CNS STIMULANT EFFECT OF N-ACYLDERIVATIVES OF GLUTAMIC AND ASPARTIC ACIDS

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In the course of searching for biologically active substances among derivatives of glutamic and aspartic acid by employing experiments on radioligand binding [4], we have shown that certain N- and O-derivatives of these stimulant amino acids (SAA) can interact with "glutamate-recognizing sites" of rat brain synaptic membranes. We studied the biological activity of N-acyl derivatives of glutamic and aspartic acid (I-XVII) by injecting them directly into the brain.

In accordance with generally accepted opinion [5, 8], compounds that interact with SAA receptors should contain three ionogenic groups: Two negatively charged acid groups and one positively charged amino group. The structure of the investigated compounds I-XVII is distinguished by the fact that the compounds contain only two ionogenic (carboxylic) groups and a polar amide group instead of an amino group. The purpose of the present work was to study the effect of this group's environs on the pharmacological activity of compounds.

We synthesized the following compounds having the general formula:

$$RCONHCH(COOH)(CH_2)_nCOOH,$$
I—XVII

$R=C_6H_5$ (I, II), $C_6H_5CH_2$ (III, IV), $C_6H_5OCH_2$ (V), $C_6H_5COCH_2CH_2$ (VI), $C_6H_5(CH_2)_3$ (VII), 1-$C_{10}H_7CH_2$ (VIII), 2-$C_{10}H_7CH_2$ (IX), 4-Ad$C_6H_4OCH_2$ (X), Pht-L-GluOH (XI), 2-$HOCOC_6H_4$ (XII), $(C_6H_5)_2COH$ (XIII), $(C_6H_5)_2C(OC_2H_5)$ (XIV), $(C_6H_5)_2CH$ (XV), $C_6H_5CH_2OCO$ (XVI, XVII); n=1 (I, III, XVII), 2 (II, IV—X, XII—XVI).

Compounds I-IV, XI, XVI, XVII have been described previously [3, 12].

The N-acyl derivatives of amino acids I-XVII were synthesized by starting from the amino acids themselves or from their dibenzyl salts. The initial carboxylic acids were obtained by the following procedures: β-benzoylpropionic acid [2], γ-phenylbutyric acid [6], 2-naphthylacetic [3], α,α-diphenyl-α-ethoxyacetic acid [7]. N-phthalylglutamic acid and compound XII were synthesized from phthalic anhydride and ethyl glutamate [3].

EXPERIMENTAL (CHEMICAL)

N-phenoxyacetylglutamic Acid (V). A 1.4 ml (0.01 mole) portion of isobutylchloroformate was added dropwise to a solution of 1.6 g (0.01 mole) of phenoxyacetic acid and 1.4 ml (0.01 mole) of triethylamine in 25 ml of dioxane with stirring and cooled with ice. After 5-10 min the triethylamine chlorohydrate precipitate was filtered off and washed with 5 ml of dioxane. This was added upon stirring and cooling (0°C) to a solution of 2.94 g (0.02 mole) of L-glutamic acid and 2.24 g (0.04 mole) of KOH in 5 ml of water for 30 min. The reaction mixture was stirred for another 30 min and then carefully acidified with a 5% solution of HCl to pH 2. This was then triple extracted with 50 ml each portions of ethyl acetate. The organic extract was washed with water, dried with $MgSO_4$ and the ethyl acetate was distilled to dryness. The dry residue was crystallized from alcohol with ether.

N-(β-Benzoylpropionyl)glutamic acid (VI), N-(4-phenylbutyl)glutamic acid (VII), N-(1-naphthyl)acetylglutamic acid (VII) and N-[4-(1-adamantyl)phenoxy]acetylglutamic acid (X) were obtained in a similar manner. The yields and constants of the synthesized compounds are given in Table 1.

N-(2-Naphthyl)acetylglutamic Acid (IX). A 1.2 ml portion of pivaloyl chloride was added dropwise to a solution of 1.85 g (0.01 mole) of 2-naphthylacetic acid and 1.4 ml (0.01 mole) of triethylamine in 25 ml of dioxane with cooling (0°C) and stirring. After 5-7 min the

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TABLE 1. N-Acyl Derivatives of Glutamic and Aspartic Acids

