The result as a whole indicates that adaptation to short-term stress somehow stabilizes the creatine kinase system in acute hypoxia and maintains its ability to restore the CP concentration quickly during reoxygenation; this, in turn, is accompanied by more marked superrecovery of the contractile function of the heart.

LITERATURE CITED

1. V. I. Kapel'ko, V. V. Kupriyanov, N. A. Iiovikova, et al., Fiziol. Zh. (Kiev), No. 3, 34 (1988).
2. F. Z. Meerson, V. V. Malyshev, E. N. Ekimov, et al., Vopr. Med. Khim., No. 1, 76 (1986).
3. F. Z. Meerson, M. G. Pshennikova, E. V. Shabunina, et al., Vestn. Akad. Med. Nauk SSSR, No. 6, 47 (1987).
4. W. P. Meshkova and S. E. Severin, Textbook of Practical Biochemistry [in Russian], Moscow (1979), pp. 186-189.
5. D. G. Hearse and E. B. Chain, Biochem. J., **128**, 1125 (1972).
6. H. G. Hers, Adv. Metabol. Dis., **1**, 1 (1964).
7. D. Keppler and K. Decker, Methods of Enzymatic Analysis, Vol. 3, New York (1974), pp. 1123-1131.
8. F. Z. Meerson, A. D. Dmitriev, V. I. Zayatz, et al., Myocardial Metabolism, Harwood (1987), pp. 508-512.
9. W. B. Rathbun and M. V. Betlach, Analyt. Biochem., **28**, 436 (1969).
10. G. Szasz, J. Waldestram, and M. Gruber, Clin. Chem., **25**, 446 (1979).

COMPARATIVE CYTOTOXIC ACTION OF QUINOLINIC ACID AND N-METHYL-D-ASPARTATE ON HIPPOCAMPAL NEURONS IN CULTURE

L. G. Khaspekov, É. Kida, I. V. Viktorov, and M. Mossakowski

UDC 616.831.314-091.81-02:615.917:547.831]-07

KEY WORDS: nerve cell culture; hippocampus; neurocytotoxins; quinolinic acid; N-methyl-D-aspartate

The neurocytotoxic action of glutamic acid (GA), a CNS neurotransmitter, and its endogenous analog, quinolinic acid (QA), recently discovered by many investigators, laid the foundations for the suggestion that excitatory amino acids (EAA) play a role in the pathogenesis of nervous and mental diseases, accompanied by systemic degeneration of brain neurons [4, 11]. In this connection some relevant experimental investigations have been made of the mechanisms of action of EAA on neurons of various brain structures both in vivo and in vitro.

The excitatory and neurodestructive effect of GA has been shown to be mediated by receptors of three types for its exogenous analogs: N-methyl-D-aspartate (NMDA), kainate, and quisqualate [12]. The results of recent investigations [13] have demonstrated the predominant role of receptors for NMDA in mediation of the neurocytotoxic effect of QA. Meanwhile comparison of the character of the excitatory responses of neurons to QA and NMDA suggested that these responses are mediated by different subtypes of NMDA-receptors [5, 12].

The aim of the present investigation was a comparative morphological study of the cytotoxic effect of QA and NMDA on hippocampal neurons of mouse embryos, developing in cell culture, an object widely used in recent years to study the mechanisms of the neurodestructive action of EAA [4, 9].

Laboratory of Experimental Neurocytology, Brain Institute, Academy of Medical Sciences of the USSR, Moscow. Center for Experimental and Clinical Medicine, Polish Academy of Sciences, Warsaw.

(Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Adrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 9, pp. 246-249, September, 1990. Original article submitted July 28, 1989.

