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Review

Interface between metabolic balance and reproduction in ruminants: Focus on the hypothalamus and pituitary

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ABSTRACT

This article is part of a Special Issue "Energy Balance".

The interface between metabolic regulators and the reproductive system is reviewed with special reference to the sheep. Even though sheep are ruminants with particular metabolic characteristics, there is a broad consensus across species in the way that the reproductive system is influenced by metabolic state. An update on the neuroendocrinology of reproduction indicates the need to account for the way that kisspeptin provides major drive to gonadotropin releasing hormone (GnRH) neurons and also mediates the feedback effects of gonadal steroids. The way that kisspeptin function is influenced by appetite regulating peptides (ARP) is considered. Another newly recognised factor is gonadotropin inhibitory hormone (GnIH), which has a dual function in that it suppresses reproductive function whilst also acting as an orexigen.

Our understanding of the regulation of food intake and energy expenditure has expanded exponentially in the last 3 decades and historical perspective is provided. The function of the regulatory factors and the hypothalamic cellular systems involved is reviewed with special reference to the sheep. Less is known of these systems in the cow, especially the dairy cow, in which a major fertility issue has emerged in parallel with selection for increased milk production.

Other endocrine systems – the hypothalamo-pituitary-adrenal axis, the growth hormone (GH) axis and the thyroid hormones – are influenced by metabolic state and are relevant to the interface between metabolic function and reproduction. Special consideration is given to issues such as season and lactation, where the relationship between metabolic hormones and reproductive function is altered.

Contents

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Introduction

Reproductive function and metabolic function are intimately linked for the simple reason that the former comes at a cost. This is especially the case for females, who need to source energy for pregnancy and lactation. Central regulation of both reproduction and metabolic function provides a means whereby adequate energy or energy deficit can be sensed, such that food intake and energy expenditure can be modulated. In the past three decades, our understanding of the central regulation of food intake and energy expenditure has increased due to the identification of a range of neuronal systems within the brain. In addition, the relatively recent identification of neuropeptides that modulate reproductive function has provided a new layer of knowledge. In particular, kisspeptin and gonadotropin inhibitory hormone (GnIH) stand out as key factors in the regulation of reproduction. Not surprisingly, the neural elements within the brain that control metabolic function and those that control reproduction are inter-connected.

The focus of this article is on ruminant species, particularly sheep and cattle. Other excellent reviews deal with the topic as it pertains to rodents (True et al., 2011a), but large animal models provide special opportunities to gain knowledge of the subject. In particular, the sheep is the species of choice for the real-time measurement of the secretion of gonadotropin releasing hormone (GnRH) and the blood volume and passive nature of sheep allow serial measurement of circulating hormones. Sheep are not nocturnal, as are rodents, and they are able to undergo remarkable variations in live-weight (adiposity). Nevertheless, rodent models provide opportunities to manipulate genetic makeup and also have a much shorter generation interval than ruminant animals. Excellent studies in rodent species have been reviewed in this volume (Bellefontaine N & Elias CF (2014 Minireview: Metabolic control of the reproductive physiology: Insights from genetic mouse models)). Ruminants present a special case in terms of metabolic function because their digestive system is different to that of monogastric species and they derive energy from the diet in terms of volatile fatty acids rather than carbohydrates and proteins, both of which are digested in the rumen. In spite of this, these animals have very efficient gluconeogenic function and maintain blood glucose levels within a narrow range. There is a paucity of information on the interaction between the reproductive and metabolic systems in bovine species but there are some interesting issues, especially in relation to the high-producing dairy cow.

Firstly, I outline reproductive and metabolic function in ruminants and consider the impact of altered energy balance on reproductive function, especially in terms of the synthesis and secretion of GnRH. I review information on the neural connections within the hypothalamus that allow the reproductive axis to be influenced by metabolic state. This will include information on connectivity of neural elements within the hypothalamus and how these connections translate into function. With this framework, I then consider the regulation of metabolic function in ruminants, with particular reference to hypothalamic systems. The specific cases of how growth hormone (GH) and thyroid hormones are affected by metabolic perturbation and how this affects the reproductive axis are considered and the particular implications of seasonality and lactation are reviewed. This article focusses on the hypothalamic and pituitary components of the reproductive axis, although it is recognised that metabolic state also impacts directly on the function of the gonads.

General aspects of reproduction in ruminants

Relationship between GnRH and gonadotropins

The primary driver of reproduction is GnRH, secreted from the brain into the hypophysial portal system in a pulsatile manner (Clarke and Cummins, 1982). In sheep, the majority of GnRH cells are found in the preoptic area of the brain, as in rodent species (Lehman et al., 1986). GnRH is mandatory for the synthesis and secretion of gonadotropins in the gonadotropes of the anterior pituitary, but there are important distinctions in the way this is effected (Clarke et al., 2011). Luteinising hormone (LH) is secreted in a pulsatile manner, directly reflecting the pulsatile secretion of GnRH in terms of frequency. The relationship between the amplitude of GnRH and LH pulses is somewhat more complicated, because of the following reasons:

1. There is an inverse relationship between LH pulse frequency and amplitude. If the amplitude of GnRH pulses is held constant, but the frequency is reduced, the LH response to GnRH increases. The amplitude of the LH response to GnRH is a reflection of the releasable pool of LH in the pituitary gland (Clarke and Cummins, 1985).
2. Some factors, such as cortisol (Breen et al., 2008), can act on the pituitary gland to dampen the amplitude of LH pulses. These relationships assume importance in cases where physiological state, such as body weight, alters the frequency of GnRH pulses.

Follicle stimulating hormone (FSH) synthesis and secretion is also dependent upon GnRH, but in a different way. GnRH stimulates the synthesis of FSH, but secretion does not require the pulsatile input of GnRH to the gonadotrope. Thus, in an experimental model where hypothalamic input of GnRH is eliminated in the sheep by hypothalamo-pituitary disconnection, cessation of replacement with pulsatile GnRH administration will lead to immediate loss of pulsatile LH secretion, but FSH secretion continues for days/weeks (Clarke et al., 1986a). This is because FSH may be secreted from the gonadotrope in a passive manner, in direct proportion to the releasable pool of FSH in the cell (Clarke et al., 1986a). The reason these distinctions are laboured is because manipulations of body weight can lead to changes in GnRH secretion that are more likely to be reflected in the peripheral levels of LH than those of FSH. Changes in FSH secretion are much more sluggish than changes in LH secretion for the reasons indicated above, as well as the longer half-life of FSH in plasma (Fry et al., 1987). An important point to note is that sex steroids affect the secretion of GnRH and the gonadotropins, by actions at both the hypothalamic and pituitary levels, whereas inhibin has a specific effect only at the level of the gonadotrope to lower FSH production and secretion (Clarke et al., 1986b, 1993; Findlay et al., 1987).

Another important point to note is with respect to determination of whether any regulatory factor acts on GnRH cells in the hypothalamus and/or gonadotropes in the pituitary gland. In many cases, studies have been reported in which GnRH antagonists or antibodies have been used to negate GnRH function and then an agent is administered to determine whether it acts directly on the pituitary gland. This is not a valid protocol because the function of the gonadotrope in the anterior pituitary gland has an absolute requirement for GnRH input. Without such input, gonadotrope function cannot be investigated. In other words, study of gonadotropes without appropriate pulsatile GnRH

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input do not inform us of direct action/s of any agent on gonadotropin synthesis or secretion. A more appropriate way to determine whether the pituitary gonadotropes are directly affected by a particular agent is to isolate the pituitary gland from the brain and then provide chronic pulsatile GnRH input in a rigid format. This has been done in primate models and in sheep, to investigate the direct effects of gonadal steroids (inhibitory and stimulatory) and inhibin (inhibitory) on the responsiveness to GnRH (Clarke and Cummins, 1984; Nakai et al., 1978). Another way to study direct effects on gonadotropes is to 'clamp' the pituitary with oestrogen (Breen and Karsch, 2004). One caveat with this latter approach is that the treatment with oestrogen may alter the response, at the level of the gonadotrope, to the agent being investigated.

