

## Blessing, Chapter 4. Breathing, part 2

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### DRG Neurons and Their Inputs From Slowly Adapting Pulmonary Stretch Receptors

Von Baumgarten and Kanzow (1958) recorded extracellularly in the cat medulla and found neurons with an inspiratory rhythm located "within a circumscribed region of the reticular formation closely below the tractus solitarius, 1-3 mm rostral to the obex." These neurons are now known as the DRG group. Von Baumgarten and Kanzow, and subsequent investigators, assessed the response of the neurons to inputs from slowly adapting pulmonary stretch receptors, activated by lung inflation. Some neurons ( $R\alpha$  or  $I\alpha$ ) always discharge in phase with the phrenic nerve; during lung inflation their firing starts and stops together with phrenic activity. The discharge of other neurons ( $R\beta$  or  $I\beta$ ) is increased by lung inflation, even when this procedure abolishes phrenic discharge. The discharge of  $I\beta$  neurons also increases when the lung is inflated during expiration, when the DRG respiratory neurons are normally silent. The initial findings suggested that  $I\alpha$  cells function as premotor inspiratory neurons, driving phrenic neurons, while the  $I\beta$  cells, by an inhibitory action on  $I\alpha$  cells, function to transmit information concerning lung inflation to the phrenic nucleus.

Cat DRG neurons have been demonstrated by electrophysiological recording and by intracellular filling with HRP (Berger, 1977; Berger et al., 1984; Otake et al., 1989; Voss et al., 1990). Properties of these neurons are shown in Figure 4.7.

**Figure 4.7 A,B**, Location and morphology of DRG respiratory neurons. (Modified from Berger et al., 1984, and Davies et al., 1987.) **C**, Location of nucleus tractus solitarius neurons projecting to the region of the contralateral phrenic nucleus. (Modified from Rikard-Bell et al., 1984, and Onai and Miura, 1986.) **D,E**, Intracellularly recorded membrane potential of inspiratory DRG neuron, showing discharge in response to lung inflation and axonal trajectories of intracellularly labeled inspiratory DRG neurons. (Modified from Otake et al., 1989.) All data from the cat. MP, membrane potential; Phr, phrenic nerve discharge; is, interstitial; int, intermediate; m, medial; lat, lateral; vl, ventrolateral subdivisions of nucleus tractus solitarius. Other abbreviations listed on pages xiii-xiv.

The cell soma is approximately 50 $\mu$ m in diameter. The dendritic tree extends approximately 1 mm rostral and 1 mm caudal to the soma, orientated principally within the horizontal plane so that dendrites remain ventrolateral to the tractus solitarius. Based on neuron size as judged by Nissl stains, there are approximately 40 DRG cells on each side of the medulla (Berger et al., 1984). However, this may well be an underestimation, since retrograde HRP studies in the cat indicate that 10-20 times this number of neurons project from the DRG to the region of the phrenic nucleus (Rikard-Bell et al., 1984; Onai and Miura, 1986).

Berger and colleagues located the cat DRG neurons in a region generally referred to as the ventrolateral nucleus of the nTS (see Chapter 3 for a discussion of nTS subdivisions). This particular

location may have depended partially on selection criteria, since the recording region was limited to between 1 and 2 mm ventral to the dorsal surface of the medulla (Berger, 1977). Similarly, Backman and colleagues (1984) reported intracellular recordings from DRG inspiratory neurons in the ventrolateral nTS, demonstrating occurrence of EPSPs in DRG neurons triggered from nodose ganglion cells activated by pulmonary stretch receptors. Kalia and colleagues (1979) noted inspiratory unit activity in regions "extending beyond" the ventrolateral nTS. Although many electrophysiological studies fail to provide histological evidence of the recording site, other investigators have identified neurons with increased inspiratory discharge around the tractus solitarius (Cohen and Feldman, 1984; Bennett et al., 1985; Pantaleo and Corda, 1986; Jiang et al., 1986; Davies et al., 1987) not only in the ventrolateral region (see Fig. 4.7B). Cells in these other nTS regions may therefore have properties similar to neurons in the DRG. Apparently only the area ventrolateral to the tractus solitarius contains cells that project to the region of the phrenic nucleus in the cat (Fig. 4.7C).

An intracellular study by Otake and colleagues (1989) demonstrated that axons of cat inspiratory DRG neurons course ventrally and medially, crossing the midline slightly rostral to the level of the soma and then descending to the spinal cord on the contralateral side (Fig. 4.7D,E). A small proportion of the axons have intramedullary collaterals in the region of the ventrolateral respiratory groups.

Recording of respiratory rhythms from axons in the DRG region could mistakenly be attributed to DRG neurons. When Kalia and colleagues (1979) injected HRP into the DRG they found labeled neurons in the ipsilateral nodose ganglion, indicative of uptake by afferent fibers en route to the nTS. Axons of laryngeal motoneurons pass near the ventrolateral nTS as they curve dorsally from the nucleus ambiguus, and axons of expiratory BötC neurons project to the nTS via the ventrolateral region (see section on BötC neurons, below). Collaterals of rVTG neurons also approach this region (Lipski et al., 1994). Some studies are explicitly directed toward the terminations of primary respiratory axons in the nTS region. Donoghue and colleagues (1982b) mapped medullary sites from which nodose ganglion neurons (discharging in response to lung inflation) could be antidromically activated. The major projection of these neurons was to the medial subnucleus of the nTS, not the ventrolateral subnucleus. Berger and Averill (1983) used spike-triggered averaging of field potentials to confirm the termination of afferents carrying slowly adapting stretch receptor signals in both medial and ventrolateral subnuclei. Averill and colleagues (1984) recorded from cat DRG neurons and cross correlated their discharge with nodose ganglion units adapting slowly to lung inflation. The results suggested that the relevant nodose cells monosynaptically excite DRG I $\beta$  and pump cells, but have neither excitatory or inhibitory connections with I $\alpha$  cells.

Von Euler and colleagues (1973a,b) found that DRG neurons antidromically activated from the spinal cord were not affected by electrical stimulation of the cervical vagus (below the junction with the superior laryngeal nerve). Such neurons displayed the I $\alpha$  response to lung inflation. Conversely, if a DRG inspiratory neuron could be driven by vagal stimulation it could not be antidromically activated from the spinal cord. Such cells had the I $\beta$  response to lung inflation. They retained some respiratory rhythmicity when the ventilator was momentarily turned off, indicating that their discharge did not depend solely on inputs from pulmonary mechanoreceptors. In general agreement with the

conclusions of Von Baumgarten and Kanzow, Von Euler and colleagues considered that I $\beta$  neurons mediate the Breuer-Hering inspiratory inhibitory reflex, although it was thought that I $\beta$  neurons probably do not receive direct input from pulmonary afferents.

However, a more complex picture of the function of cat DRG neurons has now emerged. The discharge pattern of some I $\beta$  neurons suggests that they may be involved in the reflex termination of inspiration, but they are most unlikely to contribute to reflex prolongation of expiration. Berger (1977) noted that moderate lung inflation often increases expiratory duration without exciting I $\beta$  neurons. Other investigators have shown that I $\beta$  neurons are mostly inactive during this period due to powerful inhibitory inputs (for details, see Cohen, 1979; Richter, 1982) now known to arise at least in part from expiratory BötC cells. The initial demonstration of monosynaptic inhibition of inspiratory neurons in the DRG by BötC expiratory neurons (Fig. 4.8) was a landmark study, the first electrophysiological demonstration of a monosynaptic neuronal connection in intramedullary respiratory circuitry.

**Figure 4.8** First demonstration, by spike-triggered averaging, of monosynaptic connections between medullary respiratory neurons. Bötzing expiratory neurons inhibit DRG inspiratory neurons. IPSP, inhibitory postsynaptic potential. (Modified from Merrill et al., 1983.)

Other DRG neurons, without a respiratory rhythm of central origin, discharge synchronously with lung inflation, exhibiting very little adaptation. Berger argued that these so-called pump (P) cells, not the rapidly adapting I $\beta$  neurons, are responsible for the prolongation of the expiratory phase in the Hering-Breuer reflex.

Kalia and Richter (1985a,b), using intraaxonal injections of HRP after intraaxonal recordings from units discharging with little adaptation following lung inflation, identified myelinated HRP-containing axons with terminal boutons in the interstitial, intermediate, ventral, and ventrolateral divisions of the nTS (Fig. 4.9).

**Figure 4.9** Projections of axons from slowly and rapidly adapting receptors in the lung to the nucleus tractus solitarius, demonstrated by intra-axonal injections of HRP after physiological identification. (Modified from Kalia and Richter, 1985a,b.) d, i, v, and vl are, respectively, dorsal, interstitial, ventral, and ventrolateral subnuclei of the nucleus tractus solitarius. Other abbreviations listed on pages xiii-xiv.

However, other anatomical studies show that primary afferents from the lung and large bronchi (where stretch receptors are located) are unlikely to provide a major innervation of the DRG neurons, since, as discussed above, many of these cells are situated ventrolateral to the tractus solitarius. Kalia and Mesulam (1980a) show that at levels just rostral to the obex, where the DRG is located, terminals of lung and the main bronchi afferents are located lateral, rather than ventrolateral, to the tractus solitarius (see Fig. 11 in Kalia and Mesulam, 1980a). The additional ventrolateral spread of the dendritic tree of DRG neurons (Fig. 4.7A) probably means that many are beyond the region of

termination of primary afferents from the lung. Some more medially projecting dendrites (Voss et al., 1990) could be directly affected by primary afferents terminating in medial nTS regions.

