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EFFECT OF ANTAGONISTS OF EXCITATORY AMINO ACIDS ON NEURODEGENERATIVE ACTION OF QUINOLINIC ACID IN VITRO COMPARED WITH THEIR ANTICONVULSANT ACTION IN SITU

L. G. Khaspekov, I. P. Lapin,
I. V. Rhyzov, and I. V. Viktorov

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The results of recent experimental investigations show that quinolinic acid (QA), one of the strongest neuroactive products of the kynurenin metabolism of tryptophan, has a marked excitatory, convulsant, and neurodegenerative action [11, 14]. This action of QA is similar in many respects with the effects of excitatory amino acids (glutamate, aspartate) and of their exogenous analogs (N-methyl-D-aspartate, ibothenate, quisqualate, cainate, etc.). As it has been shown [4, 12, 14], the QA concentration in various brain structures rises during aging and also in epilepsy, senile dementias, Alzheimer's disease, Huntington's chorea, and hepatic coma, and this may be one of the causes of destruction of neurons. Identification and study of the mechanisms of action of QA antagonists is thus an urgent problem.

Soem of the investigations to be described below were conducted on dissociated cultures of cells from various brain structures, by means of which the destructive action of QA and its analogs on living neurons could be studied at the cellular level and the protective effect of putative antagonists of this action revealed [8, 13]. Experiments to study the anticonvulsant action of these compounds in situ by their systemic administration and injection into the cerebral ventricles, preceding injection of cytotoxins, are another traditional and effective method of identifying antagonists [1, 6].

The aim of the investigation was to compare the effects of various antagonists on the neurodegenerative action of QA in dissociated cultures of hippocampal cells and on its convulsant action in situ.

Laboratory of Experimental Neurocytology, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR. Laboratory of Psychopharmacology, V. M. Bekhterev Leningrad Psychoneurologic Research Institute.

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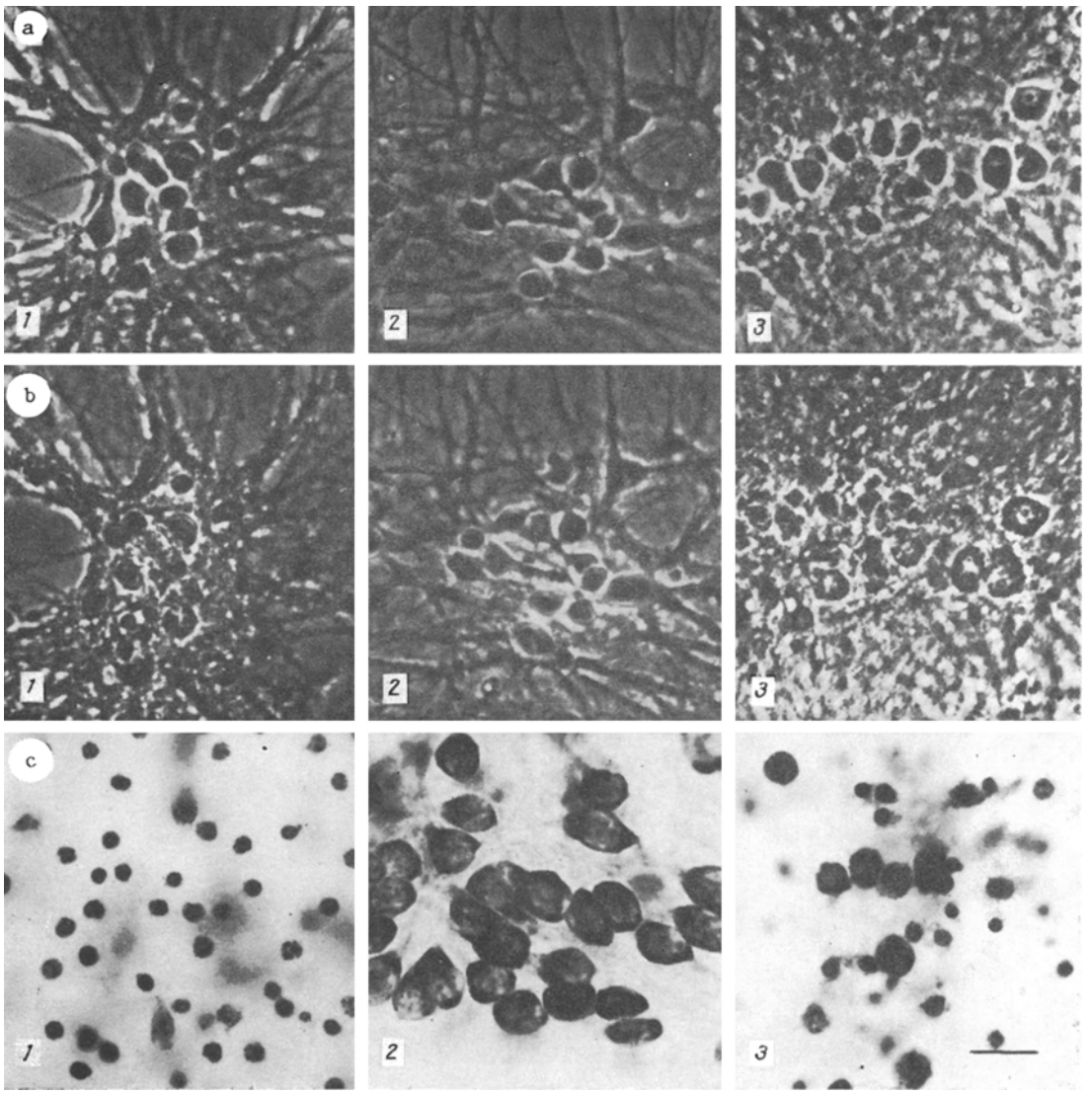

