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Hypothalamic Melanin-Concentrating Hormone and Estrogen-Induced Weight Loss

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Melanin-concentrating hormone (MCH) is an orexigenic neuropeptide produced by neurons of the lateral hypothalamic area (LHA). Because genetic MCH deficiency induces hypophagia and loss of body fat, we hypothesized that MCH neurons may represent a specific LHA pathway that, when inhibited, contributes to the pathogenesis of certain anorexia syndromes. To test this hypothesis, we measured behavioral, hormonal, and hypothalamic neuropeptide responses in two models of hyperestrogenemia in male rats, a highly reproducible anorexia paradigm. Whereas estrogen-induced weight loss engaged multiple systems that normally favor recovery of lost weight, the expected increase of MCH mRNA expression induced by energy restriction was selectively and completely abolished. These findings identify MCH neurons as specific targets of estrogen action and suggest that inhibition of these neurons may contribute to the hypophagic effect of estrogen.

Key words: MCH; estrogen; body weight; energy balance; lateral hypothalamus; anorexia; pair-feeding

The lateral hypothalamus plays a critical role in the regulation of energy intake and expenditure. Initial work performed >50 years ago demonstrated that bilateral lesions of the lateral hypothalamic area (LHA) result in profound hypophagia in rodents (Stellar, 1954), and recent progress has begun to identify candidate neuronal subpopulations that may mediate these LHA feeding effects. Neurons producing melanin concentrating hormone (MCH) represent one such subset. In rats and mice, intracerebroventricular administration of MCH induces hyperphagia, whereas MCH deficiency induced by targeted gene deletion leads to a hypophagia syndrome and loss of body fat (Qu et al., 1996; Shimada et al., 1998; Tritos et al., 1998). Thus, selective inhibition of MCH neurons might be expected to induce an anorexic response.

Converging evidence suggests that the hypothalamic arcuate nucleus (ARC), in addition to the LHA, plays a key role in the transduction of peripheral signals reflecting negative energy balance into behavioral, autonomic, and metabolic responses that favor the recovery of lost weight (Elmquist et al., 1998; Woods et al., 1998; Schwartz et al. 2000). Accordingly, hormonal responses to negative energy balance (declining plasma levels of insulin and leptin in concert with increasing levels of corticosterone) induce the ARC to increase biosynthesis and release of the coexpressed orexigenic molecules neuropeptide Y (NPY) and agouti-related protein (AgRP) and to decrease expression of pro-opiomelanocortin (POMC), the precursor of the anorexic melanocortin α-MSH (Schwartz et al., 1997; Hahn et al., 1998; Havel et al. 2000). Changes in the release of these neuropeptides from ARC neurons are hypothesized to influence energy homeostasis via effects on "second order" neurons situated in adjacent hypothalamic areas, such as the LHA and the paraventricular nucleus (PVN) (Broberger et al., 1998; Elias et al., 1998; Sawchenko, 1998). The abundance of ARC projections to MCH neurons in the LHA (Elias et al., 1998) supports the hypothesis that the MCH system lies "downstream" of ARC neurons involved in the adaptive response to weight loss. Therefore, under conditions in which MCH signaling is selectively disrupted, anorexia might be expected to persist despite intact upstream signaling events (e.g., low levels of plasma leptin and ARC POMC and high levels of plasma corticosterone and ARC NPY).

One anorexia paradigm of particular utility for investigating this hypothesis is chronic hyperestrogenemia in male rats (Wade, 1972, 1986; Mordes and Rossini, 1981; Mordes et al., 1984; Bernstein et al., 1986). This model provides reliable and sustained decreases in food intake and body weight without overt toxicity, is easily used and reversible, and allows quantification of the anorexic stimulus. Furthermore, estrogen was recently shown to inhibit hypothalamic MCH expression in ovariectomized female rats (Murray et al., 2000). We therefore hypothesized that anorexia because of hyperestrogenemia in male rats is associated with selective inhibition of MCH. To investigate this hypothesis, we measured changes in food intake, body weight, plasma hormone levels, and hypothalamic levels of MCH, NPY, AgRP, POMC, and corticotropin-releasing hormone (CRH) mRNA in two rat models of hyperestrogenemia: Leydig cell tumor implantation (whose anorexic effects are proposed to be primarily mediated by estrogen secretion) (Mordes and Rossini, 1981; Mordes et al., 1984; Bernstein et al., 1986) and chronic subcutaneous estrogen administration.

MATERIALS AND METHODS

Study animals and protocols

Animals. Adult male Wistar-Furth rats (Simonsen Laboratories, Gilroy, CA) weighing 200–300 gm were housed individually and maintained on a 12 hr light/dark cycle (lights on at 7:00 A.M.) with *ad libitum* access to drinking water and pelleted Purina lab chow (#5001), except as noted. All study protocols were approved by the University of Washington Animal Care Committee.

