NATURE AND ORIGIN OF AUTOMATIC ACTIVITY IN THE RESPIRATORY CENTER OF THE FROG

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An analysis of information concerning the activity of the respiratory center [2, 4-9, 11, 16, 18-20] indicates that in spite of more than 200 years of study there is still indecision concerning the cardinal problem of the structure of the respiratory center and the localization and origin of its automatic activity. This is explained, apparently, by the complexity of structure and physiological connections, particularly in higher animals. An investigation of the comparative physiological aspects of the problem may assist clarification of the origin of automatic activity in this region.

In connection with this problem, we investigated the functional changes which originate in different parts of the respiratory center of frogs after alteration of automatic respiratory center excitation. Frogs were selected for study because they are one of the first animals to possess a pulmonary respiratory apparatus with a specific mechanism regulating its activity. It was therefore possible to study the natute and origin of automatic activity of the respiratory center in a simplified system whose original schema was unobscured by auxiliary connections.

In the investigations of Ariens-Kappers [17], Bernard [12], and Herrick [15] it was established that impulses from the respiratory muscles and superficial mucosa of the respiratory organ of frogs pass through the VII, IX and X sensory nerves to the respiratory center. The greater part of the fibers involved are directed caudally, forming the fasciculus solitarius (isolated tract). The nucleus fasciculi solitarii is also located in the region where these nerves enter the brainstem. Herrick called this nucleus the "strategic locus" of the nervous system with regard to visceral function. In 1947 A.I. Roitbak noted: "It is extremely tempting to assume that the nucleus of the respiratory bundle is precisely the automatic center. Most of the axons from cells of this nucleus are directed to the motor nuclei of the IX and X pairs of nerves, crossing part of the network of the formatio reticularis". In 1957, Baumgarten [13] also suggested that the nucleus fasciculi solitarii is the repiratory center. Chemical or faradic stimulation at the entrance of the X nerve into the medulla oblongata of frogs with arrested respiration provokes excitation of the respiratory center, whereas stimulation of other parts of the brain under these conditions does not produce this result [5, 6, etc.]. Experiments by B.D. Kravchinskii [3] showed that it is possible to switch the automatic activity on and off, since it is the input from receptors in the aortic zone which is the factor determining the activity of the respiratory center in frogs.

METHOD

Experiments were conducted from 1956-1959 on Rana esculenta and Rana ridibunda, using animals with strong pulmonary respiration. Before each experiment the medulla oblongata of the animal was exposed. In order to study activity of the "inspiration" center (or of its efferent part), a preparation was made of the geniohyoid muscle; for study of the "expiration" center, a preparation of the external oblique muscle of the trunk was used. These muscles, respectively, are related to inspiration and expiration. Subsequent observations were made of contraction of these muscles following localized, juxtalimenal stimulation of their corresponding regions in the medulla. The frogs were fastened, spine upwards, on a plate with an opening opposite the heart and muscles of the fundus of the oral cavity. The reference electrode (silver plate 0.5 cm^2) was fastened to cranial bone. A nichrome microelectrode with glass insulation was used for stimulation. The diameter of the microelectrode tip was 30μ. Location of the electrode tip was primarily determined by the response of corresponding inspiration or expiration muscles to threshold stimulation. After the experiment additional histological verification of the location of the tip of the stimulating electrode was made (hematoxylin-stained microscopic sections). At the beginning of the experiment, the functional characteristics of stimulation of a brainstem region during automatic activity were determined for the following: 1) Onset of expiration (for inspiratory muscles); 2) onset of inspiration (for expiratory muscles). Subsequently, a small piece of cotton wool, soaked with a 2% solution of novocain, was applied to the region of bifurcation of the aorta, and the same parameter was measured every 3 minutes during the resulting cessation of respiration. Preliminary experiments indicated that placing a small piece of cotton, soaked with novocain, on the lymphatic sac does not produce cessation of respiration. Consequently, cessation of respiration in the first case was produced only as a result of blocking the input from aortic receptors, and not as a consequence of the action of novocain on the respiratory center.

RESULTS

It was found that the geniohyoid muscle (as well as other inspiratory muscles) may contract even during stimulation of related fibers of neurons located at the level of the entrance of the vagus nerve bundle. It was also found that during stimulation of the brain at the level of outflow of the II cerebrospinal root, which according to Ecker [14] corresponds to the XII pair of cranial nerves of warm-blooded animals, contraction of only the geniohyoid muscle may occur. Histological examination indicated that in the first case the tip of the stimulating electrode was located among cell bodies near the fasciculus solitarius (Fig. 1), and that in the second case the electrode tip was in a region of motor elements (ventral horn). In the latter case the fasciculus solitarius was found closer to the dorsal part of the medulla (see lower part of Fig. 3). Consequently, motor neurons of the geniohyoid muscle make connection with units situated at the level of entry of fibers of the X pair of nerves. Stimulation of the indicated region causes contraction of all inspiratory muscles. Efferent nerve cells (motorneurons) of these muscles are partially located at the same level (anterior and posterior deglutitory muscles, innervated by the vagus), and partially at the level of entry of the II cerebrospinal root (e.g., geniohyoid muscle). These characteristics were used in a special investigation of the functional characteristics of the efferent part of the respiratory center.