Sex steroid feedback regulation of GnRH and gonadotropin secretion

It has been known for many decades that the reproductive axis is a closed loop whereby sex steroids feed back to the brain and the pituitary gland to regulate GnRH and gonadotropin secretion, but it is important to consider this briefly prior to consideration of how metabolic function impacts on the hypothalamo-pituitary axis. It is generally considered that the main feedback effects of sex steroids, to regulate GnRH secretion, involve cells that express the relevant receptors in the brain. Importantly, GnRH cells do not express oestrogen receptor-α (ER-α) (Herbison et al., 1993; Lehman and Karsch, 1993; Shivers et al., 1983), progesterone (Fox et al., 1990; Leranth et al., 1992) or androgen (Herbison et al., 1996; Huang and Harlan, 1993) receptors. ER-β is expressed by GnRH cells (Hrabovszky et al., 2000, 2001) which explains the effects of estradiol at this level (Handa et al., 2012), but the main receptor thought to mediate the feedback effects of oestrogens is ER-α (Couse et al., 2003). Since the discovery of the kisspeptin in the brain, a wealth of information has accumulated to indicate that the cells that produce this peptide, in the arcuate nucleus and in the preoptic area, promulgate the feedback effects of steroids to the GnRH cells (reviewed in Clarke et al., 2011). A dual mechanism may exist, such that kisspeptin cells regulate GnRH secretion by way of action on the GnRH neuronal cell bodies and the secretory terminals in the median eminence (d'Anglemont de Tassigny et al., 2008, 2010; Smith et al., 2008b). Accordingly, discussion of the interface between metabolic and reproductive regulatory systems should involve consideration of the kisspeptin cells as will be presented below.

The secretion of GnRH is very tightly regulated by sex steroid feedback and this has been reviewed in detail in Clarke et al. (2011). In ewes, sustained elevation of estradiol-17β, progesterone or androgen levels causes negative feedback, whereas acute elevation of estradiol-17β (unopposed by progesterone) causes positive feedback in the female (Clarke, 1987; Clarke et al., 2011). On the other hand, there is little evidence to show that feedback effects are of any major consequence in relation to GnRH synthesis (Clarke and Pompolo, 2005). Cyclic changes have been reported in female rodents (Clarke and Pompolo, 2005), but there does not appear to be any effect of sex steroids on GnRH synthesis in sheep, nor are there any differences across breeding and non-breeding (anestrous) seasons (Hileman et al., 1998).

In terms of secretion, the cyclic variations in the secretion of sex steroids (estradiol-17β and progesterone) in the female have a major impact on the frequency and amplitude of GnRH pulses secreted into portal blood (Clarke, 1995). In the male, androgens negatively regulate GnRH secretion (Tilbrook et al., 1991). At the level of the pituitary gland, there is minimal inhibitory effect of progesterone (Clarke and Cummins, 1984), but oestrogen does modulate function in a variety of ways, increasing the expression of the GnRH receptor and sensitivity of the gonadotrope to GnRH and influencing the function of the cells through modulation of second messengers, intracellular free calcium and membrane ion channels (reviewed in Clarke, 2002). Regulation of the expression of gonadotropin subunit genes by sex steroids is a complex matter that differs between species. In at least some cases, it has been demonstrated, for example, that estradiol does not regulate LHβ gene expression when pulsatile GnRH input is held constant (Mercer et al., 1988). There is evidence from in vitro studies that gonadotropin subunit gene expression is affected by GnRH pulse frequency in rodents (Burger et al., 2002, 2009; Haisenleder et al., 1998, 2008) and by second messengers and transcription factors, rather than by the direct binding of liganded sex steroids to response elements on the regulatory regions of the genes (Ciccone and Kaiser, 2009).

Within the hypothalamus, sex steroid responsive neurons mediate the feedback effects of steroids as mentioned above. Whereas kisspeptin cells are integral to the feedback effects of steroids on GnRH secretion, a number of other cells in the brain also express sex steroid receptors, albeit at a lower level. Subsets of these cells may mediate the feedback effects of steroids and some of these neuronal systems are recognised as appetite regulators (Table 1), regulating function of both food intake and energy expenditure. Nevertheless, the kisspeptin cells appear to be the major upstream regulators of GnRH secretion (Clarke et al., 2011).

Kisspeptin is a potent stimulator of GnRH secretion (Smith et al., 2011). Whether this is due to action on the cell bodies of the GnRH neurons or action on the secretory terminals in the secretory zone of the median eminence (where GnRH is stored) has become a question of significance. If the latter pertains, this introduces a fundamental change in the way that we view regulation of neurosecretion because there are no direct connections between kisspeptin elements and GnRH terminals within the median eminence (d'Anglemont de Tassigny et al., 2008; Smith et al., 2011). Accordingly, the action of kisspeptin at this level would be through a novel mechanism, such as volume control. Intravenous administration of kisspeptin causes virtual immediate secretion of GnRH, even though kisspeptin does not cross the blood–brain barrier (Caraty et al., 2013), consistent with action at the level of the median eminence.

In rodents, it is apparent that the kisspeptin cells of the arcuate nucleus are the conduit for the negative feedback effects of sex steroids on GnRH secretion in both males and females (reviewed in Oakley

Table 1
Expression of ER-α in specific cell types in the ovine brain.

Neuron type	Location	% oestrogen receptor-α positive	Reference
GnRH	Pre-optic area	0	Lehman and Karsch (1993)
Dopamine (TH+)	Arcuate nucleus	3–5	Lehman and Karsch (1993) and Skinner and Herbison (1997)
	A14	25	
POMC (β-end+)	Arcuate nucleus	15–20	Lehman and Karsch (1993)
NPY	Caudal Arcuate Nucleus	10	Skinner and Herbison (1997)
Somatostatin	Ventromedial nucleus	29	Scanlan et al. (2003)
	Arcuate nucleus	13	
GABA	Pre-optic area	40	Herbison et al. (1993)
Glutamate	Arcuate/ventromedial nucleus	52–61	Pompolo et al. (2003)
	Pre-optic area	37–52	
	Bed nucleus of stria terminalis	37–58	
Kisspeptin	Preoptic area	50	Franceschini et al. (2006)
Kisspeptin/neurokinin B/dynorphin	Arcuate nucleus	100	Franceschini et al. (2006)

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et al., 2009). The positive feedback effect of estradiol that causes the preovulatory surge in rodents is mediated by the kisspeptin cells of the antero-ventral periventricular nucleus. A fundamental species difference exists, such that the positive feedback effects of estradiol in ewe are *initiated* in the kisspeptin cells of the arcuate nucleus (Smith et al., 2009) and *facilitated* by the kisspeptin cells of the lateral preoptic area (Hoffman et al., 2011). Thus, in the ewe, implants of estradiol in the mediobasal hypothalamus cause positive feedback and a full GnRH/LH surge event (Caraty et al., 1998). At this level, estradiol increases the activity of kisspeptin neurons in the arcuate nucleus, determined by c-Fos appearance in the cell nuclei (Smith et al., 2009). Kisspeptin gene expression and peptide content of the kisspeptin cells of the arcuate nucleus are increased in the late follicular phase of the estrous cycle (Estrada et al., 2006; Smith, 2009; Smith et al., 2009). Kisspeptin gene expression in the cells of the lateral preoptic area is also increased in the late follicular phase of the cycle (Smith, 2009; Smith et al., 2009) and these cells show increased c-Fos activity in the follicular phase, prior to the GnRH/LH surge (Hoffman et al., 2011).

The kisspeptin cells of the arcuate nucleus also produce neurokinin B and dynorphin, prompting the nomenclature of KNDy cells (Goodman et al., 2007; Lehman et al., 2010). Recent data from rodent and human studies suggest that neurokinin B acts in a 'paracrine' manner to regulate kisspeptin secretion from these cells (Young et al., 2013). In sheep, the KNDy cells not only mediate the positive feedback effects of estradiol, but also the negative feedback effects (Smith et al., 2009). Thus, alterations in sex steroid feedback that are caused by shifts in metabolic function would most likely involve changes in KNDy cells.

Fig. 1 summarises the feedback effects of sex steroids that regulate GnRH secretion and gonadotropin secretion in female sheep via the KNDy/kisspeptin cells of the hypothalamus and preoptic area.

