Discharge patterns of Bötzinger complex neurons during cough in the cat

FULVIA BONGIANNI, DONATELLA MUTOLO, GIOVANNI A. FONTANA, AND TITO PANTALEO
Dipartimento di Scienze Fisiologiche, Università degli Studi di Firenze, I-50134 Firenze, Italy

Discharge patterns of Bötzinger complex neurons during cough in the cat. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1015–R1024, 1998.—This study was carried out on pentobarbital sodium-anesthetized, spontaneously breathing cats to address the hypothesis that Bötzinger complex (BötC) neurons are involved in the production of the cough motor pattern induced by mechanical stimulation of the tracheobronchial tree. Phrenic nerve and abdominal muscle activities as well as intratracheal pressure were monitored; single-unit extracellular recordings from BötC neurons (n = 87) were performed. The majority of augmenting expiratory (E-Aug) neurons encountered (n = 47) displayed excitatory responses during the expulsive phases of coughing in parallel with the main components of the abdominal bursts and the corresponding increases in tracheal pressure. We also encountered E-Aug neurons markedly depressed up to complete inhibition during coughing (n = 14) as well as E-Aug neurons assuming a decremental pattern without any increase or even with some reduction in their peak activity (n = 15). During the expiratory thrusts, most decrementing expiratory neurons (n = 7) presented excitatory responses, whereas others were depressed (n = 3) or completely inhibited (n = 1). The results are consistent with the view that these neurons are involved in the generation of the cough motor pattern and, in particular, that some BötC E-Aug neurons convey excitatory drive to expiratory motoneurons and, hence, to expiratory motoneurons.

Cough is a defensive reflex aimed at removing mucus and foreign particles from the respiratory tract by the generation of large flows. Cough has been extensively studied (see Refs. 21 and 32 for review); the cough motor pattern generally consists of large-amplitude bursts of inspiratory muscle activity followed by large-amplitude bursts of expiratory muscle activity (4, 21, 32, 39). The central neural mechanisms that produce the cough motor pattern are poorly understood. Earlier studies have shown that VRG inspiratory and cVRG expiratory neurons display excitatory responses during the respective phases of coughing induced either by electrical stimulation of the superior laryngeal nerve (SLN) or by mechanical stimulation of the tracheobronchial tree in anesthetized, spontaneously breathing cats (9, 17, 31). More recent studies have investigated the behavior of medullary bulbospinal and propriobulbar neurons with different discharge patterns during fictive coughing induced either by SLN stimulation or mechanical stimulation of the tracheobronchial tree in decerebrate, paralyzed, and artificially ventilated cats (13, 28, 35, 37, 38). Although expiratory neurons of the cVRG have been extensively investigated and appear to have a more obvious role in the generation of the expiratory thrusts (9, 17, 31), at present little knowledge is available on cough-related responses of rostral expiratory neurons.

This study was undertaken to analyze the behavior of neurons with expiratory discharge patterns located in the BötC region during coughing evoked by mechanical stimulation of the tracheobronchial tree in anesthetized, spontaneously breathing cats. The question we addressed was whether these neurons, which are important components of the respiratory network (2, 10, 11), are also involved in producing the cough motor pattern. More specifically, our main purpose was to ascertain whether E-Aug neurons were activated in parallel with cough-related abdominal bursts, thus supporting the hypothesis (6, 7, 10, 12) that they may convey an expiratory drive to expiratory motoneurons and, hence, to expiratory motoneurons.

METHODS

Animal preparation. Experiments were performed on 18 adult cats of either sex (weight 2.4–3.9 kg) anesthetized with pentobarbital sodium (35 mg/kg ip, supplemented by 2–4 mg·kg⁻¹·h⁻¹ iv; Nembutal, Abbott, Saint-Remy-sur-Avre, France). Atropine (0.1 mg/kg im) and dexamethasone (2 mg/kg iv) were administered to reduce mucosal secretion in the airways and to minimize brain edema, respectively. The state of the pupil (constriction) and the absence of response to nociceptive stimulation (reflex withdrawal of hindlimb) indicated full surgical anesthesia. In addition, the adequacy of anesthesia was assessed by a stable and regular pattern of phrenic

0363-6119/98 $5.00 Copyright © 1998 the American Physiological Society R1015

Downloaded from http://ajpregu.physiology.org/ by 10.220.33.5 on October 14, 2017
discharge and by the absence of fluctuations in arterial blood pressure or phrenic nerve activity, whether spontaneous or in response to somatic nociceptive stimuli. All animal care and experimental procedures were conducted in accordance with Italian legislation and the official regulations of the European Communities Council on use of laboratory animals (directive 86/609/EEC).