Fig. 1. Neurocytotoxic action of QA and effect of KA and KN on this effect in hippocampal cell cultures. a: 1-3) Before addition of substances to nutrient medium; b, c) 2-3 h after addition of QA (b, 1, c, 1), after addition of QA preceded by KA (b, 2, c, 2) and KN (b, 3, c, 3). a, b: Living cultures, phase contrast, c) stained with cresyl violet. Here and in Fig. 2, 23rd day in vitro. Objective 20, ocular 10. Scale: 30 μ (a, b), 20μ (c).

EXPERIMENTAL METHOD

To obtain dissociated cultures by the method described previously [2] a suspension of hippocampal cells from 17-19-day C57BL/6 mouse embryos was used. On the 20th-25th day of culture QA was added to the nutrient medium in a concentration of 0.5 mM. In one series of experiemnts, N-methyl-D-aspartate (NMDA, 0.1 mM) was used as the toxin. The antagonists were added to the cultures 5-7 min before the toxins. The endogenous compounds used included kynurenic acid (KA, up to 1.5 mM) and L-kinurenin (KN, up to 1.5 mM), and the exogenous compounds included ethylimidazole-4,5-dicarboxylic acid (IÉM-1442, up to 1.5 mM; synthesized at the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, under the direction of Candidate of Chemical Sciences L. B. Piotrovskii) and D,L-2-amino-5-phosphonovalerate (APV, 0.3 mM). The cultures were investigated intravitally in phase contrast and were stained with cresyl violet.

The method of recording the convulsant effects of QA and assessing the action of antagonists on them was described previously [10]. Experiments were carried out on adult male mice weighing 18-25 g belonging to the C57BL/6 strain. The toxin (5 μg in 5 μl of physiological saline) was injected into the right lateral ventricle of the brain. The

