OPPOSITE EFFECTS OF PYRACETAM AND PROLINE ON RELEASE OF ^{3}H-D-ASPARTIC ACID FROM RAT CEREBRAL CORTICAL SYNAPTOSOMES

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Pyracetam, an α-pyrrolidone derivative, possesses antihypoxic activity and also facilitates normalization of memory functions [2]. Data now available suggest that the glutamatergic and aspartatergic systems of the brain participate in the realization of both these effects [3, 9].

The antihypoxic action of pyracetam correlates with its ability to normalize glutamic and aspartic acid levels in the brain, when raised as the result of experimental hypoxia [8]. It has been suggested that a definite role in the development of hypoxic brain damage may be played by hyperactivation of autoreceptors of excitatory amino-acid neurotransmitters [6]. In that case desensitization of these receptors might be an important component of the mechanism of action of substances with antihypoxic activity and, in particular, of pyracetam.

A distinguishing feature of the effect of pyracetam on memory processes, it has been shown, is its antagonism to the amnesic action of proline [3]. This suggested that the probable molecular mechanism of this effect may be antagonism between these structurally similar compounds at the receptor level [3]. Since we know that proline can modulate neurotransmitter release from glutamatergic terminals [7] it was logical to suppose that antagonism of pyracetam and proline may take place at the level of the presynaptic receptors that participate in the regulation of this process.

To test hypotheses on the possible role of the presynaptic stage of glutamateaspartatergic neurotransmission in the mechanism of action of pyracetam and proline, it was decided to study the effect of these substances on K^+-stimulated release of ^{3}H-D-aspartic acid, a nonmetabolized substrate of the system for uptake of L-forms of glutamic and aspartic acids [5], from rat cerebral cortical synaptosomes, and also the possible connection of these effects with glutamate (aspartate) autoreceptors.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-220 g. The P_2 fraction of unpurified synaptosomes was isolated from the ceretral cortex by the method fully described previously [1]. Oxygenated Krebs-bicarbonate buffer of the following composition (in mM) was used: NaCl 118, KCl 4.75, $CaCl_2$ 2.54, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.19, glucose 11, pH 7.4. To obtain buffer with an increased K^+ concentration (30 mM) the NaCl was replaced by the corresponding amount of KCl. To 1 ml of buffer at 37°C 50 μl of the suspension of synaptosomes was added (protein concentration 7-9 mg/ml). After 5 min, 2,3-$^3H_2$-D-aspartic acid (from Izotop, USSR, specific radioactivity 33 Ci/mmole) was added up to a final concentration of 10^{-7} M. Incubation continued for 10 min with gentle stirring, after which the suspension of synaptosomes was applied to a GF/C glass-fiber filter (Whatman, England), 20 mm in diameter, fixed in a constant-temperature chamber. The incubation medium was removed at the rate of 4 ml/min means of a "Multiperpex" peristaltic pump (LKB, Sweden), after which the synaptosomes were washed twice with buffer, 2 ml each time. Further perfusion

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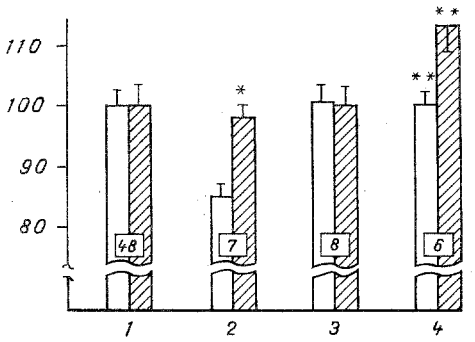

