POSSIBLE STRUCTURO-FUNCTIONAL ASSOCIATION OF PYRACETAM AND VASOPRESSIN

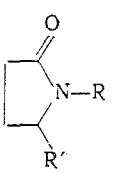
T. A. Gudasheva, R. U. Ostrovskaya,
F. B. Maksimova, S. S. Trofimov, M. Yu. Kosoi,
G. M. Molodavkin, and A. P. Skoldinov

UDC 615.214.3:547.745]:[615.357:577.175.343].074

Recently considerable attention has been attracted by nootropic compounds, which specifically activate the higher integrative functions of the brain, affecting processes of learning and memory [2]. The main drug from this group of compounds is pyracetam (nootropyl, I). There are several hypotheses on the mechanism of its action: 1) it nonspecifically accelerates neuronal metabolism on account of activation of the enzymes of ATP metabolism [10]; 2) the action of I is based on a GABAergic mechanism, on the basis of its structural relationship to the cyclic form of GABA [9]; 3) a relatively specific interaction of I with one of the subtypes of glutamate receptors has recently been detected [4]. However, up to the present time no convincing experimental evidence has been obtained of the participation of these mechanisms in the realization of the nootropic effect of I.

Earlier [1] we hypothesized that I is a synthetic analog of an as yet unknown endogenous peptide ligand of the presumed nootropic receptors, and neuropharmacological evidence of the correctness of these hypotheses was obtained. The insufficient activity of I is explained from this standpoint by the unsuitable orientation of the glycine residue. Transfer of this residue from position 1 of the ring to position 5, i.e., transition from I to the structurally close amide of L-pyroglutamylglycine (II), led to a substantial increase in the specific mnestic activity, measured in experiments with a conditioned reflex of passive avoidance (CRPA) in rats (the specific activity connotes the ratio of the maximum mnestic activity to the the corresponding dose). The replacement of glycine by β-alanine and then by GABA decreased the specific mnestic activity of the peptides. This indicates that the second amino acid of the dipeptide should probably be the α-amino acid encoded. Since the amide of L-pyroglutamyl-β-alanine (III) is still significantly more active than I [1], we suggested that the β-alanine residue can participate in the binding to the receptor and synthesize the dipeptide of L-pyroglutamic acid, the structure of which contains fragments both of glycine and of β-alanine, namely, the amide of L-pyroglutamyl-L-asparagine (IV). This dipeptide facilitated the ability

TABLE 1. Pyroglutamylpeptides



Compound	Name	R	R′
I	Pyracetam	CH_2CONH_2	H
II	pGLu-GLy-NH_2	H	$CONHCH_2CONH_2$
III	pGLu-β-ALa-NH_2	H	$CONHCH_2$ CH_2CONH_2
IV	pGLu-Asn-NH_2	H	$CONHCHCONH_2$ CH_2CONH_2
V	[pGLu⁴, Cyt⁶]-AVP-(4-9)	H	$CONHCHCONH$-Cyt-Pro-Arg-Gly-NH_2 CH_2CONH_2

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 3, pp. 271-275, March, 1988. Original article submitted March 18, 1987.

0091-150X/88/2203-0191$12.50

TABLE 2. Activity of Pyracetam and the Amide of Pyroglutamyl-asparagine in the CRPA Test

Compound	Dose		Activity, A_t, %		
	mg/kg	μmoles/kg	before training	after training	before testing
Pyracetam	200	1400	20,0*	1,0	—15,0**
P_t			<0,025	Insignificant	<0,0
L-pGlu-L-Asn-NH_2	0,1	0,5	28,0*	—36,0**	13,0**
P_t			<0,01	<0,05	<0,05
	10,0	50,0	22,0*	—	—
P_t			<0,01		
	0,01	0,05	35,0***	—	—
P_t			<0,01		

Note. *40 animals, **30 animals; ***20 animals; A_t) mnestic activity according to the parameter t [1]; P_t) significance of the parameter t, calculated according to the Wilcoxon—Mann—Whitney method; the symbol — indicates amnestic activity.

of rats to be trained in the CRPA test; its specific mnestic activity was an order of magnitude greater than that of the amide II (see Tables 1 and 2).

It is noteworthy that the amide IV corresponds structurally to the N-terminal fragment of the principal metabolite of vasopressin [pGlu^4, Cyt^6]-AVP-(4-9) (V), a peptide more active in its influence on the memory of rats in the CRPA test than that of vasopressin itself, and, moreover, not possessing the hormonal activity of the latter [5]. Thus, the synthesis and detection of mnestic activity of the amide IV bridges the gap between I and the memory peptide vasopressin. D. DeWied [8] considers the latter as a precursor of a whole series of shorter peptides, which have specific effects on different stages of learning and memory.

The amide IV, like I and the amide II, does not affect the motor activity of rats, i.e., the improvement of training in this case is not associated with a general stimulating effect. In contrast to I, the amide IV does not possess antihypoxic activity, which is evidence of its more selective action on learning and memory.

