thymus during the whole period of embryogenesis. Similarly to cells that secrete thymosin α_1, thymulin, and thymopoietin [17], thymalin-secreting cells are located in the subcapsular and medullary zones.

The observation that thymalin is present in the basal layer of the skin, epiglottis, larynx, trachea, and esophagus are in line with published data indicating the presence of thymopoietin in skin keratinocytes of adult humans [3] and in some epithelial tissues of adult mice [13].

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The Protective Effect of Sodium Hypochlorite on Morphology and Transcription of Rat Central Neurons in Acute Nembutal Poisoning

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Hypoxia plays a key role in the pathogenesis of acute barbiturate poisoning [1,3]. Accordingly, in brain tissues, which naturally have a high level of energy metabolism, barbiturates cause damage accompanied by a decrease of creatine phosphate and of the total amount of ATP, ADP, and AMP [1,2]. In addition, in CNS neurons, in particular in the pyramidal cells of the cortex, barbiturates

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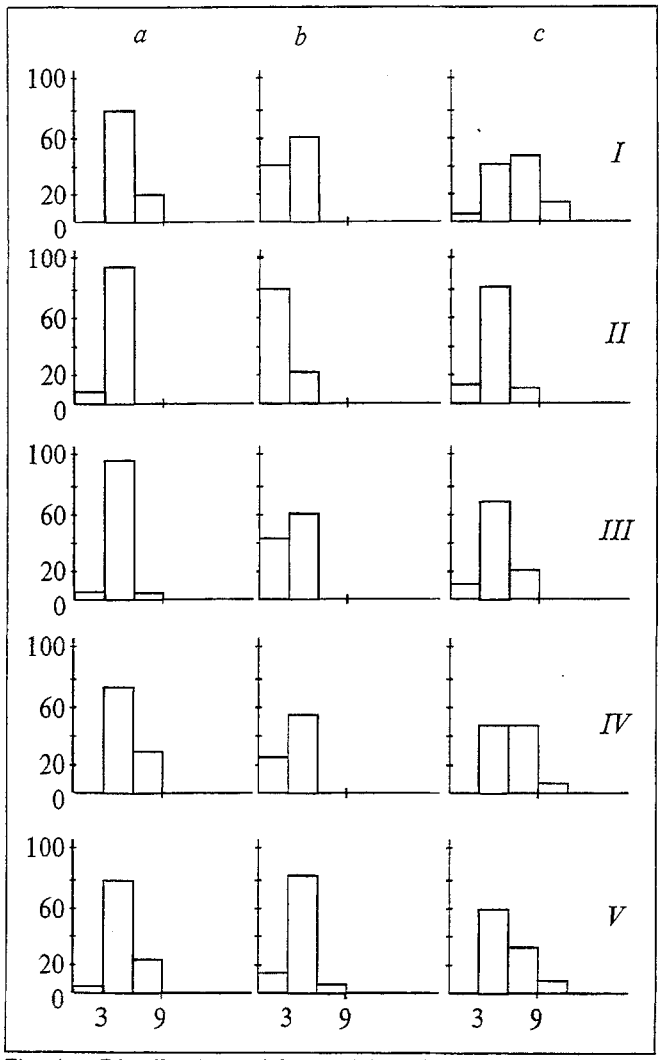

Fig. 1. Distribution of large (*a*) and medium-sized (*b*) pyramids in sensorimotor cortex and Purkinje cells (*c*) in cerebellar cortex in rat brain as a function of transcriptional activity of nuclear chromatin. Here and in Figs. 2 and 3: abscissa: level of transcription (expressed in conventional units); ordinate: fraction of cells (%). *I*) control; *II*) nembutal (500 mg/kg, sacrifice 45 min later); *III*) nembutal (500 mg/kg, sacrifice 1.5 h later); *IV*) sodium hypochlorite (3 mg/kg, sacrifice 45 min later); *V*) nembutal + hypochlorite (45 min after nembutal, sacrifice after another 45 min).

