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ABSTRACT *Purpose:* To evaluate obestatin and ghrelin effects on iris muscle contraction. *Materials and Methods:* Obestatin (10^{-5} M) or ghrelin (10^{-5} M) were tested on two consecutive carbachol- or epinephrine-elicited contractions of iris rabbit sphincter or dilator muscles. Ghrelin and obestatin effects on iris muscles basal tension were also tested, and their effects on iris sphincter EFS-elicited contraction were evaluated. *Results:* Compared with the first, tension of the second carbachol-induced contraction of the iris sphincter decreased 11.5 ± 5.5% in the vehicle group, increased 19.0 ± 10.2% in presence of obestatin, and remained unchanged by ghrelin. Epinephrine-induced contractions were not affected by obestatin or ghrelin. EFS-elicited contractions were decreased 9.3 ± 3.2% by ghrelin. Basal tension of the iris sphincter decreased 21.7 ± 3.7% in presence of ghrelin (10^{-5} M), while that of the dilator decreased 14.1 ± 5.0% in presence of obestatin (10^{-5} M). *Conclusion:* This study suggests that obestatin potentiates the cholinergic contraction of the iris sphincter and relaxes the iris dilator muscles.

KEYWORDS ghrelin; iris dilator muscle; iris sphincter muscle; obestatin; peptide hormones

INTRODUCTION

Obestatinis a 23 amino acid amidated peptide derived from the preproghrelin, discovered through a bioinformatics search. This recently discovered product of the ghrelin gene was isolated in rat stomach.[1] Obestatin activates a rodopsin type G coupled receptor (GPR-39), a member of the ghrelin receptor superfamily.[1,2] Zhang et al. identified, by RT-PCR and [^{125}I]obestatin, binding sites in multiple human and rat organs, including stomach, intestine, pituitary and hypothalamus.[1] Later Jackson et al. detected the highest levels of GPR39 mRNA in the amygdala, hippocampus, and auditory cortex. Low levels were identified in several other brain regions; however, no expression was found in the hypothalamus.[3] In the gastrointestinal system, obestatin was co-localized with acetylcholine in the myenteric plexus of the gastrointestinal system.[4] Recent reports suggested that obestatin may not be the major ligand of GPR-39.[5]

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Ghrelin is a 28 aa peptide identified in the oxyntic mucosa of the stomach. It is also found in the intestine, pituitary, and hypothalamus.[6] Ghrelin's mRNA was localized in the iris posterior epithelium and nonpigmented ciliary epithelial cells of the rat's eye.[7] Ghrelin has several metabolic and muscular functions. The muscular effects of ghrelin were observed in the cardiac, skeletal, and smooth muscle.[8] In the heart, ghrelin has been described as a negative inotrope and lusitrope.[9,10] It promotes vasodilation through an endothelium independent mechanism suggesting an action at the smooth muscle level.[11,12] On rat's gastric smooth muscle, ghrelin increases the EFS-evoked small cholinergic contraction and after-contraction, which are mediated by cholinergic and non-cholinergic excitatory activities.[13,14] Finally, on the iris smooth muscle, ghrelin has recently been implicated in the relaxation of the iris sphincter and dilator muscles. This relaxation is GHSR-1a dependent on the dilator muscle, but GHSR-1a independent on the sphincter.[7]

Obestatin and ghrelin appear to have opposite effects in metabolic and gastrointestinal (GI) functions. While ghrelin stimulates feeding in several animal species, intracerebroventricular and systemic injections of obestatin suppress body weight gain in rats.[1] Ghrelin blood levels increase in fasting conditions, decreasing after free access to food and drink, while those of obestatin are constant during fasting and feeding.[1,15] Ghrelin has a prokinetic effect and contracts the jejunal muscle strips.[13,14] However, chronically administered obestatin delays gastric emptying in a sustained fashion, while *in vitro* it acutely decreases the contractile activity of those muscle strips.[1] In GRP-39 knocked-out animals, gastric emptying is accelerated and expulsion of distal located pallets is more effective.[16] Some of the gastrointestinal and metabolic effects of obestatin were not reproduced in subsequent reports.[17] In ocular tissues, obestatin promotes the proliferation of hRPe cells (human retinal pigment epithelium) in a pathway related to ERK $^1/_2$ activation.[18]