The trachea was cannulated. Polyethylene catheters were inserted into a femoral artery and vein for monitoring arterial blood pressure and for administration of fluids and drugs, respectively. Both C5 phrenic roots were dissected free and cut distally. Both cervical vagus nerves were dissected free in the neck and maintained intact. Both SLNs (internal branches) were exposed, dissected free, and cut distally.

The animals were then placed in a prone position and held by vertebral clamps and a stereotaxic head holder. Their abdomens were supported by the base plate of the stereotaxic instrument. Their heads were ventroflexed at an angle of 45° relative to the Horsley-Clarke horizontal plane to facilitate recordings from the medulla. The dorsal surface of the medulla was widely exposed by occipital craniotomy and the dura and arachnoid membranes were removed. The posterior part of the cerebellum was removed by gentle suction to provide access to the rostral part of the medulla. All exposed tissues were covered with warm paraffin oil (37–38°C). Rectal temperature was kept at 37 ± 0.5°C using a heating pad and/or an infrared lamp controlled by a rectal thermometer probe.

Recording procedures. Efferent phrenic activity was recorded using bipolar platinum electrodes from the central stump of the cut and desheathed C5 phrenic roots. The electromyographic (EMG) activity of abdominal muscles was recorded by wire electrodes (Nichrome wires, insulated except for 1 mm at the tips, diameter 0.1 mm) inserted into the external or, less often, into the internal oblique abdominal muscles. Phrenic and abdominal activities were amplified, full-wave rectified, and “integrated” (low-pass RC filter, time constant 100 ms). Single-unit or pauci-unit extracellular recordings from the BoC (3.4–5.0 mm rostral to the obex, 2.7–3.7 mm lateral to the midline, and 3.8–5.5 mm below the dorsal medullary surface) were made with tungsten microelectrodes (5–12 MΩ impedance, as tested at 1 kHz). To discriminate single-unit action potentials before further processing, a window discriminator, which provided an output of standard pulses, was used. Discharges from single neurons were amplified and processed in the same way as phrenic and abdominal activities. In some trials the output of the window discriminator was fed to an instantaneous frequency meter to measure the discharge frequency of BoC neurons. Extracellular action potentials were in all probability recorded from cell bodies because they displayed biphasic or triphasic shapes, relatively high amplitudes (200–450 µV), and long durations (>1 ms); furthermore, recordings were stable and could be held over a long (50–100 µm) microelectrode travel (see also Ref. 20 for further references). In each experiment, some of the recording sites were marked with small cathodal electrolytic lesions (20 µA for 20 s) to facilitate later histological identification. Phrenic, abdominal, and neuronal activities were monitored as “raw” signals on an oscilloscope (model 5112; Tektronix, Beaverton, OR) and fed to an audiomonitor. Strain gauge manometers were used for monitoring arterial blood pressure and intratracheal pressure. End-tidal CO2 partial pressure (P CO2) was measured by an infrared CO2 analyzer (Datex CD-102; Normocap, Helsinki, Finland). Integrated phrenic, abdominal, and neuronal activities, as well as the signals of the other variables studied, were recorded on an eight-channel rectilinearly writing chart recorder (model 8K20; NEC San-ei, Tokyo, Japan). On some occasions, the various signals were displayed on an oscilloscope (model 5112; Tektronix) and photographed by means of a kymograph camera.

Stimulation procedures. Coughing was induced by insertion of a 0.5-mm diameter nylon fiber through the tracheal cannula until the tip was judged to be near the carina and thin bronchi (39). Repeated caudal advancements of the fiber to touch the carina on nearby airway walls were made (at ~2 times/s) over periods of 5–15 s. As far as possible, the force applied was maintained at similar levels in most experiments. However, in some trials an attempt was made to vary the force and especially the repetition rate of fiber contacts with the airway mucosa so as to obtain reflex cough responses of low and high intensity, respectively. An evaluation of the magnitude of coughs was given by the intensity of tracheal pressure changes.

