ACTA NEUROBIOL. EXP. 1976, 36: 581–592

RESPIRATORY NEURONS OF THE VENTRAL RESPIRATORY NUCLEUS OF THE RABBIT AND THEIR VAGAL CONNECTIONS

H. GROMYSZ and W. A. KARCZEWSKI

Laboratory of Neurophysiology, Medical Research Center
Polish Academy of Sciences, Warsaw, Poland

Abstract. The pattern of firing and responses to electrical stimulation of the ipsilateral vagus nerve were studied in respiratory neurons near the nucleus ambiguus (NA) in the region called "ventral respiratory nucleus". In 41 (out of 77) respiratory neurons orthodromic responses were recorded; the remaining neurons did not respond to stimulation. It is concluded that the NA neurons of the rabbit are not laryngeal motoneurons. Moreover, the short-latency excitatory response of some of these neurons, and long-latency inhibition of the other, suggest that NA neurons may be involved in the processing of vagal information.

INTRODUCTION

It is almost generally accepted that the respiratory neurons of the medulla are concentrated mainly in two regions. One of them, called by Bianchi (8) the "dorsal respiratory nucleus" is situated ventro-laterally to the solitarius complex; the other, called the "ventral respiratory nucleus" — in the close neighborhood of nucleus ambiguus. A functional organization of these groups of neurons in the cat has been investigated by many authors (3, 7–9, 13, 15, 23, 28). Nevertheless, the picture is far from being clear, although modern neurophysiological techniques like microelectrode recording, antidromic stimulation and computer-assisted analysis were used to determine the role of medullary respiratory neurons in the control of breathing.

The functional organization of the rabbit's respiratory complex is even less well known, although the rabbit used to be the most "classical"

laboratory animal in the physiology of respiration. Still, it has been demonstrated that in the rabbit respiratory activities can be found both in the medulla and in the pons (17), that these activities can be modified by inflation, deflation (12) and electrical stimulation of the vagus nerve (19) and are affected by anesthesia and P_{CO_2} (10, 17). Gromysz (17) has shown that respiratory units in the brain stem of the rabbit are grouped in two, more or less parallel, layers extending from the rostral pons down to the obex at the depth of 2–3 mm (dorsal layer) and 4–5 mm (ventral layer), 2–3 mm laterally from the midline. Still, the precise topography of the main groupings of respiratory neurons in the rabbit and their functional significance has not been clarified, and, above all, the interpretation of findings was based on stereotaxic coordinates only, without histological recognition of regions explored with microelectrodes.

The present work was undertaken in order to find the structural correlates of electrophysiological observations in the ventral respiratory "nucleus".

METHODS

The experiments were performed with male rabbits weighing from 2.5 to 3.5 kg, premedicated with neuroleptoanalgesia (1.25 mg/kg of Droperidol and 0.05 mg/kg of Fentanyl, Richter, intravenously) and anesthetized with halothane (Narcotan-Specia or Halan-Germed) 0.7 vol% in air. The animals were paralyzed with gallamine (Tricuran-Germed) and artificially ventilated. End-tidal CO_2 per cent was continuously monitored (Capnograph-Godart). Both vagus nerves and C_3 root of one phrenic nerve were dissected in the neck. The vagi were cut (in order to eliminate the volume-related input from the lungs) and one of them was placed on bipolar stimulating electrodes. The activity of the phrenic nerves was recorded through bipolar silver electrodes, amplified (Tektronix 3A9 differential amplifiers), "integrated" (time constant, 0.5 s) and recorded together with other variables on a multichannel oscilloscope (Tektronix 565 with 3A74 and 3A72 plug-in units). A polyethylene catheter was inserted into the left femoral artery and blood pressure was monitored with a Statham pressure gauge and an electromanometer (EK-4 FARUM). A special-purpose computer (ANOPS-101) driven by the phrenic nerve activity was used to trigger a pulse generator (Tönnies) at a preset moment of the central respiratory cycle. The central stump of one vagus nerve was stimulated with single pulses (or short trains of them) of a strength carefully selected to produce threshold changes in the respiratory rhythm (see also 19).

