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THE EFFECTS OF PONTINE OR BULBAR TRANSECTION ON THE RESPIRATORY PATTERN IN THE RABBIT

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Abstract. Rabbits were anesthetized with halothane, paralyzed with d-tubocurarine and mechanically ventilated at eucapnic level. The activity of both phrenic nerves was recorded before and after brainstem transection in animals with vagi intact and then cut. The effect of transection depended upon its level: midpontine transections elicited an apneustic pattern of firing in phrenic nerves prior to vagotomy and a considerable prolongation of expiratory time after vagotomy. Transections through the rostral part of the n. facialis in animals with vagi intact increased the duration of both inspiratory time and — even more — expiratory time. Vagotomy abolished the activities of phrenic nerves. Transection through the n. retrofacialis resulted in fast, irregular, low-amplitude volleys of phrenic nerves; vagotomy elicited inspiratory apneusis. Low-frequency electrical stimulation of the vagus nerve enhanced this tonic firing, whereas high-frequency stimulation interrupted it. The results indicate that medullary neurons are capable of generating the basic respiratory pattern, and that the vagal input is integrated at the bulbar level in the rabbit.

INTRODUCTION

Transection of the brainstem — a technique introduced by LeGalois (9) in 1812 — has become one of the basic approaches to the problem of localization and function of the respiratory generator. The results obtained by means of this technique did not, however, solve all controversies as to the precise localization of the respiratory centers.

Several authors (16, 17, 19, 33, 34) suggested that the basic respiratory generator is localized entirely in the medulla. However, the others (26, 27, 31, 35, 36) interpreted the phenomenon of "apneusis" as an indication that the inspiratory drive originates in an apneustic center periodically inhibited by impulses from the pneumotaxic center and vagal pulmonary stretch receptors. Among them, Pitts et al. (31) believed that the apneustic center is placed in the medulla, whereas Wang et al. (36) indicated a pontine localization.

More recently, many investigators combined the technique of transections with stimulation and recording (1–3, 5, 7, 18, 22, 30). There is now an almost general agreement that the pneumotaxic center, studied systematically by Cohen (5) and Bertrand and Hugelin (2), can be identified at the rostro-lateral pontine level in the region of nucleus parabrachialis and Kölliker–Fuse (see 6 and 29 for fuller discussion). According to this view a coagulation or separation of the rostral pons combined with vagotomy elicits apneustic breathing, i.e., a disturbance of the breathing pattern consisting in a considerable prolongation of inspiration with no, or very little, change in the duration of expiration. On the other hand, however, St. John et al. (21) reported that general anesthesia is a prerequisite for apneustic breathing, since vagotomized cats with pneumotaxic center lesions assumed normal pattern of breathing after recovery from anesthesia. Koepchen et al. (25) and Budzińska et al. (4) elicited apneustic breathing as a result of cooling or destruction of the dorsal respiratory "nucleus" of the medulla in the cat. In the rabbit, apneustic breathing can be elicited by a pharmacological blockade in the region of n. facialis (12) or a hemitransection of the brainstem at the same level (11).

Also the level of integration of the vagal input from the lungs has not been unequivocally established. Kahn and Wang (23) suggested that inspiratory neurons of the n. reticularis pontis caudalis and magnocellularis are responsible for integrating the Breuer–Hering inflation reflex, since gasping respiration with no effect of the vagal input is typical of the medullary animal.

Fadiga et al. (8) observed, however, powerful "excito-inspiratory responses" to lung inflation in medullary preparations and Hukuhara et al. (20) claim that medullary neurons are the main level of integration of the Breuer–Hering reflex.

Almost all of the above data have been obtained on cats. As shown earlier (13–15, 24), the functional organization of the rabbit's respiratory generator is different than in the cat. The present paper deals with the effects of transecting the neuraxis on the respiratory pattern and with the level of integration of the vagal input in the rabbit.

METHODS

The experiments were performed with 16 rabbits weighing from 2.5–3.5 kg, anesthetized with neuroleptanalgesia (i.v. Fentanyl and Droperidol, 0.01 and 0.5 mg/kg, respectively, Janssen) followed by halothane (0.7 vol. % in a 3 : 1 air-oxygen mixture, Halan — Germed). The animals were paralyzed with d-tubocurarine (Orion) 5 mg/kg and artificially ventilated at eupneic level ($PaCO_2$ 32–38 Torr). Arterial blood pressure (Statham P23db gauge) and end-tidal CO_2 % (Jaeger CO_2 — Test) were continuously monitored. Arterial blood samples were taken at frequent intervals for PO_2, PCO_2 and pH estimations (Radiometer BMS 1). The activity of both phrenic roots (C3) was amplified (Tektronix 3A9 differential amplifiers) and recorded as an "integrated" signal (time constant 100 ms) on a Honeywell 4408A Visicorder along with end-tidal CO_2 % and arterial blood pressure.

