MORPHOLOGY AND PATHOMORPHOLOGY

Changes in Cerebellar Purkinje Cells during Postresuscitation Period: Morphometric and Ultrastructural Analysis

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Morphometric and electron microscopic analysis of rat cerebellar Purkinje cells was carried out after external neurological recovery following 10-min systemic circulation arrest caused by clamping cardiac vascular bundle, in order to elucidate the mechanisms of delayed encephalopathies. The composition of Purkinje cell population notably changed during the postresuscitation period. Ultrastructural disorders in these neurons, persisted for 1 month after resuscitation.

Key Words: *ischemia; Purkinje cells; ultrastructure*

Complete recovery of cerebral function after severe ischemia caused by circulatory arrest is an important theoretical and practical medical problem [7].

According to clinical reports, 70% patients survived a terminal state develop neurological disorders, in particular cerebellar pathology [3]. These disorders are often latent and delayed and develop against the background of apparent neurological recovery. Experimental studies showed that Purkinje cells (PC) are the most vulnerable during clinical death of different etiology and their state correlates with the duration of ischemia and neurological recovery [1].

The aim of this work was a morphometrical and ultrastructural analysis of PC population at various terms of 1-month postresuscitation period in animals with apparent neurological recovery after 10-min cardiac arrest.

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MATERIALS AND METHODS

Experiments were carried out on 30 random-bred male albino rats (160-180 g). Ten-minute circulatory arrest was induced under ether narcosis by intrathoracal clamping of the cardiac vascular bundle [5]. Resuscitation was carried out by closed chest massage combined with jet ventilation. The neurological status of animals during the postresuscitation period was scored using a 100-point scale including 19 parameters (type of respiration, reaction to pain, turning reflex, muscle tone, hearing, vision, *etc.*) [6]. Zero score corresponded to complete neurologic recovery.

The density and composition of PC population in the lateral portion of the cerebellum (most vulnerable to ischemic damage [1]) was studied morphometrically [1] 4, 7, 14, and 30 days after cardiac arrest in animals without apparent neurological disorders. Intact age-matched animals served as the controls.

Neuronal dimorphism morphologically manifests by the presence of normal light and dark cells with different functional activity. Changes in the ratio be-

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tween of different types neurons in different exposures can serve as an indicator of brain status [8]. The numbers of light, dark, and morphologically changed neurons per mm of PC layer length were counted separately. Morphologically normal intact cells with dark nucleus and cytoplasm were considered as dark neurons. The group of morphologically changed neurons included cells with various abnormalities (pericellular edema, ischemic changes, swelling, tigrolysis, hydropic changes, and wrinkling).

Cerebellar tissue for electron microscopy was taken 7, 14, and 30 days after resuscitation, fixed in 4% glutaraldehyde, postfixed in OsO_4, and embedded in epon. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined under a Philips CM 10 electron microscope.

The data from various groups were statistically processed using parametrical and nonparametrical tests (Student, Mann—Whitney, and Fisher).

RESULTS

Examination at various terms of the postresuscitation period revealed very rapid recovery of the neurological status in all animals survived a 10-min circulatory arrest (0 score on days 4-7). Morphometric analysis revealed no essential changes in PC population on day 4 postresuscitation. On day 7, the total density of PC population in experimental rats did not differ from the control, but its composition changed: the number of light neurons decreased by 28.7% in comparison with the control and there was a tendency to an increase in the number of morphologically altered cells (by 38%). By day 14 the total density of PC population did not change, but pathological changes augmented: the density of light neurons decreased by 53.2% in comparison with the control, and that of morphologically changed neurons increased by 75.8%. On day 30 postresuscitation the pathological process involved also dark PC: their number decreased by 29.9%, while the number of morphologically intact neurons remained 49.2% above the control. Total density of the population did not differ from that in age-matched intact controls.

