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Differential BBB interactions of three ingestive peptides: Obestatin, ghrelin, and adiponectin

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ABSTRACT

Endogenous compounds, including ingestive peptides, can interact with the blood–brain barrier (BBB) in different ways. Here we used in vivo and in vitro techniques to examine the BBB permeation of the newly described satiety peptide obestatin. The fate of obestatin in blood and at the BBB was contrasted with that of adiponectin. By the sensitive multiple time-regression method, obestatin appeared to have an extremely fast influx rate to the brain whereas adiponectin did not cross the BBB. HPLC analysis, however, showed the obestatin result to be spurious, reflecting rapid degradation. Absence of BBB permeation by obestatin and adiponectin was in contrast to the saturable transport of human ghrelin reported previously. As a positive control, ghrelin showed saturable binding and endocytosis in RBE4 cerebral microvessel endothelial cells. By comparison, obestatin lacked specific binding and endocytosis, and the small amount internalized showed rapid intracellular degradation before the radioactivity was released by exocytosis. The differential interactions of obestatin, adiponectin, and ghrelin with the BBB illustrate their distinctive physiological interactions with the CNS.

1. Introduction

Among ingestive peptides that decrease feeding, some readily cross the blood–brain barrier (BBB) by a saturable transport system. These include insulin [1], leptin [2], activated urocortin [11], mahogany (1377–1428) [6], and galanin-like peptide (GALP) [8]. This is in contrast to most ingestive peptides that increase feeding (with the exception of ghrelin discussed below), which do not cross the BBB by a saturable transport system. These include neuropeptide Y (NPY) [4], orexin [5], agouti-related protein (AgRP) (83–132) [7], melanin-concentrating hormone (MCH) [9], and glucagon-like peptide (GLP)-1 [10]. If a structural function relationship in the permeation of these peptides across the BBB could be identified, this would facilitate the design of synthetic analogs for therapeutic intervention of feeding behavior.

The 23 amino acid peptide obestatin is the newest member of the endogenous ingestive peptide family [21]. The primary structure of obestatin is a homologous sequence conserved among species in preproghrelin, downstream to the appetite-stimulating peptide ghrelin [21]. Purified from the rat stomach, obestatin binds to the orphan G-protein coupled receptor GPR39, which is present in the brain [14]. Obestatin decreases food ingestion after peripheral administration, as opposed to ghrelin, which increases feeding. As the original paper focused more on biochemical assays rather than behavioral studies, it is not certain whether obestatin acts mainly in the hypothalamus, reducing the drive for ingestion, or in the area postrema and other regions, affecting taste aversion. The hypothalamus is separated from peripheral circulation by the BBB whereas the area postrema is not [19].

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In this study, we determined the pharmacokinetic features of obestatin and adiponectin, another peptide that suppresses feeding, in adult mice. Ghrelin, which has previously been shown to penetrate the BBB in vivo [3], was used for additional experiments. Adiponectin and ghrelin have opposite effects on feeding behavior [15,20]. We also tested for the presence of a possible specific transport system for obestatin in cerebral microvessel endothelial cells in culture. The findings should determine whether obestatin and adiponectin, the newly appreciated ingestive peptides from the periphery, exert significant CNS actions by permeating the BBB.

2. Materials and methods

C57 mice, 5–6 weeks old, were bred in our facility and used according to the protocol approved by the Institutional Animal Care and Use Committee. The mice were studied after anesthesia induced by intraperitoneal injection of ketamine and xylazine. Mouse/rat obestatin and human ghrelin were obtained from Phoenix Pharmaceuticals (Belmont, CA), and mouse adiponectin from Apotech (San Diego, CA). All other reagents were from Sigma (St. Louis, MO). Obestatin was radioactively labeled (radiolabeled) with ^{125}I by the chloramine-T method, the reaction being stopped at 1 min by addition of sodium metabisulfite. The specific activity of ^{125}I-obestatin was about 90–130 Ci/g for different experiments. Ghrelin and bovine serum albumin were radiolabeled with ^{131}I by the chloramine-T method with specific activities of about 20 Ci/g. Mouse adiponectin was radiolabeled with ^{125}I by the chloramine-T method with a specific activity of about 120 Ci/g. These peptides were purified by elution on Sephadex G-10 columns, and the purity was verified by both acid precipitation and reversed phase high performance liquid chromatography (HPLC).

