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INVESTIGATIONS ON THE NEURAL CONTROL OF RESPIRATION IN EXPERIMENTAL CONSTRICTION OF THE UPPER AIRWAYS

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Abstract. The adaptation of the work of the respiratory centers to suddenly arising respiratory resistance has been the object of interest of numerous investigators. Some of them believed that in this condition an essential role is played by the vagus nerves. Others considered that neurocontrol is mainly dependent on the respiratory muscles. The present study was undertaken to establish which of these two systems is the preponderantly responsible one. Experiments were made with anesthetized intact and vagotomized rabbits. Constriction of the airways was achieved by connecting narrowed glass tube to the trachea. The activity of the respiratory centers was evaluated on the basis of their main two "outputs", the activity of phrenic neurons and of the respiratory motoneurons of the vagus nerve. In intact animals as a response to constriction a decrease of the respiratory frequency and an immediate increase of neural activity were observed. An increased activity of motoneurons was also noted in vagotomized animals, however, after a latency period lasting about 15 sec. The respiratory frequency in these animals changed in the opposite direction becoming higher after 15 sec. Results indicate that the vagus nerves play an essential role in the adaptation of the respiratory centers to constriction. All other sources and ways of information may compensate respiratory resistance by intensifying the work of the respiratory muscles only after the lapse of a certain time, moreover, they are incapable of adjusting an optimal rhythm. The possible sources of information in these conditions are discussed.

INTRODUCTION

Pathological conditions in man associated with difficulty in breathing have long since been an object of interest to investigators. Davies et al. (18) described the decrease of frequency of breathing with simultaneous increase of tidal volume occurring as a consequence of constriction of the upper airways. Simonelli (67) and Killick (41) applied artificial air flow resistance in the airways and confirmed these observations, stressing, however, that tidal volume changes are much less than the frequency of breathing. They also described a decrease in minute ventilation, and the occurrence of symptoms of hypoxia and a raised carbon diaxide level in blood with prolonged constriction.

With the progress in understanding the mechanics of respiration, further classification of respiratory resistances was established. In the first place the difference between elastic and nonelastic resistance was defined.

This resistance may also be distinguished as extra- and intrapulmonary (40, 47). From the point of view of changes in the respiratory mechanics a number of humans and animals subjected to additional air flow resistance in the upper airways were investigated. The results obtained in man showed discrepancies, but the respiratory reaction had the same direction. Mostly the previoulsy described slowing down and deepening of breathing was confirmed. Most investigators maintained the constriction for several minutes and frequently they noted hypoxia and hypercapnia.

Graff et al. (36) stressed, however, that the changes in blood chemistry are not an early symptom, but occur later as the result of exhaustion of the compensating mechanisms. Beside observations on man, a number of experiments were made with animals. This made possible a more precise understanding of the nature of these phenomena and gave wider experimental opportunities. The reactions of dogs (73), cats (26) and rabbits (17) were similar. All the animals whether anesthetized or not, responded to a rapid narrowing of the upper airways as follows: the tidal volume and minute volume diminished, the rate decreased, the phase of the respiratory cycle during which resistance was increased was prolonged, pleural pressure rose considerably (in negative values), the work of breathing and bioelectric activity of respiratory muscles increased, intratracheal pressure varied widely. All these changes increased in intensity in proportion to the extent of constriction. Thus, animals reacted in the same way as anesthetized humans. The only difference as compared with non-anesthetized humans was the consistent decrease of tidal volume. All these observations led the investigators to take an interest in two main problems:

1. The factors responsible for adaptation of the organism to new conditions. This adaptation is independent of consciousness and known as "objective reaction" (12).

2. The pathways by which information concerning the occurring obstacles reaches consciousness, that is "subjective reaction" (12).

The sources and pathways of information transmitting to the centers have so far not been exactly determined.

On the basis of up-to-date knowledge two basic groups of opinions can be distinguished. The first refers to the reaction to respiratory resistance to information starting in the pulmonary receptors and conducted by the vagi nerves (e.g., see 19, 45, 46, 48, 75). In view of the fundamental importance of the vagus nerve in respiratory control, they suggested that this nerve is responsible for both the subjective and the objective reaction to the introduced resistance. The second group of investigators considers a fundamental role in respiratory muscles and some undefined receptors of the chest walls. This view was developed mainly by a Campbell group (7, 8, 10, 12) and by Bland et al. (6). These investigators suggested, moreover, that the same mechanism is responsible for the subjective reaction (12).

While the investigators of the first group based their views on the studies of Adrian (1), the second group restricting to minimum the role of the vagus nerve on those of Fleisch (27). The latter demonstrated that the introduction of inspiratory resistance increases the work of the inspiratory muscles, and expiratory resistances increased the work of the expiratory muscles. Both these responses occurred independently to the continuity or not of the vagus nerves. Further studies (6-9, 12) extended to the changes in respiratory mechanics, confirmed the Fleisch's observations. All the above-mentioned investigations, however, dealt with elastic resistance, and it can make a difference (21). For neural control of respiration under increased nonelastic resistance the school of Campbell (4, 53, 56) suggests the same principles. Their observations were, however, made exclusively on humans, and this made final evaluation difficult. In order to obtain a full picture of the problem it was necessary to perform experiments with animals, since only then observations on the effect of vagotomy could be made, and only this method could confirm or exclude the contribution of the vagus nerves.

Under the conditions of present knowledge it cannot be decided which view is correct, thus, which information pathway is of fundamental importance. The subjective answer, that is the perception of the resistance has not been unequivocally elaborated. On the one hand, patients with immobilized chest after spinalectomy at the Th-1 level (74) and with pathology of the cerebellum (51) sense additional resistance as healthy individuals. This would contradict the essential role of the respiratory muscle receptors. On the other hand, Guz et al. (39) demonstrated that blocking of both vagus nerves in humans also does not disturb perception of the additional respiratory resistances. As a model of "objective reaction" of the organism to resistance may serve the sensation associated with breath holding for any chosen length of time. After exclusion of the influence of changes in blood gas content (28), the factor forcing the individual to draw in breath may be analogous to those conditioning the perception of respiratory resistance (12). This experimental model, however, does not supply unequivocal results. The experiments of Campbell et al. (11) seem to indicate a muscular information pathway, since muscle paralysis abolishes the sensation of "breathlessness". On the other hand, an identical situation has been described by Guz et al. (39), but as a consequence of interruption of conductance in the vagus and glossopharyngeal nerves.

Thus, the sources and pathways of information reaching both respiratory centers and consciousness have not been elucidated definitively (42). Trials of establishing which of the above discussed two systems is essential for the adaptation of the respiratory centers to violently increased air flow resistance in the trachea are the subject of the present work.

The activity level of the respiratory centers was determined on the basis of discharges from two basic "outputs" from these centers, that is respiratory motoneurons of the vagus and phrenic nerves (14, 25, 38).

