DYNAMICS OF SPINAL CORD CHANGES PRODUCED BY PRENATAL ASPHYXIA

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Changes in neurons and nerve fibers of the spinal cord at the level C4-C5 were studied in rats exposed to asphyxia *in utero* and killed at different times. In the first 10 days the changes in the nerve cells progressed gradually. By the age of 1-2 months the state of most neurons is back to normal, but in each hundred fields of vision pathologically changed neurons are found, mainly in zones of the collateral circulation. Changes also are observed in the nerve fibers.

KEY WORDS: *spinal cord; prenatal asphyxia; postnatal ontogeny; nerve fibers; nerve cells.*

Acute and chronic oxygen deprivation of embryos and fetuses disturbs brain development [4-7]. Asphyxia causes a disturbance of the cerebral hemodynamics and biochemical changes in nerve tissue which, in turn, play an important role in the formation of the sequelae to prenatal asphyxia [2].

The object of this investigation was to examine the effect of severe general asphyxia in the prenatal period on the development of nerve cells and fibers of the spinal cord.

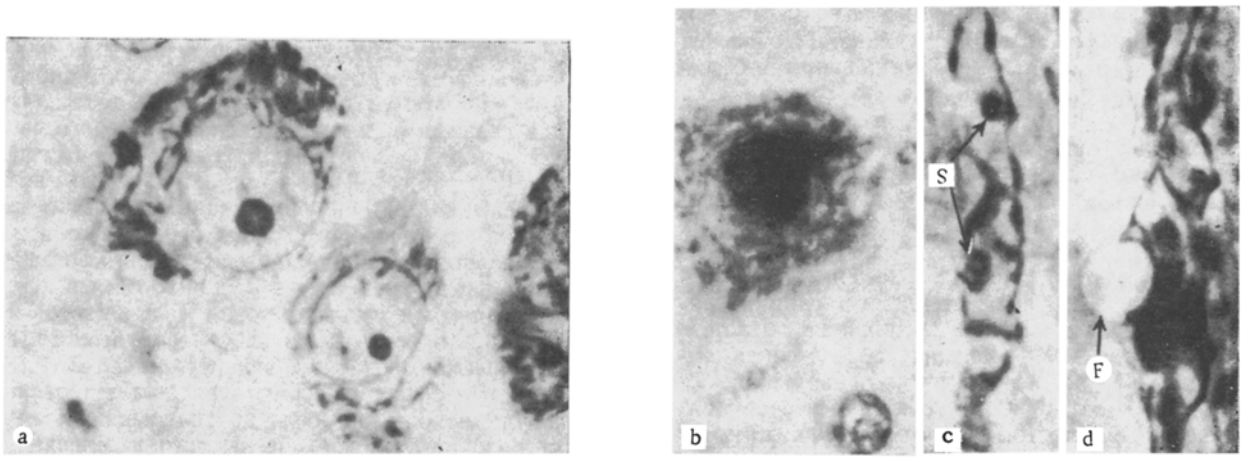

Fig. 1. Motoneurons of ventrolateral group and nerve fibers of ventral columns of spinal cord at level C4-C5 of rats aged 10 days (a) and 1 month (b, c, d) and exposed to asphyxia *in utero:* a) swelling of nuclei, decrease in size of nucleoli, disturbance of formation of Nissl's substance, partial clearing of cytoplasm; b) severe changes in nerve cell — shrunken hyperchromic nucleus merged with outlines of nucleolus; scattering of tigroid and loss of cell boundary; c) uneven distribution of myelin as spheres (S) and vacuolation of nerve fibers (F). a, b) Stained by Nissl's method, 400×; c, d) stained by Kulchitsky–Wolter method, 900×.

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EXPERIMENTAL METHOD

Albino rats were used. Asphyxia was induced in embryos at the 16th-19th day of development by compression of the umbilical vessels without removal of the fetuses from the mother [3]. The depth of asphyxia was judged from the ECG. In each case a decrease in the fetal heart rate from 180-200 to 20-25 per minute was awaited. The period of asphyxia required for this purpose varied from 18 to 46 min. Part of the experimental material was taken 3 h after asphyxia, otherwise the rats were born at term, on the 20th-21st day of pregnancy. The young rats were killed during the first day after birth, on the fifth and tenth days, and also at the age of 1 and 2 months.

