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GIP-dependent expression of hypothalamic genes

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SUMMARY

GIP (glucose dependent insulinotrophic polypeptide), originally identified as an incretin peptide synthesized in the gut, has recently been identified, along with its receptors (GIPR), in the brain. Our objective was to investigate the role of GIP in hypothalamic gene expression of biomarkers linked to regulating energy balance and feeding behavior related neurocircuitry. Rats with lateral cerebroventricular cannulas were administered 10 μg GIP or 10 μl artificial cerebrospinal fluid (*a*CSF) daily for 4 days, after which whole hypothalami were collected. Real time Taqman™ RT-PCR was used to quantitatively compare the mRNA expression levels of a set of genes in the hypothalamus. Administration of GIP resulted in up-regulation of hypothalamic mRNA levels of AVP (46.9±4.5%), CART (25.9±2.7%), CREB1 (38.5±4.5%), GABRD (67.1±11%), JAK2 (22.1±3.6%), MAPK1 (33.8±7.8%), NPY (25.3 ± 5.3%), OXT (49.1±5.1%), STAT3 (21.6±3.8%), and TH (33.9±8.5%). In a second experiment the same set of genes was evaluated in $GIPR^{-/-}$ and $GIPR^{+/?}$ mice to determine the effect of lack of GIP stimulation on gene expression. In $GIPR^{-/-}$ mice expressions of the following genes were down-regulated: AVP (27.1±7.5%), CART (28.3±3.7%), OXT (25.2±5.8%), PTGES (23.9 ± 4.5%), and STAT3 (8.8±2.3%). These results suggest that AVP, CART, OXT and STAT3 may be involved in energy balance-related hypothalamic circuits affected by GIP

Key words: GIP receptor knockout; ICV administration; mRNA expression, rats, mice

INTRODUCTION

Gastric inhibitory polypeptide (GIP) is a 42 amino acid peptide hormone, synthesized by K cells in the intestinal epithelium (Buchan et al., 1978) and released into the circulation in response to nutrient ingestion. It was renamed glucose dependent insulinotropic polypeptide, reflecting its important role as a hormone in gut physiology. GIP stimulates insulin secretion, and potentiates the action of insulin in adipose tissue and other target tissues (Moens et al., 1996, Wideman and Kieffer, 2004, Yip and Wolfe, 2000). GIP receptors were reported to be present in the pancreas, gut, adipose tissue, adrenal cortex and heart, and pituitary, as well as in several brain regions, including the cerebral cortex, hippocampus, hypothalamus and olfactory bulb (Kaplan and Vigna, 1994, Usdin et al., 1993). Expression of GIP has also been reported in these brain regions (Nyberg et al., 2005, Sondhi et al., 2006).

The secretin family of peptides, of which GIP is a member, have been reported to be involved in a variety of brain functions such as neuro-modulation, neurogenesis, brain development, cell-cycle regulation, differentiation, and cell death as well as in regulation of food intake, body weight and body temperature (Nyberg et al., 2005, Sherwood et al., 2000). GIP also appears to have a role in bone metabolism, with GIP receptor knockout ($GIPR^{-/-}$) mice exhibiting reduced bone mass, whereas transgenic mice with GIP overexpression have increased bone mass (Ding et al., 2008).

GIP regulates lipid metabolism and storage (Holst, 2004) partly through its effect on insulin secretion and the resulting increased uptake and incorporation of glucose into lipids, as well as through direct effects in adipose tissue (Hauner et al., 1988, Wasada T, 1981, Wideman and Kieffer, 2004, Yip and Wolfe, 2000). GIPR$^{-/-}$ mice were found to be resistant to diet-induced obesity and insulin resistance, and GIP has been considered as a potential pharmaceutical target in the treatment of diabetes, obesity and other metabolic disorders (Kieffer, 2003, Miyawaki et al., 2002).

The objective of this study was to determine whether central administration of GIP altered expression of genes known to be involved in regulation of food intake and body energy balance and whether GIP receptor deficiency would result in opposite changes in expression of some or all of those genes. We report that GIP administered centrally to rats reduced body weight gain and induced changes in several genes involved in energy balance regulation. Furthermore, we identified a subset of those genes showing the opposite changes in expression in GIPR$^{-/-}$ mice compared to GIPR$^{+/?}$ mice.

Material and Methods:

Male Sprague-Dawley rats (250-274 g) purchased from Harlan, Inc. (Indianapolis, IN) were used in the experiments 1 and 2. Ten week old C57BL6 GIPR $^{+/?}$ (n=20) and GIPR $^{-/-}$ mice (n=18) were obtained from Dr. Carlos Isales (Medical College of Georgia). Two pairs of homozygous mice (GIPR$^{-/-}$) with targeted disruptions of exon 4 and 5 of the GIPR gene were originally obtained from the laboratory of Dr. Yuichiro Yamada (Department of Metabolism and Clinical Nutrition, Kyoto University Graduate School of

Medicine, Kyoto, Japan), from which a breeding colony was established. The GIPR knockout mice were generated, bred, and maintained in the C57BL/6 line (Miyawaki et al., 1999). These mice were bred and maintained on a C57Bl/6 genetic background in Dr. Isales' facility. Mice were kept four animals/cage, at 25°C with a 12/12 h light/dark cycle. They had free access to a standard diet (Harlan TakLad Rodent Diet (W) 8604) and water ad libitum. Rats were housed singly in a light (12 h on/12h off) - and temperature controlled environment (22±1 °C, humidity 50%). Rats were fed standard rodent chow and provided with water *ad libitum*.