Antidromic stimulation of the vagus or SLN was used to identify vagal or laryngeal motoneurons to exclude them from this study. Bipolar silver electrodes were used for electrical stimulation of both vagi and SLN, both ipsi- and contralateral to the sites of neuronal recordings. Rectangular pulses (0.1 ms duration, intensity up to 200 µA) were delivered by a stimulus isolation unit (model SIU 5; Grass Instruments, Quincy, MA) driven by a stimulator (model 58, Grass Instruments). Stimulation was performed with single shocks or trains of stimuli (200 Hz, 40–100 ms). Current intensity was monitored as voltage drop through a series resistor using a differential amplifier and an oscilloscope. As previously reported (30), standard criteria for antidromic activation of medullary respiratory neurons were used (22), in particular, the ability to follow high-frequency stimulation (200 Hz) and collision tests. No attempt was made to investigate other axonal projections of sampled neurons, such as those to the glossopharyngeal nerve or to the pharyngeal branches of the vagus nerve (3; see Refs. 2 and 11 for review), owing to the obvious difficulties in maintaining single-unit recordings for long periods. However, because it has been reported that most BoC neurons are inhibited by SLN stimulation (5, 19, 28, 29), the effects of SLN stimulation on the ongoing activity of most of the BoC neurons encountered were also investigated. To this purpose, we used single stimuli at current intensities varying from the threshold for evoking an inhibitory response in ongoing phrenic activity (see also Ref. 5 for further references) to three times that value.

Histological control of recording sites. At the end of the experiment, the brain was perfused with 0.9% NaCl solution and then with 10% Formalin solution via a carotid artery. After at least a 48-h immersion in 10% Formalin solution, the brain was placed in a hypertonic sucrose solution. Frozen serial sections 50 µm thick were made in the frontal plane to confirm the stereotaxically located recording sites, according to the atlas of Berman (1).

Data analysis. The output signals of the instantaneous frequency meter were used for measuring peak discharge frequencies of some E-Aug neurons (n = 18) during control breathing and coughing. As already reported in this section, these signals were displayed on an oscilloscope and photographed. For each neuron tested, we considered the maximum value of peak frequency observed during control breaths and expiratory thrusts, respectively. Student’s t-test for paired samples was used in the statistical analysis. The relationship between the amplitude of abdominal integrated EMG (IEMG) activity (arbitrary units) and the peak discharge frequency of E-Aug neurons were investigated on a cough-by-cough basis by means of linear regression analysis. For this purpose, these variables were expressed as a percentage of the maxi...
mum abdominal IEMG amplitude observed in each experiment and the maximum firing rate attained by each neuron, respectively. Regression lines were compared using covariance analysis. P < 0.05 was considered significant. Reported values are means ± SE.

RESULTS

Reflex cough responses. Present observations on the cough motor pattern in response to the mechanical stimulation of the tracheobronchial tree are consistent with those of previous studies on either spontaneously breathing or paralyzed, artificially ventilated animals (4, 21). A total of 216 reflex cough responses (including repeated trials on the same neurons) in 18 cats were considered for the present description of the cough motor pattern.

As shown in Fig. 1B, mechanical stimulation of the tracheobronchial tree caused reflex cough responses consisting of repeated coughs that continued after the stimulus had stopped, often displaying a trend of decreasing intensity. Individual coughs consisted, as a rule, of large-amplitude phrenic bursts or preparatory inspirations immediately followed by bursts of abdominal muscle activity. Both the peak and the rate of rise of integrated phrenic nerve activity increased during coughing. Abdominal muscles were usually silent in control conditions; only in a few cases (10 trials in 3 cats) did they display a very low level of rhythmic activity. When cough ensued, these muscles presented bursts of activity generally characterized by early peaks and decrementing discharge patterns associated with corresponding increases in tracheal pressure (Fig. 1). During cough-related phrenic bursts, abdominal muscles were silent on some occasions (36 trials in 6 cats); in the remaining cases, they displayed different degrees of activation during both ramp and postinspiratory phrenic activity (2, 10). On some occasions (n = 6), the initial cough in a series lacked the preparatory inspiration (Fig. 1), thus fitting more appropriately the definition of expiration reflex that is typically evoked by mechanical stimulation of the vocal folds (21). Trials performed in an attempt to obtain cough responses of different intensities (8 trials in 5 cats) showed that, as a rule, multiple coughs were elicited even with minimal stimulation and that the strength of individual coughs, as well as their frequency and total number, could vary over a wide range (Fig. 1). Changes in tracheal pressure varied from barely appreciable to maximum values of 4.2 and 14.5 cmH₂O during low- and high-intensity cough responses, respectively.

End-tidal PCO₂ decreased during coughing on account of the associated hyperventilation and then returned to control levels (Fig. 1). A brief period of respiratory depression (see e.g., Fig. 1), probably related at least in part to the transient decrease in end-tidal PCO₂, usually followed the end of cough response.

