We should emphasize that the limiting factor observed in the specific activity of Streptokinase modified by a copolymer is the degree of its modification.

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SYNTHESIS AND PSYCHOTROPIC PROPERTIES OF THE TETRAPEPTIDE OF HUMAN INTERFERON-α_2-(122-125)

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UDC 615.339(578.245].012.6+615.339:578.245].017:615.214

Besides strong immunomodulating, antiviral, and anticarcinogenic properties, interferon (IFN) exhibits many other effects, including action on the central nervous system [10]. It was shown that leucocyte human EFN has a structural-functional similarity to corticotropin (ACTH) and endorphins [7], exhibits endorphin-like and ACTH-like activity [6, 11], and has a positive effect in the treatment of patients with diffuse sclerosis [8]. From these and certain, other data [9], we can assume that IFN or (and) its fragments may have an effect on training and memory processes, and also that IFN, like other protein regulators, for example, β-lipotropin, is a predecessor of a whole series of biologically active low-molecular-weight peptides.

To expose these peptides, we synthesized a Tyr-Phe-Gln-Arg tetrapeptide, with a 122-125 sequence, of the leucocytary human IFN-α_2 [12], and studied some of its neuro- and psychotropic properties. We selected this fragment on the basis of general principles of structural-functional organization of the peptide molecules, which are training and memory modulators, already established by us in [4, 5].

EXPERIMENTAL CHEMICAL SECTION

The melting points of the compounds were determined in a capillary (without correction). The homogeneity of the compounds was verified by thin-layer chromatography on silica gel plates of the firm "Merck" in the systems: tert-butanol—n-butanol—acetic acid—water, 2:2:1:1 (A); n-butanol—acetic acid—pyridine—water, 15:3:10:6 (B); and chloroform—ethanol—ethyl acetate—acetic acid, 5:2:5:0.5:0.5 (C). Ninhydrin was used for developing the chromatograms and the chlorobenzidine, Pauli, and Sakaguti reagents. The electrophoretic mobility (E) was determined on a Filtrak FN117 paper in 5 N acetic acid (pH 1.9) at a potential gradient of 30 V/cm in the course of 1.5 h. The specific rotation of the compounds was measured on the model 141 M polarimeter of the first Perkin—Elmer. The peptides were hydrolyzed by 6 N hydrochloric

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Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 19, No. 1, pp. 35-39. January, 1985. Original article submitted March 20, 1984.

0091-150X/85/1901-0024$09.50

TABLE 1. Influence of Tetrapeptide of Interferon-α_2-(122-125) on Development of a Conditional Punishment Evasion Reflex 45 min after Subcutaneous Administration

Preparation	Dose, μg/kg	Mean number of runs before achieving criterion of skill*		Mean time of training before achieving criterion of skill,* sec	
		during training	after 24 h	during training	after 24 h
		Males			
Control†		12,4±1,1	3,9±0,8	62±12	14±4
IFN peptide	20	12,1±1,4	3,7±1,2	59±14	12±5
	100	10,0±0,5	2,8±0,7	51±8	9±3
	500	16,3±2,9	3,4±0,7	97±23	28±12
		Females			
Control†		10,2±0,9	2,8±0,4	47±8	11±3
IFN peptide	100	8,9±0,7	2,2±0,3	32±6	9±3
		Males			
Control†		12,1±0,9	3,7±0,7	60±11	14±5
IFN peptide	50	4,2±0,6	1,2±0,6	27±8	5±3

*Mean values and double error of mean values are given.
†A 1-ml portion of physiological solution; in the control 14 animals were used, and in each experiment, 10.

acid at 105-110°C for 24 h; the amino acid composition of the hydrolysates was determined on the "Liquimat III" analyzer.