GnIH has emerged as an additional player in the regulation of reproduction, although there are species differences that are important to note. In sheep, the cells that produce GnIH are located in the dorsal region of the paraventricular nucleus and the dorsomedial nucleus of the hypothalamus (Clarke et al., 2008). These cells project to GnRH cells and to the external zone of the median eminence (Clarke et al., 2008). The peptide is secreted into the hypophysial portal blood and acts on pituitary gonadotropes to negatively regulate the synthesis and secretion of gonadotropins (Smith et al., 2012). Further evidence that GnIH plays a significant role in the control of reproduction in this species is the demonstration that expression of the GnIH gene is reduced during the follicular phase of the estrous cycle. Infusion of the peptide reduces pulsatile LH secretion and diminishes the oestrogen-induced LH surge in ovariectomised ewes (Clarke et al., 2012a). The extent to which the expression and secretion of GnIH is directly or indirectly affected by sex steroids is not known.

Seasonality of breeding and metabolic function in sheep

Sheep are seasonal breeders and also display seasonality of metabolic function; this provides a useful adjunct to their utility as a model for both reproduction and energy balance. The annual cycle of reproductive activity and inactivity differs between breeds (Hafez, 1952). Reproductive seasonality is discussed briefly here and the photoperiodic regulation of appetite and energy expenditure is considered below. Seasonality is due to the action of melatonin, functioning as a 'zeitgeber' of the hours of darkness, during which levels are elevated (Lincoln et al., 1981; Yellon et al., 1985). Sheep are short-day breeders, so the entry into the breeding season is prompted by declining day-length after the summer solstice. The commencement of the breeding season is governed by photo-induction (response to declining day-length) and it is terminated by photorefractoriness (cessation of response to short day photoperiod) (Robinson and Karsch, 1984). Thyroid function is also important in the determination of seasonal breeding and this is discussed below. The mechanisms underlying seasonal breeding in the sheep and the role of clock genes are extensively reviewed elsewhere (Dardente et al., 2010; Hazlerigg, 2012).

In the case of free-running GnRH and gonadotropin secretion in the ovariectomised ewe, circannual variation in the occurrence of LH pulses (reflecting GnRH pulses) is seen, with an increase in frequency at the time when normal animals would be in their breeding season (Robinson et al., 1985). Most remarkable, however, is the seasonal change in the ability of estradiol-17β to suppress GnRH and LH secretion. This was elucidated in a classic experiment by Legan et al. (1977), in which ovariectomised ewes were administered subcutaneous implants containing estradiol-17β that was released continuously over the course of a year. In a parallel series of normal animals which were experiencing the same conditions, the breeding season was defined. Most remarkably, estradiol-17β was unable to suppress LH secretion during the period of the normal breeding season, whereas total suppression was seen at the time of normal anestrus. These two studies demonstrate the respective steroid-independent and steroid (estradiol-17β) dependent effects of changing photoperiod. In any consideration of the interaction between metabolic function and reproduction, these important circannual rhythms need to be taken into account.

With the realisation that KNDy cells of the arcuate nucleus are the operative means by which sex steroids provide feedback signals to GnRH neurons in the ovine brain, the integral role in seasonality of breeding became apparent (Clarke and Smith, 2010; Smith et al., 2007). The number of kisspeptin cells detected by in situ hybridisation falls throughout the year in ewes in Australia, where the anestrous period prevails in the later months (Smith et al., 2007). This is reflected in the peptide content of cells in ovariectomised ewes with or without continuous estradiol-17β treatment (Clarke and Smith, 2010; Smith et al., 2007, 2008a). Furthermore, the number of GnRH cell bodies receiving input from kisspeptin cells is reduced in the anestrous period (Smith et al., 2008a). This points to the KnDY cells as major intermediaries in the determination of seasonal breeding.

In addition, the identification of GnIH neurons as negative regulators of reproductive function begged the question as to whether these cells play a role in seasonality of breeding. GnIH expression is upregulated in the anestrous period, as is input to GnRH cells (Smith et al., 2008a). Secretion of GnIH into hypophysial portal blood is higher in the non-breeding season (Smith et al., 2012). Both of these mechanisms contribute to reduced reproductive function in anestrus.

General aspects of the regulation of metabolic function in ruminants

Brain sensing of metabolic state — historical perspective

An appreciation of the regulation of food intake and energy expenditure was rudimentary until approximately 30 years ago. Whereas it was known that lesions in the ventromedial nucleus of the hypothalamus cause hyperphagia and obesity (Hetherington and Ranson, 1939) and lesions in the lateral hypothalamus caused hypophagia (Anand and Brobeck, 1951), the neural substrates (appetite regulating peptides — ARP) in these regions of the brain were not identified for another 30 years. In relation to farm animals, Dukes (1955) mentioned that 'deglutition' (swallowing) was under the control of a centre in the medulla oblongata, but had no knowledge of the regulation of energy balance. Vagovagal connections between the gastrointestinal tract and the brain were recognised (Blessing, 1997), as was the fact that cholecystokinin (CCK) was found in the small intestine (and in the brain) (Rehfeld, 1978). By 1981, the textbooks (Williams, 1981) mentioned reports that CCK inhibited food intake, but merely as a passing reference. This was in spite of the fact that others (Gibbs et al., 1973) had shown that CCK powerfully reduced food intake in rats. By 1983, textbooks registered that the brain regulates food intake and energy expenditure by sensing circulating factors, but none were mentioned (Berne and Levy, 1983). In a chapter on obesity (Bierman and Hirsch, 1981), it was stated that 'Since neural regulation of adipose mass appears likely…future developments of drugs

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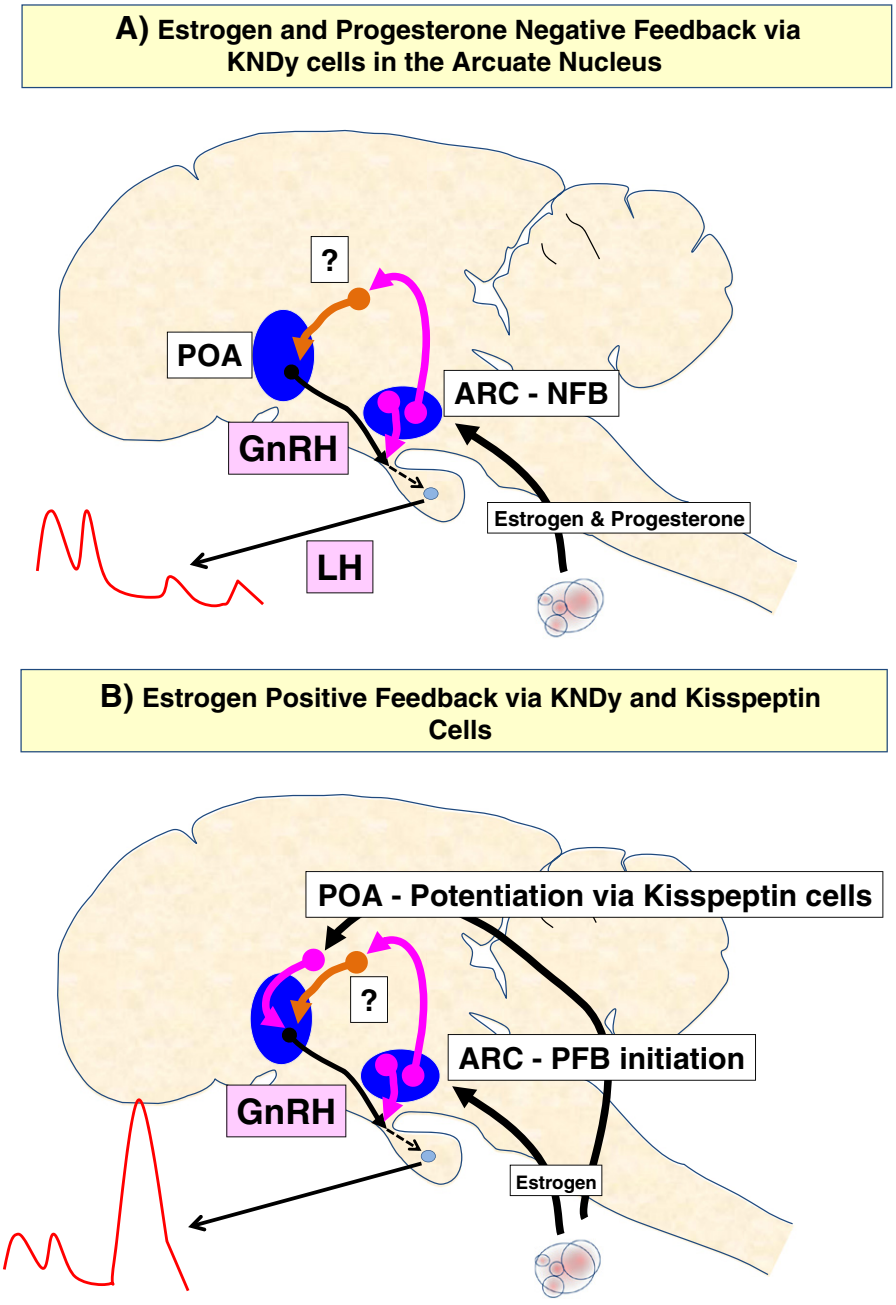