Hence, notable changes in the cerebellar PC population develop even in rats with relatively rapid neurological recovery. Though the total density of the population did not change during 1 month postresuscitation, which indicated the absence of neuronal loss, the population composition was disordered (Fig. 1). After 7 days, some light PC was morphologically transformed. By this term degenerative changes in neurons were also observed in various cerebellar structures in different models of ischemia [10,14]. In dogs exposed to 20-min global ischemia, the number of damaged neurons in the cerebellum and CA1 and CA4 hippocampal sectors increased after 7 days. Delayed cell damage is probably caused by increased immunoreactivity of phospholipase C in the cerebellum [14]. Previous studies [1] revealed no loss and notable morphological alteration in neurons on day 4 postresuscitation in rats with rapid neurological recovery after 15-min cardiac arrest. Rearrangement of neuronal populations of various compartments of the brain in animals with rapid neurological recovery after 10-min cardiac arrest is delayed, which can be attributed to inhibition of protein synthesis on day 7 of postresuscitation, while the absence of changes at the earlier postischemic period is believed to be associated with increased protein production in cortical, hippocampal, and cerebellar neurons by day 4 postresuscitation [2]. By day 14 postresuscitation, pathological changes in PC population augmented, but involved only light neurons. A notable decrease in the number of normal neurons during this period was observed in the striatum of rats exposed to 10-min brain ischemia [11]. The number of dark PC decreased only 1 month postresuscitation due to their transition into morphologically altered status, which indicated lower reactivity of dark neurons in comparison with light ones and progress of the pathological process. The data indicate delayed restructuring of PC population, which is in line with the maturation phenomenon [9].

Electron microscopy showed that cerebellar PC normally have a large nucleus with finely dispersed chromatin and a nucleolus, mitochondria with densely packed parallel crystae, well developed granular endoplasmatic reticulum (EPR), and few lysosomes (Fig. 2, *a*).

On day 7 postresuscitation, PC nuclei had normal ultrastructure, EPR cisterns in the cytoplasm were notably widened, but other organelles were relatively unchanged (Fig. 3, *a*).

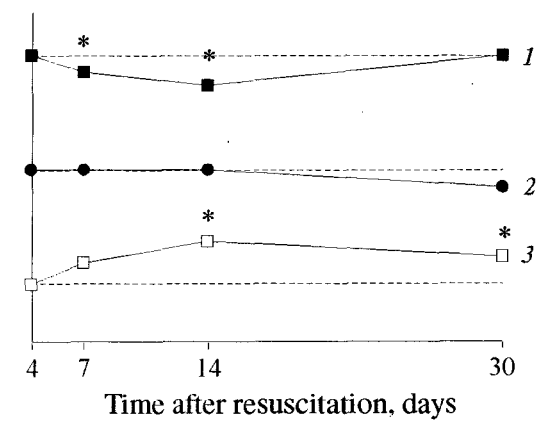

Fig. 1. Relative changes in the number of light (*1*), dark (*2*), and morphologically changed (*3*) neurons in comparison with the control (dotted line) during the postresuscitation period after 10-min cardiac arrest in rats. *$p<0.05$ *vs.* the control.