METHODS

The experiments were made with 70 rabbits of both sexes weighing 2-3 kg breathing spontaneously, immobilized on a warmed operation table. As anesthetics urethane-chlorasole 40% solution (1.2 g/kg + 50 mg/kg) was applied (one half of the doses into the marginal auricular vein and the other part intramusculary). The anesthetics were chosen according to literature data from which results that chloralose in a 500 mg/kg body weight dose neither enhances nor reduces the activity recorded from the entire brain stem (61). Neither does urethane depress noticeably the excitability of nervous reflexes, and the activity of the central nervous system under urethane anesthesia is higher as compared with that after barbiturate application (54). Urethane-chloralose anesthesia causes within 1 hr after administration a slight decrease in tidal volume, an increase of the frequency of breathing, an increase of the cardiac minute output and a fall of blood pressure to 90% of the control value. During anesthesia these changes become stabilized. Oxygen and carbon dioxide pressure in the blood and pH remain at a normal level with slight deviations (54). The experiments were started 1-1.5 hr after application of anesthetics. Preparation of the single nerve fibers and bioelectric potential recordings have been described in a previous paper (22).

Experimental constriction. Experimental constriction of the trachea was achieved by connecting to the tracheotomy tube (equal to the average trachea diameter of the rabbit = 4 mm) additional glass tube, called "experimental stricture". It was 10 mm in length and inside diameter was tapped to 1 mm half way down its length. It constricted upper airways in this place to one quarter. It is commonly used model of experimental resistances in the upper airways (4, 56, 73).

Air flow in a thus narrowed duct is mainly turbulent, that is dependent on the square of flow velocity. The pressure necessary for forcing through a definite amount of air in a definite time is expressed by the formula

$$p = K_2 \cdot \dot{V}^{o2}$$

where K_2, constant dependent on gas density, $\dot{V}^{o2}$, flow velocity (15).

The value p thus characterizes the degree of constriction. In constriction of the airways the value p is the difference in pressure arising in the trachea below the constriction during the respiratory cycle as compared with barely several-millimeter differences found in the absence of resistance. p determined for the constriction during inspiration is a negative, and in expiration a positive value (investigations performed with a water manometer). Therefore average p for experimental tube was ± 8.7 cmH_2O.

The constrictions were of a shape causing mainly turbulent flow, since in the upper airways in human pathology they are of such a character (15).

Gasometric measurements. In part of the experiments a glass cannula was inserted into the carotid artery and simultaneously heparin was administered intravenously. Blood samples were taken directly into test tubes under a paraffin layer. Saturation of arterial blood with oxygen was calculated by the oxymetric method (Oxymetr 0.57, USSR), and the carbon dioxide content in van Slyke's apparatus.

Experimental procedure. 1. Bioelectric activity of the vagus and phrenic motoneurons were recorded as follows:

a) before connecting of the experimental tube — as normal state reading;

b) at the moment of connecting experimental tube and subsequently in 15, 30, 60, 90, 120 sec;

c) continuously since the moment of disconnection up to 15 sec and subsequently 30, 60, 120 sec.

2. Analogous recordings were performed after bilateral vagotomy.

3. Since after 2 min of constriction hypercapnia was not noted, only slight hypoxia in arterial blood, as described previously (22), this group was used for demonstrating the influence of blood hypoxia due to constriction. The experiments were made with vagotomized animals as follows:

a) at the first stage the constriction was maintained for 2 min, and together with each recording the degree of arterial blood saturation with oxygen was determined;

b) at the second stage the animals were paralysed with Flaxedil, and nitrogen-air (1 : 3) mixture was applied for inhalation. Respiration of this mixture caused a fall of oxygen saturation in arterial blood to the values noted after constriction. It was called a pure hypoxia. The changes in activity were recorded at 15 sec intervals for 2-3 min and blood samples were taken in this time.

Elaboration of results. The results were elaborated by the method of Gill (32). According to this author, the following notations were used for the particular activity parameters:

τ, respiratory frequency per minute;

T, duration of discharge during one respiratory cycle or duration of active phase, expressed in seconds;

N, number of action potentials in the entire respiratory cycle (equal to number of impulses during a single discharge);

f_{ins}, maximal discharge frequency during inspiration, e.g., greatest number of impulses in 1 sec;

f_{ex}, maximal discharge frequency during expiration. This is an additional parameter not recorded in Gill's paper (32), which only deals with the activity appearing during inspiration;

SP, phase of neuron inactivity (silence period). It is as well the time between two successive bursts of discharges and is expressed in seconds.

Control measurements of the particular parameters showed certain numerical differences. This is understandable if we consider that the investigated nerve fibers run to various segments of the airways.

The phrenic neurons may also — through the particular fibers of the phrenic nerve — control various parts of the diaphragm. In view of the nonuniform initial level, it was difficult to compare the results of the particular experiments. The only way of standardization of the results was their conversion to relative values. These were obtained by dividing every parameter by the corresponding control value. Thus the control was assumed as 1, and all results below or above unity gave an easily comparable picture of the increase or decrease of the given parameter. The particular results calculated in this way allowed a global evaluation based on the mean values of the changes. Detailed statistical analysis was very difficult. As described above, there were certain discrepancies in the control results, and the numerical data after application of the stimulus exhibited a wide dispersion. The causes of this may be numerous. Of paramount importance would seem here as in control investigations — the difference in the situation of the site to which the recorded discharge bursts tend. It could be expected that, particularly in pathological conditions, various segments of the airway or parts of the diaphragm may be differently quantitatively and even qualitatively involved in the control processes.

There are, moreover, many other factors difficult to rule out, for instance, individual adaptive abilities of the particular animals consisting among other things in differences in the force of respiratory muscles and the value of the tidal volume. There may have also been slight differences in the depth of anesthesia, for it was not always possible to perform the measurements at an identical time after administration of the anesthetic. Therefore the evaluation of the results, with the use of the standard deviation for instance, might show in a part of the experiments that these results are not significant owing to the wide dispersion, whereas all the animals reacted invariably in the same direction, only with a different intensity. Therefore, in the Table listing the mean values the ranges of deviations are given.

RESULTS

Reaction of vagal respiratory motoneurons to constriction before vagotomy

The results in this experimental group are graphically presented in Fig. 1, numerical data and the range of deviations in Table I, and an exemplary experiment in Fig. 2.

The reaction of respiratory neurons of the vagus nerve to the constriction was qualitatively uniform: the duration of discharge, number of impulses in volley and maximal frequency of impulses during inspiration increased considerably. The discharge was prolonged to the phase of expiration giving a pattern of expiratory activity, although the neurons in the control did not exhibit it. The intensity of reaction varied from single expiratory impulses to 30 potentials per second. This was a characteristic feature of the reaction of the respiratory neurons of the vagus nerve (22).