Experiment 1: effects of Leydig cell tumor implantation on food intake, plasma hormones, and hypothalamic neuropeptide levels. Body weight and food intake was measured daily for each rodent. One day before tumor implantation surgery, 30 animals were weight-matched and placed into one

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of three groups ($n = 10$/group): tumor-bearing or sham-implanted controls fed *ad libitum*, or sham-implanted controls that were pair-fed to the food intake of the tumor bearing group. The pair-fed group received two daily meals of equal size (given at lights out and 4 hr into the dark cycle). The total amount of chow provided daily to pair-fed animals was equal to that consumed on the previous day by a previously assigned partner in the tumor-bearing group. This feeding regimen continued for 22 d.

Tumor implantation procedure. A LTW(m) Leydig cell tumor (graciously provided by Dr. Ilene Bernstein) was removed from a donor rat anesthetized with ketamine and xylazine (6.5 ml/10 ml, 1 ml/kg, i.p. body weight for each drug), and the excised tumor was cut into 5–10 mg fragments and placed in 0.9% sterile saline. Under sterile conditions, a tumor fragment was inserted into a small subcutaneous pocket created in the right flank of similarly anesthetized recipient rats. *Ad libitum* fed and pair-fed animals underwent the same procedure, but tumor cells were not implanted.

Blood and tissue analyses. On day 22 after implantation (1 hr after lights on), tumor-bearing and sham-implanted rats were individually briefly exposed to CO_2 and decapitated, followed by rapid brain removal and collection of trunk blood in cooled heparinized tubes. Brains and plasma were frozen at −80°C. Tumors were weighed and stored at −20°C. The mean postmortem tumor weight was 0.82 ± 0.46 gm, and in no case did tumor weight exceed 1% of total body weight at the time of killing. Given the 24 hr lag required for pair feeding, collection of blood and tissue from the pair-fed group occurred 24 hr later. Plasma levels of leptin (Linco, St. Louis, MO) and corticosterone (ICN Biomedicals, Costa Mesa, CA) were measured by RIA. Plasma insulin was assayed by a modification of the double-antibody method of Morgan and Lazarow (1963). Serum estradiol was measured using a solid phase, competitive, time-resolved immunofluorometric assay (DELFIA; Wallac Oy, Oulansalo, Finland) using europium-labeled estradiol, polyclonal anti-estradiol antibodies derived from rabbit, and a second antibody directed against rabbit IgG coated onto 96 well plates. The intra-assay coefficient of variation of this assay was 3.8%, and the lower limit of sensitivity was 0.05 nmol/l (13.6 pg/ml). Plasma glucose levels were determined by the glucose oxidase method.

Experiment 2: dose-effect of estrogen administration on food intake, body weight, and plasma hormone levels. To identify a dose of estradiol that yields plasma levels comparable with those achieved in tumor-bearing rats of experiment 1, rats were anesthetized with ketamine and xylazine and implanted (subcutaneously) with a pellet containing either vehicle only ($n = 15$) or 2.5 mg ($n = 7$), 7.5 mg ($n = 8$), or 15 mg ($n = 7$) of 17β-estradiol (Innovative Research of America, Sarasota, FL). Of the vehicle-treated rats, eight were pair-fed, as in experiment 1, to a rat of similar initial body weight in the group receiving the 15 mg estradiol dose. Food intake and body weights were measured daily for 29 d and plasma was obtained for analysis on day 29.

Experiment 3: effect of chronic 17β-estradiol administration on hypothalamic neuropeptide mRNA levels. Rats were implanted subcutaneously with a pellet containing either vehicle ($n = 10$) or 7.5 mg ($n = 8$) of 17β-estradiol, a dose selected on the basis of results from experiment 2 to approximate plasma levels of tumor-bearing rats in experiment 1, and food intake and body weights were measured daily for 22 d. Plasma and brains were obtained and analyzed as described for experiment 1.

