Transplantable Rat Glucagonomas Cause Acute Onset of Severe Anorexia and Adipsia Despite Highly Elevated NPY mRNA Levels in the Hypothalamic Arcuate Nucleus

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Abstract

We have isolated a stable, transplantable, and small glucagonoma (MSL-G-AN) associated with abrupt onset of severe anorexia occurring 2–3 wk after subcutaneous transplantation. Before onset of anorexia, food consumption is comparable to untreated controls. Anorexia is followed by adipsia and weight loss, and progresses rapidly in severity, eventually resulting in reduction of food and water intake of 100 and 80%, respectively. During the anorectic phase, the rats eventually become hypoglycemic and hypothermic. The tumor-associated anorexia shows no sex difference, and is not affected by bilateral abdominal vagotomy, indicating a direct central effect. The adipose satiety factor leptin, known to suppress food intake by reducing hypothalamic neuropeptide Y (NPY) levels, was not found to be expressed by the tumor, and circulating leptin levels were reduced twofold in the anorectic phase. A highly significant increase in hypothalamic (arcuate nucleus) NPY mRNA levels was found in anorectic rats compared with control animals. Since elevated hypothalamic NPY is among the most potent stimulators of feeding and a characteristic of most animal models of hyperphagia, we conclude that the MSL-G-AN glucagonoma releases circulating factor(s) that overrides the hypothalamic NPY-ergic system, thereby eliminating the orexigenic effect of NPY. We hypothesize a possible central role of proglucagon-derived peptides in the observed anorexia. (*J. Clin. Invest.* 1998. 101:503–510.) Key words: hunger regulation • cancer • glucagon • GLP-1 • leptin

Introduction

We have reported previously the establishment of stable, transplantable insulinomas (1) as well as glucagonomas from a multihormonal rat islet stem cell–like cell line. The glucagonoma (MSL-G-AN)[1] phenotype was identified because of the occurrence of a dramatic weight loss (2). Rats transplanted subcutaneously with this tumor developed abrupt and severe anorexia and adipsia leading to cachexia (3), but displayed an otherwise normal behavior and level of activity before becoming cachectic. Metabolic derangement due to tumor size is negligible since the glucagonomas are small, slow-growing, and constitute < 1% of total body weight at the onset of anorexia. Moreover, these tumors rarely metastasize and the rats instantly resume normal feeding and drinking upon tumor resection (2). The site of transplantation (e.g., subcutaneous, intrapancreatic, or intraperitoneal) did not affect the onset of anorexia, and similar sized insulinomas of the same clonal origin did not cause anorexia or adipsia. Recent comparative studies of the effects of subcutaneous insulinomas and glucagonomas revealed a profound and quantitative elimination of endogenous islet B and A cells, respectively (4), indicating that the glucagonoma represents a transformed counterpart of the normal islet A cell. In addition, cancer anorexia in general has been reported to be transmissible in plasma (5–7). Taken together, these observations strongly suggest that the MSL-G-AN glucagonoma secretes one or more circulating factor(s) that suppress feeding and drinking.

The proglucagon processing pattern of this glucagonoma was found to be a mixture of that of the normal pancreatic A and intestinal L cells. Both glucagon and glucagon-like peptide-1 (7–36)amide (GLP-1) as well as other proglucagon derived peptides are thus released from the tumor and circulate in the blood at high levels. Murine glucagonomas with a similar processing pattern do not cause anorexia in mice (8, 9); however, in these experiments actual circulating levels of different proglucagon-derived peptides were not known (8, 9).

The glucagonoma syndrome in humans, also characterized by elevated levels of circulating proglucagon-derived peptides, is often, but not always, associated with weight loss (10, 11). This is also true for many cancer types (12, 13). However, the cause of cancer cachexia is poorly understood, and there are several tumor-derived substances capable of causing cachexia, including lipolytic substances (14) as well as humoral agents directly or indirectly interacting with hypothalamic centers involved in appetite regulation.

