Hypertrophic Synapses as a Factor of Formation of Persistent Pathological Centers in the Brain in the Late Postresuscitation Period

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Translated from *Byuleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 5, pp. 532-535, May, 2000
Original article submitted December 20, 1999

Clinical death was modelled on albino rats. Synapses in the molecular layer of the cerebral sensorimotor cortex were investigated 1, 2, 3, 4, 5, 6, 7, 8, and 9 months postresuscitation. The total density of synapses peaked and the number of hypertrophic and perforated contacts increased 4, 7, and 9 months after resuscitation. The role of increased number of hypertrophic and perforated contacts in the pathogenesis of postresuscitation encephalopathy is discussed.

Key Words: *neocortex*; *synapses*; *postresuscitation period*; *encephalopathy*

The early postresuscitation period (PRP) is characterized by a progressive decrease in neuronal density in various brain regions and pronounced compensatory rearrangement of contacts between intact neurons [6, 7,13]. The nature and direction of these structural rearrangements are considered as structural equivalents of postresuscitation encephalopathy [6-9]. Hypertrophy and separation of active synaptic contacts (SC) play a major role in postresuscitation reorganization of synapse architectonics [7,9]. Accumulation of high-efficiency hypertrophic and perforated SC in the brain in the early PRP promotes the formation of generators of pathologically enchanced excitation assembled in dominant pathological systems [8].

The mechanism of reorganization of interneuronal contacts in the late PRP remains an important problem, because of high incidence of psychoneurological complications throughout the PRP [5] and the necessity to elaborate preventive measures against these complications.

We carried out a morphological analysis of synaptic population in the molecular layer of rat cerebral sensorimotor cortex for 9 months post-resuscitation to evaluate the dynamics of the total numerical density (TND) and relative content of hypertrophic and perforated SC.

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MATERIALS AND METHODS

Experiments were carried out on 32 male outbred albino rats weighting 150-180 g under ether narcosis. Global cerebral ischemia was modeled (heart vessel occlusion for 10 min) [4]. The resuscitation procedures included closed chest cardiac massage and artificial ventilation in a moderate hyperventilation regimen. The sensorimotor cortex for ultramicroscopy were taken 1, 2, 3, 4, 5, 6, 7, 8, and 9 months postresuscitation. The samples from 5 anesthetized and intubated animals with substernal hook insertion without occlusion of cardiac vessels saerved as the control. The brain was perfused via the ascending aorta with 1% glutaraldehyde, 4% paraformaldehyde, and 5% sucrose in 0.1 M phosphate buffer (pH 7.4) for 15 minutes. The specimens were sectioned into orientated pyramidal fragments and postfixed in the same solution for 2 h. Some specimens were contrasted with OsO_4, uranyl acetate, and lead citrate, and others with phosphorotungstic acid ethanol solution [11,14]. Then these fragments were embedded in Epon-Araldite mixture. Ultrathin tangential sections through the mole-

0007-4888/00/0005-449$25.00

cular layer of the neocortex were prepared on a NOVA-8800 ultratome (LKB). The sections were analyzed and photographed on a JEL-100 electron microscope at ×15,000. Staining with phosphorotungstic acid provides good verification of all components of specialized synaptic paramembrane cytoskeleton [11, 14], so we use it for the quantitative estimation of synaptic population in the molecular layer of the neocortex. For each endpoint, the number of neuronal SC and their total length per of 4500 μ^2 neuropil crossection area were analyzed at ×30,000 using a photomagnifier. A 3-mm scale test grid was used to measure the length of SC profiles. The slices stained with OsO_4 were used for general description of synapses.

The data were analyzed by standard statistical tests [1]. Student's *t* test was used for comparing the means.

RESULTS

In the molecular layer of the sensorimotor cortex in control animals we observed typical mature axospine and axodendritic SC characterised by asymmetrical organisation, clear and discrete dense presynaptic projections, and clear-cut thickening. Small and middle-size nonperforated SC prevailed. Hypertrophic contacts (>700 nm) constituted 6.7% of all synapses.

One month after clinical death, TND of SC decreased to 69% ($p<0.001$) of the control level, the density of hypertrophic contacts was lower by 56.3% ($p<0.001$) and that of perforated contacts was higher by 28.2% ($p<0.01$) compared to the control (Fig. 1).

In the later period, three peaks (4, 7, and 9 months postresuscitation) were observed in the dynamics of TND and the number of hypertrophic and perforated contacts (Fig. 1). The number of perforated contacts in the brain of resuscitated animals significantly exceeded the control level throughout the observation periods. The maximum increase in the content of perforated contacts (by 124.5%, $p<0.001$) was observed 4 months postresuscitation (Fig. 1). It should be emphasized that pronounced perturbations in the number of hypertrophic SC occurred during the late PRP (from 3 to 4, from 6 to 7, and from 8 to 9 months, Fig. 1).