Iris sphincter muscle contraction is mediated by acetylcholine release and influenced by cAMP and cGMP levels.[19–21] As these levels increase, cholinergic contraction decreases. This can be modulated at the pre-junctional or post-junctional sites, as well as at the intracellular signal transduction level. Pituitary adenylate cyclase-activating peptide, galanin, and somatostatin act as mediators of pre-junctional sites. The former enhances contraction[22] while the latter two decrease it.[23] At the pre-junctional site, adenosine decreases the cholinergic contraction through the activation of the A_1 pre-synaptic receptors.[24] Intracellular second messenger levels are influenced by peptides that promote cAMP production such as vasointestinal peptide (VIP),[25] substance P,[26] adrenomodulin,[27] and peptides that increase cGMP release (natriuretic peptide A or C[21] and NO[28]).

Dilator muscle contraction is elicited by the adrenergic system. The modulation of its contraction is less well characterized. Neuropeptide Y potentiates it,[29] while the pituitary adenylate cyclase-activating peptide relaxes this muscle.[22]

To further investigate this issue, we performed the present study to evaluate the role of both ghrelin and obestatin in the regulation of iris muscle contraction.

METHODS

All animal procedures were performed in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Functional Studies

Specimens Preparation

The study was performed in isolated iris sphincter ($n = 60$) and iris dilator ($n = 48$) muscles from male New Zealand white rabbits (*Oryctolagus cuniculus*; 2.0–3.0 kg). Animals were euthanized after an injection of pentobarbital sodium salt (50 mg/kg) into the marginal ear vein. The eyes were immediately enucleated and placed in modified Krebs-Ringer (KR) solution at 35°C with the following composition in mM: NaCl 98, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 2.4, KH_2PO_4 1.2, glucose 4.5, $CaCl_2 \cdot 2H_2O$ 2.5, $NaHCO_3$ 17, $C_3H_3NaO_3$ 15, and CH_3COONa 5. After removal of the cornea, the iris sphincter or dilator muscles were quickly excised and immersed in the KR solution. After dissection, the ends of each piece were tied with silk thread for mounting in a 5-ml horizontal organ bath containing the above-described solutions. One end of the specimen was connected to an electromagnetic length-tension transducer (University of Antwerp, Belgium), and the other was secured to a clip at the wall of the organ bath. We tied a 5-mm strip of the iris sphincter muscle on a radial strip of the dilator with 3 mm of basal width. All the surgical procedures were taken under microscope (Zeiss, Stemi 2000C, Germany). Solutions were bubbled with 95%

O_2 and 5% CO_2, and pH was maintained between 7.38 and 7.42.

Iris muscles were allowed to extend at a constant preload (0.5 mN). When the length remained constant for more than 15 min, they were switched to isometric conditions and the protocols initiated when muscle tension stabilized.

Experimental Protocols

Effects of Obestatin or Ghrelin on Carbachol/Epinephrine-Induced Muscle Contraction

After stabilization, the rabbit iris sphincter or dilator muscles were contracted by adding, respectively, carbachol (10^{-6} M) or epinephrine (10^{-4} M) to the organ bath. The tension developed by those agents was measured. This was recorded as the first contraction. The bathing solution was then washed out, and when a steady line was reached again, 1–5 human ghrelin (frGhr; 10^{-5} M; sphincter $n = 7$; dilator $n = 8$); obestatin (Obs; 10^{-5}M; sphincter $n = 10$; dilator $n = 8$) or the vehicle (sphincter $n = 8$; dilator $n = 8$) were added to the organ bath 5 min prior to the second carbachol- or epinephrine-elicited contraction. The differences between the first and the second contractions were then analyzed.

Effects of Obestatin and Ghrelin on Electric Field Stimulation-Elicited Contraction

After stabilization, the rabbit iris sphincter muscles were contracted by placing them in electric field stimulation (EFS) of 10 V, 100 Hz during 1 ms. Developed tension was recorded in 5 consecutive 3-min-apart contractions. After completing the acquisition in baseline conditions (1st contraction), 1–5 human ghrelin (frGhr; 10^{-5} M; $n = 10$), obestatin (Obs; 10^{-5}M; $n = 10$) or the vehicle ($n = 10$) were added to the organ bath and a new electric field stimulation elicited 15 min later (2nd contraction).