The effect of prolonged maintenance of constriction was manifested only by a higher value of some parameters of the immediate reaction, in no experiment was the occurrence of any qualitative changes in neuronal activity observed. The parameters undergoing certain modification, mainly in the first minute of constriction, were: the prolonged time of discharge with simultaneous, probably compensative, increase in the number of impulses and shortening of the silent period. The dynamics of these changes was closely connected with a simultaneous slight acceleration of breathing.

The inspiratory frequency and the expiratory activity appearing in the first breaths became somewhat more pronounced, chiefly for 30 sec, and then remained at a constant level. Inspiratory activity was of the same character as manifested in the immediate reaction. The neurons which in the latter reaction showed an inspiratory-expiratory pattern did not further change their type of reaction. After disconnecting the experimental tube most parameters returned to the control level noted in the

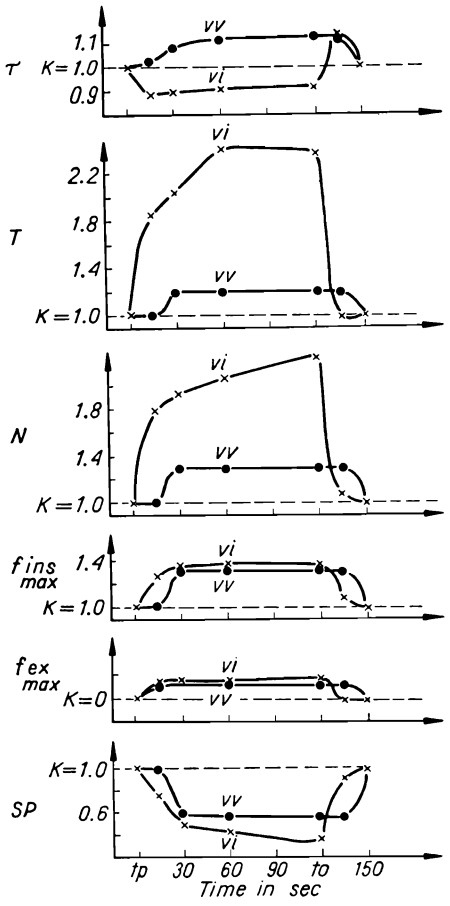

Fig. 1. Reaction of the vagal respiratory motoneurons to trachea constriction before (vi) and after (vv) vagotomy. Ordinates show relative values and dashed lines show control level. Abscissae, time in seconds. *tp*, connection of the experimental tube; *to*, disconnection of the experimental tube; τ, respiratory frequency; *T*, duration of the discharge; N, number of action potentials in volley; f_{ins}, maximal discharge frequency during inspiration. f_{ex}, maximal discharge during expiration. SP, phase of inactivity of the neurons.

TABLE I

Reaction of the vagal respiratory motoneurons to tracheal constriction before and after vagotomy. Relative values and range of deviation values. Explanation of symbols in Methods

Before vagotomy

	After connection of experimentat tube								After disconnection of experimental tube	
	15 sec		30 sec		60 sec		120 sec		15 sec	
	1	2	1	2	1	2	1	2	1	2
τ	0.85	+0.07 —0.12	0.89	+0.05 —0.10	0.90	+0.06 —0.08	0.91	+0.07 —0.09	1.13	+0.15 —0.08
T	1.85	+1.00 —0.67	2.04	+0.58 —0.41	2.40	+0.76 —0.20	2.39	+0.76 —0.68	0.95	+0.03 —0.04
N	1.80	+1.05 —0.60	1.92	+1.62 —0.51	2.03	+0.37 —0.52	2.17	+0.23 —0.48	1.08	+0.03 —0.08
f_{ins} max	1.27	+0.18 —0.10	1.36	+0.14 —0.08	1.38	+0.07 —0.08	1.38	+0.10 —0.10	1.09	+0.04 —0.04
f_{ex} max	vol 15	+5.0 imp/sec —4.0	vol 16	+5.0 imp/sec —10.0	vol 17	+10.0 imp/sec —7.0	vol 19	+8.0 imp/sec —9.0	0	
SP	0.77	+0.16 —0.34	0.49	+0.33 —0.34	0.42	+0.15 —0.13	0.36	+0.21 —0.08	0.92	+0.01 —0.01

After vagotomy

τ	1.02	+0.06 —0.02	1.08	+0.28 —0.05	1.11	+0.20 —0.08	1.12	+0.22 —0.08	1.12	+0.22 —0.08
T	1	+0.02 —0.01	1.20	+0.31 —0.14	1.20	+0.31 —0.14	1.20	+0.31 —0.14	1.20	+0.31 —0.14
N	1	+0.02 —0.02	1.30	+0.21 —0.17	1.30	+0.16 —0.18	1.30	+0.11 —0.16	1.30	+0.11 —0.14
f_{ins} max	11	+0.00 —0.00	1.32	+0.22 —0.15	1.32	+0.22 —0.15	1.33	+0.05 —0.16	1.33	+0.05 —0.16
f_{ex} max	0		vol 13	+2.5 imp/sec —5.0	vol 13	+2.5 imp/sec —5.0	vol 13	+2.5 imp/sec —5.0	vol 13	+2.5 imp/sec —5.0
SP	0.99	+0.01 —0.02	0.59	+0.25 —0.25	0.58	+0.13 —0.25	0.58	+0.13 —0.25	0.58	+0.13 —0.25

