INTERACTION OF PYRACETAM WITH SPECIFIC ^{3}H-IMIPRAMINE BINDING SITES AND GABA-BENZODIAZEPINE RECEPTOR COMPLEX OF BRAIN MEMBRANES

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Despite the extensive clinical use of pyracetam* the neurochemical mechanisms of its action have not yet been explained. Available data on its effect on the protein-synthesizing and energy-producing systems of the brain do not explain several of the specific effects of pyracetam and, in particular, its anxiolytic action, revealed in experiments on animals [3]. The writers showed previously [2] that pyracetam *in vitro* does not affect binding of the corresponding ligands with benzodiazepine, imipramine, dopamine (D_2), and serotonin (S_1 and S_2) receptors, and also with α_1- and β-adrenoreceptors of the rat brain, if the concentrations of these ligands were close to the dissociation constant (K_d) of the ligand–receptor complex. This may signify absence of competition between pyracetam and the ligands studied, but detailed study of the action of pyracetam on the affinity and maximal concentration (B_{max}) of these receptors has not been undertaken.

In the present investigation the effect of pyracetam was studied on parameters of specific binding of ^{3}H-imipramine and GABA-activated binding of ^{3}H-flunitrazepam with rat brain membranes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats and guinea pigs weighing 180-200 and 270-300 g respectively, and kept under natural conditions of light and darkness, with free access to water and food. Membranes of the unpurified synaptosome fraction were isolated from whole rat brain [5], from washed rat hippocampal membranes [7], and from guinea pig cerebellum [9] without modifications. Suspensions of rat brain membranes in appropriate buffer solutions were kept at -20°C for up to 3 weeks; guinea pig cerebellar membranes were used on the day of isolation. Specific binding of ^{3}H-imipramine by whole brain membranes [5] and GABA-activated binding of ^{3}H-flunitrazepam by washed rat hippocampal membranes [7] were studied also without modifications. Specific binding of ^{3}H-myanserine by guinea pig cerebellar membranes [9] was studied by the use of 10^{-6} M myanserine or diphenhydramine as displacing agents. In all cases the reaction was stopped by diluting the incubation mixture tenfold with the corresponding cold buffer, followed by rapid filtration of the mixture through GF/B glass fiber filters (from Whatman, England). The filters, washed and dried in air twice, were extracted overnight in 5 ml of Soviet Zhs-8 scintillator and the radioactivity of the samples was determined on an SL-4000 scintillation counter (from Intertechnique, France). ^{3}H-Imipramine (21 Ci/mmole), ^{3}H-myanserine (16.5 Ci/mmole), and ^{3}H-flunitrazepam (89 Ci/mmole) were obtained from Amersham Corporation (England). Values of K_d and B_{max} were calculated on Scatchard plots by means of an HP-33E microcalculator (USA). The significance of differences was estimated by Student's t test. The protein concentration was determined by the method in [10].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that pyracetam, in a concentration of 10^{-6} M reduced specific binding of ^{3}H-imipramine, lowering B_{max} significantly, but did not reduce the affinity of the binding sites, i.e., it behaved as a noncompetitive inhibitor of ^{3}H-imipramine binding. These data can be regarded as evidence of the heterogeneity of ^{3}H-imipramine binding sites, on the assumption that some of them, with high affinity for pyracetam, are completely blocked

*Soviet GABA analog (α-pyrrolidone derivatives)

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TABLE 1. Effect of Pyracetam on Specific Binding of ^{3}H-Imipramine and ^{3}H-Myanserine by Brain Membrane (M ± m, n = 3).

^{3}H-ligand	Test object	Parameter	Compound added	
			Control (water)	10^{-6} M pyracetam
^{3}H-imipramine	Rat brain	K_d	2,7±0,4	1,9±0,5
		B_{max}	529±12	413±27*
^{3}H-myanserine	Guinea pig cerebellum	K_d	1,66±0,30	1,58±0,24
		B_{max}	274±10	279±14

Legend. Here and in Table 2 each Scatchard graph was plotted for 6 points, each point in three repetitions. *P < 0.05. Values of K_d given in nmoles, values of B_{max} in fmoles/mg protein.

