0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(10):4696–4701

doi: 10.1210/jc.2003-030421

Oxyntomodulin Suppresses Appetite and Reduces Food Intake in Humans

MARK A. COHEN, SANDRA M. ELLIS, CAREL W. LE ROUX, RACHEL L. BATTERHAM, ADRIAN PARK, MICHAEL PATTERSON, GARY S. FROST, MOHAMMAD A. GHATEI, AND STEPHEN R. BLOOM

Departments of Metabolic Medicine (M.A.C., C.W.L.R., R.L.B., A.P., M.P., M.A.G., S.R.B.) and Dietetics (S.M.E., G.S.F.), Imperial College London, Hammersmith Hospital Campus, London, United Kingdom W12 0NN

Oxyntomodulin (OXM) is released from the gut postprandially, in proportion to energy intake, and circulating levels of OXM are elevated in several conditions associated with anorexia. Central injection of OXM reduces food intake and weight gain in rodents, suggesting that OXM signals food ingestion to hypothalamic appetite-regulating circuits. We investigated the effect of iv OXM (3.0 pmol/kg·min) on appetite and food intake in 13 healthy subjects (body mass index, 22.5 ± 0.9 kg/m^2) in a randomized, double-blind, placebo-controlled, cross-over study. Infusion of OXM significantly reduced *ad libitum* energy intake at a buffet meal (mean decrease, 19.3 ± 5.6%; $P < 0.01$) and caused a significant reduction in scores for hunger. In addition, cumulative 12-h energy intake was significantly reduced by infusion of OXM (mean decrease, 11.3 ± 6.2%; $P < 0.05$). OXM did not cause nausea or affect food palatability. Preprandial levels of the appetite-stimulatory hormone, ghrelin, were significantly suppressed by OXM (mean reduction, 44 ± 10% of postprandial decrease; $P < 0.0001$). Elevated levels of endogenous OXM associated with disorders of the gastrointestinal tract may contribute to anorexia. (*J Clin Endocrinol Metab* 88: 4696–4701, 2003)

OXYNTOMODULIN (OXM) is a 37-amino acid peptide that arises from posttranslational processing of proglucagon in intestinal cells (1) and was named after its inhibitory action on the oxyntic glands of the stomach (2). OXM contributes 50% to plasma OXM-like immunoreactivity (OLI) (3), the remaining component being OXM with a 30-amino acid N-terminal extension (4). OXM is released into the blood in response to food ingestion and in proportion to meal calorie content (5, 6). We have previously shown that injection of OXM into the brain of rats reduces *ad libitum* food intake and body weight gain (7, 8), and more recently, systemic administration of OXM (by ip injection) has also been shown to inhibit food intake in rats (Dakin, C. L., C. J. Small, R. L. Batterham, N. M. Neary, M. A. Cohen, M. Patterson, M. A. Ghatei, and S. R. Bloom, manuscript in preparation). Hence, postprandial release of OXM may signal food intake to the appetite-regulating circuits of the brain. OXM levels are markedly elevated in tropical malabsorption (9) and after jejuno-ileal bypass surgery for morbid obesity (10, 11), conditions associated with anorexia and weight loss (12, 13). Our aim was to show that elevation of plasma levels of OXM by infusion would reduce appetite and food consumption in healthy subjects.

Materials and Methods

Materials

Human OXM was synthesized by Bachem (St. Helens, UK). The *Limulus* amebocyte lysate test for pyrogen (Associates of Cape Cod, Liverpool, UK) was negative, and the peptide was sterile on culture. Saline (0.9%) was supplied by Bayer (Haywards Heath, UK), and Hemaccel by Beacon (Tunbridge Wells, UK).

Subjects

Thirteen healthy volunteers (seven men and six women), aged 19–27 yr (mean ± SEM, 20.2 ± 0.7) with a body mass index between 20.4–27.1 kg/m^2 (mean ± SEM, 22.5 ± 0.9), were recruited by advertisement at Hammersmith Hospital Campus, Imperial College London. Ethical approval was obtained from the Hammersmith Hospitals' Trust research ethics committee. The subjects gave informed written consent, and the study was performed in accordance with the Declaration of Helsinki. Criteria for exclusion included smoking, substance abuse, pregnancy, medication (except for the oral contraceptive pill), medical or psychiatric illness, and abnormalities detected on physical examination and screening investigations (electrocardiogram, full blood count, urea and electrolytes, liver function tests, and fasting glucose). Potential subjects were screened by a dietician to exclude those with a high level of restrained eating, as assessed by the Dutch Eating Behavior Questionnaire (14), and disordered eating, as assessed by the Eating Attitudes Test (15). Subjects also completed a 3-d diet diary to determine their usual eating habits before acceptance into the study. Food preferences were assessed using a nine-point hedonic scale to ensure that food offered at the buffet lunch was acceptable.