Effects of Obestatin or Ghrelin on Muscle Tension

After stabilization, muscle tension of the rabbit iris sphincter or dilator muscles was recorded in the absence and presence of increasing concentrations of 1–5 human ghrelin (frGhr; 10^{-9}–10^{-5} M; iris sphincter $n = 7$; iris dilator $n = 8$), obestatin (Obs; 10^{-9}–10^{-5}M; iris sphincter $n = 10$; iris dilator $n = 8$) or the vehicle (iris sphincter $n = 8$; iris dilator $n = 8$) to generate concentration-response curves. Effects of ghrelin, obestatin, or the vehicle on muscle tension are expressed as percent change from its initial value.

MATERIALS

Human 1–5 ghrelin (frGhr; Gly-Ser-Ser(*n*-octanoyl)-Phe-Leu-NH_2 and obestatin (FNAPFDVGIKLSGAQ-YQQHGRAL-NH_2) were obtained from Peptides International (Louisville, KY, USA). The other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Peptides were prepared in aliquots and stored at −20°C.

Statistical Analysis

Data presented as means ± SEM. The effects of ghrelin, obestatin, and vehicle on carbachol-, epinephrine-, or EFS-elicited contractions were evaluated with a paired Student's *t*-test. Comparison of muscle tension in the three experimental groups (ghrelin, obestatin, and vehicle) was performed with one-way ANOVA. Concentration-response curves in each experimental condition were evaluated with one-way repeated measures ANOVA. Effects of each concentration of ghrelin or obestatin in different experimental conditions were tested with one-way ANOVA. When significant differences were detected with any of the ANOVA tests, the Student-Newman-Keuls test was selected to perform multiple comparisons; $p < 0.05$ was accepted as significant.

RESULTS

Effects of Obestatin or Ghrelin on Carbachol-Induced Iris Sphincter Muscle Contraction

The performance of the iris sphincter muscle preparations was quite homogeneous, without significant differences between the three groups (obestatin, ghrelin, and vehicle) in active tension of the first carbachol-induced contraction, which averaged 0.99 ± 0.05 mN.

In the presence of the vehicle alone, active tension of the second carbachol-induced contraction of the iris sphincter muscle was 11.5 ± 5.5% smaller than that of the first contraction, although this difference failed to reach statistical significance ($p = 0.06$; Figs. 1 and 2). On the contrary, in the presence of obestatin, active

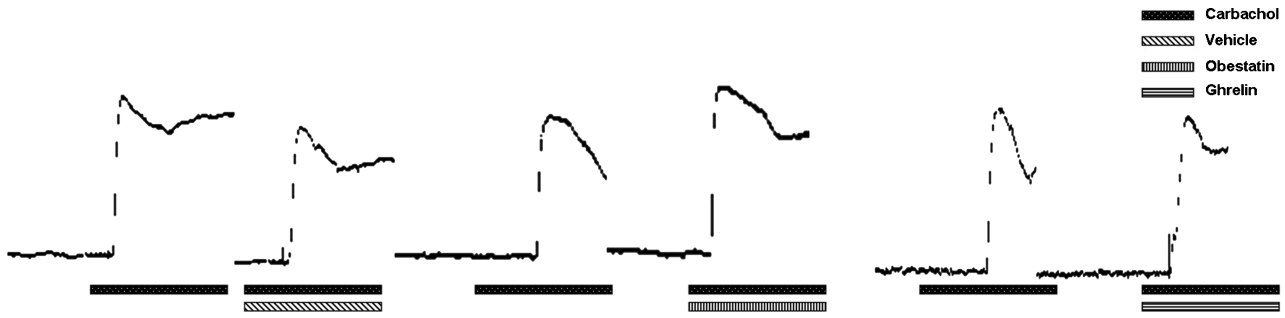

FIGURE 1 Representative example of iris sphincter muscle active tension in two consecutive carbachol-elicited contractions in the absence or presence of: (1) the vehicle (Panel A); (2) obestatin (10^{-5} M; Panel B); or (3) ghrelin (10^{-5} M; Panel C).

tension of the second carbachol-induced contraction of the iris sphincter muscle was 19.0 ± 10.2% higher than that of the first one ($p < 0.05$; Figs. 1 and 2). Finally, in the presence of ghrelin, no significant differences were detected between the active tension of the first and second carbachol-induced contractions of the rabbit iris sphincter muscle.