Effect of icv injection of GIP on food intake and weight gain in rats

Rats were implanted with chronic lateral cerebroventricular cannulas as previously described (Choi et al., 2003), and after recovery from surgery, they were randomly assigned to four groups (n=8). Control rats were administered 10 µl artificial cerebrospinal fluid (*a*CSF) at 24-hour intervals for 4 days. The GIP treated rats were administered 0.1, 1.0 or 10 µg/day GIP in 10 µl *a*CSF for 4 days. The injections were carried out using an injector cannula (C313I, Plastics One, Roanoke, VA) that protruded 1.1 mm below the tip of the guide cannula and that was connected to a Gilmont microsyringe by way of polyethylene tubing (PE20 Intramedic, Cat. #427406, BD, Sparks, MD). Food and water were available *ad libitum*. Food intake was measured daily and rats were weighed daily. All experiments and surgical procedures were performed in accordance with the guidelines approved by the Institutional Animal Care

and Use Committee of the University of Georgia. Twenty-four hours after the last icv injection, rats were killed by decapitation after a brief exposure to carbon dioxide.

Effect of icv injection of GIP on gene expression in rats

Rats were implanted with chronic lateral cerebroventricular cannulas as previously described (Choi et al., 2003), and after recovery from surgery, they were randomly divided into two groups (n=8). Control rats were administered 10 µl artificial cerebrospinal fluid (*a*CSF) at 24-hour intervals for 4 days; the GIP-treated rats were administered 10 µg/day GIP in 10 µl *a*CSF for 4 days. The dose used was selected based on the results of the previous experiment. Food and water were available *ad libitum*. Twenty-four hours after the last injection rats were killed by decapitation after a brief exposure to carbon dioxide. The brains were removed rapidly after decapitation and immediately frozen by placing them on plastic cassettes on top of powdered dry ice. Once completely frozen, they were stored at -80ºC. The brains were thawed to -20ºC before the hypothalamic dissection.

Hypothalamic gene expression in GIPR$^{-/-}$ mice

In this experiment the hypothalamic gene expression profile of GIPR$^{+/?}$ mice was compared to that from GIPR$^{-/-}$ mice. Mice were deeply anesthetized with CO_2 before decapitation. The brains were removed rapidly after decapitation and immediately frozen on powdered dry ice. They were stored at -80ºC. The brains were thawed to -20ºC

before the hypothalamic dissection. Hypothalamic blocks from two mice of the same group were randomly pooled together for RNA extraction.

Extraction of Total RNA, Reverse Transcription (RT) and Real-Time PCR

Total hypothalamic RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's protocol. The integrity of the total RNA obtained from all the samples were verified using the RNA 6000 Nano Assay and the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The RT reaction was performed on 100 ng of total RNA per sample in a 20 µl reaction mixture using the *c*DNA Archive Kit with MultiScribe™ Reverse Transcriptase (Applied Biosystems, Inc., ABI, Foster City, CA) according to the manufacturer's instructions. Reactions were incubated initially at 25°C for 10 min and subsequently at 37°C for 120 min. Quantitative PCR (Taqman™) assays chosen for the transcripts to be evaluated were from Assays-On-Demand™ (ABI), a pre-validated library of QPCR assays and were incorporated into 384-well MicroFluidic™ cards. All of the oligonucleotide primer and fluorogenic probe sets for Taqman™ real time PCR in rats and mice were from ABI (**Table 1**). Two µl of the cDNA along with 50 µl of 2 x PCR master mixes were loaded into respective channels on the microfluidic card followed by a brief centrifugation. The card was then sealed and real-time PCR and relative quantification were carried out on the ABI PRISM 7900 Sequence Detection System. The cycle conditions were: 94.5^{o}C for 15 min, followed by 40 cycles of 97°C for 30 s, 59.7^{o}C for 1 min. Data were analyzed using Sequence Detection Systems software (Applied Biosystems) and the Relative Quantification (RQ)

method, which represents the fold difference of mRNA level in rats treated with GIP relative to rats treated with control or the $GIPR^{-/-}$ mice relative to $GIPR^{+/?}$ mice. Preliminary experiments were carried out to determine the most appropriate endogenous control gene to use to correct for differences in the amounts of total RNA added to each reaction. For the rat experiment, mRNA expressions were normalized with 18 S as an endogenous control to correct the differences in the amount of total RNA added to each reaction. β-ACT was used as endogenous control in the mouse experiment. The ΔC_T values were calculated initially using C_T for a specific gene mRNA minus C_T for 18 S mRNA (in rats) and β-ACT (in the mice) in the sample. The mean mRNA expressions from the rats treated with GIP were compared with those from the *a*CSF treated rats and mRNA expressions from the $GIPR^{-/-}$ mice were compared to those from the $GIPR^{+/?}$ mice using the formula: Relative Quantification (RQ) = $2^{-\Delta\Delta CT}$ ($\Delta\Delta C_T$ is the average control group ΔC_T values minus the average experimental group ΔC_T values, and $\Delta\Delta C_T$ of 1 equates to a two-fold difference in starting amount of *c*DNA). The relative quantification values from each gene were used to compare the hypothalamic gene expression between two groups.

Statistical Analysis

Food intake and body weight gain data were analyzed using the general linear model ANOVA (Statistica v. 7.1; StatSoft, Inc., Tulsa, OK). Fishers LSD test was used to determine significance among means, with $p<0.05$ considered significant. Data are expressed as means ± SEM.

