RESPIRATORY ACTIVITY OF THE PONS AND ITS INFLUENCE ON BREATHING IN THE GUINEA PIG

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Abstract. The localization of respiratory activity in the pons of the anaesthetized guinea pigs has been studied. The respiration related units were localized in/or close to the following anatomical structures: nucleus parabrachialis, nucleus trigemini motorius and nucleus reticularis pontis caudalis. The influence of the pontine respiratory activity on the pattern of breathing was studied by the use of a reversible pharmacological block (local anaesthetic) of these structures. The application of lignocaine to all investigated sites of the pons caused the prolongation of both respiratory phases, then an effect comparable to vagotomy. In the vagotomized animal the focal block of nucleus parabrachialis has never produced apneusis (pronounced prolongation of inspiration and very short lasting expiration). Since the removal of some pontine structures involved in the breathing control caused the depression of respiration, we concluded that the respiratory activity of the pons in the guinea pigs exerts an excitatory influence on the rhythm generating mechanism in this animal.

INTRODUCTION

For a long time the existence and localization of respiratory activity in the pons has been controversial. Salmoiraghi and Burns (24) did not find any respiratory activity cranially to striae acusticae in the cat. Woldring and Drinken (31), Drinken and Woldring (9) studying the region

from corpora quadrigemia to the first spinal root in the rabbit, have recorded respiratory activity up to 3 mm rostral to the obex. That means, that they have found respiratory activity only in the medulla. However, Kahn and Wang (20), Cohen and Wang (7), Bertrand and Hugelin (2), Hukuhara (18) and Gromysz and Karczewski (17) have described both inspiratory and expiratory activities in the pons of the rabbit and cat. Hukuhara et al. (19) claimed that barbiturate anaesthesia inhibits activity in the pontine reticular formation. Caille et al. (4) have found depressive effect of pentobarbitone restricted only to reticular formation surrounding bulbar respiratory nuclei, while the respiratory activity within NPBM and in WRG was not depressed. Bertrand and Hugelin (1) suggested the application of averaging technics for the identification of pontine respiratory units, since in their study pontine units were often of low amplitude (in comparison e.g. with medullary ones) and tonic discharges of other neurones might have masked rhythmicity of respiratory units. So far, the existence of the respiratory activity within the pons in the cat and rabbit has not been questioned. This paper describes the study of the respiratory activity in the pons of the guinea pig. Special attention has been focused on the evaluation of the respiratory function of the pneumotaxic centre (Nucleus parabrachialis—NPB and Kolliker Fuse — KF complex). The importance of this nucleus in the neural control of breathing has been emphasised in many papers (1, 5, 6, 11, 12, 20 - 22, 29). It is strongly suggested that NPB is involved in the "switch off" mechanism and thereby is responsible for the termination of inspiration in bivagotomized animals (5, 11, 12, 26, 28). The lesion of NPB induces apneusis in the anaesthetized and vagotomized cat (8, 21, 28). The dependence of apneusis on the level of Sodium Pentobarbital has been shown by Webber and Peiss even in otherwise intact cat (30). Recently St. Johns et al. (27) tried to prove that NPB is able to generate respiratory rhythm without any influence from the medulla. Most of the above mentioned works have been performed with the cats. There are papers available (8, 22) concerning this problem in the guinea pig. However the method applied in that study has been considerably criticized (14, 32). For this reason we have paid more attention to NPB in our studies. Preliminary reports have been already published (15).

