EFFECT OF ANTIDEPRESSANTS ON GLUTAMATERGIC AUTOREGULATORY PRESYNAPTIC MECHANISM IN THE RAT CEREBRAL CORTEX

A. V. Prikhozhan, G. I. Kovalev, and K. S. Raevskii

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The wide distribution in the mammalian brain of neurons using glutamic (Glu) and aspartic (Asp) acids as neurotransmitters with an excitatory postsynaptic action, has drawn the attention of research workers in recent years to the possible role of excitatory amino-acid neurotransmission both in the genesis of psychopathological states and in the mechanism of action of psychotropic drugs of different classes [4, 6]. On the basis of data already published an essential role of excitatory amino-acid neurotransmission can be suggested in the mechanism of the central action of anticonvulsants, dissociative anesthetics, neuroleptics, and antiparkinsonian agents [5, 11, 12, 14]. Antidepressants have received significantly less study from this point of view. Yet grounds for an investigation of this kind could be data showing that an increase in the blood Glu concentration in patients with depressive disorders [9] is linked with accompanying treatment with antidepressants, and can be reproduced in experiments on rats [8]. However, the absence of changes in the amino-acid concentration in the cerebrospinal fluid in this case [8] does not allow the results to be interpreted as a reflection of neurospecific changes in Glu metabolism.

It has been reported that in vitro interaction between some antidepressants and the excitatory amino-acid system can be demonstrated in vitro. For instance, amitriptyline counteracted Glu-induced neuronal depolarization in the frog spinal cord, and this effect, moreover, differed from that of chlorpromazine and diazepam [7]. In experiments on cerebellar neurons in culture the inhibitory effect of another tricyclic antidepressant, namely imipramine, on Glu-stimulated cGMP synthesis was demonstrated [10]. The desmethyl analog of imipramine exhibited the properties of a noncompetitive antagonist of the N-methyl-D-aspartate subtype of Glu-Asp-receptors [13].

The writers showed previously that amitriptyline and desmethylimipramine have an inhibitory effect on K^+-stimulated release of the unmetabolized substrate of systems for uptake and release of excitatory amino acids (i.e., D-Asp) from cerebral cortical synaptosomes under perfusion conditions [14]. The aim of the present investigation was to widen the range of antidepressants studied and also to attempt to analyze the possible mechanisms of the observed effects.

EXPERIMENTAL METHOD

The method of studying K^+ (30 mM)-stimulated release of ^{3}H-D-Asp from perfused rat cerebral cortical synaptosomes was described fully by the writers previously [2, 3]. Briefly, the fraction of unpurified synaptosomes was incubated with 2,3-^{3}H-D-Asp (from "Izotop," USSR, 10^{-7} M), and then applied to glass-fibre filters, fixed into thermostated chambers. Perfusion was carried out at the rate of 1 ml/min. Release was estimated quantitatively by measuring radioactivity of the following fractions of the supernatant: fraction 1: the last 2 min of perfusion with the original buffer (basal level of release); fraction 2 — the first 2 min after addition of the buffer with 30 mM KCl, and with the substance (antidepressants, neuroleptics, analyzers) or without it (control); fraction 3 — the next 2 min of perfusion with buffer containing 30 mM K^+, and with or without the substances. The final result of each superfusion was expressed as two values: A) the ratio between radioactivities of fractions 2 and 1, B) the ratio between radioactivities of fractions 3 and 1.

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TABLE 1. Effect of Antidepressants on K^+-Stimulated Release of ^{3}H-D-Aspartic Acid from Rat Cerebral Cortical Synaptosomes ($M \pm m$, n = 6-12)

Substance	Concentration, M	Release of ^{3}H-D-aspartic acid, % of control
Control	—	100,0±2,5
Amitriptyline	10^{-6}	87,4±3,8*
	10^{-5}	81,5±2,6*
Imipramine	10^{-6}	87,4±5,7*
	10^{-5}	79,4±4,6*
Desmethylimipramine	10^{-6}	98,9±5,9
	10^{-5}	88,4±3,8*
Moclobemide	10^{-5}	105,3±4,9
	10^{-4}	84,2±3,9*
Viloxazine	10^{-5}	106,1±5,5
	10^{-4}	89,6±4,5*
Befol	10^{-4}	107,4±6,6
	$5 \cdot 10^{-4}$	88,3±4,2*

Legend. Values given indicate ratio A (see text), which was 1.74 in the control. $^*p < 0.05$ (Mann–Whitney U test).