The region of the "ventral respiratory nucleus" was explored with

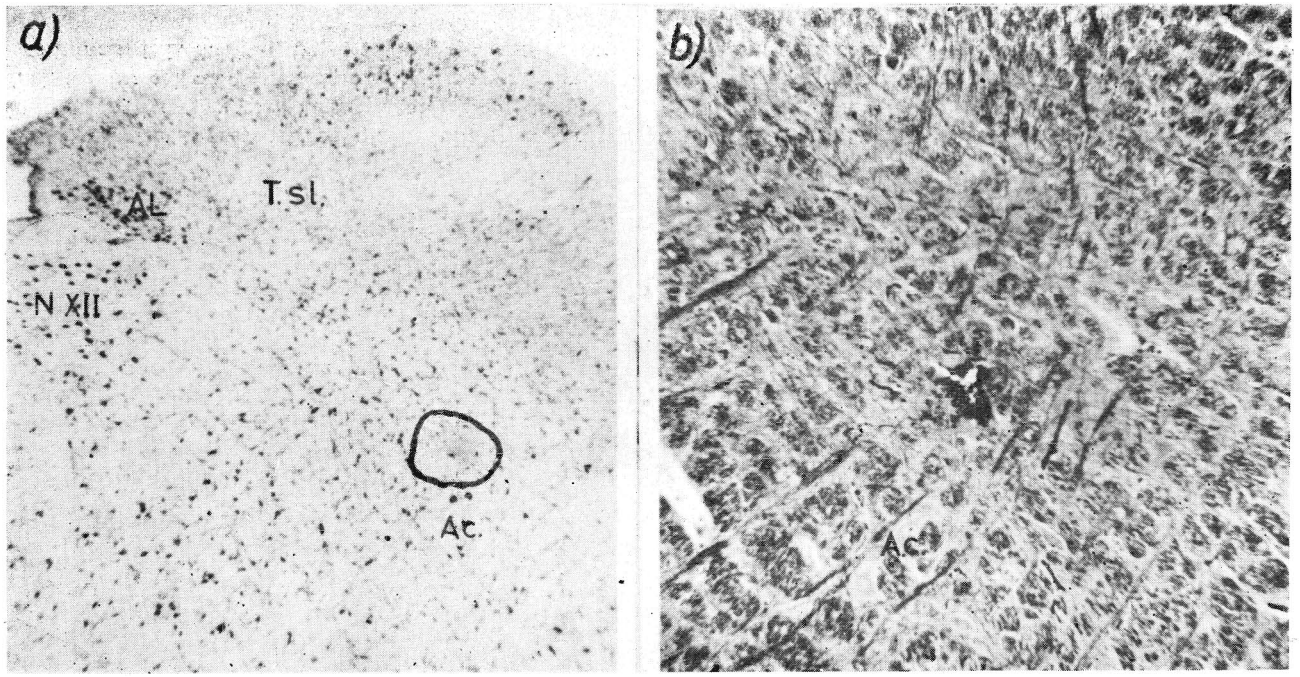

Fig. 1. Localization of the recording site performed by passing 800 μA DC through the recording microelectrode *a*, magnification 30 ×, cresyl-violet staining, region of recording encircled; *b*, magnification 100 × (Heidenhein staining). AL, n. alaris; T. sl., tractus solitarius; Ac., ambiguus; N XII, n. nerwi hypoglossi.

