EXPERIMENTAL BIOLOGY

Effect of Low-Alcohol Drinks on CNS Structures in Young Animals

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Adaptation restructuring at the level of cells and cell populations in CNS in young animals after acute intoxication with a high dose of beer was evaluated by a complex of quantitative morphometric parameters. Variants of morphological manifestations of compensatory adaptive changes were studied and variability of their combinations in the neuroglial populations of the forebrain, cerebellum, and spinal cord in young animals was revealed reflecting plastic potentialities of the studied CNS structures exposed to a destructive factor.

Key Words: *young animals; beer; CNS; neuroglia populations; adaptation restructuring*

High incidence of craving for beer in young people is a pressing problem. Beer consumption involves a chain of negative consequences caused by a combination of toxic effects of ethanol and other bioactive compounds [7-9]. Narcologists claim that beer more than any other drink determines the development of ethanol dependence in adolescents [6]. Exposure of a young organism to this factor induces reactions of the CNS which is characterized by a high plastic potential aimed at repair of the lost homeostasis; the first of these reactions are the adaptive reactions of the nervous system, predicting the outcome for the organism after the exposure [1,2,4,5,10].

We studied the adaptive rearrangements at the level of a complex of morphometric characteristics of cell populations of the studied CNS structures on a model of acute beer intoxication.

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MATERIALS AND METHODS

The study was carried out on outbred male albino rats (n=20; 110-140 g) divided into a control and an experimental groups. Experimental animals received a high dose (53.3 ml/kg) of 6% beer orally, controls received an equivalent volume of tap water. The brain was collected 6 h after the dose according to the instruction of the International Convention for use of laboratory animals (1985). The material was processed by methods used for nervous tissue processing [3].

Quantitative analysis was carried out on preparations with qualitative reactions to ribonucleoprotein complexes (using thionin by the method of Nissl modified by Viktorov). Neuronal subpopulations in the sensorimotor cortex in the forebrain (layers II+III and V), lateral nucleus (medium-sized neurons) and ganglionic layer of the cerebellum (Purkinje cells), motor nuclei of the spinal anterior horns (medium-sized neurons), and the concomitant glia cell populations were examined under a Zeiss Axioskop microscope with a videocamera using Axio Vision LE Rel. 4.3 software.

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The following parameters of neurons were evaluated: profile field area of neuronal body (S_B), cytoplasm (S_C), nucleus (S_N), and the nucleus/cytoplasm ratio (NCR). Quantitative evaluation of the basophilic substance of neuronal cytoplasm was carried out (normochromic, hyper- and hypochromic, total hyperchromic, shrunk, or ghost cells). The densities of neurons and glial cells (free and satellite) per mm^2 were evaluated at the cell population level and the glioneuronal indexes for free and satellite glia were calculated [3,5].

The results were processed using Micromed Statistica software.

RESULTS

In the population of pyramidal neurons of the sensorimotor cortex in experimental rats, adaptive reactions led to the appearance (particularly in layer V) of nerve cells with pronounced swelling of the nucleus and cytoplasm and eccentric ectopic nucleolus shifted towards the karyolemma (layers II+III, V). Redistribution of the neurons by chromatophilic substance was caused by shifts in the percent proportions among optimally functioning neurons towards the extreme variants: hyper- and hypochromic cells. These transformations attest to compensatory processes of the hypo- and hyperchromic reparative regeneration type with signs of swelling [5]. Neurons functioning at the threshold normal levels, *i.e.* total hyperchromic cells and cells with destructive manifestations (shrunk and ghost cells) appeared in similar percent proportions (Table 1).

The linear parameters of neurons significantly surpassed the normal values: S_B by 19.1%, S_N by 23.6%, S_C by 13.4% in layers II+III; S_B by 38%, S_N by 43.7%, S_C by 34.5% in layer V (Table 1). The significant increase of the linear parameters of all analyzed cell elements suggested triggering of the compensatory processes by the regeneration hypertrophy type. The neuron–glia system undergoes adaptive restructuring. The decrease in neuronal density (layer II+III by 4.7%, layer V by 7.2%) was paralleled by a 2.4-fold increase of the satellite gliocyte density in layer V and a 1.5-fold reduction of this parameter in layer V, while the numerical density of free gliocytes remained normal, which led to shifts in the glioneuronal indexes (Table 1). Increasing numbers of neurons with pronounced chromatolysis and swelling in layer V populations was responsible for cell enlargement,

TABLE 1. Morphological Characteristics of Neuronal Populations of the Sensorimotor Cortex in Young Rats in Health and After Acute Beer Intoxication (*M*±*m*)

Parameter			Layer II+III		Layer V	
			control	experiment	control	experiment
Evaluation of neuronal chromatophilic substance	normochromic		85.7±27.1	57.9±11.6*	73.4±6.2	22.9±9.7*
	hyperchromic		7.0±2.4	15.8±1.9*	14.5±5.4	32.0±10.6*
	hypochromic		5.9±1.4	15.0±1.9*	7.8±2.6	22.1±14.9*
	total hyperchromic		0.3±0.3	3.3±1.3*	4.5±2.2	3.1±3.0
	shrunk		0.3±0.3	3.0±1.1*	-	9.8±8.9*
	ghost cells		0.5±0.5	4.8±1.2*	-	10.0±5.7*
Linear parameters of neurons (μ^2) and their derivatives	S_B		71.8±10.7	85.5±12.3*	151.9±43.1	209.8±35.7*
	S_N		39.7±8.0	49.1±10.4*	60.5±13.7	86.8±18.2*
	S_C		32.1±6.9	36.4±7.1*	91.4±33.2	122.9±26.8*
	NCR		1.28±0.3	1.4±0.4*	0.7±0.1	0.73±0.20
Neuron-glia system parameters	density of neurons		4079±584	3884±577*	1182±213	1097±195*
	density of glia	free	1093±172	1154±211	2395±460	2415±266
		satellite	344±63	824±170*	567±190	366±74*
	glia-neuron index	free	0.27±0.05	0.30±0.07*	2.08±0.50	2.26±0.48*
		satellite	0.08±0.02	0.22±0.06*	0.49±0.19	0.34±0.09*

Note. Here and in Table 2: *p<0.01 *vs.* control.

appearance of cell devastation areas, reduced density of neuron distribution, and attests to high probability of predominance of the decompensation phenomena [3,10,11]. Reduced density of satellite gliocytes suggested an unfavorable prognosis [3,5,7].

