Medullary regions for neurogenesis of gasping: noeud vital or noeuds vitaux?

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St. John, Walter M. Medullary regions for neurogenesis of gasping: noeud vital or noeuds vitaux? J. Appl. Physiol. 81(5): 1865–1877, 1996.—Gasping is a critical mechanism for survival in that it serves as a mechanism for autoresuscitation when eupnea fails. Eupnea and gasping are separable patterns of automatic ventilatory activity in all mammalian species from the day of birth. The neurogenesis of the gasp is dependent on the discharge of neurons in the rostroventral medulla. This gasping center overlaps a region termed “the pre-Bötzinger complex.” Neuronal activities of this complex, characterized in an in vitro brain stem spinal cord preparation of the neonatal rat, have been hypothesized to underlie respiratory rhythm generation. Yet, the rhythmic activity of this in vitro preparation is markedly different from eupnea but identical with gasping in vivo. In eupnea, medullary neuronal activities generating the gasp and the identical rhythm of the in vitro preparation are incorporated into a portion of the pontomedullary circuit defining eupneic ventilatory activity. However, these medullary neuronal activities do not appear critical for the neurogenesis of eupnea, per se.

eupnea; in vitro; in vivo; medulla; pons

IN 1923, THOMAS LUMSDEN reported the marked and progressive changes in ventilatory activity, which he observed on exposure of experimental animals to anoxia or ischemia. For easier interpretation, records of activity of the phrenic nerve during cerebral hypoxemia are presented in Fig. 1; Lumsden’s words document the changes observed: “...after the blood is completely shut off the pneumotaxic center fails, respiration becomes slow and then apneustic in type, a long inspiratory tonus is followed by a few short failing apneuses. Very soon gasps alone occur and death results. If however,...the vertebrals are freed before gasping ceases, recovery takes place in the reverse order” (Ref. 41, p. 359–360).

These changes appeared to represent a sequential depression of the brain stem respiratory centers that Lumsden had described (40–43). This description was based on findings after rostral to caudal transections of the brain stem.

As shown in Fig. 2, Lumsden reported that eupnea was maintained after a transection at the midcollicular level. After a midpontile transection, caudal to the “pneumotaxic center”, the ventilatory pattern is altered to apneusis, marked by a sustained pause in the inspiratory position. Apneusis is replaced by gasping when all of the pons is removed. Parenthetically, Lumsden did not recognize the importance of bilateral vagotomy as a requisite to obtaining apneusis; apparently, the vagi were damaged during his dissections. Gasping can be elicited in animals having intact vagi or after bilateral vagotomy.

Lumsden felt that eupnea and gasping were fundamentally different and separable respiratory patterns: “The gasping centre ....does not appear to influence true rhythmical breathing of normal type” (Ref. 41, p. 366). For the neurogenesis of “normal type” respiration, Lumsden proposed that mechanisms of the pontile pneumotaxic center played a critical role (40–41). Concerning gasping, Lumsden presaged its importance in autoresuscitation (26, 29, 33, 35, 66, 77): “My view is that gasping is a relic of some transitory primitive respiratory process half way between gill and lung respiration as a fish gasps when taken out of water. Yet I have seen so many instances in which gasping has been sufficient to revive animals whose higher respiratory centres have temporarily failed that I feel no surprise that the faculty has persisted in the evolutionary struggle” (Ref. 41, p. 364).

These concepts of Lumsden lead to testable or, indeed, tested hypotheses. If, indeed, gasping is a “relic of some previous respiratory mechanism,” which serves as a backup when eupnea fails, then the capacity to exhibit both eupnea and gasping patterns must be present in every mammal from the day of birth. Similarly, identification and elimination of the gasping center should not eliminate eupnea. There is sig-
significant experimental support for each of these hypotheses.

DIFFERENTIATION OF EUPNEA AND GASPING

In his basic characterization, Lumsden noted that the rate of rise of inspiratory activity is much greater in gasping than in eupnea (40). In eupnea, phrenic activity increases as a "ramplike" function; in gasping, it rises almost immediately to a peak value. Hence, whereas the peak of phrenic activity is reached at the end of the burst in eupnea, it is much earlier in neural inspiration in gasping (24, 77, 81, 90) (Figs. 1-3). Similarly, motor activities of other spinal and cranial nerves, which have varying discharge patterns during the eupneic inspiration, acquire the stereotypic rapidly peaking pattern in gasping. Included in these inspiratory activities are those of the intercostal, facial, hypoglossal, and recurrent laryngeal nerves (32, 79, 83). Indeed, the recurrent laryngeal branch to the thyroary-