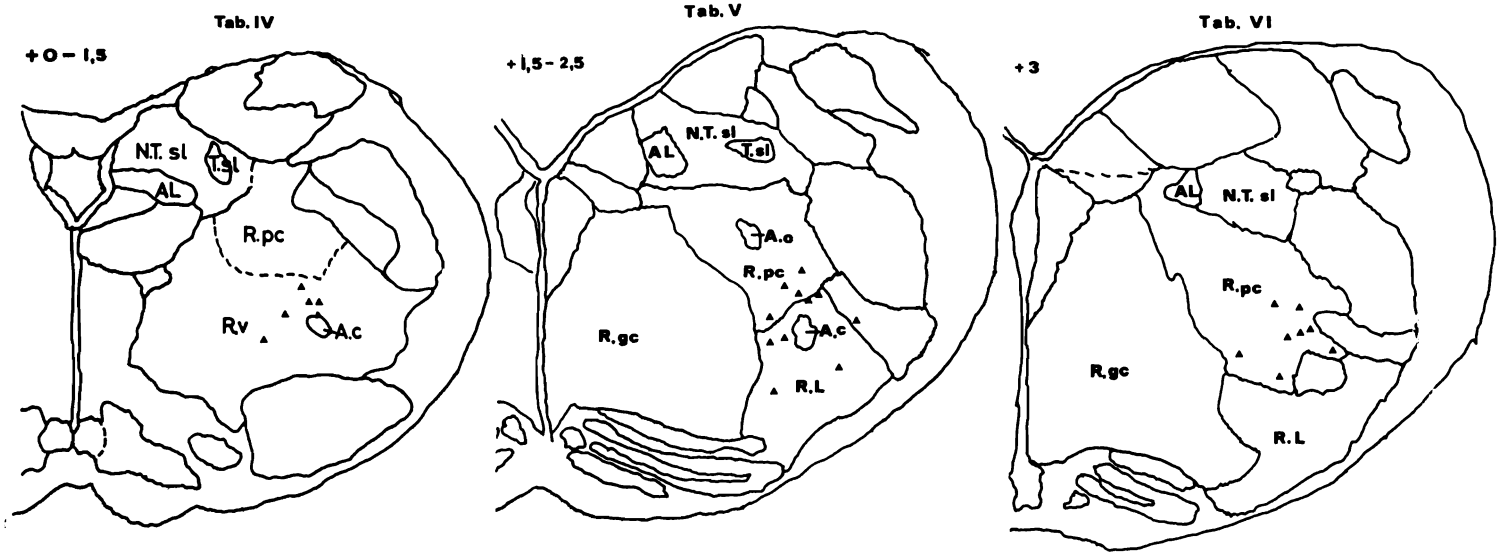

Fig. 2. Semischematic representation of medullary respiratory neurons in the rabbit. Tab. IV, V, VI are frontal sections of the medulla from the obex to 3 mm rostral. Triangles, neurons of the ventral respiratory nucleus. N.T. sl, nucleus tractus solitarii; T. sl, tractus solitarius; AL, n. alaris; R.pc; n. reticularis parvocellularis; R.v, subnucleus ret. ventralis; R.gc, n. ret. gagantocellularis; R.L, n. ret. lateralis; A.c, n. ambiguus caudalis; A.o., n. ambiguus oralis. From Messen and Olszewski (27).

glass microelectrodes (tip diameter 1–2 μm, impedance 5–15 MΩ) filled with 3M KCL. The activities were recorded extracelullarly via a cathode follower and the electrodes were introduced through a hole drilled in the occipital bone, where a steel tube was fixed. The hole was covered with agar in order to minimize the movement artifacts (16). The position of the recording electrode was estimated histologically (Heidenhein and cresyl violet staining) after passing direct current (800 μA) through the electrode. The cytoarchitectonic atlas of Messen and Olszewski (27) was used.

RESULTS

During the experiments, performed on 28 rabbits, 77 respiratory neurons were studied. The neurons were investigated in the region defined by the following stereotaxic coordinates: 3.0–4.5 mm from the dorsal surface of the medulla, 2.0–3.5 laterally from the midline and from the obex to about 3.00 mm rostrally. Histological control (Fig. 1) showed that the following structures had been penetrated: subnucleus ret. ventralis, nucleus ret. lateralis and the ventral part of the nucleus ret. parvocelullaris. Figure 2 presents schematically the distribution of respiratory activities in the explored region. As visible, the neurons are grouped around the nucleus ambiguus (NA).

It is worth noting that neurons in this region exhibited not only a large number of different patterns of firing ("early", "late", with increasing and decreasing frequency, tonic-modulated and phasic), but that they seemed to be quite densely packed. It was not unusual to pick up simultaneously two different activities (e.g., inspiratory and expiratory) at one penetration of the microelectrode. Moreover, it was sometimes difficult to resist an impression that inspiratory and expiratory activities form in this region a number of thin, alternating layers.

On the basis of the pattern of firing and of the response to shock applied to the ipsilateral vagus nerve, we have divided the inspiratory (41 neurons) and expiratory (36 neurons) units into four groups:

Group I: 14 early-inspiratory neurons. Five of them responded to a stimulus applied to the vagus with a short-latency (2 ms) excitatory response. A similar type of response (excitation followed by inhibition) was also exhibited by eight neurons with a slightly different pattern of firing, the latency being longer (5 ms) (Figs. 3 and 4). These neurons responded with excitation independently of the moment when the stimulus was applied. Only one neuron of this group responded with excitation when the stimulus was applied in expiration and with inhibition when the vagus was stimulated in inspiration (Figs. 5 and 6).

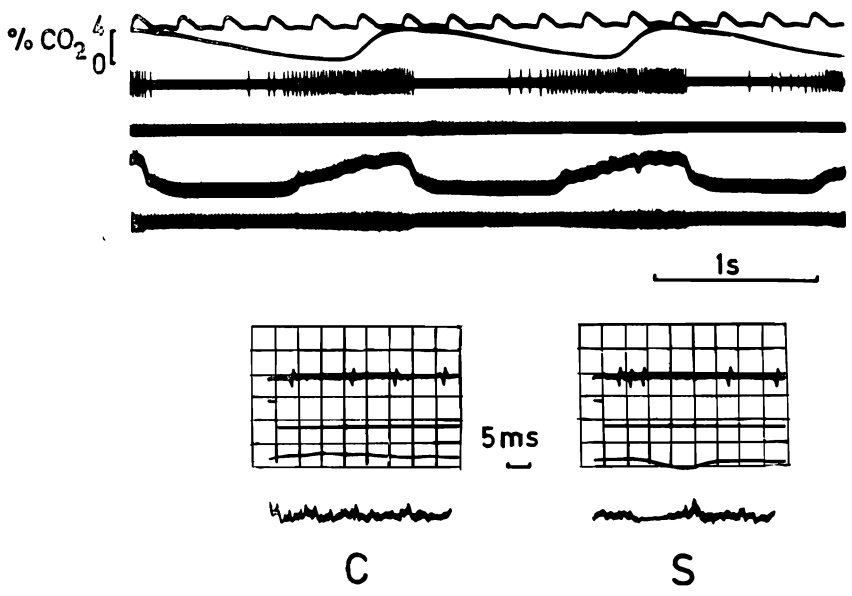

Fig. 3. The response of an early-inspiratory neuron of the ventral respiratory nucleus (VRN) to stimulus applied to the ipsilateral vagus. Upper record- traces from top to bottom: Blood pressure, end-tidal CO_2%, unit, stimulus, integrated and nonintegrated phrenic nerve activity. Lower record: C, control; S, excitatory (followed by inhibitory) response to vagal stimulation (latency of excitation 5 ms).