Fig. 1. Effect of quisqualic acid and GADE on K^+-stimulated release of ^{3}H-D-aspartic acid from rat cerebral cortical synaptosomes. Abscissa: 1) control (K^+ 30 mM), 2) quisqualic acid (10^{-3} M), 3) GADE (10^{-4} M), 4) quisqualic acid (10^{-3} M) + GADE (10^{-4} M); ordinate: index of K^+-stimulated release (in % of control). Unshaded columns – ratio of release during first 2 min of potassium stimulation to basal level of release (value of 1.74 ± 0.04 taken as 100%); shaded column – ratio of release during next 2 min of potassium stimulation to basal level of release (value of 1.34 ± 0.04 taken as 100%). Significance of differences at $P < 0.05$ level: *) compared with control, **) compared with effect of quisqualic acid (10^{-3} M). Numbers inside columns denote number of independent measurements.

was carried out at the rate of 1 ml/min. After 6 min of perfusion with the original buffer, buffer with an increased K^+ concentration (30 mM), with or without (control) the test substance, was introduced into the chamber. Release was determined quantitatively by measuring radioactivity of the following fractions of the superfusate: fraction 1) the last 2 min of perfusion with the original buffer (basal level of release); fractin 2) the first 2 min after addition of buffer containing 30 mM K^+ with or without (control) the substance; fraction 3) the next 2 min of perfusion with buffer containing 30 mM K^+, and with or without the substance. The fractions were dissolved in Bray's scintillation fluid, and radioactivity was determined in dis/min by means of an SL 4000 liquid scintillation counter (Intertechnique, France). 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. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The results of experiments with quisqualic acid, an agonist of one subtype of glutamate (aspartate) receptors [10], are given in Fig. 1. In a concentration of 10^{-3} M quisqualic acid significantly reduced release of the label. Evidence in support of the receptor nature of this effect is given by the fact that it did not develop in the presence of glutamic acid diethyl ester (GADE), an antagonist of quisqualate receptors, in a concentration of 10^{-4} M. Pyracetam in concentrations of 10^{-6} and 10^{-5} M significantly reduced the K^+-stimulated release of D-aspartic acid, but in higher concentrations it had no appreciable effect on the process studied (Fig. 2). The similarity and identical direction of action of pyracetam and quisqualic acid revealed by these experiments suggested the possible role of quisqualate receptors in the mechanism of action of pyracetam. To test this hypothesis an attempt was made to abolish the effect of pyracetam (10^{-5} M) by means of GADE. The results of these experiments showed that pyracetam under the conditions used behaves as an agonist of the quisqualate subtype of glutamate (aspartate) autoreceptors (Fig. 2). We know that long-term administration of the agonist to animals causes desensitization of the corresponding receptors [4]. As regards pyracetam it is logical to suggest that its long-term administration, which is usually necessary in order to achieve a therapeutic effect, may lead to a decrease in sensitivity of glutamate (aspartate) autoreceptors of the quisqualate subtype.

TABLE 1. Effect of Proline on K^+-Stimulated ^3H-D-Aspartic Acid Release from Rat Cerebral Cortical Synaptosomes (M ± m)

Substance	Concentration, M	Number of experiments	^3H-D-Aspartic acid release, % of control	
			A	B
Control		50	100,0±2,5	100,0±2,8
Proline	10^{-5}	8	105,5±3,4	113,6±3,3*
	$5 \cdot 10^{-4}$	8	113,8±4,6*	127,1±4,6*
GADE	10^{-4}	8	101,0±3,0	100,0±3,0
Proline+GADE	$10^{-5}+10^{-4}$	7	114,9±5,4	112,5±5,9
Proline+GADE	$5 \cdot 10^{-4}+10^{-4}$	7	114,0±5,4	123,2±4,7
Pyracetam	10^{-4}	5	94,5±4,7	95,9±4,6
Proline + pyracetam	$10^{-5}+10^{-4}$	8	103,0±5,6**	95,6±6,6**

Legend. A and B) See "Experimental Method." Values taken as 100%: for A 1.735 ± 0.043, for B 1.335 ± 0.038. *P < 0.05 compared with control, **P < 0.05 compared with effect of proline in corresponding concentrations.