TABLE 2. Effect of Pyracetam and Mebicar on Basal and GABA-Activated Binding of ^{3}H-Flunitrazepam with Rat Hippocampal Membrane (M ± m, n = 3)

Compounds added	Without GABA		With 10^{-5} M GABA	
	K_d	B_{max}	K_d	B_{max}
Control (Water)	1,52±0,04	1,52±0,08	0,76±0,07	1,67±0,04
Pyracetam, 10^{-4} M	1,53±0,06	1,62±0,07	1,08±0,01*	1,84±0,08*
Mebicar, 10^{-5} M	1,48±0,08	1,60±0,07	0,99±0,04*	1,50±0,07

Legend. Concentration of pyracetam chosen on the basis of results in [8] showing that after a single injection of 300 mg/kg of pyracetam its concentration in the brain may reach 0.4 mM.

by it in a concentration of 10^{-6} M. Besides affinity for its own specific imipramine receptors, which are sites of allosteric regulation of serotonin reuptake systems, imipramine is also known to have comparable affinity for H_1-histamine and desmethylimipramine receptors [4]. We have shown that pyracetam, in a concentration of 10^{-5} M, does not induce significant changes in the rate of accumulation of ^{3}H-serotonin (and also ^{3}H-dopamine and ^{3}H-GABA) by rat brain synaptosomes during incubation for 5 min (this part of the work was done by V. O. Nikuradze in the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow). We postulated that pyracetam interacts with a subpopulation of ^{3}H-imipramine-binding sites are not linked with serotonin transport.

It was shown previously [9] that specific binding of ^{3}H-myanserine with guinea pig cerebellar membranes is realized only by histamine H_1-receptors. As the data in Table 1 show, pyracetam does not change the characteristics of specific binding of ^{3}H-myanserine with guinea pig cerebellar membranes. Histamine H_1-receptors are probably not the subpopulation of ^{3}H-imipramine-binding sites with which pyracetam interacts *in vitro*.

As a test system to study the mechanisms of the anxiolytic action of pyracetam, we chose GABA-activated binding of ^{3}H-flunitrazepam with thoroughly washed rat hippocampal membranes. It has been shown that GABA increases the affinity of specific binding of agonists with benzodiazepine receptors, virtually does not change affinity of antagonists, and reduces binding of so-called reversed agonists, i.e., compounds with an anxiogenic action [6].

Pyracetam in a concentration of 10^{-4} M, like the atypical tranquilizer mebicar, did not change specific binding of ^{3}H-flunitrazepam with hippocampal membranes in the absence of GABA, but in the presence of 10^{-5} M GABA it behaved as a competitor for benzodiazepine receptors, reducing K_d for the flunitrazepam–receptor complex (Table 2).

In experiments with a modified conflict-inducing drinking test, the writers showed [1] that pyracetam and mebicar, in doses of 300 and 100 mg/kg respectively, significantly (n = 7, $P \leq 0.05$) increased the number of times the rats visited the drinking bowls, accompanied by painful electrical stimulation (2.9 ± 0.6 in the control, 5.5 ± 0.7 and 10.8 ± 1.4 in groups of animals receiving pyracetam and mebicar respectively), although the anxiolytic action of

these compounds was not abolished by injection of the benzodiazepine antagonist Ro-15 1788 (10 mg/kg intraperitoneally, 15 min before testing). In this case the mean number of "punishable" approaches to the drinking bowl was 2.8 ± 0.6 in the control and 6.2 ± 0.6 and 10.2 ± 1.4 in groups of animals receiving pyracetam and mebicar respectively, compared with 2.4 ± 0.5 in rats receiving diazepam (5 g/kg 40 min before testing).

Thus pyracetam and mebicar in experiments *in vivo* on normal animals can exert their anxiolytic action without the participation of benzodiazepine receptors. The possibility remains that either the interaction of pyracetam and mebicar with benzodiazepine receptors which we observed *in vitro* has a different interpretation than competition of these compounds with specific binding sites of ^{3}H-flunitrazepam, or in experiments on normal animals *in vivo* GABA-benzodiazepine receptor complex does not accept pyracetam and mebicar, for it contains endogenous inhibitors of GABA-modulating action. Whatever the case, the results are evidence that the anxiolytic action of pyracetam (and mebicar) may be realized with the participation of this supramolecular compex.

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CEREBROVASCULAR EFFECTS OF MET- AND LEU-ENKEPHALINS

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The important role of central adrenergic and GABA-ergic mechanisms in the control of the cerebral circulation has recently been discovered [3]. Considering the extensive data in the literature on interaction of neuropeptides with adrenergic and GABA-ergic brain systems it is evidently important to study the effect of opioid peptides on the cerebral hemodynamics.

We found no information in the literature on the cerebrovascular effects of enkephalins. Meanwhile the effects of naloxone, an antagonist of opiate receptors, on the cerebral circulation has been reported in patients with neurological disturbances [5-10]. The possible role of opioid peptides in the development of cerebrovascular disorders is discussed in these publications.

The aim of the present investigation was to study the effect of Met- and Leu-enkephalins on the cerebral circulation and on neurogenic cerebrovascular reactions. The effects of Leu-enkephalin also were studied when GABA-receptors were blocked by bicuculline and opiate receptors by naloxone.

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