MECHANISM OF ANTIDEPRESSANT AND LEARNING-STIMULATING EFFECTS OF THYROTROPIN RELEASING HORMONE AND ITS ANALOGS

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The physiological activity of thyrotropin releasing hormone (THR) (L-pyroglutamyl-L-histidyl-L-proline-amide), and in particular, its role in various neuromodulator processes, is the reason for the search for derivatives of this tripeptide. One of the aims of this research is to create derivatives which possess the universal activating (ergotropic) property characteristic of TRH, but without its hormonal activity [1, 11]. Another aim is to elucidate the role of the different fragments of the tripeptide in the broad spectrum of neurotropic effects of this compound. Pyroglutamate was initially considered to be an essential fragment for the manifestation of all types of TRH activity. However, preservation of neurotropic activity in a TRH analog containing orotic acid at the N-end [11], and also ability of analogs containing an m-(chloroacetyl)benzoyl group [10] to bind with TRH receptors, make analysis of the role of pyroglutamate for manifestation of the various types of TRH activity essential. The realization of certain types of TRH activity, such as antagonism with ethanol [13] and with tetrahydrocannabinol [8], is linked by most investigators with the C-terminal fragment of TRH (histidyl-proline). Data on the role of TRH fragments in the realization of its antidepressant activity are contradictory. According to some investigators [9, 13] it also is due to the histidyl-proline fragment, but others are of the opinion that pyroglutamic acid dipeptides, lacking this fragment, preserve their antidepressant properties.

In accordance with the facts described above it was decided to study pyroglutamic acid derivatives, synthesized by the writers, lacking the histidyl-proline fragment, and containing GABA (with several lipophilic radicals or its homolog, β-alanine, as the second amino acid.

EXPERIMENTAL METHOD

The dipeptides used were synthesized by the method of activated esters from the tetrachlorophenyl ester of pyroglutamic acid and the corresponding alkyl ester of the second amino acid. The amides of the dipeptides were obtained by ammonolysis of the esters.

To assess the antidepressant activity of the synthesized compounds and of TRH as the standard preparation, their influence was studied on the motor activity-stimulating effect of amphetamine. Experiments were carried out on noninbred male albino mice weighing 18-24 g, using an "Optovarimex" multichannel recorder (from Columbus, USA). There were four series of experiments: I) mice received two injections of 0.9% NaCl solution in a volume of 0.1 ml/10 g body weight at an interval of 1 min; II) the mice were given an injection of TRH or one of its four dipeptide analogs in a dose equimolar with 5 mg/kg of TRH, and 1 min later, an injection of 0.9% NaCl solution; III) the mice received amphetamine in a dose of 5 mg/kg, followed 1 min later by 0.9% NaCl solution; IV) mice received an injection of TRH or its derivatives, followed after 1 min by an injection of amphetamine. Amphetamine was injected subcutaneously, the oligopeptides and NaCl solution intraperitoneally. Recording of the parameters began 15 min after the 2nd injection and continued for 30 min; activity of 10 mice was recorded simultaneously. Three such groups of 10 mice were used to study each combination of

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TABLE 1. Effect of Pyroglutamate Derivatives on Spontaneous and Amphetamine-Stimulated Motor Activity

Compound	Dose, mg/kg	II/I, %	IV/III, %
pGlu-His-PrO-NH_2	5	135	208
pGlu-β-Ala-NH_2	3	38	83
pGlu-GABA-NH_2	3	39	59
pGlu-GABA-OC_4H_9	4	49	78
pGlu-GABA-$OC_{16}H_{33}$	6	67	84

Legend. II/I) Averaged (for three groups, 10 mice in each group) ratio of parameters of motor activity of animals receiving pyroglutamate derivatives (II) to control value on that same day (I), taken as 100%; IV/III) averaged (for three groups, 10 mice in each group) ratio of parameters of motor activity of animals receiving test peptide together with amphetamine (IV) to activity induced by amphetamine alone (III), taken as 100%.

preparations, with daily repetition of the control series (I and III). In view of evidence that the cetyl ester of GABA possesses anticonvulsant activity [4, 7], the compound L-pGlu-GABA-$OC_{16}H_{33}$ also was studied on a model of convulsions evoked by electric shocks applied to the mice through corneal electrodes (50 V) or induced by bicuculline (3 mg/kg subcutaneously).

In view of previous data on the ability of TRH to stimulate the formation of a conditioned passive avoidance reflex (CPAR) in rats [2], and also because of the absence of information in the literature on any connection between the structure of TRH and this type of activity, it was decided to reinvestigate the compounds on a model of CPAR [9]. Rats were placed in the larger, illuminated compartment of a double section chamber. The latent period of the first visit of the animal to the dark compartment and the length of the animal's stay in it during the period of exposure (180 sec) were recorded. At the end of this time the rat was subjected to unavoidable painful electrical stimulation (50 V) through the electrode floor (five stimuli, 1 sec in duration, each after an interval of 2 sec). These parameters of learning were chosen so that about 50% of the animals learned in the control. It was thus possible to discover whether the ability of the rats to learn was impaired or improved under the influence of the test compounds. The degree of preservation of CPAR was assessed after 24 h on the basis of the number of animals which had not visited the dark compartment of the chamber, the decrease in their length of stay in it, and the difference in the degree of preservation of the CPAR, calculated by the formula:

$$\frac{\Delta t}{t_1} \times 100,$$

where $\Delta t = t_1 - t_2$, i.e., the difference between the length of animals' stay in the dark compartment of the chamber before learning (t_1) and during testing of preservation of the CPAR 24 h later (t_2). The higher the value of this parameter, the better the rats were trained. All these parameters were compared with the corresponding values in control groups.

