SYNTHESIS OF N,N'-DIACYL BIS-GLUTAMIC ACID DERIVATIVES AND THEIR INFLUENCE ON THE RECEPTOR BINDING OF ^{3}H-L-GLUTAMATE

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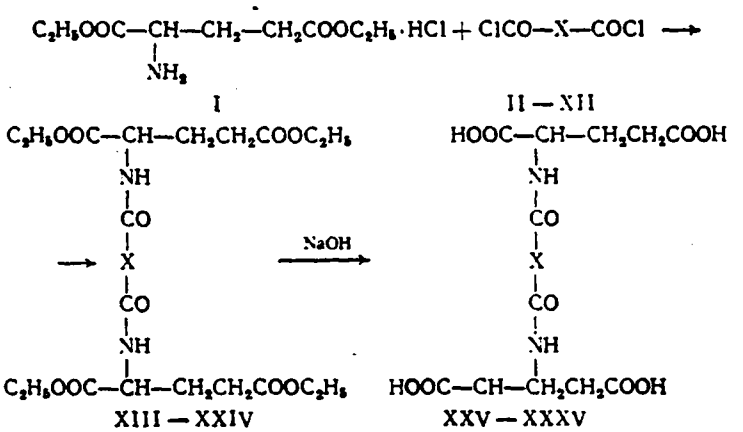
Glutamic acid is known as one of the main stimulating types of neuromediators, participating in the transfer of nerve impulses in the CNS [10]. However, postsynaptic glutamate receptors have been much less studied than the receptors of other neuromediators, such as, for example, acetylcholine [8] and catecholamines [4, 5]. The lack of highly specific agonists and antagonists of glutamate receptors poses a serious drawback to their comprehensive investigation. Since the pharmacological properties of CNS glutamate receptors have been little investigated, it is difficult to produce new drugs designed for directed action on glutamate-stimulated points of contact in the brain of mammals, affected preferentially during epilepsy [11].

These facts were the basis for a search for compounds that effectively inhibit the receptor binding of glutamate with synaptic membranes. The search for such compounds was carried out among different classes of compounds, such as the closest analogs of glutamic acid (GAMA, glutamine, α-keto- glutaric acid, etc.), N-substituted derivatives of glutamic acid, derivatives of glutamic and aspartic acids and lipids [6], and heterocyclic compounds possessing points of similarity to glutamic acid. The investigations carried out showed that many of these compounds do not influence the inhibition of the binding reaction of a radiolabeled glutamate with the synaptic fraction of the membranes of rat brain cells.

We therefore surmised that the most effective analogs of glutamic acid should be those derivatives of amino acid having a linear structure, and with at least one free carboxyl group.

We synthesized various diamides of aliphatic and aromatic dicarboxylic acids with glutamic acid (XXV-XXXV) and studied their influence on the receptor binding of ^{3}H-L-glutamate with synaptic membranes, secreted from the brain of rats.

The synthesis of the compounds was carried out according to the following scheme:



where X = 0 (II, XIII), CH_2 (III, XIV, XXV); $(CH_2)_2$ (IV, XV, XXVI); $(CH_2)_3$ — L-isomer (XVI, XXVII); $(CH_2)_3$ (V, XVII, XXVIII); $(CH_2)_4$ (VI, XVIII, XXIX); $(CH_2)_5$ (VII, XIX, XXX); $(CH_2)_7$ (VIII, XX); $(CH_2)_8$ (IX, XXI, XXXI); o-C_6H_4 (X, XXII, XXXII); m-C_6H_4 (XI, XXIII, XXXIII, XXXIV — racemate); p-C_6H_4 (XII, XXIV, XXXV).

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TABLE 1. Di[1,3-di(ethoxycarbonylprop-1-yl]amides of Polymethyl and Benzenedicarboxylic Acids (XIII-XXIV) and Di[1,3-di(hydroxycarbonyl)prop-1-yl] Amides of Polymethylene- and Benzenedicarbocarboxylic Acids (XXV-XXXV)

