

Overview of central targets for appetite regulation

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ABSTRACT: Feed intake is the cornerstone of animal productivity. The consequences of inadequate intake include inhibited growth, delayed puberty, infertility, reduced milk production, and lowered resistance to parasites and disease. Factors that influence intake include age, metabolic demand (gestation, lactation, and level of physical activity), thermal environment, photoperiod, disease, and psychosocial stress. Under some conditions intake is adequate for health and well-being but is limiting for optimum performance and productivity. The role of the central nervous system, particularly the hypothalamus, has long been recognized in the control of appetite. A variety of hormones and neurotransmitters are involved in the modulation of neural

pathways that stimulate or suppress appetite. Complementary DNA sequences and clones for these regulatory factors in livestock are rapidly being generated, providing new tools for physiological studies and the production of recombinant/synthetic hormones and analogs. An understanding of the interactions between environmental factors and the mechanisms of appetite control is fundamental to the development of practical approaches to optimize feed intake, which will usher in a new era of research in redefining the limits of productivity. An important component of this future research will be to ensure that enhanced intake is used efficiently for the production of high-quality food products.

Key Words: Appetite, Feed Intake, Livestock

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Introduction

The natural drive to eat is determined by complex interactions between biological mechanisms of appetite control and responses to challenges from the physical environment (Figure 1). Suppressed intake and performance associated with environmental, social, and disease stress are well recognized (Forbes, 1995; Wenk, 1998; Matteri et al., 2000a). When intake fails to meet the basic energy needs for maintenance and survival, potent adaptive mechanisms terminate nonessential functions such as growth, reproduction, and lactation. Under optimum conditions, nutritional intake is adequate for basal metabolic needs, growth, development, reproduction, and controlled deposition of energy stores (fat). Although animal managers strive to keep stress to a minimum and to provide adequate amounts of high-quality feed and water, targeted improvements in appetite and intake would further enhance recovery from disease and stress and overcome satiety-related limitations of growth and reproduction at full genetic potential.

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Importance of Feed Intake

Reproduction

The dependency of reproduction on adequate nutrition has long been recognized. Inadequate nutrition delays puberty and inhibits adult reproductive function (Armstrong and Britt, 1987; Randel, 1990; Keisler et al., 1999). A critical period for optimizing nutrition is during lactation, when low energy intake significantly impairs rebreeding efficiency (Aherne and Williams, 1992; Vizcarra et al., 1998; de Vries and Veerkamp, 2000). Although the majority of existing studies have evaluated nutrition-reproduction interactions in females, male reproduction also is dependent on adequate intake (Brown, 1994). A well-established consequence of undernutrition is reduced gonadotropin secretion (Brogan et al., 1999). Conversely, improvements in feed intake are positively associated with LH secretion in swine (Koketsu et al., 1998) and sheep (Holmberg and Malven, 1997). Increasing intake beyond maintenance levels in the period prior to mating (flushing) is also known to enhance reproductive efficiency (Thomas et al., 1987; Ahern and Williams, 1992).

Lactation

Optimizing feed intake during lactation is a recognized management goal (Grant, 1990; Wondra, 1998).

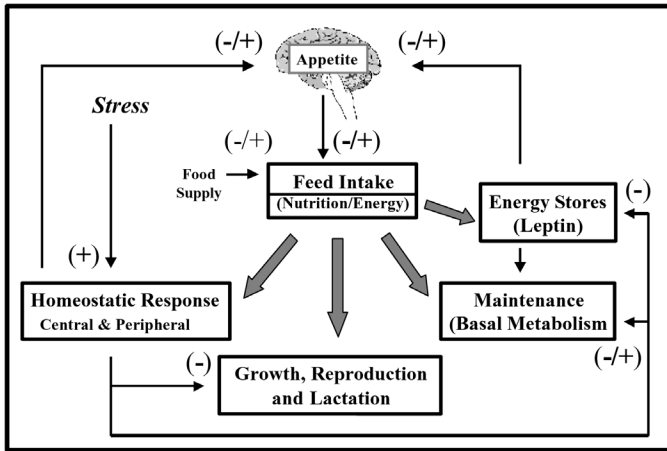


Figure 1. Interactions between physiological mechanisms of appetite control, adaptation to stress (homeostatic response), and distribution of dietary energy for homeostasis, fat deposition, basal metabolism, and productivity (growth, reproduction, and lactation).