Fig. 1. Panel A: negative feedback (NFB). During most of the estrous cycle (luteal phase), oestrogen and progesterone act on the KnDy cells of the arcuate nucleus (ARC) to reduce kisspeptin peptide production, leading to reduced GnRH and LH secretion. Reduced action of kisspeptin at the level of the median eminence could lead to reduced 'drive' of GnRH secretion. There is no effect of sex steroids during NFB mode on the kisspeptin cells of the lateral preoptic area (POA). Cells of the ARC do not directly contact GnRH cell bodies in the preoptic area (POA), but could exert influence at this level via an interneuron (?). This model does not exclude feedback effects via other neuronal projections to the GnRH cell bodies, dendrites or terminals in the median eminence. Ovariectomy releases the NFB effect and GnRH/LH pulse patterns become free running. Panel B: positive feedback. This involves a neuroendocrine switch that occurs in the follicular phase of the estrous cycle. As oestrogen levels reach a threshold level and are unopposed by progesterone, an initiation event occurs within the KNDy cells of the ARC, which culminates in a surge of GnRH and LH secretion after a delay. At the time of the GnRH/LH surge, the kisspeptin cells of the lateral POA are also activated, leading to potentiation of the effect of oestrogen to elicit a surge event. Kisspeptin released from KNDy cells may act on the GnRH terminals of the median eminence but also on the GnRH cells in the POA, through at least one unidentified (?) interneuron.

altering such mechanisms holds promise'. This has proven to be the case across all species studied. Work by Baile and associates provided a wealth of information on the regulation of food intake and energy balance in farm animals (Baile and Forbes, 1974), recognising that sex steroids, glucocorticoids and GH were important regulators. Importantly, this work highlighted the role of the central nervous system. It was indicated that:

> "Some factors may affect gastrointestinal function and thus perhaps the satiety-hunger signals. Some may influence lipid metabolism and may possibly affect feeding via the feedback system from lipid depots. Others almost certainly affect CNS function so as to impinge on the action of the centres controlling feeding".

This statement was made 20 years prior to the discovery of leptin!

The modern era of the science of energy balance may be thought of as beginning with the identification of a number of ARP in the brain, as well as the discovery of hormones produced in the stomach, fat and other organs that act on the brain to regulate food intake and energy

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expenditure. Neuropeptide Y (NPY) was discovered in 1981 (Tatemoto et al., 1982) and by 1984, this was shown to be a potent orexigen (Clark et al., 1984; Levine and Morley, 1984). This was followed by the identification of other orexigenic and anorexigenic neuropeptides, including orexins (ORX), melanin concentrating hormone (MCH), cocaine and amphetamine-regulated transcript (CART), melanocortins, opioids and agouti related protein (AgRP) (reviewed in Langhans et al., 2009). Actually, pro-opiomelanocortin (POMC) was purified earlier (Roberts and Herbert, 1977) and it was also known that β-endorphin (β-end) was encoded by POMC, but it was not until much later that the role that β-end and α-melanocyte stimulating hormone (α-MSH) play in the regulation of metabolic function was recognised. In 1990, it was noted by Brady et al. (1990) that food restriction or food deprivation increased the expression of the NPY gene and reduced the expression of the POMC gene in rats, but it was not until 1997 that the inhibitory role of melanocortins in regulation of feeding was fully appreciated (Fan et al., 1997).

The expanded list of neuropeptides that regulate food intake and energy expenditure rewrote the textbooks. Importantly, the dual role of these peptides to regulate both sides of the metabolic equation was recognised. The notion of adaptive thermogenesis had been understood for many years (Rothe, 1975), but it took some time to recognise that the so called ARP also regulated energy expenditure. It was demonstrated that NPY promoted white fat lipid storage and reduced brown fat activity (Billington et al., 1991) and the opposite effect was shown with melanocortins, derived from POMC (Haynes et al., 1999). Whilst it was recognised many years earlier that brown adipose tissue (BAT) was responsible for non-shivering thermogenesis (Hayward and Lyman, 1967) and this discrete tissue bed became known as the hibernation gland, the mechanism for this important means of dissipating energy was not identified until 1980 (Klingenberg et al., 1980). These authors described the uncoupling process within mitochondria, and the entity responsible was known as thermogenin for some time (Jacobsson et al., 1994); it later became known as uncoupling protein-1 (UCP-1) (Matthias et al., 1999). The function of UCP-1 in BAT is controlled by the sympathetic nervous system through the β-adrenergic system, particularly via β3 receptors. UCP-1 essentially 'steals' electrons diverting energy away from the production of ATP to the generation and dissipation of heat (Susulic et al., 1995). A poly-synaptic pathway exists from the ARP neurons of the hypothalamus to white (Adler et al., 2012) and brown (Oldfield et al., 2002) fat. The only domestic species in which similar studies have been done is the pig, in which it was shown that polysynaptic pathways exist between the leptin receptor expressing cells of the hypothalamus and the peri-renal fat (Czaja et al., 2003). This is the means of central regulation of peripheral thermogenesis and coordination of the regulation of food intake and energy expenditure. Recent data indicate that skeletal muscle has 'thermogenic' properties, also under the control of the sympathetic nervous system (Henry et al., 2011).

Not only do we appreciate that food intake and energy expenditure are regulated by various centres in the hypothalamus, through the function of ARP, but it is also understood that other systems in the brain, such as those involved in reward and stress responses, also impact upon energy balance. In fact, virtually every neurotransmitter and every neuropeptide in the brain have some effect on food intake and/or energy expenditure! The logical consequence of this is that an alteration in the function of any of these factors may lead to correction through another factor, which is why single gene knockouts may not be particularly informative in terms of integrated function. Whilst knockouts and overexpression of particular peptides can provide valuable information on the function of the targeted factor, the overall process of metabolic balance involves the integrated function of many peptides/systems. A classical example of this is the original studies on NPY gene knockout animals, which had normal food intake, were of normal body weight under resting conditions (Erickson et al., 1996) and had normal endocrine function (Erickson et al., 1997). This was explained, at least in part, by compensatory upregulation of AgRP function (Marsh et al., 1999). On the other hand, overexpression of NPY led to hyperphagia and obesity (Kaga et al., 2001). A later paper emphasised the importance of the background upon which a knockout is performed, showing that, in C57BL/6 mice, NPY ablation caused impaired feeding response following a fast (Segal-Lieberman et al., 2003). Other work showed impaired response when knockout animals were placed on a high fat diet (Patel et al., 2006) and extensive analysis of the roles of specific Y-receptor subtypes (Herzog, 2003; Zhang et al., 2011) substantiated the central role that NPY plays in the regulation of appetite and energy expenditure. Nevertheless, a valuable lesson was learnt about the way that knockout technology informs us of function!

Endocrine consequence of altered body weight in sheep

Reduction in body weight by food restriction in sheep leads to the changes seen in Table 2. Interestingly, there is little information on the changes in the transition from normal to 'obese' state, but the lean condition provides a useful model because it allows one to overlay treatment with leptin or other metabolic factors. In terms of reproductive function, the plasma levels of LH are the most sensitive to lean condition (Fig. 2) and this may be due to the lowering of circulating levels of factors such as leptin (see below). In this respect, lowered levels of leptin in the lean condition might be regarded as being a signal of inadequate energy stores that is detected by the brain.