![Fig. 1. Progressive changes in phrenic activity of adult cat after exposure to 1% CO. Animals were decerebrate, vagotomized, paralyzed, and artificially ventilated. Activity before exposure (Control) for stated times during exposure and 10 min after termination of exposure (Recovery) is shown. Apneusis and gasping were recorded after 17.5 and 24.5 min of exposure, respectively. (Data from Zhou et al. (92))](image)

![Fig. 2. Patterns of automatic ventilatory activity after transections of brain stem. Schematic drawings are of integrated phrenic activity. Eupnea is recorded after a midcollicular transection (level E). After a rostral pontile transection (level A), apneusis is obtained. Gasping is recorded after a transection at pontomedullary junction (level G). Note that duration of apneustic inspiration can be many minutes. IC, inferior colliculus; BP, brachium pontis.](image)
tenoid muscle of the larynx, which has a purely expiratory discharge in eupnea, becomes purely inspiratory in gasping (83).

Expiratory activities of spinal nerves are also greatly reduced or totally eliminated with the change from eupnea to gasping (20, 83, 92). It is important to emphasize that during the establishment of gasping in hypoxia or ischemia expiratory activities may transiently maintain the same discharge patterns as in eupnea. Even when steady-state gasping is established, some phasic expiratory activities may remain, albeit at reduced levels (see Refs. 79, 83).

Underlying the change in respiratory-modulated cranial and spinal neural activities, the discharge of medullary respiratory neurons is greatly altered in gasping. Compared with eupnea, patterns of neuronal activities exhibit less variation when gasping commences. Hence, inspiratory bulbospinal neuronal activities, which discharge throughout various portions of the eupneic inspiration, all commence activity just before or after the onset of the phrenic burst in gasping; some additional inspiratory neuronal activities are recruited. Also, some tonic and respiratory-modulated phase-spanning neuronal activities of eupnea become inspiratory in gasping, whereas others cease their discharge (23, 92). In addition to these tonic and phase-spanning neurons, expiratory bulbospinal, laryngeal, and nonantidromically activated neurons decline or cease activity with the change from eupnea to gasping (23, 92). These changes in respiratory-modulated neuronal activities in gasping are different from those resulting from an increase in ventilatory activity in eupnea. In hypercapnia, discharge frequencies of all respiratory modulated neuronal activities typically increase (7, 19, 75, 78, 82). Most neuronal discharge frequencies also increase in hypoxia, although some expiratory activities do decline from levels in hyperoxia and normoxia (75, 77, 81). Hence, differences in neuro-

Fig. 3. Eupnea and gasping in neonatal rat. Animals were decerebrate, vagotomized, paralyzed, and artificially ventilated. Records are of integrated phrenic (Phr) activity during eupnea in hyperoxia and in anoxia-induced gasping. Time constant of integrator = 60 ms on and 100 ms off. Records were obtained from an animal on the day of birth (0 day) and others at 2, 4, and 7 days after birth. [From Wang et al. (90), reproduced by permission of the Physiological Society of the United Kingdom.]
nal activities between eupnea and gasping are not equitable simply with an increase in ventilatory drive.

A final distinction between eupnea and gasping is in the high-frequency oscillations in inspiratory neural activities (64, 65, 87). Although the source is undefined, some investigators consider these oscillations as “signatures” of the basic mechanisms underlying the generation of the respiratory rhythm. Of importance to the present discussion is the observation that the peak frequencies of these oscillations, which are 70–90 Hz in eupnea, are shifted to values approximating 120 Hz in gasping (64, 87).

There are some data that imply that gasping may only represent a variant of eupnea or the reverse (39, 44, 48, 77). When activities of spinal nerves and/or muscles of respiration are recorded in asphyxia, the change from eupnea to gasping appears in some trials to be progressive. Yet, it must be recalled that, once generated, eupnea and gasping activate common cranial and spinal nerves. Moreover, the seeming progression from eupnea to gasping in asphyxia might reflect the law of initial values. Hence, when the rate of rise of inspiratory activity is extremely high in eupnea, it cannot become much higher in gasping.

