CHANGES IN THE ACETYLCHOLINE – CHOLINESTERASE SYSTEM IN THE COURSE OF EXPERIMENTAL BOTULISM

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UDC 616.981.553-092.9-092-07:616-008.948+616-008.931:577.152.311

After injection of type B botulinus toxin into rabbits in a dose of 5000 MLD/kg body weight the acetylcholine content rises both in the CNS (cerebral cortex, spinal cord) and in peripheral organs and tissues (blood, muscles, lungs, diaphragm). After injection of a dose of 25 MLD/kg body weight (the minimal lethal dose) the acetylcholine content rises only in the peripheral organs and tissues. Cholinesterase activity is virtually unchanged during the poisoning. It is postulated that the changes in the acetylcholine–cholinesterase system take place because of the effect of the toxin on the secretion of acetylcholine.

KEY WORDS: botulinus toxin; acetylcholine; cholinesterase; brain and muscle tissue and blood; secretion of mediators.

Since the transmission of nervous impulses is closely dependent on acetylcholine (AC) metabolism, the action of various factors on the nervous system may be reflected in one of the stages of AC metabolism. Synthesis of the mediator, its hydrolysis, or its liberation from the bound form may be altered. Botulinus toxin (BT) has been found to damage mainly the cholinergic nervous system [1, 5, 8], and to block transmission from the nerve to the effector organ. However, views of invesigators on the effect of BT on individual stages of AC metabolism are contradictory. There is evidence both of inhibition of cholinesterase (CE) [12] and also of the absence of this effect [15, 18]. There is likewise no unanimity regarding the changes in the AC content in the organs and tissues during botulinus poisoning (BP). Some workers state that the AC content in the tissues is increased [10, 13, 14], others that it is reduced [11].

An investigation was accordingly carried out to study the relationship between the changes in two indices of AC metabolism – the AC content and CE activity in the organs and tissues – during experimental BP.

EXPERIMENTAL METHOD

Experiments were carried out on 84 rabbits of the chinchilla breed weighing 2.5-3 kg. Type B BT, containing 100,000 MLD for albino mice per milligram of the dry substance, was injected intravenously (into the marginal vein of the ear) in doses of 5000 and 25 MLD/kg body weight, causing death of the animals 3 and 96 h later, respectively.

The CE activity of the blood, cerebral cortex, spinal cord, striated muscles, diaphragm, and lungs was determined by Hestrin's method in Panyukov's modification [3]. The AC content was determined at the same time in the same organs and tissues. AC was extracted by Crossland's method [6] and determined by Barletta's method [4]. The results were subjected to statistical analysis and differences were considered to be significant for which $P < 0.05$ [2].

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 1, pp. 25-27, January, 1976. Original article submitted October 7, 1974.

TABLE 1. AC Content and CE Activity in Blood and Tissues of Rabbits Following Injection of a Dose of Toxin Causing Death in 3 h (M ± m)

Test object	Control	Time after injection of toxin (in min)		
		60	120	180
Blood	60,0±4,4 / 10,1±0,6	93,0±5,1 / 7,5±0,5	123,4±7,1 / 12,7±1,0	160,3±16,2* / 7,6±0,5
Brain	37,3±1,2 / 63,9±3,8	40,0±1,5 / 62,5±9,0	54,3±4,1 / 75,0±5,2	68,0±6,7* / 56,0±5,3
Spinal cord	35,0±2,6 / 41,6±2,7	34,3±1,5 / 37,3±1,2	54,1±2,8* / 45,5±3,3	56,3±4,6* / 40,2±2,7
Muscles	76,2±1,6 / 5,1±0,7	84,3±2,9 / 5,2±0,8	101,4±5,1* / 5,3±0,3	120,2±8,4* / 4,2±0,5
Diaphragm	70,4±2,1 / 5,6±0,6	80,1±2,2 / 4,9±0,9	93,2±4,0* / 5,0±0,8	102,4±6,0* / 4,4±0,6
Lungs	46,2±1,9 / 7,9±0,6	50,0±2,0 / 6,1±0,9	59,2±2,4 / 7,8±0,9	68,1±4,1* / 7,3±0,4

Legend: Here and in Table 2, numerator shows acetylcholine content in blood (in μg %) and tissues (in μg/g); denomenator shows cholinesterase activity in mg AC/g tissue/h. Data for which $P < 0.05$ are marked by an asterisk.

