Repeated ICV administration of oxyntomodulin causes a greater reduction in body weight gain than in pair-fed rats

Catherine L. Dakin, Caroline J. Small, Adrian J. Park, Asha Seth, Mohammad A. Ghatei, and Stephen R. Bloom

Endocrine Unit, Imperial College Faculty of Medicine, Hammersmith Hospital, London W12 0NN, United Kingdom

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Repeated ICV administration of oxyntomodulin causes a greater reduction in body weight gain than in pair-fed rats. *Am J Physiol Endocrinol Metab* 283: E1173–E1177, 2002. First published July 30, 2002; 10.1152/ajpendo.00233.2002.—Oxyntomodulin (OXM) is a product of proglucagon processing in the intestine and the central nervous system. We reported that intracerebroventricular (ICV) and intranuclear administration of OXM caused an inhibition of food intake in rats (Dakin CL, Gunn I, Small CJ, Edwards CM, Hay DL, Smith DM, Ghatei MA, and Bloom SR. *Endocrinology* 142: 4244–4250, 2001). In this study, we investigated the effect of twice-daily ICV administration of OXM, 1 nmol, for 7 days. A pair-fed control was included. These animals were restricted to the food intake of the OXM group but injected twice daily with saline. OXM-treated animals gained significantly less weight than either control group (day 8: OXM, 12.2 ± 1.9 g vs. pair fed, 21.0 ± 2.1 g; *P* < 0.005). OXM treatment caused a reduction in epididymal white adipose tissue (OXM, 1.13 ± 0.03 g vs. pair fed, 1.29 ± 0.04 g; *P* < 0.05) and interscapular brown adipose tissue (OXM, 0.15 ± 0.01 g vs. pair fed, 0.18 ± 0.01 g; *P* < 0.05) and increased core temperature compared with saline control, suggestive of enhanced energy expenditure. The food restriction-induced suppression in plasma TSH, seen in the pair-fed group, was prevented by OXM, potentially via increased release of hypothalamic TRH. In summary, ICV OXM causes reduced body weight gain and body adiposity following chronic administration.

Glucagon-like peptide-1; energy expenditure; core temperature

OXYNTOMODULIN (OXM) IS A PRODUCT OF POSTTRANSLATIONAL PROCESSING OF PROGLUCAGON IN THE INTESTINE AND CENTRAL NERVOUS SYSTEM (CNS). Its amino acid sequence is highly conserved among species, which suggests an important physiological role. OXM is released from intestinal L cells in response to nutrient intake and is known to be a potent inhibitor of gastric acid secretion and gastric emptying in rodents and humans (7, 22). OXM is also present in the hypothalamus, an area of the brain known to be involved in the regulation of appetite, body weight, and endocrine function. However, since its characterization and isolation in the early 1980s, little has been reported about the role of OXM within the CNS despite the apparent abundance of OXM peptide in the hypothalamus (3, 16).

We have recently shown (6) that a single injection of OXM into the third cerebral ventricle (ICV) and into the hypothalamic paraventricular nucleus (PVN) causes a robust and sustained reduction of food intake in 24-h-fasted rats, associated with an increase in activity, indicating that the anorectic effect is not due to behavioral abnormalities.

Glucagon-like peptide-1 (GLP-1) is another product of proglucagon processing in the intestine and the CNS and is a potential satiety factor (25, 26) and regulator of energy homeostasis (10, 11, 14). It has been hypothesized that OXM mediates its actions via the GLP-1 receptor (6, 12, 21). We have previously reported (6) that the magnitude of the inhibition of food intake by GLP-1 and OXM is similar. Moreover, the anorectic actions of OXM are completely reversed by co-administration of the high-affinity GLP-1 receptor antagonist exendin-9–39. This supports the hypothesis that OXM works via the GLP-1 receptor.

Repeated daily ICV injections of GLP-1 caused a significant inhibition in cumulative body weight gain in rats fed to a 4-h schedule (18). Conversely, chronic antagonism of the GLP-1 receptor by administration of exendin-9–39 caused an increase in daily food intake and body weight gain (18). The aim of this experiment was to administer OXM ICV twice daily for 7 days and to examine its effect on daily food intake, body weight gain, adiposity, core temperature [as a marker of metabolic rate (2, 13)], and the hypothalamic-pituitary-thyroid axis. Furthermore, we aimed to determine whether any observed actions of OXM were attributable solely to the reduction in food intake or whether there were additional mechanisms involved, for example, changes in energy expenditure. This was achieved by the inclusion of a food-restricted “pair-fed” group. These animals were ICV injected with saline but received only the mean food intake of the OXM-treated group.

