SYNTHESIS OF THE TETRAPEPTIDE TUFTSIN AND ITS EFFECT ON THE CENTRAL NERVOUS SYSTEM

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The tetrapeptide tuftsin (Thr-Lys-Pro-Arg) was first isolated and described in 1972 as a phagocytosis-stimulating factor [1]. Then analogs of it were synthesized and their participation in the phagocytotic function was investigated [2, 3]. We have synthesized tuftsin with the aim of further studying its pharmacological properties and its influence on the central nervous system.

EXPERIMENTAL CHEMISTRY

Tuftsin was obtained on the basis of the method of synthesizing arginine-containing peptides [4]. All the peptides synthesized were amorphous substances having no clear melting points. The processes of synthesis and of eliminating protective groups, and also the purity of the peptides obtained were checked by high-voltage electrophoresis on Filtrak FN-12 paper in a buffer consisting of 2% acetic acid at a voltage of about 40 V/cm. All the amino acids used had the L configuration.

Benzyloxycarbonylproplylarginine (I). To a solution of 4.8 g (0.013 mole) of the p-nitrophenyl ester of benzyloxycarbonylproline [5] in 10 ml of DMFA was added 1.9 g (0.01 mole) of arginine (free base) monohydrate. After being stirred for 2 days, the mixture was diluted with dry ethyl acetate, and the precipitate was filtered off and dried to give 3.9 g (96%) of (I). Found, %: C 56.16; H 6.54; N 17.40. $C_{19}H_{27}N_5O_5$. Calculated, %: C 56.28; H 6.71; N 17.27.

(α-Benzyloxycarbonyl-ε-tert-butoxycarbonyllysyl)prolylarginine (II). Compound (I) (2.1 g; 0.005 mole) was hydrogenated in methanol over palladium on carbon. After the end of hydrogenation, the catalyst was filtered off, and the filtrate was evaporated to dryness. The residue was treated with a solution of 3.3 g (0.0065 mole) of the p-nitrophenyl ester of α-benzyloxycarbonyl-ε-tert-butoxycarbonyllysine [6] in a few milliliters of DMFA. The mixture was stirred until the solids had dissolved completely and was kept for an additional day, and then dry ether was added. The precipitate was filtered off and dissolved in a small amount of chloroform, and the solution was washed with water to eliminate any unchanged amino component. Then it was dried with anhydrous sodium sulfate, evaporated to small volume, and treated with dry ether. The precipitate that deposited was filtered off and dried in a vacuum desiccator to give 2.2 g (70%) of (II). Found, %: C 56.72; H 7.56; N 15.58. $C_{30}H_{47}N_7O_8$. Calculated, %: C 56.86; H 7.47; N 15.47.

Benzyloxycarbonylthreonyl(ε-tert-butoxycarbonyllysyl)prolylarginine (III). Compound (II) (0.64 g; 0.001 mole) was hydrogenated in methanol over palladium on carbon. After the end of hydrogenation, the filtering off of the catalyst, and the evaporation of the filtrate to dryness, the residue was treated with a solution of 0.65 g (0.0013 mole) of the pentachlorophenyl ester of benzyloxycarbonylthreonine [7] in a few milliliters of DMFA. After stirring until the solids had dissolved completely and keeping for 2 days, the reaction mixture was diluted with dry ether; the oil that separated out was dissolved in a small amount of methanol and the solution was treated with activated carbon, filtered, evaporated to small volume, and treated with dry ether. The resulting precipitate was filtered off and dissolved in n-butanol, and the solution was washed with water to eliminate unchanged amino component. The butanolic solution was evaporated to small volume and the residue was diluted with ether. The resulting precipitate was filtered off and dried in a vacuum desiccator to give 0.5 g (68%)

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of (III). Found, %: C 55.44; H 7.53; N 15.35. $C_{34}H_{54}N_8O_{10}$. Calculated, %: C 55.57; H 7.41; N 15.25.

Threonyllysylprolylarginine (IV). A current of dry hydrogen bromide was passed for 20 min through a solution of 0.37 g (0.005 mole) of (III) in a few milliliters of absolute trifluoroacetic acid, and then the solution was evaporated in a rotary vacuum evaporator. The residue was treated with dry ether, and the resulting precipitate was filtered off, washed with ether, dried in a vacuum deiccator, and dissolved in a small amount of water, and the solution was treated with Dowex 1×8 anion-exchange resin in the OH^- form until the reaction for bromide ion (test with silver nitrate) was negative. The resin was filtered off, the filtrate was evaporated to dryness, and the residue was treated with isopropanol. Evaporation with isopropanol was repeated several times. Then the residue was treated with ether and the resulting precipitate was filtered off and dried in a vacuum desiccator to give 0.2 g (80%) of (IV). $[\alpha]_D^{20}$ 62° (c 1, 5% acetic acid); according to the literature, $[\alpha]_D^{22}$ 60.7° (c 0.98, 5% acetic acid, [2]; $[\alpha]_D^{22}$ 60.8° (c 0.6, 5% acetic acid) [8].