Neuronal activity during coughing. We succeeded in recording the activity of 87 single units of the BoëtC during coughing induced by mechanical stimulation of the tracheobronchial tree; they were located, as confirmed by histological analysis, in the vicinity of the retrofacial nucleus, medial or ventromedial to it (Fig. 2), i.e., in an area well defined and illustrated in previous studies (12, 23, 25, 29). The neuronal types analyzed included 76 E-Aug neurons and 11 decrementing (E-Dec) neurons.

In agreement with previous results (5, 19, 28, 29), SLN stimulation caused inhibitory effects on the ongoing discharge of most neurons encountered in the BoëtC; single stimuli induced silent periods, quite similar to those we have previously described (5, 29), in the discharge of 56 of 68 E-Aug neurons tested. Comparable inhibitory periods in response to single stimuli were observed in five of eight E-Dec neurons tested. The firing of the other tested neurons (12 E-Aug neurons and 3 E-Dec neurons) was not affected by SLN stimulation.

The majority of E-Aug units (n = 47, 61.8%) presented excitatory responses during the expulsive phase of coughing, i.e., in parallel with the abdominal bursts and the corresponding increases in tracheal pressure; their discharge patterns closely resembled those of abdominal activity during the expiratory thrusts. Similar excitatory responses were displayed by neurons of
this group during the occasional expiratory thrusts \((n = 6)\) lacking preparatory inspiration. A separate analysis of these responses was not performed. The behavior of these neurons during coughing is illustrated in Fig. 1 and in more detail in Fig. 3, where raw signals of phrenic, abdominal, and neuronal activities have been shown. In Fig. 3, a photomicrograph of a coronal section through the medulla oblongata is provided as an example of the histological identification of one recording site. A quantitative evaluation of the discharge frequency was made for 12 neurons using an instantaneous frequency meter (see e.g., Fig. 3). Peak discharge frequency increased \((P < 0.0001)\) from control values of \(98.8 \pm 4.8 \text{ spikes/s (range 70–125 spikes/s)}\) to maximum values of \(316 \pm 37.2 \text{ spikes/s (range 186–565 spikes/s)}\) during the expiratory thrusts of coughing. Unlike abdominal muscles, the firing of these neurons was confined to the expiratory phase of coughing (Figs. 1 and 3). Peak neuronal activity increased in parallel with the intensity of expiratory thrusts (Figs. 1 and 3) during the same cough response as well as during separate cough responses of different intensities. The relationship between the amplitude of abdominal IEMG activity and the peak frequency of the corresponding neuronal discharge was investigated for the same 12 neurons. A positive linear relationship \((r = 0.89, P < 0.0001)\) between the two variables was found (Fig. 4). Conditions for pooling data collected before and after stimulus cessation were preliminarily ascertained by performing separate regression analysis for each set of data and providing evidence of the absence of significant differences between the two regression lines. During high-intensity cough efforts induced by maximal stimulus strength (8 trials performed on different neurons, in 5 preparations), recruitment of latent unidentified expiratory neurons was observed on four occasions. These neurons displayed short bursts of action potentials concomitant with peak activity in abdominal muscles.

We also encountered E-Aug neurons \((n = 14, 18.4\%)\) with similar characteristics of discharge in control conditions but which were markedly depressed up to complete inhibition during coughing. Nine of them

---

**Fig. 2.** Representative transverse sections of medulla showing prevailing location of sites where E-Aug (●) and decrementing expiratory (E-Dec, ▲) neurons were recorded. Horsley-Clarke frontal planes and approximate distances in millimeters from obex are indicated at left of each section. Outlines of maps derive from atlas of Berman (1). Some relevant structures are schematically represented. IO, inferior olive; P, pyramidal tract; RFN, retrofacial nucleus; SST, spinal trigeminal tract.

**Fig. 3.** Discharge pattern of 1 E-Aug neuron displaying excitatory responses during coughing and histological control of recording site. A: control breath followed by 3 coughs. B: photomicrograph of coronal section through medulla oblongata at \(-4.5 \text{ mm rostral to obex, showing small electrolytic lesion (open arrow) which marks recording site in region medial to retrofacial nucleus. Phr N, phrenic neurogram; Böt IF, instantaneous frequency of Bötzinger neuronal discharge; Böt NA, Bötzinger neuronal activity; Abd EMG, abdominal electromyogram.}
were completely inhibited or presented an irregular and low-level burst activity (Fig. 5). On the other hand, the remaining five neurons maintained an augmenting discharge pattern, albeit at lower frequency (Fig. 6A); their firing, clearly unrelated to the abdominal bursts and peak neuronal activity, thus providing evidence of parallel increase of these variables during coughing.