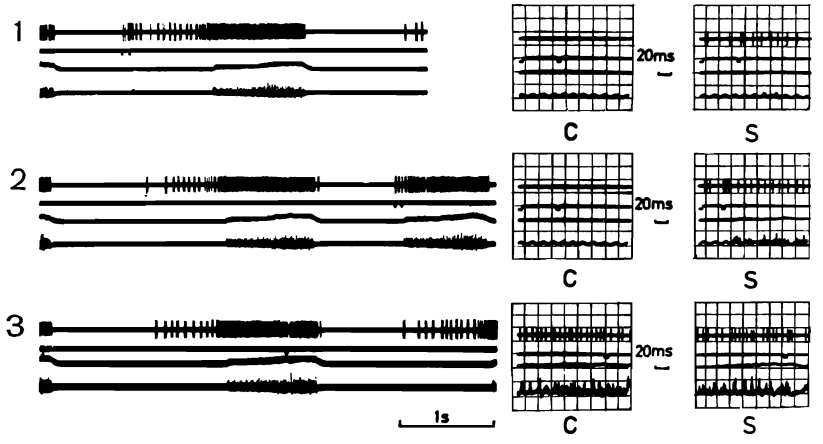

Fig. 4. The response of early-inspiratory neuron of the VRN to stimulus applied to the ipsilateral vagus. In *1*, *2* and *3*, traces from top to bottom: Early-inspiratory neuron activity, stimulus, integrated and nonintegrated phrenic nerve activity. C, controls; S, excitatory responses to vagal stimulation (latency 5 ms). In *1*, stimulus applied in part I of expiratory phase; in 2, stimulus applied in part II of expiratory phase; in 3, stimulus applied in inspiratory phase.

Group II: This group was formed by 27 neurons firing in step with the phrenic discharge. Ten of these inspiratory neurons (mainly those with increasing frequency of firing) responded with inhibition to shock applied to the vagus nerve. The latency of an inhibitory response is obviously difficult to determine, but judging from the control frequency of firing, it varied from 4 to 8 ms (Fig. 7). The other neurons (17) did not respond to stimulation.

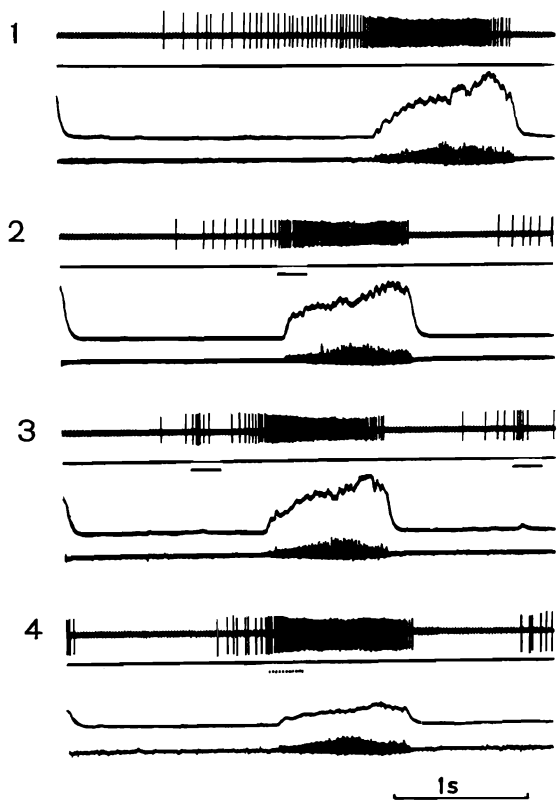

Fig. 5. The response of early-inspiratory neuron of the VRN to stimulus applied to the ipsilateral vagus. In all records, traces from top to bottom as in Fig. 4. *1*, control; *2*, stimulus (100 imp/s) applied in part II of the expiratory phase; *3*, stimulus (100 imp/s) applied in I part of the expiratory phase; *4*. stimulus (30 imp/s) applied in part II of the expiratory phase.

Group III: consisted of 33 expiratory neurons. Fourteen of them responded with inhibition to shock applied to the ipsilateral vagus after a latency of about 3 to 7 ms (Fig. 8). The remaining 19 neurons did not respond.

Group IV: consisted of three expiratory neurons with a characteristic, decremental pattern of firing. These neurons gave an excitatory response (latency 5 and 7 ms) which was followed by inhibition (Fig. 9).

