The fact that the programming of the CBG level by androgens in male rats occurs in the prepubertal period, characterized by a quite high degree of differentiation, is a new fact that indicates adaptability of highly organized structures and which broadens our ideas on general and special principles governing the formation of sexual dimorphism of various features. Moreover, this fact may also be of practical importance, for the possibility of irreversible actions of sex hormones on various functions of the body must be taken into account when sex hormone therapy is given prepubertally in man. Under these circumstances the administration of large doses both of androgens and of estrogens may lead to serious and irreversible changes not only of sex-dependent functions of the brain, but also of the liver.

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VITAMIN E DEPRESSES THE NEUROCYTOTOXIC ACTION OF KAINIC ACID IN CEREBELLAR GRANULE-CELL CULTURES

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KEY WORDS: cerebellar granule cells; kainic acid; α-tocopherol

The study of the mechanisms of action of neurocytotoxins — excitatory amino acids interacting with the glutamate receptor (glutamate, N-methyl-D-aspartate, quisqualic, ibotenic, kainic, and quinolinic acids) — is developing extremely rapidly at the present time. Interest in this problem is largely determined by the fact that the action of these neurotoxins on brain cells is regarded as the most adequate model with which to study the pathogenesis of various neurodegenerative diseases (Parkinson's and Alzheimer's diseases, Huntington's chorea, epilepsy, and also cerebral ischemia) [5, 11, 12]. In this connection analysis of the mechanism of action of these neurotoxins and their antagonists is essential if optimal pharmacological approaches are to be found to the prevention and treatment of neurodegenerative diseases of the human CNS.

The mechanism of action of the above-mentioned neurotoxins is known to be a complex cascade process. It begins with interaction of the neurotoxin with the glutamate receptor and activation of the Ca^{2+}-channel associated with the receptor, and it ends with activation of proteolytic and lipolytic Ca^{2+}-dependent enzymes as a result of entry of Ca^{2+} (calcium death) [4, 14]. The intermediate stages of this cascade mechanism have received less study. Data obtained recently point to the involvement of active forms of oxygen in the realization of the neurotoxic action of kainic acid on neurons in culture [6]. Since membrane lipids constitute one of the principal oxidation substrates during the action of active forms of oxygen [13], and since the state of the lipid bilayer of the outer cytoplasmic membrane determines the low intracellular Ca^{2+} concentration, it was natural to suggest that destruction of membrane lipids may be an important step in damage to neurons under the influence of kainic acid. Since vitamin E is a universal stabilizer of neuron membranes [2, 3], it was decided to study the effect of α-tocopherol on the neurotoxic action of kainic acid, using monolayer cultures of cerebellar granule cells.