Contrary to the findings of von Euler and colleagues (1973a,b), many I $\beta$  neurons, as well as I $\alpha$  neurons, can be antidromically activated from the spinal cord (Lipski et al., 1979; Jiang et al., 1987). When the more sensitive "no lung inflation during inspiration" test is used to eliminate phasic input transiently from pulmonary stretch receptors, different I $\alpha$  and I $\beta$  subgroups are defined. Lipski and colleagues (1983) used spike-triggered averaging to examine the effect of the discharge of I $\alpha$  and I $\beta$  DRG inspiratory neurons on phrenic motoneurons. Evidence for monosynaptic excitation of phrenic motoneurons was obtained for neurons in both categories, but the overall proportion of DRG neurons making monosynaptic connections with phrenic motoneurons could not be assessed. Duffin and Lipski (1987) used antidromic activation procedures to demonstrate that approximately 80% of inspiratory DRG neurons project to contralateral T<sub>3</sub>-T<sub>5</sub> spinal segments, with many neurons proving to have axon collaterals at the spinal level. Intracellular recordings were made from inspiratory intercostal neurons, and membrane depolarizations indicative of EPSPs could be recorded with DRG neurons as a trigger. Although the degree of convergence and divergence of monosynaptic connections between brainstem and intercostal inspiratory neurons is difficult to determine, it appears that monosynaptic excitatory connections to inspiratory intercostal neurons arise more from DRG inspiratory neurons than from rVRG inspiratory neurons.

Rat DRG respiratory neurons were thought to be absent or sparse (Saether et al., 1987; Ezure et al., 1988; Zheng et al., 1991a), but a careful study has now confirmed the presence of a group of principally inspiratory neurons in this species (DeCastro et al., 1994), situated ventral and ventrolateral to the tractus solitarius, at the level of the area postrema and just rostral to this level (Fig. 4.10). Most of the neurons discharged during inspiration, with either I $\alpha$  or I $\beta$ , or pump neuron characteristics (Fig. 4.10).

**Figure 4.10** Location, and response to lung inflation, of inspiratory neurons in rat DRG (vagi intact). The I $\alpha$  neuron discharges in phase with phrenic nerve discharge, regardless of input from lung inflation receptors. The I $\beta$  neuron responds to lung inflation, but discharges in phase with centrally generated phrenic nerve activity during lung deflation (three asterisks), as indicated by the reduced tracheal pressure (TP). The discharge of the pump neuron reflects inputs from lung inflation receptors, with no centrally generated rhythm. (Modified from de Castro et al., 1994.) Abbreviations listed on pages xiii-xiv.

Some of the I $\alpha$  neurons, but no I $\beta$  neurons could be antidromically activated from the cervical spinal cord. The conduction velocity was 4-12 m/s, suggesting that the neurons may be small, possibly explaining why several authors have found it difficult to identify them electrophysiologically. Few of the neurons could be antidromically activated from below the C<sub>2</sub> segment. Further studies of this question seem indicated, since the pseudorabies transneuronal virus evidence suggests that some DRG neurons do project to the phrenic nucleus in the rat.

In a study of the central pathway mediating the lung-inflation reflex in the rat, Bonham et al. (1993) and Bonham and McCrimmon (1990) used focal injections of excitatory amino acids in the anesthetized rat to determine nTS sites at which phrenic discharge was inhibited in a manner similar to that elicited by lung inflation. Such sites were located just medial to the tractus solitarius at the level of the obex and just caudal to this level (Fig. 4.11).

**Figure 4.11 A**, nTS site at which excitation of neurons causes inhibition of phrenic nerve activity similar to that observed in the Breuer-Hering lung inflation reflex. **B**, Cobalt-induced synaptic inactivation at the same site blocks the Breuer-Hering reflex. (Modified from Bonham and McCrimmon, 1990.) Abbreviations listed on pages xiii-xiv.

Extracellular recordings in this region identified neurons that discharged in phase with lung inflation and were silent when lung inflation was withheld (P cells). Interruption of synaptic transmission using local injections of cobalt chloride or excitatory amino acid antagonists in the same medial nTS region impaired the apnea normally produced by maintaining lung inflation. These results suggest that the Breuer-Hering reflex in the rat depends on inputs to secondary afferent neurons located in the nTS just medial to the tractus at the level of the area postrema. These neurons are not located in the same anatomical region as the rat DRG inspiratory neurons described by De Castro and colleagues (1994). The functional role of DRG inspiratory neurons remains undetermined. As Merrill (1981) noted for the I $\alpha$  neurons in the cat, it is surprising to find neurons situated so close to the primary afferents, yet discharging with a pattern closely related to the motor output of the respiratory system. Speck and Feldman (1982) found little change in respiratory rhythm after bilateral lesions of the DRG region in the cat. With unilateral lesions there was a reduction in amplitude of the contralateral phrenic discharge, consistent with the idea that DRG cells form part of the population of medullary cells whose activity increases phrenic discharge. This role seems understandable for the I $\alpha$  neurons, but they are not obviously affected by primary respiratory afferents. The excitatory action of the spinal projections of the I $\beta$  neurons is difficult to interpret, since the discharge of these cells is often out of phase with the phrenic neurogram when the paralyzed animal is ventilated with a constant frequency pump (not triggered by the respiratory cycle). McCrimmon and colleagues (1987) made lesions in the ventrolateral division of the nTS in the cat. The inspiratory-termination component of the Breuer-Hering reflex (elicited by vagal stimulation with parameters activating pulmonary stretch receptor afferents) was largely unaffected by the lesions. The findings are reminiscent of an early study by Allen (1926), who found that breathing in the guinea pig was relatively unaffected by large lesions in the dorsomedial medulla.

Thus, although inspiratory DRG neurons were among the first respiratory neurons to be characterized, there remains much controversy concerning their place in the medullary regulation of respiration. They are located at the border of the dorsomedullary region receiving direct inputs from primary respiratory afferents, and the evidence for monosynaptic inputs from primary afferents is surprisingly variable. They may receive direct inputs from slowly adapting pulmonary stretch receptors, but they probably do not receive direct inputs from chemoreceptors or from airway irritant

receptors (see below). They do not project widely within the medulla. Many do project to the spinal cord, perhaps to the phrenic nucleus, but these projections are not essential for the continuation of relatively normal central respiration, at least in anesthetized artificially ventilated animals.

### **Retrotrapezoid Respiratory Neurons**

Neurons in the retrotrapezoid nucleus (RTN) form a compact group situated between the ventral surface of the caudal pons and the facial nucleus, extending as far rostral as the caudal border of the superior olive. The cells lie on the dorsomedial edge of the spinothalamic tract. RTN neurons are of interest to respiratory physiology because of their axonal projections to the region of DRG and VRG groups (Pearce et al., 1989a; Smith et al., 1989b). In its caudal region, the relationship between the RTN and the subretrofacial nucleus (McAllen, 1986a,b) is not entirely clear, although the two are said to be distinct (Connelly et al., 1990). The neurons are small, making it difficult to record their discharge rhythms. The neurons exhibit various respiratory patterns. RTN neurons may mediate some of the respiratory and cardiovascular changes occurring after application of pharmacological agents to the "intermediate area" of the ventral surface of the ventrolateral medulla. They contain many fos-positive neurons after cats are exposed to hypercapnia (Teppema et al., 1994).

### **Bötzinger (BötC) Neurons**

The BötC of respiratory neurons was identified around 1980 when anatomical studies established that neurons in a rostral medullary region, ventral and ventromedial to the compact formation of the nucleus ambiguus (retrofacial nucleus), project to the contralateral DRG (Kalia et al., 1979). Lipski and Merrill (1980) recorded extracellularly from this region in the cat, sampling neurons with a clear respiratory modulation of their discharge. The neurons were then tested for a projection to the contralateral nTS, using antidromic electrophysiological techniques. Contrary to the assumption of Kalia and colleagues (1979), nearly all the antidromically activated neurons were spontaneously active during the expiratory phase of respiration. These rostral expiratory neurons are now referred to as Bötzinger complex neurons. The term (recalling a German vineyard) was adopted to commemorate a meeting organized by Richter in Heidelberg in 1980 and was first used in the literature in the electrophysiological analysis of Lipski and Merrill. Otake and colleagues (1987) argue that BötC neurons can be identified electrophysiologically by their augmenting expiratory pattern of discharge, without the necessity for other supporting evidence. However, the validity of this assumption may depend on the experimental situation, including whether the vagi are cut. Other investigators note that the anatomical region containing the BötC group also includes a few inspiratory neurons. The relevant recordings may have been from cranial motoneurons with an inspiratory rhythm (Zheng et al., 1991b, 1992a; Bryant et al., 1993), and it may be simplest to limit the definition of the BötC to the expiratory neurons in the region. The responses of BötC neurons to hypoxia, lung inflation, and baroreceptor inputs have been documented and discussed by Kanjhan and colleagues (1995). This study, together with the work of Pilowsky and colleagues (1990b), documents the anatomical relationships of the BötC neurons to the presympathetic vasomotor neurons that also occur in the rostral ventrolateral medulla.

Antidromic mapping studies in the cat indicate that axons of augmenting expiratory BötC cells project to the rVRG, the cVRG, the rostral pons, and the spinal cord, as well as to the DRG (for references, see Jiang and Lipski, 1990). Merrill and colleagues (1983) used spike-triggered averaging to establish that the expiratory BötC neurons monosynaptically inhibit the inspiratory DRG cells (Fig. 4.8). Similar techniques (Merrill and Fedorko, 1984; Fedorko et al., 1989a; Jiang and Lipski, 1990) have established that BötC neurons monosynaptically inhibit inspiratory rVRG cells and phrenic motoneurons (Fig. 4.12).