METHODS

The experiments were performed with 19 guinea pigs of either sex, weighing 400 - 730 g, intraperitoneally anaesthetized with urethane in the dose of 1 g/kg. Tracheostomy and cannulation of the jugular vein

were routinely performed. Both vagus nerves were dissected and encircled with loose ligatures. The right phrenic nerve was cut and prepared for the recording of its activity with a bipolar electrode. A classical equipment (A. C. preamplifier and oscilloscope Tektronix type 565) has been used for the recording of end-tidal CO_2, pontine RRUs and phrenic nerve activity. In the majority of experiments the phrenic nerve activity was "integrated" with a diode pump smoothing circuit (time constant about 100 ms.). End-tidal CO_2 was monitored with the use of CO_2 Test (Jaeger). A further preparation was performed on the animal fixed in a stereotaxic apparatus. An occipital craniotomy was made and the cerebellum and medulla were exposed. The surface of the cerebellum was covered with semi-liquid agar. At this stage of the experiment the animal was paralysed with Tricuran (100 mg in total dose i. v.) and artificially ventilated. The extracellular respiratory activity found in the pons was recorded with a glass microelectrode filled with 3M KCL solution. The tip of the electrode was 1 μm in diameter and had resistance within the range of 5 - 10 MΩ. In the majority of experiments the vagal nerves were intact, whereas in others both vagi were cut before or after inducement of lignocaine block. In some experiments with intact vagi the influence of phasic vagal input was tested by swithing off the respiratory pump. A local anaesthetic was injected to each place from which the respiratory activity was recorded. For this purpose the recording electrode was replaced by a micropippette filled with 2⁰/o lignocaine solution coloured by Alcian blue (Loba Chimie). About 2 - 5 μl of Lignocaine have been slowly injected (further details of method see — 16). At the end of the experiment the brain was perfused with 10⁰/o formaldehyde solution. Then the brain-stem was removed and the pons was kept overnight in the same fixative solution. Next the pons was cut on the freezing microtome into 50 μm slices. These slices were immersed in glicerin — water solution in 2 : 1 proportion and photographed in 10 × magnification (see Fig. 4). Anatomical localization of the lignocaine injections was done on the base of a stereotaxic atlas of the guinea pig brain-stem (Gromysz et al. unpublished) and with the help of an atlas of rabbits brain-stem (23).

RESULTS

The distribution and characteristic of respiratory activities. Respiration related units were found within the area expanded between 1 - 3 mm laterally from the midline and 3.5 mm rostrally from the stereotaxic zero. Post experimental localization of the respiratory active sites is shown in Fig. 1. Most often the respiratory units were found within

nucleus parabrachialis (NPb filled circles), subsequently in nucleus trigemini motorius (NVmot — squares) and finally in nucleus reticularis pontis caudalis (R.Pc — triangles). Altogether twenty four single and multiunit activities have been recorded and analysed. The classification of the RRU-s in relation to the phase of respiratory cycle was based
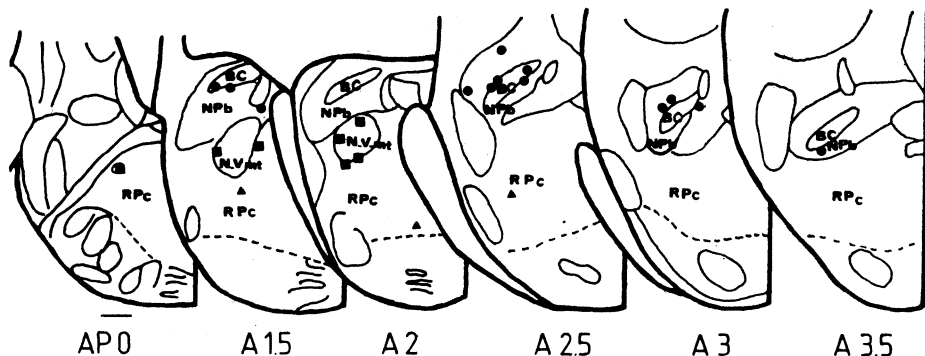

