

**The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of November 12, 2009 ):**

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:  
<http://www.sciencemag.org/cgi/content/full/315/5813/766c>

**Supporting Online Material** can be found at:  
<http://www.sciencemag.org/cgi/content/full/315/5813/766c/DC1>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:  
<http://www.sciencemag.org/cgi/content/full/315/5813/766c#related-content>

This article **cites 2 articles**, 1 of which can be accessed for free:  
<http://www.sciencemag.org/cgi/content/full/315/5813/766c#otherarticles>

This article has been **cited by** 1 articles hosted by HighWire Press; see:  
<http://www.sciencemag.org/cgi/content/full/315/5813/766c#otherarticles>

This article appears in the following **subject collections**:  
Medicine, Diseases  
<http://www.sciencemag.org/cgi/collection/medicine>  
Technical Comments  
[http://www.sciencemag.org/cgi/collection/tech\\_comment](http://www.sciencemag.org/cgi/collection/tech_comment)

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:  
<http://www.sciencemag.org/about/permissions.dtl>

# Comment on “Obestatin, a Peptide Encoded by the Ghrelin Gene, Opposes Ghrelin's Effects on Food Intake”

N. Chartrel,<sup>1\*</sup> R. Alvear-Perez,<sup>2\*</sup> J. Leprince,<sup>1</sup> X. Iturriz,<sup>2</sup> A. Reaux-Le Goazigo,<sup>2</sup> V. Audinot,<sup>3</sup> P. Chomarat,<sup>3</sup> F. Coge,<sup>3</sup> O. Nosjean,<sup>3</sup> M. Rodriguez,<sup>3</sup> J. P. Galizzi,<sup>3</sup> J. A. Boutin,<sup>3†</sup> H. Vaudry,<sup>1†</sup> C. Llorens-Cortes<sup>2†</sup>

Zhang *et al.* (Research Articles, 11 November 2005, p. 996) reported that obestatin, a peptide derived from the ghrelin precursor, activated the orphan G protein–coupled receptor GPR39. However, we found that I<sup>125</sup>-obestatin does not bind GPR39 and observed no effects of obestatin on GPR39-transfected cells in various functional assays (cyclic adenosine monophosphate production, calcium mobilization, and GPR39 internalization). Our results indicate that obestatin is not the cognate ligand for GPR39.

Zhang *et al.* (1) reported the identification, using a bioinformatic approach, of a novel neuropeptide designated obestatin, which is derived from the ghrelin precursor (1). They subsequently purified obestatin from a rat stomach extract and reported that obestatin is a cognate ligand for the orphan G protein–coupled receptor (GPCR) GPR39. Here, we provide independent evidence that obestatin does not interact with GPR39.

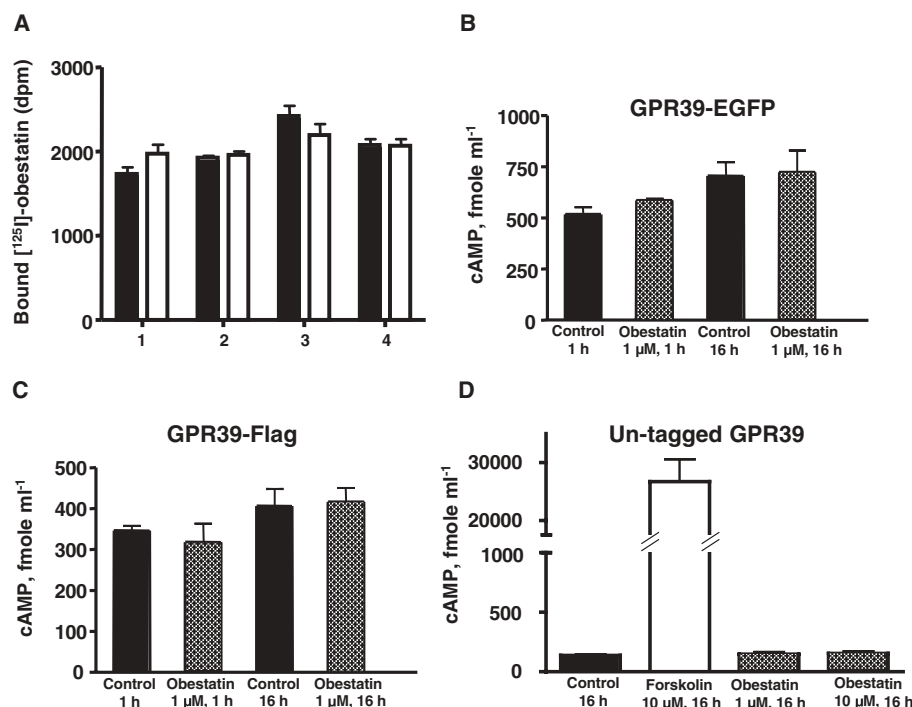
To investigate whether obestatin is the natural ligand of GPR39, we transfected Chinese hamster ovary (CHO) cells with human GPR39 cDNA [accession no. AAC26082, corresponding to the GPR39 sequence reported in (1) (fig. S1)]. We established two stably transfected CHO cell lines expressing either the Flag-tagged or the enhanced green fluorescent protein (EGFP)-tagged human GPR39. The integration of human GPR39 cDNA in the genome of the two cell lines and the presence of the GPR39 mRNA were confirmed (fig. S2 A and B), and the correct expression of the receptor at the cell surface was visualized by confocal microscopy (fig. S3 A and D). We subsequently synthesized human obestatin and thoroughly characterized the synthetic peptide by mass spectrometry and microsequencing. Its effects were then tested in parallel with those of a commercial source of synthetic human obestatin (Phoenix Pharmaceuticals, Inc., Belmont, California).

