The role of leptin in nutritional status and reproductive function

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Infertility associated with suboptimal nutrition is a major concern among livestock producers. Undernourished prepubertal animals will not enter puberty until they are well fed; similarly, adult, normally cyclic females will stop cycling when faced with extreme undernutrition. Work in our laboratory has focused on how body fat (or adiposity) of an animal can communicate to the brain and regulate reproductive competence. In 1994, the discovery in rodents of the obese (ob) gene product leptin, secreted as a hormone from adipocytes, provided a unique opportunity to understand and hence regulate whole body compositional changes. There is now evidence that similar mechanisms are functioning in livestock species in which food intake, body composition, and reproductive performance are of considerable economic importance. Leptin has been reported to be a potent regulator of food intake and reproduction in rodents. There is evidence indicating that at least some of the effects of leptin occur through receptor-mediated regulation of the hypothalamic protein neuropeptide Y (NPY). NPY is a potent stimulator of food intake, is present at high concentrations in feed-restricted cattle and ewes, and is an inhibitor of LH secretion in these livestock species. In our investigations in sheep, we have cloned a partial cDNA corresponding to the ovine long-form leptin receptor, presumably the only fully active form, and have localized the long-form leptin receptor in the ventromedial and arcuate nuclei of the hypothalamus. Leptin receptor mRNA expression was colocalized with NPY mRNAcontaining cell bodies in those regions. We have also determined that hypothalamic leptin receptor expression is greater in feed-restricted ewes than in well-fed ewes. These observations provide a foundation for future investigations into the nutritional modulators of reproduction in livestock.

Introduction

The ancient sculpture known as *Venus of Willendorf* (Fig. 1) illustrates man's long known association between nutritional status and fertility. What is not known, however, is the mechanism by which the nutritional status of an animal regulates reproductive processes. In 1953, Kennedy acknowledged the association between nutrition and reproduction and proposed the 'lipostat' or 'setpoint' theory, which asserted that the reproductive performance of an animal was related positively to the animal's body-fat mass. Kennedy's work was followed by many studies which included the classical ventromedial hypothalamic (VMH) lesioning studies by Hervey (1958). Hervey concluded that VMH lesions ablated the satiety centre and resulted in mice that exhibited hyperphagia, obesity and infertility.

In addition, the parabiotic-mice trials of Coleman *et al.* (reviewed in 1978) were extraordinarily insightful. These researchers worked with two strains of obese mice, notably the *ob/ob* mice (identified as the first genetic anomaly of its kind; Ingalis *et al.*, 1950), and the *db/db* (or diabetic) mice. The consequence of parabiosing *ob/ob* and *db/db* mice with each other or with wild-type mice are illustrated in Fig. 2. Mating of wild-type strains of mice with *ob/ob* or *db/db* mice resulted in offspring expressing simple Mendelian probabilities for the recessive traits. As a result of the lesioning and



Fig. 1. Venus of Willendorf – a symbol of the Mother and Fertility Goddess. (ca. ~25 000 years old).



Fig. 2. Consequence of parabiosing *ob/ob* and *db/db* mice together or with wild-type mice.



Fig. 3. Three-dimensional structural model of leptin.

parabiotic trials, it was concluded that blood-borne factors were responsible for communicating the animal's body composition to its brain. Elucidation (at least in part) of that 'body-to-brain' signal required more than two decades of additional work.

In 1994, Friedman and co-workers (Zhang *et al.*, 1994) used positional cloning techniques to identify the 167 amino acid protein product of the *ob* gene which was named leptin (derived from the Greek term 'leptos' meaning 'thin'). Subsequent nuclear magnetic resonance analysis of a crystalline form of leptin (E100; Zhang *et al.*, 1997) revealed that it was present as a quadra-helical protein (Fig. 3). Structurally, leptin contains a single disulfide bond which links cysteines within the C and D helices and which has proven critical to the biological activity of leptin.

Originally, Friedman and colleagues (Zhang *et al.*, 1994) reported that leptin was secreted exclusively by adipocytes; however, the list of tissues expressing leptin now includes the placental trophoblast, the mammary epithelium in primates and rodents, and avian liver. Unique to each of these tissues are their lipogenic–lipolytic capabilities. The importance of the lipogenic–lipolytic mechanisms in these tissues and particularly in the liver of oviparous and ovoviviparous animals (to include fish, birds, reptiles, select mammals (spp. Monotremata) and others), likely portends future reports of species-specific detection of leptin expression, particularly in liver or mammary epithelial tissue.

