ACTION OF QUINOLINIC ACID ON NEURONS IN DISSOCIATED CELL CULTURES FROM VARIOUS BRAIN STRUCTURES

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Recent investigations [4, 9, 10, 11] have shown that quinolinic acid (QA), a tryptophan metabolite, has a neurodestructive action, similar in many respects to the cytotoxic effect of excitatory amino acids. It has also been found that the QA level in the animal brain increases with age [8]. On the basis of these data it has been suggested that QA, as an endogenous neurotoxin, can play the role of pathogenetic factor in several neurodegenerative diseases, such as Huntington's chorea and Alzheimer's disease [3, 7, 14]. A culture of brain cells is a model which can be used to study destructive changes in living neurons under the influence of QA. In the investigation described below we used dissociated cultures of cells from the embryonic mouse hippocampus, septum, and neocortex.

EXPERIMENTAL METHODS

Cultures were prepared from cells of the above-mentioned brain structures of 17-19-day mouse embryos, obtained by enzymic and mechanical dissociation and suspended in nutrient medium [1]. Two drops of a suspension containing hippocampal or neocortical cells or a mixture of hippocampal and septal cells were applied to each of a number of coverslips coated with collagen (up to 15,000-20,000 cells per coverslip). During the first 5-6 days the cultures were incubated in modified Petri dishes [2] in a humidified incubator filled with a gas mixture of 5% CO_2 + 95% air at 35.5°C. Each coverslip with culture was then transferred into a plastic dish 40 mm in diameter containing 1 ml of nutrient medium and incubated under the same conditions without any subsequent change of medium. At different times of culture, QA was added to the nutrient medium, all of which was removed from each dish, up to a final concentration of 250 or 500 μM, after which the medium was returned to the same dish. The cultures were investigated intravitally by the phase-contrast method in an MBI-13 inverted microscope, and then stained by Nissl's method or histochemically for acetylcholinesterase [5], and counterstained with cresyl violet.

EXPERIMENTAL RESULTS

During culture, morphogenetic processes typical of brain cells when developing under dissociated culture conditions were observed: growth of processes from isolated nerve cells and from glioneuronal aggregates, the formation of axoglial bundles between aggregates, and the development of morphological varieties of nerve and glial cells. After the second week, in vitro neurons could be seen in the cultures, where they were localized in aggregates (Fig. 1a) and also independently (Fig. 2a).

The cytotoxic action of QA after its addition to the nutrient medium in a concentration of 500 μM on the 20th-22nd days of culture of hippocampal and neocortical cells was apparent after only 30-40 min, and after 1.5-2 h degeneration of neurons was observed (Fig. 1b, II, c, II).

In the control cultures, no appreciable morphological changes were observed in the neurons at this period of culture (Fig. 1a). In a lower QA concentration (250 μM) degeneration of the neurons developed much more slowly, and began toward the end of the 1st day. As a rule

