Ischemia-Induced Changes in Synaptoarchitectonics of Brain Cortex and Their Correction with Ascovertin and *Leuzea* Extract

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Peroral administration of 70 mg/kg ascovertin and 150 mg/kg *Leuzea* extract to rats with cerebral ischemia for 5 days prevented destructive changes and decrease in the density of synapses in the cerebral cortex. These preparations activated compensatory and reparative mechanisms underlying plasticity of the synaptic pool, which was realized through hypertrophy and destruction of synaptic contacts. Ascovertin possessed more pronounced cerebroprotective activity than *Leuzea* extract.

Key Words: *cerebral cortex synapses; ischemia; ascovertin; diquertin; ascorbic acid; Leuzea extract*

The type and dynamics of postischemic ultrastructural changes in interneuronal synapses of the cerebral cortex were extensively studied [2,8,9]. However, changes in synaptoarchitectonics of the cortex in contralateral hemispheres during asymmetrical occlusion of carotid vessels and their correction with preparations modulating rheological properties of the blood are poorly understood. Hemorheological therapy of ischemic insult was pathogenetically substantiated [12]. An insufficient number and low efficiency of hemorheological drugs determine the necessity of studying and elaborating new preparations [11,13]. Our previous studies showed that ascovertin (complex of diquertin and ascorbic acid) decreases the severity of ischemic injuries in rats with experimental cerebral ischemia (CI) [6]. These data indicate that hemorheological preparations hold much promise as antiischemic drugs.

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Here we studied the effects of ascovertin and *Leuzea* extract on changes in synaptoarchitectonics of the cerebral cortex in rats with CI.

MATERIALS AND METHODS

Experiments were performed on 45 adult male and female Wistar rats weighing 250-300 g and receiving a standard diet. Intact rats (n=8) served as the control. CI was induced in rats of groups 1 (n=19), 2 (n=9), and 3 (n=9) under ether anesthesia. The left common carotid artery was ligated, and blood flow in the right common carotid artery was 50% reduced [3]. Group 1 rats received no drugs. Group 2 and 3 rats received 70 mg/kg ascovertin and 150 mg/kg *Leuzea* extract, respectively.

Ascovertin contains total flavonoid extract from Mongolian and Siberian larch trees and ascorbic acid (ratio 1:2:5). Dry *Leuzea* extract is prepared from *Rhaponticum carthamoide* roots and rhizomes by extraction with 40% ethanol. These preparations were administered daily in 1% starch gel for 5 days starting from day 1 after CI (through a gastric tube). Group 1

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rats received an equivalent volume of starch gel. Ascorbic acid was added to diquertin to increase its antioxidant activity [6]. Doses of preparations were selected taking into account their maximum effects on rheological properties of the blood.

The animals were decapitated under ether anesthesia on day 5. For electron microscopy, samples of the cerebral cortex from the anterior parietal area (PA_S) [7] were fixed with 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4), postfixed with 1% OsO_4, dehydrated in alcohols of increasing concentrations, and embedded in Araldite [7]. For visualization of filaments in subsynaptic units, cerebral cortex specimens were contrasted with 5% phosphotungstic acid (PTA) in absolute alcohol for 3 h during dehydration (without treatment with OsO_4). Semithin sections were stained with toluidine blue. Ultrathin sections of osmium-treated samples were contrasted with uranyl acetate and lead citrate and examined under JEM 7A and JEM 100 CX II electron microscopes. We photographed 15 randomly selected fields of view in 5 sections of each hemisphere (layer IV). We estimated the number of synapses and density of PTA-positive contacts per 100 μ^2 neuropil using a photographic enlarger (final magnification 30,000). The length of synaptic active zones was determined using a test grid (increment 3 mm). We measured the density of asymmetrical and symmetrical flat, positively or negatively curved, hypertrophic (length of the synaptic active zone more than 700 nm), and perforated synapses [8]. The percent of destructed synapses was estimated in osmium-treated sections. The results were analyzed by Mann—Whitney test.