Inadequate nutrition during lactation limits milk yield in beef (Lalman et al., 2000) and dairy (de Vries and Veerkamp, 2000) cattle. Feed intake drops by about one-third in the week before parturition in dairy cattle (Grant and Albright, 1995). Energy intake during early lactation in cattle does not meet metabolic demands until approximately 40 d after parturition (de Vries and Veerkamp, 2000). The association between intake and milk production is also well-recognized in swine; sow feed intake during lactation is a recognized influence on milk yield and the associated nutritional status of pigs and rebreeding performance (Britt, 1986; Tokach and Dial, 1992; Koketsu et al., 1996). Increasing ambient temperature decreases intake in lactating sows, with a suppression of pig growth at temperatures above 25°C (Quiniou and Noblet, 1999).

Growth

Feed intake is an important determinant of growth. The neonatal period of livestock is characterized by an exceptional relative growth rate and efficiency (Harrell et al., 1993). Growth advantages realized early in life can be maintained and have a positive impact on later growth and efficiency (Clutter and Nielsen, 1987; Mahan et al., 1998). Accordingly, considerable interest exists in optimizing neonatal intake by minimizing adverse effects of stressors and by enhancing energy intake. High-fat milk replacer has been tested in order to increase energy intake in preweaning calves, but natural mechanisms of satiety actually reduce intake and growth in animals on the high-energy diet (Kuehn et al., 1994). Sow milk production often limits piglet nutrition and growth (Versteegen et al., 1985). Pigs provided ad libitum access to milk replacer were significantly heavier than sow-fed pigs at weaning and reached market weight 10 d sooner than their sow-

reared counterparts (Harrell et al., 1993). No differences in carcass characteristics were found between artificially fed and sow-fed pigs. Preliminary results from a study in the laboratory of Gary Allee (University of Missouri-Columbia) have shown that feeding newly weaned pigs at 80, 100, and 120% of NRC requirements via gastric cannulas has a positive linear relationship with growth (Dyer et al., 1998). Increasing feed intake may also benefit growth beyond the neonatal period. Feeding 4-mo-old pigs 120% of ad libitum intake for 23 d produced a 40% improvement in growth with no adverse effect on fat vs lean tissue accretion (Pekas, 1985). Feed efficiency in the superalimented animals showed a tendency for improvement (7.3%) over pigs given ad libitum access to feed.

Other Considerations

As our knowledge of appetite control develops, we may find novel applications for feed intake enhancement under conditions of exogenously stimulated and naturally accelerated growth rate and efficiency. Injections of porcine somatotropin (**pST**) significantly increase the efficiency and rate of growth; however, suppression of appetite is a common side effect. Pigs treated with pST eat 10 to 20% less than their untreated counterparts (Roberts and Azain, 1997; Ji et al., 1998). Administration of recombinant pST from d 12 to 29 of lactation can increase milk production and piglet growth; however, treated sows eat less and consequently lose more body weight and backfat than control sows (Harkins et al., 1989). Enhanced (or unsuppressed) feed intake during somatotropin treatment would provide useful insights into the limiting factors of growth during periods of high growth rate and efficiency. Interestingly, somatotropin treatment has been shown to increase levels of messenger RNA (mRNA) for leptin (an appetite-suppressing hormone described below) in chickens and cattle (Ashwell et al., 1999; Houseknecht et al., 2000).

Compensatory growth often accompanies recovery from a period of inadequate nutrition, such as when a stressor is removed (Hahn, 1987) or refeeding following feed restriction. Compensatory growth is characterized by significantly elevated growth rates and feed efficiencies and is often accompanied by increased feed intake to the level limited by satiety (Kabbali et al., 1992; Sainz et al., 1995; Choi et al., 1997). In human infants, compensatory growth is accompanied by a doubling of normal intake (Casey and Arnold, 1985). Accordingly, induced compensatory growth could serve as a valuable model for studying appetite regulation. The ability to overcome natural mechanisms of satiety during compensatory growth could improve the effectiveness of stair-step feeding strategies (limit feeding followed by ad libitum intake), as well as facilitate the process of recovering from stress or illness.