On the other hand, the difference between adequate and excess leptin levels in the obese condition is less relevant in terms of reproductive function. In a model of growth restriction in early life, reduced LH secretion is observed and this is due to reduction in the frequency of GnRH pulses detected in hypophysial portal blood, at one year of age (I'Anson et al., 2000). This demonstrates that the metabolic condition of nutritional deficit has a direct effect at the level of the brain to affect GnRH secretion. Interestingly, in this singular study of GnRH secretion in animals with compromised reproductive function due to food-restriction, the amplitude of GnRH pulses was increased (not statistically significant), but the amplitude of the LH pulses in peripheral plasma was reduced. This suggests that the metabolic function of the animals compromised gonadotrope function, but this requires definitive investigation. There are no studies of GnRH secretion in animals that are made lean or obese in adulthood, but there is reduced frequency LH pulses seen in peripheral plasma of lean ovariectomised animals (Henry et al., 2000; Thomas et al., 1990). This is not due to any substantial reduction in the expression of genes encoding the gonadotropin α- and β-subunits in the lean condition (Thomas

Table 2
Changes in hormone levels seen in sheep with alteration in body weight (by food restriction or supplementary feeding).
The information presented here is taken from Archer et al. (2002), Estienne et al. (1990), Henry et al. (2000), Henry et al. (2001a), Kurose et al. (2005), Thomas et al. (1990), and Tilbrook et al. (2008).

Hormone	Lean	Fat	Comment
LH	↓	↓	Only seen after gonadectomy. Amplitude of pulses is reduced in fat condition
FSH	↔	↔	
GH	↑	↓	
Cortisol	↓	↑	Comparison between lean and fat and difference is seen only when stressed
T4	↓	?	
T3	↓	?	
Prolactin	↔	↔	
Insulin	↓	↑	
Leptin	↓	↑	
Glucose	↓	↔	
Ghrelin	↔	↑	This differs from the results obtained in other species, including humans
Free fatty acids	↓	↑	

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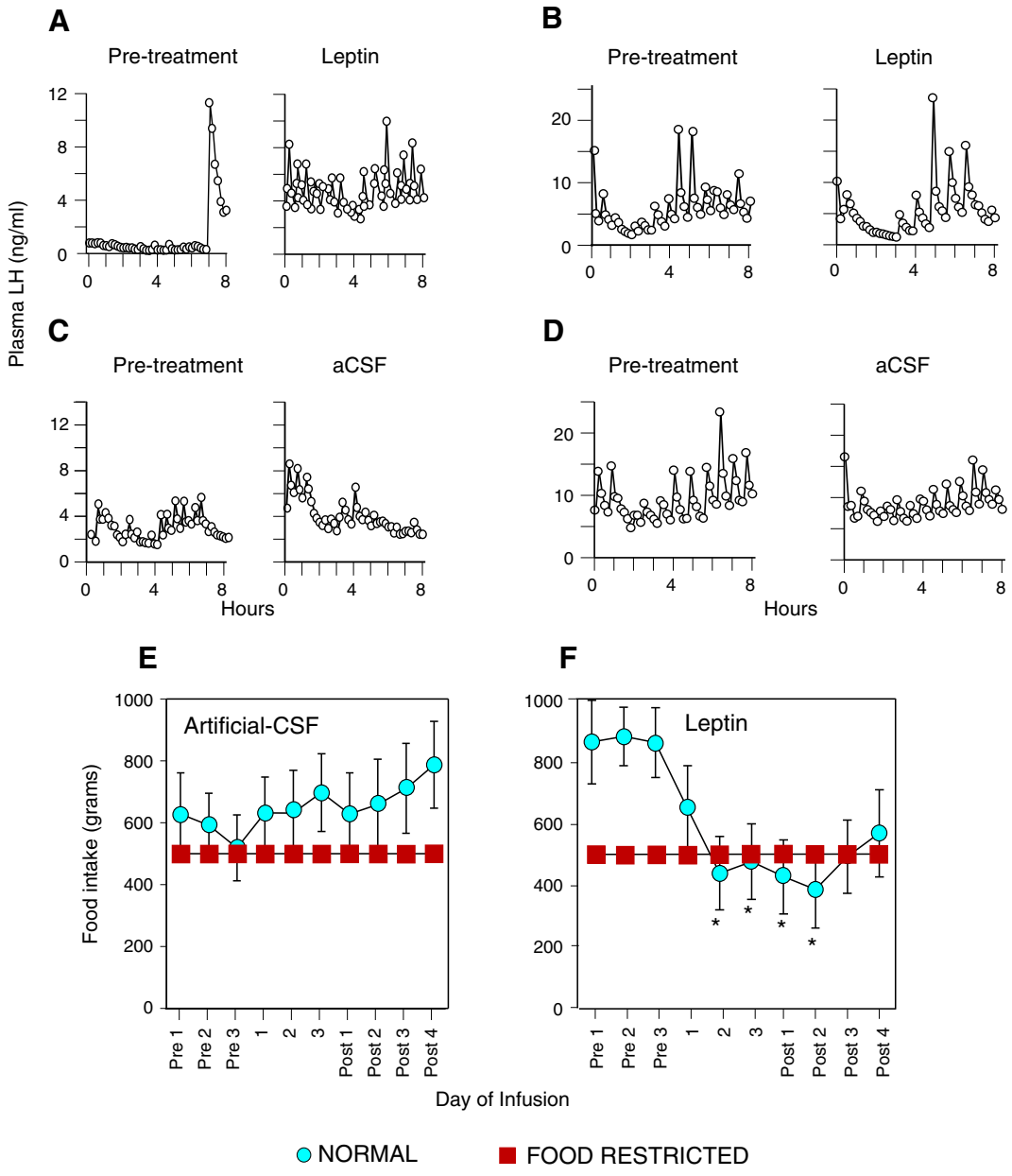

Fig. 2. Leptin activates the reproductive neuroendocrine axis in hypogonadotropic lean ovariectomised ewes with no reduction in food intake, whereas in ovariectomised ewes of normal body weight, leptin reduces food intake and has no effect on LH secretion. The sheep were sampled before and after 3 days of treatment with 4 μg/h leptin or artificial cerebrospinal fluid (aCSF) by intracerebroventricular infusion. Panel A shows the leptin effect on LH levels in lean animals and Panel B shows the lack of effect on LH levels in animals of normal body weight. Panels C and D show lack of LH responses to control (aCSF) infusions. Panel E shows the lack of effect of leptin on food intake in lean animals and panel F shows reduction in food intake when leptin is given to animals of normal body weight. *P < 0.05 vs pretreatment.
Adapted from Henry et al. (2001a).

et al., 1990) and begs the question as to whether there is an effect on GnRH receptor levels and/or post-receptor signalling in the gonadotropes of the lean animals. In sheep, FSH levels may be reduced in the lean condition (Thomas et al., 1990) but, in some studies, the levels of this gonadotropin were found to be similar in lean and fat animals (Henry et al., 2001a).

The changes that occur in GH and thyroid hormone levels will be discussed later. Basal plasma levels of cortisol are increased in response to a fast, but are not higher in animals made lean by food restriction — these effects are different to those seen in rodents and humans (Henry and Clarke, 2007). Notably, the relatively fat sheep shows a greater cortisol response to stress than the relatively lean animal in terms of adrenocorticotropin (ACTH) and cortisol secretion (Fig. 3), as well as adrenalin secretion (Tilbrook et al., 2008).

The changes in plasma levels of sex steroids with altered body weight are not indicated here because this is a complex issue, related to sequestration of sex steroids in fat. Most studies that have investigated the effect of altered body condition on the reproductive axis in sheep have been conducted in gonadectomised animals, because the effects on gonadotropin levels are more exaggerated than the changes seen in gonad-intact animals. Food restriction increases ER-α levels in the preoptic area of the lean ovariectomised ewe but reduces the levels in the ventromedial nucleus (Hileman et al., 1999). Determining the feedback effects of oestrogen in different metabolic states is, however, a complicated issue because clearance of estradiol-17β from plasma is reduced in lean sheep (Renquist et al., 2008a) and careful attention needs to be paid to plasma levels of the steroid to compare effects in animals of different body conditions. When plasma levels of estradiol-17β were equalised in animals of