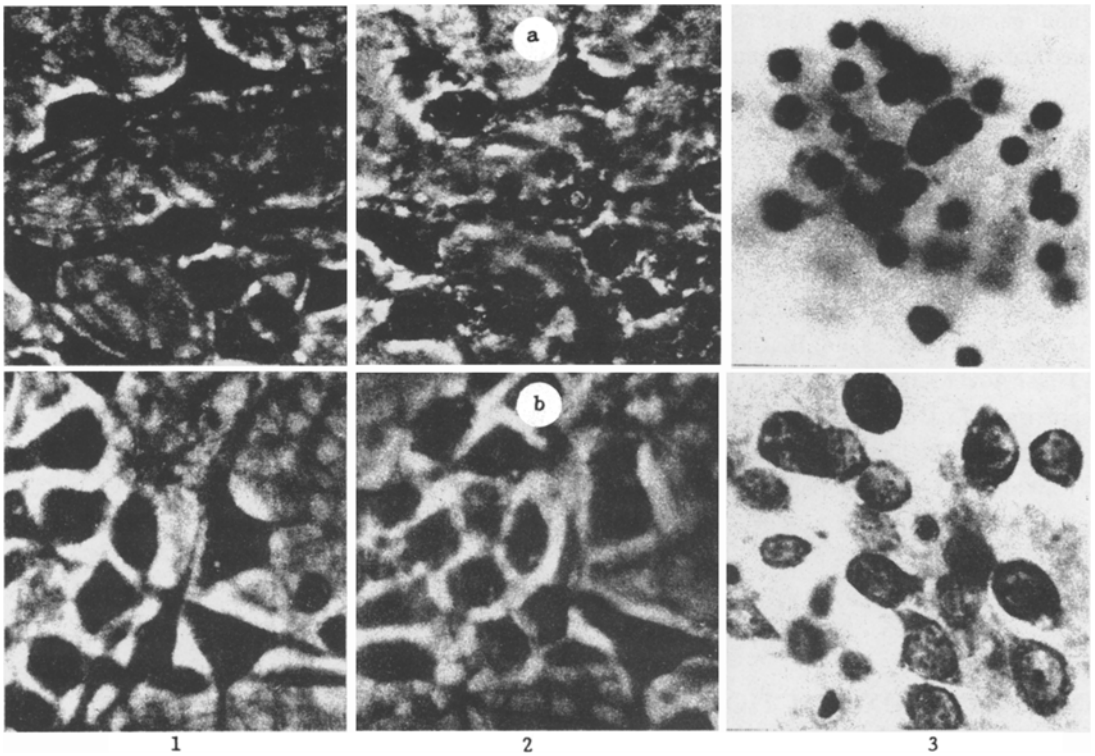

Fig. 1. Action of NMDA and QA on embryonic hippocampal neurons in culture: a) 1 h before (1) and 16 h (2) and 24 h (3) after addition of NMDA (0.1 mM) on 10th day in vitro to nutrient medium; b) 1 h before (1) and 16 h (2) and 10 days (3) after addition of QA (0.5 mM) on 10th day in vitro to nutrient medium. a1,2 and b1,2) living cultures, phase contrast; a3 and b3) stained with cresyl violet. Objective 40, ocular 10. Scale 30 μ.

EXPERIMENTAL METHOD

Dissociated cultures of hippocampal cells from 17-19-day mouse embryos of the C57B1/6 Strain were obtained by the method described by the writers previously [2]. On the 10th day of culture NMDA (0.1 mM) or QA (0.5 mM) was added to the nutrient medium. The living cultures were studied 14-16 h after addition of the toxin and photographed in phase contrast, after which they were stained with Cresyl violet or treated for electron microscopy [6].

EXPERIMENTAL RESULTS

Active growth and fasciculation of islets of nerve and glial cells were observed in vitro in the control cultures during the first 10-12 days. During this time, most of the hippocampal cells in culture were located in glioneuronal aggregates, in which the neuron bodies and their processes were clearly distinguishable (Fig. 1: a1, b1). The light-microscopic and ultrastructural characteristics of the neurons during development for 3-4 weeks in control cultures were described in more detail previously [2, 6].

