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ELECTRON-CYTOCHEMICAL AND MORPHOMETRIC INVESTIGATION OF CEREBRAL CORTICAL SYNAPSES DURING POSTMORTEM AUTOLYSIS

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A close study of the structure of interneuronal junctions in the brain after death is essential in order to elucidate early postmortem autolytic changes in the CNS. The time course of ultrastructural changes in synapses during postmortem autolysis has been described [1-3]. However, the state of the system of neurofilamentous subsynaptic units (SSU) which, as we know, plays an important role in maintaining the integrative function of synapses, was not examined in the investigations cited. The ability of the SSU to change rapidly under various influences has been demonstrated [6, 7].

The aim of this investigation was to study structural changes in SSU of cerebral cortical synapses of rats during postmortem autolysis.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male albino rats weighing 170-230 g, anesthetized with ether, at a temperature of 20°C. Three intact animals served as the control, and nine rats were killed by clamping the intubation tube. Cardiac arrest took place 5 min 30 sec after the beginning asphyxia. The brain was perfused with a mixture of 4% formaldehyde solution and 1% glutaraldehyde solution in phosphate buffer, pH 7.4, with sucrose (5%). Pieces of sensomotor cortex for orientation purposes were embedded in a plane-parallel arrangement in Araldite 30 and 90 min and 6 h after death. To reveal paramembranous synaptic concentrations of neurofilaments, at the dehydration stage in 100° ethanol the material was stained in a 5% solution of phosphotungstic acid (PTA) for 3 h. Ultrathin sections of the molecular layer of the neocortex were cut in a tangential plane and studied in the EMV-100LM electron microscope. Twenty random fields of neuropil from one animal were photographed under standard magnification of 15,000. Morphometry was carried out on negatives on a "Belarus'-2" enlarger with final magnification of 30,000. The total number of PTA-positive contacts and the number of definite and indefinite synapses, symmetrical relative to the SSU system and of asymmetrical contacts per 100 μ^2 of neuropil were determined. Depending on the abundance of dense projections (DP) of the presynaptic grid (PG) all the definite synapses were divided into four types: A, B, C, and D [5]. Synapses with a straight and curved active zone of contact (AZC) also were distinguished. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

On staining of the cortex with PTA mainly a paramembranous specialization of the neurofilaments of interneuronal synapses was observed in the form of SSU (Fig. 1a). In asymmetri-

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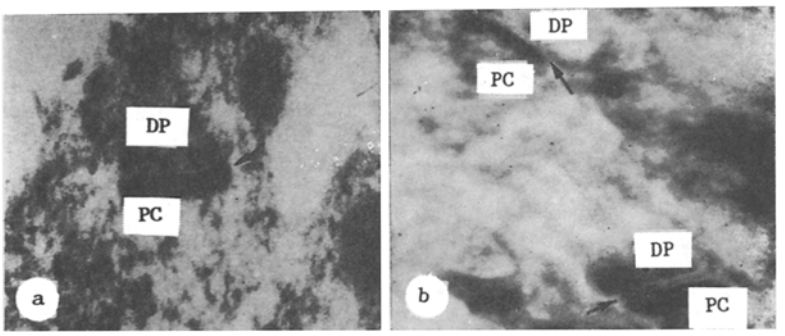

Fig. 1. Specialized paramembranous neurofilamentous formations of cerebral cortical synapses of a rat during postmortem autolysis. a) Type A synapse with distinct, completely formed structures of SSU (control); b) indistinct homogeneous staining of paramembranous structures of pre- and postsynaptic zone and substance of synaptic space (6 h). 52,000 ×. Arrow indicates substance of synaptic space. Stained with PTA.

TABLE 1. Number of PTA-Positive Synapses (per 100 μ^2 of neuropil) in Molecular Layer of Cerebral Cortex of Rats during Postmortem Autolysis ($\overline{X} + S_{\overline{X}}$)