induce primary histotoxic damage which develops even in the absence of marked disorders of the hemodynamics and stems primarily from disturbances in water metabolism [2,3]. The complex of morphological changes in central neurons caused by barbiturate poisoning corresponds, on the whole, to the picture of toxohypoxic damage reported in the literature. In addition, a decrease of RNA and of the total and alkaline proteins (to the point of disappearance of the latter) has been described for damaged cells [2]. However, the functional state of the nucleus, in particular, the processes of transcription, in central neurons remains unclear in barbiturate poisoning. Another important aspect is the search for the most efficient routes and methods of detoxication of the organism. Here, simple electrochemical catalytic systems with an efficiency approximating the natural oxidative systems of the liver, but with other oxygen carriers, hold much promise. Sodium hypochlorite may serve as one such carrier [4]. The aim of the present investigation was to study the effect of sodium hypochlorite on the general morphology and transcriptional activity in different types of brain neurons and on the processes of transcription in the central neurons in cases of intoxication with high doses of nembutal.

MATERIALS AND METHODS

Male white rats weighing 200 g were injected with sodium hypochlorite in a dose of 3 mg/kg 45 min after pretreatment with nembutal (500 mg/kg). The control groups were as follows: intact animals; rats treated with nembutal only in the above dose and killed 45 min or 1.5 h after injection; animals which received hypochlorite alone in the mentioned dose and were sacrificed 45 min later. Medium-sized and large pyramids of the sensorimotor cortex along with Purkinje, basket, and grain cells were studied in the rat cerebellar cortex. The general morphological assessment was performed on slides stained with methylene blue after Nissl. Transcriptional activity was examined by the Moore historadioautographic method [5], based on recording the activity of endogenous RNA-polymerases directly on histological specimens (slides). For the RNA-polymerase reaction, 8-μ sections of the rat brain and cerebellar cortex were obtained with a histocryotome at -20°C, air-dried, and fixed in an alcohol-acetone mixture (1:1) during 5 min at 4°C. Slides were stored at -20°C until use. The slides were then covered with 0.02 ml of a mixture of the following composition (μmol): Tris-HCl buffer (pH 7.9) 100, sucrose 150, ammonium sulfate 80, 2-mercaptoethanol 12, ^{3}H-UTP 0.02, nonlabeled (cold) triphosphates 0.6 each, $MgCl_2$ 8, $MnCl_2$ 2. The slides were incubated at 37°C for 30 min. The reaction was stopped by careful washing of the slides in distilled water and then the samples were additionally fixed for 30 min with ethanol - acetic acid (3:1). Nonbinding triphosphates were removed with 5% trichloroacetic acid (15 min, at 4°C), after which the slides were washed for 30-60 min under running water, dried, covered with type M emulsion, and exposed for 10 days. The level of nuclear template activity was assessed as the number of granules of reduced silver which were counted visually. For estimation of the reliability of differences between the mean