Gene expression data are shown as means ± SEM of relative quantification (RQ) values from the Sequence Detection System (SDS, ABI-Perkin Elmer, Foster City, CA) files for

all the genes. Statistical significance was assessed by general linear model ANOVA for multiple comparisons between the means for the different treatment/genotype groups. The Benjamini–Hochberg procedure was used to control for false discovery rate. A $p<0.05$ was considered to be significant. Multivariate analysis between genes was performed with Pearson correlation.

RESULTS

Effect of icv administration of GIP on food intake and body weight of rats.

GIP administered icv for 4 days in rats had no effect on food intake (data not shown). However, 10 µg/day GIP caused a significant loss of body weight compared to control and the other GIP treatments (Fig. 1). This dose was then selected for determination of effects on hypothalamic gene expression.

Effect of icv administration of GIP on hypothalamic gene expression in rats

Increased hypothalamic mRNA expression levels were found for AVP (46.9±4.5%), CART (25.9±2.7%), CREB1 (38.5±4.5%), GABRD (67.1±11%), JAK2 (22.1±3.6%), MAPK1 (33.8±7.8%), NPY (25.3 ± 5.3%), OXT (49.1±5.1%), STAT3 (21.6±3.8%), and TH (33.9±8.5%). after 4 days of icv injections of 10 µg/day GIP in rats **(Table 2)**.

Correlation between CREB1 and MAPK1 mRNA expression levels in GIP-treated rats

The mRNA levels of both CREB1 and MAPK1 showed a proportional increase with a positive correlation (Correlation coefficient, $r = 0.87$, $p<0.001$) in the hypothalamus of GIP-treated rats.

Hypothalamic gene expression in GIPR$^{-/-}$ and GIPR$^{+/?}$ mice

The hypothalamic mRNA levels of some biomarkers were lower in hypothalami of GIPR$^{-/-}$ mice when compared with those of wild-type controls. Relative to GIPR$^{+/?}$ mice, expression levels of hypothalamic mRNAs of AVP (27.1$\pm$7.5%), CART (28.3$\pm$3.7%), OXT (25.2$\pm$5.8%), PTGES (23.9 ± 4.5%), and STAT3 (8.8$\pm$2.3%) in GIPR$^{-/-}$ mice were down-regulated (**Table 2**).

Hypothalamic mRNA expression of MAPK1 (38.2$\pm$4.0%, $p < 0.01$) and NPY (31.6+8.5%, $p < 0.01$) was increased in GIPR$^{-/-}$ mice relative to GIPR$^{+/?}$ mice.

DISCUSSION

GIP is considered to be one of the principle incretin factors of the enteroinsular axis system which has been implicated to play a critical role in the pathogenesis of diabetes and obesity (Wideman and Kieffer, 2004, Yip and Wolfe, 2000). The current study was designed to investigate the alterations in expression of hypothalamic genes involved in feeding behavior and energy balance regulation in rats treated icv with GIP and in GIPR deficient mice.

AVP is suggested to trigger a variety of effects relating to regulation of water, glucose and electrolytes and neuroendocrine functions and may also play a role in energy balance regulation (Bray, 2000, Landgraf, 2006). The structurally-related neuropeptide oxytocin (OXT) has also been implicated in feeding, complex social behaviors and anxiety-associated behaviors (Bale et al., 2001, Sabatier et al., 2007). Higher levels of AVP and

OXT mRNA expression were found following icv GIP treatment in rats. Furthermore, both AVP and OXT expressions were lower in $GIPR^{-/-}$ mice, thus suggesting that these hypothalamic neuropeptides may mediate GIP-induced behavioral changes related to energy balance regulation.

Cocaine- and amphetamine-regulated transcript (CART) is highly expressed in the hypothalamus, particularly in the paraventricular (PVN) and arcuate (ARC) nuclei (Couceyro et al., 1997), which are critical in the regulation of energy homeostasis. CART was originally reported to be an anorectic neuropeptide, based on icv administration of CART(55-102) (Kristensen et al., 1998, Stanley et al., 2001) and on overexpression of CART after administration of recombinant adeno-associated virus (rAAV)-CART into the third ventricle in diet-induced obese rats (Qing and Chen, 2007). However, icv administration of CART is also associated with motor disturbances which may interfere with feeding (Aja et al., 2001). In contrast, direct administration of CART (55–102) into discrete hypothalamic nuclei (Abbott et al., 2001) and over-expression of CART in the ARC (Kong et al., 2003) and PVN (Smith et al., 2008) have been shown to increase food intake and weight gain.

In our study hypothalamic CART expression was increased in rats treated icv with GIP and decreased in mice deficient in GIPR. Although GIP did not affect food intake in the rats, it did cause weight loss, and a previous study has shown that overexpression of GIP in mice resulted in enhanced locomotor behavior and no increase in anxiety behavior (Ding et al., 2006). Thus, the changes in whole hypothalamic CART expression may be

related to the effect of GIP on energy balance regulation, but probably not through changes in anxiety or locomotor behavior.

STAT proteins are a family of cytoplasmic transcription factors activated by tyrosine phosphorylation and dimerization in response to cytokines, growth factors and other extracellular ligands (Dandoy-Dron et al., 1995, Sano et al., 1999). In particular, STAT3 has been shown to play an important role in the regulation of energy balance. STAT3 is a known downstream component of both leptin and ciliary neurotrophic factor (CNTF) action (Hubschle et al., 2001, Lambert et al., 2001). Mice with STAT3 deletion in leptin receptor positive neurons of the hypothalamus become hyperphagic after weaning, have increased body weight and body fat, and exhibit impaired glucose tolerance (Cui et al., 2004). While phosphorylation levels of STAT3 are believed to be more critical than variations of mRNA level, our previous study showed that mRNA levels of STAT3 in the arcuate nucleus/median eminence of the hypothalamus were significantly up-regulated in rats with ICV treatment of leptin or CNTF (Ambati et al., 2007). The results from the present study show increased expression of STAT3 in the hypothalamus of rats treated icv with GIP and decreased expression of hypothalamic STAT3 in mice deficient in GIPR. Thus, STAT3 may also be a downstream transcription factor involved in the central effect of GIP on energy balance regulation.