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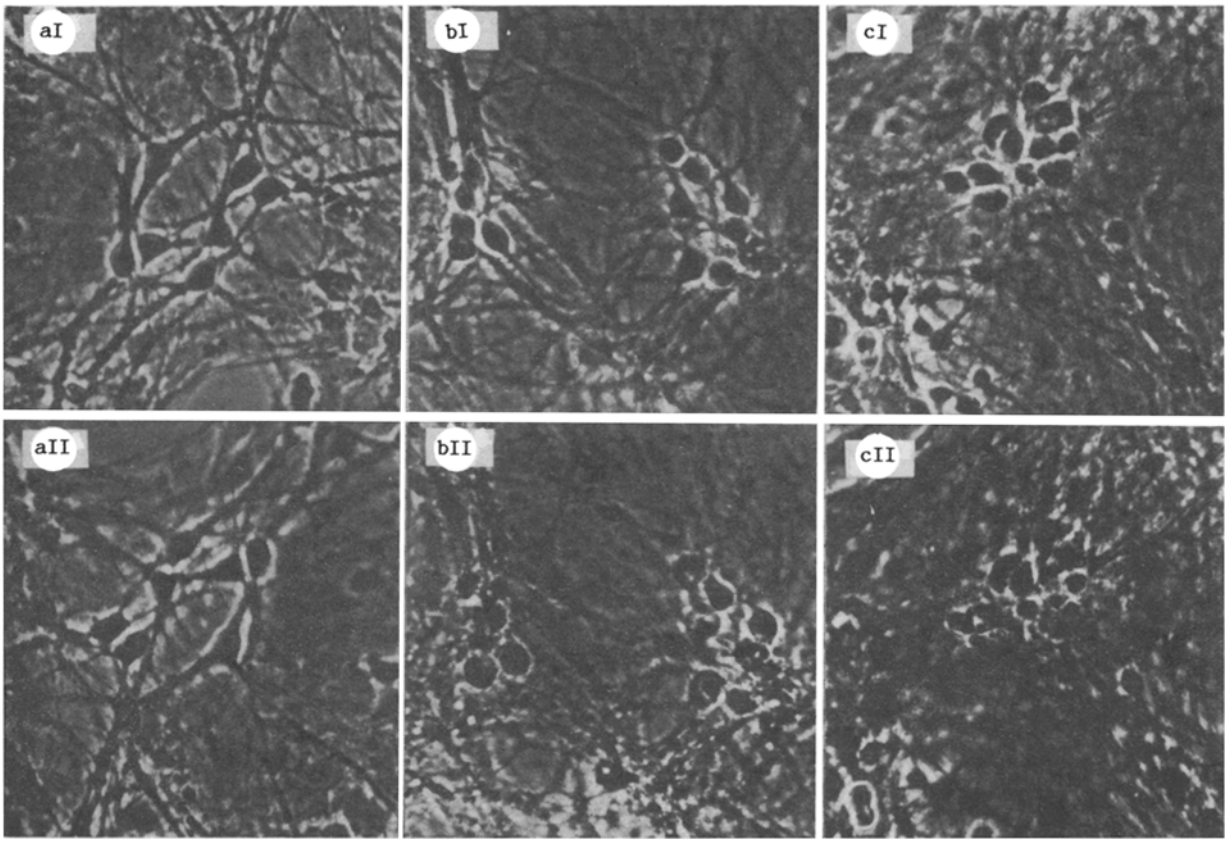

Fig. 1. Destructive action of QA in neurons in dissociated cultures. a) Group of neurons in aggregate of control culture of hippocampal cells: I) 21st day in vitro, II) 23rd day in vitro. b, c) Groups of neurons in aggregates of culture of hippocampal (b) and neocortical (c) cells: I) before addition of QA, II) degeneration of neurons 2 h after addition of QA (500 μM). 21st day in vitro. Living cultures, phase contrast. Objective 20, ocular 10. Scale 50 μ.

neurons located in glioneuronal aggregates were exposed to the action of QA, whereas individual nerve cells did not degenerate (Fig. 2a).

When cultures subjected to the action of QA in the late stages of development were stained by Nissl's method, degeneration and lysis of neurons were found in the aggregates, but were never found in cultures to which QA was added in the earlier stages (before 2 weeks) in vitro. In this period QA in a concentration of 500 μM had no appreciable destructive effect on nerve cells in culture (Fig. 2b), whose subsequent development did not differ from the development of neurons in control cultures.

The features of the cytotoxic action of QA on neurons in dissociated cultures of hippocampal and neocortical cells described above were similar.

On combined culture of hippocampal and septal cells, large single neurons staining positively for acetylcholinesterase could be seen after 2-3 weeks in the peripheral zone of the glioneuronal aggregates (Fig. 2c, I). Under the influence of QA these cells remained intact for a long time, whereas other neurons in the composition of the aggregates degenerated (Fig. 2c, II).

The data given above demonstrate features distinguishing the nonspecific cytotoxic effect of QA, because the amount of it (40-80 μg) added to the nutrient medium was much more than the quantity of QA (5-10 μg) which had a selective destructive action on neurons of different brain structures in vivo [11, 12]. The results also are evidence that the neurocytotoxic action of QA in vitro depends both on the character of intercellular relations established in dissociated culture and on the period of its development. According to data in the literature, QA predominantly causes destruction of neurons which have mature synaptic connections with afferent fibers, whose presynaptic endings remain undamaged, in the whole brain