In general, 41 neurons (out of 77) responded to a stimulus applied to the ipsilateral vagus: 36 neurons did not respond.

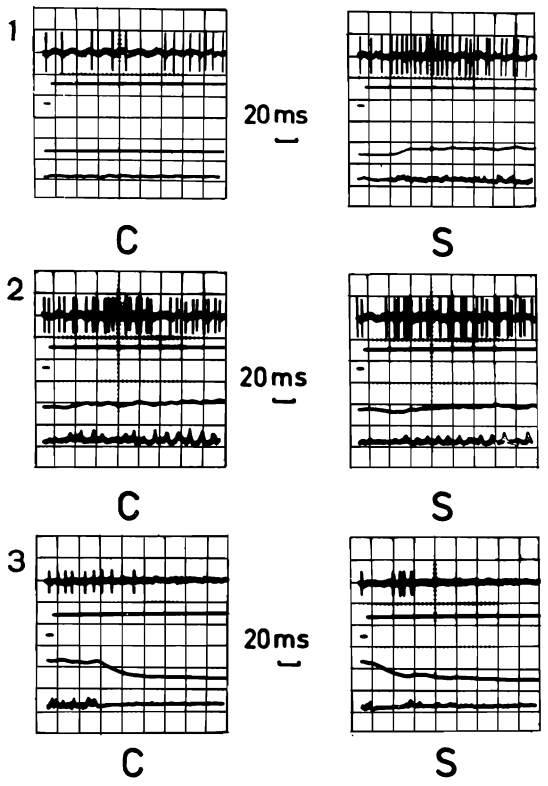

Fig. 6. Activity of the same neuron as in Fig. 5. C, controls; S in *1*, excitatory response to vagal stimulation (stimulus applied in part II of the expiratory phase); S in *2* and *3*, inhibitory response to vagal stimulation (stimulus applied in the inspiratory phase).

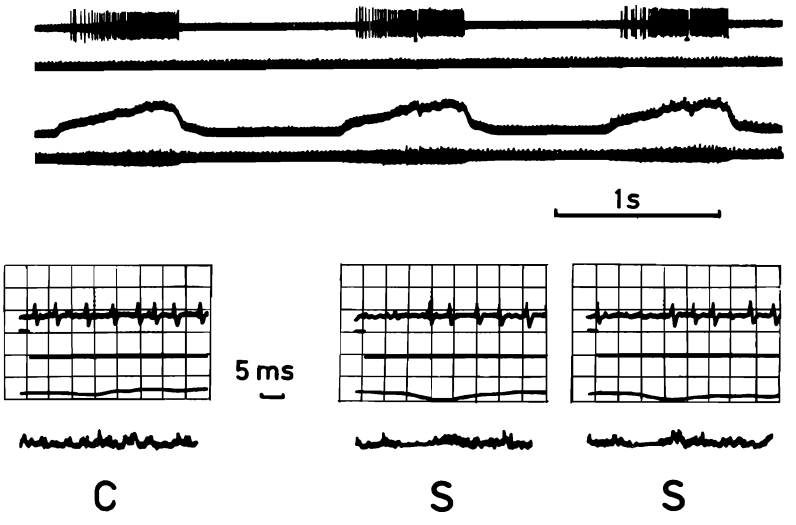

Fig. 7. The response of an inspiratory neuron of the VRN to stimulus applied to the ipsilateral vagus. Upper record, traces as in Fig 4. Lower record: C, control; S, inhibitory responses to vagal stimulation (latency 4–8 ms).

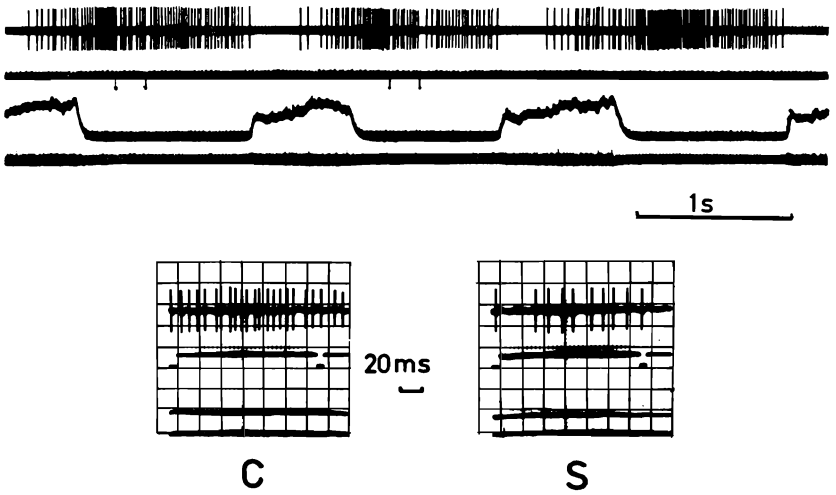

Fig. 8. The response of expiratory neuron of the VRN to stimulus applied to the ipsilateral vagus. Upper record, traces as in Fig. 4. Lower record: C, control; S, inhibitory response to vagal stimulation (latency 3–7 ms).