Another set of observations, which is consistent with a single mechanism underlying the neurogenesis of eupnea and gasping, is that electrical stimulation of both the carotid sinus and superior laryngeal nerves causes qualitatively similar changes in the frequency of phrenic bursts during both patterns (48). Again, however, many common elements of the medullary respiratory network are active during both ventilatory patterns. Because both the carotid sinus and superior laryngeal nerves terminate on these components (16, 19), some qualitatively similar responses might be expected.

RELEASE OF MEDULLARY MECHANISMS FOR GASPING

The pontile inhibition of medullary mechanisms for gasping would appear to be powerful, since during progressive transections no gasping is observed so long as any anatomical connections remain between pons and medulla (76). Similarly, hypoxia causes a progressive rostral to caudal depression of brain stem function (42, 43, 77). These findings, after brain stem transection and hypoxia, should not be equated with the death of pontile neurons being the necessary requisite for the release of gasping. Indeed, as Lumsden has pointed out, reestablishment of oxygenation, once gasping commences, can result in the reestablishment of eupnea (42, 43).

The sequence of hypoxia-induced changes leading to apnea appears to represent an “active” process involving changes in the release of neurotransmitters in the brain stem. Hence, the primary apnea does not represent a failure of energy production by the neuron (4, 49, 54). There is evidence of such an impairment of energy production once gasping commences. However, a direct linkage between metabolic insufficiency and gasping is tenuous, since, once begun, gasping continues for some period after oxygenation is restored (49). Indeed, in recovery from hypoxia, the presence of mixed respira-

EUPNEA AND GASPING AS FUNCTIONS OF AGE

From the numerous studies in adult animals, it is evident that gasping often represents the last ventilatory effort before death. Similarly, mechanisms for gasping can be recruited at birth. Indeed, in any case in which hypoxemia is present, “gasing is a natural requisite to normal breathing” (34).

The ability of animals to exhibit eupnea and gasping at birth obviously means that the neuronal mechanism underlying each must be functional. There is significant evidence that such mechanisms may, in fact, be functional long before birth.

Fetuses in utero exhibit periodic “respiratory movements” during the rapid-eye-movement stage of sleep. These movements disappear in hypoxia unless the hypoxia is extended or severe. Respiratory movements then reappear with a gasping pattern (34, 66).

If gasping can be observed in the fetus and at birth, it might be expected that eupnea and gasping would also be clearly distinguishable patterns in the newborn. Indeed, in every mammalian species examined, the ventilatory pattern can be altered from eupnea to gasping in severe hypoxia, anoxia, or ischemia. Included in these species are those that are very immature at birth, such as mice, rats, and rabbits, and those more fully developed, including lambs, piglets, monkeys, and humans (1, 2, 27, 29, 33–35, 39, 44, 74, 90). As in adult animals, the phrenic activity in eupnea in newborn animals exhibits the typical ramplike rise. Similarly, as in adults, gasping is characterized by the rapid rate of rise of activity early in inspiration. Ex-
amples of eupnea and gasping in neonatal rats are shown in Fig. 3.

The ability of newborn animals to alter the ventilatory pattern from eupnea to gasping is considered critical for survival. In extreme hypoxia or anoxia, the newborn typically exhibits a biphasic response with ventilatory activity first increasing and then declining to apnea. This primary apnea is interrupted by gasping. If animals are allowed to breathe air or oxygen once gasping commences, recovery of eupnea and survival occurs in most, but not all, animals. This ability of gasping to reverse a life-threatening episode has been termed “autoresuscitation” (29, 35, 39, 74, 77, 90).

IDENTIFICATION OF A MEDULLARY GASPING CENTER

Patterns of ventilatory activity in medullary preparations. Beginning in the late 1930s, a number of investigators, including Breckenridge, Hof, Magoun, Pitts, Ranson, Stella, and Wang attempted to establish what type of ventilatory activity could be supported by various components of brain stem respiratory centers (see Ref. 77). Much discussion was devoted to whether, as Lumsden had proposed, pontile mechanisms exercised a primary function in the neurogenesis of eupnea. Also, the contrary question was debated as to whether eupnea and apneusis represented variants of gasping (see discussion in Ref. 88); Barcroft proposed such a “kernel” theory in his elegant essay in 1938 (2). Finally, the question was considered as to whether “normal” ventilatory activity could be supported by medullary mechanisms alone. In 1990, I reviewed this material in some detail and concluded that “gassing is the one pattern of automatic ventilatory activity which can be obtained in a reproducible manner in the medullary preparation” (77). I believe that this conclusion is still entirely valid both for in vivo and, as discussed below, in vitro preparations.

