

AD 675832

TRANSLATION NO. 2286

DATE: Feb 1966

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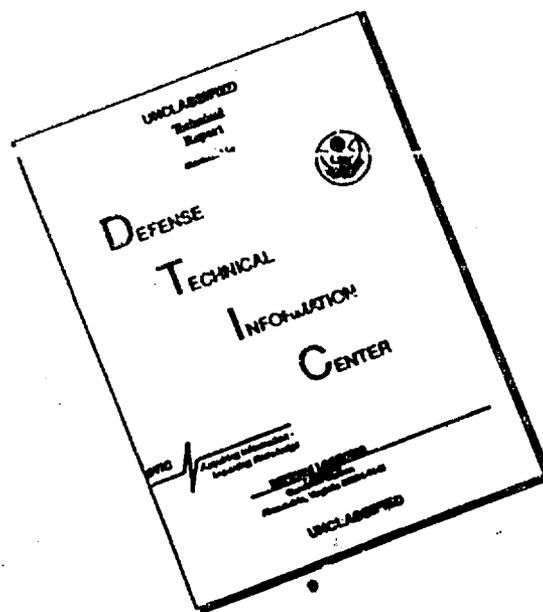
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Arkhiv Patologii (Moskva), 18 (1), 1956, pp.86-91

Mechanism of Action of Botulinal Toxin on Respiration

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(Received December 1, 1954)

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The botulinal toxin causes acute respiratory changes along with injuries to various organs and physiological systems. In the course of botulinal intoxication, functional disorders occur in the upper respiratory tracts (YA.YA. GALPERIN), together with injuries affecting pulmonary tissues (I.I. ZILBERG) and also changes in the respiratory movements in patients at various stages of the development of botulism (A.M. KORITSKII). It is a known fact that death in botulism disease follows due to respiratory paralysis. Even if established facts about the effects of botulinal toxin on various functions of the organs and on physiological systems do exist, the mechanism of action of botulinal toxin should not be regarded as clarified, as the matter stands today. In pathogenesis of botulism some authors (YU.A. PETROVSKII, T.I. BATURENKO, YA.A. NAULENKO, K.I. MATVEEV, et al.) attach importance to the vasoconstrictive effects of the toxin, while others (E.A. KURAEV et al.) are concerned with the injuries involving the endothelial cells of vessels.

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Presently, investigators in a greater number consider the injuries involving the nervous system as a controlling factor in the pathogenesis of botulism, yet the opinions differ pertinently to the point of assault of botulinal toxin. Foreign investigators (DAVIES, MORGAN, WRIGHT et al.) broadly deny any direct effect of botulinal toxin on the central nervous system.

In order to determine the significance of injuries to the nervous system in connection with respiratory disturbances caused by botulism, we studied the characteristics of changes in respiratory movements and in the mechanism of action of botulinal toxin on the respiratory neuroreflex control. It has been established recently that the "pathogenic action of microbes, toxins and viruses is not confined, by far, to their effects on receptors, but all branches of the nervous system are objectives of their action, as well as numerous organs and tissues" (A.D.ADO).

We shall attempt to explain the question: what injuries in branches of the nervous system are linked with disorders in respiratory movements during botulinal intoxication?

We performed three series of experiments on rabbits, female cats and kittens. We determined in the first series of experiments the nature of changes in respiratory movements during botulinal intoxication; in the second series we established the changes in the irritation threshold of the vagus, sciatic and phrenic nerves during botulinal intoxication; in the third series we studied the changes in the irritation threshold of the vagus nerve after administration of botulinal toxin to myelencephalon.