The spinal cords of 58 control and 61 experimental animals were studied. Continuous series of longitudinal and transverse sections through the cervical, thoracic, and lumbar divisions of the spinal cord were stained by Nissl's method, with hematoxylin-eosin, and by the Feulgen and Kulchitsky–Wolter methods.

Areas of cross section of the bodies of the nerve cells and their nuclei and nucleoli and also the nucleo-cytoplasmic ratio were calculated for motoneurons in the nucleus of the phrenic nerve, cells of the ventrolateral group, and cells of the intermediate zone at the level of C4-C5. From 30 to 50 nerve cells were counted in each cell group.

EXPERIMENTAL RESULTS

No marked changes were observed in the nerve cells of the spinal cord 3 h after asphyxia. However, in the experimental young rats taken on the first day after birth, slight swelling of the nuclei and a decrease in the cytoplasm to nucleus ratio could be seen in motoneurons 3-4 days after asphyxia. The small cells were indistinguishable from cells in control animals (Table 1).

By the age of 5 days the swelling of the cell nuclei had increased in degree, the area of cross section of the motoneurons in the phrenic nerve nucleus and the ventrolateral group was reduced and, at the same time, there was a slight decrease in the size of the nucleolus of the cells of the ventrolateral group. In the small cells of the intermediate zone swelling of the nuclei also was observed and the Nissl's substance was visible only at the periphery of the cell body. Similar changes also took place in the remaining nerve cells of the gray matter of the spinal cord (Table 1).

By the age of 10 days the nuclei of most cells still remained swollen, the nucleoli were reduced in size, and the neurons were smaller than in the control animals; Nissl's substance could be seen only at the periphery of the cell body in clumps. In some small cells no masses of tigroid whatsoever could be seen, the cytoplasm was stained homogeneously, and some part of it appeared optically empty (Table 1; Fig. 1a). At this age for the first time dying small nerve cells could be seen.

By the 30th and 60th days of life the size and structure of the overwhelming majority of spinal neurons were returning to normal and were almost the same as in the control animals. However, in every hundred fields of vision there were 5.5% of pathologically changed large nerve cells, 10.5% of similarly changed medium-sized cells and 21.6% of pathologically changed small cells. In these cells scattering of the tigroid, endocellular edema, a decrease in size and pale staining of the nucleolus, and shrinking of the nucleus were observed. In some cells there was vacuolation of the cytoplasm and dendrites; some cells showed changes indicative of a severe degree of damage, with shrinking and intense staining of the nucleus and an indistinguishable nucleolus. Some ghost cells were observed (Table 1; Fig. 1b).

At this age the pathologically changed nerve cells were not yet scattered throughout the cross section of the spinal cord, as in the younger animals, but were seen mainly at the periphery of the gray matter and in the intermediate zone. These areas are zones of collateral circulation. They lie at the junction between the territories of distribution of the arteries of the gray and white matter and of the central arteries and arteries of the posterior horns.

On examination of sections through the spinal cord of animals aged 1 and 2 months stained by the Kulchitsky–Wolter method, vacuolation and an irregular distribution of myelin in the form of spheres were observed in some nerve fibers, evidence of disintegration of the myelin sheath. Abnormal nerve fibers were not localized in particular conducting systems of the spinal cord (Fig. 1c, d).

TABLE 1. Changes in Area of Cross Section of Cell Bodies, Cytoplasm to Nucleus Ratio, and Area of Nucleoli in Spinal Cord at Level C4 during Development of Control and Experimental Animals (M ± m)