GABRD is a receptor for gamma-aminobutyric acid, an inhibitory neuropeptide involved in a number of hypothalamic and higher brain functions, including neuronal network development, cognition and speech (Windpassinger et al., 2002). CREB1 protein is a transcription factor and usually considered a marker of neuron activation (Zachariou et

al., 2001). In rats treated icv with GIP, GABRD and CREB1 mRNA expression was increased, suggesting elevated neuronal activity as a response to GIP treatment. Interestingly, proportionate increase in mRNA expression levels of CREB1 and MAPK1 in the hypothalamus of GIP-treated rats indicated a possible MAPK-1 signaling-dependent neuron activation by CREB1 (Euskirchen et al., 2004, Pandey, 2003). CREB1 plays a regulatory role in the nervous system and is widely believed to promote neuronal survival, precursor proliferation and other neuronal-related functions (Rodriguez and Ferrer, 2007). Phosphorylation of CREB1 through MAPK signaling is a critical event in the network of neuronal activation, neuronal proliferation, differentiation and synaptic function. Activation of this network also stimulates neurite outgrowth in the central nervous system and also in neuronal cell-lines (Ma'ayan et al., 2009). Furthermore, there is evidence that phosphorylation of CREB regulates NPY expression in the brain (Pandey, 2003).

In conclusion, our data suggest that GIP and its receptor may be involved in energy balance regulation by the hypothalamus as evident by the changes in expression levels of genes such as AVP, CART, OXT, and STAT3.

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REFERENCES

ABBOTT CR, ROSSI M, WREN AM, MURPHY KG, KENNEDY AR, STANLEY SA, ZOLLNER AN, MORGAN DG, MORGAN I, GHATEI MA, SMALL CJ, AND BLOOM SR. Evidence of an orexigenic role for cocaine- and amphetamine-regulated transcript after administration into discrete hypothalamic nuclei. *Endocrinology* **142**: 3457-3463, 2001.

AJA S, SCHWARTZ GJ, KUHAR MJ, AND MORAN TH. Intracerebroventricular CART peptide reduces rat ingestive behavior and alters licking microstructure. *Am J Physiol Regul Integr Comp Physiol* **280**: R1613-1619, 2001.

ALTAREJOS JY AND MONTMINY M. CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat Rev Mol Cell Biol* **12**: 141-151, 2011.

AMBATI S, DUAN J, CHOI YH, HARTZELL DL, DELLA-FERA MA, AND BAILE CA. ICV vs. VMH injection of leptin: Comparative effects on hypothalamic gene expression. *Behav Brain Res* **196**: 279-285, 2009.

AMBATI S, DUAN J, DUFF E, CHOI YH, HARTZELL DL, DELLA-FERA MA, AND BAILE CA. Gene expression in arcuate nucleus of rats treated with leptin or ciliary neurotrophic factor. *Biofactors* **31**: 133-144, 2007.

ASAKAWA A, INUI A, YUZURIHA H, NAGATA T, KAGA T, UENO N, FUJINO MA, AND KASUGA M. Cocaine-amphetamine-regulated transcript influences energy metabolism, anxiety and gastric emptying in mice. *Horm Metab Res* **33**: 554-558, 2001.

AUERNHAMMER CJ, BOUSQUET C, CHESNOKOVA V, AND MELMED S. SOCS proteins: modulators of neuroimmunoendocrine functions. Impact on corticotroph LIF signaling. *Ann N Y Acad Sci* **917**: 658-664, 2000.

BALE TL, DAVIS AM, AUGER AP, DORSA DM, AND MCCARTHY MM. CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior. *J Neurosci* **21**: 2546-2552, 2001.

BOSTON BA. Pro-opiomelanocortin and weight regulation: from mice to men. *J Pediatr Endocrinol Metab* **14 Suppl 6**: 1409-1416, 2001.

BRAY GA. Afferent signals regulating food intake. *Proc Nutr Soc* **59**: 373-384., 2000.

BUCHAN AM, POLAK JM, CAPELLA C, SOLCIA E, AND PEARSE AG. Electronimmunocytochemical evidence for the K cell localization of gastric inhibitory polypeptide (GIP) in man. *Histochemistry* **56**: 37-44, 1978.

CARLEZON WA, JR., DUMAN RS, AND NESTLER EJ. The many faces of CREB. *Trends Neurosci* **28**: 436-445, 2005.

CARVALHEIRA JB, SILOTO RM, IGNACCHITTI I, BRENELLI SL, CARVALHO CR, LEITE A, VELLOSO LA, GONTIJO JA, AND SAAD MJ. Insulin modulates leptin-induced STAT3 activation in rat hypothalamus. *FEBS Lett* **500**: 119-124, 2001.

CHENG CY, CHU JY, AND CHOW BK. Central and peripheral administration of secretin inhibits food intake in mice through the activation of the melanocortin system. *Neuropsychopharmacology* **36**: 459-471, 2011.

CHOI YH, LI C, PAGE K, WESTBY A, DELLA-FERA MA, LIN J, HARTZELL DL, AND BAILE CA. Melanocortin receptors mediate leptin effects on feeding and body weight but not adipose apoptosis. *Physiol Behav* **79**: 795-801, 2003.