Gassing center in adult cats and rats. By the end of the 1970s, it was well accepted that, in cats, respiratory-modulated neuronal activities were concentrated in two medullary regions: the dorsal and ventral respiratory nuclei. The former is located dorsomedially, in approximation to the nucleus tractus solitarii. The ventral medullary respiratory nucleus extends from the cervical level to that of the pontomedullary junction and approximates the nucleus ambiguus, nucleus retroambiguous, and, at its rostral end, the retrofacial nucleus (16, 19).

Because it is unequivocal that gasping is generated and supported by medullary mechanisms, we hypothesized that a critical region for its neurogenesis could be identified. In a 1984 study, in which decerebrate cats were used, neurons in the dorsal and ventral medullary respiratory nuclei were destroyed by injections of the neurotoxin kainic acid (79). Gassing was not eliminated despite numerous injections into the entire extent of the dorsal respiratory nucleus. Similarly, much of the ventral respiratory nucleus was found not to be essential for the gasp to be generated. However, injections into the lateral tegmental field, just medial to the ventral nucleus, eliminated gasping. In a subsequent study, using more discrete injections of kainic acid (22), we localized this “gassing center” in cats as extending from dorsomedial to ventrolateral to the nucleus ambiguus (Fig. 4). A comparable region for the gasping center near the nucleus ambiguus was also established in adult rats (24) (Fig. 4).

Importantly, neither in cats (22, 79) nor in rats (24) did lesions of the gasping center alter the eupneic rhythm. This inability to alter eupnea by medullary lesions was consistent with previous findings. Thus Speck and Feldman (73) found that multiple lesions which, in sum, destroyed most of the dorsal and ventral respiratory nuclei in anesthetized cats, caused no changes in the respiratory rhythm as the amplitude of respiratory-modulated neural activities gradually fell. Concerning the ventral nucleus, these investigators emphasized the completeness of lesions, which were 250–900 µm in diameter and extended from 1.0 mm caudal to 5.0 mm rostral to the obex. Hence, at the most rostral extreme, the so-called Bützing complex would be destroyed. In reviewing this work, Feldman notes: “Thus maintenance of normal rhythm without interruption after bilateral DRG-VRG (dorsal and ventral respiratory group or nuclei) lesions indicates that these lesioned areas, including both cell and fibers of passage, need not be intact for respiratory-rhythm generation” (Ref. 19, p. 481). In a subsequent study, Speck and Beck (72) again produced extensive lesions of the dorsal and ventral medullary respiratory nuclei but in decerebrate animals, and still caused no marked changes in eupnea.

In summary, we have identified a region, extending from dorsomedial to ventrolateral to the nucleus ambiguous, which is critical for the neurogenesis of gasping. In agreement with the work of others (19, 72, 73), neither this gasping center nor the neighboring portions of the ventral respiratory nucleus appear essential for the neurogenesis of eupnea. However, these findings and their interpretation have become confirmed by the results derived from an in vitro brain stem-spinal cord preparation of the neonatal rat.

IN VITRO BRAIN STEM-SPINAL CORD PREPARATION

In 1983, Suzue (84) described an extraordinary preparation for the study of ventilatory activity. This preparation, presented in detail in a 1984 publication (85), was a brain stem and spinal cord of the neonatal rat, which was removed from the animal and maintained without circulation or perfusion in a medium. Activities of spinal and cranial nerves exhibited synchronous discharges that were related to movements of the thorax, when the latter was removed with the spinal cord. These periodic synchronized discharges could continue for hours.

In this initial description, Suzue discussed explicitly the limitations of this in vitro preparation. Foremost among these is the relationship of its synchronized discharges with eupnea of in vivo animals. Differences included a respiratory frequency, which was an order of magnitude slower than that of eupnea, and a much more rapid rate of rise of inspiratory activity. Concern-
ing inspiratory activity, another difference from eupnea was that stimuli, such as alterations of pH of the in vitro solution, changed only the frequency and not the peak height of phrenic bursts. Also, as opposed to eupnea, the appearance of these bursts was not altered after a complete brain stem transection at the pontomedullary junction. Based on these considerations, Suzue noted: “the periodic rhythm may be correlated to gasping rather than the normal respiratory rhythm. The low frequency of the rhythm in the present preparation may be at least partly attributed to the absence of the afferent input...” (85).

