Genesis and Control of the Respiratory Rhythm in Adult Mammals

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The neural mechanisms responsible for respiratory rhythmogenesis in mammals were studied first in vivo in adults and subsequently in vitro in neonates. In vitro data have suggested that the pacemaker neurons are the kernel of the respiratory network. These data are reviewed, and their relevance to adults is discussed.

Several billion mammals, including people, manage to stay alive by breathing rhythmically; however, little was known about how this feat is actually achieved from birth to death until some useful in vitro data became available. For several decades, all attempts to investigate this vital function were based on the use of electrophysiological, pharmacological, and anatomic in vivo approaches on adult animals, mainly cats (1). The activity of several thousands of neurons firing in phase with respiration was recorded; the extent and the location of the so-called respiratory centers in the brain stem were mapped out; the responses of respiratory muscles, nerves, and neurons to various stimuli were studied; and the effects of applying various drugs were tested. Although several theoretical models were proposed, the actual mechanisms responsible for respiratory rhythmogenesis were largely misunderstood until quite recently. In vitro studies performed on neonatal rodents showed, however, that a special set of neurons located within the rostral ventrolateral medulla act as inspiratory pacemaker neurons and may play the leading role in respiratory rhythmogenesis (8, 14). On the one hand, the reasons why in vivo adult studies have failed to explain respiratory rhythmogenesis are easily understandable, because breathing is such a complex motor activity involving several regulatory processes. On the other hand, the possibility of eliminating peripheral inputs and gaining direct access to the extracellular medium under in vivo conditions greatly simplified the problem. However, one cannot help wondering whether the in vitro approach did not yield a rather oversimplified picture at first since the overall mechanisms involved in respiratory rhythm generation are probably far more complex than what these data seemed to suggest. In this review, we propose to describe the great complexity of the respiratory act and the way in which it is regulated before reviewing the main data obtained both in vivo and in vitro on the respiratory network, based on a selection of the latest studies on the subject.

Complexity of the respiratory motor act

Respiration is a complex rhythmic motor act involving several tens of muscles, which belong to three main functional groups. First, there is the diaphragm. This muscle, inserted between the lower ribs, acts like a pump moving up and down within the rib cage. Its contractions are directly responsible for sending air into the lungs. Secondly, there are several pairs of muscles, such as the internal and external intercostal, elevator costae, and abdominal muscles. Although they are often classified as “accessory” respiratory muscles, they play an important role since they stiffen the rib cage and thus determine the efficiency of the diaphragm contractions. Thirdly, the upper airway muscles such as the laryngeal, pharyngeal, and genioglossus muscles control the rhythmic opening and closure of the upper airways. Their contractions regulate the rate at which the air flows in and out of the lungs in much the same way as a valve (Fig. 1).

The motoneurons commanding the respiratory muscles are located at different levels of the central nervous system. The upper airway motoneurons are located at the cranial level; the phrenic motoneurons that control the diaphragm are located in the cervical cord ventral horn, and the rib cage and abdominal motoneurons are located in the ventral horn of the thoracic and lumbar segments. All of these motoneurons are rhythmically driven by a central command exerted by the so-called respiratory centers, a network of interneurons housed in the brain stem. This central drive is conveyed to the respiratory motoneurons by both mono- and paucisynaptic pathways at the cervical interneurons. Although the frequency of breathing is determined only in the brain stem, its magnitude depends on both the strength of the central command and that of the other inputs impinging on the respiratory motoneurons. These inputs can be of both central (cortical inputs) and peripheral (homonymous and heteronymous proprioceptive inputs) origin, and they can act over both the motoneurons and the respiratory centers (Fig. 1).

Coordination of the contraction of the various respiratory muscles

An efficient respiratory act cannot be achieved without the coordinated contraction of all the respiratory muscles, and this coordination is carried out at both the central and peripheral levels.