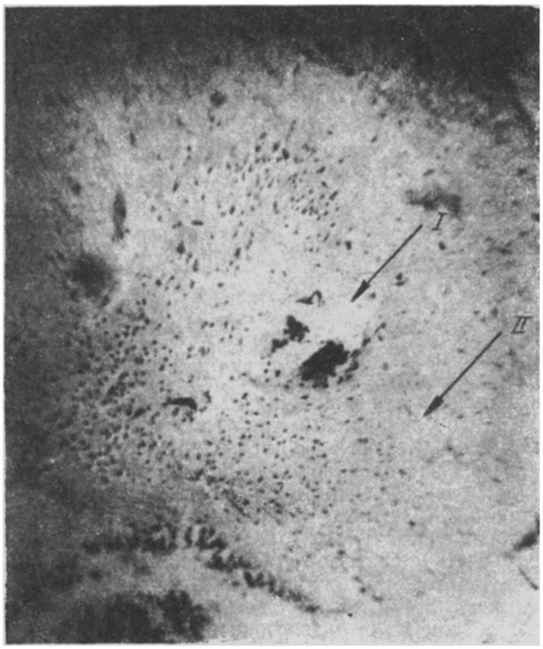

Fig. 1. Medulla oblongata of frog (experiment of March 26, 1959). Section at level of entry of IX and X pairs of cranial nerves. Region of the inspiration center. Microphotograph taken with a 9 x 12 photographic attachment. Ocular 4 x, objective 12 x. Hematoxylin stain. I) Location of tip of stimulating electrode. II) Fasciculus solitarius.

Fig. 2. Medulla oblongata of frog. Microscopic section at level immediately above entry of II cerebrospinal root (dorsal part). Region of the expiration center. Photography, magnification, stains and marks as indicated in Fig. 1.

The nerve elements of expiratory muscles are caudally disposed, and just above the level of entry of the II cerebrospinal roots. Stimulation of this region caused contraction of expiratory muscles, particularly the external oblique muscle of the trunk. Histological examination indicated that the tip of the microelectrode during responses of this type was located in the dorsal part of the medulla near the fasciculus solitarius (Fig. 2).

The motor neurons of expiratory muscles are located at the level of entry of the III-VIII pairs of cranial nerves. Thus the histological controls in conjuction with local stimulation indicated the presence of two centers in the medulla of the frog, located near the fasciculus solitarius and connected with excitation of inspiratory and expiratory muscles. This is consistent with previous studies of the respiratory center of warm-blooded animals by a series of Russian and foreign authors (cf. references cited in first paragraph).

The existence, however, of these centers does not solve the problem of the origin of their excitation. In connection with this, by means of novocain block of aortic zone input, we measured physiological parameters of the indicated regions during cessation of the automatic activity of the respiratory center. In experiment No. 3 (March 26, 1959) measurements were made in the inspiration region. The location of the tip of the stimulating microelectrode is illustrated in Fig. 1. Immediately prior to the effect of novocain, the rheobase was 11 v, chronaxie 0.37σ, and accomodation constant (λ) 183σ. These values were changed as soon as 1.5 minutes after the onset of the novocain effect. At 15 minutes the rheobase was 19 v, chronaxie 2.22σ, accomodation constant (λ) 77σ. Similar changes in excitability, accomodation, and chronaxie were observed in the other experiments. Mean values for 25 experiments are given in Table 1. The mean rheobase was increased 2 times, chronaxie was lengthened 4-10 times, and the accomodation constant (λ) was sharply reduced. Measurement of parameters in the expiration region and in the efferent part of the respiratory center revealed no abrupt changes even throughout the first hour following cessation of impulses from the aortic zone. Thus, for example, in experiment No. 2 (March 13, 1959) the rheobase in the efferent region immediately prior to novocain block was 7 v, chronaxie 0.18σ, and accomodation constant (λ) 100σ. Following novacaine block the rheobase was 8.5 v, chronaxie 0.2σ, and accomodation constant (λ) 113σ. The location of the tip of the electrode is illustrated in Fig. 3. Mean values for 25 experiments are given in Table 1.

