BEHAVIORAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF L-PYROGLUTAMYL-D-ALANINE AMIDE, A PYRACETAM ANALOG

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It was shown previously that the amide of L-pyroglutamyl-D-alanine (pGlu-D-Ala-NH_2, compound I), like other dipeptides based on pyroglutamic acid, which are structural analogs of pyracetam*, improves the ability of animals to be trained in passive avoidance [3].

Since the presence of a D-amino acid can increase the resistance of dipeptides to the action of hydrolytic enzymes, the aim of the present investigation was to study the stability of this particular compound, and to give fuller characteristics of its effect on training ability and an electrophysiologic analysis of its action on integrative functions of the brain.

EXPERIMENTAL METHOD

The stability of compound I was studied in vitro at 37°C by the method of H-NMR-spectroscopy [6], using a WM-500 spectrometer (Bruker, West Germany) with a working frequency of 500 MHz. Signals were assigned in the spectra by the double resonance method. Blood serum was obtained as described previously [6]. Preparations of membranes from the brush border of enterocytes were generously provided by members of the staff of the Laboratory of Experimental Therapy, Research Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. The concentration of the peptide in the samples was 0.5 mM. The effect of compound I on the ability of rats to be trained was estimated by studying its effect on conditioned active and passive avoidance reflexes (CAAR and CPAR, respectively). A CAAR was produced in a shuttle box: in the course of one experiment combinations of a conditioned photic stimulus and an unconditioned painful electrical stimulus were presented until the criterion of training was reached (10 consecutive correct responses), but the total number of combinations did not exceed 60. Compound I was injected 15 min before the experiment began. The CPAR was formed by a modified method [9, 11] in a two-section chamber, with painful electrical stimulation applied in the initially preferred dark compartment. Preservation of the CPAR was tested after 24 h. The effect of compound I was investigated previously by the writers [3], but only by preliminary administration of the compound (schedule I). Since the CPAR was produced with a single reinforcement, so that injection of the dipeptide could be timed to a particular phase of learning, in the present investigation the effect of the compound was studied when administered immediately after reflex formation (schedule II) or 15 min before testing (schedule III). Since data on the effect of pyracetam on learning, with respect to CAAR and CPAR models, are contradictory [2, 8-10], we compared the action of the dipeptide (1 mg/kg, intraperitoneally) and of pyracetam (200 mg/kg, intraperitoneally) as standard nootropic agent. In the experiments with CAAR pyracetam was injected 30 min before the beginning of training, in the experiments with CPAR immediately after training or 60 min before testing preservation of the reflex. Electrophysiological analysis of the action of compound I was carried out on unanesthetized, curarized (listhenon) cats, artificially ventilated. The technique of the preparatory operation was described previously [5]. Somatosensory potentials evoked by electrical stimulation of the facial nerve were re-

*α-pyrrolidone acetamide.

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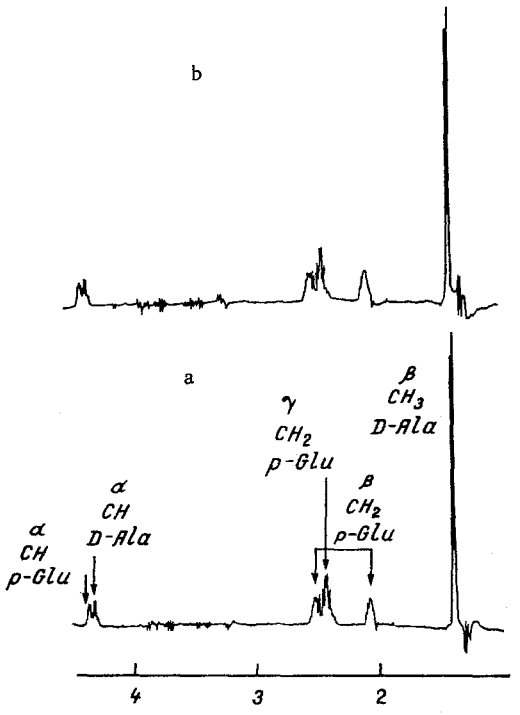