Protocol

The study design was a double-blind, placebo-controlled, cross-over (Fig. 1). Each subject received an iv infusion of OXM (3.0 pmol/kg·min) and a control infusion of saline (0.9%), at least 7 d apart, in random order. Female subjects were studied in the follicular phase of the menstrual cycle to control for the potential effect of cycle phase on food intake and appetite sensation (16). Subjects completed a diary of food consumption for 2 d preinfusion and 24 h postinfusion. They refrained from alcohol and strenuous exercise for 24 h before and after the study day. Subjects maintained a similar diet for 48 h before each infusion. They consumed an identical meal and then fasted from 2100 h on the night before each infusion. They were permitted to drink water. Subjects were studied in pairs and were separated by a screen. Throughout the study they were encouraged to relax by reading or watching video films. Clocks were

Abbreviations: GLP-1, Glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; OLI, oxyntomodulin-like immunoreactivity; OXM, oxyntomodulin; PYY, peptide YY; VAS, visual analog scales.

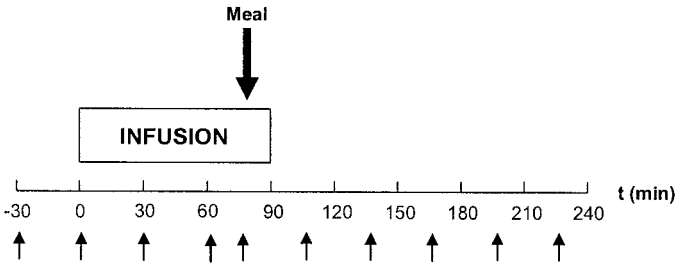

FIG. 1. Protocol for investigation of the effects of OXM infusion on appetite and food intake in humans. The scale represents time (t) in minutes. OXM (3.0 pmol/kg·min) or saline was infused for a 90-min period (t_0 to t_{90}). The buffet meal, denoted by the *bold arrow*, was presented at t_{75}. The timing of blood samples and visual analog scales is indicated by *arrows* below the scale at −30, 0, 30, 60, 75, 105, 135, 165, 195, and 225 min.

removed from the study room to limit the potential effect of awareness of time on expectation of food and appetite sensation.

OXM was dissolved in saline containing Hemaccel (5%, vol/vol) to reduce adsorption of peptide to the syringe and tubing. The infusion was administered via an iv cannula in a forearm vein for 90 min (t_0 to t_{90}). Blood samples were collected from a cannula sited in the contralateral forearm into lithium-heparin tubes (LIP Ltd., Cambridge, UK) containing 5000 kallikrein inhibitor units (0.2 ml) aprotinin (Trasylol, Bayer) and stored on ice. After centrifugation, plasma was immediately separated and stored at −70 C until analysis. Blood samples were taken at −30, 0, 30, 60, 75, 105, 135, 165, 195, and 225 min. Immediately before blood sampling, subjects completed visual analog scales (VAS) rating hunger, satiety, prospective food consumption, and nausea. These consisted of 100-mm scales with the text expressing the most positive and the most negative rating anchored at each end (17). The blood pressure of the subjects was measured every 15 min, and their electrocardiograms were continuously monitored.

At 15 min before termination of the infusion (t_{75}), subjects were offered a buffet meal, which was provided in excess such that all appetites would be satisfied. Water was freely available. Energy intake was determined by weighing food and water pre- and postprandially. The duration of the meal was timed, and subjects rated the palatability of the food by VAS. Subjects remained in the study room until t_{240}. They continued to complete VAS until 0900 h the following morning and recorded their food intake in diaries for 24 h after the buffet meal (until 1300 h the following day). Food diaries were analyzed by a dietician blinded to the study, and energy intake was calculated with the aid of the Dietplan program (Forestfield Software Ltd., West Sussex, UK).

Analysis of OXM degradation in human plasma

To determine whether infused OXM is converted into other molecular forms, plasma taken at the plateau of OXM infusion (t_{60}) was fractionated with Sephadex G-50 Superfine on a 60 × 0.9-cm column (Amersham Pharmacia Biotech, Uppsala, Sweden). The column was eluted at a flow rate of 3.2 ml/h at 4 C in 0.06 M phosphate buffer containing 0.2 M NaCl and 0.3% (vol/vol) BSA, and 0.7-ml fractions were collected. To determine the relative elution coefficient (K_{av}) of OLI dextran blue (molecular weight, 2,000,000; 30 mg/ml; K_{av} = 0), horse heart cytochrome *c* (molecular weight, 12,384; 30 mg/ml; K_{av} = 0.26) and [^{125}I]Na (K_{av} = 1.0) were added to each sample (n = 3), and samples were loaded in a volume of 0.8 ml. The elution profile of plasma OXM was determined by RIA for OLI.