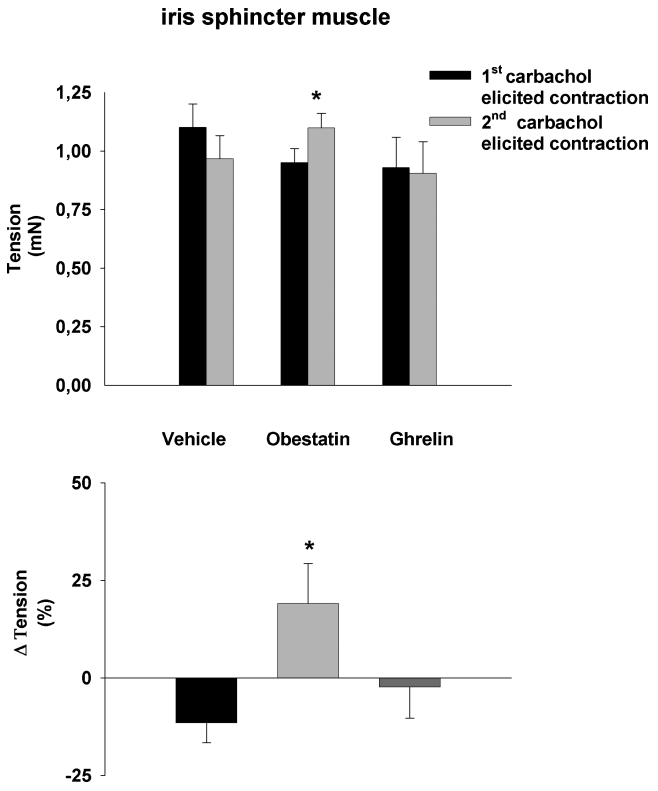

FIGURE 2 Iris sphincter muscle tension (upper panel) of two consecutive carbachol-induced (10^{-6} M) contractions in the absence (first contraction, left column) or presence (second contraction, right column) of the vehicle alone ($n = 8$), obestatin (10^{-5} M; $n = 10$) or ghrelin (10^{-5} M; $n = 7$). The lower panel represents the percent tension variation from the first to the second contraction in the same experimental conditions ($p < 0.05$: *second versus first contraction).

Effects of Obestatin or Ghrelin on Epinephrine-Induced Iris Dilator Muscle Contraction

Active tension of the first epinephrine-induced iris dilator muscle contraction was also not significantly different among the three groups (obestatin, ghrelin, and vehicle), averaging 0.37 ± 0.04 mN.

In the presence of the vehicle alone, the second epinephrine-induced contraction developed an active tension 10.7 ± 2.7% lower than the first ($p < 0.05$; Fig. 3). In the presence of either obestatin or ghrelin,