Fig. 1. Schematic representation of the pontine structures explored in the present study showing the localization of the lignocaine blockades. AP 0 means the stereotaxic zero, and A 3.5 mm is most rostral from this level. In all cross-sections, dots represent region of NPb, squares represent NV mot, triangles represent RPc.
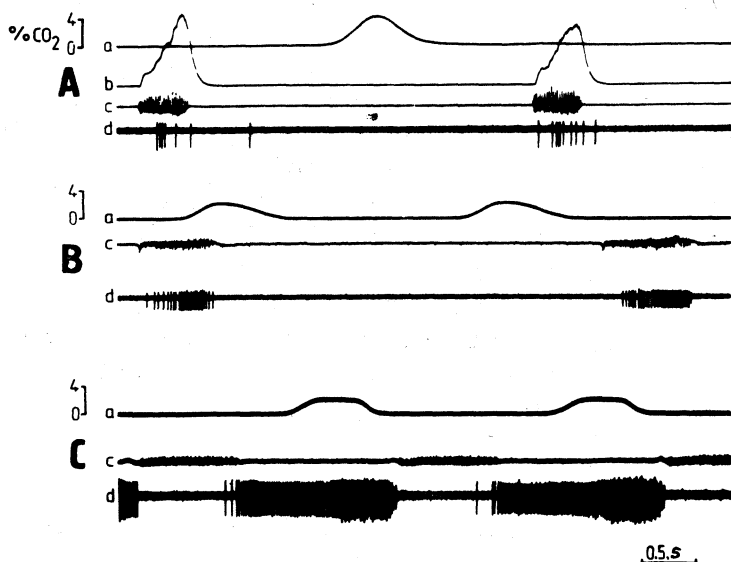