Concomitant with the identification of leptin, leptin receptors were cloned and are now known to occur in at least five variably spliced forms (*OB*-Ra-e; Li *et al.*, 1998). The high structural similarity of leptin receptors to cytokine receptors has led to numerous reports referring to leptin as a member of the cytokine family. When leptin binds to its receptor, receptor dimerization occurs; this is also characteristic of members of the growth hormone–cytokine family of receptors (Devos *et al.*, 1997; Girard, 1997; Liu *et al.*, 1997; Nakashima *et al.*, 1997). However, only one of the leptin receptors, referred to as *Ob*-Rb, or the 'long-form' of the leptin receptor is known to both span the cellular membrane and possess a 302 amino acid cytoplasmic domain. The G-protein-like signal transduction mechanism of the long-form of the leptin receptor is mediated most likely via



Fig. 4. Leptin receptor expression (arrow) in the ventromedial hypothalamus of feed-restricted (a) versus wellfed (b) ewes.

activation of janus kinase (JAK)-2/signal transducer and activator of transcription (STAT)-3, -5 or-6 pathways (Bjorbaek *et al.*, 1997). At least three of the short-forms of the leptin receptors (*Ob*-Ra, c, and d) also possess cytoplasmic domains. However, their cytoplasmic domains are truncated to 30–40 amino acids. Initially the short-form leptin receptors were believed not to be involved in signal transduction, but to mediate transmembrane movement or clearance of leptin. However, recent investigations (Murakami *et al.*, 1997; Yamashita *et al.*, 1998) have provided evidence that the short forms of the leptin receptors (*Ob*-Ra, c, and d) may indeed possess functional signal transduction capabilities (ex. via JAK/mitogen-activated protein kinase (MAPK) or phosphotidyl inositol [PI]-3 kinase activation). The leptin receptor denoted as Ob-Re is completely devoid of a cytoplasmic domain and is believed to occur in several circulating forms, possibly as soluble binding proteins (Gavrilova *et al.*, 1997; Lollmann *et al.*, 1997; Li et *al.*, 1998).

Initially leptin receptors were cloned from the choroid plexus (Tartaglia *et al.*, 1995). The significance of the choroid plexus is that it is one of the circumventricular organs within the brain where the brain is said to be 'permeable' to the exchange of substances with the peripheral blood supply. Location of leptin receptors in these neuroanatomical tissues may be a potential point of control in the regulation of the 'body-to-brain' signal. Indeed, there is evidence to support the hypothesis that the leptin transport process in the circumventricular organs is specific and saturable (Banks *et al.*, 1996; Caro *et al.*, 1996; Schwartz *et al.*, 1996; Diamond *et al.*, 1997; Corp *et al.*, 1998; Karonen *et al.*, 1998). In addition to the choroid plexus, leptin receptor expression has been localized in the brain to the ventromedial hypothalamus (Fig. 4), arcuate nucleus, hippocampus, thalamus, piriform cortex and anterior pituitary, and peripherally in tissues including adipocytes, liver, pancreas, fetal cartilage–bone, hair follicles, ovary, testis, uterus, heart, skeletal muscle, lung, lymph nodes, thyroid, adrenals, kidney, spleen and prostate gland (Hoggard *et al.*, 1997; Mendiola *et al.*, 1997; Zamorano *et al.*, 1997). The function, if any, of leptin in many of these tissues has yet to be determined.



Fig. 5. Relationship between human body fat mass content and serum concentration of leptin.

Role of Leptin in Nutritional Status

All systems that perform work require fuel to function and physiological processes are not exceptions. However, unlike mechanical engines, animals possess the ability to integrate what they know about their environment and physiological status to anticipate their need for additional energy and to partition the energy among the systems in need. In order for an animal to anticipate its need for fuel and partition it appropriately, some variable or combination of variables (that is, inputs to the system) must reflect the well-being or status of each of the components of the system and the amount of fuel contained within the animal. Consequently, the fuel itself may serve as a dynamic indicator of the well-being or status of the animal by providing instantaneous yet discrete (yes versus no) assessments of status.

The fuel is important in determining whether the system functions or not, but how robust the system operates must be determined by some indicator(s) of the quality or quantity of fuel present within the animal. The issue of fuel quality implies that there are several sources of fuel which differ in energetic or satiating capabilities; and this is known to be true. For example, the brain is heavily dependent on glucose, its preferred energy substrate, but the brain can also function (within limitations) on reserves of ketones (Owen *et al.*, 1967). Another example is that fatty acids can vary greatly in carbon-length, structural branching, and proportion of hydrogen saturation, all of which can influence the insulinogenic response within the animal. Consequently, although the quality of fuel is a mechanism by which physiological responses can be affected, it has so far received limited attention but will probably be an important area of study in the future.