After recording all variables in control conditions, the brainstem was transected with a segment of a razor blade mounted in the holder of a micromanipulator. The cerebellum was left intact. The transections were made at levels from 9 mm to 3 mm rostral to obex. The animals in which brain oedema, excessive bleeding or a rapid fall in blood pressure developed after transection were rejected.

Physiological variables were recorded 5 and 10 min after each transection. Bilateral cervical vagotomy was then performed and 10 min later recording was repeated. One vagus nerve was then placed on electrodes and electrically stimulated (Cobrabid stimulator with isolation unit, frequency from 20 to 100 Hz, pulse duration 0.5 ms, threshold intensity for rhythm changes). Each experiment was completed by fixing the brain in a 10% formaldehyde solution. After three days serial frozen sections were made (50 μm) and examined under a microscope to check the localization and completeness of the transections. The microscopic pictures were confronted with Meessen and Olszewski's (28) and our own (H. Gromysz and K. Ruszczyk — unpubl.) stereotaxic atlases.

RESULTS

Figure 1 presents schematically the levels of transections and their effects on the phrenic pattern of activity.

Midpontine transections performed 7.5 to 9 mm rostral to obex (four experiments, one asterisk in Fig. 1) in animals with intact vagi elicited a considerable prolongation of inspiratory time (T_I) with little change in the expiratory time (T_E), i.e., a classical inspiratory apneusis (Fig. 2*B*). Vagotomy resulted in a large increase in T_E (Fig. 2*C*).

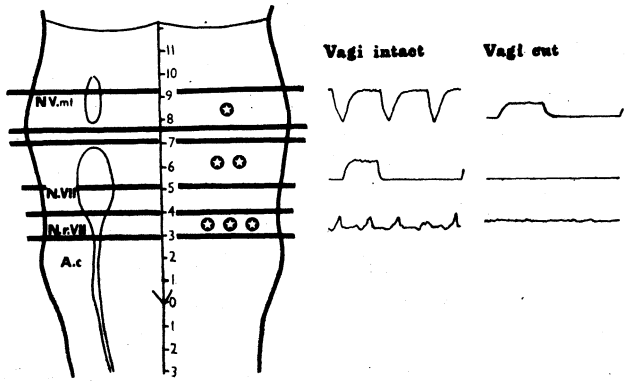

Fig. 1. Schematic representation of transverse sections of the brainstem and their effects on phrenic discharges. Left half of the diagram shows some structures of the brainstem: N.V. mt, nucleus motorius n. trigemini; N. VII, nucleus facialis; N.r. VII, nucleus retrofacialis; A.c., nucleus ambiguus (see 28 for terminology). Right half: one asterisk, four transection experiments; two asterisks, seven transection experiments; three asterisks, two transection experiments. Right-hand side of the diagram, schematic pattern of phrenic discharges after transection; vertical scale, mm rostral and caudal to obex (V).

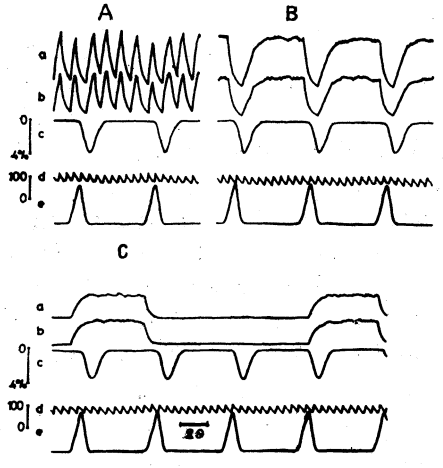

Fig. 2. The effect of midpontine transection on the activity of phrenic nerves. A, before transection; B, 10 min after transection; C, 20 min after transection and 10 min after vagotomy. Traces from top to bottom: a and b, "integrated" activity of the left and right phrenic nerves; c, end-tidal CO_2%; d, arterial blood pressure; e, stroke volume (arbitrary units).

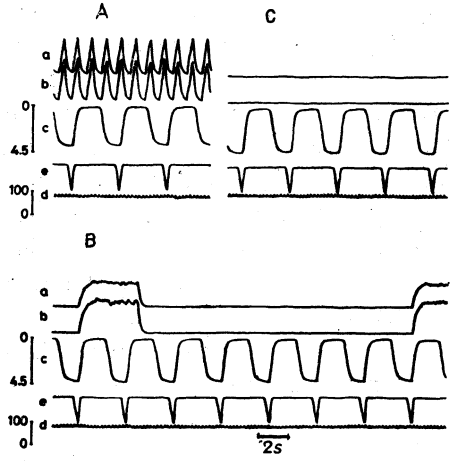

Fig. 3. The effect of transverse brainstem section at the level of the rostral part of n. facialis on the activity of phrenic nerves. A, before transection; B, 10 min after transection; C, 20 min after transection and 10 min after vagotomy. Traces as in Fig. 2.

Transections at the level of rostral part of nucleus facialis (5 to 7 mm rostral to obex, seven experiments — region marked with two asterisks in Fig. 1) reduced the central respiratory rhythm mainly via prolongation of T_E in animals with intact vagi (Fig. 3*B*). Phrenic activity was abolished by vagotomy (Fig. 3*C*). Afferent electrical low-frequency (20–30 Hz) stimulation of vagus nerve elecited apneusis or apneustic breathing, switching to higher frequencies (100 Hz) inhibited this activity.