Hormone measurements

All samples were assayed in duplicate and within one assay to eliminate interassay variation. Plasma OLI, glucagon, peptide YY (PYY), insulin, glucagon-like peptide-1 (GLP-1), and ghrelin were measured using established in-house RIAs. The OLI assay (5) could detect changes of 10 pmol/liter (95% confidence limit) with an intraassay variation of 5.7%. The PYY assay (18) could detect changes of 2 pmol/liter (95% confidence limit) with an intraassay variation of 5.8%. The PYY antibody was specific for the C-terminal of PYY and reacted fully with human PYY-(3–36). The insulin assay (19) could detect changes of 6 pmol/liter (95% confidence limit) with an intraassay variation of 5.4%. The GLP-1 assay (19) could detect changes of 8 pmol/liter (95% confidence limit) with an intraassay variation of 6.1%. The GLP-1 antibody was specific for N-terminal amidated GLP-1 and did not cross-react with GLP-1-(1–37), GLP-1-(1–36), or GLP-1-(7–37). The ghrelin assay (20) could detect changes of 10 pmol/liter (95% confidence limit) with an intraassay variation of 9.5%. Plasma leptin was measured using a human leptin RIA kit (Linco Research, Inc., St. Charles, MO).

Statistical analyses

Combined data are represented as the mean ± SEM. The integrated area under the curve was calculated using the trapezoid rule. Comparisons of areas under the curve and food consumption between OXM and saline groups were by paired *t* test. VAS were compared using the Wilcoxon signed-rank test. $P < 0.05$ was considered significant.

Results

Effects of OXM infusion on energy intake

OXM infusion significantly reduced energy intake at the buffet meal by 19.3 ± 5.6% (reduction *vs.* saline, 921 ± 251 kJ; $P < 0.01$). Twelve of the 13 subjects studied showed a decrease in energy intake with OXM infusion (Fig. 2). There was no obvious cause for the failure of response in one subject. OXM infusion significantly reduced cumulative 12-h energy intake by 11.3 ± 6.2% (reduction *vs.* saline, 1528 ± 666 kJ; $P < 0.05$; Fig. 3). Cumulative 24-h energy intake was not significantly altered (saline, 12,740 ± 1,532 kJ; OXM, 11,589 ± 1,243 kJ). OXM did not change water consumption or the proportion of energy obtained from different macronutrients, either at the buffet meal or in the subsequent 24 h.

Effects of OXM infusion on appetite and palatability

During infusion of saline, VAS for hunger did not change significantly throughout the fasting period (Fig. 4), whereas OXM infusion caused a significant fall in hunger (incremental area under the curve t_0 to t_{75}: saline, +273 ± 128 mm/min; OXM, −374 ± 185 mm/min; $P < 0.05$). The decrease in hunger after the buffet meal was similar on saline and OXM infusion days, and hunger scores remained similar thereafter. The duration of the meal was significantly reduced by

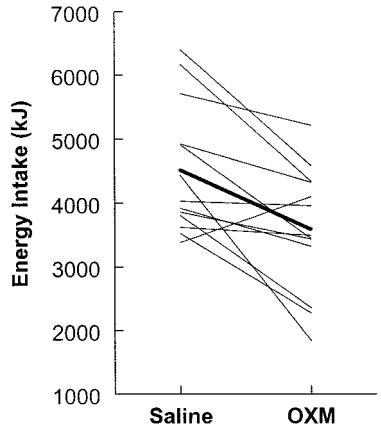

FIG. 2. Energy intake at the buffet meal for the infusion of OXM and saline. Each *line* represents the energy intake of an individual subject for saline and OXM infusions. The *bold line* denotes the mean energy intake for all volunteers (n = 13).

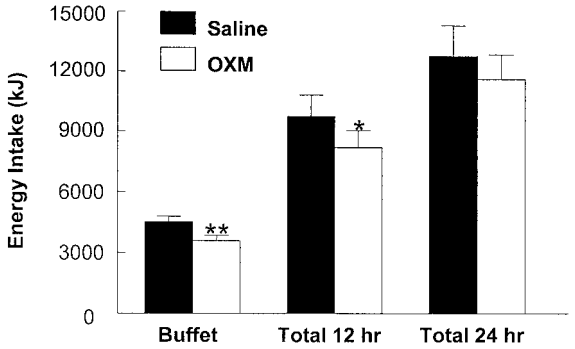

FIG. 3. Mean energy intake for the infusion of OXM and saline. The mean energy intake ± SEM for buffet meal, cumulative 12-h energy intake (until midnight on infusion day), and cumulative 24-h energy intake (until 1300 h on the postinfusion day; n = 13) are shown. *, $P < 0.05$; **, $P < 0.01$ (OXM *vs.* saline).