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The changes in the nature of respiratory movements due to the effects of botulinal toxin were observed in experiments on rabbits and kittens without narcosis and with the urethane narcosis (urethane was administered subcutaneously, in a 10% solution, allowing 1.8 gm per 1 kg of the animal weight). The recording of the respiratory movements was traced on the kymograph drum with the aid of HAREY'S capsule connected with the tracheal tube. Botulinal toxin (type A, series 14; obtained from H.P. GANALEI'S Institute of Epidemiology and Microbiology; a fatal dose for a white mouse 0.0025 mg) was administered intravenously, allowing 2 mg per 1 kg of animal's weight. The respiratory movements were traced up to the moment of administration of botulinal toxin and throughout fixed intervals of time in the course of 4 to 5 hours after administration of the toxin.

As a result of conducted experiments, it has been determined that, after administration of botulinal toxin, the latter caused a frequency increase after 30 to 45 minutes and a depression of respiratory movements. In 2 to 3 hours after administration of the toxin, the amplitude and the number of respiratory movements declined, also the exhalation action became protracted and then recurrent respiration was recorded. Death of animals resulted due to respiratory paralysis. The contraction of the heart muscle was discontinued no sooner than 10 to 15 minutes after the respiratory standstill.

Having determined the nature of the respiratory changes effected by botulinal toxin, we conducted experiments intended to clarify

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the mechanism of those changes.

We performed on rabbits and female cats 18 experiments designed to determine the irritation threshold of the vagus nerve up to and following the administration of botulinum toxin. We placed platinum electrodes on the central end of the left vagus nerve transected in the neck. The irritations were accomplished with a current from induction coil (storage battery 2 volts) and the irritation of the nerve lasted 6 seconds. The experiments designed to establish the irritation threshold of the vagus nerve were conducted for this reason that, following a determination of the vagus nerve irritation threshold, one could form an opinion about the functional condition of the respiratory center, inasmuch as the first inhibition of respiratory movements appears at the vagus nerve (M.V. SERGIYEVSKI et al.). The experiments included one-sided and two-sided transections of the vagus nerves. We believed we could judge the functional condition of the respiratory center according to the threshold value of the central end of the vagus nerve at which the respiratory inhibition occurs. Table 1 and figure 1 show the threshold changes, as observed after irritation of the vagus nerve in rabbits and during botulinum intoxication.

Following the administration of botulinum toxin, the latter caused, at first, a decline in the threshold value of the vagus nerve irritation in most rabbits. But, after 2 to 3 hours following administration of the toxin, a decline of the vagus nerve irritation threshold value changed to an increase, thus, in order to obtain a respiratory inhibition, we had to decrease the range between the

Table 1

Changes in the Threshold Value of the Vagus Nerve Irritations in Rabbits Under the Influence of Botuline Intoxication (After Intravenous Administration of the Toxin)

Rabbit's number	Date of experiment	Prior to administration of the toxin	Irritation threshold of the vagus nerve (in cm ranges of coils after administration of the toxin)						
			In 10 to 30 minutes	In 1 to 1 1/2 hrs	In 1 1/2 to 2 hrs	In 2 to 2 1/2 hours	In 2 1/2 to 3 hours	In 3 to 4 hours	
9	Apr 7, 1954	31 1/2	15	16	12*)				
10	Apr 9	25	30	29	30	30	30	20*)	
11	Apr 10	14	16	25	30	17	17	12	5*)
12	Apr 15	26	29	32	28**)				
13	Apr 16	13	15	17	16	9#)			
15	Apr 22	16	16	20	21	20	20	16	16**
16	May 5,	19	19	22	17	13	13	4*)	
17	May 6	7	11	15	15	15	15	14*)	
25	May 22	14	17	16	16	16	16	16	16**

*) - The threshold value was determined to the last minutes of rabbit's life.

***) - The threshold value was not determined to the last minutes of rabbit's life.

coils. The irritation threshold reached in many experiments the initial magnitude.

Figure 1 - Changes in the functional condition of the respiratory center induced by botulinal intoxication in a rabbit No.11. A - Irritation threshold of the left vagus nerve prior to administration of botulinal toxin (14 cm range of coils); B - Irritation threshold of the left vagus nerve in 1½ hours after administration of botulinal toxin (30 cm range of coils); C - Irritation threshold of the left vagus nerve after 3 hours following administration of botulinal toxin (12 cm range of coils). Time mark 3 seconds.