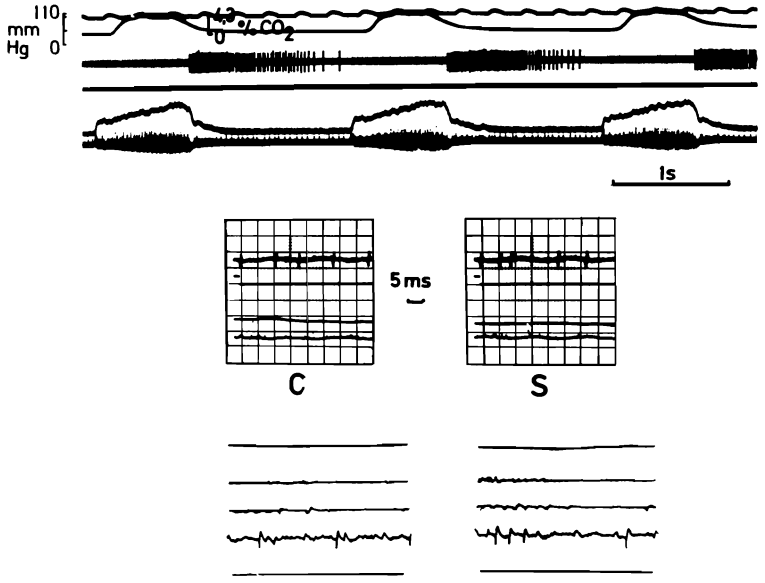

Fig. 9. Responses of expiratory neurons of the VRN to stimulus applied to the ipsilateral vagus. Upper record, traces as in Fig. 3. Middle and lower records: C, controls; S, excitatory (followed by inhibitory) responses of two expiratory neurons to vagal stimulation (latency 7 and ms).

The activity of the phrenic nerve responded uniformly with inhibition after a latency of 8 to 9 ms (see Figs. 3 and 7). The duration of this inhibition increased gradually from 7 to 11.5 ms as inspiratory discharge

progressed. A single shock (or a short volley of pulses) applied to the vagus during the last part of the expiratory pause precipitated the next inspiration after a latency decreasing from 38 to 26 ms.

DISCUSSION

Several years of microelectrode investigations on respiratory neurons did not yield unequivocal results. There are many controversies concerning not only the role of individual groups of neurons in the respiratory complex, but even their localization in the medulla. Dirken and Woldring (14) and Achard and Bucher (1) have reported that inspiratory neurons in the rabbit are localized rostrally, and expiratory neurons caudally to obex. These observations have been confirmed on cats by Haber et al. (21), Nelson (29), Batsel (2), Baumgarten et al. (3, 4) and Merrill (26). On the other hand, Salmoiraghi and Burns (31) and Hukuhara et al. (24) claim in relation to cats, and Bystrzycka et al. (11) in relation to rabbits that inspiratory and expiratory neurons are intermingled in the reticular formation of the medulla. These data were confirmed for cats also by Bianchi (8) who suggests, however, that inspiratory neurons are grouped rostrally and expiratory units caudally to obex.

Even this short review of literature shows that the picture is far from being clear, even in the same species. Presumably the discrepancies result either from different experimental conditions (e.g., pentobarbitone anesthesia used by some of the authors is believed to reduce the number of active respiratory units (6, 25)) or from the absence of histological control (the majority of authors relied upon the stereotaxic coordinates). It is certain, however, that both in the cat (3, 5, 8, 15) and in the rabbit (20) one can demonstrate the "dorsal respiratory nucleus" which seems to be purely inspiratory. Since this structure is localized rostrally to obex, one can get the impression that inspiratory neurons dominate above the obex. Since stereotaxic coordinates alone may give confusing results (and interpretation) we suggest that histological control should never be avoided in studies of the respiratory complex of the brain stem. When this is taken into account, the electrophysiological characteristics of the ventral respiratory "nucleus" in the rabbit are very similar to those described in the cat by Bianchi (8), Bianchi and Barillot (9) and Hilaire and Monteau (22).

There might be species differences as far as the dorsal respiratory "nucleus" is concerned. A group of neurons firing predominantly in inspiration was found ventro-laterally to nucleus tractus solitarii both in the cat (3, 8, 15) and in the rabbit (20). The group of big neurons descri-

bed by Baumgarten et al. (3) in the cat, which was supposed to play an important role in the control of respiration (15), has not been found in the rabbit (20) and does not seem to be always present in the cat [1].