After addition of NMDA (0.1 mM) to the nutrient medium on the 10th day in vitro, intravital changes of nerve cell structure were observed as early as during the first few hours, and after 14-16 h destruction was considerable (Fig. 1, a2). On ultrastructural investigation (Fig. 2a) massive vacuolation of the perikaryon and fragmentation of the nuclear chromatin were discovered in the neurons. In individual nerve cells vacuoles were found also in the nucleoplasm, and some of them were located between membranes of the split nuclear membrane. In cells with the most marked destructive changes, disappearance of the mitochondria, dispersion of ribosomes, and lipid granules and concentrations of electron-dense material could be found. Intensive swelling of neuronal processes was observed in the neuropil (Fig. 2b). Glial cells as a rule remained intact, but the bodies and processes of some of them contained vacuoles, lipid inclusions, and glycogen particles. Further culture during the next 24 h was accompanied by total degeneration of the neurons (Fig. 1, a3). A similar cytotoxic reaction of NMDA was observed after its addition to the nutrient medium at later periods also in vitro (up to 4 weeks). Preliminary addition of

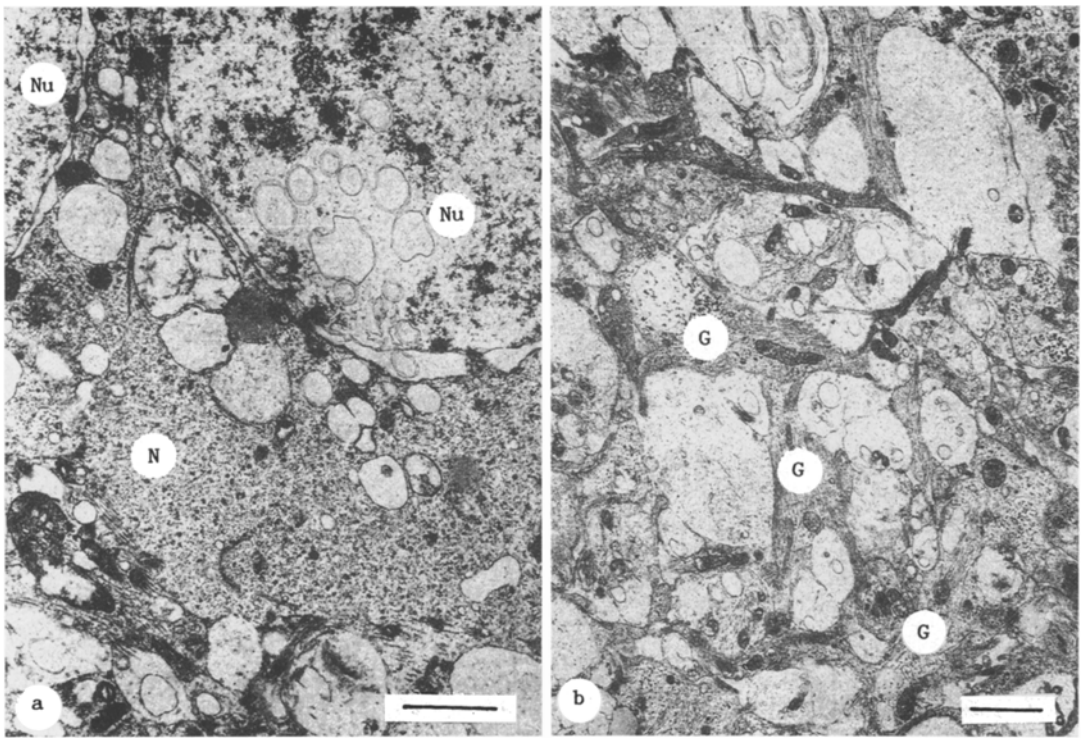

Fig. 2. Ultrastructure of hippocampal cells in culture 16 h after addition of NMDA (0.1 mM) on 10th day in vitro. a) Massive vacuolation of perikaryon and nucleoplasm of a neuron (N), fragmentation of chromatin and splitting of nuclear membrane (N). 6000×; b) swelling and vacuolation of neuronal processes in neuropil, vacuoles, lipid inclusions, and glycogen particles in glial cells (G). 5000×. Scale 3 μ.