We observed in some experiments, e.g. in rabbit No.9, a respiratory standstill without a prior decrease of the vagus nerve irritation threshold value.

At times, a respiratory standstill occurred in the presence of comparatively low threshold values of the vagus nerve irritation (rabbit No.17).

In some experiments (25, 11, 15) we noticed a distorted reaction following the irritation of the central end of the vagus nerve. Thus, 30 minutes after intravenous administration of botulinal toxin, the effect in a rabbit No.25, after irritation of the central end of the vagus nerve, was identical in the presence of

17 cm, as well as with the 19 cm range of coil. Then, 45 minutes after administration of the toxin, the effect after irritation of the central end of the vagus nerve was stronger in the presence of 20 cm range of coil, than with the 19 cm range of coil.

Subsequently, we run tests to determine a threshold value of the sciatic nerve up to and after administration of botulinal toxin.

Many authors described the respiratory reaction on irritation of the sciatic nerve by inductive current. The reaction produces depressed respiration and increased respiratory frequency. If the strength of current is increased, the respiratory delay is evident with the exhalation. According to literature data (N.A. MISLAVSKI et al.), this reaction, as well as the reaction on irritation of the vagus nerve, alternate according to the functional condition of the respiratory center. We conducted experiments on rabbits without narcosis. We placed platinum electrodes on the central end of the left sciatic nerve, transected in the hip. The irritations were produced with inductive current (storage battery 2 volts), and the irritation time was 6 seconds.

At first, after intravenous administration of botulinal toxin, the threshold value of the sciatic nerve irritation declined, as in the vagus nerve irritation. For example, prior to administration of botulinal toxin to rabbit No.31, the threshold value of the sciatic nerve irritation was equal to the 9 cm range of coils; after 30 minutes - to 12 cm, after 1 1/2 hours - to 15 cm and after 2 hours - to 10 cm. With respect to rabbit No.32, the threshold value of the sciatic nerve irritation was 10 cm prior to administration of

botulinal toxin; after 30 minutes - 15 cm and after 1 hour - 17 cm (see figure 2). It should be mentioned that the respiratory paralysis in rabbits of this series of experiments occurred, in most cases, with a declined threshold value of the sciatic nerve irri-

Figure 2 - Changes in the functional condition of the respiratory center effected by botulinal intoxication in rabbit No.32. A - Threshold value of the left sciatic nerve irritation prior to administration of botulinal toxin (10 cm range of coils); B - Threshold value of the left sciatic nerve irritation 1 hour after administration of botulinal toxin (15 cm range of coils).

tation, if compared with the threshold value noted prior to administration of the toxin.

Many authors (A.M. KORITSKII et al.) assume that the reason for the onset of respiratory disorders in botulism is the injury affecting the diaphragm. In order to verify this assumption, we performed experiments on rabbits (without narcosis) to determine a threshold value of the phrenic nerve irritation at various times after administration of botulinal toxin. The recording of respiration was effected with the aid of MAREY'S capsule connected to the tracheal tube. We dissected and transected in the neck the right phrenic nerve and we placed platinum electrodes on its pe-

ripheral end. The irritations were accomplished with current from induction coil (storage battery 2 volts).

We noticed during the experiments that the threshold value of the phrenic nerve irritation changed but slightly in the course of the entire investigation; even a few minutes before death of rabbits it remained almost the same as before the administration of botulinal toxin. We quote here one record pertinent to our experiments.

Record of January 26, 1955. A rabbit weighing 2,450 gm was tied to a bench at 10:15 o'clock. At 10:45, under local anesthesia, we dissected the trachea and inserted a tracheal tube; we dissected the right phrenic nerve in the neck.