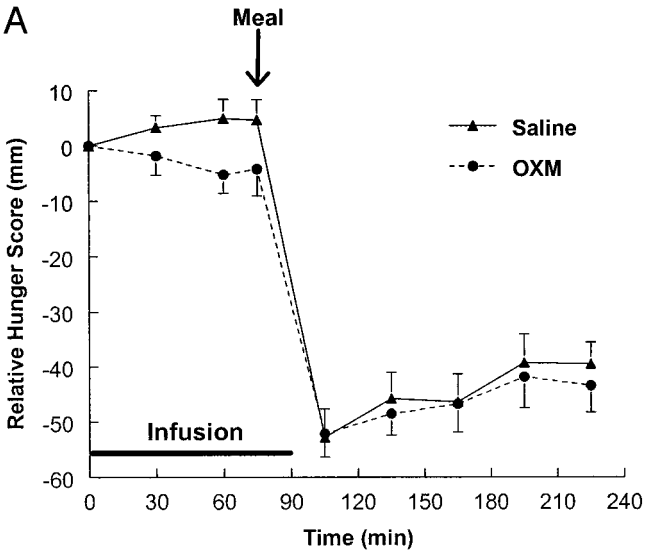

FIG. 4. Hunger scores for infusion of OXM and saline. VAS expressed as change from baseline (millimeters). The period of infusion of OXM or saline is shown by the *bold horizontal line*. Presentation of the buffet meal at t_{75} is denoted by an *arrow* (n = 13).

OXM (saline, 19.2 ± 1.3 min; OXM, 15.1 ± 1.8 min; $P < 0.05$). There was no significant effect of OXM on VAS for satiety, prospective food consumption, nausea, or meal palatability (data not shown).

Plasma levels of OLI

Infusion of OXM elevated plasma OLI from 62 ± 5 pmol/liter to a peak of 907 ± 32 pmol/liter at t_{60} (Fig. 5). In comparison, on the saline infusion day, consumption of the buffet meal led to a peak postprandial OLI level of 151 ± 18 pmol/liter at 195 min. Gel permeation analysis of plasma samples during OXM infusion (Fig. 6) demonstrated a single immunoreactive peak eluting in the same position as synthetic OXM (K_{av} = 0.6). Thus, intact full-length OXM was the principle circulating form.

Effects of OXM infusion on plasma ghrelin

During the saline infusion, plasma ghrelin levels increased throughout the fasting period (t_0, 461 ± 32 pmol/liter; t_{75}, 484 ± 35 pmol/liter) and decreased postprandially (t_{225}, 357 ± 28 pmol/liter). However, during infusion of OXM, fasting levels of ghrelin decreased before the meal (t_0, 482 ± 33 pmol/liter; t_{75}, 435 ± 35 pmol/liter), and there was a further postprandial reduction in ghrelin (t_{225}, 356 ± 31 pmol/liter). Hence, plasma ghrelin before the buffet meal was significantly reduced by OXM infusion compared with saline (mean change in ghrelin from t_0 to t_{75}: saline, +24 ± 10 pmol/liter; OXM, −47 ± 11 pmol/liter; $P < 0.0001$; Fig. 7). The suppression of plasma ghrelin due to OXM infusion represents 44 ± 10% of the postprandial decrease in ghrelin on the corresponding saline infusion day (mean postprandial decrease, 155 ± 19 pmol/liter).

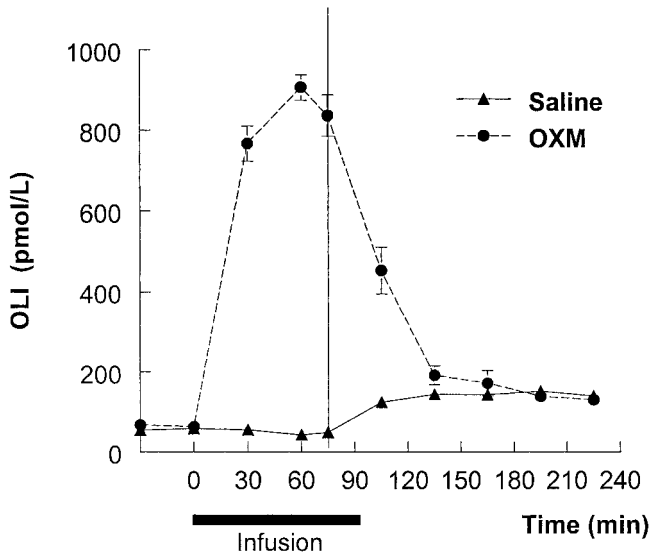

FIG. 5. Plasma OLI for infusions of OXM and saline. OLI (picomoles per liter) for infusions of OXM or saline. Infusion from t_0 to t_{90} is denoted by the *bold horizontal line*. Presentation of the buffet meal at t_{75} is represented by the *vertical line* (n = 13).

