DETERMINATION OF CEREBROSIDES IN DIFFERENT PARTS OF THE BRAIN OF RABBITS SUFFERING FROM GENERALIZED TETANUS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 4, pp. 66-70, April, 1961
Original article submitted April 14, 1960

The study of the cerebrosides of the brain has led to the suggestion that these substances may, in certain functional states of the central nervous system, serve as energy-producing substrates for the brain [1, 2, 3]. We have advanced this as an explanation of the fall in cerebroside content of the brain of rabbits in a state of generalized tetanus [4].

In order to ascertain whether these changes affect the whole brain, or parts of it only, we determined the cerebroside content separately for the left and right hemispheres, the cerebellum, the brain stem, cortical grey and white matter, and the subcortical formations of the cerebral hemispheres of the brains of rabbits in health and in generalized tetanus.

METHODS

A lethal dose of tetanus toxin was injected into the left hind leg of rabbits. The animals were sacrificed 7-8 days later, when they were in a pre-agonal state. The brain was washed free of blood, and was dissected into its various parts.

These were all treated in the same way. The tissues were ground up with trichloroacetic acid, the homogenate was centrifuged, the sediment of proteins and lipids was washed with distilled water, and then extracted in a Soxhlet apparatus. Extraction was performed sequentially with acetone, ether, and 1:1 chloroform-methanol mixture, extracting for 12 hours in each case.

The residue after elimination of the solvents was hydrolyzed with 10% sulfuric acid for 2 hours on a boiling water bath. Galactose was determined in the hydrolyzate by the anthrone method. The cerebroside content was derived from the galactose content thus found. In all cases we express the galactose content as percentages of the dry protein content of the given material, this being the most stable reference basis for this type of experiment.

Parallel determinations of galactose were made for material from normal animals and for those in a state of generalized tetanus. We found considerable individual variations between the galactose contents of the same parts of the brain, even for normal animals. The widest variations were found for the grey and the white matter of the cortex. Fairly standard results were, however, obtained for the cerebroside content of the whole brain of normal animals [4].

TABLE 1

Cerebroside Galactose Content of Rabbit Cerebellum

Wt. of galactose (mg)	Protein content (mg dry weight)	Galactose content (as % dry protein content)
	Normal	
3,40	115,2	2,94
2,83	115,6	2,44
3,00	138,8	2,16
2,33	93,2	2,50
2,86	127,1	2,25
2,77	100,1	2,78
3,37	125,4	2,68
	Mean	2,53
	Tetanus	
3,10	107,9	2,87
2,99	96,8	3,09
3,60	152,4	2,36
3,42	129,6	2,63
2,80	102,7	2,72
3,12	109,1	2,85
3,86	138,8	2,78
	Mean	2,75

TABLE 2

Cerebroside Galactose Content of the Brain Stem of Rabbits

Galactose (mg)	Protein content (mg dry wt.)	Galactose (as % of dry protein content)
	Normal	
9,50	254,4	3,73
7,00	217,4	3,22
8,70	243,2	3,55
8,25	214,9	3,83
9,66	241,0	4,08
8,58	183,7	4,67
Mean		3,85
	Tetanus	
10,00	250,2	3,90
8,24	224,3	3,67
6,00	159,1	3,77
7,92	198,1	3,99
7,50	168,7	4,44
9,40	239,6	3,92
6,84	179,2	3,81
Mean		3,92

TABLE 3

Cerebroside Galactose Content of the Cerebral Hemisphere of Rabbits

Right hemisphere			Left hemisphere		
galactose (mg)	protein content (mg dry weight)	galactose (as % of dry protein content)	galactose (mg)	protein content (mg dry weight)	galactose (as % of dry protein content)
		Normal			
6,24	300,6	2,07	5,74	302,5	1,89
6,60	296,2	2,25	6,60	283,2	2,33
6,84	306,1	2,23	7,06	294,3	2,29
6,74	304,5	2,21	7,00	306,6	2,33
6,12	274,8	2,22	6,48	278,9	2,32
5,76	263,3	2,18	5,76	231,7	2,48
6,00	277,9	2,15	5,62	284,5	1,97
Mean		2,18	Mean		2,22
		Tetanus			
6,00	343,5	1,74	6,98	327,2	2,11
4,38	262,3	1,66	5,40	264,1	2,04
5,75	287,4	2,00	6,12	263,6	2,32
4,90	279,4	1,74	6,56	275,0	2,38
4,70	261,4	1,71	5,72	258,5	2,21
5,46	278,4	1,95	6,26	278,0	2,25
4,43	255,0	1,73	4,70	227,9	2,06
Mean		1,79	Mean		2,19

