Exp Brain Res (1989) 78:624–632

Experimental Brain Research

Evidence for respiratory interneurones in the C3-C5 cervical spinal cord in the decorticate rabbit

R. Palisses, L. Perségol, and D. Viala
Laboratoire de Neurophysiologie, Faculté des Sciences Mirande, Institut des Neurosciences, CNRS-URA 9, F-21004 Dijon, France

Summary. In mammals, it has long been considered that the bulbo-spinal inspiratory drive provided a direct (monosynaptic) excitation of phrenic motoneurones (Phr Mns). Although such connections have been demonstrated, recent indirect data strongly suggested that the main inspiratory drive is polysynaptic. We tried to directly demonstrate relay respiratory interneurones at the C3-C6 spinal cord level where the Phr Mn pool is located. The experiments were performed on decorticate, unanaesthetized, bilaterally vagotomized and curarized rabbits and the firing pattern of spinal interneurones was compared to the phrenic bursting. Dorsally and dorso-medially to the Phr Mn pool, different classes of inspiratory (54%) and expiratory (46%) interneurones could be identified in the ventral horn. Three classes of inspiratory interneurones were characterized and classified as "I all" (26%), "I late" (43%) and "I tonic" (29%) according to the terminology used by other authors for the bulbospinal inspiratory neurones which drive the spinal respiratory motoneurones. The expiratory interneurones could also be divided into 3 classes: "E all" (48%), "E late" (10%) and "E tonic" (41%). This first direct evidence of inspiratory interneurones at the C3-C6 spinal cord levels can account for the major polysynaptic excitation of the Phr Mns while the presence of numerous expiratory interneurones at this level suggests a polysynaptic bulbo-spinal inhibitory action onto the Phr Mns. These classes of inspiratory and expiratory interneurones did not always coincide with the bulbo-spinal classes of neurones described elsewhere. Unless these discrepancies are due to the different experimental conditions, they may indicate that some of these interneurones are not just relay target cells and they suggest that they might behave as integrative operators between the medullary drive and the Phr Mns.

Key words: Respiration – Cervical cord – Spinal interneurones – Phrenic motoneurones

Offprint requests to: R. Palisses (address see above)

Introduction

The central respiratory drive to phrenic and intercostal motoneurones is provided by bulbo-spinal neurone activation from the "dorsal respiratory group" (DRG) in the ventrolateral part of the nucleus tractus solitarius and from the "ventral respiratory group" (VRG) which includes nucleus ambigus (NA), retroambigus (NRA) and the Bötzinger nucleus. Whereas the inspiratory bulbospinal drive originates from both DRG and VRG (Nakayama and von Baumgarten 1964; Bianchi 1971), the expiratory drive emerges from the VRG (Feldman et al. 1985; Merrill and Lipski 1987). These results obtained in the cat somewhat differ from those in the rabbit: Jiang et al. (1987) thus claim that both the inspiratory and the expiratory drives originate from the DRG.

More recently, interest has been focused on the spinal target of these command neurones. Based on electrophysiological and/or anatomical studies, inspiratory bulbo-spinal neurones from the DRG were shown to connect monosynaptically with phrenic motoneurones, Phr Mns (Cohen et al. 1974; Hilaire and Monteau 1976; Cohen 1979; Graham and Duffin 1982; Feldman and Speck 1983; Lipski and Merrill 1983; Fedorko et al. 1983; Cohen and Feldman 1984; Davies et al. 1985a,b), and with inspiratory intercostal motoneurones, IC Mns (Hilaire and Monteau 1976; Davies et al. 1985a,b; Feldman et al. 1985; Duffin and Lipski 1987). It is

the same for the descending inspiratory neurones from the VRG with Phr Mns (Cohen et al. 1974; Hilaire and Monteau 1976; Feldman and Speck 1983; Lipski and Merrill 1983) and IC Mns (Kirkwood and Sears 1973; Feldman et al. 1985; Merrill and Lipski 1987). Nevertheless, recent quantitative assessment of such connections suggested that "the net depolarization provided by this source is only a small fraction of the total needed to secure motoneurone discharge" (assessed as 22% for Phr Mns and 8% for IC Mns, according to Davies et al. 1985b), thus implying that bulbo-spinal respiratory inputs are mostly upon interneurones. Direct evidence for such interneurones with bursty firing in phase with respiration has been given by Pitts (1942) and Kirkwood et al. (1988) in the thoracic cord. These pools of interneurones may allow interactions between local and descending inputs at the thoracic level and thus provide a further means for integration.

If such inspiratory interneurones were also present in the cervical cord, they might have still a different significance. Spinal respiration has been described in the cat (Aoki et al. 1978, 1980) and in the rabbit (Viala et al. 1980; Viala and Freton 1983). While in the Cl spinal cat preparation the spontaneous respiratory-like activity recorded from the Phr nerves was abolished after a C2-C3 transection (which suggests that this activity is generated at C1-C3 levels), spinal phrenic bursting could again be obtained after such a transection in the rabbit, during pharmacological activation, suggesting the presence of a "spinal respiration generator" within the C3-C7 spinal cord. The latter spinal pattern is not observed in the intact animal, since it is probably controlled and entrained by the medullary pattern. If so, (i) respiratory interneurones should be characterized in the C2-C7 spinal cord zone of non spinal preparations, and (ii) part of these interneurones should belong to the "spinal respiration generator". The data presented here deal with the first aspect of this demonstration. Different categories of respiratory interneurones could be found in the C3-C6 cervical cord of the decorticate rabbit preparation. These results have already been published as a preliminary note (Palisses and Viala 1987).