Study of the typical morphological forms of adaptation restructuring by the status of chromatophilic substance in the cerebellar neuron populations showed redistribution of optimally functioning neurocytes towards obvious predominance of dark (hyperchromic) cells (Table 2). The hyperchromic type of reparative regeneration aimed at restoration of damaged neuroplasm manifested in this case. However, some neurons in the ganglionic layer and dentate nucleus populations transformed into a borderline status (total hyperchromic). The appearance of shrunk cells (neurons with manifest destructive changes) was typical of the cerebellar neuronal populations (Table 2). The S_B, S_N, and S_C decreased significantly in comparison with the values in the intact group (Table 2). According to the classification of adaptive changes in the neurocyte perikaryon, the changes in the cerebellar neuronal populations combined the typical forms of morphological changeability of the nervous tissue elements, manifesting by atrophy, on the one hand, and by hyperchromic reparative regeneration, on the other. The compensatory adaptive changes in response to the exposure (hyperchromatism, low activity of the synthesized substrate utilization, emergence of pyknomorphic cells, shrinkage of the neurons) could be regarded as a variant of adaptation processes bordering the degeneration [3,5]. In the cerebellar neuron–glia system, dark small Purkinje's cells were more tightly arranged and formed compact rows after the exposure. Adaptation changes in the glia populations manifested by intense proliferation of gliocytes. Due to cell proliferation the density of free glia increased by 42% in the ganglionic layer and by 68.5% in the dentate nucleus, the density of satellite glia increased by 51% and 83%, respectively, in comparison with the control (Table 2). The perineuronal satellitosis predominated round hyperchromic, total hyperchromic cells, and neurons with signs of destruction.

The adaptation restructuring in the populations of medium-sized neurons in the spinal anterior horn motor nuclei evaluated by the reactions of the neurocyte body components combined two forms (similarly as in the cerebellum): hyperchromic reparative regeneration and regeneration hypertrophy (Table 2). The compensatory adaptive changes in the glial populations manifested by a drastic intensification of proliferative processes aimed at restoration of exhausted neurons with degenerative and necrotic manifestations, this

TABLE 2. Morphological Characteristics of Cerebellar and Spinal Neuronal Populations in Young Rats in Health and After Acute Beer Intoxication ($M \pm m$)

Parameter			Purkinje's cells		Cerebellar lateral nucleus neurons		Spinal anterior horn motor nuclear neurons	
			control	experiment	control	experiment	control	experiment
Evaluation of neuronal chromatophilic substance	normochromic		69.8±1.3	32.7±1.8*	69.6±3.6	33.5±2.5*	72.7±2.4	23.3±9.2*
	hyperchromic		14.7±1.1	38.2±2.0*	14.2±1.4	38.0±2.4*	13.9±1.7	33.5±13.7*
	hypochromic		15.1±0.9	8.2±0.8*	15.5±1.2	12.6±1.6*	12.1±1.7	4.8±2.3*
	total hyperchromic		-	15.5±1.5*	0.4±0.2	7.6±0.9*	1.2±0.7	21.6±6.9*
	shrunk		0.2±0.1	4.6±0.7*	-	4.8±0.7*	-	13.1±5.1*
	ghost cells		0.2±0.1	0.7±0.2*	0.3±0.1	3.2±0.5*	-	3.5±2.5*
Linear parameters of neurons (μ^2) and their derivatives	S_B		119.2±17.3	114.9±23.0*	145.7±29.5	137.5±37.8*	99.5±30.1	113.7±21.4
	S_N		40.2±9.6	38.6±10.6*	47.7±11.2	46.0±14.1	26.7±8.6	31.5±11.1
	S_C		78.9±15.4	76.3±18.4*	97.9±22.6	91.5±34.1*	63.6±19.1	82.2±20.9
	NCR		0.52±0.10	0.52±0.10*	0.5±0.1	0.54±0.30	0.45±0.10	0.42±0.20
Neuron-glia system parameters	density of neurons		4688±868	5486±1511*	602±182	627±103	83.4±34.1	114.7±52.4*
	density of glia	free	9820±2228	13920±2591*	2378±574	4009±379*	2838±494	4996±1012*
		satellite	2870±886	4333±1250*	315±126	575±103*	53±28.8	177±128*
	glia-neuron index	free	2.15±0.60	2.79±1.20*	4.25±1.50	7.82±7.30*	41.0±20.6	55.4±34.1*
		satellite	0.62±0.20	0.83±0.20*	0.53±0.10	0.93±0.10*	0.76±0.60	1.73±1.20*

explaining the fact of pronounced perineuronal satellitosis in the motor neuron populations.

Hence, the found combinations of typical forms of adaptation restructuring at the level of cell populations in CNS structures of young animals in response to acute beer intoxication demonstrated the degree of reversible development of the shifts, the relationships between variants of adaptive changes in the structures and the active factor, and, speaking about prevention, suggested an explaination of the negative effects of low-ethanol drinks on the growing organism.

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