In situ *hybridization*

Tissue preparation. Frozen brains were sectioned in a coronal plane at 14 μm with a cryostat, mounted on RNase-free slides, and treated with 4% paraformaldehyde, acetic anhydride, ethanol, and chloroform. For each animal, six slides (12 brain sections) of hypothalamus in the region 2.0–3.5 mm posterior to bregma were selected for hybridization for each riboprobe. Sections for quantitation of POMC mRNA were selected from the ARC rostral to the ventromedial nucleus (VMN), whereas those used for NPY and AgRP mRNA hybridization contained the VMN and corresponded to the midregion of the ARC. Sections for MCH hybridization were selected from an area just rostral to slides chosen for POMC. Particular care was taken to anatomically match sections among animals for each probe by an investigator blinded to study conditions. For each experiment, all brain slices were concurrently prepared for hybridization and used in the same assay. Riboprobes from cDNA templates for NPY, AgRP, POMC, and MCH mRNAs were transcribed in the presence of 33P-UTP. Unincorporated label was separated using a QIAquick Nucleotide Removal Kit (Qiagen, Santa Clarita, CA). The hybridization signal on film autoradiograms in the ARC of each brain slice was quantified using an MCID image analysis system (Imaging Research, St. Catherine's, Ontario, Canada), as described previously (Schwartz et al., 1997). Both the hybridization density and image area were measured, the product of which was calculated as an index of mRNA levels from the mean of six to eight sections per animal (Schwartz et al., 1997).

Statistical analysis. Group mean values were compared by one-way ANOVA for experiments 1 and 2, using Fisher's test for multiple comparisons of between-group differences. For Experiment 3, group mean values were compared with a two-tailed unpaired Student's t test. The null hypothesis of equal means was rejected at the $p = 0.05$ level of significance. Data are expressed as mean ± SEM.

Table 1. Physiologic and hormonal responses to Leydig cell tumor-mediated hyperestrogenemia from experiment 1

	Sham	Tumor bearing	Pair-fed
Daily food intake, gm	20.3 ± 0.4^b	13.5 ± 0.7^a	13.8 ± 0.7^a
Daily food intake, % vehicle	100 ± 2.1^b	66 ± 3.6^a	68 ± 3.5^a
Final body weight, gm	305 ± 5^b	235 ± 5^{ab}	261 ± 4^a
Final body weight, % vehicle	100 ± 1.5^b	77 ± 1.5^{ab}	86 ± 1.1^a
Plasma values			
Estradiol, pg/ml	38 ± 21	305 ± 77^{ab}	20 ± 2
Leptin, ng/ml	4.5 ± 0.3^b	1.7 ± 0.3^{ab}	3.3 ± 0.3^a
Insulin, pmol/l	555 ± 39^b	380 ± 47^{ab}	565 ± 61^a
Glucose, mg/dl	134 ± 1.4	136 ± 5.0	136 ± 5.7
Corticosterone, μg/dl	6 ± 4^b	59 ± 15^a	75 ± 21^a

$^a p < 0.05$ versus sham rodents; $^b p < 0.05$ versus pair-fed rodents.

RESULTS

Experiment 1: effects of Leydig cell tumor implantation on food intake, plasma hormones, and hypothalamic neuropeptide levels

To investigate the mechanisms underlying anorexia induced by subcutaneous implantation of estrogen-secreting Leydig cell tumor cells, we studied three groups of male Wistar rats for 22 d: Leydig cell tumor-bearing, sham-implanted controls that were pair-fed to the intake of tumor-bearing group, and sham-implanted controls fed *ad libitum*. Relative to the *ad libitum* fed controls, mean daily food intake and final body weight of the tumor-bearing rats were reduced by 34 and 23% ($p < 0.05$ for both), respectively (Table 1). Whereas food consumption was designed to be comparable in pair-fed and tumor-bearing groups, final body weights were significantly lower in tumor-bearing rodents, suggesting an increase in energy expenditure relative to pair-fed animals. Mean plasma levels of 17β-estradiol were ~10-fold greater in the tumor-bearing rats than those measured in either sham-implanted group. As expected, leptin levels declined in both tumor-bearing and pair-fed groups relative to *ad libitum* fed controls (4.5 ± 0.3 ng/ml), but were significantly lower in tumor-bearing (1.7 ± 0.3 ng/ml; $p < 0.05$ vs *ad libitum* fed controls) than in pair-fed rats (3.3 ± 0.3 ng/ml; $p < 0.05$ vs *ad libitum* fed controls and tumor-bearing rodents). Plasma insulin levels were also significantly decreased in tumor-bearing rodents, but not in pair-fed animals, whereas corticosterone levels were significantly elevated in both tumor-bearing and pair-fed animals. Nonfasting plasma glucose levels were similar across groups (Table 1).