Methods

Experiments were carried out on 35 rabbits (2.5–3 kg body weight). Thirty were used for electrophysiological experiments and 5 for anatomical studies (phrenic motoneurone mapping with horseradish peroxidase, HRP).

Electrophysiological experiments

Surgery. After general anaesthesia with sodium pentobarbitate (Pentobarbital, 35 mg/kg, i.v.), the animal was tracheotomized. A jugular cannula was inserted for further injections and a carotid cannula for blood pressure recording. Both vagal nerves were prepared so that they could be severed when required. Dexamethasone (Soludecadron 0.3 mg/kg, i.v.) was administrated to minimize brain oedema. Then the animal was curarized with gallamine triethiodide (Flaxedil 5 mg/kg, i.v.) and artificially ventilated. The head of the animal was fixed in a stereotaxic frame and an extensive decortication was performed after craniotomy (decorticate preparations were used for comparison with previous studies performed using the same conditions). The remaining exposed brain was protected with Agar. The phrenic nerves were isolated bilaterally through a dorsal neck approach and were maintained in mineral oil poured into a pool formed by the skin flaps. The rostral part of the vertebral column was rigidly maintained in a horizontal position with two clamps placed on C2 and Th1 vertebral bodies. A laminectomy was performed at C3-C5 vertebral levels to lay the C3-C6 cord levels open. The dura was removed and the spinal cord immediately covered with mineral oil maintained at 38°C. In addition to the bilateral vagotomy, a spinal transection at C7 level was achieved after lidocaïne (Xylocaïne, 1% adrenaline) infiltration in order to prevent afferent inputs related to passive chest movements from reaching the cervical cord; lidocaïne was used to minimize the effects of mechanical stimulation.

In some cases, a bilateral pneumothorax was carried out to reduce mechanical artefacts due to pulmonary inflations.

Recordings. Recordings only began 6 h after the onset of surgery, i.e. when the main effects of anaesthesia had subsided. Phrenic nerve activities were recorded from their central cut ends with a pair of silver electrodes connected to short-time constant amplifiers (bandwith 100 Hz–10 kHz). Spinal "respiratory" units were recorded extracellularly with stainless steel microelectrodes (resistance 2–3 MΩ at 1000 Hz A.C. current) in the cervical C3-C6 spinal cord. Such electrodes allow the activity of neurones of large and smaller size to be recorded. The electrodes were driven by a microdrive (Narishige) through the spinal cord.

During the experiment, all recordings were monitored on a multitrace oscilloscope and were also fed into a multichannel tape recorder (Schlumberger MP 5522) to allow further processing and film recording. Phrenic nerve activity could be rectified and filtered and unit activities sent into an instantaneous frequency (i.f) device before being monitored on a polygraph (Siemens, Oscillomink 8). For each recorded unit, the respirator was turned off for 4–5 respiratory periods in order to exclude a possible artefactual rhythmicity due to chest movements. Heart rate, blood pressure and end-tidal CO_2 (Beckman LB3) were constantly monitored. The tidal volume of respiration was adjusted to maintain a relatively constant end-tidal CO_2 (3.8–4%) for a constant frequency of the respirator (0.68 Hz).

Stimulations. The phrenic nerve ipsilateral to the spinal recorded unit could be electrically stimulated (0.1 ms; 2–5 V) through the recording electrodes to identify phrenic motoneurones by their antidromic action potentials and their disappearance when using the collision test. The identification of interneural activity was based on the lack of antidromic response to ipsilateral phrenic nerve stimulation during expiration and during inspiration. Several types of additional observations contributed to the conclusion that the units recorded within the grey matter of the spinal cord were cell bodies and not passing axons. First, the record stability, second, the spike amplitude (up to several hundred μVolts) and third, the waveform of the spike, which is usually multiphasic for body cell activity whereas positive spikes

represent the activity recorded from axons (Nelson 1959). Moreover, the discharges could be recorded over a range of electrode positions (20 to 30 μm) without loss or destruction of the unit, whereas fibre spikes appeared abruptly and disappeared with slight displacement of the electrode.

Mapping procedure. Some of the medullary "respiratory" interneurones were localized by injecting a current through the tip of the microelectrode at the recorded site (negative current of 50 μA for 15 s). Post mortem, the explored segments of the spinal cord were fixed by immersion in a solution containing 30% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and potassium hexacyanoferrate at 1% to reveal the labeling (Green's method, 1958). One week later the tissues were treated for histology (50 μm cross-sections) and counterstained with a 1% neutral red solution.

Neuroanatomical experiments

In 5 rabbits we used the retrograde transport of horseradish peroxidase (HRP) to label the Phr Mn pool. Under aseptic surgical conditions, one phrenic nerve was isolated by ventral neck approach and severed; the proximal end was crushed with fine forceps and small quantities of dry HRP (6–8 mg) were applied on this crushed part of the nerve (Duron et al. 1979). After a 72 h survival period, the animals were deeply anaesthetized (Nembutal 40 mg/kg i.v.) and first perfused with saline then with a fixative solution (2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4) through a cardiac cannula inserted into the left ventricle. After a cervical laminectomy, the C3-C6 cord was removed and placed in the same fixative solution for 2 days, then in a 0.1 M phosphate buffer (pH 7.4) solution plus 30% sucrose for 4 days. The tissues were then sectioned and revealed according to Mesulam et al's protocol (1980) and finally counterstained with a 1% neutral red solution.