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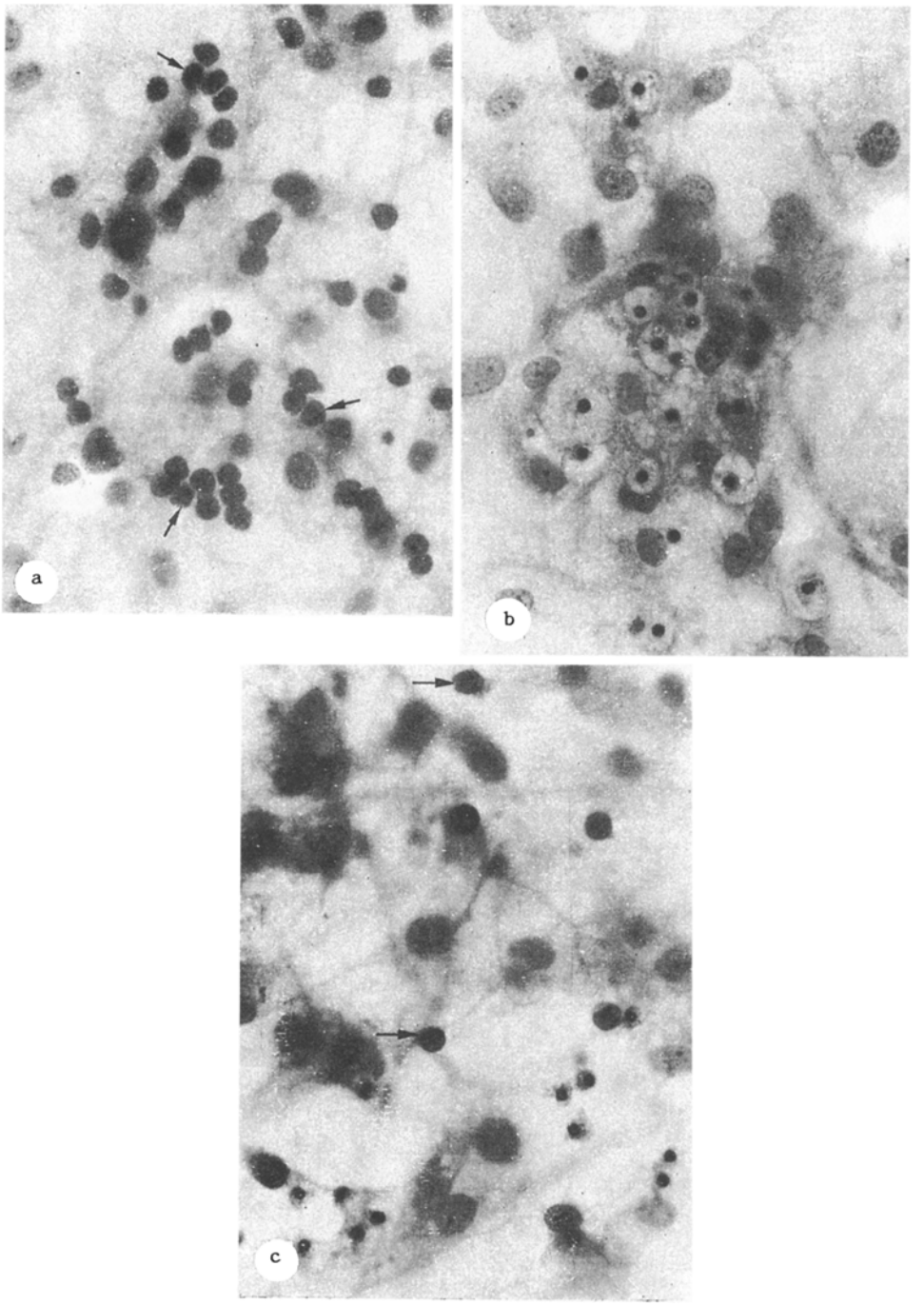

Fig. 1. Granule cells in monolayer-dissociated culture of cerebellum of 6-day-old rats. Seventh day of culture. Stained with vanadium-hematoxylin. Scale 20 μ. a) Control culture. Stained nuclei of normal granule cells (arrows). Larger nuclei of glial cells can be seen; b) changes in granule cells under influence of 10^{-4} M kainic acid. Marked edema of granule cells and compressed, darkly stained nuclei of degenerated neurons can be seen; glial cells are intact; c) effect of α-tocopherol on granule cells treated with 10^{-4} M kainic acid. Besides degenerated granule cells, unchanged neurons can still be seen in the culture (arrows).

EXPERIMENTAL METHOD

A suspension of cerebellar granule cells from Wistar rats aged 4-6 days was obtained by a modified enzymic dissociation method [15] and cultured on slides covered with poly-L-lysine [1] or with dry collagen [10], in plastic dishes by the usual laboratory method.

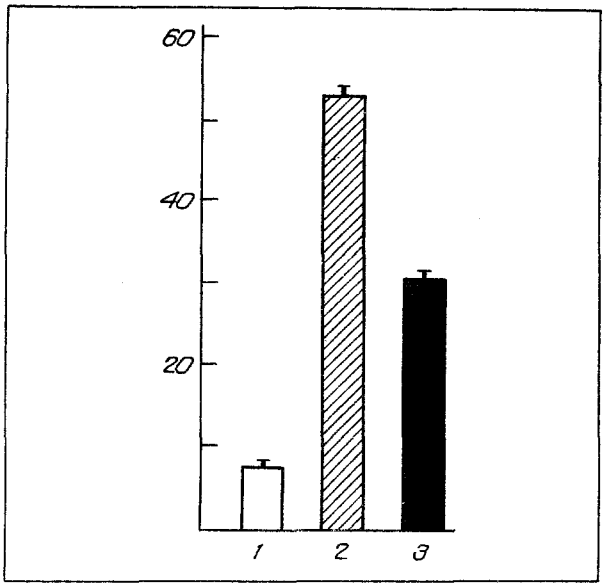

Fig. 2. Effect of α-tocopherol on destruction of cerebellar granule cells of 6-day-old rats, induced by kainic acid. Ordinate, number of degenerated granule cells (in per cent of total number of cells). 1) Control, 2) 10^{-4} M kainic acid, 3) $5 \cdot 10^{-4}$ M α-tocopherol + 10^{-4} M kainic acid; $p < 0.01$.