RESULTS

CI was accompanied by "light destruction" of interneuronal synapses [2], which was manifested in swelling of the presynaptic zone and reduction of its electron density, destruction and decrease in the count of vesicles, impairment of organelles (*e.g.*, mitochondria), vacuolization, and appearance of threadlike and fine-grained materials (Fig. 1, *a*). Synapses more rarely underwent "dark destruction", which was characterized by the appearance of highly osmiophilic pre- and postsynaptic zones, agglutination, and destruction of vesicles hardly detectable in the electron-dense axoplasm (Fig. 1, *b*). Examination of osmium-treated samples showed that during CI the count of destructed synapses increased in the right and, particularly, in the left cerebral cortex (Table 1), where ischemic injuries were much more pronounced [3]. CI decreased the total density of PTA-positive contacts in the right and left hemispheres to 66.1 and 46.9% of the control, respectively. This was associated with a decrease in the density of functionally mature asymmetrical synapses in the left and right hemispheres to 63.6 and 35.8%, respectively. The density of functionally immature symmetrical contacts in the right and left hemispheres decreased to 71.9 and 71% of the control, respectively. Treatment with ascovertin abolished the development of destructive changes in synapses in both hemispheres and prevented the decrease in the density of asymmetrical and symmetrical contacts in the right hemisphere. In the left hemisphere test parameters increased in animals treated with ascovertin (compared to untreated rats), but were above the control. The effects of *Leuzea* extract were less pronounced. This preparation changed the count of destructed synapses only in the right hemisphere compared to that in group 1 rats.

Curvature of synapses reflects their functional activity. Previous studies showed that flat synapses were inactive, while curved contacts intensively functioned. Moreover, positively curved synapses were char-

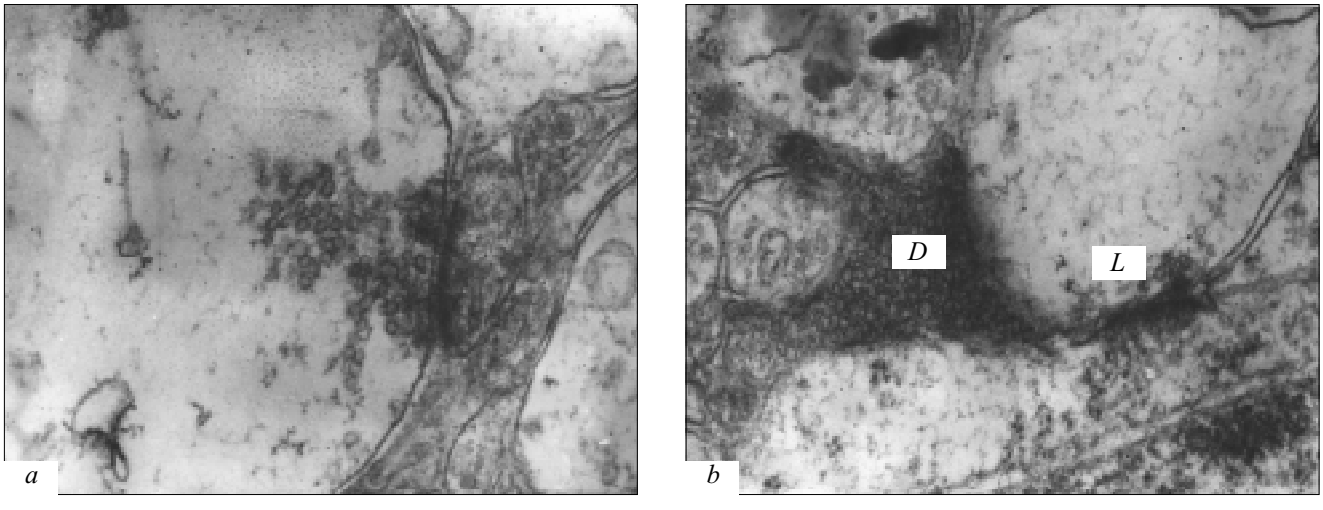

Fig. 1. Light (*a*) and dark (*D*) and light (*L*) destruction (*b*) of interneuronal synapses in cerebral cortex during ischemia (×36,000).

TABLE 1. Effects of Daily Peroral Treatment with 70 mg/kg Ascovertin and 150 mg/kg *Leuzea* Extract for 5 Days on Synaptoarchitectonics in Rats with Cerebral Ischemia (per 100 μ^2 Cortical Layer IV Neuropil, *M*±*m*)