Results

Evidence for different categories of respiratory interneurones in the C3-C6 cervical cord

Spontaneously firing interneurones recorded from the cervical cord were considered as respiratory interneurones: (i) if their activity was modulated in phase with the central respiratory pattern and (ii) if they were not invaded or monosynaptically activated by antidromic stimulation of the ipsilateral phrenic nerve (as phrenic motoneurones and "Renshaw" type cells would respectively be). They were characterized by their firing pattern and classified according to Gauthier and Monteau's terminology (1984): respiratory units were phasic when their firing burst was limited to one phase of the respiratory period (T) and tonic when their continuous firing displayed a periodic modulation with the respiratory rhythm; in addition, they were termed "inspiratory" or "expiratory" according to the timing of their increased firing rate within the respiratory period assessed on the phrenic nerve record.

1) Inspiratory interneurones. Of 63 respiratory interneurones which were recorded, 34 belonged to the inspiratory groups. They could be divided into 3 different functional categories:

— All *I all* interneurones (Fig. $1B_1$) had a quite comparable firing pattern. They became active at the very beginning of the phrenic burst; their discharge frequency progressively increased until the end of T_I (inspiratory period) and then decreased more or less rapidly to stop at the end of the post inspiratory period T_{E1} (Fig. 1A). They were completely silent during the expiratory period T_{E2} (Fig. $1B_1$). From frequencies close to 40 Hz at inspiratory onset, these units usually reached 100 Hz as maximal values (Fig. $1B_2$). Of 34 inspiratory units, 9 belonged to this category (26%).

— The *I late* interneurones (Fig. $1C_1$) started firing 100–600 ms after T_I onset, depending on the unit. Figure $1C_2$, D and E shows the activity of 3 interneurones which belong to this type. Like the I all interneurones, their firing rate rapidly rose and reached a maximum at T_I ending but, at variance with them, they became silent immediately after that time of maximal activity. About half these units had a relatively low maximal frequency, close to 50 Hz (examples in Fig. $1C_2$, E), whereas the others reached higher frequencies (130 Hz in Fig. $1C_2$). Two particularly late units, one of them which is shown in Fig. 1E, had a firing reduced to a few spikes towards T_I ending. This class of inspiratory interneurones was the most represented (43%, i.e. 15 units from 34).

— The tonic inspiratory interneurones (*I ton*), shown in Fig. 2 A_1–A_2, fired throughout the respiratory period with a clear phase modulation: the minimal instantaneous frequency (20–50 Hz) was seen during T_{E2} or at least during its latest part. Thereafter, their discharge frequency progressively increased from T_I onset to T_I ending where the maximal instantaneous frequency was reached (maximal frequencies between 100 and 130 Hz). The unit returned to its minimal firing rate with a gradual slowing down of its frequency either limited to T_{E1} for some units or overlapping with T_{E2} for others (Fig. $2A_2$). 10 interneurones out of 34 (29%) had this pattern.

2) In addition to isolated activity, multiunit inspiratory discharges could be followed with the microelectrode for several hundred micrometers. Throughout this distance, the number of units in the multiunit activities could vary, and consequently induce a modification of discharge amplitude. It is noteworthy that they always occurred in phase with the phrenic inspiratory burst. This multiunit firing was useful to delimitate the dorso-ventral area of the cord where the rhythmic interneurones could be found. An example of such record can be seen on Fig. 2C and another one on Fig. 3C where this kind