**Figure 4.12** Inhibitory effect of augmenting BötC neurons on discharge of phrenic motoneurons (A) and on inspiratory rVRG neurons (B), demonstrated by spike-triggered averaging. ipsp, inhibitory postsynaptic potential. (Modified from Merrill and Fedorko, 1984, and from Jiang and Lipski, 1990.)

In bulbospinal cVRG expiratory neurons (see below), there is inspiratory IPSP activity and expiratory depolarization (Ballantyne and Richter, 1986). Considering the similarity of the expiratory discharge patterns of BötC neurons and cVRG neurons, some authors have considered that an excitatory input from BötC neurons contributes substantially to the discharge of the cVRG neuron (Merrill, 1981; Long and Duffin, 1986; Bongiani et al., 1990), an idea that is consistent with the presence of numerous axon collaterals from BötC expiratory neurons to the cVRG region (see Fig 4.13B). However, such an excitatory projection was not confirmed in the careful spike-triggered averaging study conducted by Jiang and Lipski (1990). These authors did document inhibitory effects of BötC neurons on cVRG discharge. Inhibition of cVRG neurons is considered further in the appropriate section below.

Otake and colleagues (1987, 1988) used a combination of electrophysiological and neuroanatomical techniques to study the morphology of BötC neurons antidromically activated from the contralateral ventral brainstem, from the DRG region, or (rarely) from the spinal cord. The dendrites of these BötC neurons spread radially for approximately 600  $\mu\text{m}$ . The stem axons coursed dorsomedially and bifurcated. One branch crossed the midline at approximately the same rostrocaudal level as its somata, presumably projecting to the contralateral BötC. Other branches descended ventromedial to the retrofacial nucleus and the nucleus ambiguus. Boutons were distributed to neurons in these regions. Transverse and horizontal reconstructions of one BötC augmenting expiratory neuron in the cat is shown in Figure 4.13A.

**Figure 4.13** Discharge patterns and morphology of Bötzingen augmenting expiratory neurons. The transverse and horizontal views of the neuron in **A** (from cat) are modified from Otake et al. (1987). The sagittal view of the neuron in **B** (from rat) is modified from Bryant et al. (1993). Abbreviations listed on pages xiii-xiv.

Bryant and colleagues (1993) identified rat BötC neurons using intracellular injections of biotin, after electrophysiological identification. The neurons were small (about 20  $\mu\text{m}$  diameter) and difficult to

impair. Morphological or electrophysiological evidence for a spinal projection was found in only a small proportion of the cells, but extensive axonal arborizations were demonstrated in the ipsilateral medulla, with some contralateral projections. The stem axon bifurcated into a branch that coursed dorsomedially and a descending branch that issued numerous collaterals to target sites within the ventrolateral medulla. A sagittal reconstruction of one BötC augmenting expiratory neuron from this study is shown in Figure 4.13B.

BötC neurons are particularly interesting because of their extensive intramedullary branching. The well-documented inhibitory effects of their activity on other respiratory neurons suggests that they may have a role in the definition and organization of the respiratory rhythm. The physiological evidence suggests that the BötC augmenting expiratory cells contain an inhibitory neurotransmitter. Given its general inhibitory role, GABA is a likely candidate. This neurotransmitter is present in neurons in the BötC region of the rat (Ruggiero et al., 1985a; Kihara and Kubo, 1989; Livingston and Berger, 1989), and physiologically uncharacterized GABA neurons in a similar region in the rabbit project contralaterally (Blessing, 1990), consistent with the physiologically determined projections of BötC neurons. However, so far there are no intracellular electrophysiological/structural studies that demonstrate the presence of GABA in BötC perikarya. Moreover, GABA is widely distributed, being present in various neurons in the ventrolateral medulla. Ezure and colleagues consider that inhibitory decrementing BötC expiratory neurons play an important role in the transition between inspiration and expiration, particularly since the neurons are excited by lung inflation (see review by Ezure, 1990).

### **cVRG Expiratory Neurons**

Neurons with an expiratory rhythm in the caudal ventrolateral medulla, distributed from the level of the obex to the first cervical rootless, were first electrophysiologically identified in Wang's laboratory (Haber et al., 1957) in experiments in anesthetized vagotomized cats. These neurons are now assigned to the cVRG. An example of a cVRG neuron with its typical incrementing discharge pattern commencing in the second half of expiration is shown in Figure 4.14A. Bainton and Kirkwood (1979) showed in the anesthetized cat that the discharge of cVRG bulbospinal expiratory neurons decreases as PCO<sub>2</sub> is reduced from 30 to 20 mm Hg in phase with decreased neural activity in expiratory intercostal nerves. The normally phasic discharge becomes more tonic as PCO<sub>2</sub> is lowered.

**Figure 4.14 A**, Intracellular recordings of cVRG expiratory neurons in the anesthetized vagotomized cat. Initial hyperpolarization gradually diminishes in intensity, and an augmenting discharge commences late in expiration. (Modified from Anders et al., 1991.) **B**, Axonal projections of identified HRP-filled cVRG neurons. (Modified from Arita et al., 1987.) MP, membrane potential. Other abbreviations listed on pages xiii-xiv.

Most of the cVRG neurons have an axonal projection to the thoracic spinal cord, and their discharge is at least partially responsible for maintaining the expiratory rhythms present in thoracic expiratory neurons. Kirkwood and Sears (1973) and Cohen and colleagues (1985) argued for an excitatory

monosynaptic connection between the cVRG neurons and the thoracic expiratory motoneurons. However, Merrill and Lipski (1987) found EPSPs in only 2 of 57 expiratory thoracic motoneurons examined by spike-triggered averaging and no IPSPs in inspiratory thoracic motoneurons. Most cVRG expiratory neurons thus appeared to synapse on spinal interneurons, not on motoneurons. Kirkwood (1995) extended his initial cross-correlation analysis study and supplemented it with spike-triggered averaging of extracellularly recorded spinal neuronal potentials. On balance, it appears that perhaps 10% of the expiratory neurons receive monosynaptic input from cVRG cells. Excitatory control of the pool of thoracic expiratory neurons appears to depend on cVRG-mediated excitation of spinal interneurons. A few neurons with an inspiratory rhythm have been identified in cVRG. These include early inspiratory cells whose membrane potential is almost a mirror image of that of the cVRG expiratory cells (Arita et al., 1987). In contrast to the expiratory cells, the inspiratory cVRG neurons cannot be antidromically activated from the spinal cord.

Arita and colleagues (1987) made intracellular recordings from cVRG expiratory neurons in decerebrated, spontaneously breathing cats, antidromically activating the cells from the C3 segment. Most neurons exhibited an augmenting pattern of discharge, associated with ramp-like depolarizing potentials during expiration and rapid hyperpolarization with onset of phrenic nerve discharge at the termination of expiration. Regulation of these membrane potentials may include contributions from rVRG neurons (see below for a description of this group). The hyperpolarization may reflect inhibition originating in the activity of inspiratory neurons in the rostral rVRG (perhaps in the pre-BötC group, see below), as indicated by spike-triggered averaging of cVRG membrane potentials using the discharge of inspiratory rVRG neurons as a trigger (Anders et al., 1991). Although the rVRG neurons could be responsible for the inhibitory effects, their discharge pattern is not strongly related to the hyperpolarization of the expiratory cVRG neurons, so that the IPSPs may reflect rVRG-mediated excitation of inhibitory interneurons, possibly located within the cVRG region.

Intracellular fills with HRP by Arita and colleagues (1987) indicate that the axons of cVRG neurons project rostrally and dorsomedially for approximately 2 mm after emerging from the soma, then turn caudally and ventrally, crossing the midline of the medulla almost at the same rostrocaudal level as the soma (Fig. 4.14B). In the cat, axon collaterals were not observed along the length of the stained portion. Electrophysiological evidence suggests that projections to the phrenic nucleus are sparse (Merrill and Fedorko, 1984). Thus it appears that the effects of cVRG neuronal discharge are likely to be confined principally to thoracic spinal neurons.

The morphology of augmenting expiratory neurons in the rat cVRG has been investigated by Zheng and colleagues (1992a). Only one neuron was antidromically activated from the spinal cord. Two nonantidromically activated neurons, with augmenting expiratory rhythms, were filled with HRP and demonstrated to have anatomical projections similar to those observed for bulbospinal cVRG augmenting expiratory neurons in cat, without medullary collaterals. Other intracellularly labeled cells and axons did give off collaterals in the medulla, but these were not augmenting expiratory cVRG cells.

The weight of evidence therefore indicates that expiratory cVRG bulbospinal neurons have an executive function, transmitting excitation to spinal expiratory neurons without making a major contribution to the medullary integration of respiratory control.

### **rVRG Augmenting Inspiratory Neurons**

Bianchi (1971) was one of the first to investigate medullary respiratory neurons using antidromic activation to identify bulbospinal respiratory neurons in the ventrolateral medulla of the anesthetized cat. Hilaire and Montreau (1976), using similar experimental paradigms, located bulbospinal neurons with respiratory rhythms in the vicinity of the nucleus ambiguus at the level of the obex and just rostral to this level (Ezure, 1990). These neurons, discovered before the BötC neurons were identified, are assigned to the rVRG. When cranial nerve motoneurons are excluded from consideration, the majority of neurons with a respiratory rhythm in the rVRG region discharge during inspiration. Most rVRG respiratory neurons have an augmenting pattern of discharge, commencing around the beginning of phrenic activity or in late expiration and ceasing either at the peak or at the end of phrenic discharge.