Assessments of the quantity of fuel present within an animal has been long sought and has resulted in the development of a variety of electromechanical approaches, including body-condition scores, skin fold thickness, dual-energy X-ray absorptiometry (DEXA), K⁴⁰ counting, and ultrasonography, all of which are focused, in essence, on estimating fat mass. The discovery of leptin and subsequent development of methods to assess it in primates and rodents has provided



Fig. 6. NPY mRNA expression (arrows) in the ventromedial hypothalamus of feed-restricted (a) versus well-fed (b) ewes.

investigators with a relatively simple and accurate indicator of body-fat mass in these species (Fig. 5; Maffei *et al.*, 1995; Campfield *et al.*, 1996; Blum *et al.*, 1997; Perry *et al.*, 1997; Shimizu *et al.*, 1997; Langendonk *et al.*, 1998). Unfortunately, this relatively easily applied 'tool' has resulted in a large number of reports of replicated efforts and clinical associations between fat-mass and a variety of weakly linked conditions. The few quality studies reported so far have allowed us to focus on the relationship between fat-mass and physiological function and shape understanding of the regulatory processes involved in body 'fat and function'.

Similar tests for assessing leptin in livestock species have been developed with opportunities to: (1) monitor more accurately the 'fuel reserves' that an animal possesses in order to facilitate management of the animal and (2) potentially provide meat producers, processors, and consumers with an objective quantifier of meat quality.

As a result of years of focus on fundamental hypothesis driven research, it has been established that when animals lose body fuel reserves or fat mass, as in starvation conditions, satiety centres within the brain stimulate appetite (Hoebel, 1997; Hirschberg, 1998). Neurochemically, this is mediated, at least in part, if not predominately, by an increase in brain content of neuropeptide Y (NPY; Fig. 6; Tomaszuk *et al.*, 1996; Kalra, 1997; Yu *et al.*, 1997; Xu *et al.*, 1998). It is also important to note here that NPY is not being considered as the sole mediator of these processes, as there must be other collateral mechanisms to ensure the redundancy and thus preservation of this axis of communication. However, the significance of the focus on NPY is that there is evidence to support a pivotal role for NPY in regulating both nutritional status and reproductive function.

Neuropeptide Y is a 37 amino acid peptide, which is known to be one of the most potent stimulators of appetite in a broad range of species. But how secretion of NPY is regulated, or more specifically which somatic signals arise outside of the brain that are capable of communicating quantity or quality of body fuel reserves to the NPY neurones are not known. The answer may be that when many of the traditional indicators of nutritional status increase, especially insulin, secretion of NPY decreases (Kalra, 1997). In addition, there are direct effects of leptin on NPY neurones, as leptin receptors have been colocalized with NPY neurones in rodents (Mercer *et al.*, 1996) and sheep (Keisler *et al.*, unpublished observations; see Fig. 7). Consequently, at least one axis of the 'body-to-brain' signalling pathways is direct. The functionality of this pathway has been confirmed via intracerebroventricular infusions of leptin into both well-fed and feed-restricted ewes (Henry *et al.*, 1998; Morrison *et al.*, 1998). The mean (\pm one standard error) of feed-intake profiles of



Fig. 7. $40 \times$ magnification grey scale image of a hypothalamic NPY neurone (NPY mRNA in grey) exhibiting colocalization with leptin receptor mRNA (dark silver grains).

well-fed and feed-restricted ewes receiving vehicle or recombinant ovine leptin is shown in Fig. 8. Recombinant ovine leptin was infused in a linearly increasing dose from 0 μ g kg⁻¹ h⁻¹ on day 0 to a maximum dose of 1.25 μ g kg⁻¹ h⁻¹ on day 8. Well-fed ewes receiving leptin began to decrease intake of feed on day 4 and ceased eating by day 7 of infusion. However, feed-restricted ewes receiving leptin began to limit their intake only while receiving maximum amounts of leptin. These observations may reflect the ability of an animal to balance its presumptive desire for food against its constitutive demand for food. In well-fed ewes, leptin suppressed the animals' desire for food, while fat reserves within the animal supplied its demand for food. In contrast, the feed-restricted ewes were almost devoid of body fat and, therefore, their constitutive demand for food (for survival) superseded signals (exogenous leptin) suppressing their presumptive disinterest in eating. In essence, the message that communicated the quantity (or possibly quality) of available body fuel reserves (that is, leptin) did not concur with the flagrant absence of fuel; consequently, the thin ewes continued to eat to survive.