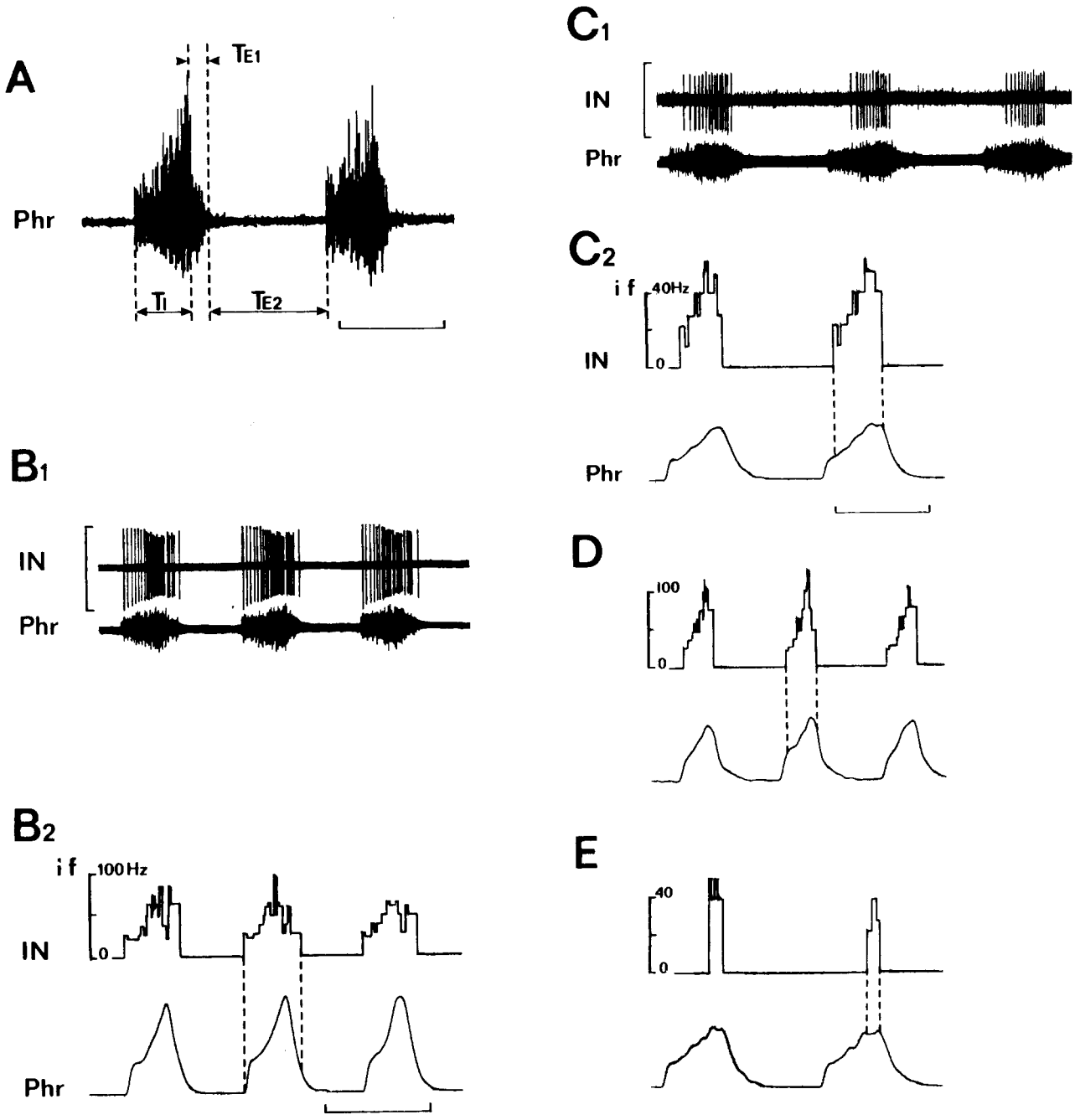

Fig. 1A–E. Firing pattern of I all and I late interneurones. **A** Spontaneous activity recorded from the phrenic nerve (Phr) with indication of the different phases of the respiratory cycle: – T_I: inspiratory phase – T_{E1}: post-inspiratory phase – T_{E2}: expiratory phase. $\mathbf{B}_1$ Simultaneous records from an I all interneurone (IN) and from the ipsilateral phrenic nerve (Phr). $\mathbf{B}_2$ Changes in the instantaneous frequency (thick letters in Hz) of the same interneurone as in $\mathbf{B}_1$ (IN upper trace) compared to the filtered phrenic activity (Phr – low trace). $\mathbf{C}_1$–**E** Different firing patterns of I late interneurones. $\mathbf{C}_1$, $\mathbf{C}_2$ Records of the same I late interneurone. Same presentation as in $\mathbf{B}_1$, $\mathbf{B}_2$. **D**, **E** Other examples of I late interneurones. Same presentation as in $\mathbf{B}_2$. Time base: 1 s; Amplitude calibration for the unit records: 330 μV

of activity was recorded at the same time as an expiratory unit firing.

3) Expiratory interneurones were also found in this part of the spinal cord and they could be divided into different classes:

— the tonic expiratory interneurones (*E ton*, Fig. 2B_1 and B_2) had a tonic, phase modulated activity. They fired at low frequency since the maximal i.f. during T_{E1} hardly outnumbered 40 Hz. 3 section parts can be distinguished in the activation