The hypothalamic paraventricular nucleus (PVN) is central in the regulation of metabolism and eating behavior with microinjections of a variety of substances into this region eliciting manifest changes in eating and drinking. Neuropeptide Y (NPY) is the most powerful stimulator of feeding identified in this way (15, 16) and has been shown to have a hypothermic effect (17). The PVN is innervated by NPY-containing nerve terminals, some of which originate from cell bodies in the arcuate nucleus (ARC), which is a major site of hypothalamic NPY

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1. *Abbreviations used in this paper:* ARC, arcuate nucleus; GLP-1, glucagon-like peptide-1 (7-36)amide; MSL-G-AN, glucagonoma; NEDH, New England Deaconess hospital rat; NPY, neuropeptide Y; PVN, paraventricular nucleus; RT-PCR, reverse transcription polymerase chain reaction.

synthesis (18–20). An arcuato-paraventricular and dorsomedial hypothalamic NPY-containing system is considered to connect peripheral stimuli with central regulation of feeding behavior and metabolism (21). Several studies have shown a correlation between the level of NPY mRNA in the ARC and satiety. NPY mRNA in the ARC is elevated in food-deprived rats (22, 23), in obese hyperphagic Zucker rats (22, 24–26), and in streptozotocin-induced hyperphagia (27–30). Thus, a tight correlation normally seems to exist between the sensation of hunger and the transcriptional activity of the NPY gene in the ARC. Also the hyperphagic *ob/ob* mice lacking the functional adipocyte-derived leptin (31) express elevated hypothalamic NPY mRNA levels (32). In these animals, peripheral (33, 34) and central (35) administration of recombinant leptin causes satiety and weight loss through reduction of hypothalamic NPY levels (36, 37).

The aims of this study were to further characterize the nature of the onset of anorexia and adipsia, to analyze circulating hormone levels (GLP-1, glucagon, insulin, corticosterone, and leptin), and to quantify NPY mRNA levels in the ARC in order to clarify the central mechanisms involved in glucagonoma-induced anorexia/adipsia.

Methods

Animal studies

New England Deaconess hospital (NEDH) rats (Møllegaard, Lille Skensved, Denmark) were transplanted with the glucagonoma line MSL-G-AN as described previously (2, 3).

Study 1. Of two groups of eight animals (four males and four females in each), one group served as a control, while the other was transplanted with the anorectic tumor line MSL-G-AN as described previously (2). The rats were housed individually in normal cages, and body weight and food and water intake were recorded daily. Transplanted rats were followed from 10 d after the transplant and killed when anorexia had resulted in a weight loss of $\sim$ 30%. The control group was followed for a period of 10 d, beginning 2 d after individual caging. Blood glucose was measured once a week and at the end of the experiment for the transplanted rats, and on days 6 and 10 for the control group. Body rectal temperature was measured three times a week for all rats and at the end of the experiment. It should be noted that the transplant procedure is a single subcutaneous injection of as little as 50 μl of RPMI 1640 containing a tumor fragment of $<$ 1 mg of tumor tissue. This manipulation has no effect on the animals and the corresponding control group was therefore not sham-operated.

Study 2. A group of nine MSL-G-AN transplanted rats was killed in the anorectic state and their brains were collected for in situ hybridization (see below). Six nontransplanted rats served as controls. Plasma was collected from a total of 15 anorectic and 10 control rats for measurements of insulin, glucagon, and GLP-1.

Statistical analysis for comparison of blood glucose levels and body temperatures was carried out using a Student's *t* test. Hormone levels between glucagonoma transplanted and control animals were analyzed using the two-tailed, unpaired Mann-Whitney U test.

Data calculation from study 1

Due to variation in onset of weight loss (probably caused by variation in the size of transplanted tumor fragments) the data set for each individual rat was adjusted around day 0, defined as the day preceding constant daily weight loss. For comparison of individual body weight values, transplanted rats were normalized to their maximal weight (100%) and control rat body weights were normalized to the weight recorded on day 4 (100%).

The mean value of food and water intake before day 0 (100%) was used to normalize data from each transplanted rat, while the mean value over the 10-d test period was used for control rats. Since there was no gender difference between the normalized values, the data were pooled and expressed as mean±SD for all eight rats in the two groups.

Blood glucose and plasma hormone levels

Blood glucose was measured using a Hypocount blood glucose meter (model MX-B; Hypoguard, Woodbridge, UK) and plasma hormone levels were measured by radioimmunoassay as described for glucagon (38), GLP-1 (39), and insulin (40). Leptin measurements were carried out on a separate group of anorectic (n = 8) and control (n = 6) rats using a rat leptin RIA kit (Linco Research Inc., St. Charles, MO). Corticosterone levels were measured with a kit from ICH Biomedicals Inc. (Costa Mesa, CA).