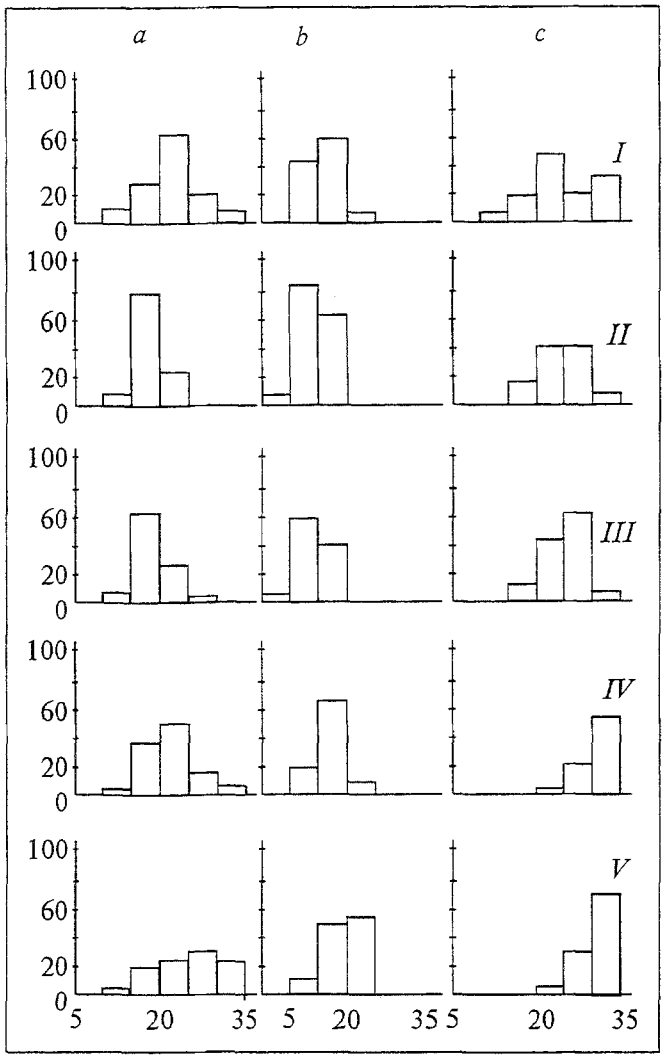

Fig. 2. Distribution of large (*a*) and medium-sized (*b*) pyramids in sensorimotor cortex and Purkinje cells (*c*) in cerebellar cortex in rat brain as a function of transcriptional activity of extranucleolar chromatin.

values of transcription the Wilcoxon test was used and between the histograms the ϕ test was used.

RESULTS

Nembutal induced structural damage to all the macroneurons, resulting in the appearance of a definite spectrum of morphologically changed cells which is largely consistent with the description of acute toxohypoxic encephalopathy for which polymorphism of cell changes is characteristic (vacuolization, different forms of chromatolysis, impregnation of nuclei with dye, a decrease of the nucleus-cytoplasmic ratios due to shriveling of nuclei, hyperchromia of cytoplasm, and so on). The latter applies more to the cortical pyramidal neurons, whereas the Purkinje cells mostly demonstrated other changes in shape, with a clearly marked dynamics of the morphological shifts depending on the time of nembutal administration. Vacuolized neurons prevailed over other cell changes in the neuronal population 45 min after injection, while 1.5 h later the majority of cells were in a state of total chromatolysis.

The changes in grain and basket cells in the cortex were not defined readily due to the very high nucleus-cytoplasmic ratio, i. e., the very small volume of cytoplasm in these cells.

The mean level of transcriptional activity of nuclear chromatin 45 min after nembutal injection was lowered (Table 1) compared to the norm due to an increase of the fraction of cells with weak labeling and to a decrease of the fraction with strong labeling (Figs. 1, 2, and 3 - *II*) in all types of neurons under study. After the next 1.5 h the level of transcription remained low in all cell types except the grain cells, where it was restored to the control level (Figs. 1, 2, and 3 - *III*).