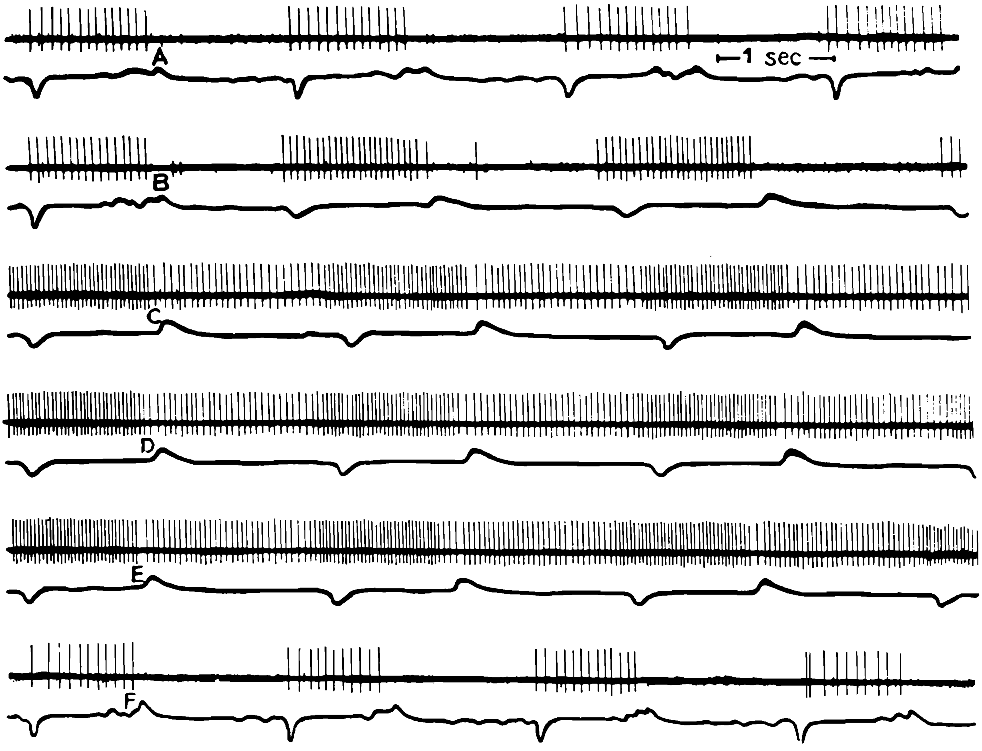

Fig. 2. Changes in activity of the single vagal respiratory motoneuron during trachael constriction before vagotomy. Lower trace respiratory curve. Inspiration downwards. *A*, record before connection of the experimental tube; *B*, connection of the experimental tube between the first and second breath; *C*, 30 sec later; *D*, 60 sec later; *E*, 120 sec later; *F*, record after disconnection of the experimental tube.

first several respirations (on the average up to 15 sec). Full compensation was observed only after stabilization of the respiratory rhythm that is on the average after 30 sec.

Reaction of respiratory motoneurons of the vagus nerve to constriction in vagotomized animals

The results from this group of experiments are graphically presented in Fig. 1, the numerical data and range of deviations in Table I, and an exemplary experiment in Fig. 3.

Contrary to the direct response of the neuron which did not show any changes in activity in the first few respirations (on the average for 15 sec) (22), prolonged maintenance of the constriction produced significant changes.

As regards respiratory rhythm these changes occurred in opposite

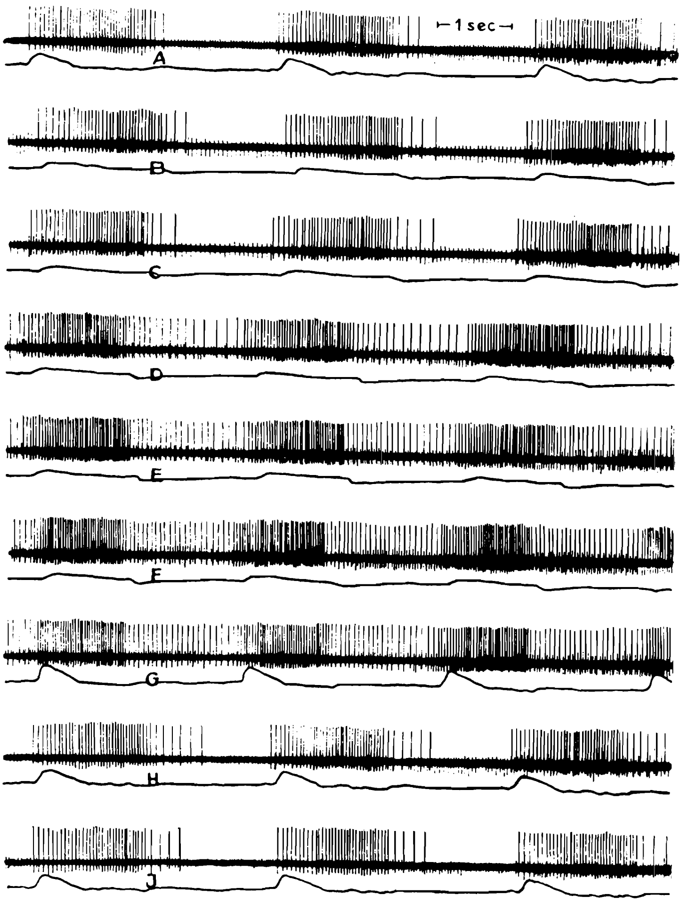

Fig. 3. Changes in activity of the single vagal respiratory motoneuron during tracheal constriction in vagotomized animals. Lower trace respiratory curve, inspiration upwards. *A*, control record before connection of the experimental tube; *B*, immediately after connection; *C*, 15 sec later; *D*, 30 sec later; *E*, 60 sec later; *F*, 120 sec later; *G*, 15 sec after disconnection of the experimental tube; *H*, 30 sec later; *J*, 60 sec later.

direction as compared with those observed in animals with intact vagus nerves.

In all experiments accelerated breathing was noted. As seen in Fig. 1, this acceleration was proportionally even greater than the corresponding slowing down before vagotomy. Although the mean value was 12, sometimes the velocity reached even 30⁰/₀ in relation to the control value. After 1 min of constriction, the respiratory frequency stabilized at a certain level. Neither was a further acceleration of respiratory rhythm observed in experiments in which the constriction lasted 1 hr. Between the 15th and 30th sec a drastic increase of activity occurred associated with changes in all the parameters. These changes occurred in the same direction as those observed before vagotomy: the duration of discharge was prolonged, and the number of action potentials increased. The inspiratory frequency of the neuron increased and expiratory activity appeared. Since these changes occurred simultaneously with the previously described considerable acceleration of the respiratory frequency, they greatly reduce the silent phase. The same as before vagotomy, in some experiments this phase disappeared completely and was replaced by continuous modulated activity. It is noteworthy that the increase in time of discharge and in the number of impulses was lower than when the second vagus nerve remained intact. This may perhaps have been due to the greatly shortened respiratory cycle, whereas before vagotomy it was greatly prolonged. The almost identical level of inspiratory and expiratory activity was striking, both increased notwithstanding whether the vagus nerves were intact or not. It is also interesting that in vagotomized animals the changes were more stable.

Elimination of the constriction produced no immediate response, that is it did not change the enhanced activity of the neurons. However, the system began to return to the control level as late as after a period of 15 sec and recovered after 30 sec.

Comparative studies on the effects of hypoxia due to constriction and pure hypoxia on the activity of respiratory neurons of the vagus nerve (Fig. 4)

Maintenance of constriction for 2 min reduced the oxygen level in arterial blood from an average value of 91% to 89% within 30 sec and to 88% later on. No changes in the arterial blood CO_2 were noted. In some experiments, in spite of distinct changes in the activity of the motoneurons of the vagus, hypoxia was not found. There were, however, experiments in which oxygen saturation fell to 86%.

If we compare the changes of activity of respiratory neurons of the vagus nerve in response to constriction with the influence of analogous level of pure hypoxia, the incommensurably intensive reaction in the former case is noteworthy. True hypoxia to an 85% level gave only slight activity changes. Only values below 80% caused enhanced activity of the vagal motoneurons, as described by Widdicombe (70), and manifested in the present experiments by an increased inspiratory frequency and greater number of action potentials and sometimes the appearance of expiratory activity. Hypoxia of this level was, however, never noted in the case of constriction.