Some investigators who have adopted the in vitro preparation have maintained strongly that “the respiratory pattern in vitro is not gasping, although it may share some common mechanisms” (71). In fact, experimental evidence appears substantial that the ventilatory pattern exhibited by the in vitro brain stem-spinal preparation of the neonatal rat is, indeed, gasping. Rather than consider publications in chronological order, evidence of gasping in this preparation will be considered by topic.

Brain stem transections. To explain the absence of change in pattern after a transection at the pontomedullary junction, the pons was considered to play little, if any, role in ventilatory regulation in rats (9, 31, 50, 71). This conclusion was based on the absence of apneusis in adult rats after transections at mid-to-caudal pontile levels (50). These studies had not recognized the findings of Wang et al. in 1957 (89) that the depth and duration of apneusis decline as transections are made at progressively more caudal pontile levels. Indeed, it is now established that apneusis, with prolonged inspiratory and expiratory phases, is obtained after discrete lesions of the rostral pontile pneumotaxic center and vagotomy in both adult (25, 37, 52, 91) and neonatal rats (21).

Patterns of neural activities. Activity of the cervical roots of the in vitro preparation has a stereotyped “rapidly peaking-slowly decrementing” pattern (9, 15, 28, 31, 47, 53, 55–59, 70, 71) (Fig. 5). Other cranial and spinal nerves display this same pattern, which is very similar to gasping of adult animals in vivo. However, some investigators have maintained that this pattern is also displayed by the in vivo neonate after vagotomy (71).

In a comparison of in vitro and in vivo preparations, Smith et al. (71) present electromyographic recordings of diaphragmatic activity in spontaneously breathing animals after vagotomy. In animals younger than 4 days, the diaphragmatic activity is similar to that of the in vitro preparation, whereas in rats older than 7 days, this activity is as the ramplike rise of the adult (Fig. 5). Smith et al. concluded that vagotomy had transformed the pattern of motor output and that this transformation is age dependent. A similar alteration to a gasp-like output after bilateral vagotomy has been reported by Murakoshi et al. (53) However, as opposed to Smith et al. (71), Murakoshi et al. (53) report such a transformation for rats of ages 4–27 days. Finally, in spontaneously breathing unanesthetized rats of ages 0–11 days, Fedorko et al. (18) found that all animals breathed with a “gasp-type” pattern after vagotomy.

As far as a transformation of the ventilatory pattern by vagotomy is concerned, a problem in terminology
exists in that “gaslike” should not be considered as synonymous with “gasping.” Although expanded experimental records are not presented by Murakoshi et al. (53) and Fedorko et al. (18), records in the latter report do appear to show peak tidal volume being reached at the end of inspiration, as in eupnea, and not at the beginning, as in gasping. More importantly, Fedorko et al. point out that respiratory minute volume falls greatly in all neonatal rats after vagotomy and “if alveolar ventilation is similarly affected, may well compromise metabolic function, and this can result in respiratory failure.”

Experimental records of Smith et al. (71) clearly show gasping in vivo after vagotomy. However, it is probable that, rather than a transformation resulting from vagotomy per se, the gasping reflects an inability of these animals to support eupnea. Hence, vagotomy and surgical interventions may compromise ventilation to such a degree that hypoxemia is induced, which, in turn, causes an alteration from eupnea to gasping.

In a recent study (90), we have reported that neonatal rats, from the day of birth, have a ramp-like rise of phrenic and inspiratory activities in eupnea. While the rate of rise may increase, this ramp-like pattern is maintained after vagotomy and also sectioning of the carotid sinus nerves. With exposure to anoxia, this ramp-like pattern is converted to the rapidly peaking-slowly decrementing pattern, which is characteristic of gasping and the in vitro preparation (cf. Figs. 3 and 5).

Expiratory activities also support the concept that the in vitro preparation is exhibiting gasping. As noted above, a reduction in expiratory activities is a common feature of gasping (20, 83, 90, 92). In a compilation of recordings from a number of in vitro preparations, Smith et al. (71) show both early and late expiratory discharges of cranial and spinal nerves. Compared with recordings from in vivo neonatal and adult animals, such expiratory activities are much reduced. Moreover, such expiratory neural activities were apparently not consistently recorded in the in vitro preparation, with no expiratory activities of the vagus being discernible in other figures of Smith et al. or Brockhaus et al. (5). Smith et al. (71) note that “the E-phase activity on a given nerve occurred intermittently in any preparation.” Another indication that the in vitro preparation is exhibiting gasping is the temperature at which these preparations are examined. When we reduced the body temperature of in vivo rats toward 27°C, eupneic ventilatory activity was severely distorted and disappeared entirely in most animals. This activity returned in anoxia with a gasplike pattern, identical to that of the in vitro preparation (90).