Compound	Yield, %	mp, °C	$[\alpha]_D^{20}$ (s; sovlent)	Empirical formula
V	68	104—106	−15° (2; water	$C_{13}H_{15}NO_6$
VI	43	92—95	−14° (2; water	$C_{15}H_{17}NO_6 \cdot H_2O$
VII	65	126—129	+2° (2; water	$C_{15}H_{19}NO_5$. DCHA salt
VIII	30	210—211	−15° (0,2; water—ethanol, 4:1)	$C_{17}H_{17}NO_5$
IX	35	168—170	−18° (1, water—ethanol, 1:1)	$C_{17}H_{17}NO_5$
X	39	184—186	+5° (1; water—ethanol, 1:1)	$C_{23}H_{29}NO_6$
XII	88	300	—	$C_{13}H_{10}NNa_3O_7 \cdot H_2O$
XIII	62	70 (dec.)	−27° (0,3; water—ethanol, 2:1)	$C_{19}H_{19}NO_6$
XIV	40	134—136	−20° (0,5; water—ethanol, 2:1)	$C_{21}H_{23}NO_6$
XV	58	111—112	−49° (0,5; water	$C_{19}H_{19}NO_5$

triethylamine chlorohydrate was filtered off and washed with 5 ml of dioxane. This was added upon stirring and cooling with ice to a solution of 5.0 g of the dibenzyl tosylate of L-glutamic acid and 1.4 ml of triethylamine in 10 ml of DMFA for 30 min. The cooling was stopped and the reaction mixture was stirred for 30 min at room temperature as the reaction was controlled by TLC. The solvents were vacuum-distilled to dryness and the residue was dissolved in 100 ml of ethyl acetate and successively washed with 50 ml of a 5% NaOH solution, water, 50 ml of a 5% H_2SO_4 solution, and water. The solution was dried over $MgSO_4$ and the ethyl acetate was distilled off to dryness. The residue was dissolved in ethanol (50 ml). A 10 ml portion of a 10% acetic acid solution was added along with a catalyst (10% Pd/C) and then a hydrogen stream was passed through the reaction mixture with constant stirring for 3-6 h. Completeness of the reaction was checked by TLC. The catalyst was filtered off, the solvents were vacuum-distilled to dryness, and the residue was crystallized from water. Yield was 35% (total from two stages); see Table 1 for constants.

N(2-Carboxybenzoyl)glutamic Acid (XII). A 2.35 g (0.0016 mole) portion of phthalic anhydride was added in small quantities to an ether solution of 3.22 g (0.0016 mole) of diethyl glutamate. A precipitate begins to form when a glass tube is rubbed against the container wall. The mixture is left overnight. The resultant precipitate was filtered off, washed on a filter with anhydrous ether. This is followed by crystallization from benzene with light petroleum ether. Yield was 4.7 g, mp 92-93°C.

The product was hydrolyzed with a 10% aq. NaOH solution for 1-4 h while stirring at room temperature. After the water was vacuum-distilled the dry residue was triturated with anhydrous alcohol. This resulted in sodium N-(carboxybenzoyl)glutamate. Yield was 88%.

N-(α,α-Diphenyl-α-ethoxyacetyl)glutamic Acid (XIV). A 2.6 g (0.01 mole) portion of α,α-diphenyl-α-ethoxyacetic acid was dissolved in 10 ml of DMFA to which 3 g (0.02 mole) NOBT was added, followed by cooling with ice to 0°C. A 1.4 ml (0.01 mole) portion of triethylamine was added to a solution of 5 g (0.01) mole of glutamic dibenzyl tosylate in 10 ml of DMFA with stirring and cooling. The solutions were decanted. A 2.06 g (0.01 mole) portion of dicycloglutamic acid was then sprinkled on the mixture with stirring and cooling. The mixture was stirred for 1 h at 0°C, and for 24 h at room temperature with TLC control. A 5 ml portion of glacial acetic acid was added after which the mixture was stirred for 0.5 h. Dicyclohexylurea was filtered off and the filtrate was vacuum-evaporated to dryness. The dry residue was dissolved in 100 ml of ethyl acetate and successively washed with 50 ml of a 5% $NaHCO_3$ solution, water, 50 ml of a 5% H_2SO_4 solution, and again with water. The product was dried over $MgSO_4$. The ethyl acetate was distilled off to dryness and the benzyl groups were removed by the same method as described for compound IX. Yield was 40% (in two stages).

α,α-Diphenyl-α-chloroacetic Anhydride. A 100 g portion of benzylic acid dampened with 50 ml of chloroform was added to a suspension of 200 g of phosphorus pentachloride and chloroform with stirring and cooling with ice. The cooling is stopped and mixture is stirred for 2 h at room temperature and for 2 h in a boiling water bath, and left overnight. The solvent and phosphorus oxychloride were vacuum-distilled off. The residue began to crystallize upon standing. The crystals were triturated in 50 ml of hexane, filtered off, and washed with hexane on a filter. Yield was 71 g (82.5%), mp 50-51°C (from heptane).