First, the central inspiratory command activates the respiratory motoneurons in a predetermined sequential order. The upper airway motoneurons are recruited first, before those of
the diaphragm and the rib cage. This facilitates the opening of the glottis before the decrease in the tracheal pressure resulting from diaphragm contraction, thus preventing the occurrence of any upper airway collapses. Collapses of this kind resulting from defective coordination between the upper airway valve and the inspiratory pump muscles are responsible for the most frequent respiratory disorders, which are obstructive apneas. In addition, because the diaphragm is inserted between the lower ribs, its contractions might pull in the ribs and thus decrease the transversal diameter of the rib cage during inspiration, making the contraction of the diaphragm less efficient. This is prevented from occurring by the cocontraction of the accessory muscles, which hold the ribs in a fixed position during the contraction of the diaphragm. If the coordination between diaphragm and intercostal contractions is impaired, the respiratory act becomes less efficient, as occasionally observed in preterm babies.

On the other hand, proprioceptive receptors in the respiratory muscles are also involved in linking up the various motoneuron pools. The regulatory system, including segmental and plurisegmental spinal loops and the spinal circuitry, coordinates the activity of agonist and antagonist muscles. In addition, long loops, including spinal and central respiratory interneurons, may adjust the command to the respiratory motoneurons. For example, the activation of intercostal proprioceptive afferents during respiratory contractions contributes to regulating the contractions of the diaphragm and vice versa.

The main systems of respiratory regulation

Several factors are involved in adapting the central command to meet the physiological requirements and protecting the respiratory system from various risks.

First of all, because the purpose of respiration is to promote blood oxygenation and \(O_2\) delivery to the whole body, any blood hypoxia is rapidly detected at the peripheral level, mainly by the carotid bodies. These peripheral chemoreceptors in turn adjust the central respiratory drive to the motoneurons in terms of its magnitude and frequency. If the resulting increase in ventilation fails to restore blood normoxia, central hypoxia will develop and depress the respiratory network.

Secondly, the feedback mechanisms that serve to maintain normal breathing rely on the \(CO_2\) levels as well as the \(pH\) levels. Although the carotid bodies can participate in this regulation, the central \(CO_2\) and/or \(pH\) sensors are located at or close to the surface of the rostral ventrolateral medulla, although it has been suggested that they may have a more widespread pattern of anatomic distribution. The overall \(CO_2\) level constitutes the basis of one major “drive” to the central pattern generator (11), and this chemoreceptor function may be an intrinsic feature of the central pattern generator that drives breathing in air or water (16). The arterial pressure changes also regulate breathing via baroreceptor inputs to the respiratory centers and via central interactions between cardiovascular and respiratory networks. It is noteworthy that respiratory and cardiovascular networks are located in overlapping brain stem areas.

Thirdly, the regulation of ventilation protects the respiratory effectors, i.e., the lungs and the respiratory muscles. The lungs are inflated during inspiration, and this activates pulmonary stretch receptors, which, via the vagus nerve, excite interneurons located in the nucleus of the solitary tract, and these interneurons in turn inhibit the central inspiratory drive. This inhibitory loop, known as the Brüer-Hering reflex, helps to prevent overinflation, which might damage the lungs. In addition, the tracheal receptors, which are activated by any abnormal irritative substance present in the airways, also play a crucial role consisting of inducing coughing. In addition, the respiratory muscles are protected by proprioceptive afferents in the same way as in the case of nonrespiratory motoneurons.

Interactions between respiratory and nonrespiratory functions

Under physiological conditions, the respiratory muscles are frequently involved in nonrespiratory behavior (Fig. 1). For example, the respiratory muscles are involved in vocalization and swallowing, and the central structures controlling these types of behavior exert a potent control on both the respiratory centers and the respiratory motoneurons. In some mammals, the respiratory muscles subservice thermal regulation. During hypothermia, the frequency and amplitude of the respiratory movements, as well as the respiratory regulation, are modified to deal with the main priority, which is no longer delivering but maintaining the central temperature within a normal range. The other cases in which respiratory muscles are involved in nonrespiratory functions include vomiting, defecation, and parturition. At the same time, breathing is also modulated by complex functions such as emotional exercise, sleep, etc., which are controlled at the cortical level and in other parts of the central nervous system. Transneuronal tracing was carried out by injecting rabies virus into the respiratory muscles of adult mice, and the results confirmed that several structures, such as raphe nuclei, locus coeruleus, area central, thalamic and hypothalamic nuclei, cerebellum, and some cortical areas, are connected to the brain stem respiratory centers (5). All of this means that the discharge patterns of respiratory muscles, motoneurons, and central interneurons are affected by many factors other than breathing, under in vivo conditions. It is therefore difficult to interpret the respiratory changes induced experimentally by stimulating central structures or by performing lesions or applying drugs to these structures. It is also difficult to ascertain whether the structures in question actually belong to the respiratory network or to nonrespiratory centers interacting with respiration. The general complexity of the breathing act may in fact explain why the results of in vivo studies have not sufficed to account for the respiratory rhythmogenesis (Fig. 1).
Respiratory rhythmogenesis