The results of *in situ* hybridization performed on coronal brain sections are shown in Figures 1 and 2. Relative to *ad libitum* fed controls, NPY and AgRP mRNA expression were comparably increased in the pair-fed and tumor-bearing groups (Figs. 1*a*, 2), whereas POMC and CRH levels were reduced in these groups (Fig. 1*b*). As with body weight and plasma leptin levels, POMC mRNA levels in the tumor-bearing group were reduced to a significantly greater extent than was observed in the pair-fed group. The direction of changes of each of these neuropeptide mRNAs was similar in tumor-bearing and pair-fed groups and consistent with a compensatory response to chronic energy restriction. In contrast, expression of MCH mRNA in the LHA differed markedly between tumor-bearing and pair-fed groups. Thus, whereas MCH gene expression was markedly increased by pair-feeding (585% ± 117 of *ad libitum* fed controls, $p < 0.05$ vs *ad libitum* fed controls and tumor-bearing rodents), MCH levels in the tumor-bearing group were nonsignificantly lower than in *ad libitum* fed rats (74% ± 17 of *ad libitum* fed rodents; $p = 0.19$ for *ad libitum* fed controls vs tumor-bearing animals) (Figs. 1*c*, 2). MCH mRNA levels in the LHA of pair-fed rats were therefore eightfold higher than in the tumor-bearing rats ($p < 0.05$).

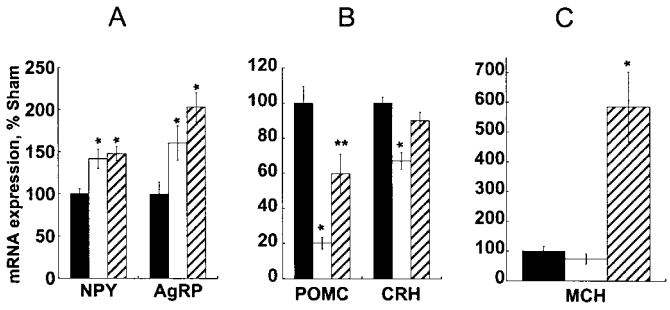

Figure 1. Hypothalamic neuropeptide mRNA levels as measured by *in situ* hybridization from animals in experiment 1. Results from Leydig cell tumor-bearing (*open bar*), pair-fed (*diagonal hatching*), and sham-implanted (Sham; *solid bar*) rodents presented as a percentage of mRNA expression relative to sham-implanted rodents fed *ad libitum*. Pair-fed rodents were sham-implanted, and their food intake was matched to that of the tumor-bearing group. *NPY,* Neuropeptide Y; *AgRP,* Agouti-related protein; *POMC,* pro-opiomelanocortin; *CRH,* corticotropin-releasing hormone; *MCH,* melanin concentrating hormone. $^{*}p < 0.05$ versus Sham; $^{**}p < 0.05$ versus tumor bearing.

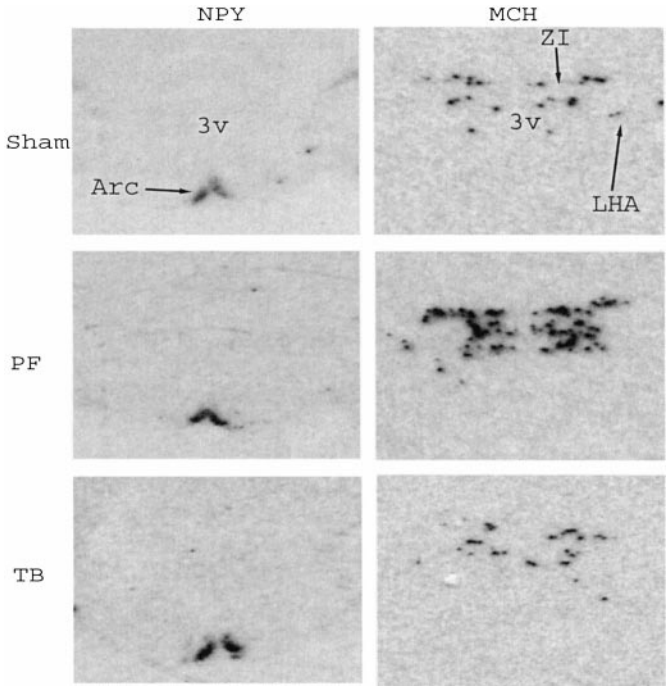

Figure 2. Representative autoradiograms of hypothalamic neuropeptide Y (*NPY*) and melanin-concentrating hormone (*MCH*) mRNA by *in situ* hybridization in Leydig cell tumor-bearing (*TB*), pair-fed (*PF*), and sham-implanted (*Sham*) rodents of experiment 1. NPY mRNA levels were visibly increased in tumor-bearing and pair-fed controls relative to Sham animals, whereas MCH mRNA expression was reduced in tumor-bearing compared with pair-fed animals. *Arc,* Arcuate nucleus of the hypothalamus; *ZI,* zona incerta; *LHA,* lateral hypothalamic area; *3v,* third cerebral ventricle.