Evidence for monosynaptic excitatory connections between rVRG neurons and spinal respiratory motoneurons was obtained in the cat by summing gross discharges from phrenic (C<sub>5</sub> ventral root) or inspiratory intercostal nerves and using cross-correlation analysis to relate them to activity of rVRG cells (Cohen et al., 1974). Recent analyses using cross-correlation analysis conclude that only a minority of the synaptic depolarization of phrenic and inspiratory intercostal motoneurons derives from monosynaptic inputs from inspiratory neurons in either the rVRG or the DRG (Davies et al., 1985a,b). The technical limitations of the cross-correlation studies have been mentioned above, and spike-triggered averaging studies have yielded different conclusions. Fedorko, Hoskin, and Duffin (1989b) applied this technique to augmenting rVRG inspiratory neurons antidromically activated from the contralateral C<sub>5</sub> segment in the cat. Two thirds of the neurons could be antidromically activated, with a conduction velocity of the descending axons of 11-40 m/s. When rVRG spikes were used as a trigger, phrenic motoneurons showed EPSPs underlying a step/ ramp depolarization during inspiration followed by abrupt hyperpolarization at the start of the expiratory phase. Fifteen of 53 phrenic motoneurons displayed unitary EPSPs with amplitudes and rise times appropriate for a monosynaptic connection. None showed IPSPs. The study argues for a significant monosynaptic excitatory input from augmenting rVRG inspiratory neurons to phrenic motoneurons.

Ezure and Manabe (1989) made extracellular recordings of the discharges of bulbospinal augmentary inspiratory rVRG neurons in the cat and, with these as a trigger, the intracellular potentials of contralateral inspiratory rVRG neurons were averaged after the impaled neurons were quickly tested for their projection to the spinal cord, superior laryngeal nerve, or vagus nerve. In 4 of 137 pairs examined, monosynaptic EPSPs resulting from discharge of the contralateral rVRG neuron were detected in ipsilateral bulbospinal rVRG neurons. Ezure and Manabe argue that their results support the idea that, during their active phases, bulbospinal rVRG neurons excite each other via medullary collaterals.

Morphological details of cat rVRG inspiratory neurons have been determined by Sasaki and colleagues (1989) following appropriate intracellular recording and antidromic activation from the C<sub>4</sub>-C<sub>5</sub> level of the spinal cord. Animals survived from 3-36 hours after the intracellular injection. The average somal size was approximately 35 µm, with a tendency to a dorsomedial-ventrolateral orientation. Most of the axons crossed the midline approximately at the rostrocaudal level of the somata. A number of intramedullary collaterals were noted. An example of a neuron with an augmenting inspiratory pattern of discharge is shown in Figure 4.15.

**Figure 4.15** Horizontal section through the cat medulla demonstrating morphology of a number of antidromically activated bulbospinal augmenting inspiratory neurons in the rVRG of the cat. MP, membrane potential; Phr, phrenic nerve discharge. (Modified from Sasaki et al., 1989.) Other abbreviations listed on pages xiii-xiv.

Electrophysiological studies in the rat also demonstrate neurons (otherwise uncharacterized) with inspiratory discharge patterns in the vicinity of the nucleus ambiguus (Ezure et al., 1988) so that an rVRG appears to exist in this species. Antidromic activation from the phrenic nucleus has been demonstrated for some of these cells, but no spike-triggered averaging studies confirming monosynaptic inputs to phrenic motoneurons are available in the rat. Intracellular electrophysiological and morphological studies in the rat have been completed (Zheng et al., 1992b; Lipski et al., 1994). An example of a respiratory neuron exhibiting large depolarizing shifts of the membrane potential during inspiration in the vagotomized decerebrate animal is shown in Figure 4.16A.

**Figure 4.16** Transverse section through the rat medulla, just rostral to the obex, showing two electrophysiologically identified, intracellularly filled, rVRG augmenting inspiratory neurons. The neuron in **A** could not be antidromically activated from the cervical spinal cord. The neuron in **B** could be antidromically activated from the spinal cord, and the intracellular fill revealed descending axons, one being traced to the vicinity of the dendrites of an intracellularly filled phrenic motoneuron. MP, membrane potential; phr, phrenic nerve discharge; TP, tracheal pressure. (A, modified from Zheng et al., 1992b; B, from Lipski et al., 1994.)

This neuron, located dorsomedial to the nucleus ambiguus, just rostral to the obex, was not antidromically activated from the spinal cord or from cranial nerves. The long, thin perikaryon gave off radially distributed dendrites up to 1 mm long. The axon ran dorsomedially, giving off numerous collaterals, many of which arborized in the ipsilateral and contralateral hypoglossal nuclei, and in the dorsal motor nucleus of the vagus, confirming that the cell was not a somatic motoneuron. A second example of the morphology of an rVRG inspiratory neuron is shown in Figure 4.16B, from the work of Lipski and colleagues (1994). Intracellularly injected neurobiotin was used to identify spinal projections of electrophysiologically characterized rVRG neurons in the vagotomized anesthetized rat. Neurons in rVRG, with augmenting inspiratory discharge patterns, were antidromically activated from the ventrolateral fasciculus of the spinal cord at C<sub>3</sub>. Conduction velocity

was between 5 and 15 m/s. Animals were maintained under deep anesthesia for some hours after the injection so as to facilitate tracing of long axonal projections. During inspiration the neuron in Figure 4.16B underwent large depolarizing membrane potential shifts, associated with augmenting neuronal discharge. Fine axon collaterals were found within the medulla, bilaterally in VRG and BötC regions and in the hypoglossal nucleus. The morphological reconstruction revealed two spinally projecting axons, as well as collaterals innervating the ipsilateral hypoglossal nucleus. In other identified rVRG augmenting neurons described in this study, the stem axon arborized into two main branches in the medulla, rostral to the parent cell, with each branch descending on opposite sides in the ventrolateral columns to the spinal cord, giving off collaterals in the C<sub>3</sub>-C<sub>5</sub> segments. Many labeled axon varicosities were found near dendrites of phrenic motoneurons (also displayed by intracellular fills after antidromic activation from the phrenic nerve).

Other neuroanatomical studies, including transneuronal viral transport studies, support a direct input to phrenic motoneurons from rVRG cells in the rat (see Fig 4.3 and discussion earlier in this chapter). Whether activity of rVRG neurons is necessary for inspiration to occur has not yet been established. Budzinska and colleagues (1985) showed that focal cold blocks in structures located ventrolaterally in the rostral medulla, perhaps rostral to the rVRG neurons, consistently caused apnea or deep depression of inspiratory motor output. The appearance of apnea was independent of the arterial PCO<sub>2</sub>. Gromysz and colleagues (quoted by Speck and Feldman, 1982) found that respiratory patterns in the rabbit were altered by lesions within the rVRG. However, there were no marked changes in the respiratory pattern in the cat when bilateral electrolytic lesions were made in the rVRG region (Speck and Feldman, 1982). Jung and colleagues (1991) found that injection of an excitatory amino acid receptor antagonist (kynurenic acid) into the more caudal portion of the rVRG region caused cessation of respiration in anesthetized rats.

Thus the physiological role of bulbospinal inspiratory rVRG neurons is still far from clear. Some authors view these cells as phrenic premotor neurons without the extensive intramedullary connections required for an important integrative role in the regulation of respiration. Certainly, rVRG augmenting inspiratory neurons are not considered to be pacemaker cells in their own right.

### **Pre-Bötzinger Respiratory Neurons in the Rostral rVRG**

Not all inspiratory neurons in the rVRG region exhibit an augmenting pattern of discharge. Some exhibit constant rapid discharge during the inspiratory phase. Others discharge at the onset of phrenic activity, just as activity in the expiratory population terminates, and gradually cease firing as inspiration proceeds. These latter neurons, called early-burst inspiratory neurons by Merrill (1974, 1981), are shown as decrementing (I-DEC) cells in Figure 4.1 and are also referred to as pre-I neurons. Merrill used the antidromic mapping technique to suggest that early-burst neurons have extensive collateral arborizations in the medulla, without projections to the spinal cord. The neurons were judged to be fairly small because they were hard to hold during extracellular recordings and their spike amplitudes were low. Merrill considered that the early-burst inspiratory activity of these neurons was responsible for the periodic inhibition that silences expiratory respiratory neurons during

inspiration. He was circumspect concerning a respiratory pacemaker role for the early-burst neurons, noting that they appeared to receive an excitatory drive during inspiration.

A useful discussion of these pre-I propriobulbar respiratory interneurons, written before the definition of the pre-BötC (see below), is that of Duffin and Aweida (1990). Important physiological studies of pre-I neurons, without detailed attention to anatomical localization, were carried out in neonatal rat brainstem spinal cord preparations by Onimaru, Homma, and colleagues (see references of Onimaru, 1995).

The possible existence of pacemaker neurons just caudal to the BötC was suggested by the study of Smith and colleagues (1991), described later in this chapter in the section on the origin of respiratory rhythms. The region is now referred to as the pre-Bötzing complex of respiratory neurons (pre-BötC). In vivo studies in cats (Connelly et al., 1992; Schwarzacher et al., 1995) have demonstrated that the region contains a concentration of neurons with properties similar to those described by Merrill. The neurons commence firing very early in inspiration, or before the onset of inspiration, and exhibit non-augmenting discharge patterns as phrenic discharge augments during inspiration. The anatomical location of the pre-BötC neurons and examples of their discharge are shown in Figure 4.17A. It is proposed that pre-BötC neurons are key elements in respiratory rhythm generation.