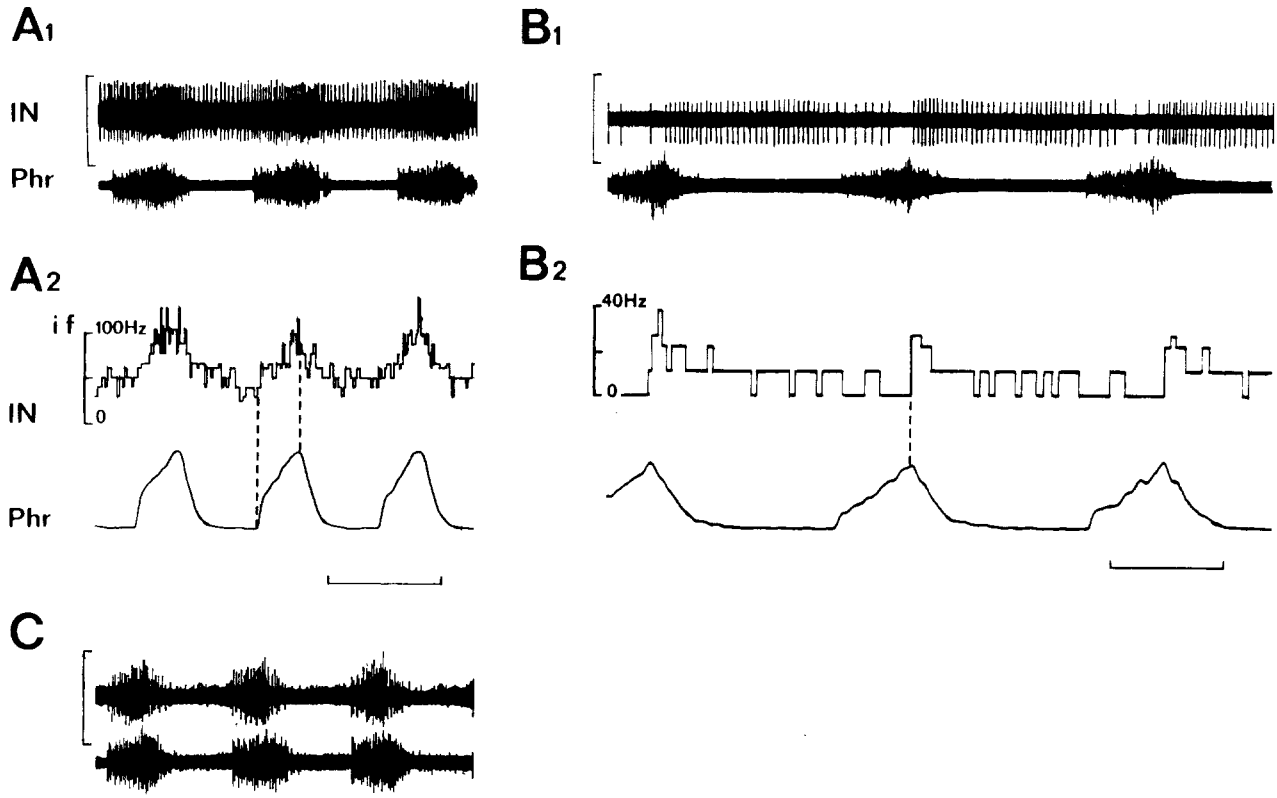

Fig. 2A–C. Firing pattern of I tonic and E tonic interneurones and multiunit record. A_1, A_2 Records of the same I ton interneurone. Same presentation as in Fig. 1 B_1, B_2. B_1, B_2 Records of the same E ton interneurone. Same presentation as in Fig. 1 B_1, B_2 except for the amplitude calibration: 130 μV. C Simultaneous record of a multiunit inspiratory activity (upper trace) in the cervical cord and of phrenic nerve activity (low trace). Same presentation as in Fig. 1 B_1

pattern. The firing rate was minimal during T_I (3–10 Hz), with a slowing down near T_I termination which could lead to a silent period during the last 100 ms sampling. The maximal firing rate occurred just after, from T_{E1} onset, and it was mainly limited to T_{E1}. During T_{E2}, the unit discharged at a stable, intermediate frequency (about 10 Hz). 12 out of 29 expiratory units belonged to this category (i.e. 41%).

— the *E all* (Fig. 3 A, B) interneurones started firing during T_{E1} and went on at a low and regular frequency (mean value 20 Hz) until the end of T_{E2}. The unit presented in Fig. 3 A, B shows the typical discharge pattern of such interneurones. Their maximal i.f. never exceeded 25–30 Hz. They were frequently observed since they corresponded to 48% of the expiratory interneurones (14 out of 29).

— the late expiratory interneurones (*E late*, Fig. 3 C, D) displayed a progressively increasing discharge that started during T_{E2} and stopped with its end, at which point firing rate was maximal (the maximal i.f. reached 200 Hz in Fig. 3D). Only 3 recorded units displayed such a firing pattern, accounting for only a small proportion (10%) of all the expiratory units.

From these electrophysiological experiments, no particular spinal localization of these different categories of interneurones could be observed. Inspiratory and expiratory activities could even be recorded simultaneously (Fig. 3C). Several respiratory interneurones could sometimes be met during the same track (maximal: 3). The field potential was most often absent or, when present, was of low amplitude, indicating a location of the interneurones dorsally to the Phr Mn pool. A large amplitude field potential was only observed for a few units close to the Phr Mn pool.

Compared localization of the respiratory interneurones and the phrenic motoneurone pool

A few experiments were performed to localize the Phr Mn pool labeled by retrograde migration of HRP in rabbits of the same weight as those used in the physiological experiments. In agreement with Ullah's work (1978) (also in the rabbit), the phrenic nucleus was found to extend from the C4 to at least half of the C6 spinal segments (Fig. 4B). On cross-sections (Fig. 4A) the phrenic pool was located in an

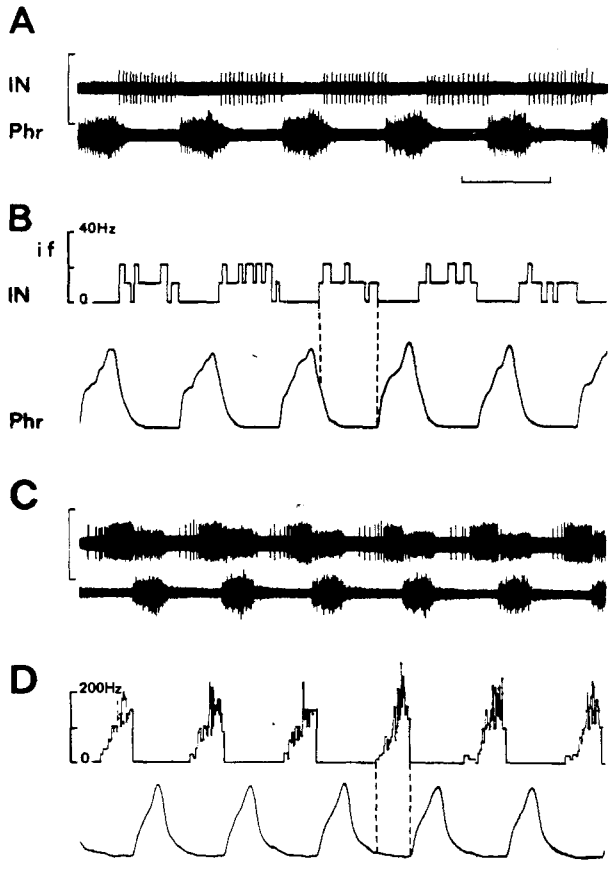

Fig. 3A–D. Firing pattern of E all and E late interneurones. **A, B** Records of the same E all interneurone. Same presentation as in Fig. 1 B_1, B_2. **C, D** Records of the same E late interneurone. Same presentation as in Fig. 1 B_1, B_2

intermediate position in the ventral horn, between the ventrolateral and the ventromedial columns in the grey matter and appeared as a cluster of 2–6 motoneurones. The lateral and dorso-ventral coordinates were fairly constant and later on, this pool could be identified on stained cross-sections without any HRP labeling.