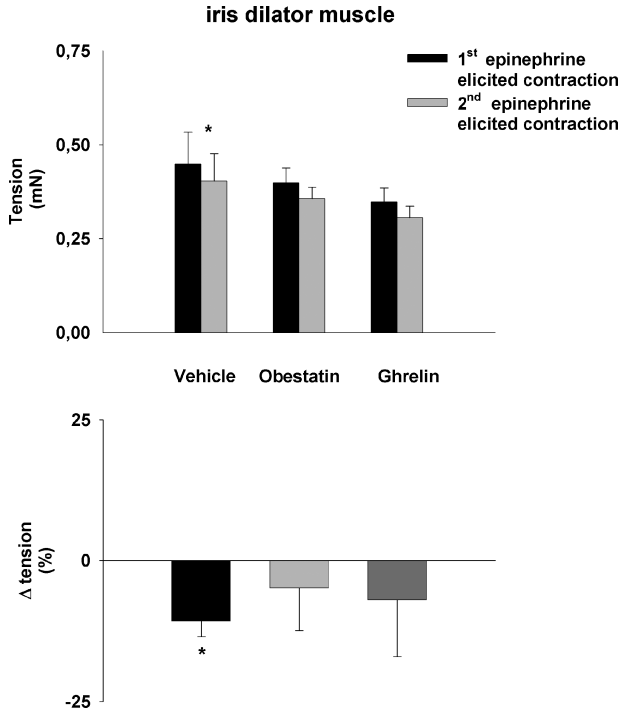

FIGURE 3 Iris dilator muscle tension (upper panel) of two consecutive epinephrine-induced (10^{-4} M) contractions in the absence (first contraction, left column) or presence (second contraction, right column) of the vehicle alone ($n = 8$), obestatin (10^{-5} M; $n = 8$) or ghrelin (10^{-5} M; $n = 8$). The lower panel represents the percent tension variation from the first to the second contraction in the same experimental conditions ($p < 0.05$: *second versus first contraction).

no significant differences between the active tension of the first and second epinephrine-induced contractions were detected (Fig. 3).

Effects of Obestatin and Ghrelin on EFS Elicited Contraction

In response to electric field stimulation (EFS), the first contraction of the iris sphincter muscle was similar in the three experimental groups (obestatin, ghrelin, and vehicle) and averaged 0.86 ± 0.06 mN.

In the presence of the vehicle alone, the first and second EFS-elicited contractions developed similar active tensions. The same happened in the presence of obestatin. With ghrelin, results were different. The active tension of the second EFS-induced contraction was 9.3 ± 3.2% smaller than the first ($p < 0.05$; Fig. 4).

Effects of Obestatin and Ghrelin on Muscle Tension

Baseline tension of the iris sphincter muscle preparations was quite stable and not significantly different

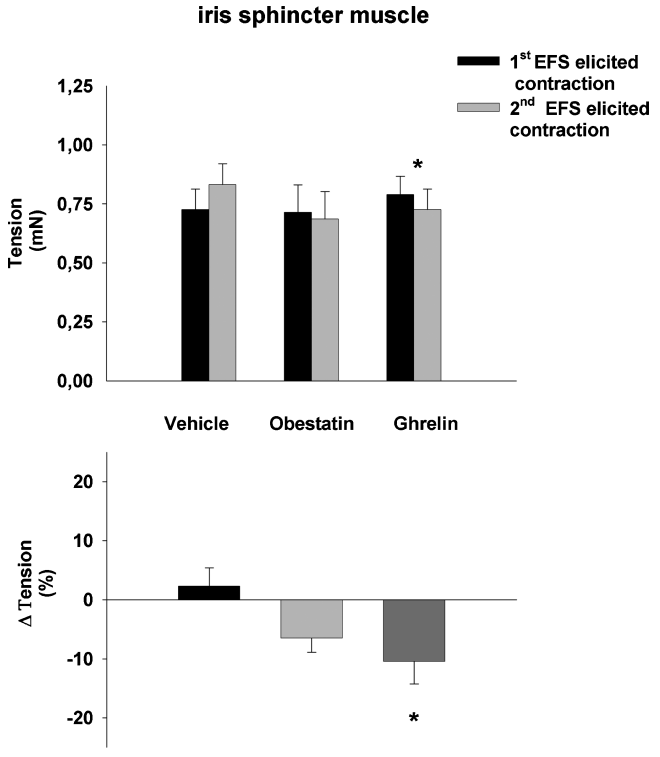

FIGURE 4 **Iris sphincter muscle tension (upper panel) of two consecutive EFS-elicited contractions in the absence (first contraction, left column) or presence (second contractions, right column) of the vehicle alone ($n = 8$), obestatin (10^{-5} M; $n = 8$) or ghrelin (10^{-5} M; $n = 8$). The lower panel represents the percent tension variation from the first to the second contraction in the same experimental conditions ($p < 0.05$: **second versus first contraction).**