In the experiments to study the effect of vitamin E on cerebellar granule cells subjected to the action of kainic acid, we used 7-8-day cultures, divided into three groups: 1) control cultures incubated consecutively in Simms' salt solution (SSS) for 2 h and 30 min in salt solution with the addition of $CaCl_2$ (Ca^{2+}-SS) of the following composition: 8.0 g NaCl, 0.2 g KCl, 0.25 g $CaCl_2$, 0.005 g NaH_2PO_4, 1.0 g $NaHCO_3$, and 2.0 g glucose to 1 liter; 2) cultures treated with kainic acid (10^{-4} M, 30 min), added to the Ca^{2+}-SS; 3) cultures treated consecutively with α-tocopherol ($5 \cdot 10^{-4}$ M) in SSS (2 h, 37°C) and kainic acid (10^{-4} M, 30 min, in Ca^{2+}-SS).

Since the stock solution of α-tocopherol was made up in 96° alcohol, alcohol was added to the incubation media of the cultures of Groups 1 and 2 in a volume equal to the volume of alcohol in the α-tocopherol solution. The scheme of the experiment for all groups of cultures was the same and it included: 1) washing the cultures with SSS; 2) incubation consecutively in salt solutions containing (Groups 2 and 3) and not containing (Group 1) the biologically active substances under investigation; 3) washing in SSS, 4) culture in nutrient medium for 4-24 h (35.5 ± 0.5°C), 5) fixation with a mixture of alcohol, formalin, and acetic acid (7:2:1), and 6) staining with vanadium-hematoxylin.

The number of intact granule cells and the number of pycnotic nuclei were counted in the histological preparation thus obtained. Counting was carried out in 35-40 randomly chosen fields of vision on each slide. In each of the eight experiments in one group no fewer than three cultures were used. The results were subjected to statistical analysis and expressed as the number of dying cells (mean ± error of the mean). Significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

The neuron population in dissociated cerebellar cultures of 4-6-day old rats consisted of neurons of only one type, namely granule cells. Because of the small size of the perikaryon (6-8 μ), the high refractoriness, and the typical neuronal arrangement on the surface of the glial monolayer, the granule cells could easily be identified intravitally under phase contrast.

Intravital observation of the control cultures (Group 1) revealed no changes in the cells. During the study of cultures treated with kainic acid (Groups 2 and 3) some cells were enlarged in volume and lost their refractoriness 20-30 min after addition of the kainate. After 2 h the edema of these neurons was intensified and compression of the nuclei could be observed; intravitally this took the form of highly refractory round structures 3-4 μ in diameter.

The study of histological preparations of the control cultures revealed many intact round granule cells with deeply stained nucleus, containing particles of dispersed chromatin (Fig. 1a). In cultures treated with kainate (Groups 2 and 3), besides unchanged granule cells, other cells could be seen which were greatly enlarged in volume or completely destroyed, and replaced by darkly stained pycnotic nuclei (Fig. 1b, c). The ratio (in per cent) of pycnotic nuclei to total number of granule cells varied in cultures of Group 1 from 4.7 to 9%, in cultures of Group 2 from 28 to 85%, and in cultures of Group 3 from 11 to 78.4%. It must be pointed out that all the changes mentioned above under the influence of kainate were observed only if Ca^{2+} was present in the incubation solution, in agreement with the known data on the Ca^{2+}-dependent character of the neurotoxic action of kainic acid.

The results in Fig. 2 show that kainic acid caused death of a certain number of granule cells in dissociated cultures of the cerebellum, in agreement with results published previously, obtained by different experimental methods of assessment of neuronal death [7]. Preliminary treatment of the cerebellar granule cells in culture with α-tocopherol leads to a statistically significant decrease in the number of degenerated neurons, subjected to the action of kainic acid. The variability of the relative percentages of intact neurons and neurons destroyed by the action of kainate, and which was also found in cultures treated beforehand with α-tocopherol, is the result of differences in the differentiation of these neurons at the time that the cerebellar tissue was taken for dissociation, and of their sensitivity to the action of excitatory amino acids, which are glutamate analogs [8, 9].

The results thus demonstrate the definite protective effect of α-tocopherol against the action of kainic acid on cerebellar granule-cells in culture. Meanwhile, considering the universal character of the stabilizing action of vitamin E on neuronal membranes, which is manifested both during activation of lipid peroxidation and during enzymic hydrolysis of membrane phospholipids by phospholipase A_2 [2, 3], further research is needed in order to discover the molecular mechanism lying at the basis of the protective effect described above.

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