Part of the microelectrode recordings were performed at C5 spinal level in order to determine the lateral and dorso-ventral extent of the interneurones. On stained cross-sections, the blue points that allowed the interneurones to be localized were found from 500–300 μm dorsal to the phrenic nucleus down to the phrenic nucleus itself. Laterally, these interneurones extended from 700 to 1000 μm from the midline, an area which is broader than the motoneurone extension, mainly on its medial side (Fig. 4A). This explains why the motoneurones were not necessarily encountered during the electrophysiological experiments. These interneurones were finally found in a definite area of the ventral cord. The rostro-caudal extension of the respiratory interneurones was determined by systematic unit recording at the same laterality (0.9 mm, which corresponds to the center of the active zone), in the C3-C7 spinal cord every 3 mm along the spinal axis and with more accuracy close to the rostral and caudal limits. The longitudinal extension of these respiratory units coincided with that of the motoneurones, extending from rostral C4 to C6, although somewhat more restricted than the motoneuronal pool at the C6 level (Fig 4B).

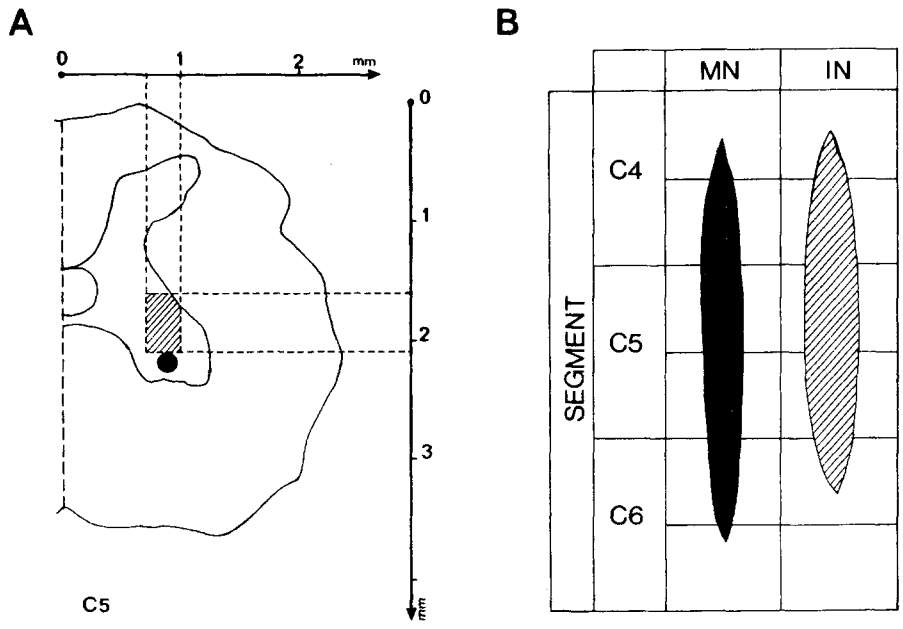

Fig. 4A, B. Compared localizations of the respiratory interneurones (hatched area) and of the phrenic pool of motoneurones (black area) on a half cross-section of the spinal cord at C5 level in **A**. Schematic representation of the rostro-caudal and lateral extension of the respiratory interneurones versus the phrenic pool of motoneurones at C4-C6 levels in **B**

Discussion

These results provide the first direct evidence for respiratory interneurones in the C4-C6 spinal cord, i.e. in a part of the cord roughly corresponding to the rostro-caudal extent of the phrenic nucleus (Ullah 1978; Rikard-Bell and Bystrzycka 1980; Duron et al. 1979). The different criteria used to differentiate the fibres from the soma ensured some security in the identification. The shape, the duration of spike and the activity maintained when moving the electrode over a few tens of micrometers confirm the presence of respiratory interneurones in addition to respiratory fibres in this cervical part of the cord. These interneurones do not belong to the Renshaw interneurones described in the cat (Hilaire et al. 1983; Lipski et al. 1985; Hilaire et al. 1986) since they were not monosynaptically excited by the antidromic stimulation of the ipsilateral nerve. In addition, they were located dorsally to the phrenic pool of Mns and not inside as shown by the size of the field potentials and by the labeling observed on cross-sections. Since the unitary activities constitutive of the multiunit discharges could not be followed separately, it cannot be decided whether they were only recorded over a few microns (as fibres are) or over longer distances (as somas are). It is likely that some of them originate from a bundle of bulbo-spinal respiratory axons entering in the grey matter and running in a dorso-ventral direction towards the Phr Mns. The ventro-lateral location of the bulbo-spinal pathways within the white matter (Jiang et al. 1987) is consistent with such a fibre entrance towards the Phr Mn target. Some of these multiunit inspiratory activities might also originate from interneurones of small diameter which are more readily recorded with steel microelectrodes than with glass micropipettes.

The different functional classes of interneurones that we could identify at this cervical level may be compared to the categories of respiratory bulbospinal neurones (BSNs), described by Bianchi (1971) and Gauthier and Monteau (1984) in the cat and by Jiang et al. (1987) in the rabbit, since they are supposed to induce the respiratory drive at the spinal level. We shall first consider the inspiratory neurones. The I all and the I late interneurones identified in the cervical cord are probably driven by the corresponding BSNs shown by Bianchi (1971) Gauthier and Monteau (1984) and by Jiang et al. (1987) and used as relays of the medullary inspiratory command to the Phr Mns that display one or other discharge pattern. Their firing pattern and the maximal frequencies they can reach are consistent with such a close transmission of information. It is nonetheless surprising that the spinal I late interneurones are much more numerous than the I all (43% against 26%) because (i) a reverse proportion of I late and I all bulbospinal neurones (Bianchi 1971; Gauthier and Monteau 1984; Jiang et al. 1987) and (ii) a relatively equal ratio of I all (53%) and I late (47%) Phr Mns (Hilaire et al. 1972) have been observed. Our results are not necessarily inconsistent with the others if we consider that the multiunit inspiratory activities that were recorded in the cervical cord can be expected to originate in part from inspiratory interneurones; all these multiunit activities have not been taken into account in the proportions which they could largely modify.