Compound	Yield, %	mp, °C	Solvent for crystallization	Found, %			Empirical formula	Calculated, %		
				C	H	N		C	H	N
XIII	37,0	87—90	Acetone–water	52,01	7,01	6,04	$C_{28}H_{32}N_2O_{16}$	52,16	7,00	6,08
XIV	31,0	69—72	Benzene–hexane	53,12	7,48	5,95	$C_{31}H_{34}N_2O_{16}$	53,15	7,22	5,90
XV	38,3	98—9,5	Benzene–hexane	54,29	7,57	5,62	$C_{32}H_{36}N_2O_{16}$	54,08	7,43	5,73
XVI*	36,4	105	Benzene–hexane	54,52	7,52	5,63	$C_{32}H_{36}N_2O_{16}$	54,08	7,43	5,73
XVII	30,7	90—4	Benzene–hexane	55,24	7,60	5,56	$C_{33}H_{38}N_2O_{16}$	54,96	7,62	5,57
XVIII	31,1	106—8	Acetone–water	55,93	8,06	5,45	$C_{24}H_{40}N_2O_{16}$	55,79	7,80	5,42
XIX	36,2	92—5	Acetone–water	57,16	8,09	5,15	$C_{26}H_{44}N_2O_{16}$	57,33	8,14	5,14
XX	44,3	67—9	Benzene–hexane	58,08	8,32	4,92	$C_{27}H_{46}N_2O_{16}$	58,01	8,30	5,01
XXI	52,9	75—7	Acetone–water	58,69	8,47	4,80	$C_{28}H_{48}N_2O_{16}$	58,72	8,45	4,89
XXII	23,5	85—8	Benzene-hexane-	56,36	6,99	5,19	$C_{28}H_{34}N_2O_{16}$	58,20	6,76	5,22
XXIV	65,0	94—9	Benzene–hexane ether	57,87	6,93	5,31	$C_{28}H_{34}N_2O_{16}$	58,20	6,76	5,22
XXV	46,2	178—83	Ethanol–ether	42,96	5,00	8,00	$C_{12}H_{16}N_2O_{10}$	43,09	5,01	7,73
XXVI	55,8	175—8		44,71	5,37	7,14	$C_{14}H_{20}N_2O_{10}$	44,68	5,36	7,44
XXVII**	53,2	177—9		44,55	5,43	7,48	$C_{14}H_{20}N_2O_{10}$	44,68	5,36	7,44
XXVIII	51,3	154—7		46,02	5,75	6,94	$C_{15}H_{22}N_2O_{10}$	46,15	5,68	7,18
XXIX	60,0	212—5		47,60	6,08	7,06	$C_{16}H_{24}N_2O_{10}$	47,52	5,92	6,93
XXX	84,8	149—52		50,26	6,61	6,31	$C_{18}H_{28}N_2O_{10}$	49,99	6,53	6,48
XXXI	72,5	155—7	Water	32,23	7,09	5,94	$C_{10}H_{22}N_2O_{10}$	52,16	7,00	6,08
XXXII	27,0	125—7	Water–ethyl acetate	50,90	4,66	6,70	$C_{19}H_{20}N_2O_{16}$	50,94	4,75	6,60
XXXIII	25,8	184—8	Acetone–ethyl acetate	50,97	4,77	6,81	$C_{19}H_{20}N_2O_{16}$	50,94	4,75	6,60
XXXIV***	9,8	142—7	Ethyl acetate	50,79	4,76	6,32	$C_{19}H_{20}N_2O_{16}$	50,94	4,75	6,60
XXXV	63,9	143—8	Ether–ethanol	50,97	4,74	6,57	$C_{19}H_{20}N_2O_{16}$	50,94	4,75	6,60

Note. One asterisk: $[\alpha]_D^{29} = 27.74°$; two asterisks: $[\alpha]_D^{22} = -29.38°$; three asterisks: racemate.

Diethyl glutamate hydrochloride (I) was obtained by the method in [9]. Di[1,3-di(ethoxycarbonyl)prop-1-yl] amides of polymethylene- and benzenedicarboxylic acids (XIII-XXIV) were obtained by reacting dichlorides of the corresponding acids (II-XII) with I in chloroform in the presence of triethylamine. The presence of two asymmetric centers in the molecules of amides (XIII-XXIV) implies that a mixture of diastereomers was obtained. From the mixture were isolated meso-isomers, which were crystalline compounds. The remaining mixtures, consisting mainly of racemates, were in the form of viscous oils, which crystallized very slowly (in the course of one year).

A substituted amide of adipic acid (XVIII) was also obtained by the azide method: by the reaction of adipic acid azide [7] with I.