Vagotomy

In a final set of experiments, male adult rats were anaesthetized with avertine (1 ml/100 g body weight) prepared as follows: 2 ml of avertine stock solution (6.67 g 2,2,2-tribromethanol dissolved in 3.33 ml 3-pentanol; stored in dark at room temperature) was added to 8 ml 96% ethanol and 90 ml isotonic saline (0.9% NaCl in H_2O). Using a dissecting microscope the vagus nerves were bilaterally, surgically sectioned just below the diaphragm. After recovery, the animals were implanted with segments of the anorectic tumor line and caged individually. Animals were followed as in study 1. The completeness of the vagotomy procedure was confirmed by the marked increase in size of the stomach in nonimplanted animals.

NPY in situ hybridization

3–5 d after onset of severe anorexia, nine glucagonoma transplanted rats and six normally feeding control rats were anaesthetized with pentobarbital sodium (50 mg/kg body wt) intraperitoneally and decapitated. Their brains were rapidly removed, frozen on dry ice, and stored at −80°C until sectioning. Serial coronal sections through the ARC (12 μm) were cut in a cryostat and thaw-mounted onto gelatin-coated slides, and stored at −80°C until use.

The sections were fixed in 4% (wt/vol) paraformaldehyde, rinsed twice in 50 mM phosphate buffered saline, and processed in parallel using the in situ hybridization procedure as described previously (41). Briefly, the sections were prepared for hybridization by acetylation in 0.1 M triethanolamine, 0.9% NaCl (pH 8.0) containing 0.25% acetic anhydride (vol/vol) for 10 min at room temperature, followed by delipidation in an ascending gradient of ethanol (70, 80, 90, 100%) for 2 min each, and terminating with a wash in chloroform for 2 min. Two different synthetic antisense DNA oligonucleotide probes complementary to different parts of the rat NPY mRNA (42) were used: 5′-GGG GGC ATT TTC TGT GCT TTC TCT CAT TAA GAG ATC TGA AAT CAG TGT-3′ (25) and 5′-GGA GTA GTA TCT GGC CAT GTC CTC TGC TGG CGC GTC-3′ (43). The probes were labeled with ^{35}S alpha-thio-ATP ($>$ 3,000 Ci/mmol; NEN, Boston, MA) to a specific activity of 2.0×10^9 degradations per min/mol using terminal deoxynucleotidyl transferase (Boehringer Mannheim, Mannheim, Germany). Hybridization was carried out in a buffer containing 25% (vol/vol) formamide, 4 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2), 1× Denhardt's solution, 10% (wt/vol) dextran sulphate, 10 mM dithiothreitol, 0.5 mg/ml salmon sperm DNA, and 250 mg/ml yeast tRNA at 37°C. After hybridization, slides were washed in 1 × SSC four times (15 min each) at 55°C and twice (30 min each) at room temperature. The slides were then dipped into water, blown dry, and either exposed to Amersham Hyperfilm^R (Amersham, Little Chalfort, UK) for 3 wk, or dipped into Amersham LM-1^R nuclear emulsion and exposed for 3 wk before being developed for microscopic localization of NPY mRNA transcripts. Some brain sections were stained using cresyl violet to facilitate morphological analysis. The x-ray autoradiograms were quantified using an Image analysis system (model 1.49 G; Wayne Rasband, National Institutes of

Health, Bethesda, MD), with measurement of grain densities using ^{35}S-brain paste standards as a reference.

The density was measured over the ARC of three individual sections from the same animal. The mean density of these three observations was used for statistical analysis using the two-tailed unpaired Mann-Whitney U test.

Comparative reverse transcription-PCR (RT-PCR)

Total RNA was extracted from tissues using RNAzol (Cinna/Biotecx, Houston, TX), following the manufacturer's instructions. Total RNA was diluted to 0.2 μg/μl in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. First-strand cDNA was prepared using a Superscript RT kit and random hexamers (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's instructions. cDNA was diluted 1:2 with H_2O, and PCR was carried out using 3 μl diluted cDNA and a PCR mix containing *Taq* DNA polymerase (2.5 U) and buffer (Promega Corp., Madison, WI), and a dNTP mix (40 mM each except dCTP 20 mM, final concentrations) (Pharmacia, Uppsala, Sweden) containing 2.5 μCi of alpha-^{32}P-dCTP (3,000 Ci/mmol) (Amersham) in a 50 μl reaction volume, following the instructions from Promega. Two primer sets (10 pmol of each primer) were used simultaneously, one set specific for rat *ob* cDNA (44) (5′ primer: 5′-TCA CAC ACG CAG TCG GTA TCC G-3′ and 3′ primer: 5′-GAC GCC ATC CAG GCT CTC TGG-3′) giving rise to a 266-bp fragment, and the other set specific for glucose-6-phosphate dehydrogenase, used as internal standard (5′ primer: 5′-GAC CTG CAG AGC TCC AAT CAA C-3′ and 3′ primer: 5′-CAC GAC CCT CAG TAC CAA ACC C-3′) giving rise to a 214-bp fragment. PCR conditions were 94°C for 1 min (denaturation) followed by 25 cycles of 94°C, 30 s; 55°C, 30 s; 72°C, 30 s. PCR products were electrophoretically separated on a 6% (wt/vol) polyacrylamide gel, containing 7 M urea and exposed on x-ray film.