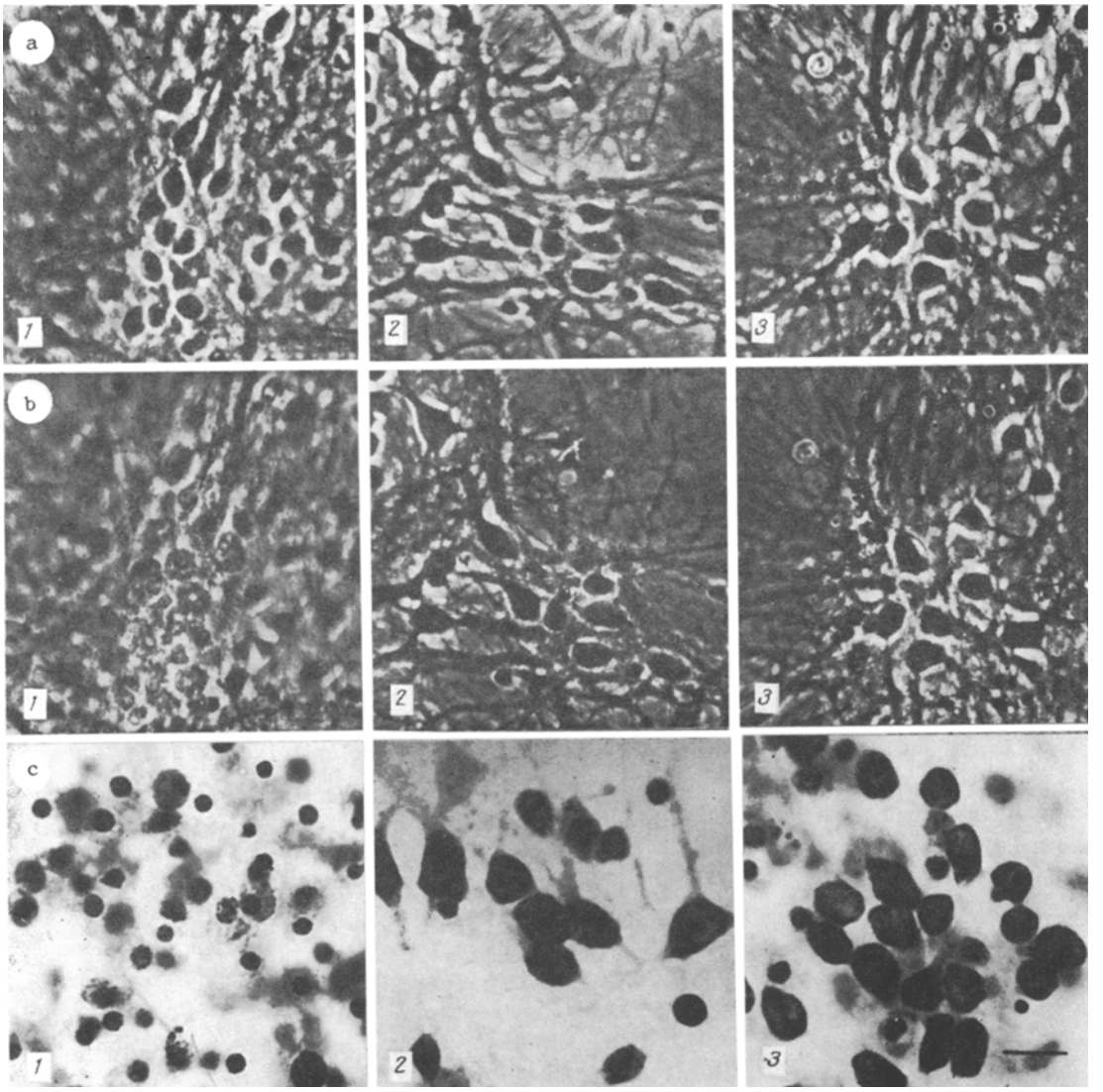

Fig. 2. Effect of IÉM-1442 and of APV on neurocytotoxic effect in hippocampal cell cultures. a, 1-3) Before addition of substances to nutrient medium; b, c) 2-3 h after addition: QA preceded by IÉM-1442 (b, 1, c, 1), QA preceded by APV (b, 2, c, 2). NMDA preceded by APV (b, 3, c, 3). 24th day in vitro.

antagonists: KA (1-5μg), KN (10-25 μg), and IÉM-1442 (2.5-5 μg) were injected into the ventricle 5 min before the toxin. Besides the substances mentioned, NMDA (0.25 μg) and glutamate (25 μg) also were used as convulsants, and proline (10-50 μg), cerulein (1-5 ng), and magnesium sulfate (which was injected intraperitoneally in a dose of 100-500 mg/kg 30 min before the injection of QA into the cerebral ventricle) were used as antagonists.