Ethyl Ester of N^{α}-tert-Butoxycarbonyl-L-tyrosyl-L-phenylalanine (I). A 37.10-g portion (0.134 mole) of Boc-L-tyrosine, 30.83 g (0.134 mole) of L-phenylalanine ethyl ester hydrochloride, and 20.25 g (0.150 mole) of 1-hydroxyenzotriazole are dissolved in a mixture of 100 ml of dimethylformamide (DMFA) and 100 ml of ethyl acetate. The solution is cooled to −10°C, and 18.76 g (0.134 mole) of triethylamine and a solution of 27.65 g (0.134 mole) of dicyclohexylcarbodiimide in 100 ml of ethyl acetate are added with stirring. After 3 h, the reaction mixture is placed in a refrigerator (0°C) for 20 h, the precipitate is filtered, and the filtrate is diluted with a saturated solution of sodium chloride (up to phase separation). The organic layer is separated and washed with a 0.5 N sulfuric acid, water, a 5% solution of sodium hydrocarbonate, and then again with water. The solution is dried over anhydrous sodium sulfate, evaporated, and diluted with hexane. The product that crystallizes out is filtered, washed with hexane, dried, and recrystallized from a mixture of ethanol and water. The yield of I is 43.50 g (71%), mp 139-141°C, $[\alpha]_D^{18}$ −12.9° (c 1, DMFA), R_f 0.87 (A) 0.87 (B), 0.94 (C). Found, %: C 65.96; H 7.14; N 6.37. $C_{25}H_{32}N_2O_6$. Calculated, %: C 65.66; H 7.07; N 6.14.

Hydrazide of N^{α}-tert-Butoxycarbonyl-L-tyrosyl-L-phenylalanine (II). A 16.50-g portion (0.036 mole) of compound I is dissolved in 100 ml of ethanol, 11.6 ml of anhydrous hydrazine are added, and the reaction mixture is held for 48 h at room temperature and then diluted with ethyl acetate and hexane, dried, and recrystallized from a mixture of DMFA and ethyl acetate. The yield of II is 14.20 g (87%), mp 212-214•C, $[\alpha]_D^{18}$ 27.1° (c 1, DMFA), R_f 0.96 (A), 0.84 (B), 0.91 (C). Found, %: C 61.40; H 6.70; N 12.65. $C_{29}H_{30}N_4O_5$•0.5 H_2O. Calculated, %: C 61.18; H 6.92; N 12.41.

N^{α}-tert-Butoxycarbonyl-L-glutamyl-L-arginine (III). A 14.70-g portion (0.040 mole) of p-nitrophenyl ester of Boc-L-glutamine is dissolved in 130 ml of DMFA. A solution of 6.40 g (0.036 mole) of L-arginine in 20 ml of water is added, and the mixture is stirred for 48 h. The gel formed is ground with dioxane and ether, and dried. After crystallization from a mixture of DMFA and ethyl acetate, 14.67 g (97%, based on arginine) of III are obtained, mp 150°C (dec), $[\alpha]_D^{20}$ −3.3° (c 0.5, DMFA), R_f 0.37 (A), 0.48 (B), 0.00 (C). Found, %: C 47.21; H 7.20; N 20.03. $C_{16}H_{30}N_6O_6$•0.5 H_2O. Calculated, %: C 46.93; H 7.63; N 20.52.

L-Glutamyl-L-arginine Hydrochloride (IV). A 3.70-g portion (0.009 mole) of dipeptide III is dissolved in 20 ml of acetic acid. Then 20 ml of a 5.2 N solution of HCl in dioxane is added, the mixture is stirred for 1 h, and then diluted with dry ether. The oil that separates out is thoroughly triturated with fresh portions of ether up to the formation of a

TABLE 2. Reproduction of Conditional Punishment Escape Reflex in Rats 24 h after Dosed Training, Followed by Introduction of IFN-α_2-(122-125) Tetrapeptide

Preparation	Dose, μg/kg	Mean number of runs before achieving criterion of skill	Mean time of training before achieving criterion of skill, sec
		Males	
Control*		4,6±0,7	22±6
IFN peptide	100	2,8±0,4	14±3
	500	2,6±0,5	13±4
		Females	
Control*		3,9±0,6	19±5
IFN peptide	100	2,4±0,6	12±6
		Males	
Control*		4,9±0,4	20±7
AVP	20	2,2±0,3	8±2

*A 1-ml portion of physiological solution; in the control 14 animals were used and in each experiment, 12.

floculent precipitate, which is rapidly filtered, and dried in a vacuum exciccator over P_2O_5–KOH. The yield of IV is 3.02 g (99%); a hygroscopic substance, R_f 0.03 (A), 0.11 (B).