**Figure 4.17 A**, Discharge patterns of two pre-I respiratory neurons in the pre-BötC of the cat. The neuron in the top panel shows an augmenting discharge during late expiration, with an abrupt increase in discharge directly before the onset of the inspiratory phase and a decrementing pattern during the early inspiratory period. The second shows, in addition to the pre-I discharge, a second peak of increased discharge during late inspiration. (Modified from Schwarzacher et al., 1995.) **B,C**, Horizontal and transverse views (respectively) of the same intracellularly filled VRG non-augmenting inspiratory neuron in the cat. The inset at bottom left shows the constant inspiratory discharge of this cell, and the inset at bottom right shows the location of the cell in the ventrolateral medulla. Collaterals marked a-d correspond in the two views. (Modified from Otake et al., 1990.) MP, membrane potential; phr, phrenic discharge. Other abbreviations listed on pages xiii-xiv.

Given the difficulty of making extracellular recordings from early-burst decrementing neurons, it is not surprising that intracellular reconstruction anatomical studies are not available. Otake and colleagues (1990) managed to fill cat ventrolateral medullary neurons intracellularly with nonaugmenting patterns of discharge. The neurons were small with axon collaterals in the nucleus ambiguus region and projections to the dorsomedial medulla and to the contralateral medulla. One such neuron exhibiting a constant pattern of discharge is shown in Figure 4.17B,C. Constant-pattern inspiratory neurons may induce monosynaptic EPSPs in augmenting rVRG inspiratory neurons (Ezure et al., 1989) and thus may play a part in respiratory rhythmogenesis.

The possible role of pre-BötC neurons in the generation of the respiratory rhythm is further discussed later in this chapter, where interesting work from in vitro preparations is described.

## **Superior Laryngeal Nerve Afferents and Their Effects on Medullary Respiratory Groups**

The internal branch of the superior laryngeal nerve (SLN) is predominantly or wholly an afferent nerve, containing, in the cat, about 2,200 myelinated fibers. This is almost as many as the approximately 3,000 myelinated fibers present in the afferent cervical vagus in this species (Bartlett, 1989). Many studies have used electrical stimulation of this nerve to probe central pathways. However, especially in lightly anesthetized animals, such stimulation can evoke a variety of responses, including apnea, tight closure of the glottis, coughing (forced expiration preceded by a brief inspiratory effort), and swallowing. The most prominent reflex response to chemical or physical stimulation of laryngeal mucosa is immediate tight closure of the glottis, associated with apnea rather than inspiration (Bartlett, 1989). Upper airway occlusion stimulates SLN receptors whose activity leads to reflex activation of pharyngeal muscles maintaining airway patency, a response partially mediated through activation of hypoglossal motoneurons (for references, see Jiang et al., 1991). Thus interpretation of electrical stimulation studies may be difficult, as illustrated by considering the carefully done studies of Lipski and colleagues (Bellingham et al., 1989; Jiang et al., 1991; Jiang and Lipski, 1992). These authors recorded intracellularly from phrenic motoneurons and from various classes of intramedullary respiratory neurons, assessing their responses to electrical stimulation of the SLN in the cat. Stimulation evoked EPSPs in most bulbospinal inspiratory DRG neurons (augmenting pattern of membrane depolarization during inspiration), although IPSPs were observed in a small proportion and in all nonbulbospinal DRG cells (propriobulbar inspiratory neurons). Conduction velocity in afferent fibers (1.5 to 2 m/s, with theoretical synaptic delay of 0.5 ms) suggested that the EPSPs reflect monosynaptic inputs from primary afferents. DRG neurons are thus likely to form the central link in an excitatory disynaptic pathway linking superior laryngeal nerve input with the contralateral phrenic neurons. This pathway could mediate the short latency increase in contralateral phrenic neuronal discharge observed with stimulation of the SLN, since lesions of the DRG abolish this response (McCrimmon et al., 1987).

However, in the studies of Lipski and colleagues, stimulation of the SLN had a mainly inhibitory action on phrenic motoneurons and on inspiratory rVRG neurons. The central neurons mediating SLN-evoked inhibition of phrenic and inspiratory rVRG neurons have not been identified. Lesioning the DRG region does not affect the response, so it is unlikely that DRG neurons are involved (McCrimmon et al., 1987). This is as expected, since descending projections of DRG neurons excite phrenic motoneurons, and DRG neurons are not thought to have extensive intramedullary collaterals. BötC expiratory neurons are likely theoretical candidates for mediation of inhibitory effects, but in the study of Jiang and Lipski (1992) neurons in this class were themselves inhibited by stimulation of the SLN. Lesions of respiratory regions in the rostral dorsolateral pons do not abolish the inhibition of phrenic discharge evoked by stimulation of the superior laryngeal nerve (Karius et al., 1991). Perhaps there are as yet unidentified interneurons in the nTS with inhibitory projections to the rVRG inspiratory cells that drive the phrenic motoneurons. A short latency excitation of phrenic discharge following stimulation of the SLN was abolished by the lesions, possibly indicating the DRG neurons in the cat might form part of the CNS pathway mediating reflexes such as cough initiated by laryngeal

irritation. Bellingham and Lipski (1992) have shown that fast (15-38 m/s) conducting fibers in the SLN project to the ventrolateral region containing the DRG neurons. Many DRG inspiratory neurons discharged at short latency following superior laryngeal nerve stimulation. The response of the P cells to ipsilateral SLN stimulation may be of importance in explaining the sustained apnea that can occur with stimulation of the larynx.

Most cVRG expiratory bulbospinal neurons are inhibited by electrical stimulation of the SLN. Motoneurons innervating intrinsic laryngeal muscles were excited by stimulation of the SLN, but whether this response underlies the adduction of the cords induced by SLN stimulation is unclear. Both expiratory and inspiratory laryngeal motoneurons were excited, and the latter class are usually associated with abduction of the cords. Hypoglossal motoneurons were excited, possibly reflecting SLN-mediated reflex activation of pharyngeal muscles maintaining airway patency.

### **Chemoreceptor Secondary Afferent Neurons and Their Interaction with Brainstem Regulation of Respiration**

Decerebration studies have established that brainstem circuitry is sufficient to mediate respiratory responses to stimulation of peripheral chemoreceptors (Korner, 1971; Tenney and Ou, 1977). Early studies identifying medullary neurons receiving direct inputs from primary chemoreceptor afferents concentrated on electrophysiological recordings from neurons with obvious respiratory rhythms, including the DRG cells. Attention was focused on medullary respiratory neurons mediating "gating" of the effects of chemoreceptor inputs (see below). Surprisingly few functional studies have characterized all CNS neurons receiving direct inputs from chemoreceptors. Similarly, few functional studies have aimed to determine the central mechanisms whereby more prolonged chemoreceptor stimuli affect respiration. Many studies have relied on electrical stimulation of the carotid sinus nerve. This can be a useful procedure, but physiological stimuli are necessary to separate chemoreceptor and baroreceptor inputs.

Vardhan and colleagues (1993) showed that pharmacological blockade of a restricted area in the commissural region of the nTS blocked respiratory responses to peripheral chemoreceptor stimulation in the rat. Davies and Edwards (1975) studied CNS termination of chemoreceptor afferents using carotid sinus nerve stimulation and cyanide activation of the carotid body in the cat. They detected stimulus-related field potentials and increases in extracellular activity in the ventrolateral nTS region and in the VRG region, arguing from latency considerations that neurons in both these regions receive chemoreceptor inputs. The results are interesting in view of neuroanatomical evidence that some primary chemoreceptor afferents do terminate in the ventrolateral medulla (see section above), possibly directly innervating rVRG or cVRG neurons. Lipski et al. (1975) found that carotid sinus nerve fibers could easily be antidromically activated by stimulation of the nTS region in the vicinity of the tractus solitarius. In contrast, they were unable to activate carotid sinus nerve fibers antidromically from the ventrolateral medulla. This may have reflected the sparsity of projections to the ventrolateral medulla compared with the nTS, or, as the authors propose, the projection to the ventrolateral medulla may have been polysynaptic.

Lipski et al. (1977) discuss earlier work and report their own confirmation that  $I\alpha$  and  $I\beta$  DRG neurons in the cat respond to chemoreceptor stimulation with a  $CO_2$  saturated Krebs solution in a "gated" manner, analogous to the response of the phrenic nerve; brief chemoreceptor stimulation applied during inspiration enhanced both activities, but the same stimulus applied in expiration had little effect. There was no subthreshold excitation of the DRG neurons from chemoreceptor stimulation during expiration. Indeed, an inhibitory effect was sometimes observed. This evidence suggested a lack of direct chemoreceptor inputs to DRG cells or a prevalence of inhibition during this phase. In agreement with this conclusion, and perhaps somewhat surprisingly, it did not prove possible to activate DRG neurons by single, double, or triple electrical pulses to the carotid sinus nerve. Slightly different results were obtained by Lawson and colleagues (1989), who observed augmented and prolonged inspiratory membrane depolarization of  $I\alpha$  DRG neurons in response both to local peripheral chemoreceptor activation (5%  $CO_2$  solution to lingual artery) and to carotid sinus nerve stimulation. This response occurred in phase with phrenic nerve activation. Averaging of the DRG neuron membrane potential during carotid sinus nerve stimulation did not reveal short latency postsynaptic potentials. The authors concluded that  $I\alpha$  DRG neurons do not receive monosynaptic inputs from chemoreceptor afferents. Three DRG  $I\beta$  neurons were studied. Activation of carotid chemoreceptors caused slight transient membrane depolarization, followed by hyperpolarization, even though phrenic nerve activity was enhanced. Again, no monosynaptic inputs from chemoreceptors were detected. It seems peripheral chemoreceptors affect DRG neurons principally by oligosynaptically evoked excitation.