In vivo adult mammals. In anesthetized adult cats, it has been established (1, 17) that the respiratory cycle can be divided into three successive stages: 1) stage I (inspiration), during which the diaphragm and the other inspiratory muscles of the rib cage contract; 2) stage I-E (corresponding to the transition between inspiration and expiration), during which the inspiratory muscles progressively cease to contract while the expiratory laryngeal muscles contract to control the airflow out of the lungs; and 3) stage E (active expiration), when the expiratory intercostal and abdominal muscles contract (Fig. 2A).

On the basis of recordings of the activity of brain stem neurons that were rhythmically active in phase with respiration, six different types of neurons have been defined on the basis of their firing patterns, their membrane potential changes, and their synaptic inputs (1, 17). As shown in Fig. 2A, four types of neurons fire during inspiration: the Pre-I neurons, which fire at the transition between expiration and inspiration; the Early-I neurons, which fire from the beginning to the middle of inspiration; the Late-I neurons, which fire throughout inspiration; and the Late-I neurons, which are active at the very end of inspiration. During the IE phase, Post-I (or Early-E) neurons are activated. Lastly, the E neurons discharge during expiration. The activity of these neurons can be recorded at various brain stem loci. At the medullary level, respiratory neurons of all kinds are gathered in the vicinity of the nucleus ambiguous and the surrounding reticular formation, constituting the so-called ventral respiratory group (VRG). Some rather densely packed expiratory neurons are to be found in the rostral part of the VRG, forming the Bötzinger group. Among the VRG, some inspiratory and expiratory bulbospinal neurons send their axons toward the spinal cord. The inspiratory bulbospinal neurons excite the inspiratory motoneurons via spinal interneurons, and numerous expiratory bulbospinal neurons from the Bötzinger group inhibit phrenic motoneurons during expiration. Some VRG neurons are motoneurons innervating the upper airway muscles. There are some other VRG neurons with their axons entirely located within the brain stem; these neurons, which are called propriobulbar interneurons, might participate in setting up the central drive. In the dorsal part of the medulla, a dense group of inspiratory neurons forms the dorsal respiratory group (DRG) in the vicinity of the nucleus of the solitary tract, close to the entry of peripheral afferents from baro- and chemoreceptors and from the lungs. Most of the DRG neurons are inspiratory bulbospinal neurons having mono- or paucisynaptic excitatory effects on the phrenic motoneurons (1). The inspiratory neurons of the DRG are unlikely to be simply “output” neurons of the respiratory centers, because they often excite other inspiratory neurons and because their massive antidromic activation can affect the respiratory rhythm in cats. In adult rats, however, the existence of a DRG is questionable.

At the pontine level, respiratory neurons have been encountered in the Kölliker-Fuse and Parabrachialis areas. These neurons often fire tonically with a respiratory modulation, especially at the transition phases between inspiration and expiration. They constitute the “pneumotaxic centers” that were first believed to play an important role in the genesis of respiratory rhythm, because in anesthetized and vagotomized cats lesion of the dorsal pons induced apneusis, i.e., very-long-lasting inspirations (>5 s) interrupted by short expirations. However, because apneusis does not occur without vagotomy and anesthesia, and because it is questionable whether it occurs in species other than the cat, less attention is being paid at the present time to the role of the pontine area in rhythmogenesis.

