Vestibuloprotective Activity of Seal Serum Proteins

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Cat and rat experiments show that the protein fraction isolated from blood serum of the Greenland seal has a protective activity against motion sickness. This activity is comparable to that of the classical vestibuloprotector scopolamine and is greater than that of diprazine. Radioligand assay of the receptor binding showed that the serum protein fraction has the highest affinity for α_2-adrenoreceptors, μ-opioid, and benzodiazepine receptors.

Key Words: *serum protein fraction; motion sickness; opioid and benzodiazepam receptors*

Previously we reported that a peptide-protein substance isolated from the internal organs of the Greenland seal exhibits a definite vestibuloprotective activity in the cat [2]. It is known that the endogenous opioid system plays an important role in the pathogenesis of motion sickness (MS) in both humans and animals [4,5]. Rat experiments have shown that systemic or central administration of the protein fraction isolated from blood serum of the Greenland seal (BSGS) modulates the effects of opiates (in particular, the analgetic effect) and opioid peptides [1,3]. It can therefore be assumed that BSGS modulates the development of MS in animals.

In this work we studied the vestibuloprotective activity of proteins isolated from the serum of Greenland seal and the presumed mechanism of action of BSGS (a radioligand binding assay).

MATERIALS AND METHODS

The serum protein fraction was obtained as described previously [1]. Motion sickness was simulated in cats weighing 2.9-4.2 kg and Wistar rats weighing 180-250 g by rotating them in a modified NASA apparatus (USA) [6] in two perpendicular planes at a frequency of 0.33 Hz. In cats, the degree of MS was evaluated visually and expressed according to a published scale [7]. In rats, MS was evaluated by the amount of food eaten during 24 h after rotation (a modified method [8]). BSGS, the control vestibuloprotector diprazine, and normal saline were injected intraperitoneally 30-45 min prior to rotation. Another vestibuloprotector, scopolamine, was injected subcutaneously. The effect of BSGS on rat brain opioid and benzodiazepine receptors was studied in a separate series of experiments. Immediately after decapitation the brain was frozen in liquid nitrogen and stored at -70°C until use. The brain was homogenized in a Teflon/glass homogenizer (10 volumes of 0.32 M sucrose) followed by destruction in a polytron (position 9.3×10 sec). The homogenate was centrifuged for 10 min at 2500g. The supernatant was centrifuged for 30 min at 20,000 g, and the pellet was resuspended in 10 volumes of 50 mM Tris-HCl buffer (pH 7.7) and centrifuged for 30 min at 20,000 min. Centrifugation was performed another 7 times. The incubation mixture consisted of 200 μl suspension of crude fraction of synaptic membranes, 30 μl of an

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TABLE 1. Vestibuloprotective Activity of Several Drugs Evaluated by the Amount of Food Eaten by Rats during a 24-h Period ($M \pm m$)

Preparation, mg/kg	Number of animals	Food consumption after rotation, % of background
Control (isotonic saline)	8	63±6
Scopolamine, 0.01	6	71±13
Scopolamine, 0.1	6	86±7
Diprazine, 10.0	5	71±7*
Diprazine, 50.0	5	81±5*
BSGS, 0.1	6	70±6
BSGS, 1.0	6	126±23*
BSGS subfraction, 0.1	6	92±7***
BSGS subfraction, 0.4	6	93±8**

Note. The amount of food consumed before the experiment was taken as 100% (baseline). One asterisk indicates $p<0.05$, two asterisks $p<0.02$, and three asterisks $p<0.01$ (difference from the control is statistically significant, Student's t test.

TABLE 2. Effect of Serum Protein Fraction on the Binding of Labeled Ligands to Specific Receptors of Rat Brain Synaptic Membranes

Receptor	Inhibition constant, μg/ml
α_2-adrenoreceptors	3.2
μ-opioid	59.3
S-2-serotonin	No inhibition
Benzodiazepine	5.8

isotope solution of the corresponding concentration, and 30 μl of the test compounds in the corresponding concentration. The following ligands were used: ^{3}H-flunitrazepam (85 Ci/mM) at a concentration of 2 nM, ^{3}H-naloxone (61 Ci/mM), ^{3}H-spiperone (88 Ci/mM), and ^{3}H-clonidine (23.2 Ci/mM) at a concentration of 1 nM. When nonspecific binding was assessed, the substituting agents were: diazepam, morphine, pirenperone, and clonidine at a concentration of 10^{-6} M. The peptide-protein compounds were studied in the concentration range 0.1-1000 μg/ml. All solutions were prepared using 50 mM Tris-HCl buffer. Incubation was carried out at 25°C for 30 min. The reaction was stopped by the addition of 10 mM Tris-HCL buffer followed by filtration through a GF/F glass filter and 7 washings with the same buffer in an Automash-2000 harvester (Dynatech). Radiation was measured in an Intertechnique SL-3000 scintillation counter. Protein concentration in the samples was determined by the method of Peterson. The inhibition constant and IC_{50} were determined using designated software (IC_{50}).

RESULTS

At a dose of 0.3 mg/kg BSGS mitigated (59%, $p<0.05$) the manifestations of vestibulosympathetic disorders in cats to almost the same degree as did scopolamine in a dose of 0.1 mg/kg (55%, $p<0.05$). Consequently, in the cat BSGS exhibits a vestibuloprotective activity comparable to that of the vestibuloprotector scopolamine.

Similar results were obtained in rats (Table 1). It can be seen from the table that scopolamine and diprazine were effective only at high doses (0.1 and 50 mg/kg, respectively). At a dose of 0.1 mg/kg BSGS had no vestibuloprotective activity; however, such activity became pronounced at 10-fold higher doses of BSGS. A marked vestibuloprotective effect was observed at low doses (0.1 and 0.4 mg/kg) of BSGS subfractions. Thus, the vestibuloprotective activity of BSGS is greater than that of scopolamine.

Radioligand assay showed that BSGS has a high affinity for α_2-adrenoreceptors, μ-opioid, and benzodiazepine receptors, a low affinity for other brain receptors, and no affinity for 5-OT_2 receptors (Table 2).

It was previously found that in the dose range 1-5 mg/kg (intraperitoneally) [1,3] BSGS has no effect on alertness and emotional reactivity of rats in the open field test. Meanwhile, when administered centrally or systemically, BSGS almost completely blocks (mice and rats) or markedly attenuates (rabbits) morphine analgesia.

It may be assumed that an opioid component is involved in the mechanism responsible for the vestibuloprotective effect of BSGS. The participation of brain catecholamines and benzodiazepine brain receptors cannot be excluded eihter.

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