More dorsomedially placed nTS neurons, with no obvious respiratory rhythm, were also studied by Lawson and colleagues. Many of these cells responded with a slowly rising depolarization to stimulation of the peripheral chemoreceptors, and the authors speculate that these neurons may represent the major group of secondary afferent neurons activated by inputs from peripheral chemoreceptors. Similar neurons have been studied by Spyer and colleagues (1990) and by Mifflin (1992). The study by Mifflin concentrated on nTS neurons situated dorsomedial to the tractus solitarius, with membrane potentials not fluctuating in phase with either central respiratory activity or lung inflation. Neurons that could be driven from the superior laryngeal nerve were excluded from analysis. Approximately 90% of responding neurons were excited by stimulation of peripheral chemoreceptors. These neurons discharge spontaneously, either irregularly or relatively regularly at frequencies up to 10 Hz. Most neurons exhibited EPSPs in response to electrical stimulation of the carotid sinus nerve, some exhibited EPSP-IPSP sequences, but none exhibited only IPSPs. The short latency (2-4 ms) suggested monosynaptic inputs in many cases. Some neurons were depolarized by both chemoreceptor and baroreceptor inputs (Fig. 4.18A).

**Figure 4.18 A**, Effect of carotid chemoreceptor stimulation, baroreceptor stimulation, and carotid sinus nerve stimulation on the membrane potential of an intracellularly recorded neuron in the dorsomedial nucleus tractus solitarius. (Modified from Mifflin, 1992.) **B**, Effect of carotid chemoreceptor stimulation on the membrane potential and discharge rate of an intracellularly recorded rVRG neuron. (Modified from Lawson et al., 1989.)

Mifflin (1993), using intracellular recordings in cat nTS, identified neurons depolarized by activation of peripheral chemoreceptors. The neurons did not display a respiratory rhythm, and their activity was not influenced by pulmonary stretch receptors. An extracellular recording study in the commissural portion of the rat nTS has identified neurons that increase their discharge rate in response to peripheral chemoreceptor activation, without a response to baroreceptor stimulation (Chitravanshi and Sapru, 1995). These neurons did not display any rhythm related to phrenic nerve discharge.

St. John (1981) studied the responses of ventral medullary respiratory neurons to peripheral chemoreceptor stimulation (intracarotid nicotine or cyanide). The neurons were not classified by antidromic activation, and the variety of responses emphasizes the need for careful neuronal identification. One would expect bulbospinal rVRG augmenting inspiratory neurons to increase their discharge during peripheral chemoreceptor stimulation that activates phrenic nerve discharge. Such a result was observed in the careful study of Lawson and colleagues (1989). These authors identified cat rVRG inspiratory neurons by their rhythm and by their response to antidromic activation from the spinal cord. The membrane potential of such neurons exhibited early, increased, and prolonged inspiratory depolarization, with increased discharge frequency, in response to both peripheral chemoreceptor activation (Fig 4.18B) and carotid sinus nerve stimulation. This increased activity was reflected in the increased phrenic neurogram activity. Carotid sinus nerve stimulation late in expiration caused membrane hyperpolarization. Neither St. John nor Lawson et al. consider the possibility of direct chemoreceptor inputs to rVRG inspiratory neurons. The anatomical findings discussed earlier in this chapter indicate that this could occur.

Lipski and colleagues (1984) examined the effect of peripheral carotid chemoreceptor excitation on medullary expiratory neurons in cats. Five out of nine BötC expiratory neurons showed no change in peak discharge frequency in response to peripheral chemoreceptor stimulation applied in the second half of expiration. The other four units increased both their peak firing frequency and their duration of the firing burst. Expiratory cVRG neurons exhibited a similar variety of responses, with inhibition observed in a few cells. Since BötC expiratory neurons inhibit inspiratory DRG neurons, the decreased excitability occurring in DRG neurons in response to peripheral chemoreceptor stimuli applied in expiration may reflect activation of the BötC expiratory neurons. The pathway whereby primary afferent information reaches the BötC neurons is not known. Presumably there is a direct projection from nTS secondary afferent neurons. As discussed above, the anatomical evidence suggests that cVRG neurons and caudal rVRG neurons could receive a monosynaptic input from primary afferents projecting directly to the ventrolateral medulla.

### **Medullary Neurons with Selective Chemoreceptor Function**

All neurons are ultimately affected by lack of oxygen and by an acidotic environment. However, it appears that the medulla oblongata and (possibly) the pons contain structures, presumably neurons, that are particularly sensitive to hypoxia and increased PCO<sub>2</sub> or decreased pH so that even in animals with peripheral chemoreceptor denervation these conditions cause marked effects on respiration.

Richter and colleagues (1991) have investigated the effects of systemic hypoxia in cats with chemoreceptors either intact or denervated. Systemic hypoxia had effects similar to those seen with cerebral ischemia induced by occlusion of carotid and vertebral arteries. In chemoreceptor-denervated animals moderate hypoxia caused phrenic nerve discharge to increase in amplitude and frequency. When hypoxic or ischemic conditions were maintained for more than 1-2 minutes, phrenic nerve activity gradually diminished and then ceased. Various respiratory neurons were monitored extracellularly, intracellularly, or intraaxonally. Some neurons were identified by antidromic activation from the spinal cord or from the vagus nerve. Similar responses to hypoxia were obtained in bulbospinal and nonbulbospinal respiratory neurons. Neurons in the VRG - inspiratory, postinspiratory, and expiratory - increased their activity during the period of enhanced phrenic nerve activity. Their activity decreased with decreasing phrenic activity, finally disappearing. The neurons sometimes discharged again with the appearance of gasp-like activity in the phrenic nerve. Richter and colleagues consider that neurons, possibly GABA-ergic neurons, responsible for causing IPSPs in respiratory neurons are particularly vulnerable to hypoxia. Their intracellular analysis suggests that depression of respiratory activity results not from a decrease in neuronal excitability (as judged by the response to antidromic activation), but, somewhat paradoxically, from a reduction in periodic inhibition of neuronal activity. The findings are compared with those of St. John and Bianchi (1985), who studied respiratory effects of hypoxia in animals with intact peripheral chemoreceptors. Hypoxia eventually causes cessation of phrenic discharge in these animals, and central respiratory neurons also display persistent excitability after they cease firing. Thus Richter and colleagues argue that hypoxia impairs respiration by removing vital inhibitory elements, thereby causing an "arrhythmia" of the respiratory network.

In 1958 Loeschcke and coworkers described the marked respiratory effects of applying pieces of filter paper soaked in solutions of different pH, or different drugs, to the ventral surface of the medulla (see references in Loeschcke, 1982). The adequate central chemoreceptor stimulus for breathing was suggested to be pH rather than  $PCO_2$ . Three bilateral ventromedullary zones were defined (Trough et al., 1973; Loeschcke, 1982): a rostral region (labeled M for Mitchell), an intermediate region (labeled S for Schläfke), and a caudal region (labeled L for Loeschcke). Application of chemosensitive stimuli in these regions caused stimulation of respiration. These regions are depicted in Figure 4.19.

**Figure 4.19** Regions on the ventral surface of the medulla from which marked respiratory changes can be elicited. Area M is for Mitchell, area S is for Schläfke, and area L is for Loeschcke. (Modified from Trough et al., 1973, and from Loeschcke, 1982.)

There is now an extensive literature dealing with the various properties of these zones. The studies concentrate on the "ventral surface," and there has not been sufficient effort to integrate the findings with those from studies that consider the medulla as a three-dimensional structure containing neurons with specific axonal connections. There is still no agreement as to the location or the identity of the structures (presumably neurons) responsible for the respiratory effects. Nor is it clear whether the

natural physiological stimulus to respiration is the  $PCO_2/pH$  of the cerebrospinal fluid bathing the ventral surface or the  $PCO_2/pH/PO_2$  of vertebral artery blood affecting the interstitial fluid bathing the chemosensitive structures. The dendrites of many respiratory neurons in the BötC, pre-BötC, rVRG, and cVRG groups approach within the required 200-300  $\mu m$  of the ventral surface of the medulla. McAllen (1986a) used focal injections (30-50 nl) of excitatory amino acids to identify cat ventrolateral medullary neurons that, when stimulated, increased phrenic nerve activity. The injections were made not more than 1 mm deep to the ventral surface. A rostral group of respiratory-stimulating neurons was found beneath the ventral surface corresponding to the rostral (M) area. However, stimulation of neurons ventral to the intermediate (S) area caused inhibition of phrenic activity. Stimulation in the caudal region (L, medial to the hypoglossal rootless) had no effect on respiration, although more lateral stimulation did increase phrenic activity.

Coates and colleagues (1993) used small (1 nl) intramedullary injections of acetazolamide to inhibit carbonic anhydrase and thereby produce local tissue acidosis, confirmed by measurement with an electrode placed at the site of the injection. The injection sites were marked by including fluorescent beads with the injectate. Change in phrenic nerve activity was assessed in paralyzed, mechanically ventilated rats and cats. Increases in phrenic nerve activity were obtained with injections into the cat ventrolateral medulla, within 0.8 mm of the ventral surface and, 3-4 mm lateral to the midline, from the caudal extent of the inferior olive to the caudal extent of the superior olive (involving territory in caudal, intermediate, and rostral ventral surface areas). Increases in phrenic activity were also observed with injections into the nTS and just dorsolateral to this nucleus and into the region of the locus coeruleus. The medullary sites yielding positive phrenic nerve responses are in reasonable agreement with sites containing fos-positive neurons in rats exposed to high levels of  $CO_2$  in the inspired air (Sato et al., 1992).