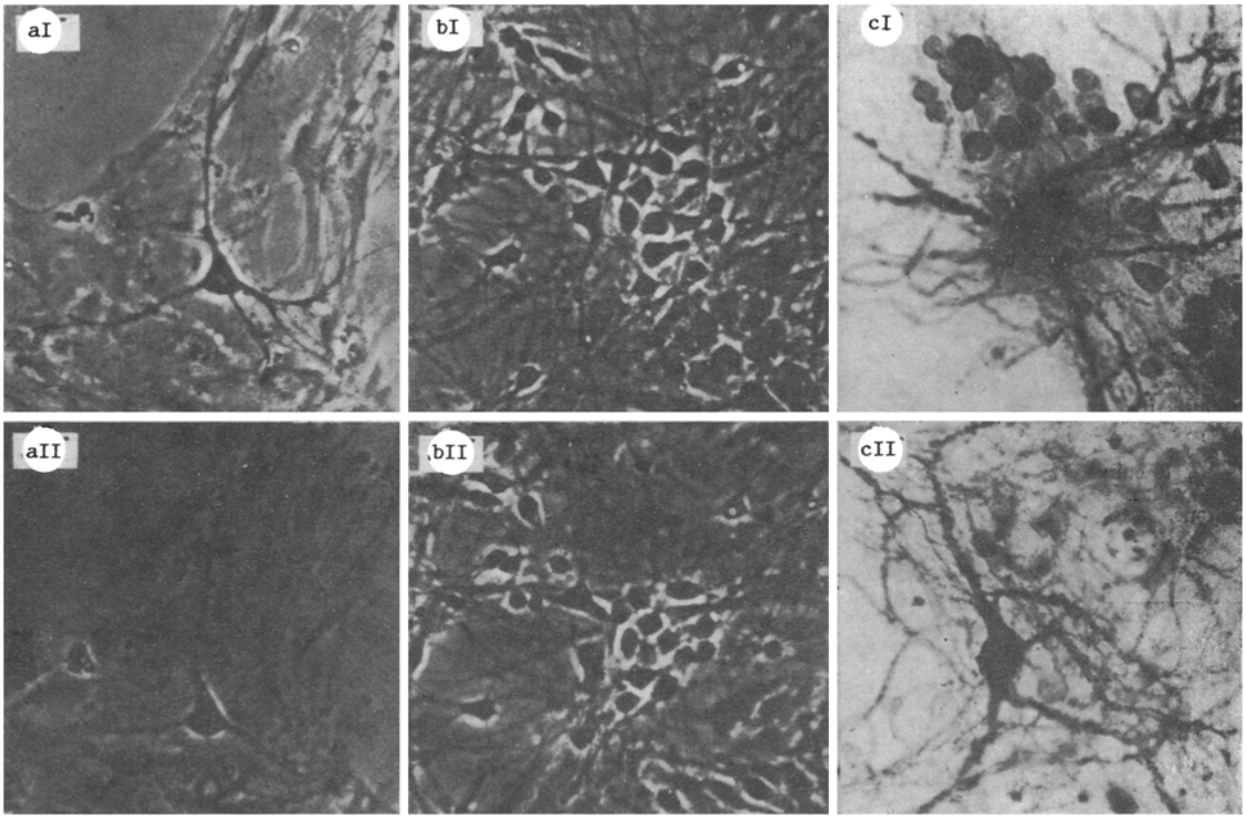

Fig. 2. Absence of destructive action of QA on neurons in dissociated cultures. a) Solitary neuron in culture of neocortical cells before (I) and 24 h after (II) addition of QA (500 μM). 21st day in vitro; b) group of neurons in aggregate of hippocampal cell culture before (I) and 24 h after (II) addition of QA (500 μM). 10th day in vitro; C: I) cholinergic neuron in aggregate among other neurons in combined culture of hippocampal and septal cells. 21st day in vitro, II) degeneration of neurons in aggregate containing intact cholinergic neuron, 48 h after addition of QA (500 μM). 23rd day in vitro. a, b) Living cultures, phase contrast; c) stained for acetylcholinesterase and counterstained with cresyl violet. a, b) Objective 20, ocular 20. Scale 50 μ; c) objective 40, ocular 10. Scale 70 μ.

and in organotypical cultures of nerve tissue [13, 15]. Our experiments showed that QA in the early stages of culture had no cytotoxic effect, evidently due to the absence or immaturity of synaptic connections between the neurons. The destructive action of QA on hippocampal and neocortical neurons localized in aggregates, observed in the later period in vitro, may be attributed to the presence of fully formed synapses on these neurons with afferent fibers reaching the aggregates in the composition of the axoglial bundles.

In combined cultures of hippocampal and septal cells, cholinergic neurons located in the peripheral zone of aggregates could remain intact when exposed to the action of QA because they are not specific targets of hippocampal neurons and are not innervated by them. The results of the experiments in vivo also indicate that septal neurons are less vulnerable to the destructive action of QA than neurons of other cholinergic brain structures [6, 11].