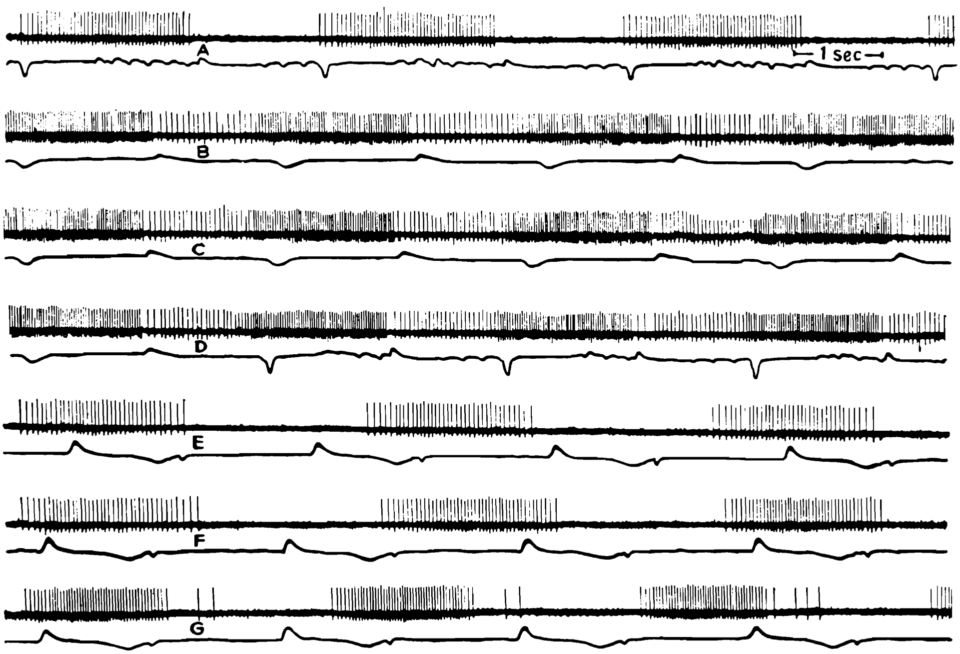

Fig. 4. Comparative records of the changes of activity of the single vagal motoneuron during constriction and pure hypoxia in vagotomized rabbits. *A-C* on lower trace respiratory curve, inspiration upwards. *D-G* on lower trace rhythm of respiratory pump. *A*, control record oxygen saturation 94%; *B*, 30 sec duration of constriction oxygen saturation 93%; *C*, 120 sec of constriction, oxygen saturation 89%. *D*, 15 sec after disconnection of experimental tube, oxygen saturation 93%. *E*, Control record during artificial ventilation, oxygen saturation 92%; *F*, lowered oxygen saturation to 85%; *G*, lowered oxygen saturation to 70%.

Reaction of phrenic motoneurons to constriction before vagotomy

The results in this experimental group are graphically presented in Fig. 5, the numerical data in Table II, and exemplary experiments in Fig. 6. One of the immediate reactions to connecting of experimental tube was the already described slowing down of breathing. It was more pronounced than in the previous experiments since both the vagus nerves were intact.

Simultaneously with the immediate change of respiratory rhythm all the parameters of neuronal activity of the phrenic nerve increased. The reaction occurred without exception and always in the same direction, although the intensity varied. Prolongation of discharge duration (*T*) and of the silence period of the neuron (SP) was noted. This was closely connected with the prolongation of the phase of inspiration and expiration. The volley of impulses from the neurons of the phrenic nerve, in

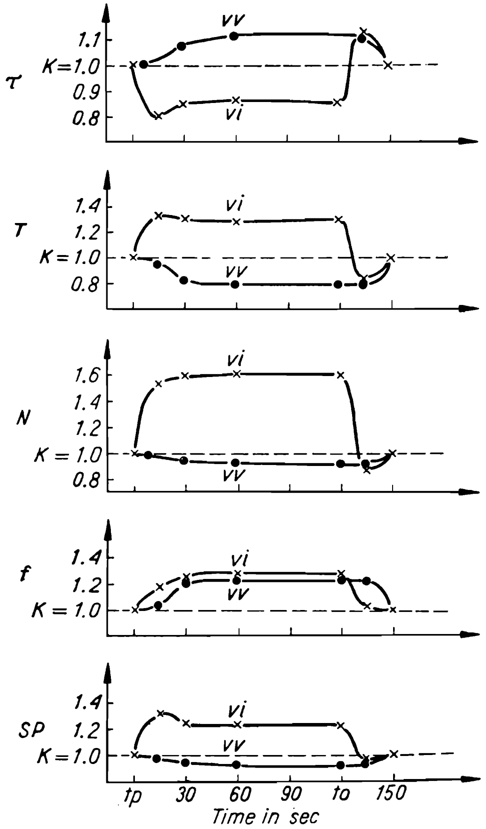

Fig. 5. Reaction of the phrenic motoneurons to trachael constriction before (vi) and after (vv) vagotomy. Symbols same as in Fig. 1.

contrast to that from the respiratory neurons of the vagus nerve, was never so prolonged as to extend to the expiratory phase in the experiments. A common feature, on the other hand, was the regularly noted increase in the number of action potentials, causing a considerably higher impulse frequency in the volley.

As in the previously described experimental subgroup of this type, maintenance of the constriction for 2 min did not produce any major changes. The activity of the phrenic nerve neurons, enhanced already in the immediate response, became slightly more intensive in the first 30 sec and then remained at a constant level. This was due to a slight shortening of the discharge duration and of the silence period. It was connected with a simultaneous acceleration of the respiratory rhythm as previously described. The increased number of action potentials — on the average by 5% — caused an increased impulse frequency. After disconnection of the constriction a drastic change of the most active parameters occurred beginning with the first breath. Most of them

TABLE II

Reaction to the phrenic motoneurons to trachael constriction before and after vagotomy. Relative values and range of deviation values. Explanation of symbols in Methods.