Other E-Aug neurons (n = 15, 19.7%) assumed decrementing discharge patterns during coughing, with early peaks corresponding to the peak activity in abdominal muscles. Their decrementing discharges paralleled a decrementing abdominal activity and were generally, but not always, confined to the expiratory phase of coughing. Peak neuronal activity could attain a level similar to that of control breaths during high-intensity expiratory thrusts, but it was always depressed during less-intense cough efforts (Fig. 6B). The analysis performed on the discharge frequencies of six units revealed that the maximum value of peak frequency during coughing (78.2 ± 6.4 spikes/s) was slightly less (P < 0.05) than in control breaths (90.3 ± 6.7 spikes/s).

During coughing, 7 of the 11 E-Dec neurons sampled displayed various degrees of activation, as revealed by increases in peak and/or rate of rise of their integrated activity (Fig. 7A). The other E-Dec neurons were either depressed (n = 3, Fig. 7B) or completely inhibited (n = 1, not illustrated). No attempt was made to perform a quantitative analysis of the discharge frequency of these neurons.

**DISCUSSION**

This study extends previous observations on the activity of medullary respiratory neurons during cough (9, 13, 17, 28, 31, 35); it is the first to provide a description of the behavior of a relatively large number of Bötzinger neurons with expiratory (E-Aug, E-Dec) discharge patterns during coughing induced by mechanical stimulation of the tracheobronchial tree in anesthetized, spontaneously breathing cats. A quantitative evaluation of the discharge frequency of some of these neurons (E-Aug) has also been provided.

Bötzinger neurons display various cough-related alterations in their discharge patterns, supporting the view that they play different functional roles in the genesis of cough motor responses. In particular, some Bötzinger neurons may have a role in the generation of the expulsive phase of coughing by conveying an excitatory drive to caudal expiratory neurons and, hence, to expiratory motoneurons. In fact, the majority of E-Aug neurons were excited during the expulsive phase of coughing, in parallel with the burst of abdominal EMG activity and the corresponding increases in tracheal pressure (see also Ref. 35).

The locations and discharge patterns of the sampled neurons suggest that they belong to the same Bötzinger population of propriobulbar and bulbospinal neurons previously studied; these neurons were located in the vicinity of the retrofacial nucleus, medial or ventromedial to it (Fig. 2), and presented in most instances expiratory activity with augmenting discharge patterns (2, 3, 18, 23, 25). In addition, inhibitory responses to single-shock stimulation of the SLN further characterize these neurons as belonging to the Bötzinger (5, 19, 28, 29). Respiratory motoneurons with axonal projections...
in the vagus or the SLN were excluded from the present study. However, because we did not stimulate the relatively inaccessible glossopharyngeal nerves and/or the pharyngeal branches of the vagus nerve, in some instances we could also have sampled pharyngeal motoneurons (2, 3, 11). Nevertheless, we believe this is uncommon because these motoneurons have mainly been encountered in decerebrate preparations. In the anesthetized cat, spontaneous respiration-related activity of most cranial motoneurons is depressed much more than that of other respiratory neurons; these motoneurons seldom fire spontaneously and are especially sensitive to barbiturates (2, 10, 11). On the other hand, pharyngeal motoneurons with expiratory discharge patterns are rarely encountered in the rostral medulla even in decerebrate cats (3). In addition, they have been shown to be localized in the retrofacial nucleus itself rather than medial or ventromedial to it (14). For these reasons, we are confident that we recorded mainly from propriobulbar or bulbospinal neurons located in the vicinity of the retrofacial nucleus, to which the term BöC is properly applied (2, 10, 11).