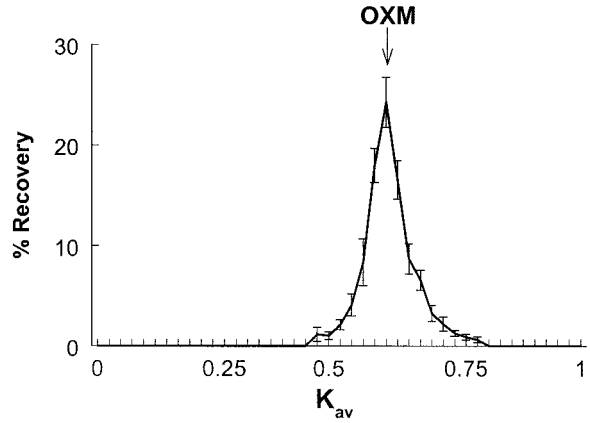

FIG. 6. Sephadex G-50 column chromatography of plasma during OXM infusion. The percentage total recovery of OLI against K_{av} for plasma samples taken at plateau of OXM infusion (t_{60}). The elution position of synthetic OXM is indicated by an *arrow* above the single immunoreactive peak (n = 3).

Effects of OXM infusion on plasma hormones

There was no significant effect of OXM infusion on fasting plasma levels of PYY, insulin, glucagon, GLP-1, or leptin

(Table 1). Plasma concentrations of leptin in female subjects were higher than in males, as previously reported. As expected, there was a significant postprandial rise in PYY for both saline (t_{75}, 14 ± 1 pmol/liter; t_{225}, 28 ± 2 pmol/liter; $P < 0.0001$) and OXM (t_{75}, 14 ± 1 pmol/liter; t_{225}, 29 ± 2 pmol/liter; $P < 0.0001$) infusions. There was also a significant postprandial rise in plasma insulin (saline: t_{75}, 36 ± 5 pmol/liter; t_{225}, 242 ± 30 pmol/liter; $P < 0.0001$; OXM: t_{75}, 37 ± 5 pmol/liter; t_{225}, 219 ± 38 pmol/liter; $P < 0.0001$) and GLP-1 (saline: t_{75}, 47 ± 6 pmol/liter; t_{225}, 86 ± 7 pmol/liter; $P < 0.0001$; OXM: t_{75}, 41 ± 6 pmol/liter; t_{225}, 86 ± 11 pmol/liter; $P < 0.0001$). There was no significant difference between saline and OXM infusions in the magnitude of the postprandial rise in PYY, insulin, or GLP-1. In addition, there was no significant postprandial change in plasma glucagon or leptin.

Discussion

In recent years much progress has been made in understanding body weight regulation by the hypothalamus (21). The brain receives feedback signals of nutritional status, such as leptin, secreted in proportion to adipose tissue mass (22), and ghrelin, which is released from the stomach in response to fasting (23, 24). The reduction in appetite that follows the consumption of a meal does not occur when nutrients are administered iv (25), suggesting that food ingestion leads to gut-derived signals that reduce appetite. OXM is released postprandially from the same specialized endocrine cells of the distal gut as PYY (26) and GLP-1 (27). We recently demonstrated that PYY signals food ingestion to the hypothalamus, and infusion of physiological levels of PYY significantly reduces food intake in human subjects (28). GLP-1 may also be involved in the regulation of food intake (29), and infusions of GLP-1 have been shown to inhibit appetite in man (30). Circulating levels of OXM rise within 30 min of a meal and remain elevated for several hours (5, 31). In rodents, we have shown that injection of OXM directly into the hypothalamus (7, 8) or systemically, by ip injection (Dakin, C. L., C. J. Small, R. L. Batterham, N. M. Neary, M. A. Cohen, M. Patterson, M. A. Ghatei, and S. R. Bloom, manuscript in preparation), reduces *ad libitum* food intake. These findings suggest that postprandial release of OXM may be a feedback signal to brain circuits that regulate food intake.