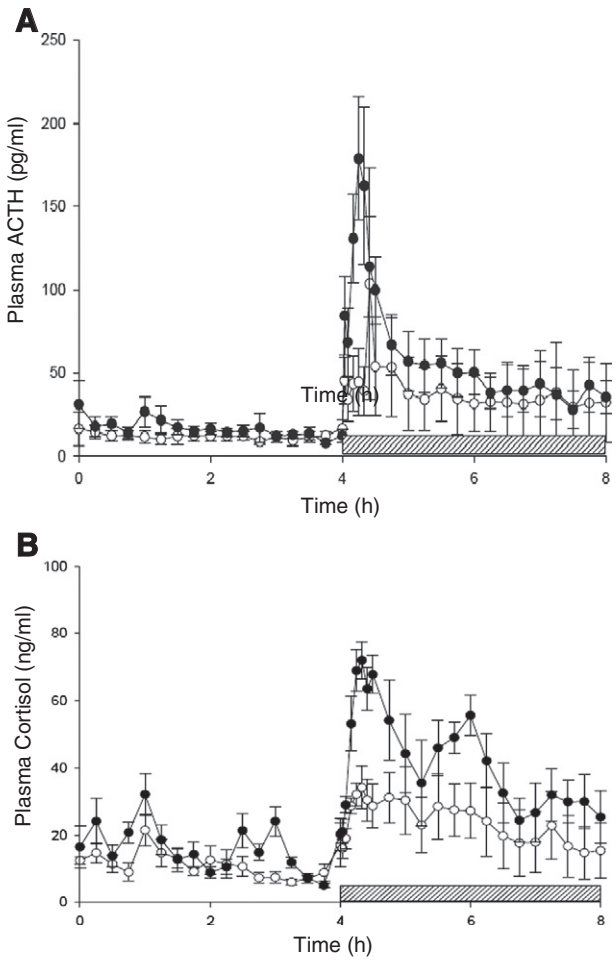

Fig. 3. Effect of isolation-restraint stress in ewes of the lean (open circles) or obese (closed circles) condition. Panel A — plasma ACTH concentrations, Panel B — plasma cortisol concentrations. Data are means ± SEM and responses of both hormones to the stressor were significantly different in fat and lean animals ($P < 0.05$).
Adapted from Tilbrook et al. (2008).

lean and normal body conditions, enhanced negative feedback on the secretion of LH was observed in the lean animals (Renquist et al., 2008b).

Appetite regulating peptides in the ovine hypothalamus

In ruminants, as in other species, metabolic function is regulated by brain elements that regulate food intake and energy expenditure. These systems respond to factors originating from peripheral organs and tissues that produce modulatory factors such as leptin and ghrelin. In terms of central regulation of food intake and energy expenditure, the arcuate nucleus is of fundamental importance (Cone et al., 2001). Recent data showed that this hypothalamic centre is, at least in part, outside the blood brain barrier (Ciofi, 2011) and permeability changes with metabolic state (Langlet et al., 2013a). A comprehensive review of integrated regulation of appetite in domestic animals has been provided recently (Sartin et al., 2010) and will not be recapitulated here. Alternatively, specific issues that relate to the nexus between ARP and reproductive function in a model ruminant, the sheep, will be discussed below. In sheep, as in other species, neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) cells in the arcuate nucleus appear to be integral regulators of metabolic function. NPY is a potent appetite stimulant in sheep (Miner et al., 1989, 1990), but there are no data to indicate an effect of this neuropeptide in the regulation of energy expenditure, as has been shown in rodents (Billington et al., 1991; Shi et al., 2013). As indicated above, NPY cells also produce AgRP, which acts as an orexigenic factor, through blockade of the melanocortin signalling system in the brain (Fong et al., 1997). In sheep, AgRP is also a potent orexigen (Wagner et al., 2004).

Melanocortins, produced by post-transcriptional processing of the POMC precursor in cells of the arcuate nucleus, act to suppress appetite and stimulate energy expenditure in rodents (Cone, 2005; Fan et al., 1997; Haynes et al., 1999; Mountjoy, 2010). Again, the same has not been demonstrated in sheep. Importantly, the post-translational products (ACTH, endorphins and melanocortins) are produced by virtue of the action of a range of enzymes (Mountjoy, 2010). The acetylation of melanocortins is necessary for function (Guo et al., 2004), but acetylation of endorphins reduces function (Mountjoy, 2010). Melanocortins act through sub-types of the melanocortin receptors (MC3R and MC4R) in the brain, to regulate the function of a number of 'appetite-regulating peptide' neurons, although the function of the latter seems more critical than that of the former in relation to regulate of metabolic function (Mountjoy, 2010). Although often not considered in the greater scheme of overall control of appetite/energy expenditure/adiposity, opioids are also important regulatory peptides, stimulating food intake in sheep (Obese et al., 2007). The question arises as to why one gene (POMC) can encode for peptides that either stimulate (β-end) or inhibit (melanocortin) food intake and the answer probably lies in the fact that post-translational processing of the POMC precursor is the point of regulation (see below); this is a worthy area of investigation.

ORX and melanin-concentrating hormone (MCH) are produced in the lateral hypothalamus and stimulate food intake in sheep (Sartin et al., 2001, 2008; Whitlock et al., 2005), as in other species. Connectivity between the melanocortin and NPY cells of the ARC and the ORX and MCH cells of the lateral hypothalamus has been demonstrated in rats (Elias et al., 1998, 1999; Elmquist, 2001), but the notion that there is 'first-order' (NPY/AgRP and POMC) signalling to 'second-order' neurons (ORX, MCH etc.) is not well established for large animals. Interestingly, virtually all of the ORX and MCH cells in the ovine brain express the leptin receptor (Iqbal et al., 2001b) and leptin signalling to these so-called 'second-order' neurons occurs in the absence of the arcuate nucleus in the sheep brain (Qi et al., 2010). Anterograde tracing shows that there are projections from the arcuate nucleus and the ventromedial nucleus to the perifornical area and the paraventricular nucleus of the hypothalamus, but there are few, if any, projections to the lateral hypothalamus, where ORX and MCH neurons are found (Fig. 4). This, coupled with the ability of the cells of the lateral hypothalamus to respond to leptin in an independent manner, strongly suggests that the first-order/second-order model for metabolic regulation of neuronal function within the hypothalamus is not tenable, for the sheep at least. There may be important signalling from the arcuate nucleus to the paraventricular nucleus but this requires investigation in non-rodent models.

Peripheral regulators of food intake/energy expenditure and reproduction

Many hormones have been identified as regulators of food intake and insulin is prominent amongst these. The story, however, is not simple, as outlined by Morley (1987). The acute effect of insulin is to enhance feeding, whereas the chronic effect is to reduce eating. Clearly such effects also involve changes in circulating glucose levels. A classic study (Coleman and Hummel, 1969), using parabiosis of mice, revealed that unidentified circulating factors existed. Thus, by joining the circulation of animals that were obese db/db mutants to normal animals, the latter died of starvation. It was concluded that this was due to high levels of a circulating satiety factor in the db/db mutants. Pairing of ob/ob obese mice with db/db mice showed that the former reduced their food intake and became lean, again leading to the conclusion that there was a satiety factor that was present in the db/db mice that was absent in the ob/ob mice. The ob gene was eventually cloned in 1994, with the encoded protein being named leptin (Zhang et al., 1994). Although the ob gene had been recognised for many years, it was not until this classic finding that obesity in ob/ob mice was

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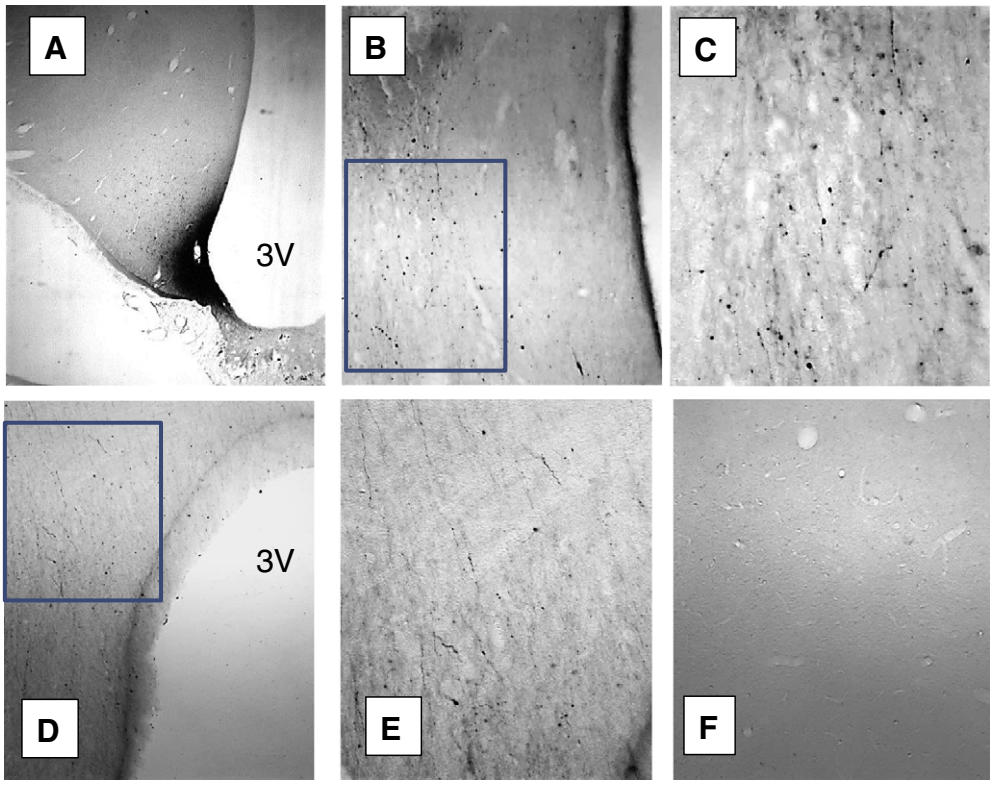