The tonic inspiratory interneurones (I ton) represent 29% of the inspiratory interneurones, while bulbo-spinal neurones with the same pattern were rarely encountered (less than 4% according to Bianchi 1971) and even not at all (Gauthier and Monteau 1984) in the cat. Only one category of BSNs, the "continuous I units" mentioned by Jiang et al. 1987 could belong to the I ton category in the rabbit. This class of BSNs is not important and can hardly be responsible for the excitation of the large number of I ton spinal interneurones that even prevails on I all interneurones (26%). One possible explanation would be that I ton interneurones display a spontaneous spinal tonic firing modulated by a phasic excitation from I all bulbo-spinal neurones. This would be another way to account for the relatively low proportion of I all interneurones compared to the predominant percentage of I all BSNs. A few other BSNs described in the rabbit, the "decrementing whole I" and the "constant whole I" have no equivalent at the spinal level.

The evidence of different classes of expiratory interneurones as numerous as the inspiratory interneurones was unexpected in this part of the cord where the respiratory efferent activities from the Phr Mns are purely inspiratory ones. The main conclusion is that these interneurones are involved in the inhibition of the Phr Mns during expiration and add their action to the monosynaptic bulbospinal expiratory inhibition described by Fedorko and Merrill (1984). We tried to compare the spinal expiratory interneurones to the expiratory BSNs described by Bianchi (1971) and Gauthier and Monteau (1984) in the cat and by Jiang et a. (1987) in the rabbit. In the cat, only two classes of expiratory BSNs have been disclosed, the E all and the E late, the latter class being more common than the first. In fact, Bianchi (1971) considers them together because they all have the same progressively increasing firing rate although they start firing at different times after the end of T_I. In spite of the lack of details, more numerous classes of expiratory BSNs have been

mentioned in the rabbit: the main groups are represented by "augmenting whole E" units that probably belong to the E all and E late groups in the cat, and by the E1 (postinspiratory) units. The E late class of interneurones we could record in the C3-C6 spinal cord is the only one which fits with the E all-E late BSNs described by others. It suggests that these interneurones relay the drive from the corresponding BSNs and inhibit the Phr Mns. Nonetheless, the low proportion of E late spinal interneurones (10%) is at variance with the important representation of E all plus E late BSNs.

The interneurones that we classified as E all cannot be mixed up with the E late since they fire earlier (during T_{E1}) and maintain a constant firing rate at low frequency. This E all class of interneurones is the most frequently encountered (48%) and has only a few equivalent BSNs in the rabbit and no equivalent in the cat. If these E all interneurones are spontaneously active at spinal level, one can assume that their respiratory modulation originates from a periodic inhibition achieved by the numerous I all BSNs; their discharge timing is consistent with such an effect on the E all interneurones.

When considering the E tonic spinal interneurones which are also numerous (41%), their cyclic modulation of activity can be explained neither by a single bulbo-spinal excitatory drive (from expiratory BSNs), nor by an inhibitory drive (from inspiratory BSNs).

We need to be cautious when comparing our results to others obtained in different experimental conditions (rabbit versus cat and/or unanaesthetized versus anaesthetized). Taken together, this comparison at the medullary and at the spinal levels nonetheless suggest that: (i) some of the spinal interneurones are likely to be relays of the medullary drive; (ii) some of them receive more complex commands and could be involved in integrative processes from supra-spinal and thoracic inputs as suggested by Kirkwood et al. (1988) for the respiratory interneurones found in the thoracic spinal cord. One might finally consider a possible role of the cervical respiratory interneurones, shown in this paper, as relay neurones towards the accessory respiratory Mns, which are silent during anaesthesia. The Mns to the sternocleidomastoid and to the trapezius muscles, respectively located at C1-C2 and at C1-C5 spinal levels (Satomi et al. 1985), can hardly be the target of such C3-C6 respiratory interneurones because even in unanaesthetized preparations, they are usually silent and they display synchronous discharges with the phrenic ones only during special activities that involve respiration such as sneezing (Satomi et al. 1984). At variance, all the interneurones we have recorded had an uninterrupted periodic activity entirely locked to the phrenic cycling. It remains to be seen whether some of these spinal interneurones belong to the spinal respiration generator (Viala et al. 1980; Viala and Freton 1983). This problem will have to be studied in another mammalian species because of the important survival problems encountered in the rabbit after the cervical vertebral column was placed in a horizontal position.

Acknowledgement. We are endebted to Dr. Buser for his valuable review of the manuscript.

References

Aoki M, Mori S, Kawahara K, Watanabe H, Ebata N (1980) Generation of spontaneous respiratory rhythm in high spinal cats. Brain Res 202: 51–63

Aoki M, Watanabe H, Ebata N, Mori S (1978) Spontaneous respiratory activity in spinal cats. Brain Res 157: 376–381

Bianchi A (1971) Localisation et étude des neurones respiratoires bulbaires. J Physiol (Paris) 63: 5–40

Cohen MI (1979) Neurogenesis of respiratory rhythm in the mammal. Physiol Rev 59: 1105–1173

Cohen MI, Piercey MF, Gootman PM, Wolotsky P (1974) Synaptic connections between medullary inspiratory neurons and phrenic motoneurons as revealed by cross correlation. Brain Res 81: 319–324

Cohen MI, Feldman JL (1984) Discharge properties of dorsal medullary inspiratory neurons: relation to pulmonary afferent and phrenic efferent discharge. J. Neurophysiol 51: 753–776

Davies JG McF, Kirkwood PA, Sears TA (1985a) The detection of monosynaptic connexions from inspiratory bulbospinal neurones to inspiratory motoneurones in the cat. J Physiol (Lond) 368: 33–62