Address for reprint requests and other correspondence: S. R. Bloom, Endocrine Unit, Imperial College Faculty of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom (E-mail: s.bloom@ic.ac.uk).

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MATERIALS AND METHODS

Peptides and chemicals. Porcine OXM was purchased from IAP BioChem Pharma (Laval, Canada). All other chemicals were purchased from Merck Eurolab (Leicestershire, UK) unless otherwise stated.

Animals and surgery. Adult male Wistar rats (Imperial College, Hammersmith Hospital) were maintained in individual cages under controlled conditions of temperature (21–23°C) and light (lights on at 0700, lights off at 1900) with ad libitum access to food (RM1 Diet, Special Diet Services UK, Witham, UK) and water. Animals were handled daily after recovery from surgery until completion of the study. All animal procedures undertaken were approved by the British Home Office Animals (Scientific Procedures) Act 1986 (Project License PIL 70/5281). Animals had permanent stainless steel guide cannulae (Plastics One, Roanoke, VA) stereotactically implanted into the lateral cerebral ventricle (8) [1.5 mm posterior to bregma, 0.5 mm lateral to the mid sagittal line, 2.5 mm below the outer surface of the skull (19)], which were kept patent until injection by insertion of a dummy stylet. OXM or saline was administered as previously described (8). From the time of surgery, animals were housed singly throughout the experiment.

Experimental protocol. After a 7-day postsurgery recovery period, 45 ICV-cannulated animals were randomized by weight into three groups (n = 15 per group): group 1 (saline controls), which received twice-daily ICV injections of saline (at 0800 and 1830) for 7 days with ad libitum access to food; group 2 (OXM-treated), which received twice-daily ICV injections of OXM (1 nmol at 0800 and 1830) for 7 days with ad libitum access to food, the dose chosen being one that we (6) have previously shown to cause a potent, but not maximal, inhibition of food intake; and group 3 (food-restricted pair-fed controls), which received twice-daily ICV injections of saline (at 0800 and 1830) for 7 days but were food restricted to receive the average daily food intake of the OXM-treated group during both light and dark phases.

The pair-fed animals received their food immediately after each injection, food being provided for the light and dark phases. The aim was to ensure that the food intake of the different light phases was matched and that the entire daily amount of food was not consumed at one time.

The bioactivity of OXM is not retained when in solution at 37°C. Therefore, it was not possible to use Alzet osmotic minipumps to deliver the peptide throughout the study. For this reason, repeated ICV injections were required. Fresh OXM peptide was used on each occasion to prevent any potential loss of bioactivity.

Food intake (g) was measured before each injection and 2 h after injection to validate the bioactivity of the peptide used. Furthermore, whereas food intake was measured at lights on and just before lights off, the average light- and dark-phase food intake by the OXM-treated group could be calculated for the saline-treated pair-fed group. Body weight (g) was recorded daily from 3 days before the start of the experiment until its completion.

Measurement of body temperature in the rats was accomplished using a rectal probe thermocouple thermometer (Physiotemp, model BAT-12). The lubricated probe was inserted 3.5 cm until a stable temperature reading was obtained. Rectal temperature (°C) was measured at 1030 from 2 days before the onset of the study until completion of the study. Results are expressed as changes in temperature (ΔT), compared with the constant temperature of the vehicle-treated rats (37.4°C). It was observed that this procedure did not cause distress.

The morning after the last injection, the animals were decapitated (between 0830 and 0930). Their trunk blood was collected in lithium-heparin-coated plastic tubes containing 0.6 mg of aprotinin (Trasylool; Bayer, Haywards Heath, UK). The samples were centrifuged, and the plasma was separated and stored at −20°C until time of assay. Epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were removed and weighed.