Results

Onset of tumor-induced weight loss. Transplanted rats carrying MSL-G-AN glucagonomas all developed severe weight loss after variable periods. The onset ranged from 16 to 25 d after transplantation, with no correlation to sex or initial body weight at the time of transplantation. Variation in the time of onset is ascribed to biological variation (e.g., quality and actual size of the tumor fragment, as well as the process of vascularization). The changes in relative body weight are shown for control and MSL-G-AN transplanted rats relative to the day of onset of weight loss (Fig. 1 *A*). It is demonstrated that the tumor has minimal or no effect on weight gain before acute onset of weight loss, as weight gain, food, and water intake in the transplanted group follow that of nontreated NEDH controls. After onset of anorexia, the rats lose weight at a constant high rate equivalent to as much as 3–4% per day of their maximal body weight. Despite the decline in weight, anorectic rats appeared normally active.

Onset of anorexia and adipsia in relation to weight loss. Changes in food and water intake for control and transplanted rats are shown in Fig. 1, *B* and *C*. A dramatic decrease in food intake leading to an almost complete absence of feeding was observed within a few days after onset of anorexia (Fig. 1 *B*). This was accompanied by an almost as severe reduction in water intake, stabilizing at ~ 20% of normal water consumption (Fig. 1 *C*). When comparing the mean values of body weight, food, and water intake for the transplanted rats, it was evident that anorexia preceded adipsia and then weight loss in sequential order (Fig. 2). Body temperature was slightly, but significantly, lowered in the late stage of anorexia compared with control values (37.4±0.8°C vs. 38.6±0.6°C, $n = 8$ in each group, $0.01 < P < 0.05$). Blood glucose values were slightly decreased during the anorectic phase. End-point mean blood glucose in the anorectic group was 3.05±0.85 vs. 4.93±1.15 mM ($P < 0.005$) in the control group.