Fig. 4. Projections from the arcuate nucleus to perifornical area (PFA), paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA) in the ewe, determined by anterograde tracing with biotinylated dextran amine injection into the arcuate nucleus. The sections were taken from the brain of an animal that was previously described in Pompolo et al. (2001). Panel A shows the injection site in the arcuate nucleus, Panel B shows varicose fibres in PFA and Panel C shows a higher magnification of the boxed area in Panel B. Panel D shows varicose fibres in the PVN and Panel E shows a higher magnification of the boxed area in Panel D. Panel F shows the lack of projections to the LHA.
Adapted from I.J. Clarke (unpublished data).

identified as a mutation in the leptin gene. The obesity phenotype of db/db mice was revealed as a mutation in the leptin receptor (Tartaglia et al., 1995). These studies were carried out in rodents but leptin signalling and physiology are clearly the same in ruminants.

Leptin produced by white adipose cells circulates to act as an appetite suppressant in sheep, as in other species (Henry et al., 1999). Centrally administered leptin also increases energy expenditure, by amplifying the post-prandial thermogenic output of muscle and fat in sheep (Henry et al., 2008). This is associated with activation via the sympathetic nervous system (B.A. Henry and I.J. Clarke, unpublished data), but the exact intracellular mechanisms that pertain in regulation of heat production in muscle have not been elucidated.

Leptin treatment of ovariectomised ewes that were hypo-gonadotropic, due to lean condition induced by dietary restriction, restored pulsatile LH secretion to normal (Henry et al., 2001a) (Figs. 2A, B). In the case of the hypo-gonadotropic, lean ewe, leptin treatment

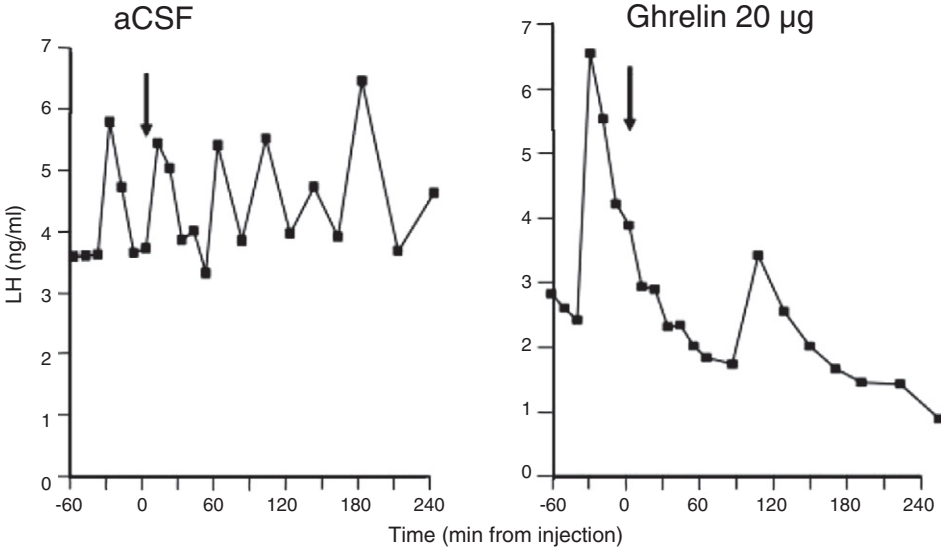

Fig. 5. Effect of intracerebroventricular injection of ghrelin on plasma LH concentrations in ovariectomised ewes.
Adapted from Iqbal et al. (2006).

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did not suppress food intake (Henry et al., 2001a), indicating their strong appetite drive in food restricted animals. In animals of normal body weight and ad libitum feeding, the effect of leptin was to suppress food intake but there was no effect on free running LH secretion (Figs. 2C, D), which is interpreted to mean that leptin levels were signalling adequacy of body stores in these animals and further elevation of leptin was not of any consequence in terms of the reproductive axis (Henry et al., 2001a). Intravenous leptin injections stimulated plasma LH levels in oestrogen-treated, ovariectomised cows (Zieba et al., 2003), which may be due to direct positive effects at the level of the pituitary gland (Amstalden et al., 2003). Leptin treatment can also prevent the fasting-induced reduction in plasma LH levels in heifers, enhancing the pituitary response to GnRH (Maciel et al., 2004). Overall, these studies suggest that an adequate level of circulating leptin is required for normal function of the hypothalamo-pituitary axis that drives reproduction.

The discovery of ghrelin, which was achieved through screening for ligands which activated the receptor for GH releasing peptide (Kojima et al., 1999), was also a milestone. This receptor had been identified earlier (Howard et al., 1996) and it was not until later that it became apparent that ghrelin is a circulating orexigen, potently stimulating feeding (Tschop et al., 2000; Wren et al., 2000); the same is true for sheep (Grouselle et al., 2008).

Elegant studies in sheep demonstrated that, with programmed meal-feeding, the appetite-stimulant ghrelin is secreted from the oxyntic glands of the stomach in anticipation of a meal (Sugino et al., 2002). In ruminants, the ghrelin-producing cells are found in the abomasum (Hayashida et al., 2001). Ghrelin is most well known as a gut-derived hormone that acts centrally to stimulate appetite, but it also has potent effects to stimulate GH secretion and suppress pulsatile LH secretion in sheep (Iqbal et al., 2006) (Fig. 5). This result conforms to the general rule that factors which stimulate food intake generally suppress reproductive function and this will be discussed in greater detail below.

Insulin is another circulating hormone that appears to be essential for appropriate function of the reproductive axis. Central infusion of insulin stimulates LH secretion in sheep (Berne and Levy, 1983) and insulin levels fall in early lactation in dairy cows (Wathes et al., 2007a,b); this could contribute to low fertility at this time.

Although leptin, ghrelin and insulin are major regulators of ARP neurons in the hypothalamus that regulate food intake and energy expenditure, many other factors, such as sugars, fatty acids, cytokines and other circulating entities also act in a coordinated manner at this level as well described in recent reviews (Langhans et al., 2009; Reichenbach et al., 2012) (Fig. 6). From this, it is obvious that the regulation of food intake and energy expenditure is multifaceted and complicated. In order to understand this and to manipulate it in any way, one needs to adopt an approach that takes account of as many factors as possible. Within the brain, these factors act on a multitude of orexigenic or anorexigenic ARP. It is clear that single factor knockouts can inform us of the function of the particular gene in question, but it is equally obvious that metabolic regulation involves a number of factors acting in concert.

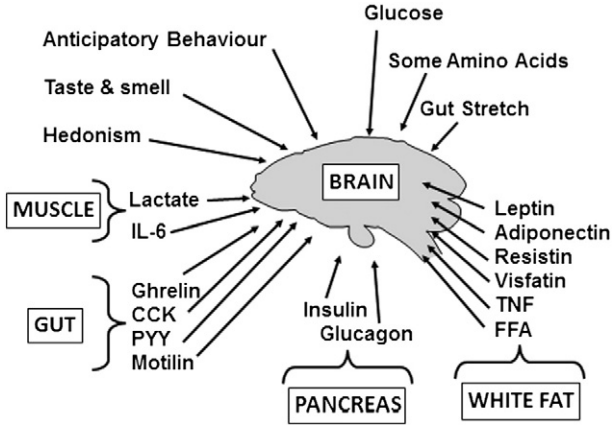

Fig. 6. Factors that influence food intake and energy expenditure by action within the brain. IL-6 — interleukin 6; CCK — cholecystokinin; PYY — peptide YY; TNF — tumour necrosis factor; FFA — free fatty acids.