EXPERIMENTAL RESULTS

Table 1 gives data on the effect of several antidepressants, both typical and atypical, on K^+-stimulated release of ^{3}H-D-Asp from cerebral cortical synaptosomes. Clearly, according to this parameter the tricyclic antidepressants amitriptyline, imipramine, and desmethylimipramine exhibit much stronger activity than antidepressants with a different chemical structure (moclobemide, befol, viloxazine). For instance, imipramine and amitriptyline inhibited ^{3}H-D-Asp release in a concentration as low as 10^{-6} M, whereas the inhibitory effect of befol was significant only with a concentration of 0.5 mM or higher. Somewhat higher activity than that of befol was exhibited by moclobemide, which is chemically closely similar to it, and also viloxazine.

As the writers showed previously, release of excitatory amino acids in the rat cerebral cortex is under the control of an autoregulatory mechanism, one component of which is evidently the quisqualate subtype of Glu/Asp receptors [2]. The fact that the effects of quisqualic acid, the specific agonist of this subtype of receptors, on the one hand, and of the antidepressants studied in the present investigation, on the other hand, are similar in direction suggests that an autoregulatory mechanism may be involved in the action of the latter substances on the process of release of excitatory amino acids. To test this hypothesis experiments were carried out with the combined use of antidepressants and an antagonist of quisqualate receptors, namely the diethyl ester of Glu (DEEG), which, as the writers showed previously, can abolish the inhibitory effect of quisqualenic acid on the stimulated release of ^{3}H-D-Asp in a concentration of 10^{-4} M, at which it has no intrinsic effect on the release of the label [2].

The results of experiments to study the effect of DEEG on the inhibitory properties of the test antidepressants relative to stimulated ^{3}H-D-Asp release are given in Fig. 1. They show that DEEG completely abolishes the effect of typical antidepressants, namely amitriptyline, imipramine, and desmethylimipramine, and in the case of atypical preparations (moclobemide, viloxazine, and befol) the effect of their combined administration with DEEG exceeds the control figures, i.e., the effect of antidepressants is reversed. Relative to this parameter, befol was closer to the typical antidepressants.

It was interesting to study to what degree the observed antagonism with DEEG is specific for antidepressants as a special class of psychopharmacological agents. It was found previously that the effect of the tranquilizer phenazepam, which also inhibits stimulated release of ^{3}H-D-Asp, is insensitive to DEEG [1, 2]. The neuroleptics also inhibit release of excitatory amino acids [3, 14], although the mechanism of this effect has not yet been studied; at the same time, we do not know whether an autoregulatory mechanism (quisqualate-sensitive) is involved in its realization. In the present study we investigated the effect of carbidine, a Soviet atypical neuroleptic, with an antidepressive component in its spectrum of action [1]. The plan of the experiment was similar to that described above for antidepressants. For comparison, effects of two typical neuroleptics (haloperidol and chlorpromazine), which are known to be without antidepressive properties, were examined. The results of these experiments are illustrated in Fig. 2.

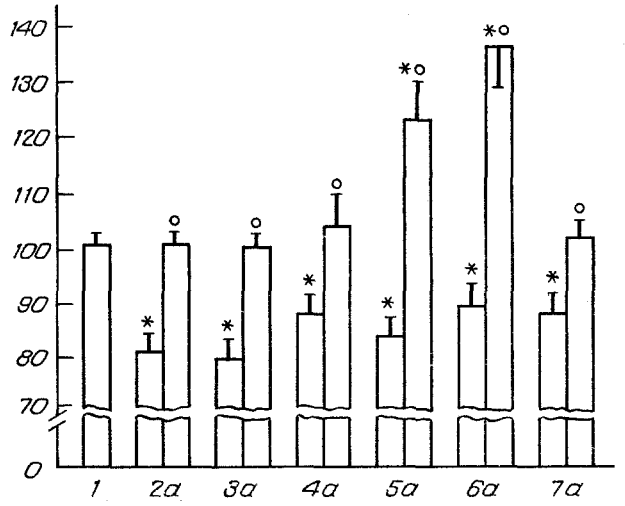

Fig. 1

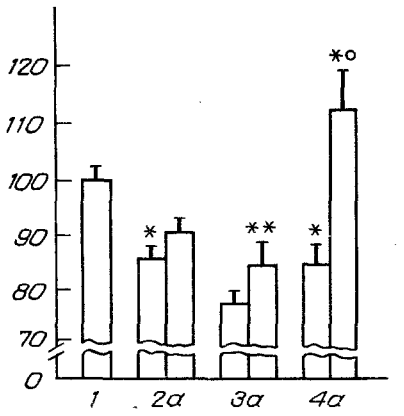

Fig. 2

Fig. 1. Effect of diethyl ether of glutamic acid on inhibition of K^+-stimulated release of ^{3}H-D-aspartic acid from rat cerebral cortical synaptosomes by antidepressants. Abscissa: 1) control (30 mM KCl); 2) amitriptyline, 10^{-5} M; 3) imipramine, 10^{-5} M; 4) desmethylimipramine, 10^{-5} M, 5) moclobemide, 10^{-4} M; 6) viloxazine, 10^{-4} M; bifol, $5 \cdot 10^{-4}$ M; a) corresponding antidepressant + DEEG, 10^{-4} M; ordinate, degree of secretion of label (in % of control). Value of A = 1.74 taken as 100%. Significance of differences at $p < 0.05$ level (Mann—Whitney U test): asterisks — from control, ○) from effect of corresponding antidepressant.