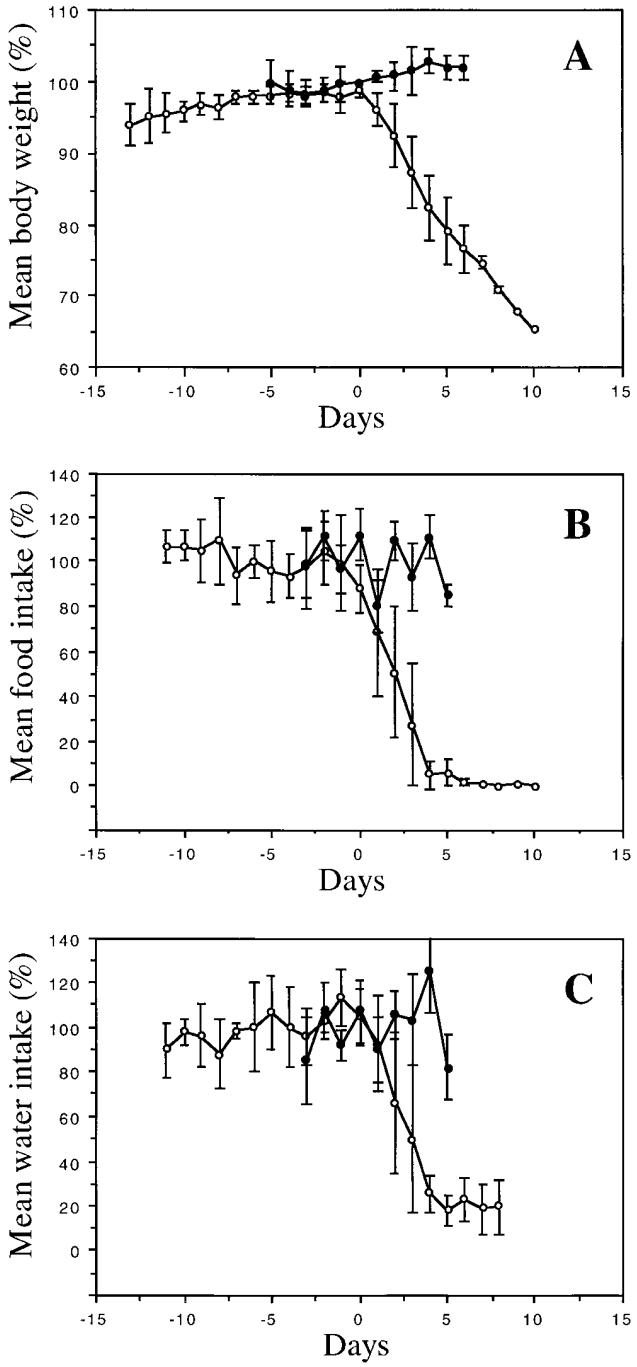

Figure 1. Glucagonoma-associated anorexia. Effects of anorectic tumors on body weight (*A*), food intake (*B*), and water intake (*C*). Open circles represent rats transplanted with MSL-G-AN, closed circles are control rats ($n = 8$ in each group). Values are normalized as described in Methods (data calculation from study 1). The time point of anorexia onset is day 0.

No effect of vagotomy. A group of five rats was vagotomized before transplantation. These rats developed anorexia

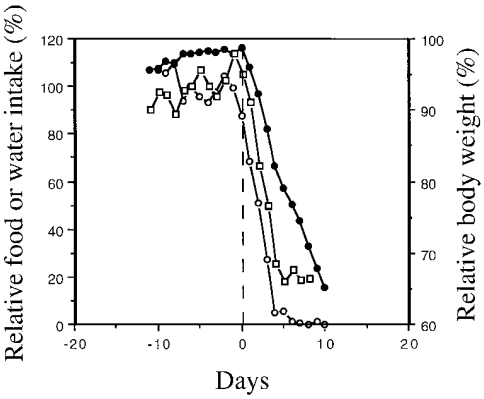

Figure 2. Anorexia and weight loss. Overlay plot of mean values for food intake (*open circles*), water intake (*open squares*), and relative body weight (*filled circles*) from the group of anorectic rats (Fig. 1). It is evident that onset of weight loss follows anorexia by 2 d and adipsia by 1 d.

with exactly the same characteristics as transplanted NEDH control rats. Onset of anorexia ranged from day 11 to day 20 with a mean of 15±3.3 d and was preceded by a period characterized by weight gain. After onset of anorexia, individual weight loss curves were indistinguishable from tumor-transplanted nonvagotomized rats (Fig. 1 *A*) with maximal weight loss of 3.8±0.7% per d of maximal body weight.

Circulating hormone levels. Anorectic rats showed highly elevated levels of circulating glucagon and GLP-1 immunoreactivity compared to controls (Fig. 3). Interestingly, all transplanted rats also became hypoinsulinemic (Fig. 3), most likely reflecting similarity between the anorectic and starved state. Corticosterone levels of anorectic rats were 226±56 ng/ml (n = 5) and thus well within the normal range of 50–400 ng/ml of control rats.

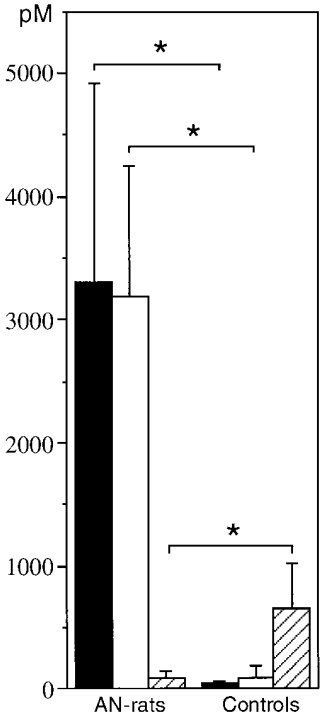

Figure 3. Circulating hormone levels. Mean values of circulating hormones±SD are shown for glucagon (*black bars*), GLP-1 (*white bars*), and insulin (*hatched bars*). Significant hyper-GLP-1/glucagonemia is evident in MSL-G-AN while insulin levels are significantly reduced. *$P < 0.0001$.

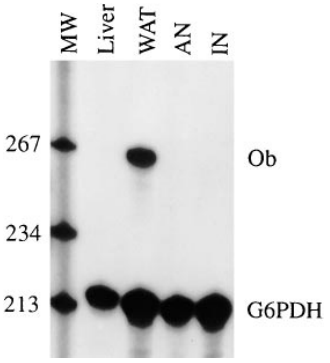

Figure 4. Leptin mRNA expression. Comparative RT-PCR demonstrating the selective presence of leptin (*Ob*) mRNA in rat white fat (*WAT*), its absence in rat liver as well as in the anorectic MSL-G-AN glucagonoma (*AN*), and nonanorectic insulinoma (*IN*). Molecular marker DNA is seen in the left lane. G6PDH was used as an internal control of RNA (cDNA) quality.

