BIOGENIC AMINE CONCENTRATIONS AND PATHOMORPHOLOGICAL CHANGES IN ORGANS AND TISSUES DURING HEMATOGENOUS SPREAD AND FIXATION OF BOTULINUS TOXIN

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Experiments on cats showed that 30 min after intravenous injection of type C botulinus toxin the concentrations of catecholamines and histamine were reduced and the serotonin level increased in various structures of the brain and spinal cord and in some of the internal organs. Changes in metabolism of biogenic amines were combined with definite pathomorphological changes, namely acute swelling, chromatolysis, and death of single spinal cord and brain neurons, degenerative changes in the internal organs, and increased permeability of tissue-blood barriers. The marked biochemical and pathomorphological changes in the spinal cord and brain, where only minimal concentrations of the toxin were detected during the period of its spread, suggest that the pathogenic action of botulinus toxin is effected through a disturbance of the metabolism of biologically active compounds.

KEY WORDS: botulinus toxin; biogenic amines; pathmorphology.

Efforts by Soviet and other pathophysiologists to solve problems in the pathogenesis of botulism have been aimed mainly at studying the functional activity of different components of spinal reflect arcs [2-4, 7, 10, 11]. Meanwhile information is very limited on the metabolism of biologically active compounds — catecholamines, histamine, and serotonin — the basic regulators of permeability of tissue-blood barriers, modulators of acetylcholine liberation, chemical transmitters of nervous excitation, and so on.

In a previous communication it was stated [6] that the appearance of clinical manifestations of botulism is associated with marked disturbances of biogenic amine metabolism, which considerably potentiate the lethal effect of the toxin.

No solution has yet been found to the problem of by what mechanism high-molecular-weight botulinus toxins, which are protein in nature and do not possess sufficiently high pathogenic enzyme activity, can penetrate through the tissue–blood barriers and, in particular, the blood–brain barrier and, having accumulated in minimal concentration in nerve tissue, can exert a maximal neurotropic action. In the study of the metabolism of biologically active compounds during the period of hematogenous spread and fixation of botulinus toxin in the tissues, the answers to these questions can be obtained.

EXPERIMENTAL METHOD

Experiments were carried out on cats poisoned by intravenous injection of type C botulinus toxin in a dose of 2.5 mg/kg and on appropriate controls. Various morphological and biochemical investigations were carried out 30 min after injection of the toxin. The catecholamine concentrations were determined in various structures of the nervous system and internal organs by the fluorometric trihydroxyindole method [1], the histamine concentration was determined spectrofluorometrically with orthophthalic aldehyde [12], and serotonin was determined, also by a spectrofluorometric method, using ninhydrin [9]. In parallel experiments pieces from various organs were fixed in 10% formalin solution and in Carnoy's fluid. Paraffin sections were stained with hematoxylin-eosin. Polysaccharides and glycogen were revealed by the PAS reaction [5]. Mast cells were counted and the degree of chromatolysis assessed in sections of the duodenum and various parts of the nervous system stained with toluidine blue. When the

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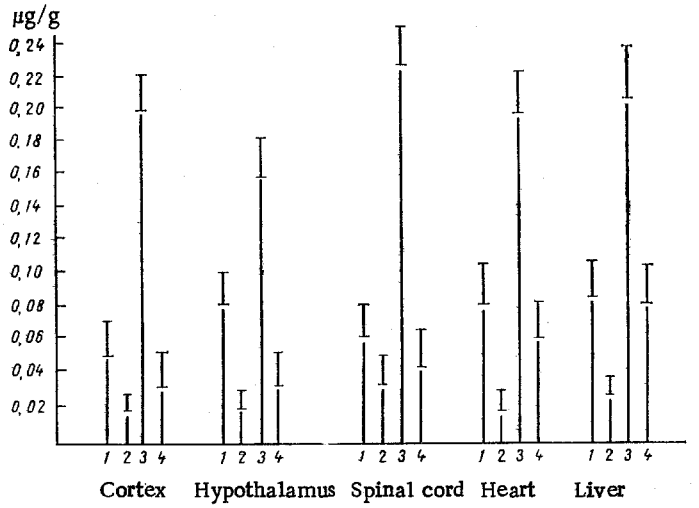

Fig. 1. Catecholamine concentrations in organs of cats 30 min after injection of botulinus toxin. Adrenalin: 1) control, 2) poisoning; noadrenalin: 3) control; 4) poisoning. Changes in concentrations of catecholamines were similar to those shown in the cerebellum, sciatic nerves, gastrocnemius muscles, and kidneys during botulinus poisoning. Ordinate, concentration of catecholamines (in μg/g).

mast cells were counted in the muscular coat of the duodenum the degree of their differentiation into immature, mature, degranulating, and disintegrating was taken into account. This appearance of the mast cells also was estimated quantitatively [8].