2.1. Multiple time-regression analyses

To determine whether obestatin enters the mouse brain and whether it does so by saturable transport, the following four groups of mice were studied (8 mice/group): (1) ^{125}I-obestatin only; (2) ^{125}I-obestatin with the inclusion of 1 μg/mouse of unlabeled obestatin; (3) ^{125}I-obestatin with the inclusion of 1 μg/mouse of unlabeled human ghrelin; and (4) ^{125}I-obestatin with the inclusion of 1 μg/mouse of unlabeled leptin. About 30,000 cpm/μl of ^{125}I-obestatin was delivered to the isolated left jugular vein in 100 μl of lactated Ringer's with 1% BSA (LR/BSA) at time 0. At various times between 1 and 20 min, blood was collected by dissection of the right common carotid artery. The mouse was decapitated immediately afterward. The radioactivity was measured in the whole brain and 50 μl of serum, and the brain/serum ratio of ^{125}I-obestatin and ^{131}I-albumin in each gram of brain was calculated separately. Based on the exponential decay pattern of serum radioactivity, the exposure time was calculated for each time point. The exposure time is the integral of serum radioactivity over time divided by the serum radioactivity at a given time [12]. The linear regression correlation between the brain/serum ratio and exposure time was determined with Prism GraphPad Statistical Software (San Diego, CA). The unidirectional influx transfer rate (K_i) was determined from the slope of the linear regression line, and the initial volume of distribution (V_i) was determined from the intercept. Differences in regression lines between the groups were compared by the least square method with the GraphPad program.

2.2. Capillary depletion

Two groups of mice were studied (n = 4/group): those receiving ^{125}I-obestatin and ^{131}I-albumin i.v., with blood collection from the abdominal aorta and decapitation 10 min later, and those receiving ^{125}I-obestatin and ^{131}I-albumin i.v., with blood collection after intracardial perfusion of 30 ml of PBS and decapitation at 10 min. The cerebral cortex of the mouse was dissected, weighed, and homogenized in capillary buffer followed by thorough mixing with 26% dextran as described previously [16]. The homogenate was centrifuged at 9000 × g for 30 min at 4 °C to separate the capillary and brain parenchyma. Radioactivity was measured in the total cortex, parenchyma, and capillaries, and that in the cerebral vasculature was calculated by comparison of the values of perfused and non-perfused brains.

2.3. Degradation assays of mouse samples by HPLC

Each mouse received an i.v. injection of 3–4 μCi of ^{125}I-obestatin in 100 μl of injection at time 0. At 10 or 20 min, arterial blood and brain were obtained and processed on ice. The brain was homogenized in 1 ml of LR/BSA containing Complete Protease Inhibitor cocktail (Sigma). To correct for ex vivo degradation, a processing control was generated by adding ^{125}I-obestatin into the blood-collection tube and brain homogenate. About 30,000 cpm of brain supernatant or serum was used for reversed phase HPLC. The positive control consisted of ^{125}I-obestatin stock solution immediately after radiolabeling. The mobile phase of HPLC was acetonitrile with 0.1% trifluoroacetic acid increasing from 10 to 70% over 40 min. One milliliter fractions were collected.

2.4. Binding and endocytosis assays in RBE4 cerebral microvessel endothelial cells

RBE4 cells (kind gift from Dr. Pierre-Olivier Couraud, Institute of Cochin, Paris, France) were grown to confluency in collagen-coated 12-well plates as described previously. Triplicates of wells were used for each time point in each group. For binding assays, the cells were kept on ice during the entire course of the study (3 h). For endocytosis assays, the cells were pre-equilibrated in transport buffer (αMEM + F10 with 20 mM of HEPES and 1% of bovine serum albumin, pH 7.4) 30 min before the radiotracers were added. At time 0, ^{125}I-obestatin and ^{131}I-ghrelin (about 200,000 cpm/ml each) were added in 0.38 ml of transport buffer. The plates were incubated in a shaking water bath at 37 °C for the desired time points (0, 5, 10, 20, 30, and 60 min). For both types of assays, the radiotracer was quickly removed at the end of incubation, and the cells were washed with cold PBS to remove the unbound ligands. Cell surface binding was determined by the use of stop/strip buffer, and the amount of ligands internalized was determined after cell lysis and collection of the lysis buffer, as previously described