Before vagotomy

	After connection of experimental tube								After disconnection of experimental tube	
	15 sec		30 sec		60 sec		120 sec		15 sec	
	1	2	1	2	1	2	1	2	1	2
τ	0.80	+0.07 —0.10	0.85	+0.06 —0.04	0.86	+0.09 —0.04	0.85	+0.08 —0.03	1.11	+0.09 —0.07
T	1.32	+0.13 —0.18	1.29	+0.20 —0.19	1.28	+0.22 —0.20	1.29	+0.30 —0.23	0.83	+0.13 —0.18
N	1.53	+0.28 —0.28	1.59	+0.35 —0.37	1.60	+0.40 —0.40	1.60	+0.34 —0.42	0.86	+0.14 —0.07
f	1.17	+0.04 —0.05	1.25	+0.06 —0.10	1.28	+0.14 —0.11	1.27	+0.09 —0.10	1.06	+0.07 —0.06
SP	1.32	+0.18 —0.24	1.24	+0.14 —0.14	1.23	+0.20 —0.14	1.23	+0.20 —0.14	0.95	+0.10 —0.10
After vagotomy										
τ	1.02	+0.06 —0.02	1.08	+0.20 —0.05	1.11	+0.20 —0.08	1.12	+0.22 —0.08	1.12	+0.22 —0.08
T	0.97	+0.06 —0.05	0.82	+0.04 —0.10	0.70	+0.10 —0.09	0.79	+0.18 —0.13	0.79	+0.10 —0.08
N	0.98	+0.05 —0.06	0.94	+0.06 —0.07	0.93	+0.07 —0.06	0.93	+0.07 —0.07	0.93	+0.07 —0.07
f	1.02	+0.02 —0.02	1.20	+0.16 —0.10	1.22	+0.10 —0.10	1.23	+0.10 —0.11	1.23	+0.15 —0.11
SP	0.97	+0.07 —0.04	0.95	+0.05 —0.07	0.92	+0.08 —0.12	0.93	+0.07 —0.06	0.93	+0.07 —0.06

changed in the opposite direction as compared with the situation before disconnection. Thus, a sudden respiratory frequency acceleration occurred associated with a considerable reduction of discharge duration and of the silence period of the neuron. The number of action potentials in the volley decreased below the control value. This decrease was, however, percentually smaller than the corresponding shortening of volley dura-

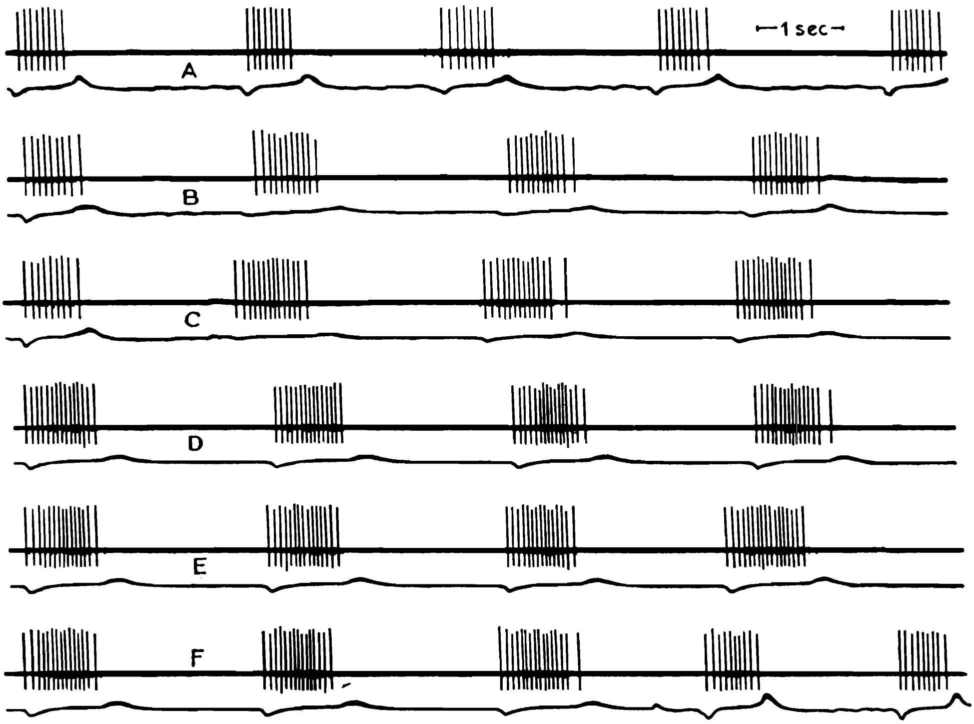

Fig. 6. Changes in activity of the single phrenic neuron during tracheal constriction before vagotomy. Inspiration downwards. *A*, a record before connection of the experimental tube; *B* and *C* immediate reaction to the connection, connection between first and second breath; *D*, 30 sec duration of the constriction; *E*, 60 sec later; *F*, 120 sec of the constriction and disconnection of the experimental tube between third and fourth breath.

tion, the impulse frequency remaining further at a level higher than the controlled one, but much lower than during constriction. The reaction of this type was probably compensatory and only after about 30 sec all the parameters returned to the control value.

Reaction of phrenic nerve neurons to the constriction in vagotomized animals

The results for this experimental group are presented graphically in Fig. 5, the numerical data and ranges of deviation in Table II, and an exemplary experiment in Fig. 7.

As in the analogous group of experiments concerning the activity of respiratory motoneurons of the vagus nerve, constriction lasting 2 min caused significant changes. There was no response at first for about 15 sec then a significant change in almost all the parameters occurred. As compared with the case of efferent fibers of the vagus, in which the reaction after 15 sec occurred in the same direction as in the nonvagotomized animals, the phrenic neurons under conditions of vagotomy, exhibited a different response as far as some parameters are concerned. Discharge duration and the silence period were considerably shortened.