EXPERIMENTAL RESULTS

The experiments showed that 30 min after injection of the toxin the concentrations of adrenalin and noradrenalin fell sharply in the cortex, hypothalamus, spinal cord, heart, and liver (Fig. 1). Simlar changes took place in the cerebellum, sciatic nerve, gastrocnemius muscles, and kidneys ($P<0.001$). Only the noradrenalin concentration in the adrenals fell, and the adrenalin concentration remained unchanged. Meanwhile the histamine level fell in the cortex, hypothalamus, sciatic nerves, muscle, small intestine, lungs, heart, kidneys, and blood. Only in the liver, medulla, and spinal cord was the histamine concentration the same as in the control. Unlike catecholamines and histamine, the serotonin concentration rose sharply both in the certain internal organs and in various parts of the nervous system (Table 1).

To determine the specificity of changes discovered in the biogenic amine level control experiments were carried out in which botulinus toxin, inactivated by boiling, was injected in the same doses and dilutions as in the experimental group. As the results showed, injection of boiled toxin had no effect on the catecholamine concentration in the tissues. The concentrations of histamine and serotonin either were unchanged or did not change significantly.

The next step was to compare the disturbances in the biogenic amine concentrations in the tissues thus revealed with their pathomorphology. The greatest changes were found in various parts of the brain. For instance, in the motor cortex severe swelling of individual neurons and lysis of their nuclei were observed. Perinuclear translucency and homogenization of the cytoplasm of other cells were seen, with disappearance of Nissl granules and absence of nucleoli. The changes were maximal in the layer of pyramidal cells. The brain substance was anemic, and solitary fresh perivascular hemorrhages were seen.

Marked edema of the white matter, anemia of the vessels, and dilatation of the perivascular spaces were found in the medulla. Chromatolysis and diffuse basophilia of the cytoplasm developed in most nerve cells. The nuclei in some cells could not be seen or their outlines were indicated.

Some neurons in the hypothalamus were swollen, with indistinct outlines, absence of nuclei, and a marked picture of chromatolysis. Other nerve cells were shrunken, they stained

TABLE 1. Concentrations of Histamine and Serotonin, in μg/g, in Organs and Tissues of Cats 30 min after Injection of Botulinus Toxin (M±m)

Organs, tissues	Histamine		Serotonin	
	control	poisoning	control	poisoning
Cerebral cortex	0,45±0,02	0,28±0,01*	0,80±0,28	2,2±0,28*
Hypothalamus	0,71±0,05	0,31±0,01*	1,72±0,18	3,5±0,39*
Medulla	0,37±0,05	0,49±0,06	1,23±0,14	2,2±0,21*
Spinal cord	0,44±0,03	0,48±0,05	1,50±0,15	3,5±0,34*
Sciatic nerve	1,84±0,21	0,93±0,012*	1,91±0,26	3,3±0,33*
Gastrocnemius muscle	1,02±0,11	0,43±0,01*	1,13±0,22	1,7±0,11*
Heart	1,27±0,13	0,50±0,05*	1,15±0,15	1,9±0,14*
Lungs	18,21±1,06	4,10±0,48*	2,10±0,21	8,7±0,50*
Liver	0,82±0,10	0,62±0,07	2,11±0,16	7,2±0,5*
Kidneys	0,66±0,8	0,37±0,02*	1,72±0,23	7,5±0,80*
Small intestine	16,47±1,27	5,30±0,31*	2,92±0,31	10,2±0,66*
Blood	0,187±0,027	0,12±0,006*	0,24±0,017	0,46±0,02*

Legend. 10-15 animals used in each series of experiments. *) $P<0.05$.

diffusely with basic dyes, and their nuclei were pycnotic or indistinguishable. The small vessels and capillaries were in a state of marked congestion.

Marked edema was present in the white matter of the spinal cord, with numerous tiny cavities. Narrow spaces also were formed around single neurons in the gray matter. The small vessels were anemic, their lumen frequently in a state of collapse. The bodies of the motor neurons were swollen, with marked signs of chromatolysis. Tigroid was absent in some cells, the outlines of the nuclei were hardly visible, and in some cells they were invisible.