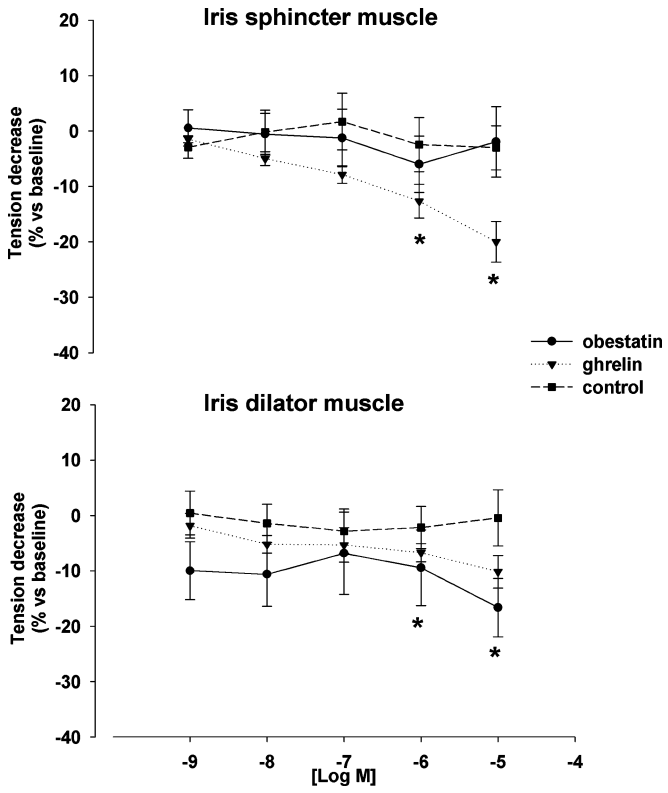

FIGURE 5 **Concentration-response curves of obestatin (10^{-9}–10^{-5}M) and ghrelin (10^{-9}–10^{-5}M) on the basal tension of the iris sphincter (upper panel) and dilator (lower panel) muscles ($p < 0.05$: *versus control).**

between the three groups (obestatin, ghrelin, and vehicle), averaging 0.49 ± 0.03 mN. The upper panel of Figure 5 shows concentration-response curves of iris sphincter muscle tension in the three groups. Only ghrelin significantly altered muscle tension, which decreased 21.7 ± 3.7% at 10^{-5} M ($p < 0.05$ versus control).

Baseline tension of the iris dilator muscle was also stable and similar in the three groups, averaging 0.36 ± 0.01 mN. In this preparation, only obestatin significantly decreased muscle tension, which averaged 14.1 ± 5.0% at 10^{-5}M ($p < 0.05$ versus control; Fig. 5, lower panel).

DISCUSSION

The present study describes the effects of obestatin and ghrelin on the contraction and relaxation of the iris sphincter and dilator muscles. In the sphincter, obestatin potentiates the cholinergic contraction elicited by carbachol, while ghrelin does not have any effect. On the other hand, ghrelin decreases basal tension of the iris sphincter in a concentration-dependent manner, while obestatin decreases basal tension of the iris dilator. Finally, in the EFS-elicited contraction of

the iris sphincter, ghrelin decreases the developed tension while obestatin does not affect it.

In the GI system, obestatin was located by immunohystochemistry in the gastric mucosa cells and in the myenteric plexus cells and fibers.[4] In nearly all cells of the guinea pig myenteric plexus, where obestatin was expressed, acetylcholine was co-localized. Obestatin was therefore proposed to be a regulator of the cholinergic system in the GI tract.[4] The present study, having shown that obestatin potentiates carbachol-elicited contraction of the iris sphincter muscle, suggests that it may also be a regulator of the cholinergic system in the iris. Taken together, the fact that in the presence of obestatin, active tension of the carbachol-induced contraction increased 19.0 ± 10.2%, while in the presence of the vehicle it decreased 11.5 ± 5.5%, the potentiation of the cholinergic contraction by obestatin averages more than 30%. Even though ghrelin did not significantly affect the carbachol-induced contraction, it has been previously shown, in the same experimental preparation, that it relaxes the carbachol precontracted iris sphincter muscle,[7] again suggesting opposite roles for ghrelin and obestatin. The relaxing effect of ghrelin was also evident in the present study, where we observed a decrease of the tension of the iris sphincter muscle even in the absence of precontraction. Interestingly, for the same concentrations, the relaxing effect of ghrelin had similar relative orders of magnitude in non-precontracted and precontracted muscles.

The first component of the iris sphincter muscle contraction in response to EFS has been related to prejunctional release of acetylcholine.[30] The present study showed that such response was decreased by ghrelin and not affected by obestatin.