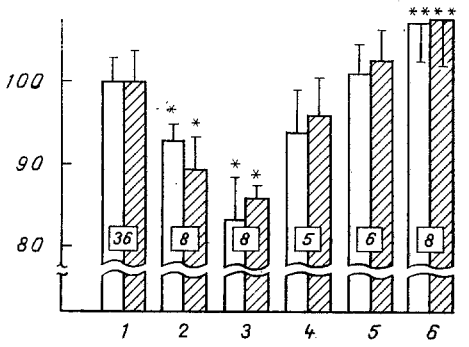

Fig. 2. Effect of pyracetam on K^+-stimulated release of ^3H-D-aspartic acid from rat cerebral cortical synaptosomes. Abscissa: 1) control (K^+ 30 mM); 2-5) pyracetam in concentrations of 10^{-6}, 10^{-5}, 10^{-4}, and 5×10^{-4} M, respectively; 6) pyracetam (10^{-5} M) + GADE (10^{-4} M); ordinate – the same as in Fig. 1. **P < 0.05 compared with effect of 10^{-5} M pyracetam.

With the aid of this mechanism pyracetam could counteract hyperactivation of the autoreceptors, resulting from accumulation of excessive amounts of excitatory amino acid neurotransmitters in the synaptic space during hypoxia [6], and thereby preventing death of presynaptic nerve fibers. This mechanism may perhaps explain one aspect of the antihypoxic action of pyracetam.

Proline (10^{-5} and 5×10^{-4} M), unlike pyracetam, considerably increased D-aspartic acid release (Table 1). Pyracetam, in a concentration which by itself did not affect this process (10^{-4} M), clearly abolished the effect of proline (10^{-5} M). Since GADE did not influence the effect of proline, it could be concluded that the phenomenon of antagonism which was found is evidently unconnected with quisqualate receptors. Antagonism of pyracetam to the action of proline on this process may perhaps take place at the level of synthesis of the glutamic acid mediator pool from proline [9]. Another possible explanation of the results may be the suggestion that proline receptors exist on terminals of glutamate/aspartatergic neurons, relative to which pyracetam behaves as an antagonist. The absence of a specific antagonist or proline receptors did not allow us to test this hypothesis.

Thus pyracetam reduces K^+-stimulated ^3H-D-aspartic acid release from rat cerebral synaptosomes, playing the role of agonist of glutamate (aspartate) autoreceptors of quisqualate subtype, and this may be of significant importance for manifestation of the antihypoxic action of the compound. Pyracetam can also abolish the proline-induced intensificatin of D-aspartic acid release by a mechanism unconnected with quisqualate receptors, evidence of the existence of functional antagonism between these two substances, with their opposite action on memory processes.

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PHARMACOLOGIC PREVENTION OF CISPLATIN NEPHROTOXICITY IN RATS

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Cisplatin is a new and effective chemotherapeutic agent used in the treatment of malignant tumors [1, 3], but it often disturbs renal function [7]. In order to prevent the nephrotoxic action of the preparation various methods are used to increase diuresis [3, 6, 7], and these have alleviated but not prevented kidney damage. Since administration of cisplatin causes severe damage to cells of the straight part of the proximal tubule of the nephron [5], an important role in the mechanism of production of renal failure could be played by its entry into those cells of the tubule that are able to secrete organic substances. In this case pharmacologic screening of the secretory apparatus during the peak period of cisplatin excretion would give protection against renal failure. The investigation described below was undertaken to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on 164 albino rats of both sexes weighing from 75 to 250 g. Cisplatin (DDP) was injected intraperitoneally in a dose of 0.5 mg/100 g body weight. The state of the renal function was assessed by administering water to the rats by gastric tube in a dose of 5 ml/100 g body weight, the animals were placed in individual constraining cages, and the urine excreted during 2 h was collected. Sodium and potassium levels in the blood serum and urine were determined on a Flapho-4 flame photometer, calcium and

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