EFFECT OF PEPTIDE FRACTIONS FROM HYDROBIONTS ON VESTIBULO-AUTONOMIC DISORDERS IN CATS

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UDC 615.324:574.52]036.8:616.859.1+616.859.1-085.324:547.52

KEY WORDS: peptides for hydrobionts; vestibulo-autonomic disorders

The writers showed previously that an undecapeptide, found for the first time and isolated from Hydra (the peptide has different names depending on its source: Hydra head activator, Hydra peptide morphogen, Hydra undecapeptide), exhibits marked vestibuloprotective activity in a model of motion sickness (MS) [1] in cats. This fact served as the basis for an investigation into the presence of vestibuloprotective properties in various other peptide fractions obtained from hydrobionts on the same model.

EXPERIMENTAL METHOD

Experiments (75) were carried out on 18 male cats weighing 3.1-4.2 kg. The model of MS was created by the method in [2, 4] and the duration of MS was 1 h. The intensity of vestibulo-autonomic reactions (licking, salivation, urination, defecation, vomiting, etc.) was assessed in points [4]. All substances except scopolamine were injected intraperitoneally 20-30 min before induction of MS (scopolamine was injected subcutaneously 30 min before MS). In a special series of experiments (five) the central administration of a pentapeptide 5 min before the beginning of vestibular stimulation was used. Fuller details of the technique were described previously[1].

The following peptide fractions, isolated from the tissues and blood of the harp seal Phoca groenlandica (preparations 1-11), and also the pentapeptide H-Lys-Glu-Val-Val-Gly-OH, synthesized by the method described previously [3], were used.

Preparations 1-5 were obtained from the spleen of the harp seal. The minced spleen was extracted with acetic acid solution and the extract was neutralized with an aqueous solution of ammonia. The dissolved part was separated by centrifugation and lyophilized. The resulting preparation 1 was dissolved in 0.01 M ammonium-acetate buffer, pH 7.0, and the extract was separated by centrifugation and lyophilized, to yield preparation 2. Preparation 1 was dissolved in 0.1 M ammonium-acetate buffer, pH 5.5, the residue was separated by centrifugation, and the supernatant was lyophilized, yielding preparation 3. The remaining residue was treated with 0.1 M ammonium-acetate buffer, pH 7.2, mixed for 10 min, and the insoluble part was separated by centrifugation. The residue was treated with 0.1 M ammonium-acetate buffer, pH 8.5 and extracted for 10 min during mixing. The solution was separated by centrifugation and lyophilized, yielding preparation 4. The minced spleen was extracted with a 1-M aqueous solution of acetic acid, after which the reaction mixture was neutralized with an aqueous solution of ammonia and the extract was separated from the insoluble part of the biological material and applied to a column, packed with carboxymethyl-cellulose; the first fractions,which were stained red or reddish brown, were discarded, but the yellow fraction was collected and lyophilized, yielding preparation 5.

Preparations 6-9 were obtained from the seal heart. Minced seal heart was extracted with acetic acid at 4°C and neutralized with an aqueous solution of ammonia. The soluble part was separated by centrifugation and passed through the ion-exchanger carboxymethyl-cellulose, equilibrated with 0.01 M ammonium-phosphate buffer, pH 7.0, and the column was washed with the same buffer solution; substances adsorbed on the column were eluted with 9.5 M ammonium-phosphate buffer, pH 7.5. The solution was dialyzed against distilled water and applied to a column (230 × 26 mm), packed with carboxymethylcellulose, equilibrated with

Institute for Medico-biological Problems, Ministry of Health of the USSR. Research Laboratory of Biologically Active Substances for Hydrobionts, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 44-46, January, 1990. Original article submitted June 14, 1989.

 0007-4888/90/0001-0056$12.50

TABLE 1. Effect of Peptide Fractions from Hydrobionts on Vestibulo-autonomic Disorders in Cats (M ± m)

Preparation (dose, mg/kg)	Intensity of symptoms of MS, points
Control (isotonic NaCl solution)	12,1±0,5
Scopolamine (0,01—0,15)	5,4±1,3*
Preparations: 1 (1,0—1,5)	10,0±0,4
2 (0,3—1,0)	10,0±1,7
3 (1,0—2,0)	8,5±0,3
4 (0,3—1,0)	3,7±2,1*
5 (1,0—1,5)	9,0±0,7
6 (1,0—2,0)	2,0±1,4*
7 (0,3—1,0)	12,0±1,6
8 (0,3—1,0)	2,7±0,7*
9 (0,3—1,0)	6,0±2,3
10 (0,5—1,0)	10,1±2,5
11 (0,3—0,5)	5,0±0,7*
Pentapeptide (0.3-0.5)	2,5±0,3**
Pentapeptide (10-50 µg into fourth ventricle)	2,0±0,5**

Legend: *$p \leq 0.01$ compared with control. **$p < 0.05$ compared with scopolamine (Student's test).

0.05 M ammonium-phosphate buffer, pH 7.0. The substances were eluted from the column with 0.25 M ammonium-phosphate buffer, pH 7.0 (rate of elution 1.5 ml/min). Fractions eluted at 60-70, 210-220, 240-260, and 275-295 ml were collected. The collected fractions were desalted by dialysis and lyophilized, yielding preparations 6-9 respectively.

Preparations 10 and 11 were obtained by isoelectric focusing of whole seal blood plasma. The fraction obtained over the range of pH 4.0-7.0 was separated, dialyzed, and chromatographed on a column with Pearl HW-40, and fractions with mol. wt. of ≤ 10 and ≈ 8.5 kilodaltons were collected. The collected fractions were dialyzed and lyophilized, yielding preparations 11 and 12, respectively.

EXPERIMENTAL RESULTS

Data on the effect of the peptide fractions from hydrobionts on vestibulo-autonomic disturbances in cats compared with the action of the test drug scopolamine, are given in Table 1. They show that preparations 4, 6, 8, and 11, and also the pentapeptide, when injected systematically, had a statistically significant vestibuloprotective action, comparable with that of scopolamine. The other preparations (1-3, 5, 7, 9, and 10) had no action against MS. The fact will be noted that the pentapeptide, in doses of 0.3-0.5 mg/kg, was the most effective of all the preparations studied against MS, for it was even more active than scopolamine ($p < 0.05$).

Taking the above results into consideration, a special series of experiments was undertaken to assess the vestibuloprotective properties of the pentapeptide when administered centrally. When injected into the fourth ventricle in doses of 10-50 µg it was found to exhibit strong activity against MS, exceeding scopolamine in this respect. It must also be noted that the preparation was about equally effective when given systematically and centrally (Table 1). Consequently, the protective action of the pentapeptide against MS may be considered to be due chiefly in its effect on the brain.

The results of this investigation thus show that a number of peptide fractions from hydrobionts possess vestibuloprotective properties on a model of MS in cats. In our view the pentapeptide is the most promising of these substances for further intensive study with a view to the development of new agents for the prevention and treatment of MS.

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