COOGAN AN AND PIGGINS HD. MAP kinases in the mammalian circadian system--key regulators of clock function. *J Neurochem* **90**: 769-775, 2004.

COUCEYRO PR, KOYLU EO, AND KUHAR MJ. Further studies on the anatomical distribution of CART by in situ hybridization. *J Chem Neuroanat* **12**: 229-241, 1997.

CUI Y, HUANG L, ELEFTERIOU F, YANG G, SHELTON JM, GILES JE, OZ OK, POURBAHRAMI T, LU CY, RICHARDSON JA, KARSENTY G, AND LI C. Essential role of STAT3 in body weight and glucose homeostasis. *Mol Cell Biol* **24**: 258-269, 2004.

DANDOY-DRON F, ITIER JM, MONTHIOUX E, BUCCHINI D, AND JAMI J. Tissue-specific expression of the rat insulin 1 gene in vivo requires both the enhancer and promoter regions. *Differentiation* **58**: 291-295, 1995.

DING KH, SHI XM, ZHONG Q, KANG B, XIE D, BOLLAG WB, BOLLAG RJ, HILL W, WASHINGTON W, MI QS, INSOGNA K, CHUTKAN N, HAMRICK M, AND ISALES CM. Impact of glucose-dependent insulinotropic peptide on age-induced bone loss. *J Bone Miner Res* **23**: 536-543, 2008.

DING KH, ZHONG Q, XIE D, CHEN HX, DELLA-FERA MA, BOLLAG RJ, BOLLAG WB, GUJRAL R, KANG B, SRIDHAR S, BAILE C, CURL W, AND ISALES CM. Effects of glucose-dependent insulinotropic peptide on behavior. *Peptides* **27**: 2750-2755, 2006.

DUMAN CH AND DUMAN RS. Neurobiology and treatment of anxiety: signal transduction and neural plasticity. *Handb Exp Pharmacol*: 305-334, 2005.

ELEFTERIOU F, AHN JD, TAKEDA S, STARBUCK M, YANG X, LIU X, KONDO H, RICHARDS WG, BANNON TW, NODA M, CLEMENT K, VAISSE C, AND KARSENTY G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature* **434**: 514-520, 2005.

EUSKIRCHEN G, ROYCE TE, BERTONE P, MARTONE R, RINN JL, NELSON FK, SAYWARD F, LUSCOMBE NM, MILLER P, GERSTEIN M, WEISSMAN S, AND SNYDER M. CREB binds to multiple loci on human chromosome 22. *Mol Cell Biol* **24**: 3804-3814, 2004.

GAO Q, MEZEI G, NIE Y, RAO Y, CHOI CS, BECHMANN I, LERANTH C, TORAN-ALLERAND D, PRIEST CA, ROBERTS JL, GAO XB, MOBBS C, SHULMAN GI, DIANO S, AND HORVATH TL. Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. *Nat Med* **13**: 89-94, 2007.

GEORGESCU D, SEARS RM, HOMMEL JD, BARROT M, BOLANOS CA, MARSH DJ, BEDNAREK MA, BIBB JA, MARATOS-FLIER E, NESTLER EJ, AND DILEONE RJ. The hypothalamic neuropeptide melanin-concentrating hormone acts in the nucleus accumbens to modulate feeding behavior and forced-swim performance. *J Neurosci* **25**: 2933-2940, 2005.

HAUNER H, GLATTING G, KAMINSKA D, AND PFEIFFER EF. Effects of gastric inhibitory polypeptide on glucose and lipid metabolism of isolated rat adipocytes. *Ann Nutr Metab* **32**: 282-288, 1988.

HEGYI K, FULOP K, KOVACS K, TOTH S, AND FALUS A. Leptin-induced signal transduction pathways. *Cell Biol Int* **28**: 159-169, 2004.

HOLST JJ. On the physiology of GIP and GLP-1. *Horm Metab Res* **36**: 747-754, 2004.

HOMMEL JD, TRINKO R, SEARS RM, GEORGESCU D, LIU ZW, GAO XB, THURMON JJ, MARINELLI M, AND DILEONE RJ. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* **51**: 801-810, 2006.

HUBSCHLE T, THOM E, WATSON A, ROTH J, KLAUS S, AND MEYERHOF W. Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. *J Neurosci* **21**: 2413-2424, 2001.

KALRA SP, DUBE MG, SAHU A, PHELPS CP, AND KALRA PS. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc Natl Acad Sci U S A* **88**: 10931-10935, 1991.

KALRA SP AND KALRA PS. Neuropeptide Y: a physiological orexigen modulated by the feedback action of ghrelin and leptin. *Endocrine* **22**: 49-56, 2003.

KAPLAN AM AND VIGNA SR. Gastric inhibitory polypeptide (GIP) binding sites in rat brain. *Peptides* **15**: 297-302, 1994.

KIEFFER TJ. GIP or not GIP? That is the question. *Trends in pharmacological sciences* **24**: 110-112, 2003.

KONG WM, STANLEY S, GARDINER J, ABBOTT C, MURPHY K, SETH A, CONNOLEY I, GHATEI M, STEPHENS D, AND BLOOM S. A role for arcuate cocaine and amphetamine-regulated transcript in hyperphagia, thermogenesis, and cold adaptation. *FASEB J* **17**: 1688-1690, 2003.

KRISTENSEN P, JUDGE ME, THIM L, RIBEL U, CHRISTJANSEN KN, WULFF BS, CLAUSEN JT, JENSEN PB, MADSEN OD, VRANG N, LARSEN PJ, AND HASTRUP S. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* **393**: 72-76, 1998.