The results are thus in agreement with existing views on synaptic mechanisms of pathomorphological changes in neurons under the influence of QA and they widen the prospects for the search for antagonists preventing the destructive action of this endogenous neurotoxin.

LITERATURE CITED

1. I. V. Viktorov, Textbook of Nerve Tissue Culture [in Russian], Moscow (1976), pp. 30-38.
2. I. V. Viktorov and E. M. Kenarskaya, Byull. Éksp. Biol. Med., No. 8, 119 (1975).
3. F. M. Beal, N. W. Kowall, D. W. Ellison, et al., Nature, 321, 168 (1986).
4. R. J. Boegman, S. R. El-Defrawy, K. Jhamandas, et al., Neurobiol. Aging, 6, 331 (1985).

5. A. El Badavi and E. A. Shenk, J. Histochem. Cytochem., 15, 580 (1967).
6. S. R. El-Defrawy, F. Coloma, K. Jhamandas, et al., Neurobiol. Aging, 6, 325 (1985).
7. A. C. Foster, W. O. Whetsell, E. D. Bird, and R. Schwarcz, Brain Res., 336, 207 (1985).
8. F. Moroni, G. Lombardi, G. Moneti, and C. Aldinio, Neurosci. Lett., 47, 51 (1984).
9. M. N. Perkins and T. W. Stone, Exp. Neurol., 88, 570 (1965).
10. R. Schwarcz, A. C. Foster, E. D. French, et al., Life Sci., 35, 19 (1984).
11. R. Schwarcz and C. Köhler, Neurosci. Lett., 38, 85 (1983).
12. R. Schwarcz, C. Speciale, E. Okuno, et al., Excitatory Amino Acids and Epilepsy, eds. R. Schwarcz and Y. Ben-Ari, New York (1986), pp. 197-212.
13. R. Schwarcz, W. O. Whetsell, and R. M. Mangano, Science, 219, 316 (1983).
14. T. W. Stone and H. H. Connick, Neuroscience, 15, 597 (1985).
15. W. O. Whetsell and R. Schwarcz, J. Neural Transmiss., 19, 53 (1983).

EFFECT OF COMBINED PROTEIN AND CALORIC DEFICIENCY ON SYNAPTIC ULTRASTRUCTURE IN THE MOLECULAR LAYER OF THE CEREBELLAR CORTEX OF DEVELOPING AND ADULT ANIMALS

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Because of the particular features of its late cytogenesis the cerebellum is more sensitive than any other part of the brain to the action of unfavorable external environmental factors [9-11]. In previous investigations the writers showed that long-term combined protein and caloric deficiency leads to an increase in the number of dark pyriform neurons in developing and adult animals, and also causes ultrastructural changes both in cells and in ultrastructural components of the neuropil of the molecular layer of the cerebellar cortex [5, 6]. Investigation of the structural organization of interneuronal connections under conditions of depressed cellular metabolism is of great interest in connection with the study of compensatory and adaptive reactions reflecting intracellular regeneration [8].

The aim of this investigation was a quantitative electron-microscopic study of disturbances of synaptic ultrastructure in the molecular layer of the cerebellar cortex of young and adult mice, receiving a diet deficient in protein and calories.

EXPERIMENTAL METHOD

CBA mice were used. Protein–caloric deficiency was created by reducing the quantity of nutrients by introducing an extra volume of cellulose into the diet. The content of ingredients in the experimental diet was 50% of that of the control diet. In age group 1, nursing females 10 days after giving birth to their young were switched to a low-protein diet, and from the 22nd through the 40th days, young mice fed themselves on a deficient diet. The animals were killed 1 month after the beginning of starvation. In age group 2, mice aged 2 months received the same synthetic diet for 1 month and were then sacrificed. Under pentobarbital anesthesia the brain was fixed by intravital perfusion through the ascending aorta with a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4, 0.1 M). Sagittal sections of the dermis of the cerebellum were cut, prefixed in 2% OsO_4 solution in the same buffer, stained with uranyl acetate, and embedded in Araldite [5]. The electron-microscopic data were subjected to quantitative analysis by visual classification of synapses in accordance with a number of features [4]. Each synapse was assessed relative to six morphological features, which were rated in points: from minimal (1 point) to maximal (5 points).

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