Experiment 2: dose-effect of estrogen administration on food intake, body weight, and plasma hormone levels

To identify a dose of estradiol that achieves plasma levels comparable with those detected in tumor-bearing rats in experiment 1 and to assess its effects on food intake and body weight, rats were monitored for 29 d after subcutaneous implantation with sustained-release pellets containing either vehicle or 17β-estradiol in one of three doses (2.5, 7.5, and 15 mg). Relative to vehicle-implanted, *ad libitum* fed controls, mean daily food intake in each of the estrogen-treated groups was reduced by ~25%, an effect detected on the first day of treatment (Table 2). The magnitude of the food intake and body weight changes did not differ across the three estradiol groups and approximated that seen in the tumor-bearing group of experiment 1. As in experiment 1, the mean final body weight of vehicle-treated, pair-fed rats in experiment 2 was higher than that of any of the estrogen-treated groups, suggesting increased energy efficiency of the pair-fed rats relative to those receiving estradiol. Plasma estradiol levels in rats receiving the 2.5 mg pellet (169 ± 42 pg/ml) were lower than those of tumor-bearing rats in experiment 1 (305 ± 77 pg/ml), whereas the 7.5 and 15 mg pellets yielded plasma estradiol levels higher than in tumor-bearing rats (624 ± 46 and 822 ± 149 pg/ml, respectively). As in experiment 1, estradiol-induced weight loss was associated with reduced plasma leptin and insulin levels and increased corticosterone levels (Table 2), and these effects (like those on food intake and body weight) were not dose-related. Unlike the response of tumor-bearing or pair-fed rats of experiment 1, plasma glucose levels decreased by 7–10% in each of the estradiol-treated groups.

Experiment 3: effect of chronic 17-β estradiol administration on hypothalamic neuropeptide mRNA levels

To investigate effects on hypothalamic gene expression of chronic hyperestrogenemia at levels comparable with those detected in the tumor-bearing group in experiment 1, male Wistar rats were implanted subcutaneously with pellets containing either vehicle or 7.5 mg of 17β-estradiol (selected on the basis of data from experiment 2). After 22 d, estradiol-treated rats had sustained a 33% reduction in final body weight relative to vehicle-implanted controls ($p < 0.05$) (Table 3). As in experiment 2, estradiol levels were markedly elevated in the treated group relative to controls (1046 ± 105 vs 8.5 ± 0.5 pg/ml; $p < 0.05$), whereas plasma leptin and glucose levels were significantly reduced. Plasma corticosterone levels were increased in the estradiol-treated group, but the effect did not reach statistical significance. As measured by *in situ* hybridization, NPY and AgRP mRNA expression in the ARC of estradiol-treated rats increased by 69.7 ± 10.9% ($p < 0.05$) and 124 ± 18% ($p < 0.05$) relative to controls, whereas POMC mRNA hybridization was reduced by 47.6% ($p < 0.05$) (Fig. 3*a,b*). This profile of NPY, AgRP, and POMC mRNA expression was similar to that seen in the tumor-bearing rats of experiment 1. Similarly, MCH mRNA levels in the LHA of estradiol-treated animals were nonsignificantly below control values, despite pronounced weight loss (Fig. 3*c*). Thus, the 7.5 mg dose of estradiol elicited behavioral, hormonal, and hypothalamic neuropeptide responses similar to those seen in experiment 1, including selective blockade of the effect of weight loss to increase hypothalamic expression of MCH mRNA.

DISCUSSION

A critical role for LHA neurons, particularly those producing MCH, in energy homeostasis is suggested by the hypophagic and lean phenotype arising from targeted gene deletion of this neuropeptide (Shimada et al., 1998). We therefore hypothesized that decreased MCH signaling contributes to the pathogenesis of certain forms of anorexia. Here we report the complete abolition of the effect of chronic negative energy balance to increase LHA MCH expression in two rat models of estrogen-mediated anorexia, despite the preservation of key hormonal (decreased plasma leptin and insulin levels with increased plasma levels of corticosterone) and hypothalamic (increased expression of NPY and AgRP mRNA along with decreased expression of CRH and POMC mRNA) responses to weight loss. These findings identify MCH neurons as specific targets of estrogen action that may contribute to its anorexic effects.