Fig. 2. Pontine respiratory related units; A and B inspiratory and C — expiratory activities. Traces are: a, end-tidal CO_2; b, integrated phrenic nerve activity, c, mass phrenic nerve activity and d, pontine respiratory units.
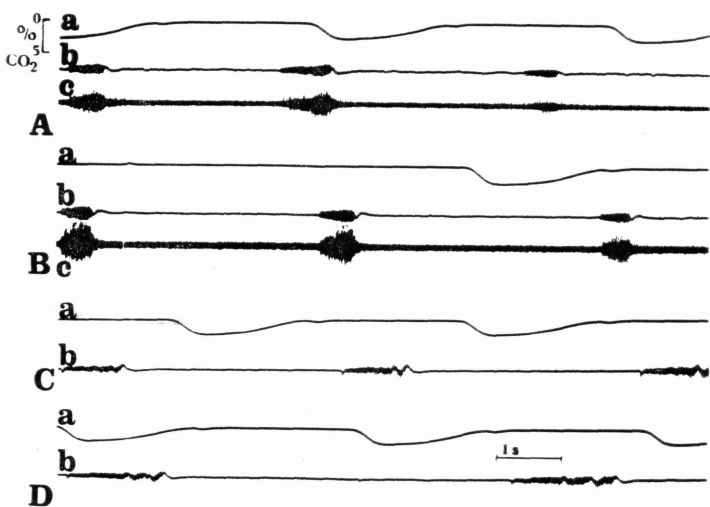
Fig. 3. Multifiber activities recorded within the nucleus parabrachialis. *A*, control; *B*, switching of the respiratory pump (note the prolongation of expiratory phase and recruitment of inspiratory units): *C*, bilateral vagotomy: *D*, blockade of NVmot (note on C and D prolongation of both respiratory phases). *a*, end-tidal CO_2, *b*, — mass phrenic nerve activity and c-RU-s.
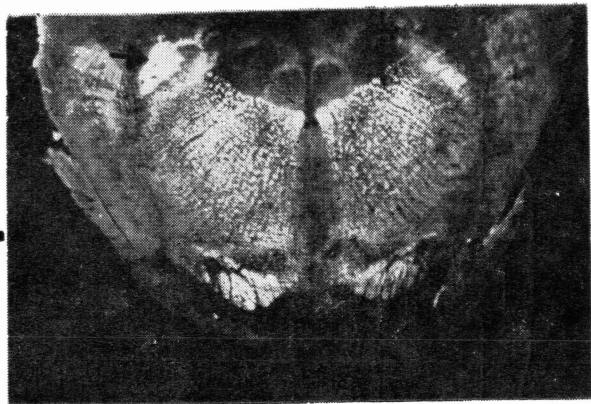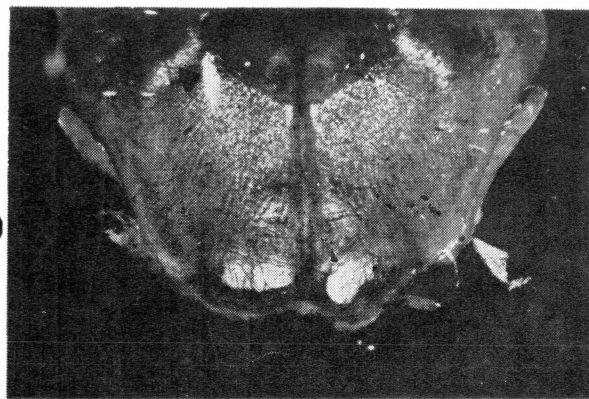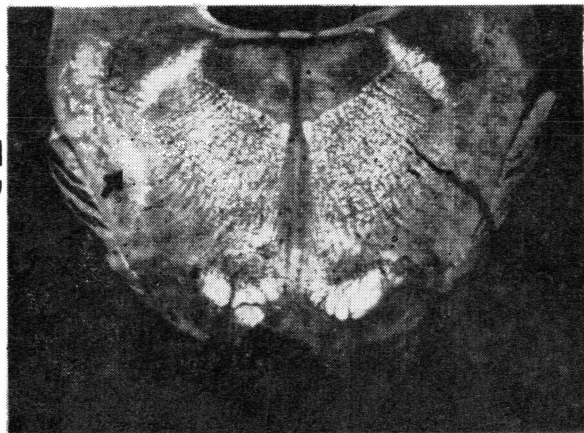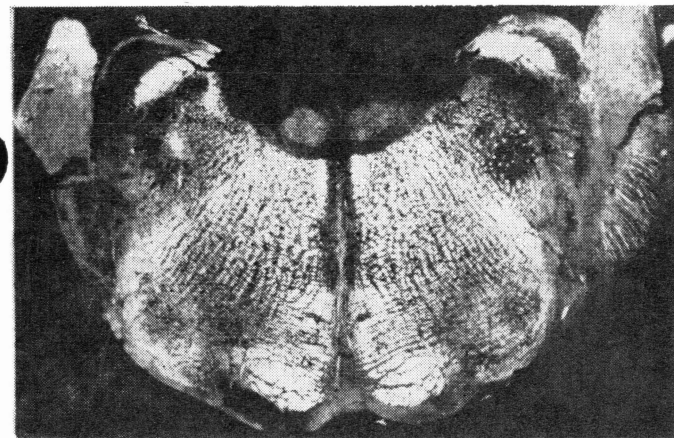
Fig. 4. Sites of lignocaine blockades indicated by arrows. *A* and *B*, — NPb regions; *C*, R.Pc and *D*, — NVmont.