Hypothalamic explant static incubation system. To further investigate the effect of OXM on the hypothalamo-pituitary-thyroid axis, a static incubation system was used as described previously (24). Male Wistar rats were killed by decapitation (between 0900 and 1000), and the whole brain was removed immediately. The brain was mounted ventral surface uppermost and placed in a vibrating microtome (Microfield Scientific, Dartmouth, UK). A 1.7-mm slice was taken from the basal hypothalamus, blocked lateral to the Circle of Willis, and incubated in chambers containing 1 ml of artificial cerebrospinal fluid (aCSF) (24), which was equilibrated with 95% O2 and 5% CO2. The hypothalamic slice encompassed the medial preoptic area, PVN, dorsomedial nucleus, ventromedial nucleus, lateral hypothalamus, and arcuate nucleus. The tubes were placed on a platform in a water bath maintained at 37°C. After an initial 2-h equilibration period, each explant was incubated for 45 min in 600 μl of aCSF (basal period) before being challenged with a test period (OXM, 100 nM dose, representing a concentration 10 times that of its IC50 for the GLP-1 receptor) for 45 min (6). The viability of the tissue was confirmed by a final 45-min exposure to aCSF containing 56 mM KCl. At the end of each experimental period, the aCSF was removed and stored at −20°C until measurement of TRH immunoreactivity by radioimmunoassay.

Assays. Free triiodothyronine (free T3) and free thyroxine (free T4) were measured using solid-phase radioimmunoassay (DPC, Los Angeles, CA). TSH levels in plasma were assayed using reagents and methods provided by the National Institute of Diabetes and Digestive and Kidney Diseases and the National Hormone and Pituitary Program (Dr. A. Parlow, Harbor University of California, Los Angeles Medical Center) as previously described (1). TRH levels in aCSF were assayed using reagents kindly provided by H. M. Fraser (Medical Research Centre, Reproductive Biology Unit, Edinburgh, Scotland), as described previously (1).

Statistical analysis. Cumulative food intake, body weight gain, and change in rectal temperature were analyzed by ANOVA with a post hoc least significant difference test. The gain, and change in rectal temperature were analyzed by paired Student t-test. In all cases, P < 0.05 was considered to be statistically significant.

RESULTS

Effect of chronic OXM on food intake and body weight. Daily food intake was lower in OXM-treated animals from day 1 with no tachyphylaxis (day 7: OXM, 182 ± 1.9 g vs. saline, 202 ± 1.5 g, P < 0.005; Fig. 1). Animals treated with OXM gained weight significantly slower than those treated with saline (day 8: OXM, 12.2 ± 1.9 g vs. saline, 24.0 ± 2.1 g, P < 0.005; Fig. 2). Animals treated with saline but restricted to the mean food intake of the OXM-treated group gained less weight than saline-injected ad libitum-fed animals; however, this difference was not statistically
significant [day 8: pair fed, 21.0 ± 2.1 g gain vs. saline, 24.0 ± 2.1 g gain, \( P = \) not significant (NS)]. Pair-fed animals ate all of the food that was offered each day during both the light and the dark phases.

**Effect of chronic ICV OXM on rectal temperature.** The temperature of saline-treated animals was 37.4°C throughout the experiment, and the temperature of saline in OXM-treated and pair-fed groups was calculated relative to 37.4°C. After the first injection of OXM, there was a dramatic hyperthermic response, which was sustained until day 3 of the study (day 3: \( \Delta T \) OXM = +0.5 ± 0.04°C vs. saline control, \( P < 0.05 \)). On day 4, the temperature of OXM-treated animals fell, although it was still higher than that of those treated with saline. This significant hyperthermic response was still evident on the final day of the study (day 7: \( \Delta T \) OXM, +0.1 ± 0.04°C vs. saline control, \( P < 0.05 \); Fig. 3). Toward the end of the study, the pair-fed animals exhibited a significant hyperthermic response compared with saline-injected ad libitum-fed controls (day 7: \( \Delta T \) pair fed, −0.4 ± 0.06°C vs. ad libitum-fed control, \( P < 0.05 \)).

**Effect of chronic ICV OXM on adiposity.** Epididymal WAT and interscapular BAT weighed significantly less in OXM-treated animals than in saline-treated control animals [WAT: OXM, 1.13 ± 0.03 g vs. saline, 1.31 ± 0.05 g (\( P < 0.05 \)); BAT: OXM, 0.15 ± 0.01 g vs. saline, 0.20 ± 0.01 g (\( P < 0.05 \); Fig. 4)]. However, the fat pads of pair-fed animals were not significantly different in weight compared with those from saline-treated animals [WAT pair fed: 1.29 ± 0.04 g vs. saline (\( P = NS \)); BAT pair fed: 0.18 ± 0.01 g vs. saline (\( P = NS \)]. The alterations in BAT weight might be a result of decreased adiposity after OXM treatment. Functional analysis of the BAT was beyond the scope of the current investigation.