Hyperestrogenemic rats displayed similar or exaggerated hormonal responses to weight loss as compared with pair-fed controls. Plasma leptin and insulin concentrations were significantly below those of both *ad libitum*-fed and pair-fed controls in experiment 1, whereas corticosterone elevations paralleled those seen in pair-fed rodents. Thus, hyperestrogenemia had no apparent adverse affect on the recruitment of adaptive hormonal responses to energy deficit. Moreover, the ability of these hormonal cues to engage ARC and PVN neuronal responses that accompany energy deficit

Table 2. Dose effect of 17β-estradiol administration on food intake, body weight, and plasma hormone levels from experiment 2

	Vehicle	2.5 mg	7.5 mg	15 mg	Pair-fed
Daily food intake, gm	19.7 ± 0.4^{bc}	14.5 ± 0.4^{a}	13.7 ± 0.4^{a}	14.2 ± 0.3^{a}	14.2^{a}
Daily food intake, % vehicle	100 ± 2.0^{bc}	74 ± 1.8^{a}	69 ± 2.3^{a}	72 ± 1.6^{a}	72^{a}
Final body weight, gm	313 ± 3^{bc}	238 ± 4^{ab}	230 ± 3^{ab}	230 ± 3^{ab}	258 ± 6^{a}
Final body weight, % vehicle	100 ± 0.9^{bc}	76.0 ± 1.5^{ab}	73.3 ± 0.8^{ab}	73.5 ± 1.0^{ab}	82.4 ± 2.1^{ac}
Plasma values					
Estradiol, pg/ml	29 ± 18	169 ± 42^{b}	624 ± 46^{abc}	822 ± 150^{abc}	13 ± 1^{c}
Leptin, ng/ml	4.5 ± 0.3^{c}	1.9 ± 0.2^{ab}	2.3 ± 0.2^{ab}	2.1 ± 0.2^{ab}	3.8 ± 0.4^{c}
Insulin, pmol/l	248 ± 24^{bc}	123 ± 19^{a}	204 ± 21^{c}	182 ± 36	162 ± 26^{a}
Glucose, mg/dl	137 ± 2.6^{c}	127 ± 1.8^{ab}	122 ± 2.1^{ab}	122 ± 2.0^{ab}	134 ± 4.0^{c}
Corticosterone, μg/dl	3.4 ± 1.1^{c}	13.1 ± 4.1^{a}	18.9 ± 4.0^{ab}	19.9 ± 2.7^{ab}	7.2 ± 1.9

$^{a}p < 0.05$ versus vehicle; $^{b}p < 0.05$ versus pair-fed; $^{c}p < 0.05$ versus 2.5 mg.

Table 3. Effect of chronic 17β-estradiol administration on body weight and plasma hormone levels from experiment 3

	Vehicle	7.5 mg estradiol
Final body weight, gm	399 ± 6	267 ± 6^{a}
Final body weight, % vehicle	100 ± 1.6	67 ± 1.5^{a}
Plasma values		
Estradiol, pg/ml	8.5 ± 0.5	1046 ± 105^{a}
Leptin, ng/ml	5.8 ± 0.7	3.3 ± 0.4^{a}
Glucose, mg/dl	137 ± 2	113 ± 2^{a}
Corticosterone, μg/dl	11 ± 9	67 ± 31^{ns}

$^{a}p < 0.05$ versus vehicle; ^{ns}p = not significant.

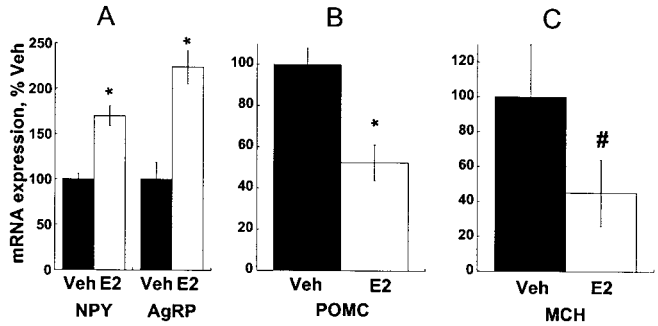

Figure 3. Hypothalamic mRNA levels as measured by *in situ* hybridization in rodents bearing a subcutaneous pellet releasing 17β-estradiol (*E2*) or vehicle (*Veh*) (experiment 3). Results (mean ± SEM) are presented as a percentage of mRNA expression relative to Veh. The pellets released 7.5 mg of E2 over 21 d. $^{*}p < 0.05$ versus Veh; $^{\#}p = 0.19$.

appeared to be intact in the hyperestrogenemic groups. Tumor-bearing rodents of experiment 1 displayed 45–100% increases in NPY and AgRP mRNA relative to *ad libitum* fed control rodents, an effect similar to that observed in the pair-fed group, whereas POMC and CRH mRNA expression was inhibited to an even greater extent in hyperestrogenemic versus pair-fed rodents. This combination of hypothalamic neuropeptide responses was also observed in estradiol-treated rodents in experiment 3. Thus, anorexia persisted in hyperestrogenemic animals, despite the apparent preservation of a set of compensatory hormonal and medial hypothalamic responses expected to potently stimulate food intake.