LAMBERT PD, ANDERSON KD, SLEEMAN MW, WONG V, TAN J, HIJARUNGURU A, CORCORAN TL, MURRAY JD, THABET KE, YANCOPOULOS GD, AND WIEGAND SJ. Ciliary neurotrophic factor activates leptin-like pathways and

reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. *Proc Natl Acad Sci U S A* **98**: 4652-4657, 2001.

LANDGRAF R. The involvement of the vasopressin system in stress-related disorders. *CNS Neurol Disord Drug Targets* **5**: 167-179, 2006.

LEE AK, MOJTAHED-JABERI M, KYRIAKOU T, ALDECOA-OTALORA ASTARLOA E, ARNO M, MARSHALL NJ, BRAIN SD, AND O'DELL SD. Effect of high-fat feeding on expression of genes controlling availability of dopamine in mouse hypothalamus. *Nutrition* **26:**411-22.

LI G, MOBBS CV, AND SCARPACE PJ. Central pro-opiomelanocortin gene delivery results in hypophagia, reduced visceral adiposity, and improved insulin sensitivity in genetically obese Zucker rats. *Diabetes* **52**: 1951-1957, 2003.

MA'AYAN A, JENKINS SL, BARASH A, AND IYENGAR R. Neuro2A differentiation by Galphai/o pathway. *Sci Signal* **2**: cm1, 2009.

MIYAWAKI K, YAMADA Y, BAN N, IHARA Y, TSUKIYAMA K, ZHOU H, FUJIMOTO S, OKU A, TSUDA K, TOYOKUNI S, HIAI H, MIZUNOYA W, FUSHIKI T, HOLST JJ, MAKINO M, TASHITA A, KOBARA Y, TSUBAMOTO Y, JINNOUCHI T, JOMORI T, AND SEINO Y. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* **8**: 738-742, 2002.

MIYAWAKI K, YAMADA Y, YANO H, NIWA H, BAN N, IHARA Y, KUBOTA A, FUJIMOTO S, KAJIKAWA M, KUROE A, TSUDA K, HASHIMOTO H, YAMASHITA T, JOMORI T, TASHIRO F, MIYAZAKI J, AND SEINO Y. Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc Natl Acad Sci U S A* **96**: 14843-14847, 1999.

MOENS K, HEIMBERG H, FLAMEZ D, HUYPENS P, QUARTIER E, LING Z, PIPELEERS D, GREMLICH S, THORENS B, AND SCHUIT F. Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. *Diabetes* **45**: 257-261, 1996.

MORRIS DL AND RUI L. Recent advances in understanding leptin signaling and leptin resistance. *Am J Physiol Endocrinol Metab* **297**: E1247-1259, 2009.

NYBERG J, ANDERSON MF, MEISTER B, ALBORN AM, STROM AK, BREDERLAU A, ILLERSKOG AC, NILSSON O, KIEFFER TJ, HIETALA MA, RICKSTEN A, AND ERIKSSON PS. Glucose-dependent insulinotropic polypeptide is expressed in adult hippocampus and induces progenitor cell proliferation. *J Neurosci* **25**: 1816-1825, 2005.

OHINATA K, TAKAGI K, BIYAJIMA K, FUJIWARA Y, FUKUMOTO S, EGUCHI N, URADE Y, ASAKAWA A, FUJIMIYA M, INUI A, AND YOSHIKAWA M. Central prostaglandin D(2) stimulates food intake via the neuropeptide Y system in mice. *FEBS Lett* **582**: 679-684, 2008.

PANDEY SC. Anxiety and alcohol abuse disorders: a common role for CREB and its target, the neuropeptide Y gene. *Trends in pharmacological sciences* **24**: 456-460, 2003.

QING K AND CHEN Y. Central CART gene delivery by recombinant AAV vector attenuates body weight gain in diet-induced-obese rats. *Regul Pept* **140**: 21-26, 2007.

RAHMOUNI K, MORGAN DA, MORGAN GM, LIU X, SIGMUND CD, MARK AL, AND HAYNES WG. Hypothalamic PI3K and MAPK differentially mediate regional sympathetic activation to insulin. *J Clin Invest* **114**: 652-658, 2004.

RODRIGUEZ A AND FERRER I. Expression of transcription factors CREB and c-Fos in the brains of terminal Creutzfeldt-Jakob disease cases. *Neurosci Lett* **421**: 10-15, 2007.

SABATIER N, ROWE I, AND LENG G. Central release of oxytocin and the ventromedial hypothalamus. *Biochem Soc Trans* **35**: 1247-1251, 2007.

SANDBERG EM, MA X, VONDERLINDEN D, GODENY MD, AND SAYESKI PP. Jak2 tyrosine kinase mediates angiotensin II-dependent inactivation of ERK2 via induction of mitogen-activated protein kinase phosphatase 1. *J Biol Chem* **279**: 1956-1967, 2004.

SANO S, ITAMI S, TAKEDA K, TARUTANI M, YAMAGUCHI Y, MIURA H, YOSHIKAWA K, AKIRA S, AND TAKEDA J. Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J* **18**: 4657-4668, 1999.

SHERWOOD NM, KRUECKL SL, AND MCRORY JE. The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. *Endocr Rev* **21**: 619-670, 2000.

SMITH KL, GARDINER JV, WARD HL, KONG WM, MURPHY KG, MARTIN NM, GHATEI MA, AND BLOOM SR. Overexpression of CART in the PVN increases food intake and weight gain in rats. *Obesity (Silver Spring)* **16**: 2239-2244, 2008.