**Effect of ICV chronic OXM on parameters of the thyroid axis.** Repeated ICV injection of OXM for 7 days did not affect plasma levels of TSH. However, pair feeding did lead to a significant suppression of plasma TSH levels [OXM, 2.2 ± 0.15 ng/ml (\( P = NS \)); pair fed, 1.7 ± 0.12 ng/ml (\( P < 0.05 \)) vs. saline, 2.3 ± 0.13 ng/ml; Fig. 5]. There was no effect on plasma free T3 or free T4 (not shown).

**Effect of OXM on TRH release from hypothalamic explants.** Incubating OXM (100 nM) with hypothalamic explants caused a significant increase in TRH release [basal, 52.9 ± 14.2 fmol/explant vs. OXM, 107 ± 12.9 fmol/explant (\( P < 0.01 \); Fig. 6)]. Explant viability, as confirmed by potassium stimulation was >80%. Nonviable explants were excluded from data analysis.
DISCUSSION

Twice-daily injection of ICV administration of OXM for 7 days caused a reduction in daily food intake with no tachyphylaxis. Furthermore, there was a significant reduction in body weight gain that was associated with a decrease in adipose tissue. Animals were observed daily and showed no signs of illness or behavioral abnormality. In the pair-fed group, animals gained slightly less weight than control animals, although this was not statistically significant. However, the OXM-treated animals gained significantly less weight than either of the two control groups. Furthermore, the reduction in WAT and BAT observed after chronic ICV OXM administration was not observed in the pair-fed group. This is suggestive of OXM causing weight loss and reduced body adiposity by mechanisms additional to its effects on food intake.

It is known that, during fasting and chronic food restriction, the expression of prepro-TRH in the PVN is suppressed. This reduces the secretion of TSH from the pituitary, which, in turn, causes a general reduction in thyroid function (4, 5, 20). In this experiment, pair feeding caused a significant suppression in plasma TSH. Whereas OXM-treated and pair-fed animals received the same daily food intake, it was expected that the plasma TSH levels would also be reduced in OXM-treated animals; however, this was not the case. It has previously been reported that α-melanocyte-stimulating hormone (α-MSH), a product of proopiomelanocortin processing in the CNS, prevents the suppression of TSH caused by fasting (9). This effect is thought to be due to α-MSH increasing the expression and release of TRH in the PVN, leading to a subsequent increase in plasma TSH. To establish whether OXM is also acting in a similar manner, we measured the release of TRH from hypothalamic explants incubated with OXM and found it to be significantly stimulated. This, therefore, provides a potential mechanism through which OXM is preventing the suppression in plasma TSH seen as a result of chronic food restriction.

Core temperature is known to be linked to energy expenditure. We measured rectal temperature daily throughout the study and found that pair-fed animals tended to exhibit a slightly hypothermic response, which was statistically significant by the final day of the study. It has been shown previously that starvation
and chronic food restriction in rats can lead to a reduction in body core temperature (17, 23). One explanation for this hypothermic response to a low energy intake is that there is a change in temperature to conserve energy. Our findings are consistent with these reports. Chronic ICV OXM administration caused a sustained hyperthermic response despite a reduced food intake. Although initially more dramatic, this increase in rectal temperature was sustained throughout the study. Previous studies have shown that administration of anorectic peptides, for example, leptin (15) and corticotropin-releasing factor (13), cause an increase in core temperature. This would suggest that OXM is raising the thermoregulatory set point and thereby increasing energy expenditure.

In conclusion, our findings indicate that, like GLP-1, chronic administration of OXM can reduce body weight gain and that this effect is not due solely to the reduction in daily food intake. There is a decrease in white adipose tissue and an increase in core temperature in OXM-treated animals that is not seen in pair-fed animals, suggesting that, in addition to inhibiting food intake, OXM might also enhance energy expenditure. Chronic CNS OXM administration decreases food intake and body weight gain and might represent a novel hypothalamic target for the treatment of obesity.

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