Although the PVN, like the LHA, contains second-order neurons involved in energy homeostasis (such as those containing CRH), the finding that electrolytic lesions of the PVN do not attenuate estrogen-induced anorexia (Butera et al., 1992) suggests that key neurons that mediate the effects of estrogen on energy homeostasis must be located elsewhere. Our demonstration that hyperestrogenemia selectively and completely suppressed the expected increase of MCH mRNA expression in response to progressive weight loss, whereas important ARC and PVN responses were unimpeded, is consistent with the model illustrated in Figure 4, in which estrogen-sensitive MCH neurons in the LHA are "downstream" in a pathway used by ARC neurons to trigger feeding responses (Elias et al., 1998; Elmquist et al., 1998; Sawchenko, 1998; Schwartz et al., 2000). According to this model, interventions that disrupt MCH signaling can cause sustained anorexia by interrupting the pathway used by ARC neurons to control feeding behavior.

This hypothesis is supported by the finding that MCH-deficient mice have reduced ARC POMC production, yet continue to undereat, and by growing evidence that ARC neurons can directly influence LHA activity (Shimada et al., 1998). Recent work has demonstrated rich connections between NPY- and POMC-containing neurons of the ARC and MCH neurons in the LHA (Broberger et al., 1998; Elias et al., 1998) and that pharmacological blockade of melanocortin-4 receptors, which are concentrated in the LHA, increases MCH mRNA expression (Hanada et al., 2000). Furthermore, leptin-deficient *ob/ob* mice display increased MCH mRNA expression (Qu et al., 1996), despite relatively low expression of leptin receptor in the LHA (Schwartz et al., 1996). Thus, leptin appears to influence MCH neurons via an indirect mechanism. The recent demonstration that ARC-derived leptin-responsive neurons project to the LHA provides a plausible mechanism, whereby leptin-deficiency increases MCH gene expression (Elias et al., 1999).

Our data are in agreement with the recently demonstrated effect of estrogen administration to decrease hypothalamic MCH mRNA levels in female rats rendered estrogen-deficient by ovariectomy (Murray et al., 2000). Thus, estrogen deficiency in female rats is associated with increased hypothalamic MCH gene expression that may, in turn, contribute to the accompanying hyperphagia and weight gain in this setting (Wade and Gray, 1979). We found in male rats that, in contrast to the sixfold increase of MCH mRNA expression observed in pair-fed controls, hyperestrogenemic animals mounted MCH responses no different from *ad libitum* fed controls. Together, these observations establish MCH neurons as targets of estrogen action. Our data further suggest that supraphysiological levels of estrogen can override the ability of weight loss and decreased leptin signaling to induce MCH expression. These findings also suggest, but do not establish, a specific contribution of MCH signaling to the alterations of energy balance observed in settings of estrogen excess and deficiency. Further work is warranted to test the hypotheses that MCH neurons mediate the feeding effects observed with physiological, as well as supraphysiological, alterations in estrogen status.

The hypothesis that estrogen receptors mediate the effects of estrogen on energy balance is supported by the observation that these receptors are expressed at high levels in medial and lateral hypothalamic areas implicated in energy homeostasis (Couse et al., 1997). However, whereas Sar et al. (1990) reported the colocalization of NPY and estrogen receptor in a subpopulation of ARC