Transections at the level nucleus retrofacialis (3 to 4 mm rostral to obex, two experiments — three asterisks in Fig. 1) elicited fast, low-amplitude, irregular discharges is the phrenic nerves before vagotomy. Temporary apnoea (respirator turned off) elicited instantly apneustic firing (Fig. 4*C*). The same effect was produced by vagotomy.

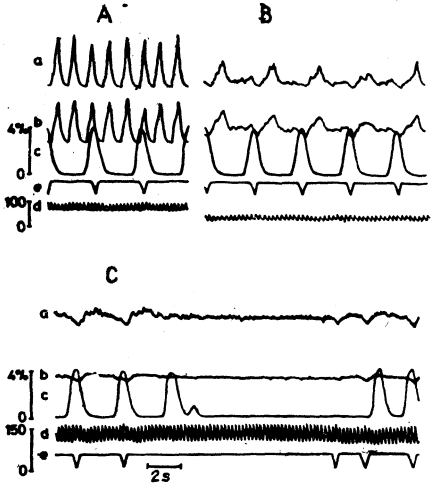

Fig. 4. The effect of transverse brainstem section at the level of n. retrofacialis on the activity of phrenic nerves. A, before transection; B, 10 min after transection; C, respiratory pump switched off. Traces as in Fig. 2.

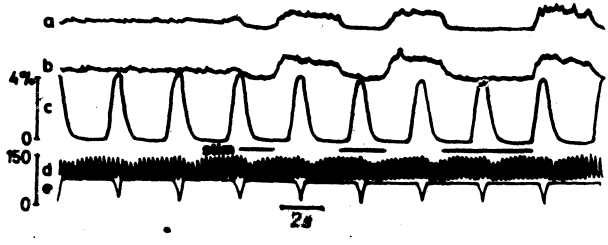

Fig. 5. The effect of vagal stimulation (100 imp/s) on the tonic activity of phrenic nerves in an animal after the same section as in Fig. 4 and after vagotomy. Note inhibition of activity during stimulation. Traces as in Fig. 2.

Tonic or phasic electrical low-frequency stimulation of vagus nerve enhanced the tonic firing. Phasic high-frequency stimulation interrupted the tonic inspiratory firing (Fig. 5).

DISCUSSION

Comparing the present results with those described in the literature, we have to take into account species differences, experimental conditions and various interpretations of similar findings. The existence in the lower pons of an apneustic center (36) periodically inhibited by a pneumotaxic center (2, 5) and vagal input has not been confirmed by microelectrode recordings. Large clusters of inspiratory activities can be found both in the cat (6, 29) and in the rabbit (12) in the medulla oblongata. This rather confirms the view of Pitts et al. (31) that the apneustic "center" is localized in the medulla and not pons. Our results support this view. The medullary vagotomized rabbit generates tonic inspiratory activity. On the other hand, intact vagi are a pre-requisite of apneustic breathing after a midpontine transection.

Transections through the rostral part of nucleus facialis elicit an expiratory "bias". This may be due to expiratory neurons in the caudal part of this nucleus; it has been shown (12) that a pharmacological blockade of this region "disinhibits" inspiration. A transection made caudal to this structure results in a low-amplitude, fast rhythmic firing in the phrenic nerves.

It has been shown that also in the cat (10, 26) and man (32) apneustic breathing can develop with vagi intact when the pneumotaxic "center" is destroyed. The results obtained in cats are, however, extremely discrepant. Kahn and Wang (23) on one hand and Fadiga et al. (8) on the other present not only different effects of trasection on phrenic nerve activity, but also different responses to vagal input. Kahn and Wang (23) obtained "gasping" in a medullary preparation and were not able to demonstrate any vagal effects. Fadiga et al. (8) also claimed that a medullary cat generates "gasping" (their Fig. 2 does not substantiate this claim) but demonstrated an inspiratory-excitatory effect of the vagal input during inflation. These discrepancies may be due to different experimental conditions — Kahn and Wang (23) were using a special respirator adjusted to keep arterial CO_2 between 45–80 mmHg.

Our results with normocapnic rabbits are similar to those obtained by Fadiga et al. (8) in cats, as far as the effects of the transections and the vagal input are concerned. Low-frequency vagal stimulation enhanced the tonic inspiratory activity, which, in turn, was inhibited by high frequency stimulation. We were therefore dealing with responses similar to the "frequency effect" described by Wyss (37).

Discussing the apneustic pattern of breathing, one has to mention the results of St. John et al. (21) who assumed that apneustic breathing consists, first of all, in an increase of inspiratory duration above 10 s (without taking into account the T_I–T_E ratio). An analysis of their results (Tables I and III in ref. 21) shows that they were dealing with a considerable decrease in respiratory frequency, but not with a typical apneustic breathing.

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