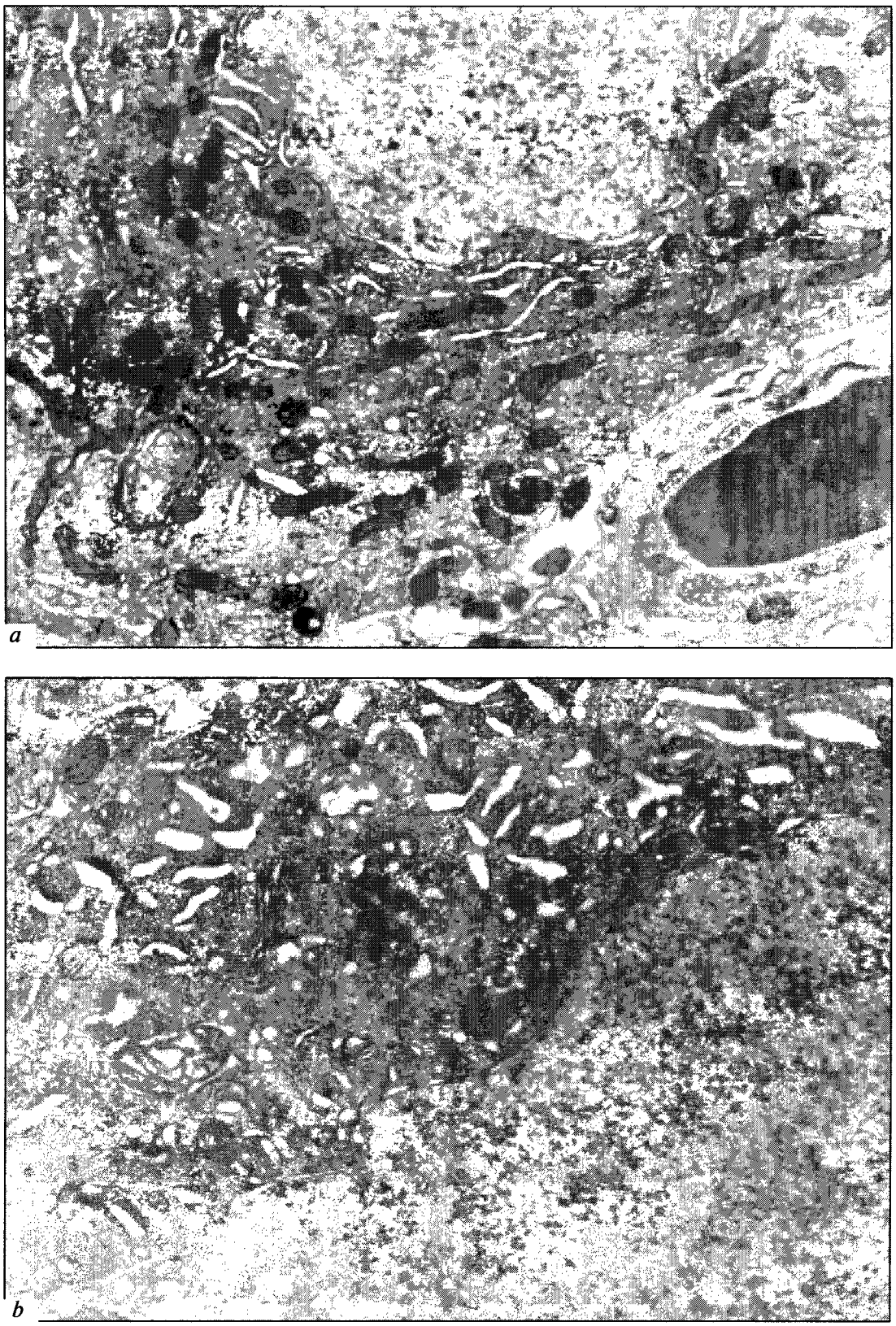

Fig. 2. Ultrastructure of Purkinje cells normally and 1 month after 10-min cardiac arrest. *a*) normal Purkinje cell, ×11,250; *b*) 1 month after resuscitation: extended cisterns of endoplasmatic reticulum, swollen mitochondria with destructed crysts and clarified matrix, ×13,050. *N*: nucleus; *M*: mitochondria.