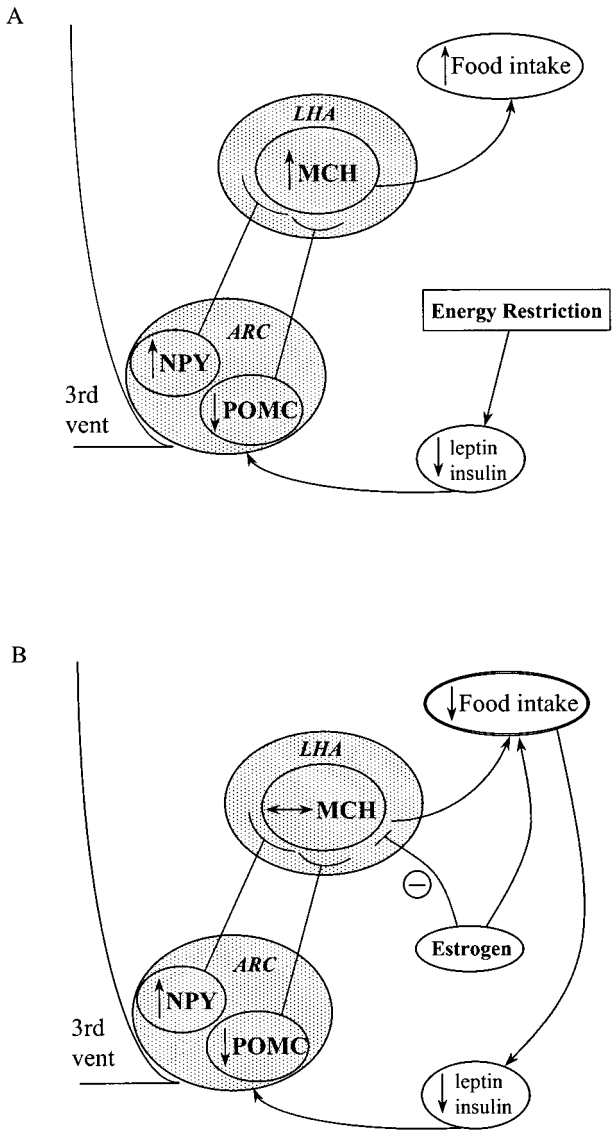

Figure 4. Proposed model of the effects of estrogen on hypothalamic neuronal pathways involved in the regulation of energy balance. *A,* In response to energy restriction, circulating leptin and insulin levels decrease, resulting in increased gene expression of orexigenic peptides (e.g., NPY) and decreased gene expression of anorexic peptides (e.g., POMC) in neurons of the ARC. These neuronal responses are proposed to increase expression of the orexigenic neuropeptide MCH in neurons of the LHA, which in turn promote increased food intake. *B,* Estrogen-mediated weight loss and anorexia also lower plasma leptin and insulin, but the expected activation of MCH neurons fails to occur in the presence of estrogen, despite the preservation of "upstream" ARC NPY and POMC neuronal responses to reduced adiposity signaling. This inhibition of MCH neurons by estrogen is hypothesized to contribute to the sustained anorexia observed with chronic estrogen exposure in male rodents. *3rd vent,* Third cerebral ventricle.

neurons, there is a paucity of data regarding the presence of estrogen receptor on other neuronal systems pertinent to energy homeostasis (Sar et al., 1990). Interestingly, deficiency of the α isoform of the estrogen receptor (ERα), which is concentrated in both the ARC and the LHA, induces obesity in mice, whereas mice deficient in estrogen receptor β (which is not expressed in the LHA) have a normal body weight (Couse and Korach, 1999), suggesting a specific role for ERα in mediating the effects of estrogen on energy balance. Whereas evidence that ERα is expressed in the LHA raises the possibility that estrogen may directly inhibit MCH neurons, the hypothesis that MCH neurons express estrogen receptor is untested (Couse et al., 1997).

The significantly higher body weights of pair-fed controls in experiments 1 and 2 (relative to hyperestrogenemic animals) suggest that hyperestrogenemia, in addition to its hypophagic effects, also impairs the ability of rodents to manifest the decrease in energy expenditure that normally accompanies weight loss. In concert with reduced food intake, this defect appears to contribute to the ability of estrogen to induce negative energy balance and deplete body fuel stores (Laudenslager et al., 1980). The hypothesis that increased hypothalamic MCH signaling contributes to adaptive decreases of energy expenditure during weight loss is a possibility that warrants additional study.

One mechanism implicated in the pathogenesis of estrogen-induced anorexia is the development of taste aversions because of illness or malaise (Bernstein et al., 1986). Whereas taste aversion may have contributed to the anorexia observed in our studies, there are few models in which sustained, marked weight loss occurs via this mechanism. For example, acute central administration of glucagon-like peptide-1 (GLP-1) reduces food intake by a mechanism that includes the development of taste aversion, but repeated administration of GLP-1 does not induce sustained reductions in food intake or body weight (van Dijk et al., 1997; Donahey et al., 1998). Thus, while taste aversions may have developed, they seem unlikely to fully explain the sustained anorexia induced by hyperestrogenemia in male rats.

The association between decreased MCH signaling and hypophagia may prove relevant to the pathogenesis of clinical anorexia syndromes, which are common and deleterious comorbidities of chronic diseases such as cancer and AIDS (Grunfeld and Feingold, 1992). Such anorexia syndromes are difficult to treat, perhaps because of our poor understanding of their pathophysiology. Although the significance of MCH in human anorexia syndromes remains uncertain, progress toward characterizing its role and that of other CNS effector systems in models of disordered food intake may eventually lead to novel treatments for these syndromes.

In conclusion, the observation that estrogen-induced anorexia in rodents is associated with decreased MCH signaling represents a first step toward identifying a potentially important role for MCH in estrogen-mediated alterations in energy homeostasis. Additional characterization of this effect will help to delineate the roles of discrete neuronal circuits involved in energy homeostasis, clarify the mechanism by which estrogen affects this process, and improve our understanding of the pathogenesis of anorexia syndromes.

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