When basic research on appetite control produces practical methods for increasing intake, it will be possi-

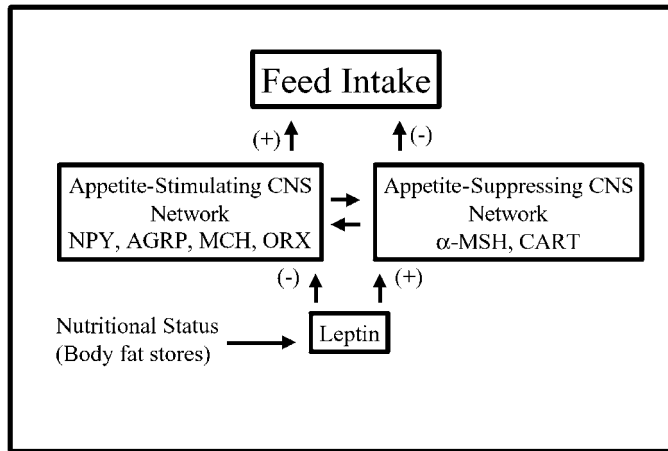


Figure 2. Conceptual diagram of interactions among a few of the many factors involved in appetite-stimulating and appetite-suppressing central nervous system (CNS) networks and regulatory input from the periphery (Leptin). Interactions among pathways exist within and between networks (see below). NPY = neuropeptide Y; AGRP = agouti-related protein; MCH = melanin-concentrating hormone; ORX = orexin; α -MSH = α -melanocyte-stimulating hormone; CART = cocaine and amphetamine-regulated transcript.

ble to study the effects of enhanced intake in combination with somatotropin treatment, compensatory growth, and other conditions of elevated growth rate and efficiency. In particular, a significant challenge in the near future will be to optimize intake to support enhanced genetic capabilities for productivity (Funk, 1993; Schinckel and de Lange, 1996).

Central Mechanisms of Appetite Control

The involvement of the brain in the control of food intake has long been recognized. Classic lesioning studies identified the hypothalamus as the site of important appetite-regulating pathways. Readers are referred to an excellent recent review of these pathways (Kalra et al., 1999). Lesions of the ventromedial nucleus (VMN), paraventricular nucleus (PVN), and dorsomedial nucleus (DMN) produce uncontrollable hunger and obesity. Conversely, lesions of the lateral hypothalamus remove the drive to eat. The idea of discrete primary brain centers for appetite control provided an important conceptual framework for early studies. We now know, however, that this early model is a great oversimplification of the complex mechanisms and pathways involved in the neural control of appetite (Rowland et al., 1996; Flier and Maratos-Flier, 1998; Kalra et al., 1999).

A variety of appetite-stimulating and appetite-suppressing hormones and neurotransmitters are produced in the central nervous system (CNS) and periphery (Figure 2; Flier and Maratos-Flier, 1998; Houseknecht et al., 1998; Kalra et al., 1999). These

compounds bind and activate their CNS receptors, triggering downstream pathways/regulators that result in appropriate changes in ingestive behavior. Complex interactions exist between pathways, such that absence of one regulatory factor may be compensated by alterations in other factors to maintain appetite regulation. A few of the appetite-stimulating neurohormones of recent interest include neuropeptide Y (NPY), the orexins, melanin-concentrating hormone (MCH), endorphins, galanin, GHRH, γ -amino butyric acid (GABA), and agouti-related protein (AGRP). Negative regulators of appetite include leptin, bombesin, glucagon-like peptide-1 (GLP-1), ciliary neurotrophic factor (CNTF), corticotropin-releasing hormone (CRH), urocortin, cholecystokinin (CCK), cocaine and amphetamine-regulated transcript (CART), and α -melanocyte-stimulating hormone (α -MSH). Cytokines also participate in suppression of appetite that occurs as a consequence of disease (Johnson, 1998; Plata-Salaman, 1998). Mechanisms of appetite suppression during disease are reviewed by Johnson (2001). With feed costs representing the primary economic input to animal production, it is important to note that many of the known appetite-regulating factors also influence feed efficiency.

Approaches for Appetite Control Research in Livestock

Most current research approaches in livestock appetite control begin with the elucidation of cDNA or genomic DNA sequences for appetite regulators. The availability of cDNA clones provides information on amino acid sequences, which then may be synthesized (peptides) or recombinantly expressed (protein hormones and receptors). The cDNA clones and sequence data for livestock orexin and leptin have resulted in the production of hormones that have been successfully used for studies in sheep and swine. Recombinant leptin is also being used for the development of new antibodies and immunoassays for cattle, sheep, swine, and poultry. Site-directed mutagenesis can be applied to cDNA clones for the structure-function analyses needed for the development of new agonists and antagonists. Examples of such compounds include leptin analogs with antagonistic or agonistic properties (Verploegen et al., 1997; Wuethrich et al., 2000). The production of cDNA clones and sequence data for livestock appetite-regulators provides primers and probes that contribute to international gene mapping programs and to the identification of polymorphisms for marker-assisted selection (Montaldo and Meza-Herrera, 1998).