A comparison of the effects of I and the amide IV on different stages of learning and memory in the CRPA test showed that I facilitates the training of rats only if it is administered before training. The administration of I immediately after training has no effect on the ability of the animals to learn, while administration before testing even worsens it. The amide IV facilitates learning if it is administered both before training and before testing of the animals. At the same time, the administration of amide IV immediately after training does not stimulate the ability of the rats to learn, but even, on the contrary, leads to an amnestic effect. According to D. DeWied's data [5, 8], the vasopressin fragments [pGlu^4, Cyt^6]-AVP-(4-9), [pGlu^4, Cyt^6]-AVP-(4-8), [Cyt^6]-AVP-(5-8) relatively specifically facilitate consolidation, whereas [Cyt^6]-AVP-(5-9) acts more selectively on recall. We should note that the authors of [8] did not study the influence of vasopressin metabolites on recall. Thus, the amide IV, like I and vasopressin metabolites, possesses specificity in action on different stages of memory.

In a comparison of the electrophysiological properties of I [7] and IV it was shown that both these substances affect the transcallosal response (TCR), increasing its amplitude. However, the electrophysiological characteristics of IV have definite peculiarities, consisting of the fact that 15-30 min after the increase in amplitude of the TCR, an inhibition both of the primary positive-negative oscillation and of the late slow wave is observed. A restoration of the amplitude of the TCR is observed 2-4 h after the administration of IV (0.1 mg/kg intraperitoneally) (see Fig. 1). Possibly this peculiarity is associated with the inhibiting action of IV at the stage of memory consolidation.

Within the investigated dose range (0.01-10 mg/kg), the mnestic activity of IV was virtually independent of the dose, which distinguished it from I, the amide II [1], vasopressin, and V but brought it closer to [pGlu^4, Cyt^6]-AVP-(4-8) [6]. In contrast to the other oligopeptides enumerated here, these two substances do not have a glycine amide residue at the C-terminus. Let us note that the presence of a bell-shaped dose dependence for vasopressin and V is associated precisely with this residue [5].

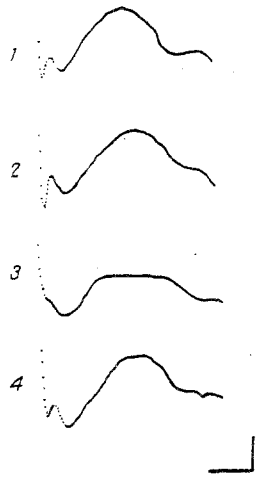


Fig. 1. Influence of substances IV on the transcallosal response of the rat parietal cortex. 1) Before administration of the substance, 2) 10 min after administration of substance IV in a dose of 0.1 mg/kg; 3) the same 30 min after administration; 4) the same 90 min after administration of the substance. Time calibration: 100 msec, amplitude 300 μV.

On the basis of the fact that the vasopressin fragments [Cyt^6]-AVP-(5-9) and [Cyt^6]-AVP-(5-8) are only slightly inferior to its major metabolite V in activity, J. Burbach et al. [11] concluded that the pyroglutamic acid residue is not essential for manifestation of the mnestic activity of V. Our data do not confirm this viewpoint, since the dipeptide IV, representing the N-terminal fragment of V, possesses high mnestic activity. It can be assumed that among the endogenously active vasopressin metabolites formed there are short N-terminal fragments of the peptide V; in particular, we do not exclude the possibility of detection of the dipeptide [pGlu^4]-AVP-(4-5), which may play a substantial role in the specific control of processes of short-term memory.

Thus, it can be assumed that pyracetam is a synthetic analog of the N-terminal fragment of the major metabolite of the memory peptide vasopressin, and the mechanism of its action on learning and memory consists of interaction with specific nootropic receptors, which play a key role in the control of memory processes, the endogenous ligands of which are vasopressin metabolites. The detection of substances, in particular, the dipeptide IV, that act specifically only at definite stages of learning indicates that different stages of memory (short-term memory, consolidation) differ chemically. The fact that the same substance induces a mnestic effect when administered before training and an amnestic effect opposite to it when administered immediately after training is evidence that the molecular mechanisms controlling the fixation of information in the short-term memory and its transfer to the long-term memory are close but not identical. In other words, we can hypothesize a heterogeneity of the population of nootropic receptors.

EXPERIMENTAL CHEMICAL

The mass spectra were recorded on an MAT-112 mass spectrometer (Varian, USA) at an ionization energy of 70 eV. The specific optical rotation was measured on an A-1-EPO automatic polarimeter.

Pentachlorophenyl Ester of L-Pyroglutamic Acid (L-pGlu-OPcp). To a suspension of 12.9 g (0.1 mole) L-pyroglutamic acid (Reanal, Hungary) in 200 ml of THF we added 32 g (0.12 mole) of pentachlorophenyl, and at 0°C 21.8 g (0.106 mole) dicyclohexylcarbodiimide in 50 ml of THF; the mixture was mixed for 1 h at 0°C and left overnight at room temperature. The precipitate was filtered off, the solvent evaporated under vacuum, and the residue recrystallized

from isopropanol. Yield 19 g (50%) L-pGlu-OPcp, mp 197-8°C, $[\alpha]_D^{25}$+15.0 (C = 2, DMFA). Literature [3]: mp 198°C, $[\alpha]_D^{20}$+18.6 (C = 1, DMFA).