Interestingly, however, ghrelin also decreases muscle basal tension, reinforcing the idea that ghrelin directly relaxes the iris sphincter muscle, as recently proposed.[7] However, from the data presented, we cannot deduce the specific site where obestatin or ghrelin act in the cholinergic and adrenergic pathway. Further investigations are needed to clarify this issue.

With regard to the iris dilator muscle, we did not find any effect of obestatin or ghrelin on the epinephrine-elicited contraction but observed a significant decrease of its basal tension in response to obestatin.

The second messenger for obestatin is not yet established. Recent studies, however, showed that in CHO cells culture overexpressing GPR39, obestatin increases cAMP levels.[1] The same second messenger was involved in the hRPE cells proliferation promoted by obestatin.[18] To what extent it plays a role in the effects reported in the present study requires further investigation.

As mentioned before, controversy has arisen by some authors due to the difficulties in reproducing the previously described effects of obestatin.[31] Given the ongoing controversy and discussion about the physiological relevance of obestatin, the present study, having shown that it has a regulatory effect of the iris contraction, adds new functions to the ghrelin/obestatin system in the eye and might help to clarify this issue.

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REFERENCES

[1] Zhang JV, Ren PG, Avsian-Kretchmer O, et al. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science.* 2005;310:996–999.

[2] McKee KK, Tan CP, Palyha OC, et al. Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors. *Genomics.* 1997;46:426–434.

[3] Jackson VR, Nothacker HP, Civelli O. GPR39 receptor expression in the mouse brain. *Neuroreport.* 2006;17:813–816.

[4] Dun SL, Brailoiu GC, Brailoiu E, Yang J, Chang JK, Dun NJ. Distribution and biological activity of obestatin in the rat. *J Endocrinol.* 2006;191:481–489.

[5] Lauwers E, Landuyt B, Arckens L, Schoofs L, Luyten W. Obestatin does not activate orphan G protein-coupled receptor GPR39. *Biochem Biophys Res Commun.* 2006;351:21–25.

[6] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature.* 1999;402:656–660.

[7] Rocha-Sousa A, Saraiva J, Henriques-Coelho T, Falcao-Reis F, Correia-Pinto J, Leite-Moreira AF. Ghrelin as a novel locally produced relaxing peptide of the iris sphincter and dilator muscles. *Exp Eye Res.* 2006;83:1179–1187.

[8] Leite-Moreira AF, Rocha-Sousa A, Henriques-Coelho T. Cardiac, skeletal and smooth muscle regulation by ghrelin *Vitamins & Hormones.* 2007;77:208–238.

[9] Bedendi I, Alloatti G, Marcantoni A, et al. Cardiac effects of ghrelin and its endogenous derivatives des-octanoyl ghrelin and des-Gln14-ghrelin. *Eur J Pharmacol.* 2003;476:87–95.

[10] Soares JB, Rocha-Sousa A, Castro-Chaves P, Henriques-Coelho T, Leite-Moreira AF. Inotropic and lusitropic effects of ghrelin and their modulation by the endocardial endothelium, NO, prostaglandins, GHS-R1a and KCa channels. *Peptides.* 2006;27:1616–1623.

[11] Kleinz MJ, Maguire JJ, Skepper JN, Davenport AP. Functional and immunocytochemical evidence for a role of ghrelin and des-octanoyl ghrelin in the regulation of vascular tone in man. *Cardiovasc Res.* 2006;69:227–235.