Having established the cDNA tools needed to monitor appetite-regulating gene expression, the next great challenge will be to elucidate the mechanisms of action and physiological roles of these compounds on feed intake and productivity. The determination of tissues in which appetite-regulators and(or) their receptors are produced is an important first step in determining mechanisms of action for newly discovered compounds.

Leptin is a classic example; this hormone is produced in adipose tissue but interacts with receptors in the brain to inhibit feed intake. Our initial work with pigs has shown a variety of tissue distribution patterns for different appetite regulators (see below). Based on our knowledge of factors that affect feed intake, the expression of CNS feed intake regulators should be altered by nutritional status, genetics, thermal stress, psychological stress, physiological/ environmental conditions that affect energy expenditure, and disease. Integration of appetite-control research into existing models of altered intake is needed. An excellent example is the disease-challenged animal (Johnson, 2001). Additionally, partnerships between animal physiology and nutrition laboratories will be extremely valuable in moving this field of research forward. Studies of known appetite regulators will provide valuable information on the physiology of appetite control in domestic animals. Cloned livestock cDNA for many hypothalamic appetite regulators and their receptors have recently been produced and have formed the basis for studies of appetite control in swine, cattle, sheep, and poultry. Related data are rapidly emerging on the regulation of gene expression for orexin, NPY, AGRP, leptin, CART, MCH, and their respective receptors.

Appetite-Regulating Factors

As indicated above, a wide variety of hormones/neurotransmitters participate in appetite control. The literature in this area will certainly grow rapidly over the next several years as the development of new technologies continues to accelerate the rate of discovery of new factors such as the appetite stimulators ghrelin and beacon (Collier et al., 2000; Tschop et al., 2000). Excellent reviews of appetite regulation are available (Rowland et al., 1996; Flier and Maratos-Flier, 1998; Kalra et al., 1999). The selected regulatory factors briefly reviewed below are intended to serve as examples of compounds of current interest. The availability of cDNA/genomic DNA (**gDNA**) clones and sequences for domestic animal genes is noted; however, at the rate of current progress many more livestock sequence entries will likely be reported by the time this paper is published. Interested readers may search the National Center for Biotechnology Information database (GenBank) at <http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=Nucleotide>.

Leptin

Leptin is a 16-kDa protein hormone produced in fat tissue in proportion to body adiposity (Houseknecht et al., 1998). The discovery of leptin in 1995 was a particularly significant event in terms of 1) identifying a major missing link involved in the signaling of energy status from the periphery to the brain and 2) stimulating a renewed research interest in the identification and manipulation of central targets for appetite regulation.

Leptin administration suppresses feed intake (Barb et al., 1998; Henry et al., 1999; Denbow et al., 2000). In general, leptin decreases the expression of hypothalamic appetite stimulators and increases the expression of appetite-suppressing neurohormones (Kalra et al., 1999; also see below). Leptin receptors have been found on neurons containing NPY, AGRP, proopiomelanocortin (**POMC**), CART, MCH, and orexin (Meister, 2000).

Neuropeptide Y

Neuropeptide Y is a 36 amino-acid peptide found throughout the peripheral and central nervous systems. GenBank NPY cDNA sequences are available for swine, cattle, sheep, chickens, and catfish. Neuropeptide Y has long been recognized as a potent stimulator of appetite (Miner et al., 1989; Leibowitz, 1995; Palmiter et al., 1998). Neuropeptide Y neurons in the brainstem project into the hypothalamus and are found in the arcuate nucleus (**ARC**) and DMN (Rowland et al., 1996; Kalra et al., 1999). Direct hypothalamic, as well as intracerebroventricular (i.c.v.), injection of NPY increases feed intake, even in sated animals (Clark et al., 1984). Central stimulation of appetite by NPY can overcome appetite suppression caused by endotoxin treatment in sheep (McMahon et al., 1999). Neuropeptide Y also acts centrally to increase respiratory quotient and decrease overall energy expenditure (Leibowitz, 1995; Marks and Waite, 1997). Appetite can be suppressed by anti-NPY antisera or antisense oligonucleotides (Shibasaki et al., 1993; Akabayashi et al., 1994). Peripherally, NPY acts as a vasoconstrictor (Malmstrom et al., 1998) but can also elevate leptin and NPY receptor mRNA levels in sheep adipose tissue (Dyer et al., 1997). Interestingly, NPY is present and inducible in human immune cells (Schwartz et al., 1994).