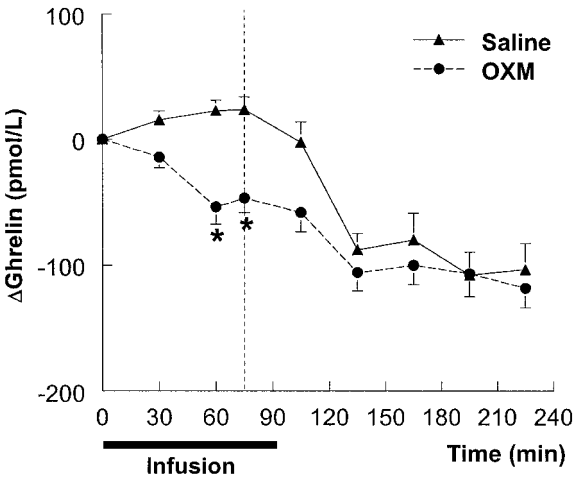

FIG. 7. Change in plasma ghrelin from baseline during infusions of OXM and saline. The change in plasma ghrelin (Δghrelin) from baseline (t_0) is shown. Infusion of OXM or saline from t_0 to t_{90} is denoted by the *bold horizontal line*. Presentation of the buffet meal at t_{75} is represented by the *vertical broken line* (n = 13). *, $P < 0.0001$ (OXM *vs.* saline).

We have now demonstrated that systemic administration of OXM significantly reduces food intake in healthy human subjects. Intravenous infusion of OXM reduced calorie intake by 19% at the buffet meal, and cumulative energy intake was decreased in the 12 h post infusion. Much smaller alterations in food consumption would lead to weight loss if sustained over the long term (32). However, there was no significant effect of OXM on cumulative 24-h energy intake. OXM did not affect enjoyment of the meal, which is important in view of its potential therapeutic use.

Ghrelin is a powerful stimulant of appetite in man (23), and preprandial rises in plasma ghrelin have been suggested to be a trigger for meal initiation (33). Hence, the novel finding that OXM infusion suppresses fasting plasma ghrelin is potentially important. Inhibition of the normal preprandial rise in ghrelin by OXM is likely to be one mechanism by which OXM infusion reduces appetite. This finding may also shed light on the poorly understood mechanism by which ghrelin levels are reduced postprandially. In rodents, fasting increases plasma ghrelin, whereas oral intake of glucose, but not water, decreases ghrelin secretion, suggesting that suppression of plasma ghrelin is related to ingestion of nutrients rather than stomach distension (34). Hence, OXM released in response to nutrient ingestion may contribute to the normal postprandial inhibition of plasma ghrelin. It is believed that only a proportion of total circulating ghrelin is the biologically active, octanoylated form (35). The effect of food con-

TABLE 1. Plasma hormones during saline and OXM infusions

Hormone	Saline			OXM		
	t_0	t_{75}	t_{225}	t_0	t_{75}	t_{225}
Ghrelin (pmol/liter)	462 ± 35	484 ± 35	157 ± 28[a]	467 ± 41	435 ± 35[b]	164 ± 31[a]
PYY (pmol/liter)	16 ± 1	14 ± 1	28 ± 2[a]	18 ± 2	14 ± 1	29 ± 2[a]
Insulin (pmol/liter)	30 ± 3	36 ± 5	242 ± 30[a]	30 ± 4	37 ± 5	219 ± 38[a]
Glucagon (pmol/liter)	12 ± 2	13 ± 2	13 ± 1	11 ± 1	13 ± 1	15 ± 3
GLP-1 (pmol/liter)	44 ± 6	47 ± 6	86 ± 7[a]	52 ± 7	41 ± 6	86 ± 11[a]
Leptin, males (ng/ml)	2.0 ± 0.3	1.9 ± 0.2	1.8 ± 0.2	2.0 ± 0.2	1.8 ± 0.2	1.8 ± 0.2
Leptin, females (ng/ml)	13.5 ± 3.7	12.5 ± 3.2	12.0 ± 3.1	12.3 ± 3.0	12.0 ± 3.1	12.3 ± 3.8

Plasma hormones (mean ± SEM) at baseline (t_0), before a meal (t_{75}), and postprandially (t_{225}) for infusions of saline and OXM.
[a] $P < 0.0001$, t_{225} *vs.* t_{75}.
[b] $P < 0.0001$, saline *vs.* OXM.

sumption and OXM infusion may be to primarily reduce levels of this active ghrelin.

Intravenous infusion of OXM has been shown to inhibit gastric emptying in humans (36). Suppression of gastric emptying may lead to increased gastric distension, which may contribute to satiety by causing a sensation of fullness. In the current study, hunger scores were significantly reduced by OXM in the fasting state, when gastric distension is unlikely to be important. Hence, the reduction in appetite during the premeal period is unlikely to result from the effects of OXM on gastric emptying. The anorectic effect of OXM does not appear to be mediated by stimulation of the release of PYY or leptin, as concentrations of these hormones were unaffected by OXM infusion.