[12] Wiley KE, Davenport AP. Comparison of vasodilators in human internal mammary artery: Ghrelin is a potent physiological antagonist of endothelin-1. *Br J Pharmacol.* 2002;136:1146–1152.
[13] Dass NB, Munonyara M, Bassil AK, et al. Growth hormone secretagogue receptors in rat and human gastrointestinal tract and the effects of ghrelin. *Neurosci.* 2003;120:443–453.
[14] Depoortere I, De Winter B, Thijs T, De Man J, Pelckmans P, Peeters T. Comparison of the gastroprokinetic effects of ghrelin, GHRP-6 and motilin in rats in vivo and in vitro. *Eur J Pharmacol.* 2005;515:160–168.
[15] Lagaud GJ, Young A, Acena A, Morton MF, Barrett TD, Shankley NP. Obestatin reduces food intake and suppresses body weight gain in rodents. *Biochem Biophys Res Commun.* 2007;357:264–269.
[16] Moechars D, Depoortere I, Moreaux B, et al. Altered gastrointestinal and metabolic function in the GPR39-obestatin receptor-knockout mouse. *Gastroenterol.* 2006;131:1131–1141.
[17] Gourcerol G, St-Pierre DH, Tache Y. Lack of obestatin effects on food intake: Should obestatin be renamed ghrelin-associated peptide (GAP)? *Regul Pept.* 2007;141:1–7.
[18] Camina JP, Campos JF, Caminos JE, Dieguez C, Casanueva FF. Obestatin-mediated proliferation of human retinal pigment epithelial cells: Regulatory mechanisms. *J Cell Physiol.* 2007;211:1–9.
[19] Tachado SD, Akhtar RA, Abdel-Latif AA. Activation of beta-adrenergic receptors causes stimulation of cyclic AMP, inhibition of inositol trisphosphate, and relaxation of bovine iris sphincter smooth muscle. Biochemical and functional interactions between the cyclic AMP and calcium signaling systems. *Invest Ophthalmol Vis Sci.* 1989;30:2232–2239.
[20] Tachado SD, Akhtar RA, Zhou CJ, Abdel-Latif AA. Effects of isoproterenol and forskolin on carbachol- and fluoroaluminate-induced polyphosphoinositide hydrolysis, inositol trisphosphate production, and contraction in bovine iris sphincter smooth muscle: Interaction between cAMP and IP3 second messenger systems. *Cellular Signaling.* 1992;4:61–75.
[21] Ding KH, Abdel-Latif AA. Actions of C-type natriuretic peptide and sodium nitroprusside on carbachol-stimulated inositol phosphate formation and contraction in ciliary and iris sphincter smooth muscles. *Invest Ophthalmol Vis Sci.* 1997;38:2629–2638.
[22] Yoshitomi T, Yamaji K, Ishikawa H, Ohnishi Y. Effect of pituitary adenylate cyclase-activating peptide on isolated rabbit iris sphincter and dilator muscles. *Invest Ophthalmol Vis Sci.* 2002;43:780–783.
[23] Yamaji K, Yoshitomi T, Usui S. Effect of somatostatin and galanin on isolated rabbit iris sphincter and dilator muscles. *Exper Eye Res.* 2003;77:609–614.
[24] Gustafsson LE, Wiklund NP. Adenosine-modulation of cholinergic and non-adrenergic non-cholinergic neurotransmission in the rabbit iris sphincter. *Br J Pharmacol.* 1986;88:197–204.
[25] Hayashi K, Masuda K. Effects of vasoactive intestinal polypeptide (VIP) and cyclic-AMP on the isolated sphincter pupillae muscles of the albino rabbit. *Japanese J Ophthalmol.* 1982;26:437–442.
[26] Marathe GK, Yousufzai SY, Abdel-Latif AA. Mediation by prostaglandins of the stimulatory effect of substance P on cyclic AMP production in dog iris sphincter smooth muscle. *Biochem Pharmacol.* 1996;52:1261–1269.
[27] Yousufzai SY, Ali N, Abdel-Latif AA. Effects of adrenomedullin on cyclic AMP formation and on relaxation in iris sphincter smooth muscle. *Invest Ophthalmol Vis Sci.* 1999;40:3245–3253.
[28] Pianka P, Oron Y, Lazar M, Geyer O. Nonadrenergic, noncholinergic relaxation of bovine iris sphincter: Role of endogenous nitric oxide. *Invest Ophthalmol Vis Sci.* 2000;41:880–886.
[29] Piccone M, Littzi J, Krupin T, Stone RA, Davis M, Wax MB. Effects of neuropeptide Y on the isolated rabbit iris dilator muscle. *Invest Ophthalmol Vis Sci.* 1988;29:330–332.
[30] Ueda N, Muramatsu I, Sakakibara Y, Fujiwara M. Noncholinergic, nonadrenergic contraction and substance P in rabbit iris sphincter muscle. *Japanese J Pharmacol.* 1981;31:1071–1079.
[31] Gourcerol G, Tache Y. Obestatin—A ghrelin-associated peptide that does not hold its promise to suppress food intake and motility. *Neurogastroenterol Motil.* 2007;19:161–165.