Hypothalamic NPY is up-regulated by undernutrition (White and Kershaw, 1990; Barker-Gibb and Clarke, 1996), increased metabolic demand caused by cold exposure (Mercer et al., 1997), lactation (Smith, 1993), or 2-deoxy-glucose (Sergeyev et al., 2000). Our preliminary findings suggest that NPY expression is positively related to body weight in normally-growing weanling pigs (Dyer et al., 1999b) but seems to be elevated in severely growth-retarded animals (Matteri, unpublished data). Neuropeptide Y expression is elevated in *ob/ob* and *db/db* mice (Wilding et al., 1993; Yamamoto et al., 2000). Leptin administration decreases NPY levels (Schwartz et al., 1996; Mercer et al., 1997) and decreases NPY-induced intake (Sahu, 1998b); however, NPY apparently does not mediate all of leptin's effects on appetite. *Ob/ob* mice that are also NPY *-/-* are still obese, albeit less obese than *ob/ob*, NPY *+/+* mice (Erickson et al., 1996). Interestingly, NPY knockout mice are not lean or hypophagic (Palmiter et al., 1998), reflecting compensation by other regulators/pathways to maintain food intake and energy homeostasis. The stimulatory effect of NPY on feed intake can be blocked by pretreatment with GLP-1, but not α -

MSH or neurotensin (Tritos et al., 1998). The appetite inhibitors CRH and MTII (type 4 melanocortin receptor [MC4R] agonist, see section on AGRP below) are able to suppress feeding in NPY $-/-$ mice (Hollopeter et al., 1998). Neuropeptide Y $-/-$ mice are extremely sensitive to the appetite-stimulating effect of AGRP (Marsh et al., 1999b).

At least six NPY receptor subtypes exist (Y1 to Y6). The Y1 and Y5 receptor subtypes are believed to play a role in appetite control. Both Y1 and Y5 antagonists inhibit feeding (Kanatani et al., 1996; Criscione et al., 1998); however, existing evidence suggests that the Y1 receptor has a predominant role in feed intake control. Nocturnal intake and post-fasting appetite are attenuated in Y1 knockout mice (Pedrazzani et al., 1998). Fasting up-regulates Y1, but not Y5, receptors in rats (Xu et al., 1998). The Y5 knockout mice show normal appetite and growth early in life (Marsh et al., 1998; Kanatani et al., 2000). Controversy still exists, however, with regard to the roles of specific NPY receptor subtypes in mediating NPY-stimulated feed intake. Kanatani et al. (2000) observed normal NPY-induced feeding, whereas another group reported a significantly reduced feeding response to NPY in Y5 knockout mice (Marsh et al., 1998). Using a variety of NPY receptor agonists and antagonists, a primary role for a single receptor subtype in regulating feeding behavior could not be demonstrated (Iyengar et al., 1999). The authors of the latter study speculated that NPY-induced feeding could be concurrently mediated by several subtypes or by a subtype yet to be discovered. The action of NPY in suppressing gonadotropin secretion and reproduction during undernutrition (Kalra et al., 1998) seems to be mediated by the Y5 receptor (Raposinho et al., 1999; Lebrethon et al., 2000b). Several NPY receptor subtypes have been cloned in swine (Y1, 2, 4, 5, and 6), sheep (Y1, 2, and 3), and cattle (Y2).

Agouti-Related Protein (AGRP)

The AGRP is a 132 amino-acid protein found primarily in the ARC (Kalra et al., 1999). Agouti-related protein is structurally and functionally similar to agouti, a protein produced in skin that lightens coat color as an antagonist to α -MSH binding at the Type 1 melanocortin receptor (Ollmann et al., 1997). Ubiquitous expression of agouti occurs in the agouti (*Ay/a*) mouse, which possesses a yellow coat color and is obese. Alpha-melanocyte-stimulating hormone suppresses appetite by a central mechanism dependent on binding to the Type 4 melanocortin receptor (**MC4R**; Marsh et al., 1999a). The MC4R is found throughout the brain, including the PVN (Mountjoy et al., 1994). The obesity of the *Ay/a* mouse is due to agouti's antagonistic binding at the MC4R (Fan et al., 1997). Agouti-related protein enhances appetite by similar antagonism of α -MSH binding to the MC4R (Marsh et al., 1999a; Edwards et al., 2000). GenBank AGRP cDNA entries exist for cattle, swine, and chickens. Porcine cDNA sequences for

MC4R have been reported (Dyer et al., 2000a; Kim et al., 2000).