Hence, in all parts of the nervous system a definite response of the nerve cells could be seen in the form of acute swelling and chromatolysis, proceeding to death of the neurons in some cases accompanied by increased vascular permeability, cerebral edema, and perivascular hemorrhages. Functional changes in the vessel (ischemia of the capillaries and congestion of the veins) also were noted. In some of the internal organs similar functional disorders of the vascular system and also degenerative changes were seen. Some of the features observed were indirect evidence of increased permeability of the tissue–blood barriers and, in particular, the appearance of erythrocytes and proteins in the lumen of individual Shumlyanskii's capsules and convoluted tubules of the kidneys, the presence of hemolyzed erythrocytes and protein masses in the lumen of the pulmonary alveoli.

The PAS reaction failed to reveal any marked changes in the glycogen contents in the skeletal muscle, heart, liver, and kidneys. However, in different parts of the nervous system, besides swelling, chromatolysis, and disappearance of the nuclei, PAS-positive material appeared in many of the neurons, further evidence of the development of degenerative changes in the early stage of poisoning, long before the appearance of its clinical manifestations. Counting the number of mast cells revealed sharp increase in disintegrating (from 8.6 to 32%, $P<0.001$) and degranulating forms (from 12.3 to 27.7%, $P<0.001$). The number of disappearing mast cells was 59.4%.

The investigations thus showed a definite parallel between the biochemical and pathomorphological disturbances arising in the period of spread and fixation of botulinus toxin in the tissues, long before the development of clinical manifestation of poisoning. These findings suggest that the pathogenic action of botulinus toxin is effected through a disturbance of metabolism of biologically active compounds, which evidently lead to an increase in the permeability of the tissue–blood barriers, so that high-molecular-weight fractions of the toxin, which do not possess the necessary enzymic activity for this purpose, can penetrate into the tissues.

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HARMFUL EFFECTS OF MICROWAVE (2400 MHz) IRRADIATION ON RATS AND SUBSEQUENT RECOVERY

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Threshold values of the power density (PD) and duration of microwave irradiation with a lethal effect of not more than 0.1% were determined in experiments on 2072 rats. The ratio between the rate of development of the harmful effect and recovery is close to an exponential function of PD of microwave irradiation. From earlier observations on mice and those now published, species differencies between mice and rats can be accepted. As regards the time of appearance of equal lethal effects, the half- and whole-recovery periods, and the ratio between the rates of injury and the rate of recovery depending on PD of microwave irradiation, mice are more sensitive than rats.

KEY WORDS: injury; recovery; species sensitivity; microwave irradiation.

Pathophysiological changes arising in rats during intensive microwave irradiation, causing death of the animals, have already been investigated experimentally [1, 2, 6, 7, 9]. The conditions of irradiation have been shown to influence the time of onset of death [8].

In the investigation described below a further study was made of the general tendencies of formation of lethal processes and recovery and the ratio between them in rats, and to compare these findings with results obtained previously [3, 5] on mice.

EXPERIMENTAL METHOD

The experiments were carried out on 2072 noninbred female rats with a mean weight of 220±12 g. The animals were irradiated in an anechoic chamber with microwaves (2400 MHz) with a power density (PD) of between 60 and 800 mW/cm^2, and in an ambient air temperature of 20-22°C. The inequality of the experimental microwave field did not exceed 2 dB. The mortality of the animals was studied. Empirical distributions were expressed algebraically.

EXPERIMENTAL RESULTS

Within the limits of the PD studied (60-800 mW/cm^2) the empirical distributions of lethal effects on the rats depending on the duration of microwave irradiation (Fig. 1) can be described sufficiently closely by equations of the type (probit analysis): $Y = 10.5566 \cdot X + 6.0070$; $Y = 20.3105 \cdot X + 2.0128$; $Y = 69.7311 \cdot X + 20.7204$; $Y = 20.4726 \cdot X - 6.6568$; $Y = 15.6766 \cdot X - 8.6621$; $Y = 15.2329 \cdot X - 14.1035$; $Y = 18.1849 \cdot X - 22.7886$, where Y is the lethal effect (in probits), and X the log of the irradiation time (in min). Just as in mice [5], with a decrease in PD the time taken to reach a prescribed effect was increased and the angle of slope of the distributions reduced. The existence of these two tendencies determines the exponential character of the relationships between PD and the duration of microwave irradiation for prescribed effects, for example 0.1, 50, and 99.9%: $\log Y = 2.6338 - 0.6918 \log X$; $\log Y = 2.7790 - 0.6741 \log X$; $\log Y = 2.9257 - 0.6549 \log X$ respectively, where log Y denotes PD (in mW/cm^2) and log X denotes the duration of microwave irradiation (in min). Analysis of these

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