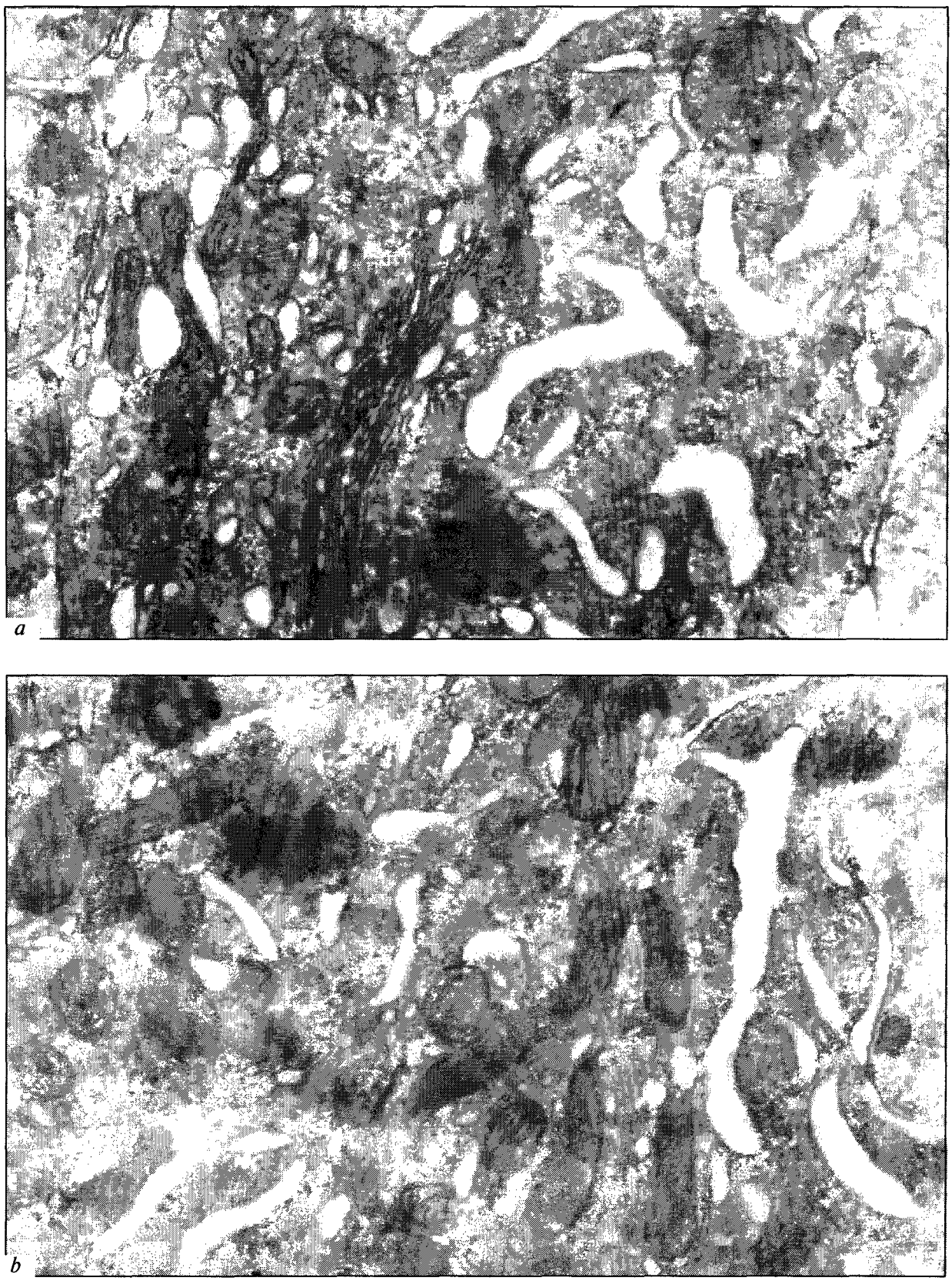

Fig. 3. Ultrastructure of cerebellar Purkinje cells on days 7 (*a*) and 14 (*b*) postresuscitation. Extended cisterns of endoplasmatic reticulum, ×35,000.

On day 14 postresuscitation, PC nuclei contained finely dispersed chromatin but deep invagination of the nucleolemma were often seen. Another ultrastructural disorder was swelling of EPR, but it was less expressed in comparison with day 7 (Fig. 3, *b*).

Thirty days after resuscitation there were no apparent changes in PC nuclei, EPR was notably widened. Swelling of mitochondria with matrix clarification, cryst deformation and destruction, and an increase in the number of lysosomes were observed (Fig. 2, *b*).

Hence, the ultrastructure of PC was essentially changed during the postresuscitation period. The changes involved mainly the cytoplasm, while the nuclei remained virtually intact. It is noteworthy that changes in PC were different and varied in intensity at various periods after resuscitation. After 7 days these changes involved only EPR whose tubules were markedly widened, which can be associated with inhibition of protein synthesis in the cell. EPR dysfunction after short-term ischemia was reported by other authors [12,13]. The ultrastructure of PC improved by day 14, but 1 month after resuscitation degenerative processes were enhanced again: numerous lysosomes and swollen mitochondria appeared.

The study showed that degenerative changes in rat cerebellar PC after cardiac arrest are characterized by a phasic pattern and develop for at least 1 month after resuscitation. Active long-term pathological process involved individual neurons and the whole population. There are many hypotheses explaining the development of degenerative processes triggered by ischemia: disorders in energy metabolism, ionic homeostasis, excitatory amino acid metabolism, formation of free radicals, modulation of gene expression, protein synthesis, *etc.* [10]. Delayed degenerative changes in neurons can be caused by reduced content of natural antioxidants and high activity of lysosomal enzymes in the brain long after resuscitation [4].

Therefore, PC population is rearranged during the postresuscitation period. A certain improvement can be followed by augmentation and development of new changes in the neuronal ultrastructure. After circulatory arrest, a complex of disorders is formed in the brain at the level of the entire neuronal population and at an ultrastructural level during the postresuscitation period, even in the absence of apparent neurological disorders. These disorders make the brain vulnerable to damage and are responsible for the formation of delayed posthypoxic encephalopathies. These findings should be taken into account in rehabilitation of patients after severe hypoxia.

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