TABLE 2. AC Content and CE Activity in Blood and Tissues of Rabbits Following Injection of the Minimal Lethal Dose of Toxin (M ± m)

Test object	Control	Time after injection of toxin (in h)			
		24	48	72	96
Blood	62,1±3,1 / 10,0±0,9	66,6±2,9 / 7,8±0,8	81,0±3,6 / 7,6±1,2	100,0±6,0* / 12,2±1,2	150,0±10,0* / 12,8±1,1
Brain	34,2±2,1 / 58,8±2,8	35,1±1,8 / 56,8±3,8	40,2±2,1 / 61,0±1,9	36,1±2,8 / 76,8±4,8	42,4±2,3 / 54,2±2,2
Spinal cord	30,4±1,3 / 41,5±1,9	31,2±1,5 / 37,8±2,2	35,0±1,6 / 50,9±3,4	36,2±1,9 / 45,3±0,7	34,3±1,4 / 49,1±0,7
Muscles	74,2±1,4 / 5,2±0,8	77,3±1,4 / 3,8±0,7	84,2±1,7 / 2,7±0,2	94,2±2,9* / 1,4±0,5*	105,4±5,4* / 1,3±0,5*
Diaphragm	71,0±1,7 / 5,0±0,5	78,1±2,0 / 4,2±0,5	65,0±2,1 / 4,0±0,4	98,2±3,2* / 2,1±0,3*	100,1±4,3* / 2,0±0,4*
Lungs	44,3±1,8 / 7,5±0,8	44,4±1,9 / 7,6±1,2	48,2±2,0 / 5,9±1,0	55,1±2,3 / 6,8±1,2	64,3±1,6* / 7,1±1,4

EXPERIMENTAL RESULTS

As the data in Table 1 show, in the course of development of BP the AC content in the organs and tissues of the rabbits rose gradually to reach a maximum at the time of the animal's death. The AC content increased both in the CNS and in the peripheral organs and tissues. However, there was no change in the CE activity.

Changes in CE activity and in the AC content after injection of the minimal dose of toxin to cause death of the animal differed somewhat from the changes following injection of a dose causing death after 180 min (Table 2). An increase in the AC content was observed only 72 h after injection of BT; no change could be found, moreover, in its content in the brain and spinal cord. The changes in CE activity also showed special features: a decrease in the striated muscles and diaphragm from the third day after injection of BT.

The AC content in the tissues could be increased both after inhibition of CE, the enzyme destroying AC, and also during an increase in the rate of synthesis or a disturbance of the processes of liberation of AC. These experiments show that BT does not affect CE activity. The exception is the CE of striated muscles and the diaphragm. However, after local injection, BT led to a decrease in CE activity of the muscles on account of its denervating action [7, 18]. This effect evidently continued to be observed also if the toxin was injected intravenously. The increase in the AC content likewise could not be the result of an increase in the rate of its synthesis. Investigations by several workers have shown that the toxin not only does not increase the rate of AC synthesis [9, 17] but, according to some evidence, it may actually inhibit it [19].

The results thus show that in botulinus poisoning the disturbance of AC metabolism takes place mainly through interference by the toxin in the process of secretion of the mediator. Certain differences were found between the action of large and small doses of the toxin on AC metabolism. In a dose of 5000 MLD the toxin increased the AC content both in the CNS and in the peripheral organs, whereas in a dose of 25 MLD it did so only in the peripheral organs and tissues.

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