Response to stimuli. In hypercapnia and/or acidosis, any change in rhythmic activity of the in vitro preparation is in frequency; the peak height of phrenic bursts is largely unaltered (55, 85). In anoxia, the in vitro preparation exhibits a transient rise in the frequency of its burst and then a gradual decline to apnea (5). These alterations of frequency only in response to stimuli are identical with responses during gasping in vivo (77, 81).

Anoxia. Researchers more than fifty years ago reported that, like all other neonatal animals, rats alter their ventilatory pattern from eupnea to gasping within seconds of exposure to anoxia (1, 74). Even when the animals are maintained in anoxia, these gasping activities persist for many minutes. There has been no consideration as to how the brain stem and spinal cord could be removed from the neonatal rat and maintained in vitro without the ventilatory pattern being altered to gasping. It is possible that, as in some animals in vivo, reestablishment of adequate oxygenation in vitro could result in a reconversion from gasping to eupnea. Yet oxygenation is not adequate.

The in vitro neonatal rat brain stem preparation, maintained in a medium equilibrated with 100% oxygen, has a core of complete anoxia (5, 55). In one study, tissue Po2 fell to zero within a maximum of 450 μm from the surface (55). Hence, anoxia during removal of the brain stem and hypoxia and anoxia of brain stem regions would appear to be compatible with gasping.

Several other mammalian in vitro preparations have been described, albeit in adult animals, in which brain stem oxygenation is maintained by an arterial perfusion system. In those having an intact pons, the inspiratory neural activities are as those of eupnea in vivo and not as the gasping patterns of the in vitro neonatal rat preparation (30, 51). However, in other preparations, the pons is removed, and only the isolated medulla and spinal cord are perfused (45). In the latter, neural activities exhibit the rapidly peaking patterns typical of gasping. Moreover, the rhythmic neural activities of these medullary preparations can continue for minutes in severe hypoxia or ischemia (45). In hypoxia or ischemia, decerebrate in vivo preparations convert the ventilatory patterns from eupnea to gasping in hypoxia, whereas in medullary in vivo preparations gasping continues with no change in pattern (24, 43, 77).

As a final point, a number of in vitro slice preparations have been developed in which, because of a smaller size, oxygenation would be improved compared with the in vivo preparation having most or all of the medulla intact (e.g., 36, 60–62, 69, 70). Although some of these preparations have a remnant of pons remaining, the rhythmic activities of these preparations are like the rapidly peaking activity of gasping and not the ramp-like rise of eupnea. However, in parallel with the development of the eupneic rhythm, these rhythmic activities recorded in vitro have been noted to undergo maturation (60–62). For example, the pattern of the rhythmic activity changes from a “decremented” activity to one having a “plateau component” (62). Yet a similar change in discharge pattern is observed in the pattern of neural activities during gasping in neonatal and adult animals (compare Figs. 1, 5, and 6 in Ref. 24 with Figs. 6 and 7 in Ref. 90). Hence, developmental changes may be evident in the brain stem neuronal circuits underlying the neurogenesis of both eupnea and gasping.

Critical medullary region. In a series of papers beginning in 1987, Onimaru, Arata, and Homma further characterized mechanisms underlying respiratory rhythm generation in vitro (56–59). These investigators found that rhythm generation was critically dependent on a region localized to the rostral medulla,
ventrolateral to the nucleus ambiguus (Fig. 6). This region was termed “pre-I” because many neurons therein discharge phasically just before and just after the phrenic burst (see also Ref. 38).

Beginning in 1990, Smith and colleagues published studies in which they also examined the region of respiratory rhythm generation in the in vitro preparation (69–71). On the basis of microtransection experiments, this rhythmic activity could be supported by a section of medulla, which “extends from the caudal end of the retrofacial nucleus to approximately 200 µm towards obex.” This critical region, named the “pre-Bötzinger” complex, was ventral and ventrolateral to the nucleus ambiguus (Fig. 6).

The pre-Bötzinger complex is considered to be caudal to the pre-I region of Onimaru et al. (70). If present in a comparable region to that in adult cats, the gasping center would be dorsal and medial to the pre-Bötzinger complex; the lateral extent of the gasping center and the medial extent of the pre-Bötzinger complex would be extremely close. Such proximity has, in fact, become overlap as the location of the pre-Bötzinger complex has been altered, based on in vivo characterizations (8, 10, 67).