The behavior of E-Aug neurons during coughing evoked by mechanical stimulation of the tracheobronchial tree clearly confirms that this population of neurons is not homogeneous (6, 18). The time course of the excitatory responses of most of these neurons (e.g., Figs. 1 and 3) and the positive linear relationship observed between the amplitude of abdominal IEMG activity and their peak discharge frequency (Fig. 4) provide correlational evidence that they are involved in
the genesis and/or in the mediation of the expiratory drive responsible for the main components of the abdominal bursts. This interpretation is in agreement with the hypothesis that bilateral axonal projections of Bötz C E-Aug neurons to the cVRG convey an excitatory input (10, 12) and it is further corroborated by the finding that the activation of Bötz C neurons achieved either by electrical microstimulation or by microinjections of excitatory amino acids exerts excitatory effects on the activity of expiratory motoneurons through the activation of expiratory cVRG neurons (6, 7). Interestingly, the recruitment of some latent neurons was observed during high-intensity cough efforts on some occasions. These neurons could be likely regarded as high-threshold Bötz C neurons and could represent an additional source of motor drive to caudal expiratory neurons. This population of silent neurons could also be involved in the mediation of respiratory effects induced by electrical and chemical stimulation of neurons located in the Bötz C region (6, 7).

The evaluation of the discharge frequency of some E-Aug neurons activated during coughing proved that these neurons could attain relatively high peak frequencies (118–565 spikes/s) during the expiratory thrusts. Similar, or even higher, peak discharge frequencies have already been observed for early and late inspiratory bulbospinal neurons of the dorsal respiratory group during laryngeal coughing (13). We believe that contamination of our single-unit recordings by action potentials of recruited neurons is unlikely. Single units were sampled on the basis of extracellular spike configuration, as observed on an oscilloscope with an expanded time scale. When a quiescent neuron was recruited, its discharge could be recognized and differentiated on the basis of spike waveform and amplitude; recruitment of latent neurons could also be revealed by interference phenomena between their spikes and the action potentials of the spontaneously active unit. For our quantitative evaluation, we have only considered recordings without signs of recruitment of silent neurons; on the other hand, the latter were observed only on some occasions during high-intensity cough efforts. In addition, because action potentials were passed through a window discriminator before further processing (see METHODS), spikes with amplitudes higher and lower than the settled window levels did not provide any output. The intensity of excitatory responses of E-Aug neurons is further emphasized by the fact that neuronal discharge frequencies increased despite decreases in respiratory duration during coughing (see e.g., Figs. 1 and 3). These neuronal responses may be due not only to a cough-related excitatory input, but also to a postinhibitory rebound greater than in control conditions; in fact, larger phrenic bursts during coughing may provide a greater inhibitory input to E-Aug neurons from central mechanisms generating inspiratory activity (see e.g., Ref. 2).

Another issue related to the discharge patterns of Bötz C E-Aug neurons during coughing can be addressed. Because airway stimulation not only preceded, but also accompanied, the initial coughs of reflex responses in our experiments (see Figs. 1, 5, and 7), the discharge patterns of Bötz C neurons could have been differentially affected by continuing airway stimulation. However, we believe this to be unlikely. In fact, neuronal discharge patterns were not altered during the relatively long stimulation period that preceded the beginning of a cough response, indicating that airway afferent inputs (32) did not exert any influence on these neurons in the absence of the development of a cough response. In addition, Bötz C neurons displayed the same behavior, i.e., excitation, inhibition, or changes in the discharge pattern, over the whole duration of the cough response, without or with airway stimulation. Nevertheless, airway stimulation during coughing could have affected the discharge patterns of excited E-Aug neurons only quantitatively, causing, for example, the development of exceedingly high or low peak frequencies compared with the intensity of abdominal IEMG activity. Indeed, the maximal expiratory bursts were usually observed during stimulation; however, single coughs could present comparable abdominal bursts both before and after stimulus cessation (Fig. 1). Moreover, the amplitude of abdominal IEMG activity, although variable throughout the complete cough response, was related to the intensity of neuronal discharges and not to the presence of airway stimulation (Figs. 1 and 4). Thus cough-related responses of Bötz C neurons appear to present similar features with and without stimulation; quantitative differences are related to the intensity of cough efforts elicited at a given moment rather than to the presence of airway stimulation per se.