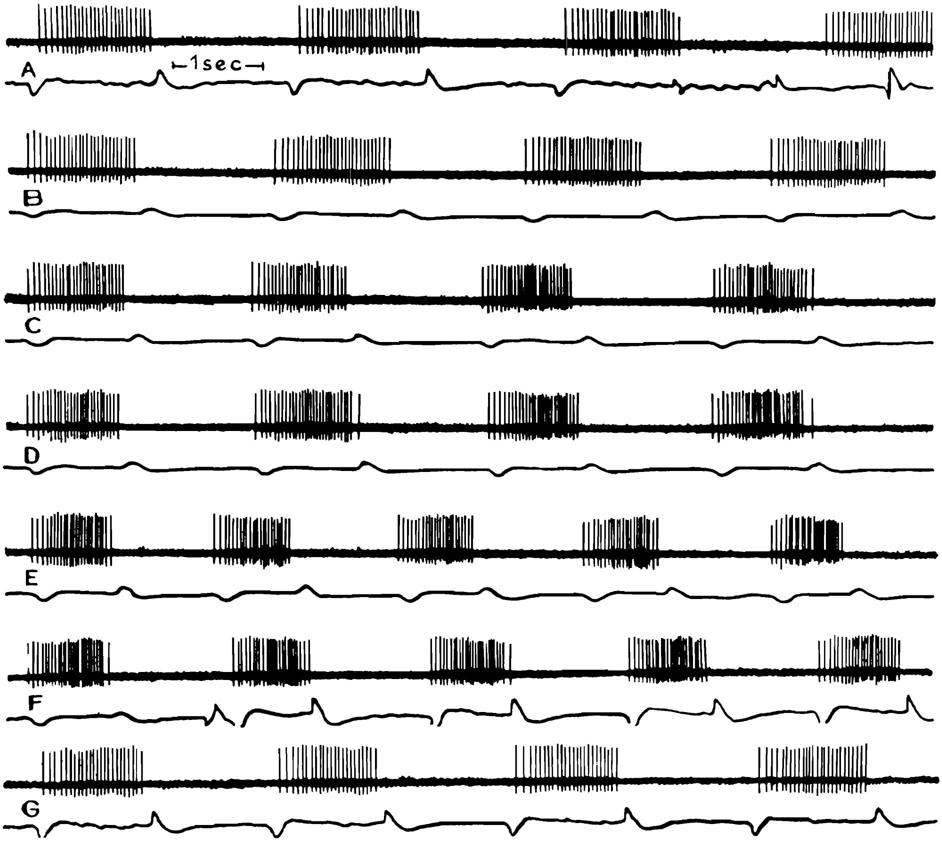

Fig. 7. Changes in activity of the single phrenic neuron during tracheal constriction in vagotomized rabbit, inspiration downwards. *A*, control record before connection of the experimental tube; *B*, 15 sec after connection; *C*, 30 sec later; *D*, 60 sec later; *E*, 120 sec later; *F*, disconnection of the experimental tube between first and second breath; *G*, 30 sec later.

The number of action potentials was slightly reduced, and this was associated with an acceleration of the respiratory frequency. The number of action potentials decreased somewhat giving a situation contrasting with that before vagotomy. The only parameter which increased analogously in intact animals was the impulse frequency in the course of the entire volley. This was the most characteristic feature of the system, and a reverse situation was never noted. In about 10% of the experiments, in which the acceleration of the respiratory rhythm was not so marked, and therefore the discharge duration less produced, the same frequency increase was obtained by an increased number of action potentials. Disconnection of the constriction did not produce any response of the phrenic nerve neurons. Similarly as in the case of vagus nerve, the system was characterized by a certain inertia, and the return of parameters to the outset values started after about 15 sec. After 30 sec the system recovered.

Comparative studies on the effects of hypoxia due to constriction and pure hypoxia on the activity of phrenic nerve neurons

The fall of the blood oxygen level from an average of 92 to 80% caused a slight acceleration of the volley rhythm, a somewhat shorter time of their duration and a decrease of the number of action potentials.

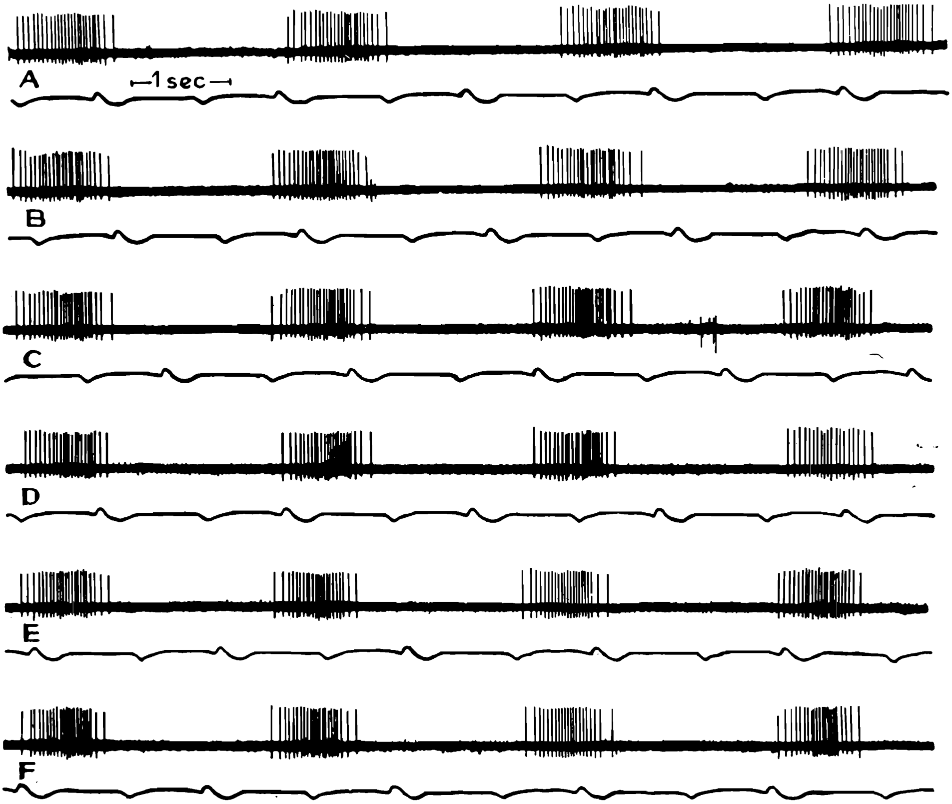

Fig. 8. Reaction of the single phrenic neuron to gradual decrease of the arterial oxygen saturation that is pure hypoxia. Lower trace rhythm of respiratory pump. *A*, oxygen saturation 92%; *B*, oxygen saturation 89%; *C*, oxygen saturation 85%; *D*, oxygen saturation 82%; *E*, oxygen saturation 78%; *F*, oxygen saturation 76%.

As a rule major changes in impulse frequency in the volley were not noted. Figure 8 shows the reaction of the fiber (described in Fig. 7) to pure hypoxia. In all experiments the changes of activity during constriction were much bigger than during a pure hypoxia.

DISCUSSION

Investigators dealing with the influence of air flow resistance and other changes of the mechanics of respiratory system on the activity of respiratory motoneurons of the vagus and phrenic nerves described the abolition of neuron responses in vagotomized animals.

It has been demonstrated that in such a case the decrease in respiratory frequency typical for increased air flow resistance does not occur (17, 44, 59). The immediate reaction was exclusively investigated, and the observations find full confirmation in the immediate response obtained in the present study. It seemed, therefore, that these results would support the view claiming that information runs exclusively via the vagus nerves. The occurrence within the period of about 15 sec. of similar changes in both the respiratory neurons of the vagus and phrenic nerve was quite unexpected. An additional surprise was the simultaneous increase in respiratory frequency. Larrabee and Knowlton (43), Glebovskiĭ and Pavlova (33) and later Tang (68) affirmed that vagotomy abolishes all reflexes accelerating respiration. This effect is connected according to Gill (32) with the elimination of the information running from the pulmonary stretch receptors. Unfortunately no papers are available dealing with the long-term influence of air flow resistance on vagotomized animals. The renewed enhancement of activity of both "outputs" of the respiratory centers together with the changes in rhythm are evidence of the arrival of new information.