EXPERIMENTAL PHARMACOLOGY

The study of psychotropic activity was performed on male mice weighing 20-22 g and male rats weighing 180-210 g. Tuftsin was administered to the mice in doses of 20-300 μg/kg, and the influence of the preparation on the spontaneous behavior of the mice [9] and also on the effects of hexenal (60 mg/kg), phenocoll (6 mg/kg), apomorphine (10 mg/kg), l-DOPA (400 mg/kg), arecoline (25 mg/kg), and reserpine (2 mg/kg) was studied. The tuftsin was administered 5 and 40 min after the administration of the test substances. All the substances were injected intraperitoneally. The measurement of spontaneous motor activity was carried out in a DAÉR-20 motor activity recorder. In the experiments on rats, the tuftsin was introduced into the head of the caudate nucleus in the brain in amounts of 10 and 20 μg in a constant volume of liquid – 0.05 and 0.005 ml. Then the changes in the behavior of the rats were observed and the existence of rotary movements was determined in a rotometer [10, 11]. The study of the influence of tuftsin on neuronal activity was carried out on identified neurons of the grape snail. For this purpose the peptide was dissolved in Ringer solution for cold-blooded animals immediately before the experiment. The volume of the bath with the preparation of the isolated central nervous system of the grape snail was 10 ml. Before the addition of the tuftsin the parameters of the activity of the nerve cells were recorded for 60 min. The intracellular take-off of the activity of the neurons was carried out by a standard method [12].

RESULTS AND DISCUSSION

In all doses and with different methods of administration, tuftsin caused excitation of the experimental animals. Stimulating action was shown in an intensification of the spontaneous activity in animals, the appearance of motor stereotypies, jumps, and an intensification of orienting-investigating behavior. The exciting function lasted for 30 ± 1.5 min and was replaced by a period of mild sedative action lasting 40 ± 1.8 min. This period was characterized by a suppression of reactions to external stimuli and by a fall in spontaneous motor activity. Table 1 gives the results of the measurement of spontaneous motor activity of mice 5 and 40 min after the administration of tuftsin.

A two-phase action of tuftsin was also shown in tests of its interaction with hexenal, phenocoll, and arecoline. Thus, when tuftsin was administered 5 min before hexenal, a weakening and shortening of the hexenal sleep was observed, and the administration of tuftsin 40 min before hexenal led to a potentiation of the soporific effect. The administration of tuftsin 5 min before the test substances intensified phenocoll stereotypy and arecoline tremor, while administration 40 min before the test substances suppressed the effect of phenocoll and did not change the action of arecoline.

When tuftsin was injected into the caudate nucleus of the brain of mice, the two-phase effect appeared more clearly than on its intraperitoneal administration. The period of excitation was particularly well defined. It began immediately after the injection of the preparation into the brain and lasted 20-30 min. In this period, the rats were very mobile, and jumps, pronounced shaking ("wet-dog movements"), and stereotypies were observed.

Unlike other oligopeptides with a stimulating action on the central nervous system (fragments of ACTH and angiotensin), tuftsin does not introduce rotational behavior on injection into the caudate nucleus. This indicates an absence of dopamine-stimulating properties in

TABLE 1. Influence of Tuftsin on the Motor Exploratory Activity of Mice

Dose of tuftsin, μg/kg	No. of movements in the first 10 min	
	5 min after the administration of tuftsin	40 min after the administration of tuftsin
50	354 (296—419)	249 (186—310)
150	398 (324—485)	214 (147—308)
300	426 (389—506)	196 (124—249)
Control	298 (211—384)	333 (203—400)

Note. The limits of variation are given in parentheses. Each group contained 20 animals.

tuftsin's activity spectrum. This conclusion was confirmed in interaction tests. It was found that tuftsin potentiates the effects of phenocoll but somewhat weakens the action of apomorphine and L-DOPA. Tuftsin leads to a decrease in the hypothermal action of reserpine.

The influence of tuftsin on identified neurons of a mollusk correlates with the facts given above. Under the action of tuftsin in a concentration of 10^{-6} M, the frequency of the spontaneous impulse activity of the nerve cells (12 take-offs) increased for 2-3 minutes, after which it decreased (to below the initial level). With a rise in the concentration to $5 \cdot 10^{-6}$ M, a decrease in the frequency of the impulse activity of the neurons was observed immediately after the addition of the substance. At tuftsin concentrations below 10^{-6} M, no inhibition phase of the neuron reaction was observed, but the activation phase was considerably weakened. The changes in frequency are based on changes in the magnitude of the rest potential observed under the action of the tuftsin. In the initial phase, activation was caused by a depolarization shift of the rest potential, after which a hyperpolarization shift was observed (10^{-6} M), accompanied by a decrease in the frequency of the impulse activity. After washing out for 20-30 min, the effect was eliminated. Thus, tuftsin exerts a two-phase action on nerve cells. The low threshold concentration of the action of tuftsin must be mentioned; it is comparable with the threshold concentration of such neuromediators as acetylcholine and serotonin.

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