Our interpretation of the functional role of E-Aug neurons activated during coughing does not contradict the finding that a subpopulation of Bötz C E-Aug neurons may have inhibitory projections to the cVRG expiratory neurons (e.g., Ref. 18). It seems reasonable to hypothesize that these inhibitory neurons should be disabled when a massive activation of caudal expiratory neurons is required, as is the case during expiratory bursts induced by mechanical stimulation of the tracheobronchial tree (9, 17, 31). Accordingly, we also encountered Bötz C E-Aug neurons (Figs. 5 and 6A) that were strongly depressed and with discharges unrelated to the activity of abdominal muscles and the corresponding increases in intratracheal pressure (see Ref. 35). Conceivably, a subpopulation of E-Aug neurons may maintain their inhibitory influences on inspiratory activity (6, 7, 12, 23–25) during the expiratory phase of coughing. In agreement with previous suggestions (see also Ref. 18 for further references), the inhibitory influences of Bötz C E-Aug neurons on both medullary inspiratory neurons and phrenic motoneurons (6, 7, 12, 23–25) may serve to prevent activation of inspiratory neurons during the expiratory phase and/or to control the duration of the expiratory phase. On the other hand, E-Dec neurons have inhibitory connections with the inspiratory neurons of the VRG and may play a role in the transition between the inspiratory and expiratory phases (11); E-Dec neurons displaying cough-related excitatory responses may inhibit inspiratory
activity during coughing, possibly in collaboration with E-Aug neurons acquiring similar decrementing discharge patterns. The present data also suggest that the BötC subpopulation of E-Dec neurons is not homogeneous.

In this context, Orem and Brooks (27) have reported that some E-Aug neurons of the retrofacial region in anesthetized cats are inactive during sneezing. This finding suggests that E-Aug neurons of the BötC may participate differently in the generation of coughing and sneezing. However, we also encountered a subpopulation of E-Aug neurons depressed or completely inhibited during coughing; these neurons may correspond to those inactive during sneezing (27). Nevertheless, it would be of interest to ascertain whether the same E-Aug neurons operate differently during coughing and sneezing.

Our results appear to be consistent with previous studies showing that, during coughing induced by mechanical stimulation of the tracheobronchial tree in spontaneously breathing anesthetized cats, the activity of both inspiratory and expiratory VRG neurons is enhanced during their respective phases of coughing (9, 17, 31); these studies also provided evidence that the same expiratory units of the cVRG (putative bulbospinal neurons) are involved in cough, sneeze, the expiration reflex, and the response to expiratory resistance (17, 31). Oku et al. (28) reported that the responses of both rostral and caudal E-Aug neurons were similar; however, at variance with our results on BötC E-Aug neurons and with previous findings on cVRG expiratory neurons (9, 17, 31), they found that the firing patterns of these neurons could not explain the bursts of abdominal nerve activity during coughing induced by SLN stimulation in decerebrate, paralyzed, and artificially ventilated cats. Thus they suggested the existence of unidentified neurons responsible for the bursts of abdominal activity during coughing. Although the problem of a still-unidentified input to spinal motoneurons remains and deserves further investigation, we believe that most of the differences between present results and those of Oku et al. (28) are possibly related to different preparations and even more to the different techniques used for eliciting the cough response in the two studies.

Differences between the cough motor pattern induced from the tracheobronchial tree and larynx have been described (21, 32). For instance, the inspiratory effort is stronger and the expiratory effort weaker in laryngeal than tracheobronchial cough. However, the major source of differences between the two studies could be the type of stimulation used by Oku et al. (28); they used electrical stimulation of the SLN, which may involve nerve fibers arising from receptors of different modalities and may evoke laryngeal reflexes other than cough, such as swallowing, apnea, and the expiration reflex (21). In fact, Oku et al. (28) induced both coughing and swallowing with the same stimulation parameters, and most coughs were accompanied by swallowing in their recordings. Thus some of their neuronal responses may reflect different inputs impinging on the same neurons.