Type of synapse	Time after death			
	control	30 min	90 min	6h
Total number	39,1±4,4	31,2±2,3	30,2±1,7	25,2±1,9
P		>0,05	>0,05	<0,05
Indefinite	15,2±3,6	15,8±1,2	28,0±1,5	16,8±1,2
P		>0,05	<0,05	>0,05
Definite	23,9±1,7	15,4±1,4	8,2±0,3	8,4±0,2
P		<0,01	<0,001	<0,001
Asymmetrical	18,4±0,6	13,7±1,0	6,9±0,2	6,1±0,1
P		<0,05	<0,01	<0,001
Type A	6,7±0,7	3,2±0,1	1,7±0,1	0,3±0,03
P		<0,05	<0,01	<0,001
Type B	6,7±0,8	6,9±0,4	3,5±0,2	2,9±0,1
P		>0,05	<0,05	<0,05
Type C	5,0±0,4	3,6±0,2	1,7±0,1	2,9±0,5
P		<0,05	<0,01	<0,05
Symmetrical Type D	5,5±0,4	1,7±0,1	1,3±0,1	2,3±0,2
P		<0,001	<0,001	<0,01
Straight	15,4±1,1	11,5±0,8	6,2±0,3	5,3±0,2
P		<0,05	<0,01	<0,01
Curved	8,5±0,9	3,9±0,3	2,0±0,2	3,1±0,4
P		<0,01	<0,01	<0,01

Legend. P — compared with control.

cal contacts in the neocortex of the control animals, discrete material of DP of the presynaptic grid was clearly identified, along with material of the synaptic space and the postsynaptic condensation (PC). The height of DP for different types of synapses varied. However, the width of DP (about 50 nm) and the distance between their centers (80-100 nm) were constant in all varieties of synapses with a formed PG. In type A synapses the height of DP was 60-90 nm. From the functional point of view they are evidently the most active synapses. The height of DP in type B synapses was approximately the same as their width, but in type C synapses the height was less than the width. Type D synapses are symmetrical with respect to the structure of SSU — immature forms with indefinite PG (DP absent). The height of PC in synapses of all types was 60-80 nm. The cerebral cortex of the control animals contained about equal numbers of synapses of types A, B, C, and D (Table 1). The ratio between the number of straight and the number of curved synapses was 1.80:1.

The total number of PTA-positive synapses 30 min after death showed a tendency to be less than in the control, there was no change in the number of indefinite synapses, but the number of definite synapses was considerably reduced (Table 1). The number of small synapses with

symmetrical organization of SSU (type D) showed the greatest decrease. Clarity of the outlines of the paramembranous neurofilamentous condensations was reduced in some synapses. The number of synapses with most abundant DP (type A) was reduced by 2.1 times. The number of synapses of intermediate types (B and C) with low, indistinct DP, however, was increased. The ratio of the number of synapses with straight and curved AZC was increased by 1.6 times due to a decrease in the number of curved synapses.

The total number of PTA-positive synapses 90 min after death still remained close to the control level (Table 1). However, the number of indefinite synapses was considerably greater. A sharp decrease in the clarity of outlines of the SSU in some synapses made their differentiation from other stained structures in the cerebral cortex more difficult. The number of type A synapses was reduced by 75%. Synapses of other types were indistinctly outlined and the homogeneity of staining of the paramembranous elements of the pre- and postsynaptic zones was increased. The ratio of the number of straight to the number of curved synapses was 3.10:1.

After 6 h there was a further intensification of autolytic processes. There was a marked decrease in the total number of PTA-positive junctions. The clarity of the outlines of SSU of the synapses was considerably reduced (Fig. 1b). Paramembranous neurofilaments of some synapses had completely disappeared. The number of type A synapses with distinct and discrete DP was sharply reduced. The number of intermediate types of synapses (B and C) still remained quite high. The ratio of the number of straight to the number of curved synapses was 1.71:1.

According to the results of the electron-cytochemical analysis, the earliest postmortem autolytic changes in cerebral cortical synapses of rats thus develop in SSU of the synapses, and more especially in DP. The SSU are predominantly protein in nature and are structurally connected with the synaptic membranes [5]. Evidently an important role in the triggering of the early dystrophic changes in the synapses during autolysis is played by marked activation of lipid peroxidation in the membranes and the hydrolytic action of lysosomal proteases [4]. The considerable decrease in the number of synapses of types A and D accompanied by the great reduction in the number of curved synapses are evidence that after 30 min the most active and the very young (functionally immature) synapses mainly undergo autolysis during the first 30 min. This leads to a decrease in the total number of definite synapses in the cortex during this period. By 90 min after death autolysis is greatly intensified in asymmetrical synapses. Transformation of some asymmetrical synapses into indefinite ones may evidently explain the marked increase in the number of the latter after 90 min, when it reflects marked dystrophic changes in a considerable group of synapses. In the late stages after death (6 h) synapses with maximally developed SSU (type A) and indefinite PTA-positive synapses develop autolysis to a greater degree, and the rate of destruction of the SSU of the less mature, definite synapses is slowed.

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