On the basis of the results of preliminary experiments TRH was selected as the reference preparation in a dose of 2 mg/kg, which stimulates learning. The dipeptides were tested in doses equimolar to 2 mg/kg of TRH. Experiments were carried out on 334 noninbred male albino rats weighing 180-240 g. All the test compounds were injected intraperitoneally in a volume of 0.2 ml/100 g body weight 15 min before learning. Bicuculline was injected subcutaneously in a dose of 1 mg/kg.

EXPERIMENTAL RESULTS

According to data in the literature TRH, in the dose used (5 mg/kg), increases spontaneous motor activity, but not significantly; it potentiated to a greater degree, however, the stimulating effect of amphetamine on motor activity (Table 1). Unlike TRH, the dipeptides of

TABLE 2. Relative Activity of TRH and Its Pyroglutamate Analogs in Rats according to the CPAR Test

Compound	Dose, mg/kg	I, %	II, %	P	Number of animals: experiment/control
pGlu--His-Pro$_2$-NH$_2$	2,0	14,5	14,4	<0,05	38/40
pGlu-β-AlA-NH$_2$	1,0	26,4	30,8	<0,01	59/59
pGlu-GABA-NH$_2$	1,1	11,6	8,5	<0,01	20/19
pGlu-GABA-OC$_4$H$_9$	1,4	24,2	37,0	<0,01	10/10
pGlu-GABA-OC$_{16}$H$_{33}$	2,2	19,2	13,6	<0,01	49/50
pGlu-GABA-OC$_{16}$H$_{33}$+ + bicuculline	2,2 + 1,0	31,9	20,6	<0,01	16/20

Legend. Comparison for each compound based on learning parameters with corresponding control taken as 100%; P) statistical significance of differences between parameters in experiment in which the given compound was used and in corresponding series of the control were assessed by the Wilcoxon–Mann–Whitney test. I) Differences in reduction of duration of stay in dark compartment of chamber by experimental and control animals during testing preservation of CPAR (t_2); II) differences in degree of training of experimental and control animals according to parameter $(\Delta t/t_1) \times 100$.

pyroglutamic acid with GABA, with its esters, or with β-alanine, which were studied did not stimulate spontaneous motor activity: when they were given in doses equimolar to 5 mg/kg of TRH, the total number of locomotor acts was actually reduced compared with the control, and the stimulating effect of amphetamine was weakened (Table 1).

It was shown previously that the cetyl ester of GABA has definite anticonvulsant activity. Despite the presence of this lipophilic fragment in the structure of the compound L-pGlu-GABA-OC$_{16}$N$_{33}$, it had no protective action either against convulsions induced by maximal electric shock or against bicuculline convulsions. This was evidently because this compound does not possess a free amino group, which, as was shown previously [7], plays an important role in the realization of the neurotropic effects of lipophilic GABA derivatives. Thus ability to potentiate the stimulating effect of amphetamine, which is a feature of TRH, disappears if the histidyl-proline complex is replaced by GABA or β-alanine. These data are additional proof of the correctness of the hypothesis [3, 15] that the 2nd and 3rd amino acids play an important role in the realization of the antidepressant activity of TRH.

Meanwhile the pyroglutamate derivatives studied exhibited activity in the animals used in the CPAR model. They increased the percentage of animals which did not visit the dark compartment of the chamber. Animals which nevertheless visited it stayed there for a shorter time than previously, evidence of an increase in their level of training compared with the control.

Data showing the relative activity of the test compounds and TRH are given in Table 2.

Since the nootropic activity of some lipophilic GABA derivatives was demonstrated previously [6], it was interesting to discover whether the action of the dipeptides, containing either GABA or β-alanine, which blocks its uptake, was due to stimulation of GABA-ergic receptors. It was shown that bicuculline, a blocker of GABA receptors, if administered before the dipeptide in a dose of 1 mg/kg, which abolishes the behavioral effects of GABA-ergic substances [5], not only did not weaken the stimulating effect of the dipeptide on learning, but on the contrary, potentiated that effect. The results suggest that realization of the stimulating effect of the test pyroglutamate and GABA dipeptides on learning in animals is linked with the presence of the pyroglutamate fragment and not of GABA.

Consequently, unlike antidepressant activity, in the manifestation of which an important role is played by the histidyl-proline complex, a more important role in realization of the learning-stimulating action of TRH and related dipeptides is played by preservation of the structure of pyroglutamate. The derivatives of pyroglutamic acid studied, irrespective of whether a third amino acid was present or absent, or whether GABA or β-alanine was used as the second amino acid, had a facilitating effect on the learning ability of rats in the CPAR. It must be emphasized that the stimulating effect of pyroglutamate derivatives on learning is not combined with stimulation of locomotor activity, as it is in the action of the classical psychostimulants. It can accordingly be concluded that the pyroglutamate derivatives studied in this investigation have a selective effect on learning ability.

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EFFECT OF BENZODIAZEPINES ON AMP-DEAMINASE AND ADENOSINE-DEAMINASE ACTIVITY IN RAT BRAIN TISSUE *IN VIVO*

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With the widespread use of tranquilizers of the 1,4-benzodiazepine series (diazepam, phenazepam,* etc.) in clinical practice the need has arisen for elucidation of the molecular mechanisms of their action.

Since benzodiazepines have no direct action on synaptic transmission, according to data in the literature their effects are linked with an indirect effect on inhibition in the CNS through GABA-ergic mechanisms [4, 6, 13]. It has been suggested that endogenous benzodiazepines of purine nature may be formed in nerve cells, and in particular, metabolic products of adenosine-5'-monophosphate (AMP) and adenosine itself, namely inosinic acid, inosine, and

*7-Bromo-1,3-dihydro-5-(2'-chlorophenyl)-2H-1,4-benzodiazepin-2-one.

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