Ubiquitous production of AGRP or MC4R deficiency in transgenic mice produces an obese phenotype similar to that of the agouti mouse (Huszar et al., 1997; Ollmann et al., 1997). Intracerebroventricular injection of AGRP, or the c-terminal peptide AGRP(83-132), stimulates feed intake (Rossi et al., 1998; Ebihara et al., 1999). Interestingly, the expression of hypothalamic AGRP and MC4R may be positively related with growth in young pigs (Dyer et al., 2000a,b). As with other stimulators of appetite, the expression of AGRP is elevated by fasting (Ebihara et al., 1999; Wilson et al., 1999). Consistent with the effects of nutritional restriction, AGRP expression is increased by the metabolic inhibitor 2-deoxy-glucose (Sergeyev et al., 2000). The suppression of feed intake produced by leptin can be blocked by AGRP (Ebihara et al., 1999). Hypothalamic AGRP expression is suppressed by leptin (Wilson et al., 1999) and is elevated in the *ob/ob* mouse (Ollmann et al., 1997). Further highlighting the complex and varied pathways controlling feed intake, appetite can be suppressed in MC4R-deficient mice by CNTF, CRH, or urocortin and enhanced by NPY (Marsh et al., 1999a). Agouti-related protein does not block the anorectic effects of CART, GLP-1, or CRH (Edwards et al., 2000). Leptin can suppress intake in lean, but not obese, MC4R knockout mice, indicating that the leptin resistance is secondary to factors associated with obesity rather than the receptor deficiency (Marsh et al., 1999a).

Using RT-PCR for AGRP mRNA in tissues from young pigs, we have produced evidence for robust AGRP expression in hypothalamus and pituitary; somewhat lower levels in thymus, adrenal, and testis; and weakly detectable signals in fat, muscle, spleen, and liver (Dyer et al., 2000b). In mice AGRP is detectable in hypothalamus and adrenal by northern blotting, and expression in both tissues is elevated in the *ob/ob* genotype (Ollmann et al., 1997). In contrast to the apparent differences in expression among tissues observed for AGRP, MC4R mRNA is readily detectable in all of the above tissues (Dyer et al., 2000a). The first reported porcine MC4R cDNA sequence was generated from fibroblast mRNA (GenBank AB021664).

Melanin-Concentrating Hormone

Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid peptide first isolated from salmon pituitary glands. The hormone derives its name from its ability to lighten skin color by concentrating melanin-containing granules in melanocytes. This hormone was subsequently discovered in mammalian brain areas associated with appetite control, specifically the zona incerta and lateral hypothalamus (Tillet et al., 1996; Viale et al., 1997). In livestock, MCH cDNA has been cloned in pigs (Matteri et al., 2000c).

Melanin-concentrating hormone expression is up-regulated by fasting (Qu et al., 1996) and by treatment with inhibitors of carbohydrate metabolism and fatty acid oxidation (Sergeyev et al., 2000). Long-term feed restriction in sheep, resulting in a lowering of body fat from 36 to 15%, significantly elevates MCH expression (Henry et al., 2000). Intracerebroventricular administration of MCH potently stimulates feeding in rats (Qu et al., 1996). Melanin-concentrating hormone knockout mice are hypophagic, lean, and have an elevated metabolic rate (Shimada et al., 1998). Hypothalamic MCH is highly expressed in *ob/ob* mice (Qu et al., 1996), and its expression is suppressed by leptin treatment (Sahu, 1998a; Bayer et al., 1999). Although the leptin-induced reduction in MCH expression could contribute to reduced feeding in normal animals, other appetite-stimulating factors are also suppressed by leptin (Sahu, 1998a). Indeed, MCH knockout mice respond to leptin treatment with an exaggerated reduction in feed intake (Shimada et al., 1998). The stimulatory effect of MCH on feed intake can be blocked by the appetite inhibitors leptin, neurotensin, GLP-1, and α -MSH (Sahu, 1998b; Tritos et al., 1998). The expression of MCH is elevated in the agouti mouse and by treatment with AGRP or the MC4R antagonist SHU9119 (Hanada et al., 2000).

In livestock, MCH receptor cDNA has been cloned in pigs (Matteri, unpublished data). The receptor for MCH corresponds to an orphan G-protein coupled receptor with similarity to somatostatin receptors (Bachner et al., 1999; Lembo et al., 1999). In mice, MCH receptor expression is elevated by fasting and the *ob/ob* genotype and suppressed by leptin treatment (Kokkotou et al., 2000).

In human tissues, MCH mRNA has been reported in thymus, brown adipose tissue, duodenum, and testis (Viale et al., 1997). In rat peripheral tissues, MCH is found in stomach, intestine, and testis (Hervieu and Nahon, 1995). Using RT-PCR with RNA samples from young pigs, we have detected MCH mRNA in the hypothalamus, but not in pituitary, fat, muscle, thymus, spleen, adrenal, testis, or liver (Matteri et al., 2000c). Interestingly, we find that MCH receptor transcripts are readily detectable in all of the above tissues (unpublished data). Outside of the CNS in mice, MCH receptor expression has been detected in adipose tissue (Kokkotou et al., 2000).