N^{α}-tert-Butoxycarbonyl-L-tyrosyl-L-phenylalanyl-L-glutamyl-L-arginine (V). A 4.42-g portion (0.010 mole) of hydrazide II is dissolved in 57 ml of DMFA. The solution is cooled to −30°C, 5 ml of 4 N solution of HCl in dioxane is added, and then 1.1 ml (0.010 mole) of tert-butylnitrite is added dropwise with vigorous stirring. After 25 min, the reaction mixture is neutralized with triethylamine, and a solution of 3.02 g (0.009 mole) of dipeptide IV in 29 ml of dimethyl sulfoxide and 1.4 ml (0.010 mole) of triethylamine are added. The mixture is stirred and held for 18 h at 5°C, and then stirred for another 5 h at room temperature and diluted with ethyl acetate. The solvents layer is decanted, and the oil dissolved in water. The solution is washed with ethyl acetate, deposited on a column with Amberlite IRC-50 (H^+), and the resin is washed with 0.25% acetic acid (500 ml). The product is eluted from the column by a 50% acetic acid (400 ml), the solution is evaporated, the oil is dissolved in ethanol and diluted by ether, and the precipitate is filtered and dried. The yield of V is 5.65 g (81%, based in IV), mp 141-143°C (dec), $[\alpha]_D^{18}$ −5.8° (c 1, DMFA), R_f 0.51 (A), 0.56 (B), 0.02 (C). Found, %: C 52.84; H 6.83; N 14.05. $C_{34}H_{48}N_8O_9 \cdot 3.5\ H_2O$. Calculated %: C 52.64; H 7.14; N 14.44.

L-Tyrosyl-L-phenylalanyl-L-glutamyl-L-arginine (VI). A 0.86-g (0.0011 mole) portion of compound V is dissolved in 10 ml of trifluoro-acetic acid and cooled to 0°C. The solution is stirred for 20 min, and evaporated. The oil is dried in a vacuum exciccator over KOH and then dissolved in 200 ml of water. Aqueous ammonia is added to pH 5.0, and the solution is deposited on a column with Amberlite IRC-50 (H^+). The elution from the column and the isolation of the peptide from the resin are carried out as for V. The solution of the peptide is evaporated to 20 ml and lyophylized. The yield of the salt-free product VI is 0.52 g.

A 0.24-g portion of the lyophylizate is dissolved in 2.5 ml of 0.2 N acetic acid, the solution is deposited on a column (107 × 3.3 cm) with Sephadex G-10 and eluted by the same solution at the rate of 93 ml/h, and 14 ml functions are collected. Detection is at 275 nm. The corresponding fractions are combined and lyophylized. The yield of VI is 127 mg/(35%), mp 139-140°C; $[\alpha]_D^{20}$ + 5.5° (c 0.2, 1 N acetic acid). R_f 0.11 (A) 0.41 (B), 0.00 (Cl. E^{His} 0.83, E^{Gly} 0.95, E^{Trp} 1.36. Amino acid composition: glutamic acid 1.05, phenylalanine 0.91, tyrosine 1.00, arginine 1.03. Found, %: C 52.25; H 6.51; N 15.40. $C_{29}H_{40}H_8O_7 \cdot C_2H_4O_2 \cdot 2H_2O$. Calculated, % C 52.54; H 6.83; N 15.80.

EXPERIMENTAL PHARMACOLOGIC SECTION

In studies on the influence of tetrapeptide VI (Tyr-Phe-Gln-Arg) on training processes, we used the method of developing in rats, weighing 180 ± 20 g each, a conditioned reflex of punishment evasion. For this purpose a special U-shaped chamber was used, with three dark compartments, each of which could be illuminated from inside by a bright lamp, and on the area's grating an electrical current was applied at a given moment. Training began when the animal was placed in the "starting" illuminated compartment, where the light was switched off after 30 sec, and the lamp in the next anticlockwise positioned compartment was switched on. Five seconds after the conditional irritant (switching over of light) was delivered, an electric current of 40 V and a frequency of 1.50 Hz was applied onto the grating of the darkened compartment areas. The current surges compelled the rat to move over the labyrinth and to choose to reside in an illuminated compartment that was "not penalized" by pain irritant. Thirty to sixty seconds after the animal had resided in the illuminated compartment selected by him, the training procedure was repeated: the light was switched on in the next anticlockwise positioned section, while the preceding compartment was darkened and a current was applied to the area's grating. The rate was considered to be trained, i.e., to have achieved a certain criterion of skill, if it completed 8-10 correct runs in succession after switching over the light without intersignal reactions, and with a latent period of evasion of 1-3 sec after switching on the current. In the course of the experiment, the mean number of runs necessary to achieve the criterion of skill, the latent period of reflex, the total and mean time of training, and the number of erroneous runs into the dark compartments were recorded. On the following day, after 24 h, the retention of the habit was verified by the same method, i.e., the required number of runs to reproduce the criterion of skill was determined,

In the second series of experiments, to study the influence or peptides directly on the consolidation of the habit in a long-term memory, the preparation was introduced immediately after a dosed training of the rats for a conditioned punishment evasion reflex of 10 runs. The retention of the habit was verified after 24 h, and in separate experiments 7 days after training; the results differed inappreciably.