Fig. 1

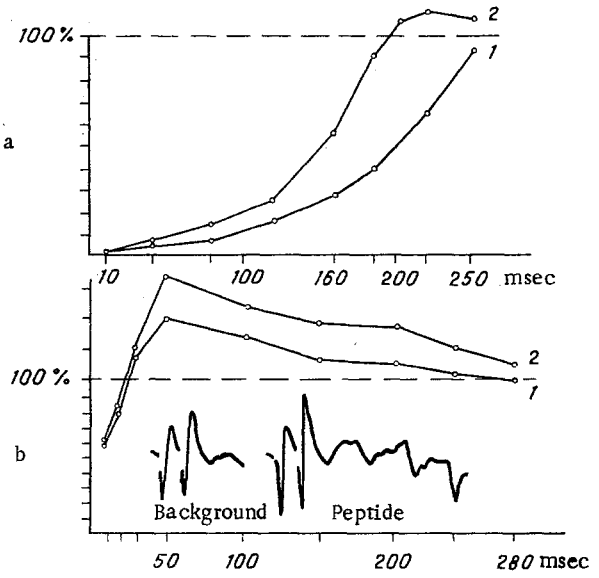

Fig. 2

Fig. 1. H-NMR spectra (500 MHz) obtained 10 min (a) and 5 h (b) after addition of compound to human blood serum (pH 7.4; TE 310 K).

Fig. 2. Recovery cycles of TCR in somatosensory (a) and parietal (b) before (1) and during (2) administration of dipeptide. Abscissa, intertrial interval (in msec); ordinate, amplitude of testing response (in % of background). Interaction with an interval of 50 msec is illustrated in Fig. 2b.

corded in the somatic projection (SI) and association areas (area 7) of the cortex. The transcallosal responses (TCR) to stimulation of the symmetrical point of the opposite hemisphere were recorded in these same zones. Stimulation was applied both as single pulses and also as paired stimulation with intervals of 10 to 300 msec between conditioning and testing stimuli. After recording of the initial electrophysiological parameters the animal was given an intraperitoneal injection of the test dipeptide in a dose of 1 mg/kg. The amplitudes of the evoked potentials were averaged for the whole group (eight animals). Differences between amplitudes of evoked potentials before and after injection of the drug were subjected to statistical analysis by the method in [4].

EXPERIMENTAL RESULTS

Since the test dipeptide was administered systematically, the longest time of contact was taken up by destructive blood enzymes. As Fig. 1 shows, the dipeptide was highly stable in the presence of blood serum enzymes. Moreover, this compound was unchanged in the presence of the enzyme system of the enterocyte brush border membranes, one of the most powerful proteolytic systems in the body (exposure 2 h).

The experiments with CAAR showed that the test dipeptide, like pyracetam, increased (compared with the control) the number of animals capable of attaining a definite level of training (Table 1). As regards an effect on the rate of formation of the reflex, only the dipeptide has a facilitatory effect. Pyracetam actually increased the number of combinations needed to attain the criterion of training a little. This may be connected with transfer of some of the rats from the number of those not yielding to training into the group of rats which, under the influence of pyracetam, learned to form a CAAR, but learned more slowly than the rats receiving compound I.

It will be clear from Table 1 that the test dipeptide improved the level of training in the CAAR achieved by reducing the time spent by the animals in the dark compartment, in which the rat had previously received painful stimulation. The effect of the dipeptide on CPAR was

TABLE 1. Effect of Compound I and Pyracetam on Conditioned Reflexes

Experimental conditions	CAAR		CPAR		
	animals achieving the criterion of training, % (1)	number of combinations before reaching criterion of training	mean length of stay of animal in dark compartment, % of control (2)		
			schedule of administration of drug		
			I (3)	II	III
Control (0,9% sodium chloride solution)	30,8 (26)	35,9 (26)	100 (40)	100 (30)	100 (30)
Pyracetam	76,9* (13)	44,0 (13)	81,3* (40)	98,7 (30)	120,7 (30)
Compound I	76,9* (13)	28,9 (13)	50,4** (20)	76,8* (30)	81,3* (30)