Thus, in the late PRP a tendency toward the increase in TND of SC had a cyclic character with three peaks at 4, 7, and 9 months after resuscitation. Published data suggest that high variability of numerical density of synapses in the brain is not typical of adult animals [11]. Our results suggest that cyclic variations in synaptic TND during the late PRP are accompanied by synchronous changes in the number of hypertrophic and perforated SC. This implies possible coexistence and parallel realization of interdependent mechanisms of hyperplasia and SC separation, which cause an increase in synaptic TND. There is evidence [7,10,14]

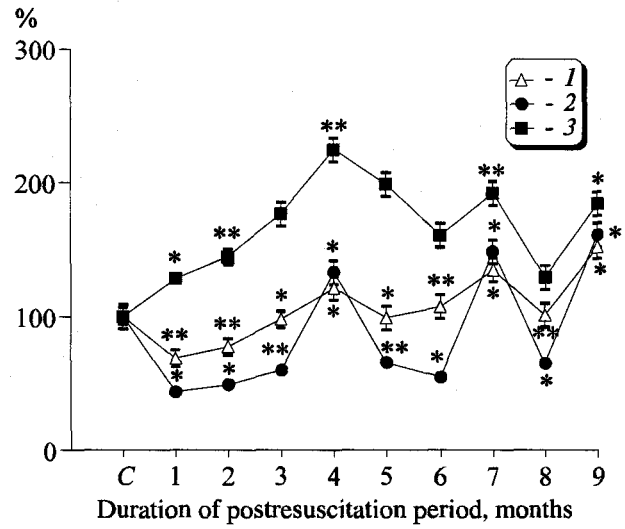

Fig. 1. Changes in total numerical density (%, *1*) and relative content of hypertrophic (*2*) and perforated (*3*) contacts in the molecular layer of cerebral sensorimotor cortex in rats during postresuscitation period. *$p<0.001$, **$p<0.01$ compared with control (C).

on possible separation of hypertrophic SC followed by an increase in TND of SC and modulation of neuronal interactions. We believe, that separation of hypertrophic contacts is a primary mechanism responsible for neuronal plasticity during normal ontogeny [11] and cerebral pathologies [7,10]. Our findings suggest that rearrangement of neuronal contacts is stimulated by resuscitation after clinical death and had a cyclic character with peaks in their activity 4, 7, and 9 months postresuscitation.

Pronounced psychoneurological manifestations of postresuscitation encephalopathy such as abnormal total behavioral activity with considerable activation-to-depression alternations and memory disturbances accompanied by degenerative, necrobiotic, and, possibly, apoptic changes in the brain were observed even after transitory arrest of systemic circulation [2,3,12]. However, a variety of psychoneurological manifestations of encephalopathy during the late PRP can not be explained by only progressive degenerative changes in the brain. Activation of compensatory mechanisms promoting the formation of high-efficient hypertrophic and perforated contacts in the resuscitated brain facilitates the development of various postresuscitative integrative disorders, which are characterized by a cyclic reorganization and imbalance between inhibition and excitation. Thus, the observed cyclic changes in the numbers of hypertrophic and perforated SC with high efficiency of impulse transmission can be responsible for continuous reorganization of neuronal contacts against the background of progressive decrease in the population of neurons, and, probably, for the formation of persistently active pathological centers in the brain during the late PRP.

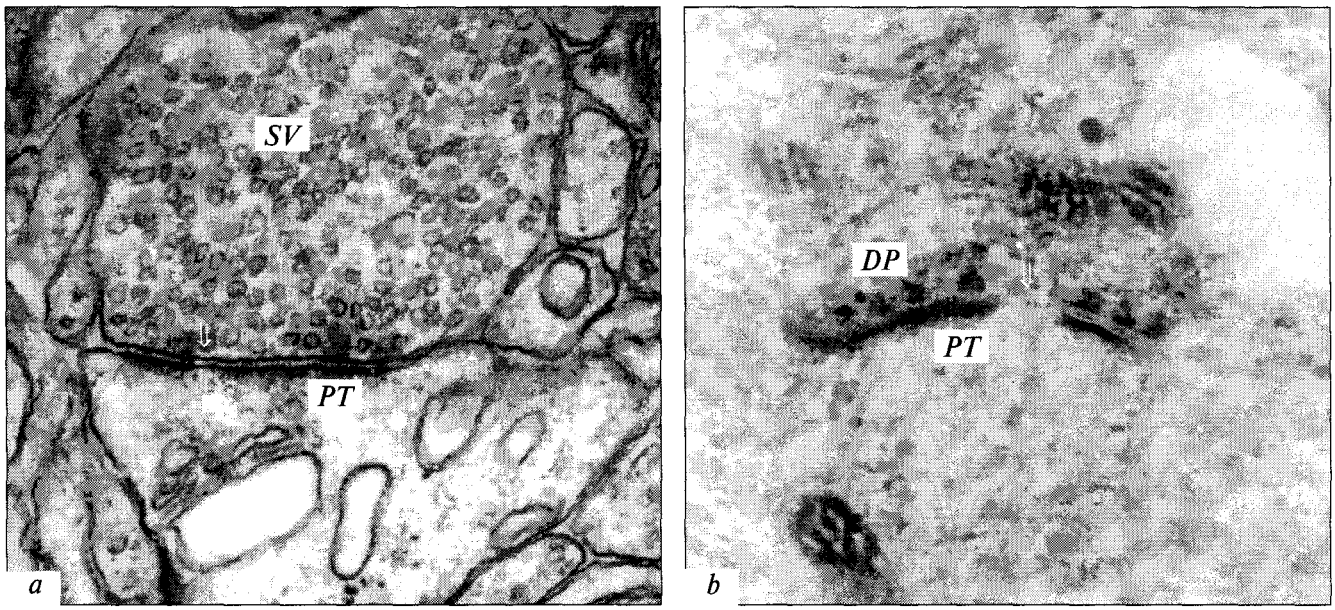

Fig. 2. Typical hypertrophic axodendritic synapses with perforated contacts in the molecular layer of cerebral sensorimotor cortex in albino rats during late postresuscitation period (4 months), ×40,000. OsO_4, uranyl acetate, and lead citrate contrasting (*a*); phosphorotungstic acid contrasting (*b*). DP: dense projections of the presynaptic part; PT: postsynaptic thickening; SV: synaptic vesicles in the presynaptic area. Arrow: perforation zone.

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