Using an analysis of previous studies, Ellenberger and Feldman (12–14) proposed that the pre-Bötzinger complex in vivo would contain a high percentage of propriobulbar neurons and would lie just caudal to the Bötzinger complex, which contains many inspiratory bulbo spinal neuronal activities. In adult rats, neuroanatomic studies identified such a population of propriobulbar neurons (10). However, at some variance with its in vitro location, the pre-Bötzinger complex in adult rats was ventral and ventromedial to the nucleus ambiguus (10) (Fig. 6). As noted above, the critical medullary region for gasping in adult rats extends from dorsomedial to ventrolateral to the nucleus ambiguus. An overlap of its lateral border with the medial border of the pre-Bötzinger complex is apparent.

In adult cats, based again on criteria of propriobulbar neurons, a pre-Bötzinger complex has been located (8, 67). However, this complex is now shown extending from dorsomedial to ventrolateral to the nucleus ambiguus (Fig. 6). A significant portion of this complex is now clearly within the ventral medullary respiratory nucleus. Even more than in rats, the dorsomedial division of this complex appears to be within the region for gasping. More overlap is evidenced by the extensive dendritic projections of pre-Bötzinger neurons within the region for gasping (63, 67).

The question arises as to whether there are, in fact, three critical medullary regions for the neurogenesis of the ventilatory activity: the pre-I region, the pre-Bötzinger complex, and the gasping center. Given the proximity and possible identity of portions of these regions, the extensive dendritic processes of neurons in these regions, and the spread of neurotoxins and damage from the center of lesions, it seems possible that there is one continuous medullary region for ventilatory neurogenesis. In this same context, the rhythmic respiratory activity generated from a brain stem slice preparation of neonatal and adult rats and mice (60–62) is dependent “upon the integrity of the nucleus ambiguus.” It would appear that the gasping center, pre-I region, and pre-Bötzinger complex are all present in this slice.

Pacemaker neurons in vitro. Onimaru et al. (57) hypothesized that the discharge of pacemaker cells underlies the neurogenesis of rhythmic activity in vitro. In support of this hypothesis, the discharge pattern of many pre-I neurons continued after blocking synaptic transmission in a low-calcium, high-magnesium solution. These findings of Onimaru et al. were confirmed by Smith, Feldman, and colleagues using medullary slices (36, 69, 70). In recordings in their pre-Bötzinger complex, as well as more caudal regions, some inspiratory neurons maintained this phasic pattern after the blockade of synaptic transmission, whereas other neurons, having tonic discharge patterns, were converted to phasic bursts.

Interrelationships of eupnea and gasping and of in vivo and in vitro preparations. Neuronal activities in a pre-Bötzinger complex have been described during eupnea in vivo in adult animals (8, 67). As in vitro, pre-I neuronal activities are considered to play a fundamental role in the neurogenesis of rhythmic activities in vivo. Yet, the pre-I patterns differ. The pre-I neuronal activities of Onimaru et al. (57) discharged just before and just after the commencement of the phrenic burst; the neuron was silent at the start of the burst. For Smith et al. (71), pre-I neuronal discharges in vitro were continuous, from the end of expiration to early inspiration. In vivo, pre-I neuronal activities may commence activities early in neural expiration and fire throughout inspiration (8, 67); these pre-I activities are synonymous with the phase-spanning expiratory-inspiratory neurons (7).

The pre-I neuronal activities in vivo may represent an entirely different population from the pre-I activities in vitro. Not only are the pre-I discharge patterns different in vitro and in vivo but many neuronal activities are recruited with the alteration from eupnea to gasping (e.g., Ref. 23).