At 11:16, we recorded a normal curve of respiration. At 11:20, the right phrenic nerve transected, its peripheral end ligated and its threshold value of irritation was determined at 15 cm range of coils, in accordance with du BOIS-REYMOND'S apparatus. At 11:53, we administered 4.9 mg of botulinal toxin into marginal vein of the ear. At 12:50, a pneumograph recording indicated an increase in the amplitude of respiratory movements and an increased respiratory frequency; the irritation of the peripheral segment of the right phrenic nerve produced its effect with 13 cm range of coils. At 14:04, the pneumograph recording indicated intensification of the inhalation and a decrease in the respiratory frequency; the irritation of the peripheral end of the phrenic nerve produced a clear intensification of the inhalation with a 14 cm range of coils. At 14:19, we noticed on the pneumogram a decrease in the amplitude of respiratory movements, also the appearance of isolated deep

inhalations and exhalations; the irritation of the peripheral end of the phrenic nerve produced intensification of the inhalation and a subsequent respiratory standstill with 13 cm range of coils. At 14:25, the amplitude of respiratory movements declined still further; the irritation of the peripheral segment of the phrenic nerve produced its effect with 14 cm range of coils. The respiration stopped at 14:30.

Thus, we almost detected an absence of changes in the excitability of the phrenic nerve during botulinal intoxication.

Subsequently, we conducted investigation of changes in the respiratory center after indirect administration of botulinal toxin into the myelencephalon, and after sprinkling the latter with botulinal toxin, as well as after a suboccipital administration of the toxin.

We conducted experiments on 15 rabbits narcotized by urethan. After dissection of the neck muscles, we cut open the atlantooccipital membrane. Botulinal toxin in a quantity of 0.1 ml (1 ml contained 1 mg of toxin) was administered 1 to 1.5 mm deep into the dorsal surface of the myelencephalon in the vicinity of obex. Other experiments included an exposure of the myelencephalon and sprinkling it with a solution of botulinal toxin (1 ml of solution contained 1 mg of toxin).

We performed some experiments for control purposes, in which we administered a physiological solution into the myelencephalon. The results of these series of experiments are included in Table 2.

Table 2
Changes in the Threshold Value of the Vagus Nerve Irritation Following Administration of the Botulism Toxin by Various Methods

Method of administration of toxin	Rabbit's number	Rabbit's weight in gm	Prior to administration of the toxin *)	Irritation threshold values						
				After administration of the toxin			Irritation threshold values			
				In 10 to 30 minutes	In 30 minutes to 1 hr	In 1 1/2 hrs to 2 hrs	In 2 to 2 1/2 hrs	In 3 to 4 hours		
Subcutaneous administration of botulism toxin	23	1,150	4	12	13	17	16	14	14	
	34	2,950	16	21	20	18	16	16	14	
	45	3,150	23	16	16	16	16	16	14	
Control with a physiological solution	50	1,950	13	12	13	13	13	13	14	
	43	3,000	13	12	0	1	5	0	0	
	37 46	1,160 2,000	13 15	10 13	1 10	1 10	10 10	10 10	11	
Control with a physiological solution	52	2,150	10	9	9	9	10	9	9	
	19	1,250	25	28	19	14				
	38 48	1,670 2,270	19 11	22 0	11 1	10 C				
Control with a physiological solution	51	1,750	15	14	15	15	15	14		

*) - The irritation time of nerves was 6 seconds.

It is evident from Table 3^{e)} that botulinal toxin administered directly into the myelencephalon causes noticeable changes in the functional condition of the respiratory center.