Orexin

A novel family of neuropeptides, orexins (also called hypocretins) were first discovered in rats and found to increase feed intake in these animals when administered directly into the brain (Sakurai et al., 1998). Subsequently, antiorexin antibody was shown to produce a dose-responsive inhibition of intake (Yamada et al., 2000). Orexin (**ORX**) mRNA is found in the subthalamus and hypothalamus (Sakurai et al., 1998). Within the hypothalamus, ORX-containing cell bodies are localized to lateral and posterior hypothalamic areas, but

ORX immunoreactive fibers are widely distributed (Date et al., 1999).

Orexin cDNA and gDNA have been cloned in pigs (Dyer et al., 1999a; Malek et al., 2000), and the ORX-A peptide has been purified and sequenced in cattle (Sakurai et al., 1998). The ORX mRNA contains codes for ORX-A and ORX-B, closely related peptides separated by three codons coding for basic amino acids. Orexin-A is 33 amino acids in length with two disulfide bonds linking Cys 6–12 and 7–14. Orexin-B consists of 28 amino acids, with no disulfide bonds. The last 10 amino acids of the c-terminus are highly conserved between the two ORX peptides. Ongoing structure-function studies are examining critical amino acid sequences for ORX receptor binding and activation (Noble et al., 2000). As in rodents, ORX seems to stimulate feed intake in domestic animals. Porcine orexin-B stimulates feed intake in sheep following intracerebroventricular administration (Sartin et al., 2001). Using the same porcine orexin-B, we found that a large i.m. dose of the synthetic orexin-B stimulated feed intake in weanling pigs (Dyer et al., 1999a). Subcutaneous injection of ORX-A or ORX-B has been shown to stimulate insulin secretion in rats (Nowak et al., 2000), further indicating a potential peripheral role for ORX peptides in regulating appetite and metabolism. Outside of the CNS, ORX expression has been detected in pituitary gland (Date et al., 2000), testis (Sakurai et al., 1998), and intestine (Kirchgessner and Liu, 1999).

Neuropeptide Y seems to mediate at least some of the appetite-stimulating effects of ORX. Administration of a selective NPY Y1 receptor antagonist can prevent the feeding response to orexins A and B (Jain et al., 2000). In another study, blockade of the Y1 receptor only partially negated the effect of ORX on feed intake, suggesting the involvement of factors other than NPY (Yamanaka et al., 2000). The expression of ORX is decreased by leptin in fasted rats (Lopez et al., 2000). In contrast to most other stimulators of feed intake, however, orexin mRNA levels are relatively low in *ob/ob* and *db/db* mice (Yamamoto et al., 1998). Levels of hypothalamic ORX mRNA are increased by feed restriction in *ob/ob* and *db/db* mice (Yamamoto et al., 2000), indicating that nutritional regulation of ORX can occur independently of leptin signaling. There also is evidence that orexin expression is not affected by pathways that involve the AGRP-MCR4 signaling pathway (Hanada et al., 2000).

Preliminary evidence suggests that ORX expression is positively correlated with piglet body weight in normally feeding piglets (Dyer et al., 1999b). In rodents, hypothalamic orexin gene expression is enhanced by fasting (Sakurai et al., 1998; Yamamoto et al., 2000) and insulin-induced hypoglycemia (Griffond et al., 1999). Insulin-induced hypoglycemia also activates ORX neurons in the LHA (Moriguchi et al., 1999). Orexin pathways may be involved in the response to environmental stressors. The level of hypothalamic ORX mRNA can be decreased by immunological challenge in neonatal

pigs (Matteri et al., 2000b). The expression of ORX in the lateral hypothalamus can be acutely stimulated by immobilization or cold stress (Ida et al., 2000).