To study the stereospecificity of binding of the preparations by glutamate receptors, we synthesized a substituted diamide of succinic acid with L-glutamic acid (XVI). To clarify the role of the unit binding two glutamate residues, we synthesized diamides of benzenedicarboxylic acids (XXII-XXIV). Diamides (XIII-XXIV) were subjected to saponification in acetone or dioxane solutions at 20°C; thus di[1,3-di(hydroxycarbonyl)prop-1-yl] amides of poly methylene- and benzenecarboxylic acids (XXV-XXXV) were isolated.

The stereoisomeric amides of isophthalic acid (XXIII) could not be separated into their isomers, and therefore, a mixture of isomers was used for the alkaline hydrolysis of the ester groups. The meso-isomer (XXXIII) and racemate (XXXIV) were obtained by the crystallization of isophthalic acid glutamides.

The condensation of I with acid dichlorides, the alkaline hydrolysis of the esters, and the homogeneity of the products obtained were monitored by TLC on Silufol plates (butanol–water–acetic acid (60:20:20) system of solvents). The plates were developed by iodine or benzidine, after being preliminarily treated by chlorine.

EXPERIMENTAL CHEMICAL PART

Preparation of di[1,3-di(ethoxycarbonyl)prop-1-yl]amides of Polymethylene- and Benzenedicarboxylic Acids (XIII-XXIV). A 0.14-mole portion of triethylamine is added with stirring and cooling by ice water to 0.06 mole of I in 50 ml of dry chloroform. Then 0.03 mole of acid dichloride (II-XII) in 10 ml of dry chloroform is added dropwise with constant stirring and cooling, and the mixture is allowed to stand overnight at 20°C and washed with ice water (the completeness of the reaction is judged from the amount of dry ethylamine hydrochloride present after evaporation of the aqueous solution). Chloroform is distilled in vacuo, ether is added to the oily precipitate, and the mixture is allowed to stand overnight. The crystals that separate are filtered, washed with ether, and recrystallized. The physical con-

TABLE 2. Inhibition of Receptor Binding of ^{3}H-L-Glutamate by Glutamic Acid Derivatives

Compound	Effectiveness of inhibition (K_i, mM)
XXV	78,0
XXVI	3,5
XXVII	7,0
XXVIII	60,0
XXIX	70,0
XXX	42,0
XXXI	56,0
XXXII	30,0
XXXIII	105,0
XXXV	Ineffective

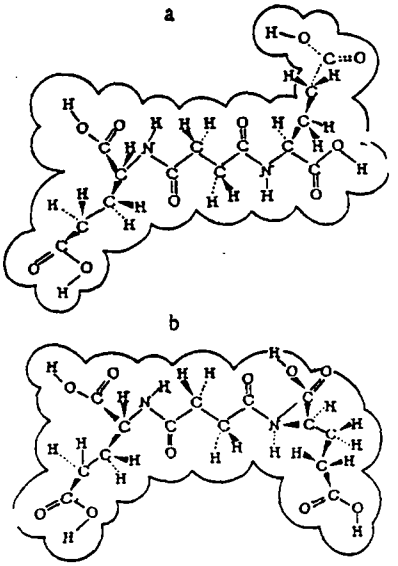

Fig. 1. Projection of Stuart model of a molecule of di[1,3-di-(hydroxycarbonyl)prop-1-yl]-succinamide on a plane. a) meso form, b) -L-isomer.

stants of the compounds obtained and the data of the elemental analysis are given in Table 1.

Preparation of Di[1,3-di-(hydroxycarbonyl)prop-1-yl]amides of Polymethylene and Benzenedicarboxylic Acids (XXV-XXXV). A 0,002-mole portion of amide XIII-XXIV is dissolved in 24 ml of acetone or dioxane and a solution of 0,008 mole of NaOH in 6 ml of water is added with stirring. The emulsion is stirred for 1.5-2 h, then allowed to settle, the solvent is decanted, fresh dry acetone or dioxane is added, and the mixture is thoroughly stirred. The operation is repeated three times. Dry acetone is added to the thickened mass and the mixture is acidified with concentrated HCl (~0,7 ml); the sodium chloride precipitate formed on grinding is filtered and washed with dry acetone. The acetone solution is evaporated in vacuo, whereupon the residual oily precipitate crystallizes. The physical constants and the analytic data are given in Table 1.