Legend. Statistical significance of differences between experimental and control groups estimated by the following methods: 1) chi-square test; 2) Wilcoxon—Mann—Whitney test (*$p < 0.05$, **$p < 0.01$); 3) data taken from [3]. Number of animals in group shown in parentheses.

manifested in all schedules of its administration. This suggests that this substance has a facilitatory action on all phases of formation of the memory engram: introduction, consolidation, and withdrawal of information. Pyracetam exhibits its own effect only if administered before training. Considering the pharmacokinetics of the drug (highest serum concentration after 1 h, equilibrium between serum and brain 4 h after injection of pyracetam) [13], it can be tentatively suggested that pyracetam exerts its maximal activity on the phase of fixation of the memory trace, although the compound may have a definite facilitatory effect also on the early phases of processing of the memory trace.

Electrophysiological analysis of the effect of the dipeptide showed that this compound, while not giving rise to significant changes in potential in area SI, increased the amplitude of TCR mainly on account of its negative phase. The amplitude of TCR in the association area increased on average by 24.3% ($p < 0.05$) but in area SI by only 12.7%. Recovery cycles of TCR in the zones tested differed in character: in area SI the cycle consisted only of a phase of depression, but in the association cortex, a phase of facilitation was present in the initial cycle. The effect of the dipeptide in the first case was expressed as weakening of depression with the appearance of facilitation in the late part of the recovery cycle (Fig. 2a), in the second case as strengthening of the initial degree of facilitation (Fig. 2b), i.e., in both cases the effect, in principle, was in the same direction.

Compound I caused the appearance of late components of somatosensory responses and also of regular discharges within the θ- and σ-rhythm bands (Fig. 2b). Comparison of the results with those of an electrophysiological study of pyracetam [1, 12] showed them to be similar. In view of the modern ideas on the genesis of the negative phase of the responses it can be tentatively suggested that compound I, like pyracetam, facilitates convergence on dendrites in the outer layers of the cortex. The late rhythmic activity may be a manifestation of intracortical reverberation. Facilitation of convergence and rhythmic activity, which are the basic mechanisms of integrative processes lying at the basis of short-term memory and the initial stages of its fixation [7], may constitute the basis for the pyracetam-like action of compound I.

The dipeptide, used in much smaller doses than pyracetam, improves training ability by facilitating all phases of memory engram processing. The effect of the dipeptide, like that of pyracetam, on training ability is selective, for neither substance causes any accompanying psychomotor excitation, which is characteristic of stimulants.

The properties of compound I are such that it can be characterized as a stable peptide analog of pyracetam with a broader spectrum of action and with higher activity.

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COMPARATIVE STUDY OF THE EFFECT OF 3-CARBOXY-β-CARBOLINE METHYLAMIDE AND DIAZEPAM ON RAT BEHAVIOR

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Discovery of the molecular mechanisms of the onset and development of anxiety states is essential for the development of concrete methods of prevention and treatment of mental disorders. In this respect a very promising line of research is the study of the effect of anxiogenic compounds on behavioral responses of animals, which could provide a new approach to the stimulation of processes characteristic of mental pathology at the biochemical level.

It has recently been shown that several compounds, which are derivatives of β-carboline, possess anxiogenic properties [12, 15]. They are behavioral antagonists of the benzodiazepines [5, 13] and compete with the latter for highly specific binding sites with the protein components of the brain (benzodiazepine receptors) [5, 12, 13, 15]. The most active representative of this class of compounds is 3-carboxy-β-carboline methylamide (FG 7142), administration of which, as experiments on animals [7, 9] and tests on human volunteers [6] have shown, induces somatic, behavioral, and endocrine syndromes characteristic of a state of fear and anxiety.

The aim of this investigation was to compare the effects of administration of diazepam and FG 7142 on a group of behavioral responses of rats, reflecting a broad spectrum of emotional states.

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