A preliminary study on the degree of emotional reaction of the rats in an "open area" facilitated the selection of emotionally stable rats for the experiment, i.e., about 80% of their number. In all the experiments, the zoosocial behavior before and after the training process was recorded, and observations of the free behavior or the animals before and after the introduction of the preparation were made by evaluating the emotional status directly when the animals were in the labyrinth.

The preparation was dissolved directly before use in a sterile physiological solution and was administered subcutaneously 30-60 min before the reflex was developed, while an isotonic solution of sodium chloride was introduced in the same volume, 1 ml subcutaneously to the control animals. Part of the investigation was concerned directly with the search for optimal doses.

RESULTS AND DISCUSSION

It was experimentally found that the optimal dose for improving the primary training was 100 µg per kg body weight. A dose of 20 µg/kg did not cause any statistically significant changes, while a dose of 500 µg/kg impaired the training (Table 1). A positive effect on the training was in decrease in the number of runs required to achieve the criterion of skill, a shorter time of training, and decrease in the number of errors and intersignal reactions. Compared with the standard, the ACTH-(4-10) fragment, which is the strongest peptide influencing the primary training, these changes are not very considerable.

It is possible that the tetrapeptide is not particularly dependent on the effects of the hormonal background, since in males and in females the effects are almost the same, although it should be noted that there is a tendency toward an intensifying effect in males compared with females. Possibly, this is because the initial level of training is higher in females than in males.

There were no changes in the free behavior when small or medium doses (20-100 µg/kg) of the tetrapeptide were introduced, while higher doses (500 µg/kg) had a weak depressant action.

The effects of IFN tetrapeptide in influencing long-term memory are presented in Table 2. They are appreciable and statistically significant, although weaker than in the case of the standard preparation 8-arginine-vasopressin (AVP) in the modulation of long-term memory:

with respect to peak effectiveness, by a factor of almost 2; with respect to the dose, by a factor of more than 5.

Experiments on retraining the rats (according to a procedure described in [1]) in the U-shaped labyrinth showed the following. The IFN tetrapeptide (in a dose of 100 μg/kg) weakly stimulates retraining and, the number of retrained rats increased inappreciably compared with control and is statistically insignificant.

A simultaneous use of IFN tetrapeptide and vasopressin in tests for long-term memory (a simultaneous injection of 20 μg/kg of AVP and 100 μg/kg of IFN tetrapeptide) led to poorer parameters than the use of vasopressin only. The relative antagonism to vasopressin, the more pronounced influence of the IFN fragment on the long-term memory compared with its influence on the short-term memory, and the absence of the effect in tests with retraining led us to assume that, in its properties, IFN tetrapeptide is closer to the group of long-term memory modulators (of the vasopressin type) than to compounds which effectively stimulate the short-term memory and the primary training (ACTH, melanotropin).

In studies with electrical shock (according to a procedure described in [3]), it was found that IFN tetrapeptide weakly protects the animals from electrical shock amnesia. Its positive influence on retrograde amnesia is somewhat stronger than that on the anterograde amnesia, i.e., it weakens the disturbances of a habit, developed before the shock. In studies on rabbits with a recording of the spontaneous bioelectrical activity (by the method in [2]), no statistically significant changes were detected, but the following tendency was clearly observed. Doses of 500 μg/kg or more gave a depressing effect with intravenous administration, increasing the low-frequency components and decreasing the high-frequency components. A dose of 100 μg/kg had a weak activating action decreasing the low-frequency components and increasing the representation of higher-frequency waves in the total encephalogram.

Preliminary results of the study on analgetic activity of IFN tetrapeptide indicates the presence of pronounced analgetic properties with intraperitoneal administration to the rats in a test with electrocutaneous irritation of a tail base.

The data of pharmacological investigations thus confirm the supposition that in the peptide chain of leucocyte IFN, sections are present which influence the training and memory processes. The Tyr-Phe-Gln-Arg tetrapeptide with 122-125 sequence is probably a minimal fragment of such a section. We concluded from the investigation that further modification of the tetrapeptide is expedient in order to find new agents with psychotropic activity.

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