It is not clear which receptor mediates the appetite-suppressant effect of OXM. OXM has been shown to bind to the receptor for GLP-1 (GLP-1R) *in vitro*, but with much lower affinity than GLP-1 (37). In rats, we have previously shown that the anorectic effect of intracerebroventricular OXM is blocked by coadministration of the GLP-1R antagonist exendin-(9–39) (7). However, exendin-(9–39) might also block the binding of OXM at a putative OXM-specific receptor.

GLP-1 is a physiological incretin hormone (19) and as such potentiates the postprandial release of insulin. OXM might also be expected to increase insulin release by binding to the GLP-1R. In this study there was no effect of OXM infusion on fasting insulin levels. However, we cannot determine the postprandial insulin-stimulating effect of OXM infusion due to the different amounts of food consumed at the buffet and the potential effect of OXM on gastric emptying.

It is unclear where circulating OXM acts to reduce appetite. The reduction of food intake by intrahypothalamic injection of OXM (7) suggests that OXM may have direct effects on hypothalamic appetite-regulating centers. Indeed, circulating OXM may gain access to the hypothalamus at sites where the blood-brain barrier is deficient, such as the arcuate nucleus (38). This region, in particular, is thought to be important in sensing and integrating peripheral signals of energy homeostasis (39). Circulating OXM may also be detected by vagal nerve endings, which transmit afferent signals to the brain stem, as has recently been shown for ghrelin (40).

Infusion of OXM produced circulating levels of OLI comparable to the elevated concentrations seen in tropical sprue (9) and after jejuno-ileal bypass surgery for morbid obesity (10, 11). Therefore, OXM may contribute to the loss of appetite and weight loss observed in these conditions. We have demonstrated the anorectic effect of elevated circulating levels of OXM. However, it is possible that lower postprandial concentrations of OXM may contribute to the physiological reduction of appetite in normal individuals.

Acknowledgments

We are most grateful to the subjects who kindly volunteered for this study. We thank Mr. Ian Grace, Imperial College London, who provided statistical advice.

Received March 11, 2003. Accepted June 14, 2003.

Address all correspondence and requests for reprints to: Prof. Stephen R. Bloom, Department of Metabolic Medicine, Imperial College London at Hammersmith Campus, Du Cane Road, London, United Kingdom W12 0NN. E-mail: s.bloom@imperial.ac.uk.

This work was supported by grants from the Medical Research Council, United Kingdom (to M.A.C.), and the Wellcome Trust (to C.L.R., R.L.B., and A.P.).

