SPECIFIC AND FUNCTIONAL DIFFERENCES IN NMDA RECEPTORS

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The mediator system of excitatory amino acids (EAA) plays an important role in the transmission and processing of information in the mammalian CNS and disturbances of its activity may be linked with psychoneurologic diseases such as epilepsy, Huntington's chorea, Alzheimer's disease, etc. [6, 7, 9]. The urgent problem has thus arisen of the use of existing drugs and the creation of new compounds which could act on this mediator system and, in particular, on receptors of EAA, for therapeutic purposes [12].

EAA receptors are now subdivided into three pharmacologically different types: N-methyl-D-aspartate (NMDA), kainate, and quisqualate [15]. NMDA receptors, with whose hyperactivation are linked the development of seizures and neurodegenerative changes in hypoxia and ischemia [1, 4, 13, 14], and kainate receptors [8] have been studied the most. Meanwhile the problem of pharmacologic correction of behavioral effects and of neurodegenerative processes, evoked by EAA in mammals of different species, has not been adequately studied.

The aim of this investigation was to study aspects of the pharmacologic correction of behavioral effects induced by EAA in mice and rats, and specifically, hyperactivity, which can be regarded as physiological regulation, and seizures, which are a pathological process.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 22-24 g and rats weighing 180-220 g. To inject the substances into the lateral ventricle, a skin incision was made on the animal's head under ether anesthesia and a hole was drilled with a fine drill with coordinates AP + 1, L 1 for mice and AP +2, L 2 for rats. The depth of insertion of the needle of the microsyringe was 2.5 and 3.5-4 mm respectively. After the operation the wound was treated with 2% procaine solution. The animals exhibited no sign of painful excitation or of unease. The animals were used in the experiment not before 2 h after the operation. The accuracy of placing of the substances was verified by injecting methylene blue. Kainic acid (KA) and N-methyl-DL-aspartate (NMA), kynurenic acid, and also 2-amino-7-phosphoheptanic acid (2,7-APH), dissolved in physiological saline, pH 7.0-7.2, were injected into the lateral ventricle in a volume of 2-3 μl. 2,5-Aminophosphovalerate (2,5-APV) and 2,7-APH were synthesized by V. V. Ragulin at the Institute of Physiologically Active Substances, Academy of Sciences of the USSR. Each dose of the substances was tested on a group of 8-10 animals. ED_{50} was determined by probit analysis [10].

EXPERIMENTAL RESULTS

Violent running with jumping, terminating in short clonic convulsions, was induced in mice by NMA in a dose of 0.2 μg. KA in a dose of 0.1 μg initially induced running with jumping, which quickly became uncoordinated, and after a few minutes clonicotonic convulsions developed and lasted 0.5-2 h.

Selective antagonists of NMDA receptors 2,5-APV and 2,7-APH caused dose-dependent prevention of convulsions induced by NMA in mice. Meanwhile these compounds in effective anticonvulsant doses did not affect running and jumping

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induced by NMA, or likewise effects induced by KA. Only in doses of 200 mg/kg or more did 2,7-APH weaken the intensity of running induced by NMA very slightly, although under these circumstances it protected virtually 100% of the animals against convulsions. Convulsions induced by NMA in mice were effectively prevented by diazepam: ED_{50} = 0.64 mg/kg. However, diazepam had no significant effect on running and jumping preceding the convulsions, even in a dose of 20 mg/kg. Haloperidol (0.5-5.0 mg/kg), phentolamine (20 mg/kg), propranolol (20 mg/kg), serotonin (200 mg/kg), atropine (0.5-5.0 mg/kg), and diphenhydramine (2.0- 5.0 mg/kg) had no effect on hyperactivity induced by NMA in mice.

Diazepam (10-20 mg/kg) inhibited hyperactivity and convulsions induced by KA in mice. Haloperidol (2.0 mg/kg) significantly shortened or abolished running induced by KA but did not affect convulsions. Serotonin (200 mg/kg) significantly depressed running but had a weaker effect on convulsions. The remaining substances had no evident influence on effects induced by KA in mice.

The behavioral effects induced by NMA and KA in rats differed somewhat from those observed in mice. Besides the initial stages of running and short clonic convulsions, NMA also induced long clonicotonic convulsions in rats, resembling kainate convulsions in mice. Meanwhile KA induced much more intensive convulsions in rats, as it also did in mice. Significant differences also were found in the character of pharmacologic correction of effects induced by NMA in rats compared with mice.

2,7-APH in a dose of 5.0 μg, when injected into the lateral ventricle, inhibited hyperactivity and convulsions induced by NMA about equally. Meanwhile diazepam, in doses of 0.5-5.0 mg/kg did not affect running and convulsions induced by NMA in rats, and only in a dose of 10 mg/kg did it inhibit both running and convulsions. Diazepam, haloperidol, and serotonin had the same action on the effects induced by KA in rats as in mice, but the remaining substances were ineffective.

Kynurenic acid, an endogenous blocker of EAA receptors, caused no change in the behavioral effects induced by NMA and KA in mice. Moreover, kynurenic acid itself led to a significant increase in motor activity and in the strength of convulsions in several cases. In rats, however, kynurenic acid inhibited hyperactivity and convulsions induced by NMA but had only a very slight influence on the effects induced by KA.