on the phrenicogram. Inspiratory, expiratory and phase-spanning activities could be found in all searched places. Examples of two types of inspiratory and one of expiratory units recorded from NPb are shown in Fig. 2. Inspiratory multiunit activity taken from NVmot is presented in Fig. 3. Out of twenty microelectrode passages through NPb (histologically confirmed), in eight cases an inspiratory activity and in four an expiratory unit were found; in the remaining eight tracks there was "respiratory silence". From NVmot we have recorded four inspiratory, three expiratory and two phase-spanning neurones. Only three expiratory units were recorded from R.Pc. In accordance with the time of the onset of inspiration, "early" and "late" units could be distinguished in inspiratory neurones. As far as the number of spikes in inspiratory volleys and the frequency of discharge are concerned, a variability in both parameters was observed. On the other hand the expiratory units started to fire with the decrement of the inspiratory phase and discharged throughout the expiratory pause.
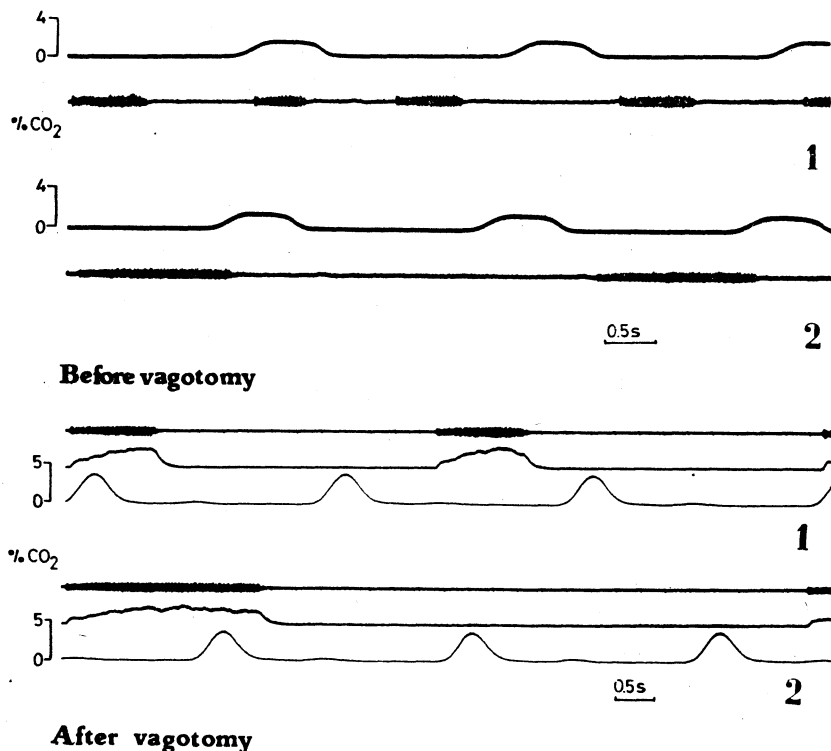
Fig. 5. Effect of focal lignocaine blockade of NPb on the duration of respiratory phases before and after bilateral vagotomy. 1, control; 2, blockade. Traces are: end-tidal CO_2, mass phrenic nerve activity before vagotomy and additionally integrated phrenic nerve activity after vagotomy.

Effect of lignocaine blockades. The scheme of localization of the focal microinjection of lignocaine is shown in Fig. 1 while Fig. 4 presents examples of photograms of brain-stem slices with the sites of injections (see arrows). Twelve out of twenty four blockades have been placed to/or near NPb. In eight instances lignocaine was injected to the intact animals and in four cases following biv̇agotomy. The effect of NPb blockades on the pattern of breathing is shown in Fig. 5 and Table I. In intact guinea pigs, as well as in vagotomized ones, both respiratory phases were prolonged and the frequency of breathing was diminished

TABLE I

Influence of lignocaine on T_I, T_E and breathing frequency. *c*, control value; *bl*, after lignocaine blockade

No.	T_I(s) c.	T_I(s) bl.	ΔT	T_E(s) c.	T_E(s) bl.	ΔE	f(min^{-1}) c.	f(min^{-1}) bl.	Δf
A before vagotomy									
1	0.90	1.50	0.60	4.25	5.40	1.15	12	7	−5
2	0.45	1.16	0.71	2.30	3.71	1.41	21	12	−9
3	1.11	1.81	0.70	2.16	4.83	1.67	18	9	−9
4	0.87	0.96	0.09	4.83	5.72	0.69	11	9	−2
5	0.80	1.20	0.40	2.47	3.80	1.33	18	12	−6
6	1.33	1.40	0.07	9.00	22.60	13.60	6	3	−3
7	0.60	1.30	0.70	2.80	4.20	1.40	18	11	−7
8	0.75	1.10	0.35	2.70	3.00	0.30	17	15	−2
Means ± SD	0.45±0.27			2.69±4.43			−5.67±2.82		
B after vagotomy									
1	0.68	1.00	0.32	5.50	6.60	1.10	10	8	−2
2	0.60	0.80	0.20	3.80	4.00	0.20	14	12	−2
3	1.70	2.50	0.80	6.20	13.00	6.80	8	4	−4
4	1.10	2.70	1.60	3.70	11.60	7.90	12	4	−8
Means ± SD	0.73±0.64			4.0±3.90			−4±2.80		