For example, we noticed in rabbit No.38 that, prior to administration of botulinal toxin, the threshold value of the respiratory center's excitability was equal to a 19 cm range of coils, after 30 minutes - to 22 cm and after 1 hour - to 10 cm. The sprinkling of myelencephalon with botulinal toxin also caused functional changes in the condition of the respiratory center. We observed in rabbit No.49 that, prior to sprinkling the myelencephalon with botulinal toxin, the threshold value of the respiratory center's excitability was equal to a 13 cm range of coils, then 1 1/2 hours later it attained the value of 1 cm range. Likewise, the suboccipital administration of botulinal toxin caused changes in the excitability of the respiratory center. We should add that in many experiments we observed the appearance of phasic phenomena in a parabolic stage in the respiratory center. Thus, in experiments on May 15 1954, we observed in rabbit No.23 that, 25 minutes after the suboccipital administration of botulinal toxin, the effect was much stronger with the irritation of the central end of the vagus nerve attained by way of a 12 cm range of coils, than with 10 cm. In experiment on October 8 1954 we observed in rabbit No.49 that 18 minutes after sprinkling the myelencephalon with botulinal toxin, the irritation effect on the central end of the vagus nerve was the

e) - Translator's Note: Table 3 is not included in the original report.

same at 11 cm, as at 12 cm range of coils.

As we mentioned before, some authors observing clinical material during botulism in connection with respiratory disorders linked the latter with injuries to diaphragm (A.M. KORITSKII) or with injuries to pulmonary capillaries (I.I. ZILBERG). Other investigators regarded injuries affecting the nerve centers as resulting from botulism. Nevertheless, we were unable to find in the literature any reports on experimental research that involved the mechanism of respiratory disorders during botulism.

The results of our experiments on animals confirmed, above all, the known facts that botulinal intoxication is accompanied by disorders in respiratory movements. Changes in the amplitude and in the frequency of respiratory movements in animals manifested themselves after 30 to 45 minutes following the administration of toxin. Death followed due to paralysis of the respiratory center.

The disturbances of respiratory movements in animals occurred simultaneously with changes in the threshold value of the vagus and sciatic nerves irritations. Our experiments involving the irritation of the central end of the vagus and sciatic nerves confirmed the determination of changes in the threshold value of irritations of the nerves discussed. Thus, the findings denote the changes in the functional condition of the respiratory center during botulism.

Hence, on the basis of the parabiologic phases (equalizing, paradoxical and retarding), detected by us in experiments No. 25, 11, 15 etc., we assumed that disorders in the functional condition of the respiratory center are the cause of the parabiologic changes in the center.

Some authors (L.G. TEREKHOVA, DAVIES et al.) used a method of the indirect effect of the stimulus on exposed myelencephalon for studying the functional condition of the respiratory center. Our experiments with a direct effect of botulinal toxin on myelencephalon (sprinkling with toxin, or administration of toxin into cerebral tissues) enabled us to observe more clearly a development of the paralytic phases.

The absence of changes in the magnitude of the threshold value in the stimuli of the phrenic nerve in botulinal intoxication indicates that the diaphragm function is not disturbed simultaneously and that the respiratory disorders during experimental botulinal intoxication are not combined with disturbances in the function of diaphragm. Consequently, our findings do not confirm the statements of some authors (BROOKS et al.) that botulinal toxin disrupts the conducting of stimulation through myoneural junctions.

Our investigations proved that, in the development of disorders in respiratory movements during botulinal intoxication, a disruption in the functional condition of the respiratory center plays its part.

Conclusions

1, Botulinal toxin causes (in animals) respiratory disorders, which, at first, manifest themselves by the increased respiratory frequency and by depression of respiration, later by a decreased frequency and by a decline in the amplitude of respiratory movements, particularly in a protraction of the exhalation action. Subsequently, a recurrent respiration ensues and the paralysis of the respiratory center follows.

2. As a result of administered botulinal toxin, changes in the reflex reaction of respiration affect the stimuli of the vagus nerve and, to some extent, those of the sciatic nerve, which is reflected in the excitability of the respiratory center during botulinal intoxication. Directly after administration of the toxin one observes an increased excitability in the respiratory centers. But, some time later, following administration of the toxin, a decline in the threshold value of the respiratory center's excitability changes to an increase. Then, one can observe paralytic phase phenomena in the respiratory center.

3. The excitability of the phrenic nerve changes slightly during botulinal intoxication.

4. The disorders in respiratory movements of animals during botulinal intoxication are combined with a development of the paralytic condition in the respiratory center.

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