In adult cats, the connectivity between the various types of respiratory neurons has been studied by performing cross-correlation analysis either between pairs of extracellular recordings or between paired extracellular and intracellular recordings and analyzing the membrane synaptic noise and the changes occurring in response to intracellular chloride injection. On the basis of these results, several models were proposed to explain respiratory rhythmogenesis, most of which were based on a process of reciprocal inhibition between the inspiratory and expiratory neurons. In the most widely recognized model (Fig. 2B), reciprocal inhibitions play the key role; glycinergetic and GABAergic inhibitory relations generate the rhythm, and few excitatory synapses are involved in the feed-
back loops maintaining the inspiratory or expiratory phases. However, this model does not fit several experimental findings. First, the existence of an active expiration may be questioned at rest and under anesthesia. In addition, in artificially ventilated cats, extensive elimination of neurons in the VRG and DRG areas depresses the amplitude of the inspiratory discharge but fails to abolish respiratory rhythm. Again in cats, polypneic respiratory rhythm is accompanied by the silencing of most of the expiratory neurons, whereas most of the inspiratory neurons continue to fire rhythmically. Furthermore, lack of glycinergic inhibition in transgenic mice does not prevent respiratory rhythmogenesis from occurring; although breathing is disturbed, it is not stopped altogether (2). Although inhibitory glycinergic effects do occur, they are therefore not essential to respiratory rhythm, and the reciprocal inhibition theory may have to be revisited. Lastly, this model is not in good agreement with recent in vitro data obtained on neonatal rodents (see Fig. 3).

In vivo preparations from neonatal mammals. It was established by Suzue (20) that, in neonatal rats, the isolated respiratory network continues to produce rhythmic bursts of potentials in the phrenic nerve in vitro (Fig. 3A). At that time, very few scientists were convinced that these in vitro preparations were actually suitable for performing respiratory network studies. Now, however, studies are being widely performed using Suzue’s model and other in vitro models of this kind, such as perfused brain stems (12), brain stem slices (9), and even restricted parts isolated from brain stem slices in which only the most indispensable part of the network is preserved. In addition, these in vitro approaches to the isolated respiratory network offer several unique advantages such as the possibility of modifying the extracellular medium, recording from respiratory neurons under much more favorable conditions, and stimulating or destroying parts of the network without inducing the changes mediated by peripheral feedback responses. In vitro, neonatal respiratory neurons have been detected in the region of the rodent VRG (but not the DRG). The most exciting result so far was the finding that some inspiratory neurons in the rostral ventral lateral medulla act as inspiratory pacemakers; they continue to produce rhythmic bursts of potentials even when the synaptic connections are blocked (Fig. 3B). These neurons play a crucial role in respiratory rhythmogenesis, because their synchronous activation during expiration in response to single-shock electrical stimulation prematurely triggers a phrenic burst, their response to locally applied drugs affects the in vitro respiratory rhythm, and their destruction by electrolytic lesion abolishes respiratory rhythmicity. Therefore these neurons may be the key components of the respiratory network, imposing the rhythm of their bursts of potentials on the rest of the network. The latter may be mainly involved in shaping the central respiratory command and defining its strength. However, the activity of the pacemaker neurons may also depend in turn on the rest of the network. Although the inspiratory pacemaker neurons do not constitute a well-defined group within the medulla, several authors have referred to it as the Pre-Bötzinger Complex (PBC). Both the electrical coupling and the excitatory chemical transmission between the pacemaker neurons certainly play an important role in the generation and/or modulation of the breathing rhythm (15). Researchers are now attempting to analyze the mechanisms underlying the special membrane properties of the pacemaker neurons and to classify them into various subtypes. However, blockade of the persistent sodium current postulated to be responsible for the rhythmic bursting of the pacemaker neurons does not alter the in vitro rhythm (3).