Several recent reviews have proposed that whereas the discharge of pacemaker neurons in the pre-Bötzinger and/or pre-I regions may be responsible for rhythm generation in vitro and in the fetus and neonate in vivo, such neuronal activities are incorporated into a circuit responsible for the neurogenesis of eupnea of adult animals in vivo (3, 11; see also Refs. 60–62). Such an incorporation is necessary since, without perturbations, only eupnea and not gasping is observed in vivo. However, this incorporation is not synonymous with the concept that the discharge of medullary pacemaker neurons serves as a kernel for the neurogenesis of all patterns of automatic ventilatory activity (70, 71). If eupnea is a variant of the gasp, why was eupnea not altered by ablation of medullary regions, which are critical for the neurogenesis of gasping? Moreover, if eupnea is a variant of the in vitro burst, why was eupnea not altered when the entire ventral medullary respiratory nucleus was destroyed in vivo in adult cats? In the adult cat, the localization of the pre-Bötzinger complex is clearly within regions that were destroyed.
in these previous in vivo experiments (8, 67, 72, 73). The caveat must be added that lesions of the gasping center and the pre-Bötzinger complex might have destroyed a sufficient population of neurons to eliminate gasping, but the residual neurons in the region might be sufficient for the neurogenesis of eupnea. Yet, for this interpretation to be correct, such a critical residual would have to be exceedingly small and missed in previous studies, which have emphasized a complete destruction of the ventral nucleus, including, retrospectively, the pre-Bötzinger complex (19, 72, 73).

NEUROGENESIS OF THE GASP

I propose that the neurogenesis of the gasp results from the discharge of pacemaker neurons in the continuous pre-I, pre-Bötzinger, and gasping regions. By strong mutual excitation, these propriobulbar neurons undergo a synchronous activation, which is transmitted to the premotor and motoneurons of medulla. This excitation by medullary mechanisms for gasping appears to be ubiquitous in that tonic neuronal activities are incorporated into the gasp; many neurons that are silent in eupnea are also recruited (6, 17, 23, 68).

Termination of the gasp would seem dependent on synaptic processes, since stimulation of medullary regions can prematurely terminate gasps (80). There is much ambiguity concerning such a process, since, as noted above, expiratory-related activities decline greatly or are eliminated in gasping. However, a complete categorization of medullary expiratory-modulated neuronal activities in eupnea and gasping remains to be performed.

In eupnea, the discharge mechanisms for gasping is suppressed or transformed by an inhibition of pontile origin. An obvious question concerns the neurotransmitters that may be responsible. In the adult, both γ-aminobutyric acid A (GABA_A) receptor agonists and glycine are endogenous neurotransmitters (46). In general, agonists of GABA_A and glycine cause depression, and antagonists cause augmentations of eupneic ventilation; the frequency is altered in some preparations (see reference lists in Refs. 30, 46). Interestingly, in a perfused brain stem preparation exhibiting eupnea, increasing doses of antagonists ultimately resulted in a breakdown of this eupneic rhythm to a mixture of eupneic and gasplike bursts (30). In contrast, the frequency of rhythmic bursts of the in vitro preparation of the neonatal rat is not altered after application of antagonists of inhibitory neurotransmitters; agonists do cause a reduction in this frequency. The discharge characteristics of individual pre-I neuronal activities are altered by antagonists of GABA and glycine (28, 53, 57, 59). These different findings concerning synaptic inhibition are again resolvable when it is recognized that the in vitro neonatal rat preparation is exhibiting gasping and not eupnea. During eupnea in both neonatal and adult animals, the inherent pacemaker-like activities of medullary neurons underlying gasping are suppressed by synaptic mechanisms arising from a pontomedullary circuit. An example would be a transformation of the late expiratory-early inspiratory pre-I discharge recorded in vitro to the phase-spanning expiratory-inspiratory pre-I discharge recorded in vivo.

This incorporation of neuronal activities underlying gasping into the pontomedullary circuit underlying eupnea would solve the enigma as to how medullary mechanisms for gasping could be seemingly quiescent for long periods and only activated when eupnea failed. Such activities are not quiescent but are fundamentally transformed by pontomedullary circuits responsible for the neurogenesis of eupnea.

NOEUD VITAL OR NOEUDS VITALS

The net sum of the experimental evidence leads to the conclusion that a medullary noeud vital is critical for the neurogenesis of gasping. How and whether neuronal activities in this medullary noeud vital play an essential role in the neurogenesis of eupnea is uncertain. An incorporation of results from in vitro studies into characteristics of the eupnea rhythm, defined in vivo in neonatal and adult animals, has required the inclusion of a series of transformations. Differences between in vitro and in vivo preparations have included discharge patterns, responses to neurotransmitters, and responses to stimuli (8, 28, 53, 57, 59–62, 70, 71). Transformations to explain such differences have included removal of sensory afferents and age-related changes. There is no doubt that the brain stem ventilatory control system does undergo maturational changes (33, 34). However, many of the transformations, which are necessary to explain differences between activities recorded in vitro and in vivo, are not
required when findings in vitro are compared with gasping in vivo. As opposed to eupnea, gasping and in vitro rhythmic activities are almost identical.

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