after the blockade. This type of response was received from all blocked sites. The effect of lignocaine injection to NVmot is shown in Fig. 3, record D. In some instances of the blockade of NPB in vagotomized guinea pigs an additional effect on inspiratory phase was observ̇ed. As it can be seen in Fig. 6, a pharmacological block provoked a characteristic "biphasic inspirations". At the crest of the first part of almost normal inspiration (only amplitude was smaller in comparison to control breaths), a transitory, but not complete inhibition of inspiration took place. The second part of inspiration was characterized by a gradual
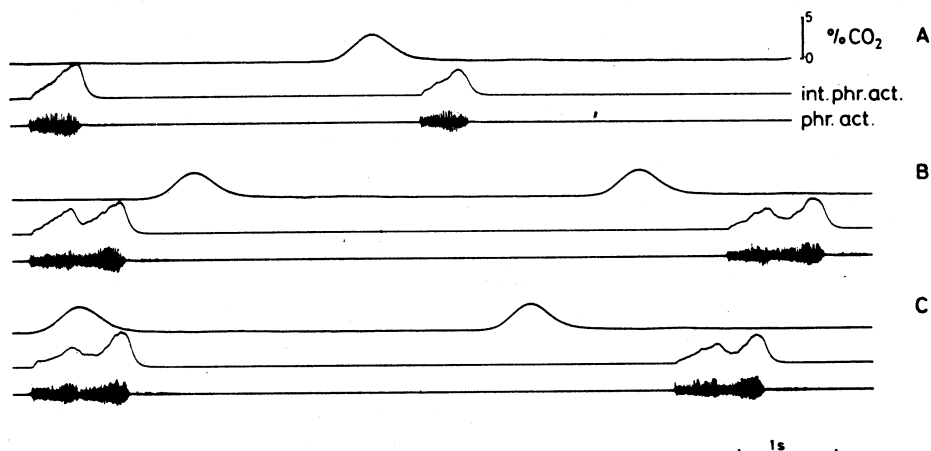
Fig. 6. Effect of lignocaine of NPb after vagotomy on the shape of phrenic nerve discharges. *A*, control; *B* and *C*, after blockade.

reinforcement of respiratory activity with increased rate of rise (see Fig. 6). At the top of this additional inspiratory excitation (now the amplitude has almost reached the control level) the inspiration was cut off. This type of response was not seen after lignocaine injection to places other than NPb. It should be added that the injection of saline never had an effect on breathing also had no effect of lignocaine injection to non respiratory sites.

DISCUSSION

Our results are in line with these works in which the respiratory activities in the pons have been described (1, 2, 4, 17, 18) and are at variance with those in which such activities have not been found (9, 24, 31). We recorded spontaneous inspiratory, expiratory and phase-spanning activities from NPb, NVmot and R.Pc. It means that there is no structure representing only one type of activity. Also the previously suggested laminar organization of the respiratory neurones (30) is not supported since the respiratory activities could be found at different depths from the surface of the brain-stem. Our results show rather that the respiratory activity is localized in certain anatomical structures of the pons. At variance with Cohen (6) we found in the pons predominantly phasic respiratory activity, while he described mainly tonic or phase spanning unit. This discrepancy may result from species differences or experimental conditions, including the depth of ana-