In the experiment of June 1, 1959, the rheobase in the expiration region prior to the action of novocain was 8 v, chronaxie 0.19σ, accomodation constant (γ) 333σ. Subsequently, the rheobase was 9.5 v, chronaxie 0.19σ, accomodation constant (γ) 375σ. Location of the microelectrode tip in this experiment is shown in Fig. 2. Mean values for 25 experiments are given in Table 1.

From all the above observations it can be concluded that automatic excitation originates in the region containing motor neurons of inspiratory muscles. The region containing motor neurons of expiratory muscles is not affected by cessation of impulses from the aorta. Under natural conditions the expiration center of the frog is excited secondarily: either by means of its reciprocal relations with the inspiration center, or, as indicated by Woldring and Dirken [19] for warm-blooded animals, as a result of its activation by receptors of the lung and proprioceptors of respiratory muscles, with the activity of these being synchronized with excitation in the inspiration center.

Of great interest for the general physiology of the nervous system is the fact that with the background of automatic activity the functional characteristics of the inspiration center are approximated by both its own efferent part and the expiration center. After exclusion of the input from the aortic zone, the characteristics of the inspiration center abruptly change, whereas in

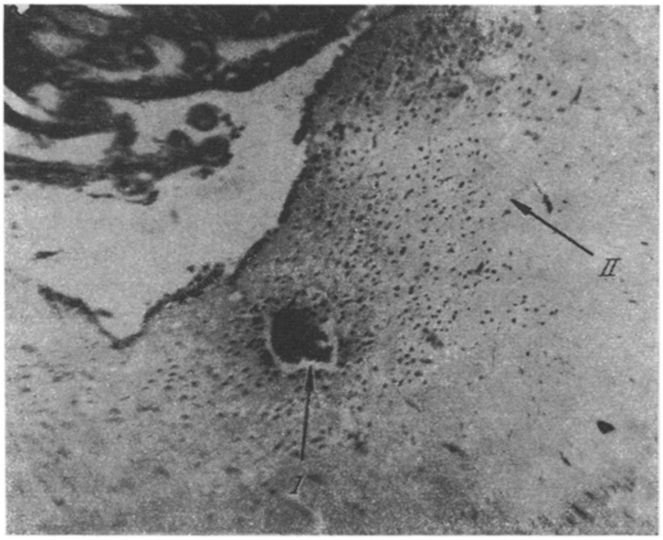

Fig. 3. Medulla oblongata of frog. Section at level of entry of II pair of cerebrospinal roots (ventral part). Efferent region of inspiration center. Magnification, stain, and marks same as Figs. 1 and 2.

Physiological Parameters of Various Regions of the Respiratory Center of the Frog with and without Automatic Activity (mean values for 25 experiments in each series)

Region of respiratory center	With background of automatic activity			During absence of automatic activity		
	rheobase (in v)	chronaxie (in σ)	accomodation constant (λ)	rheobase (in v)	chronaxie (in σ)	accomodation constant (λ)
Inspiration	8.5	0.381	153.4	16.7	3.301	110.9
Expiration	8.43	0.19	288.2	7.35	0.19	288.9
Efferent	9.03	0.338	296.8	9.4	0.386	302

other regions of the respiratory center, as mentioned above, changes are not observed. Therefore, during steady input from the aortic receptors, the inspiration region is functionally equivalent to a motor region, and it is known that motor regions are highly unstable. This instability, according to A.A. Ukhtomskii [10], can produce functional instability of afferent input, as a result of assimilation of the rhythm of the neural center. Actually, in the inspiration center, input exclusion caused an abrupt reduction of excitability, an increase in chronaxie, and a decrease in accomodation time, i.e., complex functional changes characteristic of the state of parabiosis. This agrees with the idea of N.V. Golikov [1] that "elimination of the supportive excitation of a region of low lability causes the development in it of a functional parabiosis. This occurs regardless of the absence of recurrent stimulation, and is precisely due to elimination of the instability-producing excitation".

SUMMARY

Stimulation of interneurons at the level of entrance of the vagus fasciculus near the fasciculus solitarius provokes local contraction of only "inspiratory" muscles, while stimulation of the interneurons at the level of the exit of the II spinal root, also near the dorsal portion, causes local contraction of only "expiratory" muscles. In normal automatic activity the functional characteristics of the "inspiratory" and "expiratory centers" is identical with that of the efferent region. With the exclusion of the impulsation from the aortic receptors, when automatism ceases, the rheobase in the "inspiratory center" assumes a double value, while chronaxie is increased 4–10 times, accomodation constant (λ) is cut nearly in half. At the same time the functional characteristics of both the "expiratory" center and the efferent area approach the initial one. Hence a conclusion was drawn that the automatic excitation is being formed only in the region of the "inspiratory center".

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