SONDHI S, CASTELLANO JM, CHONG VZ, ROGOZA RM, SKOBLENICK KJ, DYCK BA, GABRIELE J, THOMAS N, KI K, PRISTUPA ZB, SINGH AN, MACCRIMMON D, VORUGANTI P, FOSTER J, AND MISHRA RK. cDNA array reveals increased expression of glucose-dependent insulinotropic polypeptide following

chronic clozapine treatment: role in atypical antipsychotic drug-induced adverse metabolic effects. *Pharmacogenomics J* **6**: 131-140, 2006.

STANLEY SA, SMALL CJ, MURPHY KG, RAYES E, ABBOTT CR, SEAL LJ, MORGAN DG, SUNTER D, DAKIN CL, KIM MS, HUNTER R, KUHAR M, GHATEI MA, AND BLOOM SR. Actions of cocaine- and amphetamine-regulated transcript (CART) peptide on regulation of appetite and hypothalamo-pituitary axes in vitro and in vivo in male rats. *Brain Res* **893**: 186-194, 2001.

TIAN DR, LI XD, SHI YS, WAN Y, WANG XM, CHANG JK, YANG J, AND HAN JS. Changes of hypothalamic alpha-MSH and CART peptide expression in diet-induced obese rats. *Peptides* **25**: 2147-2153, 2004.

TURNBULL AV AND RIVIER C. Corticotropin-releasing factor, vasopressin, and prostaglandins mediate, and nitric oxide restrains, the hypothalamic-pituitary-adrenal response to acute local inflammation in the rat. *Endocrinology* **137**: 455-463, 1996.

USDIN TB, MEZEY E, BUTTON DC, BROWNSTEIN MJ, AND BONNER TI. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology* **133**: 2861-2870, 1993.

VEENEMA AH AND NEUMANN ID. Central vasopressin and oxytocin release: regulation of complex social behaviours. *Prog Brain Res* **170**: 261-276, 2008.

VILLANUEVA EC AND MYERS MG, JR. Leptin receptor signaling and the regulation of mammalian physiology. *Int J Obes (Lond)* **32 Suppl 7**: S8-12, 2008.

WASADA T MK, HARRIS V, KAWAI K, HOWARD B, AND UGER RH. Effects of gastric inhibitory polypeptide on plasma levels of chylomicron triglycerides in dogs. *J Clin Invest* **68**: 1106-1107, 1981.

WIDEMAN RD AND KIEFFER TJ. Glucose-dependent insulinotropic polypeptide as a regulator of beta cell function and fate. *Horm Metab Res* **36**: 782-786, 2004.

WINDPASSINGER C, KROISEL PM, WAGNER K, AND PETEK E. The human gamma-aminobutyric acid A receptor delta (GABRD) gene: molecular characterisation and tissue-specific expression. *Gene* **292**: 25-31, 2002.

WORTLEY KE, CHANG GQ, DAVYDOVA Z, FRIED SK, AND LEIBOWITZ SF. Cocaine- and amphetamine-regulated transcript in the arcuate nucleus stimulates lipid metabolism to control body fat accrual on a high-fat diet. *Regul Pept* **117**: 89-99, 2004.

YIP RG AND WOLFE MM. GIP biology and fat metabolism. *Life Sci* **66**: 91-103, 2000.

ZACHARIOU V, GEORGESCU D, KANSAL L, MERRIAM P, AND PICCIOTTO MR. Galanin receptor 1 gene expression is regulated by cyclic AMP through a CREB-dependent mechanism. *J Neurochem* **76**: 191-200, 2001.

ZHAO AZ, HUAN JN, GUPTA S, PAL R, AND SAHU A. A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding. *Nat Neurosci* **5**: 727-728, 2002.

Table 1: Probes used for real time PCR:

Gene symbol	Gene name	Rat ABI assay ID	Mice ABI assay ID	Function
18S		4342379-18S	-------	House-keeping gene
β-ACT	actin, beta	-------	Mm00607939_s1	House-keeping gene
AVP	arginine vasopressin	Rn00566449_m1	Mm00437761_g1	Neuroendocrine and feeding regulation (Bray, 2000, Landgraf, 2006)
CART	cocaine and amphetamine regulated transcript	Rn00567382_m1	Mm00489086_m1	Energy balance, bone metabolism, anxiety (Asakawa et al., 2001, Elefteriou et al., 2005, Kong et al., 2003, Tian et al., 2004, Wortley et al., 2004)
CREB1	cAMP responsive element binding protein 1	Rn00578829_g1	Mm00501607_m1	Learning, memory, anxiety, feeding related circuits (Altarejos and Montminy, 2011, Carlezon et al., 2005, Duman and Duman, 2005, Georgescu et al., 2005)
MAPK1	mitogen activated protein kinase 1	Rn00587719_m1	Mm00442479_m1	Sympathetic activation, leptin and insulin feeding circuits, circadian rhythm (Ambati et al., 2007, Coogan and Piggins, 2004, Hegyi et al., 2004, Rahmouni et al., 2004)
OXT	oxcytocin	Rn00564446_g1	Mm00726655_s1	social behaviors, anxiety, feeding behavior (Bale et al., 2001, Sabatier et al., 2007, Veenema and Neumann, 2008)
SCT	secretin	Rn00575360_g1	Mm00441235_g1	Feeding behavior, autism (Ambati et al., 2009, Ambati et al., 2007, Cheng et al., 2011, Lee et al., 2009)
TH	tyrosine hydroxylase	Rn00562500_m1	Mm00447546_m1	Feeding, reward (Ambati et al., 2009, Ambati et al., 2007, Lee et al., 2009)