EXPERIMENTAL PHARMACOLOGICAL PART

In the experiments nonpedigreed male rats were used, each weighing 180-200 g each. The synaptic membrane fraction from the cerebral cortex of the large hemispheres of rat brain was obtained by a modified method of gradient centrifugation [12]. The binding of ^{3}H-L-glutamate with synaptic fractions of the membranes was carried out by the method described in [2]. The synthesized compounds were selected on the basis of the data on their influence on the specific binding of ^{3}H-L-glutamate. The compounds studied were added at a known concentration to the incubation medium. The inhibition constant (K_i) was determined from the formula

$$K_i = \frac{IC_{50}}{(1 + C/K_d)},$$

where IC_{50} is the concentration of the compound studied at which a 50% inhibition of binding is obtained; C is the concentration of the tracer in the sample; K_d is the dissociation constant of the bound tracer.

In the case of a weak inhibition of the specific binding, the action of the compound was studied at a concentration of 0.1 mM.

Table 2 shows the results of the action of the synthesized glutamic acid amides (XXV-XXXV) on the receptor binding of ^{3}H-L-glutamate. It is seen that compound XXVI is most effective and in its activity it is superior by one order of magnitude to the remaining compounds. It is of interest to note that the effectiveness of compounds with an aromatic "bridge" is much lower than that of aliphatic acid derivatives, but increases with the approach of the glutamic acid residues.

From the test results of the compounds of this series, it can be assumed that in the membrane bound glutamate receptor complex there are at least two identical binding sections, separated from one another by a distance approximately equal to the length of the $-CO-CH_2-CH_2-CO-$ chain. Comparison of the activity of the L-isomer of succinic acid bisglutamide XXVII with that of the meso-isomer XXVI indicates the marked stereoselectivity of the reaction.

The highest biological activity is known to be displayed when the active ligand groups and the binding sections of the receptor macromolecule complement each other thoroughly. In the bisglutamide XXVI molecule, these active groups are the carboxylic groups.

Projection of the Stuart models for the isomeric forms of molecules of compounds XXVI and XXVII on a plane with allowance for the maximum complementarity and the forbidden positions of the groups is shown in Fig. 1. With these models, not only the distance between the active sections of the substrate support of the binding center of the glutamate receptors can be evaluated, but also the charge of the active groups on the receptor macromolecule, by means of which any possible interaction with the neuromediator must take place. Figure 1 illustrating the projection of a model of an L-isomer of XXVII on a plane, shows that only three carboxylic groups can be arranged in the plane. Hence only three carboxylic groups are complementary with the active binding sections. This probably may also explain the decrease in the activity of the L-isomer compared with that of the meso-isomer, for which all four carboxylic groups are located in the plane.

Thus, the data obtained confirm our theory that the subunits of the glutamate receptor have several neuromediator binding sites containing some of the hydrophobic amino acids in the outer zone of the membrane and forming the active site of the receptor macromolecule [1].

The predominance of the cationic charges of the amino acid residues in the active site of the macromolecule of the glutamic receptor calculated by us in [3] indicates that the first stage in the interaction of the glutamate with the recognition center of the receptor complex takes place due to remote-acting electrostatic interactions, i.e., cation–anion forces.

LITERATURE CITED

1. S. A. Dambinova, Neirokhimiya, 2, 426-440 (1983).
2. S. A. Dambinova and A. I. Gorodinskii, Biokhimiya, 49, 67-75
3. S. A. Dambinova and N. V. Khromov-Borisov, Dokl. Nauk SSSR, 274, 467-470 (1984).
4. I. V. Komissarov, Short Course of Molecular Pharmacology [in Russian], Moscow (1975), pp. 144-170.
5. A. I. Matyushin, Farmakol. Toksikol., No. 1, 107-113 (1986).
6. L. B. Piotrovskii, M. A. Dumpis, D. V. Ioffe, et al., Dokl. Akad. Nauk SSSR, 286, 235-238 (1986).
7. P. Smith, Syntheses of Organic Preparations [Russian translation], No. 8, Moscow (1958), pp. 12-16.
8. N. V. Khromov-Borisov, Short Course of Molecular Pharmacology [in Russian], Moscow (2975), pp. 113-143.
9. M. Brenner and W. Huber, Helv. Chim. Acta, 36, 1109-1115 (1953).
10. F. Fonnum, G. Neurochem., 42, 1-11 (1984).
11. B. Meldrum, The Molecular Basis of Neuropathology, A. N. Davison and R. H. S. Thompson, eds., London (1981), pp. 183-238.
12. S. R. Zukin, A. B. Young, and S. H. Snyder, Proc. Natl. Acad. Sci. USA, 71, 4802-4807 (1974).