An additional confirmation of the fact that new nervous connections are "engaged" only when the vagus nerves are eliminated is supplied by the "latency" period between the introduction of resistance and the reaction of the nerve centers. Thus we are not dealing here with an immediate spinal reflex as in the reaction of the remaining respiratory muscles. The latency period is, maybe, due to the involvement of successive nervous connections, and this, prolonging the pathway by which the excitation runs, requires a longer time. The inertia in the activity of the respiratory center after disconnection of the constriction would seem to indicate a gradual extinction of the excitation circulating in the system. Such a state has been described by Bishop (5) as "residual persistent facilitation of the inspiratory neurons". The fact of delay in the reaction of the centers, both upon introduction and disconnection of respiratory resistance, stresses the rule of the vagus nerve which enables rapid compensation of the consequences of these changes by the organism. Another problem requiring elucidation is the question whether the newly appearing source of information overtakes fully the functions of the vagus. As results from the increased activity of respiratory neurons of the vagus and phrenic nerves, the changes are both quantitative and qualitative unidirectional. This regards particularly such an important parameter as the impulse frequency in the volley, which conditions the force of contraction of the muscles of the airways (71) and the diaphragm (16, 44). One might suppose that there is complete compensation if the direction of the changes of the respiratory frequency were not reversed. Notwithstanding whether the respiratory frequency is adapted to the minimalization of the work of breathing (3) or to the force of contraction of the respiratory muscles (48), it was established that, when air flow resistance increases, only a decrease in respiratory frequency is economical (15, 48, 55). On the one hand, the muscles require a longer time for mobilization of chemical energy necessary for performance of work (2) this being associated with a specific muscle force-velocity (49). On the other hand, acceleration of respiration increases the air flow rate through the constricted site, enhancing turbulent flow, which in turn increases resistance and the work of breathing (36). Increased frequency of breathing noted in some pathological states with increased air flow resistance as for instance chronic bronchitis consists in the preponderant influence of the excessively lower lung compliance which reverses the frequency of breathing in this situation to a more economic acceleration (31). It does not, however, seem possible that constriction could produce such an important change only in vagotomized animals. Thus, it is probable that the additional sources of information are capable of ensuring a normal direction of the changes in respiratory neuron activity, and in this way good compensation of the respiratory resistance, without, however, establishing a normal rhythm. This suggestion would, however, require investigations in detail of the changes in respiratory mechanics.

The revelation of new sources of information, after exclusion of the effect of hypoxia, supports the views ascribing here a role as to the extrapulmonary receptors disseminated in the entire thoracic cage. Respiratory muscles have wide possibilities of autoregulation, and according to some authors, are as important in the neurocontrol of breathing as the vagus nerves (24).

As other skeletal muscles they react to stretching (37). This is the precisely described auto-regulation reflex are known as the follow-up-length-servo (63-65). This reflex is responsible for the enhanced work of the respiratory muscles independent of the continuity of the vagus nerves. According to the opinion of investigators of this group, the influence of the stretch reflex extends to the supraspinal structures, and in this way information on the changed situation is transmitted both to the respiratory centers and to consciousness. The problem, however, remains unexplained whether the reflex loops of the intercostal muscles run at the level of the spinal cord (58, 72) or, as suggested more and more frequently by other authors, they involve higher structures of the central nervous system (9, 57, 66). Excitation in the medullary loops spread to the neighboring intracostal structures (33) and in the case of stimulation causes an immediate response. The enhancement of the electromyographic activity of the inspiratory or expiratory muscles in response to respiratory resistance occurs in humans within milliseconds that is almost immediately (52). The delayed reaction of the motoneurons observed in the present work supports the thesis that if the same pathways supply information to the centers, this only occurs when vagal information is lacking. It would also seem that these results definitively establish the absence of autoregulation reflexes in the diaphragm. The enhanced activity of the phrenic nerve motoneurons does not appear earlier than that of the respiratory neurons of the vagus nerve this indicating that the information must first pass through the respiratory centers.

It is difficult on the basis of up-to-date knowledge to establish definitively which receptors of the thorax could take over the informative role of the vagus nerves.

Some authors stress, moreover, the possibility of information supply from the tendon receptors as well as from the ligament receptors.

Lately a certain role is more frequently assigned to the ligaments of the ribs and thoracic vertebrae (13, 51). It has been proved that the latter transmit information on many changes occurring in this system (35). These ligaments are particularly sensitive to deformation of the chest, and it seems probable that deformation does occur in the case of tracheal constriction. Savić et al. (60) demonstrated that states associated with a decreased functional residual capacity (FRC) deform most of the breathing apparatus. Judging by the decrease of inspiratory activity of the pulmonary stretch receptors such a situation occurs as a result of constriction of the upper airways (22). To support their argument the above enumerated authors described the phenomenon of respiratory rhythm acceleration in response to even passive movements of the limbs and spine in people under superficial anesthesia (20). So considering the sources and routes of information, it is difficult to disregard the sympathetic nerves which have been unfortunately little studied in this respect.

Most considerations on the kind and role of information from the thorax have been based on the observation of the time of voluntary apnea or the perception of breathlessness in humans. These systems cause great difficulties in interpretation since they require consciousness of the subject which significantly reduces their value (21, 66). The influence of the cortical structures on respiration is well known (69), as for instance the effect of pain or emotion (20), and may lead to a conscious adaptation to the investigations (30). It is also known that people under anesthesia react much less violently to respiratory resistance, probably owing to the absence of stress (53). Conclusions based on the moment of perception of resistance by man in various pathological conditions (vagotomy, spinalectomy) are not very reliable, since it has lately been found that in such cases a compensative sensing of the pressure differences by the orolaryngeal cavity occurs (13, 50).

The protagonists of the role of thoracic receptors invoke to support their views by the fact that, from among all mammals, the Hering–Breuer reflex is weakest in the man (72). Beside, however, the paper of Guz et al. (39), no effect of vagotomy has been described in man. That is perhaps why a less important role is ascribed to this reflex. There exist, however, data showing that the reflex is enhanced in anesthetized subjects (70) and in small children (62). The occurrence of identical reactions to respiratory resistance in animals and anesthetized humans (55) seems to confirm the above mentioned view. Conscious humans, as described in the introduction, increase the tidal volume. Campbell and Newsom Davis (13) expressed the view that, although the perception of breathlessness forcing to draw in air may reach consciousness by various pathways from the thoracic receptors, the respiratory drive depends exclusively on information running by the vagus nerves. These authors based their affirmation on the known relation between the duration of the voluntary time of apnea and the degree of inflation of the lungs. The greater the inflation the stronger the inhibition of the inspiratory centers, allowing longer apnea (34). It is also known that when the tidal volume is small, paralysis of the vagus nerve in man prolongs this phase (39). It would seem therefore that, in spite of the different objections, the Hering–Breuer reflex is the most important one in the control of respiration (29).

If the above thesis will find some confirmation after precise analyses, extrapolation of the results of the present study to humans would suggest that, although all other sources of information can compensate respiratory resistance by way of enhancing the work of the respiratory muscles, they cannot, however, establish an optimal rhythm in those cases. For a fuller elucidation of this problem, it is necessary to investigate the mechanics of breathing on the here presented model of prolonged influence of airway constrictions on vagotomized animals. Most reliable results could be obtained from observations of this type performed on humans, but this is very difficult both for technical and humanitarian reasons.

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