GABRD	gamma-aminobutyric acid A receptor, delta	Rn00568740_m1	Mm00433476_ml	Inhibitory neurotransmitter, cognition, speech, feeding behavior (Ambati et al., 2009, Windpassinger et al., 2002)
JAK2	Janus kinase 2	Rn00580452_ml	Mm00434561_ml	leptin signaling (Sandberg et al., 2004, Villanueva and Myers, 2008)
NPY	neuropeptide Y	Rn00561681_ml	Mm00445771_ml	Feeding behavior (Kalra et al., 1991, Kalra and Kalra, 2003)
POMC	pro-opiomelanocortin	Rn00595020_ml	Mm00435874_ml	Feeding behavior (Boston, 2001, Li et al., 2003)
PTGES	prostaglandin E synthase	Mm00456961_ml	Mm00452105_ml	Inflammation, feeding behavior(Ambati et al., 2009, Ohinata et al., 2008, Turnbull and Rivier, 1996)
SOCS3	suppressor of cytokine signaling 3	Rn00585674_sl	Mm00545913_sl	Leptin signaling, neuroimmuneendocrine modulator (Auernhammer et al., 2000, Morris and Rui, 2009)
STAT3	signal transducer and activator of transcription 3	Rn00562562_ml	Mm00456961_ml	Signal transduction in feeding related circuits (Carvalheira et al., 2001, Gao et al., 2007, Hommel et al., 2006, Zhao et al., 2002)

*POMC1, mouse; POMC2, rat

Table 2. Hypothalamic mRNA expression (mean ± SEM) of hypothalamic genes in $GIPR^{+/?}$ and $GIPR^{-/-}$ mice* and in rats after 4 days ICV injection of 0 or 10 μg/d GIP**

Gene	$GIPR^{+/?}$ mice	$GIPR^{-/-}$ mice	Rats (0 μg/d GIP ICV)	Rats (10 μg/d GIP ICV)
AVP	0.85 ± 0.09^{a}	0.62 ± 0.08^{b}	0.78 ± 0.06^{x}	1.14 ± 0.03^{y}
CART	0.7 ± 0.05^{a}	0.50 ± 0.03^{b}	0.97 ± 0.07^{a}	1.22 ± 0.02^{b}
CREB1	1.08 ± 0.02	1.04 ± 0.02	0.81 ± 0.04^{x}	1.12 ± 0.03^{y}
GABRD	1.44 ± 0.1	1.52 ± 0.05	0.89 ± 0.03^{x}	1.49 ± 0.1^{y}
JAK2	1.16 ± 0.04	1.2 ± 0.03	0.84 ± 0.06^{a}	1.03 ± 0.03^{b}
MAPK1	1.31 ± 0.06^{x}	1.82 ± 0.07^{y}	0.85 ± 0.06^{a}	1.14 ± 0.06^{b}
NPY	1.29 ± 0.06^{a}	1.7 ± 0.11^{b}	0.75 ± 0.06^{a}	0.94 ± 0.05^{b}
OXT	0.8 ± 0.06^{a}	0.6 ± 0.06^{b}	0.85 ± 0.09^{x}	1.27 ± 0.04^{y}
POMC	0.85± 0.09	0.66 ± 0.14	0.75 ± 0.07	0.85 ± 0.04
PTGES	1.17 ± 0.07^{a}	0.89 ± 0.04^{b}	0.93 ± 0.06	1.08 ± 0.06
SCT	1.1 ± 0.15	0.83 ± 0.10	0.85 ± 0.08	1.14 ± 0.01
SOCS3	1.32 ± 0.15	0.84 ± 0.06	0.90 ± 0.07	0.95 ± 0.04
STAT3	1.15 ± 0.03^{a}	1.05 ± 0.03^{b}	0.79 ± 0.06^{a}	0.96 ± 0.03^{b}
TH	0.85 ± 0.07	0.7 ± 0.04	0.76 ± 0.09^{a}	1.01 ± 0.06^{b}

* Data are normalized to β-ACT values; ** Data are normalized to the 18S values; means with different superscripts are different $^{ab}p < 0.05$; $^{xy}p < 0.01$

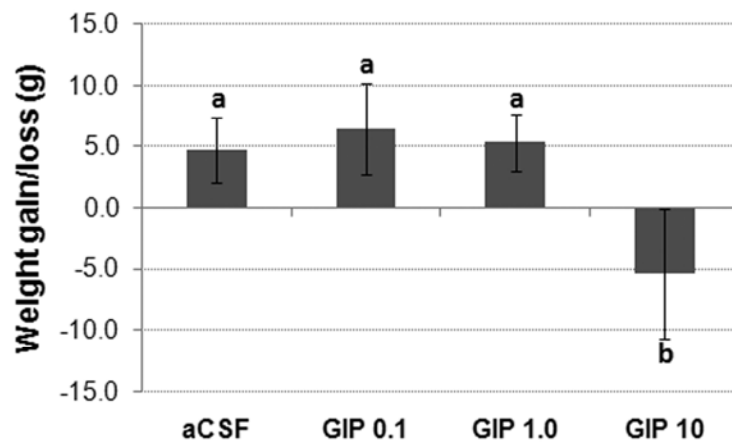

Figure 1. Weight gain/loss in rats treated daily for 4 days with control (aCSF) or 10 µg/d GIP administered into the lateral cerebral ventricle. Data are means ± SEM. Means not denoted with a common superscript are different, $^{ab}p < 0.05$.