N-(α,α-Diphenyl-α-oxyacetyl)glutamic Adid (XII). A solution of 3.6 g of α,α-diphenyl-α-chloroacetic acid in 50 ml of ethyl acetate was added to a solution 8.9 g (0.025 mole) of L-glutamic dibenzylate with stirring and cooling with ice (reaction temperature did not exceed 10-15°C. The solution was vacuum-evaporated to a volume of 40 ml and cooled. The resultant precipitate was filtered off to yield 6.2 g (92%) of N-(α,α-diphenyl-α-chloroacetyl)glutamic dibenzylate (XVIII), mp 103-105°C (from ethyl acetate).

TABLE 2. Convulsive Activity of N-acylated Aspartic and Glutamic Acids

Compound		CD_{50}, μ mole	Relative activity (L-Glu-1.00)
X	2	0,030 (0,025—0,036)	0,94 (0,78—1,13)
I	1	0,090 (0,051—0,151)	2,81 (1,70—4,64)
IV	2	0,090 (0,64—0,172)	2,81 (2,00—3,93)
III	1	0,110 (0,071—0,171)	3,44 (2,22—5,33)
VI	2	0,140 (0,096—0,204)	4,38 (3,02—6,35)
XIII	2	0,144 (0,095—0,227)	4,57 (2,97—7,10)
XIV	2	0,208 (0,161—0,205)	5,63 (5,04—6,91)
V	2	0,213 (0,159—0,288)	6,66 (4,97—8,92)
XI	2	0,270 (0,185—0,394)	8,44 (5,82—12,24)
VIII	2	0,300 (0,188—0,479)	9,38 (5,86—15,01)
II	2	0,320 (0,198—0,505)	10,00 (6,25—16,00)
XVI	2	0,370 (0,234—0,553)	11,56 (7,23—18,50)
XVII	1	0,370 (0,234—0,553)	11,56 (7,23—18,50)
VII	2	0,390 (0,233—0,596)	12,19 (7,26—20,48)
IX	2	0,450 (0,266—0,698)	14,06 (8,37—23,62)
XV	2	>0,500	

TABLE 3. Anticonvulsive Activity of N-(2-carboxylbenzoyl)-Glutamic acid (XII)

Compound	CD_{50}, millimole	Relative activity
N-methyl-D-aspartic acid	0.0017 (0.0010—0.0026)	1.00 (0.57—1.71)
+ YDGG (1.0 mM)	0.0120 (0.0087—0.0165)	7.05 (4.05—12.2)
NMDA + (XII (1.0 mM)	0.0223 (0.0176—0.0280)	13.01 (7.83—21.59)
Kainate	0.0003 (0.0002—0.0004)	1.00 (0.55—1.80)
Kainate + YDGG (1.0 mM)	0.0007 (0.0004—0.0017)	2.39 (1.19—4.78)
Kainate + (XII (1.0 mM)	0.0007 (0.0004—0.0011)	2.40 (1.31—4.36)

A 5 g portion of compound XVIII was boiled in 50 ml of water for 2 h after which 25 ml of a 5% $NaHCO_3$ solution was added and the mixture was boiled for another half hour. The mixture was cooled and extracted with ethyl acetate (three 50 ml extractions). The organic layer was washed with 30 ml of a 5% HCl solution and water, then dried over $MgSO_4$, and vacuum-dried. The benzyl groups were removed in the same manner as described above (compound IX). Yield was 2.0 g of compound XIII (62%). See Table 1 for constants.

N-(α,α-diphenylacetyl)glutamic acid (XV) was obtained in the same manner as compound XIV.

Elemental analysis data satisfied the calculated values.

EXPERIMENTAL (PHARMACOLOGICAL)

The pharmacological activity of compounds I-XVII was tested by injecting them into the cerebral lateral ventricles of Wistar line male mice weighing 18-22 g by method [10]. The compounds were tested for their ability to induce convulsions in the animals upon the injection of the substances into the brain ventricles or their ability to prevent convulsions induced by the intraventricular injection of GluOH or NMDA (N-methyl-D-aspartic acid). The intraventricular route of injection was selected in view of the test compounds' high polarity which would have prevented their penetration of the blood-brain barrier.

The test substances were dissolved in distilled water and the solution was brought up to pH 7.0 by the addition of a 1 N NaOH solution. The substances were then administered in a 5 μl volume by a semi-automatic device for 1 h. The control group was given 5 μl of a phosphate buffer (pH 7.2). Injection accuracy was controlled by the administration of methylene blue into the brain ventricles. After the preparation was injected the animals were observed for 30 min and the appearance of generalized clonic and clonic-tonic convulsions was recorded. Convulsive activity was evaluated by the CD_{50} valuers as computed by the Litchfield—Wilcoxon method [11]. Anticonvulsive activity was evaluated by the substances' ability to increase the CD_{50} of the corresponding convulsant upon joint administration. The convulsive activity of the test compounds was compared to the CD_{50} of glutamic acid.