D,L-2-amino-5-phosphonovalezate (APV), a specific antagonist of NMDA-receptors [13], to the cultures 5-7 min before addition of NMDA, in a concentration of 0.2 mM blocked the neurodestructive effect.

Addition of QA (0.5 mM) to the nutrient medium on the 10th day in vitro did not cause any subsequent appreciable morphological changes in the neuron (Fig. 1, b2,3). On electron-microscopic investigation 16 h after addition of QA (Fig. 3a,b) intact nerve cells were found, with only a few vacuoles visible in the cytoplasm and dendrites of individual neurons. Newly formed synaptic junctions remained intact. During subsequent culture the process of differentiation of the nerve cells observed in the control cultures continued and by the end of the 3rd week the morphological characteristics of the neurons in the control and in cultures treated on the 10th day in vitro with QA (Fig. 1, b3) were similar [6].

On the other hand, addition of QA later (3-4 weeks in vitro), when mature synapses were found in the cultures, in the course of 2-3 h had a marked neurodestructive effect, one of the characteristic morphological features of which, as was shown previously by the present writers [6, 14], and also by in vivo studies and in organotypical cultures of the hippocampus and other brain structures [11, 15], was damage to postsynaptic dendrites with preservation of specialized synaptic membranes and presynaptic terminals. The neurocytotoxic action of QA (like that of NMDA) was blocked by APV.

The results are evidence that active expression of receptors of NMDA-type takes place as early as in the 2nd week in vitro in a dissociated culture of embryonic hippocampal nerve cells, as is confirmed by destruction of the neurons in culture under the influence of NMDA and by the blocking of this effect by APV, a specific antagonist of NMDA-receptors. In agreement with our previous data [3, 8], and also on the basis of results obtained previously with other workers [4, 7, 13], it can be concluded with a sufficient degree of confidence that predominantly NMDA-receptors are also involved in mediation of the neurodestructive effect of QA. However, the neurocytotoxic action of QA, unlike that of NMDA at the early stages of neuronal development in vitro, when synaptic connections have not yet been definitively formed, is absent, and massive degeneration of nerve cells under the influence of QA is observed in the later stages in cultures with mature synapses, and it is accompanied by characteristic damage to postsynaptic dendrites. It can thus be tentatively suggested that the subtype of NMDA-receptors specifically mediating the neurodestructive effect of OA is expressed during maturation of interneuzonal synaptic connections and realizes its function as a component of the newly formed synaptic complex. The suggestion formulated previously by several