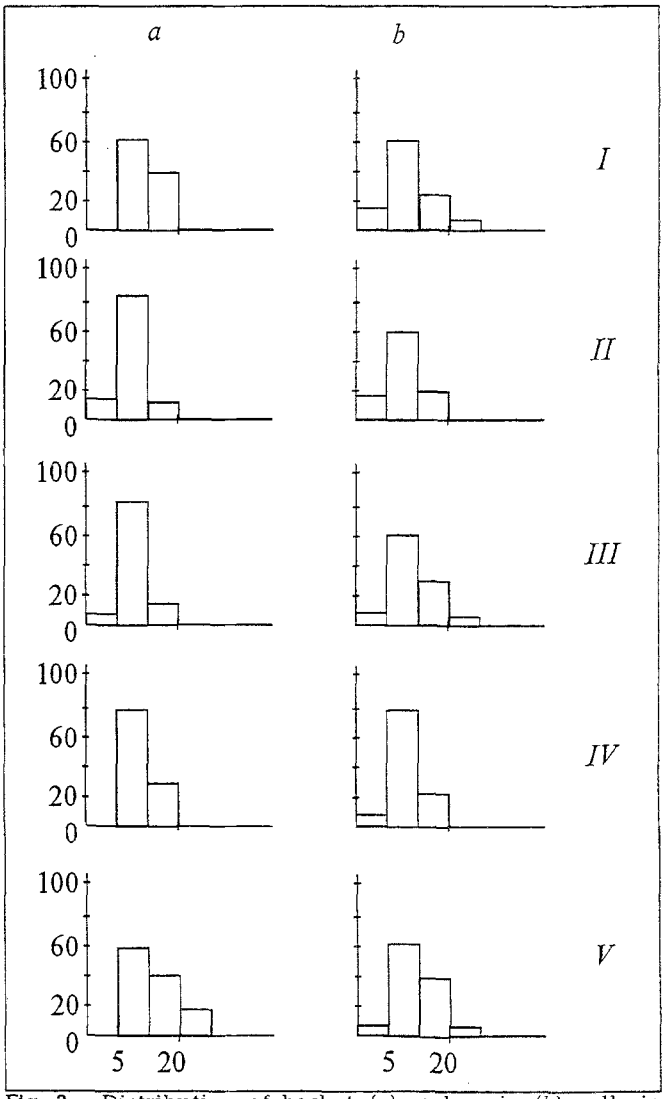

Fig. 3. Distribution of basket (*a*) and grain (*b*) cells in cerebellar cortex of rat brain as a function of transcriptional activity of chromatin.

TABLE 1. Mean Level of Transcriptional Activity of Nucleoplasmic and Nucleolar Chromatin in Neurons of Sensorimotor Cortex and Cerebellar Cortex of Rat Brain

Experimental conditions	Nucleoplasm			Nucleolus			Nucleoplasm+ nucleolus	
	LP	MP	PC	LP	MP	PC	BC	GC
C	22.8	11.5	25.5	5.7	3.8	6.7	10.2	9.3
NbI	18.5	9.9	20.6	4.5	3.2	4.6	8.1	7.6
NbII	19.2	10.1	19.5	4.9	3.7	5.2	8.2	9.4
SH	22.5	12.2	27.7	6.0	4.1	6.9	9.7	8.8
Nb+SH	26.9	14.5	26.1	5.8	4.8	6.4	10.9	9.5

Note. C: control; NbI: animals treated with nembutal only and sacrificed 45 min after injection; NbII: animals treated with nembutal only and sacrificed 1.5 h after injection; SH: animals treated with sodium hypochlorite only and sacrificed 45 min after injection; Nb+SH: animals treated with sodium hypochlorite 45 min after pretreatment with nembutal; LP: large pyramidal neurons; MP: medium pyramidal neurons; PC: Purkinje cells; BC: basket cells; GC: grain cells.

Administration of sodium hypochlorite to intact rats did not result in substantial changes in the morphology of the neuronal population under investigation, although the fraction of cells with chromatolysis slightly increased in the pyramidal neuron population, while among the Purkinje cells a small percentage of neurons with single large vacuoles appeared. The mean value of transcriptional activity in large pyramidal and basket neurons and in grain cells was not reliably altered after sodium hypochlorite administration whereas in the medium-sized pyramids and Purkinje cells it was somewhat increased due to the drop in the number of weakly labeled nuclei (Figs. 1, 2, *b*, *c* - *IV*), and the increase mentioned for the extranucleolar labeling attained statistical reliability, while not exceeding 10% (Table 1).

Sodium hypochlorite administration to animals pretreated with nembutal not only prevented the development of pathological processes, but 45 min after injection resulted in a marked normalization of the general neuronal morphology damaged by nembutal, whereas without hypochlorite pronounced pathological shifts were in evidence at this time. The mean level of transcription in this series was also higher than in animals without treatment; for neurons in the cerebellar cortex (Purkinje, grain, basket cells) this parameter matched the level of the intact control, while for the extranucleolar chromatin of pyramidal neurons in the sensorimotor cortex it significantly exceeded the norm. However, the character of distribution of nuclei depending on the labeling level was different from the norm even for proximity of the mean indexes.

Thus, sodium hypochlorite administration resulted in a marked improvement of the general morphological state of the studied population, both compared to the initial status at the time of injection, i.e., 45 min after nembutal injection, and compared to the corresponding control (1.5 h after nembutal injection).

The results obtained attest that the toxic effect of nembutal on brain neurons not only manifests itself in morphological changes but also affects cell protein synthesis at the level of transcription and ib expressed in inhibition of template activity of nuclear DNA. Sodium hypochlorite at 3 mg/kg administered 45 min after nembutal injection decreases to a great extent both the morphological signs of the toxohypoxic effect of nembutal and the inhibition of transcription. For pyramidal neurons the normalizing action of hypochlorite is transformed into a stimulating action.

The restorative effect of this drug probably derives from its capacity to activate transcription in intact neurons. This was reliably demonstrated here only for the medium-sized pyramids and Purkinje cells, evidently due to their higher sensitivity to changes in the oxygen balance.

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