## **Pontine Regions and Respiratory Control**

Lumsden (1922, 1923a,b) found that cats with inter-collicular transections displayed normal breathing patterns. When the transection was more caudal, so as to separate the rostral pons from the caudal brainstem, breathing was characterized by a sustained pause at the end of inspiration (apneustic pattern). This was attributed to removal of a "pneumotaxic" center, with preservation of a more caudal pontine "apneustic" center. After transection at the pontomedullary junction, breathing became a series of brief gasps. Later workers appreciated the importance of vagotomy for the appearance of the apneustic pattern, and it was presumed that the vagi were damaged during isolation in Lumsden's preparations (St. John, 1990). Contemporary studies have suggested that the previously defined pneumotaxic center was part of the parabrachial nuclei, especially the medial and Kölliker-Fuse subnuclei, and a study in the decerebrate rat has shown that electrolytic lesions in this region produce an apneustic breathing pattern even when the vagi are intact (Morrison et al., 1994). Lesion and stimulation studies have suggested a diffuse connection with medullary respiratory areas (St. John, 1986). Focal injections of excitatory amino acids have been used to stimulate cell bodies in the parabrachial region of the anesthetized rat (Lara et al., 1994). Stimulation of the medial parabrachial-Kölliker-Fuse area decreased the rate of phrenic discharge, and stimulation of cell bodies

in the lateral region increased this rate. The authors suggest a role for these nuclei in modifying the pattern of respiratory discharge, probably via inputs to the medullary respiratory neurons. Dick and colleagues (1994) made both extracellular and intracellular recordings from neurons in the parabrachial region of the anesthetized vagotomized cat, searching for neurons with respiratory rhythms. The whole parabrachial region was extensively searched, but neurons were not characterized according to their axonal projections. Most neurons with respiratory rhythms were in the Kölliker-Fuse region, just lateral and ventral to the ventrolateral pole of the brachium conjunctivum (superior cerebellar peduncle) and just rostral to the principal motor nucleus of V. The cells were not distributed according to their particular respiratory rhythm. No short latency EPSPs or IPSPs were evoked by electrical stimulation of the vagus, nor was the extracellularly recorded discharge rate greatly affected by this procedure. In one animal with an intact vagus, it was possible to find cells with discharge modified by lung inflation, but these neurons did not have central respiratory rhythms. Dick and colleagues (1994) provide a useful discussion of the pontine respiratory literature, emphasizing the problems of integrating pontine respiratory neurons into general theories of central respiratory control. Peripheral chemoreceptor stimulation increases the discharge of a high proportion of otherwise uncharacterized neurons in the lateral parabrachial nucleus (Hayward and Felder, 1995a). An interesting magnetic resonance imaging study has been conducted in humans breathing under conditions of increased inspiratory resistive load (Gozal et al., 1995). In the pons there was evidence for increased metabolism in regions corresponding to the parabrachial nuclei, the locus coerulei, and more ventrally in the basal pons. No signal enhancement was reported for regions in the medulla oblongata.

Guyenet and colleagues (1993) have determined whether, in the vagotomized anesthetized rat, neurons in locus coeruleus and A5 neurons receive inputs from neural circuits generating respiratory rhythms and patterns and whether the cells respond to peripheral chemoreceptor inputs. Neither locus coeruleus nor A5 cells displayed pump-related rhythms. During resting conditions approximately 50% of the neurons displayed a below average firing probability during phrenic nerve discharge and an above average discharge early in expiration. Hypoxia-induced peripheral chemoreceptor stimulation and hypercapnia-induced central stimulation activated both locus coeruleus and A5 cells, inducing a bursting discharge pattern synchronized with phrenic nerve discharge. The central pathways whereby peripheral chemoreceptor inputs affect A5 and locus neurons are not known. The process could occur via effects on central respiratory networks or via more direct inputs from second-order chemoreceptor neurons in the nTS.

In further experiments from the same laboratory (Koshiya and Guyenet, 1994a,b; Guyenet and Koshiya, 1995), inhibition of neuronal function in the A5 region by local injection of muscimol was found to reduce markedly the increases in peripheral sympathetic vasomotor tone normally observed with hypoxia-induced peripheral chemoreceptor stimulation. Similar inhibition of the caudal ventrolateral medulla did not impair the response, nor did functional impairment of the parabrachial nuclei. The sympathetic response to peripheral chemoreceptor stimulation thus seems to be mediated via the nTS and then via a pontine loop involving A5 neurons before the rVLM vasomotor neurons are activated.

## Origin of Respiratory Rhythms

As discussed in the introduction to this chapter, the presence of reasonably normal breathing in upper medullary preparations indicates that the neural circuitry for generating the respiratory rhythm must be present in the medulla. The precise location and the properties of the relevant neurons have been investigated in many ways.

The phrenic neurogram is bilaterally symmetrical, as it must be for coordinated respiration to occur. Since the time of Fluorens, researchers have studied respiratory rhythms after lesions in the midsagittal plane, thereby investigating whether each side of the medulla has its own independent rhythm generator. Useful studies have measured these rhythms in lower cranial nerves as well as in phrenic nerves. St. John (1983) showed that in decerebrate, vagotomized, ventilated cats, recurrent laryngeal nerves continue to discharge with independent respiratory rhythms after complete midsagittal brainstem division and medullopontine transection. The same procedures eliminated phrenic nerve discharge. This observation was confirmed by Kubin et al. (1987) in cats with intact vagi. The findings are consistent with the existence of independent bilateral medullary rhythm generators in the cat, with predominantly ipsilateral control of cranial motoneurons and predominantly contralateral control of phrenic motoneurons. Midsagittal division of the medulla in the rabbit and the monkey desynchronizes the phrenic neurograms without eliminating them (Janczewski and Karczewski, 1984), reflecting the importance of uncrossed bulbospinal respiratory pathways in these species. Overall, the experiments indicate that each half of the medulla has its own respiratory rhythm generator. Commissural connections synchronize and possibly amplify the rhythms.

An influential general framework for respiratory rhythmogenesis, proposed by von Euler (1986), conceptualizes the respiratory cycle in terms of successive phase transitions organized by a central pattern generator. Inspiratory activity commences due to an abrupt release of inhibition and subsequent excitation of respiratory premotor neurons. Their activity contributes to a separate neuronal mechanism that, together with inputs from lung inflation receptors, eventually terminates inspiration and switches on expiration. The theoretical concept has led to a search for neurons whose discharge, by spanning the different phases of respiration, could be responsible for the transition from phase to phase. There has been a strong emphasis on the role of inhibition, with initiation of neuronal discharge viewed as a release from inhibition rather than as resulting from intrinsic depolarization of pacemaker neurons. The idea that onset of inspiration reflects a diminution of inhibitory activity has led to a search for appropriate synaptic mechanisms, but it is not clear that other aspects of the phase transition theory are specific enough to generate illuminating testable hypotheses. Bianchi and colleagues (1995) provide an illuminating and balanced discussion of theories of respiratory rhythmogenesis, explaining the context in which much effort goes into the search for von Euler's "inspiratory off-switch." The relevant neuronal network must contain neurons that, as well as giving origin to EPSPs or IPSPs, exhibit particular intrinsic membrane properties. The interaction of the various components of the neuronal assembly produces an emergent activity that results in the various respiratory events. There must be some caution applied to the manner in which the principles of rhythmogenesis demonstrated *in vitro* are applied to intact animals.

**Possible rhythm generator properties of rVRG inspiratory neurons**

Feldman and colleagues (1990) consider that the relevant respiratory circuitry comprises a rhythm generator that controls the on and off switch of inspiratory and expiratory phases and a pattern generator that shapes neuronal discharge patterns. In early studies in vagotomized cats, the role of rVRG neurons as possible rhythm generators was investigated (Feldman et al., 1984). The upper cervical spinal cord was electrically stimulated to activate bulbospinal rVRG inspiratory neurons antidromically and thereby determine whether their discharge would reset the respiratory rhythm. This could occur by virtue of discharge of rVRG axon collaterals, possibly projecting to inspiratory timing and/or ramp generating circuitry. The spinal stimulation caused brief increases in phrenic nerve discharge, with a latency consistent with mediation by subsequent orthodromic axon potentials in antidromically activated medullary neurons. There was no phase shift or pattern change in the respiratory rhythm. This result suggests that rVRG neurons are isolated from the generators of respiratory rhythm. However, there has not been any follow-up confirmatory study documenting this potentially important finding. Some investigators consider that the antidromic stimulation may not have been sufficiently intense to discharge a sufficient proportion of the rVRG neurons. In an earlier study short trains of antidromic stimulation did produce phase shifts and resetting of the rhythm (Gauthier and Monteau, 1986). These issues are discussed in a recent review (Duffin et al., 1995). As noted in the earlier section on rVRG neurons, many investigators consider that they function principally as phrenic premotor cells.

**Possibility that specific pacemaker cells set the respiratory rhythm**

The respiratory rhythm can be viewed as resulting from oscillations of specialized pacemaker neurons, analogous to the pacemaker neurons that maintain cardiac rhythmicity. Such pacemaker neurons should maintain a rhythmic discharge when isolated from other inputs. Suzue (1984) demonstrated that a neonatal rat in vitro brainstem-spinal cord preparation could generate a respiratory rhythm. A similar rat preparation was used by Onimaru and colleagues (1992) to provide evidence that pre-inspiratory (pre-I) neurons monosynaptically excite inspiratory cells in the ventrolateral medulla. These pre-I neurons in the rostral ventrolateral medulla (defined slightly differently from the pre-I neurons investigated by Richter and colleagues) continue discharging even when synaptic transmission is eliminated by incubating the medulla in low  $\text{Ca}^{2+}$ , high  $\text{Mg}^{2+}$  solutions (Onimaru et al., 1989). Under the same synaptic blockade conditions, phrenic nerve discharge was eliminated, and inspiratory neurons ceased discharging, indicating that their activity depends on synaptic inputs. Thus the pre-I neurons are seen as primary rhythm generators, triggering respiration via an action on ventrolateral inspiratory cells (rVRG neurons?), as summarized in a recent review (Onimaru, 1995). This work would be greatly strengthened by more detailed anatomical characterization of recording sites.