As it was already mentioned, the discrepancies apply also to the role of individual groups of medullary neurons. Achard and Bucher (1) (see also 30) suggest that, generally speaking, neurons of the ventral part of the medulla are laryngeal motoneurons. These suggestions are in contrast to the findings of other authors, who have demonstrated that some of these neurons had spinal projections. Bianchi (8) suggests that this applies to about 50% of neurons and Merrill (26) mentions as much as 90%. Our results, too, do not confirm the view that neurons of the NA region are laryngeal motoneurons. In 41 (out of 77) neurons we have recorded orthodromic responses and the remaining neurons did not respond to vagal stimulation. A typical antidromic response was never seen in our experiments. This strongly implies that there are no laryngeal motoneurons in the region we have explored. Similar results were reported in the cat by Bianchi (7) and Hukuhara (23). On the other hand, however, the investigations of Cohen (13) and Hilaire and Monteau (22) imply that neurons of the ventro-lateral part of the medulla may directly control the phrenic motoneurons, although this role is more often ascribed to the dorsal "nucleus" (5, 8, 15). The former hypothesis may be supported by the results of lignocain microblockade (18). An injection into the ventral "nucleus" markedly inhibits the phrenic nerve discharge, whereas the same amount of the drug introduced into the region of NTS merely slows down the central respiratory rhythm (20). Moreover, the hypothesis of Baumgarten and Kanzov (5) seems to have one link missing: if the Rβ neurons (excited by the vagal input) inhibit the Rα neurons and, subsequently, the phrenic motoneurons, this sequence of events should be demonstrable in microelectrode recordings from this region by appropriate changes in activity and appropriate latencies. However, a synaptic inhibition of Rα neurons has never been demonstrated. In a recent paper Euler et al. (15) have obtained a synaptic excitation in Rβ neurons, but no response in Rα .In contrast to the apparently scarce number of dorsal inspiratory neurons, the abundance of respiratory activities in the ventral region might suggest a variety of functions. The early-inspiratory neurons responding with a short-latency excitation, and particularly those excited during the expiratory pause and inhibited during inspiratory phase are especially interesting. The responses

[1] The authors are greatly indebted to Professor Ewa Osetowska for examining this problem and extending great help in the interpretation of the histological material.

we found in the rabbit had been earlier observed in the cat (9). Bianchi (8) suggests that some of these neurons (about 60%) may play an important part in the genesis of respiratory rhythmicity. Their response to lung inflation (9) are similar to those we have found in the rabbit in response to electric shock applied to the vagus nerve.

The short-latency excitation in some and long-latency inhibition in other neurons of the ventral respiratory "nucleus" of the rabbit seem to reflect the processing of vagal information in the intramedullary neuronal structures.

This investigation was supported by Project 09.4.1 of the Polish Academy of Sciences.

REFERENCES

1. ACHARD, O. and BUCHER, M. V. 1954. Courants d'action bulbaire à rythm respiratoire. Helv. Physiol. Acta 12: 265–283.
2. BATSEL, H. L. 1964. Localization of bulbar respiratory center by microelectrode soonding. Exp. Neurol. 9: 410–426.
3. BAUMGARTEN, R., BALTHASAR, K. and KOEPCHEN, H. P. 1960. Über ein Substrat atmungsrhythmischer Erregungsbildung im Rautenhirn der Katze. Pflügers Arch. 270: 504–528.
4. BAUMGARTEN, R., BAUMGARTEN, A. and SCHEFFER, K. P. 1957. Beitrag zur Lokalisationsfrage bulboreticulärer respiratorischer Neurone der Katze. Pflügers Arch. 264: 217–227.
5. BAUMGARTEN, R. von, and KANZOW, E. 1958. The interaction of two types of inspiratory neurons in the region of the tractus solitarius of the cat. Arch. Ital. Biol. 96: 361–373.
6. BERTRAND, F., CAILLE, D. and VIBERT, J. F. 1976. Quantitative effects of anaesthesia on bulbo-pontine respiratory units populations. Colloquia INSERM, Amiens, p. 55–62.
7. BIANCHI, A. L. 1966. Mise en evidence d'un convergence heterosensorielle sur les neurones reticulaires bulbaires à rythme respiratoire. C. R. Soc. Biol. 160: 2387–2391.
8. BLANCHI, A. L. 1971. Localisation et étude des neurones respiratoires bulbaires. Mise en jeu antidromique par stimulation spinale ou vagale. J. Physiol. (Paris) 63: 5–40.
9. BIANCHI, A. L. and BARILLOT, J. C. 1965. Activity of medullary respiratory neurones during reflexes from the lungs in cats. Respir. Physiol. 25: 335–352.
10. BYSTRZYCKA, E., GROMYSZ, H., HUSZCZUK, A. and KARCZEWSKI, W. 1970. Influence de l'hyperventilation et de la narcose sur l'activité bioélectrique de neurones respiratoires chez les lapins. Bull. Acad. Pol. Sci. Sér. Sci. Biol. 17: 713–716.
11. BYSTRZYCKA, E., GROMYSZ, H., HUSZCZUK, A. and KARCZEWSKI, W. 1970. Etude chez le lapin de l'activité électrique des neurones respiratoires du tronc cérébral: I. Influence de la vagotomie et de l'injection d'histamine. Electroencephalogr. Clin. Neurophysiol. 29: 363–372.