EXPERIMENTAL RESULTS

The addition of QA to the nutrient medium caused destruction of neurons after 1.5-2.5 h (Fig. 1b, 1). Subsequent staining of the cultures with cresyl violet reveal complete destruction and lysis of most nerve cells (Fig. 1c, 1). NMDA also had a similar action. On the addition of endogenous tryptophan metabolites (KA and KN) to the medium, no neurocytotoxic effect was observed for a long time (up to 24 h or more). Addition of these compounds to the cell cultures before addition of QA had different effects on its neurodestructive action. KA blocked the cytotoxic action of QA (Fig. 1b, 2, c, 2), and antagonism was exhibited when QA was added to the nutrient medium even 2-3 days after the addition of KA to it. Meanwhile KN had no antagonistic action on QA (Fig. 1b, 3, c, 3). IÉM-1442, which by itself had no toxic effect, did not prevent destruction of the neurons in culture by the action of QA (Fig. 2b,

1, c, 1). The other exogenous antagonist APV, known to be a specific blocker of NMDA-receptors [14], prevented the neurocytotoxic action of QA (see Fig. 2b, 2, c, 2) and of NMDA (see Fig. 2b, 3, c, 3).

In the experiments in situ KA had no anticonvulsant action, but in higher doses it actually induced seizures itself. After preliminary injection of KN and IÉM-1422 no seizures developed after the subsequent injection of QA, The use of other antagonists in the experiments in situ showed that cerulein (against QA and NMDA), proline (against QA and glutamate), and magnesium sulfate (against QA) had an anticonvulsant effect.

As was shown previously [3], dissociated cultures of hippocampal cells from the embryonic brain reach a high level of morphological and functional development by the 3rd week in vitro, characterized by the formation of morphologic varieties of neurons typical of that structure, and of mature synaptic interneuronal connections, evidence that the model used is appropriate for the study of neurodestructive processes, induced by neurotoxins, at the cellular level. When the results now obtained are analyzed and compared with data in the literature, the first point which must be emphasized is that the destructive effect of both NMDA and QA on neurons in culture, like the convulsant action of these toxins in situ, was prevented by APV, a specific blocker of NMDA receptors [8, 15]. On systemic injection Mg^{++}, which blocks chemically excitable Ca channels that may be activated by NMDA receptors, also had an anticonvulsant effect. The facts described above confirm the existing view that the cytotoxic effects of QA are mediated by one particular subtype of glutamate receptors, namely receptors for NMDA [15]. However, the direction of action of other compounds against QA in vitro and in situ was opposite. For instance, KA, a nonspecific antagonist of NMDA receptors [15], proved to have an antidestructive effect in vitro but did not prevent the convulsant action of QA. Meanwhile there is evidence that in experiments on rats in situ KA prevented both the neurodestructive and the convulsant action of QA [1, 5, 7]. It can therefore be tentatively suggested that KA has no blocking effect on MNDA receptors of mouse brain neurons in situ.

The other endogenous metabolite of trypotophan, namely KN, had no neurodestructive effect in vitro and did not block the cytotoxic action of QA on neurons in culture, although in experiments in situ it prevented seizures evoked by intracerebral injection of QA. The absence of an antidestructive effect in cultures also was observed after the use of IÉM-1442, which prevented the convulsant action of QA.

A recent investigation [9] showed that some anticonvulsants (phenobarbital, diazepam, phenytoin), which possess anticonvulsant properties, do not block the destructive action of glutamate on neocortical neurons in culture after intraventricular injection of kainic acid, an exogenous glutamate analog. The authors cited therefore suggested that these anticonvulsants prevent seizures not by their direct, but by their indirect effect on nerve cells, and this evidently possible only in the whole brain, through the participation of complex systems of interneuronal connections. In the present experiments KN and IÉM-1442 which, as was observed following injection of QA, had an anticonvulsant effect, did not exhibit antagonism against the destructive action of QA on neurons in culture. Comparison of these results with data in the literature described above suggests that the anticonvulsant action of KN and IÉM-1442 likewise is not associated with the direct blockade of NMDA receptors. It must also be pointed out that several other biologically active substances which we used (cerulein, proline) also were characterized by an anticonvulsant effect.

The results of this investigation thus broaden our ideas on the mechanisms of action of substances of endogenous and exogenous nature, preventing the neurocytotoxic effects of quinolinic acid, and facilitate the future search for them.

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