Test object	Group of cells	Area of cross section of cell body			Cytoplasm to nucleus ratio			Area of cross section of nucleolus		
		control	experiment	P	control	experiment	P	control	experiment	P
Embryo	Motoneurons of phrenic nerve nucleus	200,0±6,2	212,0±6,7	>0,5	1,45±0,03	1,42±0,06	>0,05	5,00±0,29	5,00±0,30	—
	Cells of ventrolateral group	264,0±10,5	259,0±13,1	>0,5	1,77±0,06	1,87±0,06	>0,5	5,50±0,22	5,12±0,35	>0,5
	Cells of intermediate zone	110,0±4,3	114,0±3,4	>0,5	1,32±0,02	1,35±0,02	>0,5	3,00±0,30	3,10±0,13	>0,5
Newborn animals	Motoneurons of phrenic nerve nucleus	372,0±10,2	326,0±11,6	<0,02 >0,01	2,77±0,15	2,24±0,08	<0,001	7,04±0,03	6,75±0,03	<0,001
	Cells of ventrolateral group	300,0±7,6	312,0±12,9	>0,5	2,78±0,12	2,40±0,11	<0,02 >0,01	7,50±0,3	7,79±0,4	<0,001
	Cells of intermediate zone	125,6±4,4	121,0±4,6	>0,5	1,81±0,07	1,80±0,05	>0,5	3,28±0,15	3,40±0,09	>0,5
Aged 5 days	Motoneurons of phrenic nerve nucleus	490,0±16,0	392,0±19,8	<0,001	2,88±0,09	2,37±0,10	<0,001	9,20±0,50	8,51±0,50	>0,5
	Cells of ventrolateral group	640,0±17,6	535,0±22,3	<0,001	2,92±0,08	2,68±0,07	>0,02 <0,01	15,90±1,10	13,60±0,70	>0,5
	Cells of intermediate zone	169,0±9,1	148,0±7,1	>0,5	1,87±0,05	1,5±0,05	<0,001	5,00±0,50	3,70±0,30	<0,02
Aged 10 days	Motoneurons of phrenic nerve nucleus	773,0±20,8	555,0±4,7	<0,001	4,10±0,16	3,02±0,26	<0,001	18,0±0,90	10,00±0,50	<0,001
	Cells of ventrolateral group	815,0±28,0	704,0±20,0	<0,001	3,71±0,17	2,70±0,10	<0,001	19,3±2,30	12,30±0,60	<0,01 >0,001
	Cells of intermediate zone	187,0±5,8	170,0±5,7	>0,5	1,90±0,03	1,30±0,02	<0,001	5,80±0,25	3,30±0,23	<0,001
Aged 30 days	Motoneurons of phrenic nerve nucleus	940,0±32,6	982,0±27,4	>0,5	4,40±0,20	4,00±0,24	>0,5	18,00±0,65	16,00±0,41	<0,01 >0,001
	Cells of ventrolateral group	1302,0±36,3	1326,0±35,0	>0,2	5,40±0,20	4,90±0,27	>0,5	18,00±0,70	18,00±0,62	—
	Cells of intermediate zone	198,0±6,2	210,0±6,7	>0,5	2,30±0,40	2,10±0,09	>0,5	7,00±0,3	7,04±0,5	—

The development of myelin sheaths and the biosynthesis of myelin and its components in the white matter of the spinal cord of the rats in the course of time are known to correlate with the development of the myelinization glia. It is formed in the spinal cord during the first 10-12 days of the animal's life [1]. Presumably, during asphyxia, injury to the myelin sheath on the nerve fibers is due not only to damage to the nerve cells whose axons form these fibers, but also to damage to the myelinization glia as a result of injury to the glioblasts.

Asphyxia of the embryos in the last quarter of the intrauterine period of development thus leads to chronic pathological changes in the nerve cells and fibers of the spinal cord.

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ELECTRON-MICROSCOPIC AUTORADIOGRAPHIC INVESTIGATION OF INTRACELLULAR RNA SYNTHESIS IN THE MOUSE CEREBRAL CORTEX

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Electron-autoradiographic investigation of DNA synthesis in mouse cerebral cortical neurons showed the highest concentration of label in the nucleolus. Many grains of silver were concentrated above the nucleoplasm. The content of radioactive substances in the cytoplasm of the neurons 2.5 h after injection of uridine-5-^{3}H into the animals was lower than in other types of cells after the same length of contact with the labeled precursor. A considerable difference was observed in the number of grains of silver above serial sections of a single nucleolus and in the character of distribution of the label in neurons situated side by side.

KEY WORDS: *neurons; RNA metabolism; electron-microscopic autoradiography.*

The study of the dynamics of intracellular processes in neurons is of great importance to the further explanation of the principles governing the function of the nervous system. At the present time new prospects are being opened in this field with the opportunities offered by a combination of electron microscopy and autoradiography, whereby metabolic processes taking place in particular cell structures can be observed. One such possible line of research is the study of RNA metabolism, because the synthesis of RNA and its distribution between different parts of the cell are the chief method of regulation of intracellular processes. However, such electron-autoradiographic studies of the nervous system as have been published deal mainly with DNA synthesis during histogenesis of the brain [9], protein metabolism [6-8], and the localization of enzymes [11] and mediators [4, 5, 12].

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