Parameter		Intact control		Ischemia					
				without correction		ascovertin		*Leuzea* extract	
		RH	LH	RH	LH	RH	LH	RH	LH
Destructed synapses, % (osmium-treated sections)		6.1±1.3	5.4±1.8	28.4±5.0*	37.4±6.2*	10.7±3.5$^+$	25.0±5.3*$^+$	19.3±2.4*$^{+\#}$	32.7±2.3*$^\#$
Total synaptic density		18.9±2.1	19.6±1.7	12.5±2.0*	9.2±1.4*	17.4±1.5$^+$	13.4±2.3*$^+$	13.7±1.8*$^\#$	10.2±2.4*
Synapses	asymmetrical	13.2±1.0	13.4±2.1	8.4±1.2*	4.8±0.9*	12.7±0.7$^+$	8.1±0.6*$^+$	10.3±1.0*$^\#$	5.9±0.4*$^\#$
	symmetrical	5.7±0.7	6.2±0.9	4.1±0.5*	4.4±0.5*	4.7±1.2	5.3±1.0	3.4±0.7*	4.3±0.7*
	flat	12.7±1.5	13.0±1.1	9.2±1.1*	7.4±0.8*	11.0±1.4	7.4±0.8*	9.1±0.8*	7.0±0.8*
	positively curved	4.3±0.3	4.2±0.6	1.4±0.6*	0.9±0.4*	5.1±0.8$^+$	4.0±0.7$^+$	3.3±0.3$^+$	1.8±0.6*$^\#$
	negatively curved	1.9±0.2	2.4±0.5	1.9±0.4	0.9±0.4$^+$	1.3±0.6	2.0±0.5$^+$	1.3±0.5	1.4±0.4
	hypertrophic (>700 nm)	0.96±0.17	0.91±0.14	0.70±0.07*	0.54±0.08*	1.12±0.10$^+$	1.05±0.12$^+$	0.68±0.05*$^\#$	0.63±0.07*$^\#$
	perforated	2.16±0.24	2.23±0.18	2.01±0.33	1.70±0.26*	4.29±0.38*$^+$	3.95±0.41*$^+$	3.27±0.36*$^{+\#}$	3.02±0.38*$^{+\#}$

Note. $p<0.05$: *compared to intact animals; $^+$compared to ischemia without correction; $^\#$compared to ascovertin. RH: right hemisphere; LH: left hemisphere.

acterized by vesicular exocytosis [8,14]. In intact rats, layer IV contained 66-67, 21-23, and 10-12% flat, positively curved, and negatively curved contacts, respectively. CI was followed by changes in the density of curved and flat contacts. The density of positively curved contacts decreased most significantly (to 32.5 and 21.4% of the control in the right and left hemispheres, respectively, Table 1). Probably, positively curved contacts were transformed into negatively curved with a decrease in the total density of synapses during CI. In ascovertin-receiving rats with CI the density and ratio between positively and negatively curved synapses approached the control. The number of flat contacts decreased only in the left hemisphere with pronounced ischemic injuries. Treatment with *Leuzea* extract did not modulate the dynamics of changes in the count of flat and positively curved contacts in the left hemisphere, but increased the density of curved synapses in the right hemisphere to the baseline level.

Hypertrophy of preserved synaptic contacts and their destruction are the major mechanisms underlying changes in synaptoarchitectonics of the cerebral cortex during ischemia. These processes reflect plasticity and compensatory-adaptive reactions of synapses to ischemic injuries and decrease in their density [8]. We performed a quantitative analysis of changes in hypertrophic synapses with elongated active zones (more than 700 nm) and perforated contacts (Fig. 2). CI was followed by a decrease in the density of hypertrophic contacts in the right and left hemispheres to 73 and 59% of the control, respectively (Table 1). Ascovertin increased the density of hypertrophic synapses in the right and left hemispheres to 117 and 115% of the control, respectively. *Leuzea* extract did not change the count of hypertrophic contacts compared to that in

Fig. 2. Perforated synapse in the initial stage of destruction (arrow: perforation) during cerebral ischemia and treatment with diquertin and ascorbic acid. Staining with phosphotungstic acid (×58,000).

group 1 rats (71 and 69% in the right and left hemispheres, respectively, compared to intact animals). During ischemia the density of perforated synapses did not differ from the control in the right hemisphere, but decreased to 76% in the left hemisphere. Administration of ascovertin and *Leuzea* extract to rats with CI increased the density of perforated contacts to 77-99 and 35-51%, respectively, compared to the baseline. Our results show that *Leuzea* extract and, particularly, ascovertin promote destruction of preserved contacts in animals with CI, which indicates intensification of compensatory synaptogenesis in response to ischemic injuries and decrease in synaptic density.

Test preparations possess considerable hemorheological activity. Treatment with ascovertin and *Leuzea* extract prevents the increase in blood viscosity, intensification of erythrocyte aggregation, and decrease in erythrocyte deformability [4,5]. These changes improve microcirculation and oxygen supply to the brain. Protective properties of ascovertin during CI can be associated with antioxidant activity of its main component diquertin [10]. Moreover, ascorbic acid possesses considerable antioxidant activity and potentiates the antioxidant effect of biological flavonoids [1].

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