As to the differences in the preparations used in the two studies, it should be recalled that our cats were spontaneously breathing, whereas those of Oku et al. (28) were paralyzed and artificially ventilated. Therefore, proprioceptive spinal reflexes (see also Ref. 26 for additional references) could account for part of the abdominal activation during coughing in our experiments. However, it has been shown (4, 15) that cough can be elicited by either electrical stimulation of the SLN or mechanical stimulation of the intrathoracic trachea in paralyzed animals (fictive cough), i.e., in the absence of active or passive movement-related afferent inputs from chest wall and abdomen. In addition, the patterns of activity observed in phrenic and abdominal nerves during fictive cough have been reported to be consistent (4) or quite similar (15) to those produced during coughing in spontaneously breathing animals. These findings indicate that peripheral proprioceptive feedback does not play a prominent role in the generation of inspiratory and expiratory muscle activation during coughing. On the other hand, the presence of potent proprioceptive abdominal reflexes has been demonstrated during vomiting in decerebrate cats after removal of all supraspinal inputs or only of those arising from expiratory bulbospinal neurons in the cVRG (26). However, it has also been shown that these reflexes play only a minor role, if any, during vomiting in intact animals and that abdominal muscle activation is probably mediated primarily or exclusively by expiratory neurons in the cVRG (26). It seems plausible to infer that this is valid also for reflex cough responses in our experiments. A contribution to abdominal muscle activation during coughing from proprioceptive afferent inputs to medullary respiratory neurons also seems unlikely. Previous studies have shown that abdominal and/or intercostal nerve afferents do not have excitatory effects on E-Aug neurons of either the cVRG or the BötC (16, 34, 36); muscle spindle afferents have no effect on these expiratory neurons, whereas tendon organ afferents have an inhibitory effect. Moreover, in our experiments, cats’ abdomens were supported by the base plate of the stereotaxic instrument; this limited the resting length of abdominal muscles and therefore the strength of the proprioceptive feedback to abdominal motoneurons. In addition, our cats, like those of previous studies on the behavior of medullary neurons during coughing (9, 13, 17, 28, 31, 35), were intubated, and therefore the compressive phase of cough (21, 32) was lacking; this implies the development of lower subglottic and intra-abdominal pressures and, consequently, a reduced proprioceptive afferent input to abdominal motoneurons during coughing. Nevertheless, intubation also greatly reduces or completely abolishes the peripheral feedback from upper airways during coughing. Thus because most BötC neurons receive inhibitory afferent inputs of laryngeal origin (5, 19, 28, 29; see also present results), their discharge patterns may have been affected by this experimental procedure. It would be of interest to investigate whether...
BötC neurons display a different behavior during coughing in animals with intact airways. Partial paralysis of the diaphragm due to the cutting of both C5 phrenic roots could also have influenced proprioceptive feedback from the lower ribcage and abdominal muscles as a result of alterations in regional mechanics. However, taking into account, as discussed above, that proprioceptive afferent inputs play a minor role in the generation of the cough motor pattern, this aspect can be disregarded. All these considerations lead us to assume that during cough, the peripheral feedback from the contracting muscles provides only a minor contribution, if any, to the activation of abdominal motoneurons, which therefore are essentially or even entirely driven by medullary expiratory neurons.

Perspectives

Previous experiments performed on cats in this laboratory suggest that BötC neurons have not only inhibitory influences on inspiratory activity, but also excitatory influences on expiratory activity through their projections to the cVRG expiratory neurons (6, 7). Evidence has been provided that BötC neurons also send inhibitory projections to the cVRG (18), but their functional role is unclear. Even the role played by BötC neurons in the generation of the respiratory rhythm is not yet completely defined.

Cough can be considered as a modified respiratory act. Our experiments provide correlational evidence that BötC neurons are involved in producing the cough motor pattern; more specifically, some BötC E-Aug neurons may convey an excitatory drive to causal expiratory neurons and hence to expiratory motoneurons. Caudal expiratory neurons have also been discovered to project beyond the ends of the major respiratory motoneuron pools to the lower lumbar cord as well as to the sacral cord, where pudendal motoneurons that innervate the external urethral and anal sphincters are located (nucleus of Onuf). Thus an excitatory drive to causal expiratory neurons may play a possible role in other functions, for instance, in preventing incontinence during increased intra-abdominal pressure (see also Ref. 33 for additional references). In addition, our findings support the existence of neurons exhibiting functional flexibility (i.e., participation in several functional networks) and of “multifunctional” neural networks in the mammal brain stem (see also Ref. 13 for additional references). This neuronal flexibility might be activated by input from an appropriate afferent system. Thus the respiratory network appears to be dynamically reconfigured to generate the cough motor pattern when triggered by appropriate afferent inputs arising from the airways.

Further studies should attempt to determine whether BötC neurons play a prominent role in the genesis of the expiratory component of respiratory rhythm and cough reflexes. This can be achieved by using microinjection techniques to perform kainic acid lesions (8) as well as synaptic blockade of neural transmission in the BötC region with the antagonists of excitatory amino acids involved in respiratory rhythmogenesis (2). The authors thank Salvatore Cammarata for technical assistance and Adrio Vannucchi for preparation of the illustrations. We are grateful to Dr. Elizabeth Guerrin for the English revision of the manuscript. This study was supported by grants from the Ministero dell’ Università e della Ricerca Scientifica e Tecnologica of Italy. Address for reprint requests: T. Pantaleo, Dipartimento di Scienze Fisiologiche, Università degli Studi di Firenze, Viale G. B. Morgagni 63, I-50134 Firenze, Italy. Received 2 July 1997; accepted in final form 1 December 1997.

REFERENCES