Similar results were obtained by the use of ketamine (Calypsol, from "Gedeon Richter," Hungary), which has been shown to be a noncompetitive antagonist of NMDA receptors [3]. Ketamine effectively inhibits convulsions induced by NMA in mice (ED_{50} = 2.6 mg/kg), but only in a dose of 80 mg/kg did it inhibit running in about 50% of the animals. In rats a dose of 20 mg/kg depressed hyperactivity and convulsions induced by NMA, whereas both these effects persisted after a dose of 10 mg/kg of ketamine.

The investigations showed that hyperactivity and convulsions induced by EAA have species-specific differences and are complex processes in which other neurotransmitter systems of the CNS are also involved. We could find no significant differences in the effect of the substances tested on hyperactivity and convulsions induced by KA in mice and rats. The effect of haloperidol on hyperactivity induced by KA can be explained by the presence of kainate receptors on endings of dopaminergic neurons, and as a result of activation of these receptors by KA dopamine is released, and its action on postsynaptic neurons is blocked by haloperidol [5]. In all the remaining cases the absence of any such differences can be explained in two ways: 1) there are no selective antagonists of kainate receptors; 2) by the homogeneity of the kainate receptor population in mice and rats.

A more definite picture was observed with the use of NMA (Table 1). Convulsions induced by NMA in mice were effectively blocked by 2,5-APV, 2,7-APH, diazepam, and ketamine. Meanwhile we were unable to find any substances which would completely suppress hyperactivity induced by NMA. This points to the existence of different mechanisms inducing hyperactivity and convulsions through activation of NMDA receptors. One such mechanism could be the existence of two subtypes of NMDA receptors in mice. In rats, 2,7-APH, diazepam, and ketamine in large doses inhibited both hyperactivity and convulsions simultaneously. There was also an essential difference in the action of kynurenic acid, which was inactive in mice but inhibited hyperactivity and convulsions induced in rats by NMA. These facts indicate the functional and pharmacologic homogeneity of the NMDA receptor population in rats and their significant difference from NMDA receptors in mice.

Species-specific differences in mice and rats in the action of kynurenic acid on convulsions induced by quinolinic acid, an endogenous convulsive agent, discovered previously by Lapin and co-workers [2], may be connected with the pharmacologic properties of the NMDA receptors of these animals revealed by the present experiments, for quinolinic acid acts on NMDA receptors [11].

TABLE 1. Effect of Neurotropic Drugs on Running and Convulsions Induced in Mice and Rats

Substance	Mice		Rats
	running	convulsions	rats
	(1)	(2)	(3)
2-7 -APH	200,0	64,8 (1)	5,0*
Diazepam	20,0	0,64	10,0
Ketamine	60,0	2,6	20,0
Kynurenic acid	10,0*	(—) 10,0*	50,0*

Legend. Substances were injected intraperitoneally in all cases except where indicated by an asterisk, when they were injected into the cerebral ventricle, in micrograms per animal. Numbers in parentheses: 1) doses (in mg/kg) not affecting running in mice; 2) ED_{50} (in mg/kg) of compounds for inhibiting convulsions in mice; 3) doses (in mg/kg) which inhibited running and convulsions simultaneously in 80-90% of animals. —) No action.

Recently the question has arisen of the use of NMDA-receptor antagonists for therapeutic purposes [12]. Meanwhile there is sometimes the risk that these antagonists may influence not only pathological processes, but also normal synaptic transmission. Our experiments showed that in some species of animals these processes, i.e., pathological (convulsions) and physiological (hyperactivity), linked with activation of NMDA receptors, are mediated through different mechanisms. Whether or not this happens in more highly organized animals and, in particular, in man, requires further elucidation. Nevertheless, when potential antagonists of NMDA and other types of EAA receptors are screened, the possibility of species-specific differences in their pharmacologic properties must be taken into account.

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LITERATURE CITED

1. V. V. Grigor'ev, V. A. Nemanova, V. V. Ragulin, and E. N. Tsvetkov, Khim.-Farm. Zh., No. 3, 275 (1988).
2. I. P. Lapin and I. V. Ryzhov, Investigation of Glutamatergic Synapses [in Russian], Leningrad (1987), pp. 7-8.
3. N. A. Anis, S. C. Berry, N. R. Burton, and D. Lodge, Brit. J. Pharmacol., **79**, 565 (1983).
4. R. Dingledine, Trends Neurosci., **9**, 47 (1986).
5. B. A. Donzanti and N. Y. Uretsky, Neuropharmacology, **22**, 971 (1983).
6. G. E. Fagg, Trends Neurosci., **8**, 207 (1985).
7. F. Fonnum, J. Neurochem., **42**, 1 (1984).
8. A. C. Foster and G. E. Fagg, Brain Res. Rev., **7**, 103 (1984).
9. P. T. Francis, S. L. Lowe, A. M. Palmer, et al., Biochem. Soc. Trans., 14, 1981 (1986).
10. J. T. Litchfield and F. J. Wilcoxon, J. Pharmacol. Exp. Ther., **96**, 99 (1949).
11. H. McLennan, Neurosci. Lett., **46**, 157 (1984).
12. B. S. Meldrum, Clin. Sci., **68**, 113 (1984).
13. B. S. Meldrum, Trends Neurosci., **8**, 47 (1985).
14. B. S. Meldrum, M. C. Evans, J. H. Swan, and R. P. Simon, Med. Biol., **65**, 153 (1987).
15. J. C. Watkins and R. H. Evans, Ann. Rev. Pharmacol. Toxicol., **21**, 165 (1981).