Leptin: an unlikely cause of the observed anorexia. Comparative RT-PCR analysis for leptin expression confirmed tissue-specific expression in white fat tissue and showed that anorectic MSL-G-AN tumors did not express this potent satiety factor (Fig. 4). Further, anorectic rats displayed a twofold reduction in circulating leptin levels (1.6±0.6 ng/ml in anorectic rats vs. 3.2±0.4 ng/ml in controls).

Hypothalamic NPY mRNA levels are elevated in anorectic MSL-G-AN tumor-bearing rats. Coronal sections of the hypothalamic ARC demonstrated that NPY mRNA–expressing cells were accumulated in the medial portion of the nucleus (Fig. 5). The two probes used for in situ hybridization histochemistry produced identical patterns of expression in the ARC and the NPY-synthesizing areas of the brain represented in the same tissue section, i.e., the cerebral cortex and the amygdala (not shown), indicating that they both specifically hybridize to NPY mRNA as shown in previous studies (25, 43). Quantitative in situ hybridization for hypothalamic NPY mRNA showed significantly elevated levels (fivefold) in anorectic rats compared to controls (Fig. 6).

Individual NPY mRNA–expressing cells were identified in the emulsion-dipped sections (Fig. 5). Even though the expression after transplantation of tumor became very high, it can be seen that this is a result of higher expression within the individual cells rather than an increase in the number of cells producing NPY mRNA (Fig. 5).

Discussion

We have established and characterized a transplantable glucagonoma, MSL-G-AN, in the rat which is associated with an abrupt onset of profound anorexia and adipsia. We demonstrate the coexistence of anorexia and elevated NPY mRNA in the hypothalamic ARC. Our results suggest that MSL-G-AN tumor-derived factor(s) override or neutralize the feeding behavior normally associated with increased NPY mRNA expression in the arcuato-paraventricular pathway. The underlying mechanisms of this dual effect remain to be demonstrated, but it seems probable that the state of anorexia precedes that of increased NPY expression.

The nature of the anorectic substance remains unknown. A similar severity of anorexia/cachexia is, to our knowledge, so far only observed in two other experimental systems. First, the transplantation of experimental transgenic tumors hyperexpressing TNF/cachectin into mice (45) leads to a degree of

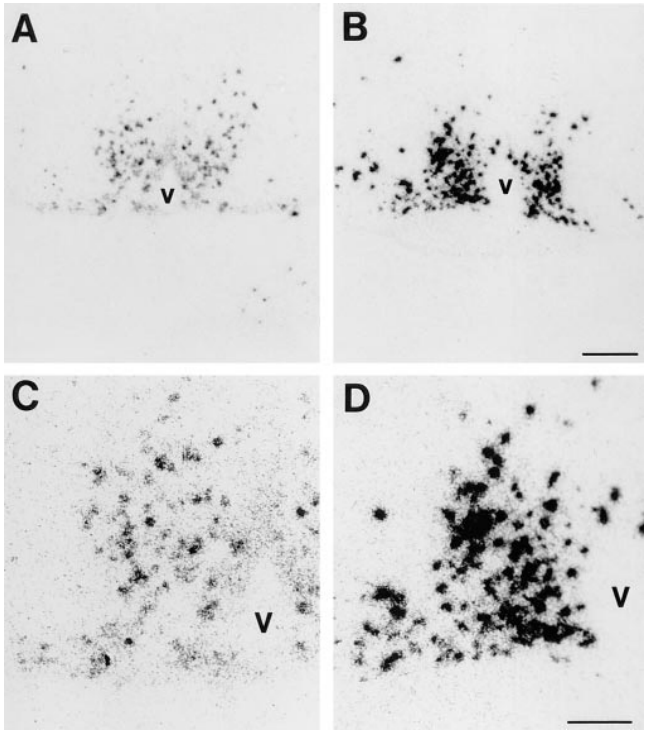

Figure 5. Autoradiographs of coronal sections of a control rat (*A* and *C*) and an anorectic rat (*B* and *D*). Clearly elevated levels of NPY mRNA can be detected in the ARC of the anorectic rat (*B*) vs. the control rat (*A*). *Bar*, 200 μm. A higher magnification (*below*) shows that the increased levels of NPY mRNA in anorectic rats is caused by an increase in expression rather than an increase in the number of cells showing NPY mRNA expression (*C* vs. *D*). *Bar*, 100 μm. V indicates the third ventricle (*A–D*).