TABLE 4

Cerebroside Galactose Content of the Gray Matter of the Cortex of the Cerebral Hemispheres

Galactose (mg)	Protein content (mg dry wt.)	Galactose (as % of dry protein content)
	Normal	
2,90	298,4	0,97
1,06	103,0	1,02
1,02	122,4	0,83
1,00	88,2	1,13
1,80	101,0	1,78
2,25	203,2	1,10
2,70	183,4	1,46
Mean		1,18
	Tetanus	
1,24	113,9	1,08
1,24	107,5	1,15
2,70	290,4	0,90
2,30	244,7	0,93
3,60	240,6	1,49
2,88	206,4	1,39
2,25	253,0	0,88
Mean		1,12

TABLE 5

Cerebroside Galactose Content of the White Matter of the Cortex of the Cerebral Hemispheres

Galactose (mg)	Protein content (mg dry wt.)	Galactose (as % of dry protein content)
	Normal	
3,50	44,0	7,90
3,80	49,9	7,65
3,84	46,9	7,16
2,19	26,0	8,42
2,00	27,6	7,24
1,55	19,4	8,01
3,13	32,2	9,66
Mean		8,01
	Tetanus	
3,46	40,7	8,50
3,24	34,6	9,30
1,55	14,0	11,70
1,25	16,5	7,50
1,15	10,6	10,80
1,96	16,2	12,00
1,45	21,1	6,83
Mean		9,52

TABLE 6

Cerebroside Galactose Content of the Subcortical Formations of the Cerebral Hemispheres

Galactose (mg)	Protein content (mg dry weight)	Galactose (as % of dry protein content)
	Normal	
5,14	247,8	2,08
7,23	302,5	2,36
9,39	317,3	2,95
7,99	375,3	2,17
7,78	385,9	2,02
8,82	370,8	2,37
6,66	359,0	1,85
Mean		2,25
	Tetanus	
5,70	367,2	1,55
6,30	397,3	1,58
4,80	277,3	1,73
4,65	303,0	1,53
5,25	390,5	1,34
5,55	397,4	1,39
5,55	340,7	1,62
Mean		1,53

RESULTS

The galactose, and hence the cerebroside, content of the cerebellum of rabbits in a state of generalized tetanus was not found to differ from that of normal rabbits (Table 1).

The data of Table 2 show that the cerebroside content of the brain stem of normal and tetanic animals was the same in both cases.

Analyses of the cerebral hemispheres showed that the cerebroside content was smaller in tetanic than in normal rabbits, and that the difference was greater for the right hemisphere (Table 3).

It should be noted that the lethal dose of toxin was administered by injection into the left hind leg.

It follows that the cerebral hemisphere contralateral to the site of injection is affected more by the toxin than is the ipsilateral one.

In the next series of experiments we analyzed anatomically different parts of the cerebral hemispheres. The data of Table 4 show that the cerebroside content of cortical gray matter was the same in normal rabbits and in those with generalized tetanus.

A rise in the cerebroside content of cortical white matter was found in a number of cases in the experimental animals, amounting on the average to 18.6% (Table 5).

The most clear-cut results were obtained for the subcortical formations of the cerebral hemispheres (Table 6). In all the experiments the cerebroside content was lower by an average of 32% than in the normal rabbits.

It may be concluded from our findings that a state of generalized tetanus leads in rabbits to a considerable depletion of the cerebroside content of the subcortical formations of the cerebral hemispheres. This effect was more marked in the hemisphere contralateral to the site of injection of toxin.

SUMMARY

The cerebroside content of the subcortical formations of the cerebral hemispheres of rabbits falls following injection of a lethal dose of tetanus toxin into a hind leg to a minimum on the 7-8th day, when the animals are moribund. This effect is more pronounced in the hemisphere contralateral to the injection site.

LITERATURE CITED

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