esthesia. Considering the pattern of discharges we have recorded various types of inspiratory neurones. As it was evidenced "early" and "late" units could be distinguished with reference to the start of the phrenic nerve discharge. Similarly, the number of spikes and intervals between the spikes varied from case to case. A more regular pattern of discharges was exhibited by the expiratory neurones. They started to fire with the end of inspiratory phase and ceased just at the beginning of the next inspiration (i.e they were active throughout the expiratory pause see Fig. 2C). The presence of RRU-s in NPb was not unexpected. However, the existence of respiratory units in other anatomical structures in the pons was not obvious. In the light of the recent works (25, 27) the finding of respiratory representation in the NVmot seems to be interesting. St. John and Bledsoe (29) have recorded the respiratory activity from the mylohyoid nerve (the branch of trigeminal nerve) which persisted after a transection of the brain-stem at the pontomedullary junction. Basing on this result, they concluded that respiratory rhythm may be generated in the pons independently from the medulla. They suggested NPB as the place for neurogenesis of eupnoe and the mylohyoid nerve as the pathway for the transmission of respiratory activity. However, their results are not in line with the earlier data of Hukuhara (19), who also recorded the respiratory activity from the trigeminal nerve as well as from other cranial nerves (facial, hypoglossal, glossopharyngeal and vagal). He found that the trigeminal and facial nuclei are very sensitive to even small doses of pentobarbital. With 2 mg of pentobarbital the activity vanished for about 60 min, while the transection of the brainstem at the pontobulbar junction irreversibly abolished activity in these nerves. The activity in the phrenic and in the hypoglossal nerves was unchanged as long as pO_2 was not below 60 mmHg. In hypoxic conditions the appearance of gasps was followed by the cessation of breathing. Hukuhara (19) has argued against the classical view that anatomical structures are responsible for the producing of apneustic or gasping pattern of breathing. He suggested that these structures could influence the neuronal network of the central respiratory mechanisms. Our results with pharmacological blockades of pontine respiratory areas would fit with the latter suggestion. The prolongation of both respiratory phases due to elimination of some pontine respiratory neurones was the most consistent effect, and was independent either from the site of the blockade (including NPb) or from vagal influence. It means that we cannot support the classical view that NPb is mainly responsible for the termination of inspiration and development of apneusis after vagotomy. Biphasic inspiration with reexcitation of the second part of inspiration described in the present work would mean that the inspiratory off-

switching mechanism, wherever it is located, must be preceded by a certain level of inspiratory excitation (see Fig. 6). One of the possible excitatory sources may be located in the NPb. This interpretation is in agreement with an earlier suggestion of Euler et al. (11) and with the model of Bertrand (3) and Feldman (13) proposed for the explanation of physiological function of the pneumotaxic centre. In their pneumotaxic oscillator model, a network of self-reexcitation between pontine inspiratory and expiratory neurones on one hand, and between medullary and pontine subset of neurones on the other hand was postulated. A further study of interaction between respiratory neurones of the rostral pons and ventral respiratory group (VRG) was performed by Segers at al. (25). However, they found only one monosynaptic connection out of 67 tested pairs of inspiratory cells and none for the expiratory cells between pons and VRG. According to these results they concluded that the influence of NPb/KF complex on the respiratory cycle may be exerted via polysynaptic pathways.

The results of our work gave evidence that the respiratory active areas of the pons have a modulatory influence on the pattern of breathing and we can postulate that pontine structures in the guinea pig involved in breathing exert an excitatory effect on rhytmogenesis.

ABBREVIATIONS

RRU-s	Respiration related units
NPb	Nucleus parabrachialis
NPbM	Nucleus parabrachialis medialis
NVmot.	Nucleus trigemini motorius
RPc	Nucleus reticularis pontis caudalis
WRG	Ventral respiratory group
KF	Kölliker Fuse

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