The pharmacological test data showed that the N-acyl derivatives of glutamic acid and aspartic acid, in the same manner as these stimulant amino acids, exhibit convulsive activity but to a lesser degree than the amino acids. This indicates that the introduction of substituents to the nitrogen atom of amino group in amino acids weakens their convulsive activity. However, from the data cited in Tables 2 and 3, one can draw certain conclusions about

the structural parameters of the molecules that determine the strength of the convulsive-causing action in this series of compounds.

In spite of the fact that the introduction of a radical into the amino group has a generally adverse effect on the ability to induce tremors, a comparison of that activity in compounds VI and VII shows that the replacement of a CO polar group in the R radical by a CH_2 methylene group results in a sharp drop in activity. A comparison of the similarly structured compounds (V and XVI, where R=$C_6H_5OCH_2$ and $C_6H_5CH_2OCO$ respectively) allows us to presume that lipophilic and polar groups must be in direct proximity to each other in the substituent on the nitrogen atom. Compounds that contain a purely lipophilic radical (VIII and VII) are the least active in the series of tested substances. At the same time the combination of a polar group with a highly lipophilic fragment (4-AdC_6H_4O-group in compound X) provides this compound with a sufficiently high degree of stimulant activity that is comparable to the activity of L-GluOH.

The activity dependence of this series of compounds on the polarity of the group situated near the aromatic radical is clearly evident if one compares compounds XIII-XV (see Table 2). The most "polar" compound XIII exhibits the greatest activity whereas the activity of compound XV, which does not have polar fragments adjacent to the aromatic ring, is very low. However, an abrupt amplification of polarity and the appearance of an ionogenic group in the molecule can result in the reversal of activity. Thus, N-phthalyl-glutamic acid XI induces tremors in mice upon intraventricular injection. An increase in this compound's polarity by opening the imide ring in N-(2-carboxybenzoyl)glutamic acid XII renders this compound with weak anticonvulsive activity. Compound XII blocks convulsions induced by kainate (Kai) and does not affect glutamic-induced tremors. Data on its anticonvulsive activity are given in Table 3.

The stimulant action of compounds I-XI, XIII-XVII is blocked by glutamic diethyl ether (100 μg, intraventricularly) which presumes the participation of a stimulant-amino acid-ergic system in the production of that effect since it has been shown [9] that glutamic diethyl ether is a specific blocking agent of glutamic-induced CNS stimulation.

Thus, derivatives of aspartic and glutamic acid containing an amino group blocked by an acyl residue retain their ability to interact with the amino acid-ergic system in the CNS. The magnitude of the compound's stimulant effect depends on the structure of the acyl radical, and particularly on the reciprocal disposition of the polar atoms or groups directly adjacent to the amide group. However, a sharp increase in polarity such as by the introduction of a tertiary carboxyl group gives compound XII blocking properties with a variable selective action against different amino acid-stimulant systems. As a whole, we observed a correlation between compounds' ability to inhibit the specific binding of ^{3}H-L-Glu to rat brain synaptic membranes and their ability to induce convulsions in mice upon intraventricular administration (compare to data on radioligand binding cited in [4]).

All of the above would indicate promising prospects for finding new pharmacologically active compounds affecting the stimulant amino acid-ergic system in the CNS among N-substituted derivatives of biamino acids.

LITERATURE CITED

1. D. Grinshteyn and M. Vinits, Chemistry of Amino Acids and Peptides [Russian translation], Moscow (1965), pp. 407-408.
2. I. Guben, Methods of Organic Chemistry [Russian translation], Moscow, Leningrad (1935), 3, Book 2, p. 461.
3. I. Guben, Methdos of Organic Chemistry [Russian translation], Moscow, Leningrad (1935), 1, Book 1, p. 1176.
4. L. B. Piotrovskii, M. A. Dumpis, D. V. Ioffe, et al., Dokl. Akad. Nauk SSSR, 286, No. 1, 235-238 (1986).
5. L. B. Piotrovskii, Khim.-farm. Zh., No. 7, 773-782 (1987).
6. Synthesis of Organic Preparations [in Russian], Moscow (1949), Coll. 2.
7. L. N. Yakhontov, L. I. Masstafanova, S. S. Liberman, et al., Khim.-farm. Zh., No. 3, 56-59 (1974).
8. A. C. Foster and G. E. Fagg, Brain Res. Rev., 7, 103-164 (1984).
9. S. Holdeman and H. McLennan, Brain Res., 45, 393-400 (1972).
10. I. P. Lapin, J. Neural Transmis., 42, 37-43 (1978).
11. J. T. Litchfield and P. Wilcoxon, J. Pharmacol. Exp. Ther., 96, 99-113 (1949).
12. T. Suyama, T. Toyoda, and S. Kanao, Yakugaku Zasshi, 85, 279-283 (1965); Chem. Abstr., 63, No. 7095 s (1965).