**Neurochemistry of respiratory neurons**

Thanks to the latest in vitro methods, it is now possible to perform pharmacological studies under satisfactory conditions, since the peripheral effects and loops can be eliminated. Several endogenous substances, such as serotonin, substance P, noradrenaline, acetylcholine, and TRH, have been found to affect the in vitro respiratory activity (see Refs. 6 and 19).
These substances may act at several levels of the respiratory network; their effects on the VRG and the motoneurons modulate the amplitude of the respiratory motor output. Their effects on the respiratory rhythm are mediated by the PBC, because the local application of drugs to the PBC affects the respiratory rhythm, and immunohistological findings have confirmed the presence of receptors to these substances in the PBC (Fig. 3D). Therefore, the numerous controls exerted on respiration in vivo may be at least partly mediated through PBC neurons, which may even mediate the respiratory response to hypoxia. Evidence exists in the literature that substance P and its neurokinin-1 receptor (NK₁) are involved in the respiratory response to hypoxia and that NK₁ receptors are expressed by the PBC inspiratory pacemaker neurons. NK₁ receptors may therefore constitute a convenient tool for locating these neurons, i.e., for “visualizing the ghost in the machine” (13).

### PBC pacemaker neurons and adult respiratory rhythm

One of the main questions that arises at the present time is whether the PBC inspiratory pacemaker neurons play the same crucial role in vivo in adult mammals as they do in the model in vitro systems described above. Much evidence has been accumulated in recent reviews suggesting that the rostral part of the VRG, which might correspond to the PBC, plays a crucial role in both young and adult mammals (8, 14). Indeed, in adult mammals 1) this area contains mainly interneurons and few output neurons; 2) blocking their activity alters breathing, whereas extensive lesions performed in the DRG and the caudal part of the VRG do not abolish the respiratory rhythm; 3) brain stem slices of young rodents containing the rostral end of the VRG can produce respiratory rhythm in vitro up to the age of 21 days; 4) the slice respiratory rhythm persists after the inhibitory synaptic transmission has been blocked; 5) possible respiratory pacemaker neurons are recorded in perfused mouse brain stem preparations (12); 6) adult rat PBC inspiratory neurons express NK₁ receptors (12); and 7) bilateral destruction of PBC neurons expressing NK₁ receptors alters the breathing pattern and the response to hypoxia (6).

On the basis of all of the above data, it seems perfectly reasonable to conclude that the PBC plays a crucial role both in vivo and in vitro, in neonates and adult mammals. However, this conclusion must be treated with all due caution. First, most of the results supporting the existence of PBC pacemaker neurons were obtained in rodents. Second, the maturation of the cellular metabolism, the membrane properties, and the synaptic processes might have functional effects on the PBC (18). Third, the variable respiratory rhythm produced by the fetal respiratory network in vitro is unlikely to originate from the PBC, because electrical stimulation applied to the PBC area has no clear-cut respiratory effects and no PBC pacemaker neurons have been detected at this age. This situation suggests that the respiratory network may generate the respiratory rhythm in several ways and that it may even be recon-
figured to produce multiple breathing patterns (8, 10, 18).

Concluding comments

It is more than likely that our understanding of the genesis of respiratory rhythm will be further improved in the near future as new approaches become available. First, respiratory network tracing methods with rabies virus will make it possible to carry out comparisons between neonatal and adult respiratory networks with a view to detecting age-related similarities and differences (5). Second, transgenic mice in which gene deletions have induced respiratory disorders limiting their survival to a few hours after birth should also help to shed more light on the role of genetic factors in respiratory rhythogenesis (4). Third, these genetic data, along with what is known about the phylogeny of various species, could be used to establish the existence of an evolutionary linkage between some air-breathing vs. aquatic animals, as suggested by the close similarities found to exist between their respiratory rhythm generators and chemosensitive brain stem areas (16).

Although the most striking results obtained so far on the genesis and control of respiratory rhythm were based on the use of neonatal in vitro preparations, it is likely that the inspiratory pacemaker neurons of the PBC may also play a role in adult mammals. This does not, however, exclude the possibility that other parts of the brain stem network and other neural mechanisms may also be part of the machine responsible for generating respiratory rhythm. It seems quite likely in the end that the ghost in the machine (13) may in fact appear in various shapes, depending on the situation.

References