-glutamate injections, vl pontine injections consistently evoked a prolongation in Té.

In the adult rat, lesions in the vl pons affect breathing in a manner similar to lesions in the dl pons (17, 24). Electrolytic lesions as well as chemical inhibition of cellular activity in the vl pons produced an apneustic breathing pattern, i.e., a pattern with prolonged Ti (17).

The vl pons may play a role in the control of breathing in the adult cat (9, 13). Fadiga et al. (9) transected the brain stem at different levels. In particular, after rostrompontine transection that separated the dl pons from the ventral pons, lung inflation shortened Ti and prolonged Té; whereas, after pontobulbar transection, lung inflation evoked phrenic nerve activity. They concluded that the vl pons was important for the integration of vagal afferent activity and the respiratory pattern generator (9). Furthermore, kainic acid injected in the lateral pons caudal and ventral to the Kölliker-Fuse nucleus prolonged Té (13). However, large injections of kainic acid in the ventromedial pons did not affect the respiratory pattern (6).

In species other than rats and cats, effective areas outside the dl pons have also been reported (14, 18). Chemical anesthetics (2% lidocaine) injected in the trigeminal motor nucleus in rabbits resulted in apneustic breathing when lung inflation was prevented (14). In fetal sheep, large lesions in the lateral pons reversed the suppression of fetal breathing movements associated with hypoxia (18).

Anatomical studies have indicated connections between the medullary nuclei involved in cardiovascular and respiratory control and the vl pons (8, 21, 23). The physiological significance of these connections remains obscure, but they appear not to be “premotor” and have been identified as third-order neurons (8). Only a few previous reports have identified structures in the lateral and vl pons as potentially playing a physiological role in control of respiration (7, 9, 16, 18). Most of these studies were performed in fetal and neonatal preparations (7, 16, 18). In these immature preparations, the vl pons inhibited respiratory rhythmically tonically (7, 16, 18). Our data indicate that this area may be an important input to the medullary respiratory network in adult animals.

Recently, physiological studies on the control of the cardiovascular system in the adult rat have shown expiratory-modulated activity in AS neurons that is increased during hypoxia (15). We have shown that chemical inhibition of cells in the vl pons affected the respiratory response to hypoxia, in particular, the posthypoxic breathing pattern (5). The prolongation of expiration that follows a brief hypoxic exposure is attenuated after bilateral vl-pontine interventions (5).

In conclusion, the paucity of available data precludes making definitive statements regarding the function of these neurons in shaping and stabilizing (22) the respiratory pattern, especially in the control of phase duration (11, 20). We believe that our previous (17) and present findings serve as a starting point for systematic investigations of this laternal pontine network involved in respiratory control in the adult rat.

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