Role of Leptin in Reproductive Function

In 1996, Barash and coworkers revealed evidence implicating a role for leptin in the reproductive axis. They reported that treatment of *ob/ob* mice with leptin increased reproductive organ weight and serum concentrations of gonadotrophins. They thus declared leptin as a 'metabolic signal to the reproductive system'. In 1997, Chehab and coworkers substantiated the involvement of leptin in the reproductive axis by reporting that treatment of wild-type mice with leptin advanced the onset of sexual maturation by 9 days. Similarly, Carro *et al.* (1997) reported that administration of leptin antiserum to ovariectomized rats led to a marked decrease in secretion of LH. The validity of the observations of Chehab *et al.* (1997) and perhaps the observations of Carro *et al.* (1997) have now



Fig. 8. Daily feed-intake of well-fed and feed-restricted ewes receiving intracerebroventricular infusions of vehicle (\blacklozenge) or recombinant ovine leptin (\blacksquare).

been challenged by the findings of Gruaz *et al.* (1998), who suggested that the action of leptin on the reproductive axis was confounded by its ability to decrease food intake and thus it is difficult to discriminate between these actions.

In addition, it is known that serum concentrations of leptin in primates and rodents: (1) are positively correlated with body weight and body mass index, (2) increase before puberty in females and to a lesser extent in males, (3) are typically three to four times greater in females than in males, and (4) are inversely related to serum concentrations of testosterone. It is also known that serum concentrations of leptin: (1) increase significantly during early pregnancy before any major changes occur in maternal body fat mass; (2) are significantly lower in newborns with intrauterine growth retardation than in newborns with normal intrauterine growth profiles and (3) are positively correlated with birth weight. In ruminants (and livestock in general), acceptable methods for assessing serum concentrations of leptin are only now being developed and information is not available so far. Furthermore, only recently has a large-scale method for the preparation of biologically active recombinant ruminant (ovine) leptin been described (Gertler *et al.*, 1998).

Irrespective of these impediments, Henry *et al.* (1998) and Morrison *et al.* (1998) infused recombinant human and ovine leptin (respectively) into the cerebroventricles of well-fed or well-fed and feed-restricted ewes, respectively. Independently, both groups of investigators reported that well-fed ewes reduced their food intake in response to leptin. This observation is consistent with the report by Henry *et al.* (1998) that hypothalamic expression of NPY (a potent orexigenic protein) was

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reduced in well-fed ewes infused with leptin. In contrast, neither Henry et al. (1998) nor Morrison et al. (1998) observed any discernible change in secretion of gonadotrophins in the well-fed ewes in response to leptin. It is possible that (1) gonadotrophin secretion in the well-fed ewes was already maximal, (2) systems governing feed intake were more responsive than systems governing reproductive responses, or (3) a further reduction in the already low hypothalamic content of NPY in the well-fed ewes was inconsequential to mechanisms governing secretion of gonadotrophins. In feed-restricted ewes (Morrison et al., 1998), cerebroventricular infusion of leptin began to reduce food intake only at maximum amounts of leptin, but again failed to alter serum concentrations of gonadotrophins. We suggest that in the feed-restricted ewes collateral mechanisms or signals (such as insulin) provide redundancy, and thus ensure the signal for food intake is maintained for the preservation of the animal. The presence of several pathways thus permits a form of checks and balances of the system. With regards to the reproductive axis, perhaps gonadotrophin secretion in the feed-restricted ewes was so inhibited that any change in NPY was not of sufficient magnitude or duration to affect an increase in gonadotrophins or collateral consequences of the feed-restricted condition prohibited an increase in gonadotrophins. Regardless of the precise mechanism of action, these observations are consistent with the hypothesis that the effects of leptin on the reproductive axis may be mediated largely, if not exclusively, by central inhibition of NPY. As stated earlier, leptin receptors have been colocalized on NPY neurones (see Fig. 7) implicating a direct body-to-brain mechanism for communicating nutritional status to the reproductive axis.

Conclusion

Although there is significant evidence implicating the role of leptin in communicating nutritional status to the reproductive axis, it is not only highly unlikely but also perilous for an animal to rely on a single mechanism to mediate these body-to-brain communications. This is not to imply that the role of leptin in this process is minimal, but rather to place the role of leptin in perspective relative to several alternative mechanisms previously examined and yet to be examined. We are only at the beginning of understanding the complexity of these 'body-to-brain' communications and their interactions and the contribution of each of the potential mediators of these processes on the local, peripheral and central tissues that constitute the animal.

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