Zhang *et al.* (1) showed that I<sup>125</sup>-obestatin binds with high affinity to crude plasma-membrane

preparations from rat jejunum, stomach, ileum, hypothalamus, pituitary, and CHO cells transfected with human GPR39. We tested the ability of commercially available I<sup>125</sup>-obestatin (Phoenix Pharmaceuticals, Inc.) to interact with GPR39 in our two GPR39-CHO cell lines. In our hands,

no specific binding was observed with I<sup>125</sup>-obestatin at a concentration of  $5 \times 10^{-10}$  M, which is half the dissociation constant reported in (1) (Fig. 1). These experiments were conducted under similar incubation conditions as those used by Zhang *et al.* (1). We also found no evidence of specific binding in crude pituitary membranes (Fig. 1A).

Zhang *et al.* (1) reported the activation of cAMP production in CHO and human embryonic kidney (HEK) 293 cells transiently transfected with human GPR39 after a 16-hour incubation with human obestatin, with maximal stimulation at obestatin concentrations between  $3 \times 10^{-8}$  and  $10^{-7}$  M. Either using the same experimental conditions or using a similar protocol with an incubation time for obestatin reduced to 1 hour, we found that obestatin ( $10^{-5}$ ,  $10^{-6}$  M) did not induce a significant increase in cAMP formation in CHO cells stably expressing the Flag- or EGFP-tagged GPR39 or in CHO cells transiently transfected with the untagged GPR39 (Fig. 1, B to D). However, treatment of the cells with  $10^{-5}$  M forskolin provoked a robust stimulation of cAMP production (Fig. 1D). In addition, incubation of CHO cells stably expressing the Flag- or EGFP-tagged GPR39 with human obestatin for 20 to 60



**Fig. 1.** Binding of obestatin on GPR39-transfected CHO cell membranes and effects of obestatin on cAMP production in CHO cells stably or transiently expressing GPR39. (A) Membranes from transfected CHO cells or from pituitary cells were tested for their ability to bind I<sup>125</sup>-obestatin using the protocol described by Zhang *et al.* (1): (1) naive CHO cells, (2) flag-tagged GPR39-expressing CHO cells, (3) EGFP-tagged GPR39-expressing CHO cells, and (4) pituitary cell membranes. Experiments were performed in triplicate and reproduced independently twice. Black bars, total binding; open bars,  $10^{-6}$  M obestatin. (B to D) CHO cells stably expressing either the EGFP-tagged (B) or the Flag-tagged (C) GPR39, or CHO cells transiently transfected with the human untagged GPR39 (D) were incubated for 1 or 16 hours in the absence or presence of  $10^{-6}$  or  $10^{-5}$  M obestatin before cAMP production assessment.

<sup>1</sup>Institut National de la Santé et de la Recherche Médicale (INSERM), U413, Laboratory of Cellular and Molecular Neuroendocrinology, and European Institute for Peptide Research (IFRMP 23), University of Rouen, 76821 Mont-Saint-Aignan, France. <sup>2</sup>INSERM, U691, and Collège de France, 75005 Paris, France. <sup>3</sup>Institut de Recherches Servier (IdRS), Centre de Recherches de Croissy, 125 Chemin de la Ronde, 78290 Croissy-sur-Seine, France.

\*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: c.llorens-cortes@college-de-france.fr (C.L.C.); hubert.vaudry@univ-rouen.fr (H.V.); jean.boutin@fr.netgrs.com (J.A.B.)

min, at doses ranging from  $10^{-9}$  to  $10^{-6}$  M, did not modify intracellular calcium concentration (fig. S3, C and F) and did not promote GPR39 internalization (fig. S3, B and E).

In conclusion, although Zhang *et al.* (1) provided convincing evidence for processing of the ghrelin precursor to generate obestatin in vivo, the lack of activity of the synthetic peptide in various

GPR39 functional assays suggests that obestatin is not the endogenous ligand of the orphan receptor GPR39. Similar observations have now been reported by other laboratories (2, 3).

#### References and Notes

1. J. V. Zhang *et al.*, *Science* **310**, 996 (2005).
2. B. Holst *et al.*, *Endocrinology* **148**, 13 (2006).

3. E. Lauwers *et al.*, *Biochem. Biophys. Res. Commun.* **351**, 21 (2006).

#### Supporting Online Material

[www.sciencemag.org/cgi/content/full/315/5813/766c/DC1](http://www.sciencemag.org/cgi/content/full/315/5813/766c/DC1)

Materials and Methods

Figs. S1 to S3

12 September 2006; accepted 14 November 2006

10.1126/science.1135047