Fig. 2. Effect of diethyl ester of glutamic acid on effects of neuroleptics on K^+-stimulated release of ^{3}H-D-aspartic acid from rat cerebral cortical synaptosomes. Abscissa: 1) control (30 mM KCl); 2) haloperidol, 10^{-5} M; 3) chlorpromazine, 10^{-5} M; 4) carbidine, 10^{-5} M; a) corresponding neuroleptic + DEEG, 10^{-4} M. Significance of differences at $p < 0.05$ level (Mann—Whitney U test): *) from control, ○) from effect of corresponding neuroleptic. Remainder of legend as to Fig. 1.

The fact will be noted that quantitatively speaking both carbidine and chlorpromazine repeated the effect of haloperidol, which we found previously, and which was reproduced in this series of experiments. However, DEEG counteracted only the effect of carbidine, while leaving the inhibitory effect of haloperidol and chlorpromazine substantially unchanged.

The results indicate that antidepressants of varied chemical structure inhibit the process of release of excitatory amino-acid neurotransmitters in the cerebral cortex of rats through the participation of a mechanism sensitive to DEEG, an antagonist of the quisqualate subtype of Glu/Asp receptors. This mechanism also is exhibited in the action of carbidine, which may be partly due to the presence of an antidepressive component in the spectrum of its psychotropic action.

It can thus be postulated that the antidepressive action of substances of varied chemical structure is effected in particular through the participation of an autoregulatory mechanism, functioning at the level of cortical endings of Glu/Asp-ergic brain neurons.

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5-HT_{1A}-RECEPTOR AGONISTS RESTORE BEHAVIOR OF RATS WHEN DISTURBED BY L-DIHYDROXYPHENYLALANINE

N. A. Bondarenko, N. A. Bondarenko, L. Baran, and A. Klodzinska

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The serotonin (5-hydroxytryptamine, 5-HT) system of the brain has a regulatory and modulating influence on the dopamine and noradrenalin system [8], which are directly involved in the expression of emotional-behavioral reactivity, sensomotor integration, and attention. An excessive increase in the concentration of exogenous dopamine (DA) and noradrenalin (NA) in the brain after injection of L-dihydroxyphenylalanine (L-dopa) causes a change in emotional reactivity, and disturbs locomotor and investigative activity and also the complex elementary rational behavior in an acute stress situation [2]. On the basis of data on the normalizing effect of the anxiolytic huspirone (which interacts with 5-HT, DA, and NA-neurotransmitter systems) and other 5-HT_{1A} agonists on behavior in stress-conflict situations [3], we studied the degree to which selective and unselective agonists of 5-HT_{1A}-receptors and also indirect 5-HT agonists can influence avoidance behavior in an acute stress situation, when disturbed by L-dopa.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180-250 g. The animals were first adapted by keeping them for 2 days in the experimental room at 20 ± 2°C, with natural alternation of daylight and darkness, and with free access to water and food. The effect of the substances on ability of the animals to solve an extrapolation problem based on escape from an acute stress situation, disturbed by L-dopa, was studied by the method developed previously [1]. The animals were tested once in the course of 2 min. The latent period (LP) of the motor reactions, the number of unsuccessful attempts to escape, and LP of deliverance were recorded and the percentage of the animals giving the corresponding pattern of behavior was calculated. At least 15 rats were used for testing each dose of the substance. A disturbance of behavior was induced by injecting Madopar in a dose of 125 mg/kg (containing 100 mg of L-dopa and 25 mg of benserazide, an inhibitor of peripheral aromatic amino-acid decarboxylase) 1 h before testing. *p*-Chlorophenylalanine (PCPA) was given over a period of 48 h in a dose of 300 mg/kg. With the exception of L-5-hydroxytryptophan, m-hydroxybenzylhydrazine (NSD-1015), mianserin and yohimbine, which were given 10 min before injection of Madopar, all the other substances were given 20 min before testing. Buspirone, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), *m*-chlorophenylpiperazine (mCPP), quinazine, 8-hydroxy-2-(di-*n*-propylamino)tetraline (80H-DPAT),

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