weight loss similar to that observed by our glucagonoma when transplanted to immunodeficient mice (2). However, we have shown that TNF is absent in the MSL-G-AN tumor (2). Second, the classical parabiosis experiments by Coleman, where the *db*/*db* mice would cause lethal anorexia of both the normal and *ob*/*ob* partners (46), predicted a satiety factor/receptor relationship which was elucidated recently by the cloning of the *ob* (31) and *db* loci (47–49) representing leptin and its receptor, respectively. Our present data exclude the involvement of leptin in the observed anorexia (see below).

Proglucagon is the dominating prohormone gene product known to be expressed in MSL-G-AN, but the role of proglucagon-derived peptides in regulation of feeding is not clear. The unusual processing of the precursor in these tumors to glucagon and GLP-1 (7–36)amide at the same time (2) represents a mixed phenotype of the pancreatic α cell and the intestinal L cell and is thus highly unphysiological. A vagally mediated postprandial reduction of spontaneous meal size by portal infusion of glucagon in the liver has been shown (50, 51) as well as an increase in spontaneous meal size by infusion of glucagon antibodies (52). These effects are, however, minor compared to the almost complete block of feeding by our glucagonoma. Given that glucagon's satiating effect is vagally mediated, the lack of effects of vagotomy in this study seem to exclude glucagon as a causative agent. Furthermore, both glucagon and GLP-1 are grossly elevated even before onset of anorexia (in the 1 nM range, data not shown) apparently with no anorectic effect even in the nonvagotomized tumor-transplanted rats (Fig. 1 *A*). This could be explained by peripheral adaptation, since a recent study showed marked downregulation of liver glucagon receptor mRNA in anorectic rats (53). Recently, intracerebroventricular administration of GLP-1 was shown to elicit a potent suppression of food intake (54–56), and moreover, GLP-1 has been shown to inhibit NPY-induced feeding (55). In contrast, peripheral administration of single high doses of GLP-1 to rats failed to elicit significant changes in food intake (3, 55, 56), in spite of the finding that peripheral GLP-1 can access the subfornical organ and the area postrema of the brain (57). However, GLP-1 is degraded extensively and very rapidly in the circulation after peripheral administration (58) which may be the main reason for the lack of effect of single injections. In agreement with this, it was shown recently that continuous infusion of physiological amounts of GLP-1 into human volunteers significantly promoted satiety and reduced food intake (59). GLP-1, therefore, remains a viable candidate for the anorectic effects of the tumors.

Hyperphagia in the leptin-deficient obese *ob*/*ob* mouse correlates with elevated hypothalamic NPY (32) and the satiety effect of recombinant leptin by both peripheral and intracerebroventricular administration (33–35) was indeed shown to be associated with downregulation of hypothalamic NPY (36, 37). Therefore the coexistence of anorexia and elevated hypothalamic NPY mRNA in the transplanted rats suggests that leptin is an unlikely candidate mediating this effect. This is further supported by the lack of leptin mRNA expression in anorectic glucagonomas combined with a twofold reduction in circulating leptin levels during the anorectic phase. Thus, low circulating leptin levels in the anorectic rats correlate with increased NPY expression in the ARC (similar to what is found in the leptin-deficient and hyperphagic *ob*/*ob* mouse). Similarly, no role of leptin was found in recent studies of several different cancers associated with cachexia (60, 61).

The NPY-containing arcuato-paraventricular pathway has drawn the most attention as the neuronal pathway responsible for regulating increased appetite for food intake (for reviews see references 62–66). In most instances characterized by in-

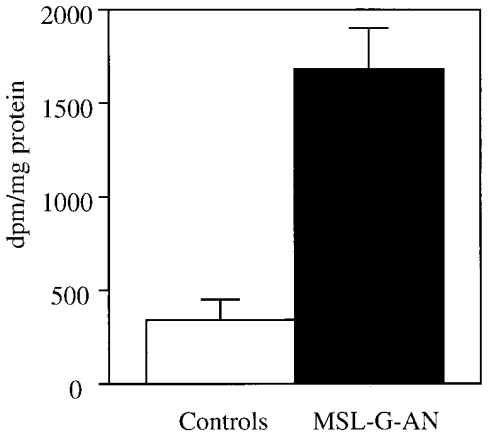

Figure 6. Quantitation of NPY mRNA levels in ARC (see Methods) demonstrates a highly significant increase in anorectic rats carrying MSL-G-AN tumors vs. normally feeding controls ($P < 0.0005$).