Two G protein-coupled receptors, Type 1 and Type 2 orexin receptors (ORXR1 and ORXR2), have been identified (Sakurai et al., 1998). Type 1 orexin receptor has a greater affinity for ORX-A than for ORX-B, whereas ORXR2 binds both ORX peptides equivalently. The ORX receptors have a wide CNS distribution, and in the hypothalamus ORXR1 is abundant in the VMN and ORXR2 in the PVN (Trivedi et al., 1998). After 20 h of fasting, ORXR1 expression is elevated in the VMN and DMH and ORXR2 expression is increased in the ARC (Lu et al., 2000). The expression of ORXR2 may be related to growth rate in young pigs (Dyer et al., 1999b). Leptin treatment reduces hypothalamic ORXR1, but not ORXR2, mRNA levels in food-deprived rats (Lopez et al., 2000). In the rat, the ORX receptors have been found in the CNS (Sakurai et al., 1998) and all lobes of the pituitary gland (Date et al., 2000). Using RT-PCR, we have amplified easily visible ORXR1 cDNA bands in hypothalamus, adrenal gland, and testis and considerably weaker signals in liver, pituitary, and adipose tissue. Also using RT-PCR, we have observed readily detectable ORXR2 mRNA in hypothalamus, pituitary, and adrenal tissue, with lower apparent levels in fat, muscle, thymus, spleen, and testis. In livestock species, ORXR1 and ORXR2 cDNA clones have been produced in pigs (Matteri et al., unpublished data).

Cocaine and Amphetamine-Regulated Transcript

Cocaine and amphetamine-regulated transcript was discovered in 1995 by differential display PCR subsequent to cocaine and amphetamine administration in rats (Douglass et al., 1995). Cocaine and amphetamine-regulated transcript and CART peptides are widely distributed throughout the CNS but are found in relatively high levels in the ARC and PVN (Kristensen et al., 1998; Lambert et al., 1998). Neuropeptide Y nerve terminals appear in close opposition with CART cell bodies in the ARC (Broberger, 1999) and PVN (Lambert et al., 1998). The CART protein is synthesized in two forms in rats (116 and 129 amino acids), but only the 116 amino acid form is found in humans. The c-terminal 41 and 48 amino acids comprise bioactive, naturally-occurring peptides, with peptide fragments within this region possessing varying degrees of activity (Lambert et al., 1998). Three disulfide bonds have been characterized in the c-terminal region of the CART protein (Thim et al., 1998). Segments of CART cDNA and gDNA have been cloned in the pig (Kenealy et al., 1999; Matteri, unpublished data). The receptor(s) for CART peptides have not been identified at this time; however, the availability of CART peptide ligands will certainly lead to receptor identification and characterization.

Localization of CART to hypothalamic regions with known functions in appetite control prompted feed intake studies that revealed appetite-suppressing activ-

ity (Kristensen et al., 1998; Kuhar and Dall Vechia, 1999). Intracerebroventricular administration of CART peptide suppresses normal intake, starvation-induced feeding, and NPY-stimulated intake in rats (Kristensen et al., 1998; Vrang et al., 1999). The anorectic effect of CART peptide is not blocked by AGRP (Edwards et al., 2000). Oppositely to the expression of appetite-stimulating factors such as NPY, AGRP, and orexin, CART expression is down-regulated by food deprivation, nearly absent in rodents with deficient leptin signaling pathways, and restored by leptin treatment in *ob/ob* mice (Kristensen et al., 1998). Cocaine and amphetamine-regulated transcript may mediate some of leptin's effects on reproduction. The increase in GnRH pulsatility caused by leptin is mimicked by CART, and partially prevented by anti-CART antibody (Lebrethon et al., 2000a,b). The pulse frequency of GnRH release from hypothalamic explants of leptin receptor-deficient (*fa/fa*) rats was unaffected by leptin but significantly accelerated by CART peptide (Lebrethon et al., 2000a).

Outside of the CNS, CART mRNA has been found in pancreas, adrenal medulla, pituitary gland, and neurons innervating the gut (Koylu et al., 1997; Couceyro et al., 1998; Jensen et al., 1999). Evidence of CART production outside of the CNS has prompted studies entailing peripheral administration, and initial evidence has shown rapid entry of CART into the brain (Kastin and Akerstrom, 1999). Cocaine and amphetamine-regulated transcript also may act directly at the pancreas to suppress insulin release (Wang et al., 2000).

Implications

The importance of feed intake in livestock is unquestioned. However, studies of appetite control in domestic species are relatively few compared with those in laboratory rodents. Worldwide interest in feed intake regulation, coupled with the advent of new research technologies and approaches, has prompted new appetite-related research in a growing number of domestic animal laboratories. New physiological data will establish model conditions that will reliably provide high contrasts between animals in the expression of appetite regulators. Transcript profiling studies, using cDNA from such animals, have been initiated. These studies offer an exciting potential for new advances in our understanding of feed intake control. New roles for known genes may be identified, as well as the detection of as yet unknown gene products. The analysis of reading frames of novel gene sequences may well reveal additional neurohormones and new central targets for appetite regulation.

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