Diethyl Ester of L-Pyroglutamyl-L-aspartic Acid L-pGlu-L-Asp × $(OEt)_2$. A mixture of 2.2 g (5.9 mmoles) L-pGlu-OPcp, 1.62 g (7.2 mmoles) L-aspartic acid diethyl ester hydrochloride (Reanal, Hungary), and 0.97 ml (7.2 mmoles) Et_3N in 30 ml of DMFA was mixed for 5 h at room temperature. The $Et_3N \cdot HCl$ precipitate was filtered off, the filtrate evaporated under vacuum, and the residue chromatographed on a column with silica gel (eluent $CHCl_3$—MeOH, 9:1). Yield 1.2 g of chromatographically homogeneous substance with R_f 0.40 (silica gel, $CHCl_3$—MeOH, 9:1), R_f 0.82 (silica gel, dioxane—water, 10:1). Mass spectrum: M^+ 300, M_{calc} 300. Found, %: C 52.01; H 6.70; N 9.43. $C_{13}H_{20}N_2O_6$. Calculated, %: C 51.99; H 6.73; N 9.32.

Amide of L-Pyroglytamyl-L-asparagine (IV). A solution of 1.2 g (4 mmoles) L-pGlu-L-Asp $(OEt)_2$ in 40 ml of MeOH, saturated with gaseous ammonia, was left overnight at room temperature. The white precipitate formed was filtered off, washed with ethanol and ether, and dried in a desiccator over P_2O_5. Yield 0.8 g (83%) IV, mp 213-214°C, R_f 0.69 (silica gel, $CHCl_3$—MeOH—25% ammonia 3:2:1), R_f 0.32 (silica gel, dioxane—water, 10:1). Mass spectrum: M^+ 242, M_{calc} 242. $[\alpha]_D^{25}$-8.0 (C = 2, water). Found, %: C 44.12; H 6.11; N 21.88. $C_9H_{14}N_4O_4 \cdot {}^1/_2 CH_3OH$. Calculated, %: C 44.18; H 6.26; N 21.68.

EXPERIMENTAL PHARMACOLOGICAL

The influence of the preparations on processes of learning and memory was studied on a modified model of CRPA in rats [6]. The investigations were conducted on noninbred male rats weighing 180-200 g. The test preparation was injected intraperitoneally in a volume of 0.2 ml per 100 g of weight 15 min before training or immediately after training, or 15 min before testing (pyracetam 60 min before testing). The animals of the control group were injected with 0.9% saline solution. For training the rat was placed in the lighted compartment of a two-section chamber. After 180 sec in the dark component, an unavoidable electro-pain stimulus was applied to it through an electrode floor with successive stimuli of alternating current (50 V, duration 1 sec each with 2 sec intervals between shocks). The retention of the CRPA was determined after 24 h. For this purpose the animal was placed in the lighted compartment and the total time of stay in it during a period of 180 sec was measured.

The influence of the preparations on the motor activity were investigated on mice with the aid of a multichannel instrument for recording motor activity (Opto-Varimex, USA).

The electrophysiological activity of the preparation was studied on the parietal cortex of male rats according to the transcallosal response procedure [7].

The antihypoxic activity of the preparation was determined on a model of normobaric hypoxia in mice with an initial oxygen content in the air of 8% by volume.

LITERATURE CITED

1. T. A. Gudasheva, R. U. Ostrovskaya, S. S. Trofimov, et al., Khim.-farm. Zh., No. 11, 1322-1329 (1985).
2. M. D. Mashkovskii, L. F. Roshchina, and A. I. Polezhaeva, Farmakol. Toksikol., No. 6, 676-683 (1977).
3. Great Britain Patent No. 1503680 (1978).
4. B. Bering, W. E. Müller, Arzneim. Forsch., 35(II), No. 9, 1350-1352 (1985).
5. J. P. H. Burbach, G. L. Kovacs, and D. De Wied, Science, 221, No. 4617, 1310-1312 (1983).
6. J. Bures and O. Buresova, J. Comp. Physiol. Psychol., 56, 268-272 (1963).
7. C. E. Giurgea and F. E. Moyersoons, Arch. Int. Pharmacodyn., 199, No. 1, 67-78 (1972).
8. G. L. Kovács, H. D. Veldhuis, D. H. G. Versteeg, and D. DeWied, Brain Res., 371, No. 1, 17-24 (1986).
9. F. E. Moyersons, C. E. Giurgea, and A. C. Evraerd, Arch. Int. Pharmacodyn., 166, No. 1, 238-251 (1967).
10. J. P. Pede, L. Schimpfessel, and R. Crokaert, Farm. Tijdschr. Belg., 50, No. 4, 298-306 (1973).
11. X.-Ch. Wang and J. P. H. Burbach, FEBS Lett., 197, No. 1, 164-168 (1986).