References

1. **Bataille D, Tatemoto K, Gespach C, Jornvall H, Rosselin G, Mutt V** 1982 Isolation of glucagon-37 (bioactive enteroglucagon/oxyntomodulin) from porcine jejuno-ileum. Characterization of the peptide. FEBS Lett 146:79–86
2. **Dubrasquet M, Bataille D, Gespach C** 1982 Oxyntomodulin (glucagon-37 or bioactive enteroglucagon): a potent inhibitor of pentagastrin-stimulated acid secretion in rats. Biosci Rep 2:391–395
3. **Kervran A, Blache P, Bataille D** 1987 Distribution of oxyntomodulin and glucagon in the gastrointestinal tract and the plasma of the rat. Endocrinology 121:704–713
4. **Holst JJ** 1997 Enteroglucagon. Annu Rev Physiol 59:257–271
5. **Ghatei MA, Uttenthal LO, Christofides ND, Bryant MG, Bloom SR** 1983 Molecular forms of human enteroglucagon in tissue and plasma: plasma responses to nutrient stimuli in health and in disorders of the upper gastrointestinal tract. J Clin Endocrinol Metab 57:488–495
6. **le Quellec A, Kervran A, Blache P, Ciurana AJ, Bataille D** 1992 Oxyntomodulin-like immunoreactivity: diurnal profile of a new potential enterogastrone. J Clin Endocrinol Metab 74:1405–1409
7. **Dakin CL, Gunn I, Small CJ, Edwards CM, Hay DL, Smith DM, Ghatei MA, Bloom SR** 2001 Oxyntomodulin inhibits food intake in the rat. Endocrinology 142:4244–4250
8. **Dakin CL, Small CJ, Park AJ, Seth A, Ghatei MA, Bloom SR** 2002 Repeated ICV administration of oxyntomodulin causes a greater reduction in body weight gain than in pair-fed rats. Am J Physiol 283:E1173–E1177
9. **Besterman HS, Cook GC, Sarson DL, Christofides ND, Bryant MG, Gregor M, Bloom SR** 1979 Gut hormones in tropical malabsorption. Br Med J 2:1252–1255
10. **Sarson DL, Scopinaro N, Bloom SR** 1981 Gut hormone changes after jejunoileal (JIB) or biliopancreatic (BPB) bypass surgery for morbid obesity. Int J Obes 5:471–480
11. **Holst JJ, Sorensen TI, Andersen AN, Stadil F, Andersen B, Lauritsen KB, Klein HC** 1979 Plasma enteroglucagon after jejunoileal bypass with 3:1 or 1:3 jejunoileal ratio. Scand J Gastroenterol 14:205–207
12. **Klipstein FA, Corcino JJ** 1977 Factors responsible for weight loss in tropical sprue. Am J Clin Nutr 30:1703–1708
13. **Payne JH, DeWind LT** 1969 Surgical treatment of obesity. Am J Surg 118: 141–147
14. **Van Strien T, Rookus MA, Bergers GP, Frijters JE, Defares PB** 1986 Life events, emotional eating and change in body mass index. Int J Obes 10:29–35
15. **Garner DM, Garfinkel PE** 1979 The Eating Attitudes Test: an index of the symptoms of anorexia nervosa. Psychol Med 9:273–279
16. **Dye L, Blundell JE** 1997 Menstrual cycle and appetite control: implications for weight regulation. Hum Reprod 12:1142–1151
17. **Flint A, Raben A, Blundell JE, Astrup A** 2000 Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 24:38–48
18. **Adrian TE, Savage AP, Sagor GR, Allen JM, Bacarese-Hamilton AJ, Tatemoto K, Polak JM, Bloom SR** 1985 Effect of peptide YY on gastric, pancreatic, and biliary function in humans. Gastroenterology 89:494–499
19. **Kreymann B, Williams G, Ghatei MA, Bloom SR** 1987 Glucagon-like peptide-1 7–36: a physiological incretin in man. Lancet 2:1300–1304
20. **English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JP** 2002 Food fails to suppress ghrelin levels in obese humans. J Clin Endocrinol Metab 87:2984
21. **Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG** 2000 Central nervous system control of food intake. Nature 404:661–671
22. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432
23. **Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR** 2001 Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86:5992
24. **Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR** 2001 Ghrelin causes hyperphagia and obesity in rats. Diabetes 50:2540–2547
25. **McCutcheon NB, Tennissen AM** 1989 Hunger and appetitive factors during total parenteral nutrition. Appetite 13:129–141
26. **Bottcher G, Sjolund K, Ekblad E, Hakanson R, Schwartz TW, Sundler F** 1984 Coexistence of peptide YY and glicentin immunoreactivity in endocrine cells of the gut. Regul Pept 8:261–266
27. **Varndell IM, Bishop AE, Sikri KL, Uttenthal LO, Bloom SR, Polak JM** 1985 Localization of glucagon-like peptide (GLP) immunoreactants in human gut and pancreas using light and electron microscopic immunocytochemistry. J Histochem Cytochem 33:1080–1086
28. **Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL,**

Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR 2002 Gut hormone PYY(3–36) physiologically inhibits food intake. Nature 418:650–654
29. **Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR** 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72
30. **Verdich C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ, Astrup A** 2001 A meta-analysis of the effect of glucagon-like peptide-1 (7–36) amide on ad libitum energy intake in humans. J Clin Endocrinol Metab 86:4382–4389
31. **Hornnes PJ, Kuhl C, Holst JJ, Lauritsen KB, Rehfeld JF, Schwartz TW** 1980 Simultaneous recording of the gastro-entero-pancreatic hormonal peptide response to food in man. Metabolism 29:777–779
32. **Schutz Y, Garrow JS** 2003 Energy and substrate balance, and weight regulation. In: Garrow JS, James WPT, Ralph A, eds. Human nutrition and dietetics. London: Churchill Livingstone; 137–148
33. **Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS** 2001 A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714–1719
34. **Tschop M, Smiley DL, Heiman ML** 2000 Ghrelin induces adiposity in rodents. Nature 407:908–913
35. **Ariyasu H, Takaya K, Hosoda H, Iwakura H, Ebihara K, Mori K, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K** 2002 Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. Endocrinology 143:3341–3350
36. **Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ** 1989 Oxyntomodulin from distal gut. Role in regulation of gastric and pancreatic functions. Dig Dis Sci 34:1411–1419
37. **Gros L, Thorens B, Bataille D, Kervran A** 1993 Glucagon-like peptide-1-(7–36) amide, oxyntomodulin, and glucagon interact with a common receptor in a somatostatin-secreting cell line. Endocrinology 133:631–638
38. **Merchenthaler I** 1991 Neurons with access to the general circulation in the central nervous system of the rat: a retrograde tracing study with fluoro-gold. Neuroscience 44:655–662
39. **Cone RD, Cowley MA, Butler AA, Fan W, Marks DL, Low MJ** 2001 The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int J Obes Relat Metab Disord 25(Suppl 5):S63–S67
40. **Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M** 2002 The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 123:1120–1128