12. BYSTRZYCKA, E., GROMYSZ, H. and HUSZCZUK, A. 1972. Studies on the Hering–Breuer inflation and deflation reflexes in rabbits. Acta Physiol. Pol. 23: 617–632.
13. COHEN, M. I. 1970. How respiratory rhythm originates: evidence from discharge patterns of brain stem respiratory neurones. *In* R. Porter (ed.) Breatching Hering–Breuer Centenary Symposium. Churchill, London, p. 125–157.
14. DIRKEN, M. N. J. and WOLDRING, S. 1951. Unit activity in bulbar respiratory center. J. Neurophysiol. 14: 211–225.
15. EULER, C. von, HAYWARD, J. N., MARTTILA, I. and WYMAN, R. J. 1973. Respiratory neurones of the ventrolateral nucleus of the solitary tract of cat: vagal input, spinal connections and morphological identifications. Brain Res. 61: 1–22.
16. GROMYSZ, H. 1968. Recording of respiratory neurones activity of the medulla by means of glass ultramicroelectrodes. Acta Physiol. Pol. 19: 521–524.
17. GROMYSZ, H. 1971. Studies on the activity of the brain stem respiratory neurones of the rabbit (in Polish). Ph. D. Thesis. Med. Res. Centre (Warsaw).
18. GROMYSZ, H. 1973. Pharmacological blockade of respiratory "centres". IRCS (73–7) 7–10–8.
19. GROMYSZ, H. and KARCZEWSKI, W. A. 1973. Responses of respiratory neurones of the rabbit to some excitatory and inhibitory stimuli. Acta Neurobiol. Exp. 33: 245–261.
20. GROMYSZ, H. and KARCZEWSKI, W. A. 1976. Responses of the brain stem respiratory neurones to stimulation of the vagal input. Colloquia INSERM, Amiens, p. 117–128.
21. HABER, E., KOHN, K. W., NGAI, S. H., HOLADAY, D. A. and WANG, S. C. 1957. Localization of spontaneous respiratory neuronal activities in the medulla oblongata of the cat: a new localization of the expiratory center. Am. J. Physiol. 190: 350–355.
22. HILAIRE, C. and MONTEAU, R. 1975. Participation de différents types de neurones bulbaires à l'élaboration de l'activité respiratoire. J. Physiol. (Paris) 70: 759–777.
23. HUKUHARA, I., Jr. 1973. Neuronal organization of the central respiratory mechanisms in the brain stem of the cat. Acta Neurobiol. Exp. 33: 219–244.
24. HUKUHARA, T., NAKAYAMA, S. and OKADA, H. 1954. Actions potentials in the normal respiratory centers and its centrifugal pathways in the medulla oblongata and spinal cord. Jap. J. Physiol. 4: 145–153.
25. HUKUHARA, T., Jr., SAJI, Y., KUMADAKI, N., KOJIMA, H., TAMAKI, H., TAKEDA, R. and SAKAI, F. 1969. Die Lokalisation von atemsynchron entladenden Neuronen in der retikulären Formation der Hirnstammes der Katze unter verschiedenen experimentellen Bedingungen. Arch. Exp. Pathol. Pharmakol. 263: 462–484.
26. MERRILL, E. G. 1970. The lateral respiratory neurones in the medulla: their association with nucleus ambiguus, nucleus retroambigualis, the spinal accessory nucleus and the spinal cord. Brain Res. 24: 11–28.
27. MESSEN, H. and OLSZEWSKI, J. 1949. A cytoarchitectonic atlas of the Rhombencephalon of the rabbit. S. Karger, Basel. 52 p.
28. NAKAYAMA, S. and BAUMGARTEN, R. 1964. Lokalisierung absteigender Atmungsbahnen im Rückenmark der Katze mittels antidromer Reizung. Pflügers Arch. 281: 231–244.
29. NELSON, J. R. 1959. Single unit activity in medullary respiratory centers of the cat. J. Neurophysiol. 22: 590–598.

30. OBERHOLZER, R. J. H. and TOFANI, W. O. 1960. The neural control of respiration. *In* J. Field (ed.), Handbook of physiology. Section I: Neurophysiology. Vol. II. Am. Physiol. Soc., Washington, p. 1111–1129.
31. SALMOIRAGHI, G. C. and BURNS, B. D. 1960. Localization and patterns of discharge of respiratory neurones in brain stem of cat. J. Neurophysiol. 23: 2–13.

Accepted 15 July 1976

Henryk GROMYSZ and Witold A. KARCZEWSKI, Laboratory of Neurophysiology, Medical Research Center, Dworkowa 3, 00-784 Warsaw, Poland.