creased NPY expression in the arcuato-paraventricular pathway, animals become grossly obese due to a combination of hyperphagia and lowered metabolism (65, 66). In addition to the acute activation of feeding behavior, NPY also activates descending pathways influencing preganglionic autonomic neurons. The overall effect of NPY upon the autonomic nervous system is, therefore, that of a shift towards a parasympathetic dominance, resulting in increased energy intake, reduced energy expenditure, and increased insulin secretion leading to obesity (67). Thus, it is surprising that, despite their highly elevated NPY mRNA expression, rats carrying the MSL-G-AN tumor suddenly become completely anorectic. This suggests that the hypothalamic NPY-sensitive neurons normally responsible for mediating hyperphagia become insensitive to NPY in the MSL-G-AN–bearing rats.

Another situation characterized by combined cachexia in ad libitum fed rats and grossly elevated NPY mRNA expression in arcuate neurons is that seen during prolonged treatment with very high doses of glucocorticoid type II receptor agonists (68, 69). Such rats are characterized by hepatic gluconeogenesis, glycogenolysis, and hyperinsulinemia leading to an overall diabetic tendency due to resulting insensitivity to insulin. Elevated levels of circulating glucocorticoids directly influence NPY gene expression in arcuate neurons, but the effect of glucocorticoids upon arcuate neurons may also be more indirect via lowered leptin release from decreased stores of body fat (68, 70). It has been shown that the hyperinsulinemia associated with elevated glucocorticoids partly counteracts increased hypothalamic NPY expression (71) thereby constituting a negative feedback on hypothalamic NPY neurons which affect insulin secretion via central autonomic pathways (72). The MSL-G-AN–bearing animals, however, display levels of circulating glucocorticoids within the normal range, and plasma insulin levels significantly lower than animals without tumors. Thus, despite similar combinations of cachexia and elevated hypothalamic NPY expression in animals with either hypercorticosteronemia or MSL-G-AN tumors, these metabolic derangements are very different and probably activate different pathways influencing arcuate NPY expression. In the MSL-G-AN–burdened rats, lowered insulin levels may, at least partially, be responsible for activation of NPY expression (25, 73–75). Thus, the hypoinsulinemic and hypoglycemic state, together with the reduced leptin levels of the anorectic animals in our study correlate with the high levels of NPY mRNA found in the ARC but the co-existence of anorexia with high NPY remains enigmatic.

Appetite control is highly complex. Most likely, the hypothalamic arcuato-paraventricular and dorsomedial nuclei function as integrators of peripheral as well as neuronal signals that regulate food intake. Therefore, several factors may be capable of mediating cancer anorexia by interaction with hypothalamic hunger regulation. This may occur through quite different mechanisms, since different types of cancer anorexia have been associated with both high (76) and low levels of NPY in the ARC (77). Our recent comparative study on the effect of transplantable insulinomas and glucagonomas on the endogenous pancreas in rats revealed a highly potent inhibition and functional elimination of the endogenous pancreatic β and A cells, respectively (4). These data suggest that the anorectic glucagonoma is a valid, transformed equivalent of the normal islet A cell phenotype. Therefore, it may be predicted that the normal A cell, or more likely the GLP-1–producing intestinal L cell, could potentially play an important, yet undefined role in appetite regulation (59).

The type of anorexia described here represents a situation where the hyperphagic action of NPY is completely blocked by an unexplained mechanism, while the expected hypothermic effect of elevated NPY seems to be intact or, alternatively, directly caused by GLP-1 (78). We suggest that the anorectic tumor secretes a factor which evidently circulates in the blood and is a potent and selective inhibitor of NPY's orexigenic and polydipsic effects. This factor exhibits its effect at the postarcuate/NPY level and is compatible with the putative function of plasma-derived GLP-1 or, alternatively, by the existence of a novel factor capable of blocking the normal hypothalamic functions of NPY. The mechanisms governing the outflow of the PVN and resulting in eating behavior are certainly interesting, and our experimental glucagonoma provides a tool with which to study such pathways in rats. Direct identification and isolation of the MSL-G-AN glucagonoma-derived anorectic factor would allow studies of its normal and pathogenic roles. A neutralizing principle for this factor might provide potential therapeutics in the treatment of certain types of cancer anorexia, while the factor itself could be of potential use in the treatment of obesity-related disorders such as non–insulin-dependent diabetes mellitus.

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