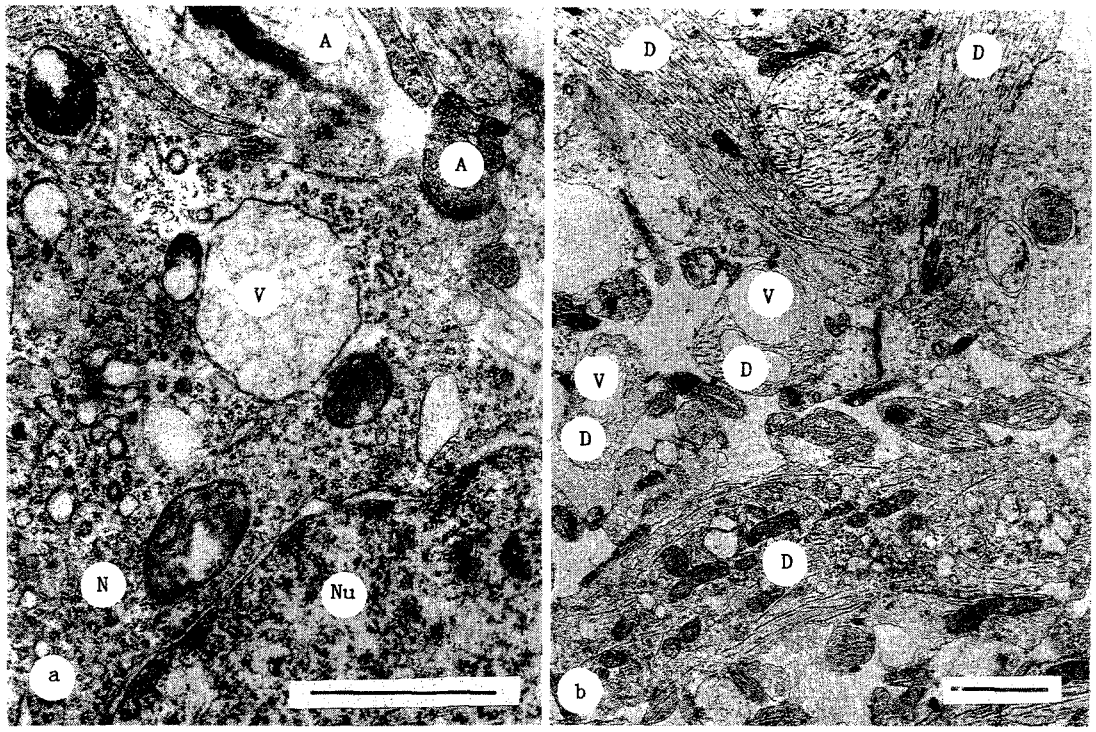

Fig. 3. Ultrastructure of hippocampal cells in culture 16 h after treatment with QA (0.5 mM) on 10th day in vitro. a) Preservation of structure of nucleus (N) and cytoplasmic organelles of a neuron (N) with partial vacuolation (V) of perikaryon. Intact axon terminals visible (A). 12,000×; b) Fragment of neuropil with vacuolation (V) of single dendrites (D). 6000×. Scale 3 μ.

workers [12, 13] regarding the probable action of QA on specific affinity sites of presynaptic terminals, activation of which may lead to excessive release of GA from them, and may thus potentiate the neurodestructive process initiated by QA, must be taken into account.

LITERATURE CITED

1. L. G. Khaspekov and I. V. Viktorov, Byull. Éksp. Biol. Med., **103**, No. 6, 738 (1987).
2. L. G. Khaspekov and I. V. Viktorov, Byull. Éksp. Biol. Med., **106**, No. 7, 103 (1988).
3. L. G. Khaspekov, I. P. Lapin, I. V. Ryzhov, and I. V. Viktorov, Byull. Éksp. Biol. Med., **108**, No. 8, 190 (1989).
4. D. W. Choi, Neuron, **1**, 623 (1988).
5. J. M. H. French-Mullen, N. Hori, and D. O. Carpenter, Neurosci. Lett., **63**, 66 (1986).
6. L. Khaspekov, E. Kida, I. Viktorov (I. Victorov), and M. J. Mossakowski, J. Neurosci. Res., **22**, 150 (1989),
7. J. P. Kim and D. W. Choi, Neuroscience, **23**, 423 (1987).
8. V. Lisy, L. G. Khaspekov, F. Stastny, and I. V. Viktorov (I. V. Victorov), Fourteenth International Congress of Biochemistry, Abstracts, Vol. 4, Prague (1988), p. 236.
9. M. P. Mattson and S. B. Kater, Brain Res., **490**, 110 (1989).
10. R. Schwarcz, W. O. Whetsell, and R. M. Mangano, Science, **210**, 316 (1983).
11. R. Schwarcz, A. C. Foster, E. D. French, et al., Life Sci., **35**, 19 (1984).
12. T. W. Stone, J. H. Connick, P. Winn, et al., Selective Neuronal Death, Chichester (1987), pp. 204-220.
13. T. W. Stone and M. R. Burton, Prog. Neurobiol., **30**, 333 (1988).
14. I. V. Viktorov (I. V. Victorov), L. G. Khaspekov, E. Kida, and M. J. Mossakowski, Clin. Neuropath., **7**, 219 (1988).
15. W. O. Whetsell, Clin. Neuropharmacol., **7**, 452 (1984).