Davies JG McF, Kirkwood PA, Sears TA (1985b) The distribution of monosynaptic connexions from inspiratory bulbospinal neurones to inspiratory motoneurones of the cat. J Physiol (Lond) 368: 63–88

Duffin J, Lipski J (1987) Monosynaptic excitation of thoracic motoneurones by inspiratory neurones of the nucleus tractus solitarius in the cat. J Physiol (Lond) 390: 415–432

Duron B, Marlot D, Larnicol N, Jung Caillol MC, Macron JM (1979) Somatotopy in the phrenic motor nucleus of the cat as revealed by retrograde transport of horseradish peroxidase. Neurosci Lett 14: 159–163

Fedorko L, Merrill EG (1984) Axonal projections from the rostral respiratory neurones of the Bötzinger complex to medulla and spinal cord. J Physiol (Lond) 350: 487–496

Fedorko L, Merrill EG, Lipski J (1983) Two descending medullary inspiratory pathways to phrenic motoneurones. Neurosci Lett 43: 285–292

Feldman JL, Loewy AD, Speck DF (1985) Projections from the ventral respiratory group to phrenic and intercostal motoneurons in cat: an autoradiographic study. J Neurosci 5: 1993–2000

Feldman JL, Speck DF (1983) Interactions among inspiratory neurons in dorsal and ventral respiratory groups in cat medulla. J Neurophysiol 49: 472–490

Gauthier P, Monteau R (1984) Inspiratory on-switch evoked by stimulation of the mesencephalon: activity of medullary respiratory neurones. Exp Brain Res 56: 475–487

Graham K, Duffin J (1982) Cross correlation of medullary dorsomedial inspiratory neurons in the cat. Exp Neurol 75: 627–643

Green JD (1958) A simple microelectrode for recording from the central nervous system. Nature 182: 962

Hilaire G, Khatib M, Monteau R (1983) Spontaneous respiratory activity of phrenic and intercostal Renshaw cells. Neurosci Lett 43: 97–102

Hilaire G, Khatib M, Monteau R (1986) Central drive on Renshaw cells coupled with phrenic motoneurons. Brain Res 376: 133–139

Hilaire G, Monteau R (1976) Connexions entre les neurones inspiratoires bulbaires et les motoneurones phréniques et intercostaux. J Physiol (Paris) 72: 987–1000

Hilaire G, Monteau R, Dussardier M (1972) Modalités du recrutement des motoneurones phréniques. J Physiol Paris 64: 457–478

Jiang C, Wu KH, Shen E (1987) Antidromic mapping of descending axons of respiratory bulbospinal neurons in the nucleus tractus solitarius of the rabbit. Brain Res 413: 189–192

Kirkwood P, Sears T (1973) Monosynaptic excitation of thoracic expiratory motoneurones from lateral respiratory neurones in the medulla of the cat. J Physiol (Lond) 234: 87P–89P

Kirkwood PA, Munson JB, Sears TA, Westgaard RH (1988) Respiratory interneurones in the thoracic spinal cord of the cat. J Physiol (Lond) 395: 161–192

Lipski J, Fyffre REW, Jodkowski J (1985) Recurrent inhibition of cat phrenic motoneurons. J Neurosci 15: 1545–1555

Lipski J, Merrill EG (1983) Inputs to intercostal motoneurones from ventrolateral medullary respiratory neurones in nembutal-anaesthetized cats. J Physiol (Lond) 339: 25–26P

Merrill EG, Lipski J (1987) Inputs to intercostal motoneurons from ventrolateral medullary respiratory neurons in the cat. J Neurophysiol 57: 1837–1853

Mesulam MM, Hegarty E, Barbas H, Carson KA, Gower EC, Knapp AG, Moss MB, Mufson EJ (1980) Additional factors influencing sensitivity in the tetramethylbenzidine method for horseradish peroxidase neurohistochemistry. J Histochem Cytochem 28: 1255–1259

Nakayama S, von Baumgarten R (1964) Lokalisierung absteigender Atmungsbahnen im Rückenmark der Katze mittels antidromer Reizung. Pflügers Arch Ges Physiol 281: 213–244

Nelson JR (1959) Single unit activity in medullary respiratory centers of cat. J Neurophysiol 22: 590–598

Palisses R, Viala D (1987) Existence d'interneurones respiratoires dans la moelle cervicale du lapin. CR Acad Sci Ser III 305: 321–324

Pitts RF (1942) The function of components of the respiratory complex. J Neurophysiol 5: 403–413

Rikard-Bell G, Bystrzycka E (1980) Localization of phrenic motor nucleus in the cat and rabbit studied with horseradish peroxidase. Brain Res 194: 479–483

Satomi H, Takahashi K, Aoki M, Kasaba T, Kurosawa Y, Otsuka K (1984) Localization of the spinal accessory nucleus in the cervical cord of cats. Neurosci Lett Suppl 17: S55

Satomi H, Takahashi K, Aoki M, Kasaba T, Kurosawa Y, Otsuka K (1985) Localization of the spinal accessory motoneurons in the cervical cord in connection with the phrenic nucleus: an HRP study in cats. Brain Res 344: 227–230

Ullah M (1978) Localization of the phrenic nucleus in the spinal cord of the rabbit. J Anat 125: 377–386

Viala D, Freton E (1983) Evidence for respiratory and locomotor pattern generators in the rabbit cervico-thoracic cord and for their interactions. Exp Brain Res 49: 247–256

Viala D, Vidal C, Freton E (1980) Mise en évidence de générateurs de rythmes locomoteurs et de rythmes "respiratoires" dans la moelle cervico-thoracique du lapin. CR Acad Sci (Paris) 291 D: 573–576

Received February 20, 1989 / Accepted June 16, 1989