Smith and colleagues (1991) transversely sectioned a neonatal brainstem-spinal rat preparation, either commencing rostrally at the pontomedullary junction and working caudally or commencing caudally at the spinomedullary junction and working rostrally. Discharge of phrenic and glossopharyngeal

rootlets was examined for the presence of respiratory rhythm. Rostral to caudal sectioning abolished phrenic rhythmic discharge at the caudal extent of the retrofacial nucleus, even though the section level was rostral to rVRG bulbospinal neurons. In other preparations, respiratory rhythm was maintained in glossopharyngeal rootlets after transection at the spinomedullary junction, and after progressively rostral sections, until the rhythm disappeared as the section level reached within 200  $\mu\text{m}$  of the caudal extent of the retrofacial nucleus. Horizontal sections that removed regions dorsal to the nucleus ambiguus did not abolish the rhythm. These findings suggested that the pacemaker neurons, or other elements critical for rhythm generation by a network, must be located in a restricted region of the ventrolateral medulla. The authors hypothesized that pre-BötC neurons constitute pacemaker neurons. The hypothesis was tested by recording from a 500  $\mu\text{m}$  thick transverse slice containing the pre-BötC neurons. Respiratory rhythms, recorded bilaterally from the attached hypoglossal rootlets, were highly correlated with the discharge of pre-BötC neurons (Fig. 4.20).

**Figure 4.20** Pacemaker model of the origin of respiratory rhythms, based on recordings from pre-BötC neurons in slice preparation from neonatal rat. **A**, The respiratory rhythm present in hypoglossal rootlets is correlated with the discharge of pre-BötC neurons. **B**, Increasing or decreasing the discharge of pre-BötC neurons causes corresponding changes in respiratory activity recorded from hypoglossal rootlets. (Modified from Smith et al., 1991.)

Experimentally increasing or decreasing the discharge of pre-BötC neurons produced corresponding changes in the respiratory discharge recorded in the hypoglossal rootlets. Intracellular recordings revealed voltage-dependent oscillatory properties of pre-BötC neurons. Blockade of synaptic inputs did not prevent rhythmical burst discharge of the neurons (Johnson et al., 1994).

These results are very encouraging, and they have been extended by the work of Di Pasquale and colleagues (1994) using an in vitro brainstem-spinal preparations from preterm fetal and newborn (0-3 day-old) rats. Such preparations generated stable respiratory rhythms that could be affected by interference with neuronal function in the rostral ventrolateral medulla. Synaptic blockade with low  $\text{Ca}^{2+}$  high  $\text{Mg}^{2+}$  solutions abolished the discharge of some, but not all, inspiratory neurons in the rostral ventrolateral medulla, suggesting the presence of pacemaker neurons in this region.

One problem is that the respiratory rhythm displayed by the in vitro preparation is different from that observed in the mature animal, perhaps similar to the gasping rhythm described by St. John (1990), and thus possibly depending on alternative medullary mechanisms. In the neonatal rat there is little postinspiratory activity in the phrenic nerve recording in either the in vivo or the in vitro preparation so that the three distinct respiratory phases are not yet developed. Even so, it has been difficult to demonstrate pacemaker properties for preBötC neurons in adult animals.

Paton and Richter (1995a,b) used "tilted" slices from rat brainstem, thereby including both the dorsomedial and the ventrolateral medulla, so that nTS, hypoglossal nucleus, and nucleus ambiguus are all in the slice. Such preparations generated respiratory rhythms that were examined by application of either glycine or strychnine. The rhythm generated by slices from animals more than 2 weeks old are much more sensitive to synaptic inhibition with glycine or excitation with strychnine.

Hypoglossal neurons show similar effects. The relative resistance of the younger animals to glycine and strychnine suggests that receptors for these agents develop as more complex synaptic inputs modify the effects of pacemaker neurons in the mature animal.

Pre-I neurons have been analyzed *in vivo* in the cat, with a view to determining whether the cells could trigger the onset of inspiration either directly, by exciting inspiratory neurons, or indirectly, by inhibiting stage 2 expiratory neurons (Schwarzacher et al., 1995). Evidence for both mechanisms was obtained. Presumably the responsible pre-I cells belong to different populations, since the first mechanism suggests an excitatory postsynaptic action and the second mechanism suggests an inhibitory one.

### **Network oscillation theories of respiratory rhythm generation**

Several versions of a network oscillation theory of respiratory rhythm generation, emphasizing the basic role of inhibitory circuitry, have been proposed by Richter and colleagues (Richter, 1982; Richter et al., 1986, 1992; Schwarzacher et al., 1991; Ogilvie et al., 1992). The general theory assumes that neural activity appropriate for respiration arises from network oscillations reflecting the particular synaptic connectivity of groups of respiratory neurons rather than the endogenous activity of a particular group of specialized pacemaker respiratory cells. The appropriate phrenic and intercostal neural activity is categorized into three basic physiological phases, designated inspiratory and postinspiratory (stage 1 during passive lung recoil expiration as phrenic activity subsides, and stage 1 during active expiration after cessation of phrenic discharge). Intracellular recordings have been made from medullary neurons discharging during these phases, and membrane potentials have been examined to identify excitatory and inhibitory synaptic inputs. Production of IPSP reversal by intracellular injections of chloride ions has emphasized the importance of strong early-inspiratory inhibitory processes, as well as relatively weak expiratory inhibition of post-inspiratory neurons. Expiratory neurons show marked early-inspiratory and post-inspiratory inhibition.

**Figure 4.21 A,B**, Network models of the origin of the respiratory rhythm. (A, modified from Ogilvie et al., 1992, and from Richter et al., 1992; B, modified from Bianchi et al., 1995.) I, inspiratory; E, expiratory. Other abbreviations given in text.

A mathematical model of the brainstem respiratory oscillator has been proposed (Fig. 4.21A). (Ogilvie et al., 1992). The primary oscillator consists of two functionally homogenous subgroups of antagonistically connected interneurons, early-inspiratory and post-inspiratory cells. The majority of the interconnections in the model are inhibitory. Inspiration starts when early-inspiratory and throughout-inspiratory neurons are released from postsynaptic inhibition, possibly arising from post-inspiratory neurons active in stage 1 of expiration (Richter and Spyer, 1990). Excitatory recurrent collaterals progressively excite the throughout-inspiratory neurons, and this inspiratory ramp discharge activates the late-inspiratory neurons. Inspiration is then terminated by post-inspiratory inhibition from early-inspiratory neurons. Expiratory discharge reflects cessation of inhibition at the

end of the post-inspiratory discharge and activation from extrinsic sources. This source of excitation, external to the network, also drives early-inspiratory and post-inspiratory neurons.

The extrinsic source of excitation is somewhat vaguely characterized as "the reticular activating system." The primary oscillator further interacts with this external source in that post-inspiratory neurons are thought to inhibit the reticular activating system during passive stage 1 expiration, thereby reducing the extrinsic excitation.

The Richter model represents a sophisticated attempt to explain respiration by a reasonably characterized neuronal network. It is currently being subjected to experimental evaluation. The authors themselves indicate several deficiencies, particularly with respect to simulation of respiratory events normally initiated by activity in afferent fibers of the appropriate cranial nerves. At present there is little direct evidence that post-inspiratory neurons inhibit other respiratory neurons (Parkes et al., 1994). The somewhat variable definition of post-inspiratory neurons is noted by Ezure (1990). There are other more general weaknesses with the model. As discussed in Chapter 3, the so-called reticular formation is an extremely nebulous entity, with dubious scientific credentials. The reticular activating system is generally postulated to be in the upper pons or the midbrain, regions not essential for maintaining respiration. Calling upon such an uncharacterized entity for such an important excitatory input is a major weakness in the Richter network theory; in this respect the model begs the question. A second weakness lies in the failure of the model to incorporate the neuroanatomical localization of the various respiratory neurons constituting the network. Neurons in the model are assigned on the basis of discharge rhythm and electrophysiological properties, with little or no regard to whether they belong to the DRG, the cVRG, the rVRG, or the BötC group.

Another possible arrangement of the various respiratory elements, suggested by Bianchi and colleagues, is depicted in Figure 4.21B. The model, like the Richter model, does not correlate the various neuronal types with their neuroanatomical localization.

## Conclusion

As noted in the introduction to this chapter, respiration serves multiple purposes, and the rhythm is easily reset in the mature individual. Neural circuitry required for such a seamless interaction between voluntary and reflex modification of pacemaker-based respiratory rhythms must be exceedingly complex. So far the various theories of rhythm and pattern generation have been unable fully to encompass this complexity. The first phase of investigation of central respiratory control was principally functional, especially electrophysiological. A new phase commenced with the application of modern neuroanatomical procedures. In the future, conclusions from these latter studies will help to "anchor" physiological studies to actual brainstem circuitry, and this will no doubt greatly accelerate our understanding of respiratory control. A simple diagram reminding us of the important players on the respiratory stage is shown in Figure 4.22.

**Figure 4.22** Schematic sagittal section of lower brainstem showing the location of medullary respiratory groups (as explained in text), together with some of their excitatory or inhibitory projections.