Impact of negative energy balance on hypothalamic ARP and peptides regulating reproduction

The effect of alteration in body weight, especially negative energy balance, on the expression of genes for ARP has been studied extensively in sheep. Early work showed that reduction in body weight, by food restriction, increased expression of the gene for NPY in the arcuate nucleus (McShane et al., 1993), as well as immunoreactive NPY peptide (Barker-Gibb and Clarke, 1996). The result for NPY has been replicated in other studies and AgRP gene expression also increases in lean animals (Adam et al., 1997). Some reports indicate that expression of the POMC gene in the arcuate nucleus is lowered in lean ewes (McShane et al., 1993). The result for POMC gene expression with altered body weight is equivocal, however (Henry et al., 2000; Kurose et al., 2005). At least for NPY cells, the upregulation in gene expression for NPY/AgRP in the ovine arcuate nucleus may be due to altered levels of expression of the leptin receptor and the ghrelin receptor (Kurose et al., 2005). No difference was found in expression of pre-pro-ORX or dynorphin gene expression in ovariectomised ewes which were either fat or lean because of dietary manipulation (Iqbal et al., 2003), but there were region-specific differences in the levels of enkephalin and CART gene expression (Henry et al., 2001b; Iqbal et al., 2003).

Interestingly, in mice at least, there are no major changes in the production of ARP in the transition from normal to overweight condition (Enriori et al., 2007). Presumably this indicates that there is little difference in the sensing of adequate or excess body stores, through changes in leptin or insulin levels (and other factors). This is in profound contrast to the effect of negative energy balance, in which leptin levels are lowered. In all species studied, including ovariectomised ewes (Henry et al., 2001a) and humans (Chan et al., 2006), leptin can restore pulsatile gonadotropin secretion in states of negative energy balance, suggesting that adequate leptin signalling is mandatory for normal reproductive function. Comparison of changes that exist in the hypothalami of sheep in normal or fat body condition requires investigation.

In sheep, we have shown that re-feeding of ovariectomised ewes that were food-restricted caused a virtually immediate restoration of pulsatile LH secretion, but this was associated with changes in oxidisable fuels and *not* an increase in leptin levels in plasma (Szymanski et al., 2007). Indeed, infusion of propionate into the mesenteric artery has a short-term (but not long-term) effect to restore LH levels in hypogonadotropic, lean ewes (Szymanski et al., 2011). This argues strongly that, in the catabolic state, the reproductive neuroendocrine system can respond to supra-physiological concentrations of leptin (as in the studies cited above), but the physiological change that occurs upon refeeding of food-restricted animals is due to changes in energy supply. The same has been argued from the study of rats (True et al., 2011c), where physiological doses of leptin were ineffective in restoring reproduction in rats on negative energy balance, but supra-physiological doses did so. The lack of effect of leptin treatment to restore low levels of kisspeptin gene expression to normal (True et al., 2011b) was an interesting result and begs the question of what might upregulate kisspeptin cells when animals exit a catabolic state. Intracerebroventricular infusion of leptin treatment can partially restore kisspeptin levels in the arcuate nucleus of lean hypogonadotropic ewes as well as restoring gonadotropin secretion (Backholer et al., 2010a), but the doses may have been supraphysiological.

Dynamic changes in the plane of nutrition are perhaps more relevant in terms of the response of ARP neurons in the hypothalamus than are levels in a static condition. Refeeding of lean hypogonadotropic ewes leads to dynamic changes in circulating insulin, ghrelin, glucose, and total ketone body concentrations (Szymanski et al., 2007) that may all

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impact on the hypothalamic and pituitary components of the HPG axis, let alone the gonads in gonad-intact animals (Scaramuzzi et al., 2011; Somchit-Assavacheep et al., 2013). Dynamic effects were investigated in an experiment on castrated male sheep (Miller et al., 2007), whereby lean animals were placed on an increasing plane of nutrition and fat animals were given a decreasing plane of nutrition; control animals were held static. The increasing plane led to a reduction in NPY/AgRP expression and an increase in POMC expression in the arcuate nucleus (relative to those on a declining plane). Those on the declining plane of nutrition showed the opposite effect. Mean plasma LH levels and LH pulse frequency were higher in the animals on increasing plane of nutrition (Miller et al., 2007). Plasma and CSF insulin and leptin levels increased in the animals on increasing plane of nutrition and the authors reasoned that these circulating factors drove the changes in ARP gene expression. The time-frame of the study was such that it was not possible to distinguish between the effects of singular effects of rising levels of leptin or insulin. Ghrelin could also be a factor that influences the gonadotropin levels in such conditions but more needs to be known about levels of this regulator in ruminants which are in different metabolic states.

Whereas marked changes in the expression of genes for ARP are seen with alteration in body weight, especially negative energy expenditure, the same is not true for GnRH gene expression or synthesis (Clarke and Pompolo, 2005). Nevertheless, reproduction is compromised in low energy states, so the point at which this is manifest is probably upstream of the GnRH neurons. As indicated above, this is due, at least in part, to reduction in the levels of kisspeptin in the arcuate nucleus (Backholer et al., 2010a). Whether this leads to changes in the levels of other relevant neuropeptides (e.g. neurokinin B) would be worthy of investigation. In terms of opioid function, altered body weight of ovariectomised ewes lead to changes in enkephalin gene expression, with upregulation or downregulation depending on the nucleus observed (Henry et al., 2000), but there is no change in expression of dynorphin or ORX genes (Iqbal et al., 2003).

GH and thyroid hormone as factors affecting metabolism and reproduction

GH in relation to metabolic state and reproduction

The GH axis and the reproductive axis are intimately linked in terms of function at various levels (Veldhuis et al., 2006). Circulating GH levels are elevated in lean sheep, due to increased expression of the gene for GH releasing hormone neurons in the arcuate nucleus and reduced somatostatin gene expression (Henry et al., 2001c) and circulating plasma GH levels are elevated in cattle (Blum et al., 1985) and sheep (Barker-Gibb and Clarke, 1996). At least part of this response is presumably due to lowered leptin action, since virtually all of the somatostatin neurons in the dorsomedial, ventromedial and arcuate nuclei of the ovine hypothalamus express leptin receptors (Iqbal et al., 2000b). Lowered leptin levels may also be a factor at the level of the pituitary gland, since in vitro GH responses to GH releasing hormone are elevated in lean animals, but this is counteracted by leptin treatment (Fig. 7). In the ovine anterior pituitary gland, 70% of somatotropes express the leptin receptor (Iqbal et al., 2000a), but the effect of leptin is only seen in the cells of the lean animals (Fig. 7). This may have significant sequelae in regard to the metabolic state of the lean animal. Non-esterified fatty acid levels in plasma tend to be lower in lean ewes (Henry et al., 2000; Szymanski et al., 2007) and free-fatty acids (FFA) reduce GH levels in ovariectomised lambs (Estienne et al., 1990), so this could also be a factor. The GH response in the lean condition may also be due to elevated ghrelin levels, which are also increased in negative energy balance in rodents (van der Lely et al., 2004) and in dairy cows under negative energy balance (Bradford and Allen, 2008).

GH is considered to be a lipolytic hormone, so the question arises as to why it should be elevated in the lean individual. GH is also an anabolic hormone, so the elevated levels in conditions of negative energy balance may be a physiological response to conserve muscle mass. Insulin induced hypoglycaemia elevates plasma GH secretion (Gale et al., 1983) and glucose levels in sheep are lower in the lean condition (Szymanski et al., 2007), consistent with the higher levels of GH. Insulin levels are also low in the lean animal (Henry et al., 2000), consistent with high GH levels at least in this condition (i.e. low insulin/high GH — see below). As for the implications regarding the reproductive function of elevated GH levels in lean animals, this is an issue for which no data exist.

It is relatively certain that insulin lowers GH levels, which explains the inverse relationship between the circulating levels of these two hormones in obese humans (Cornford et al., 2011) and the same is true for other species. This relationship appears to work in the reverse in the lean individual. The exact relationship between insulin and GH is complicated by the fact that insulin like growth factors (IGF) and IGF binding proteins also play a role. It appears that insulin and IGF-1 regulate GH synthesis and secretion by separate mechanisms (Gahete et al., 2013). The impact of these factors on the reproductive axis is salient because

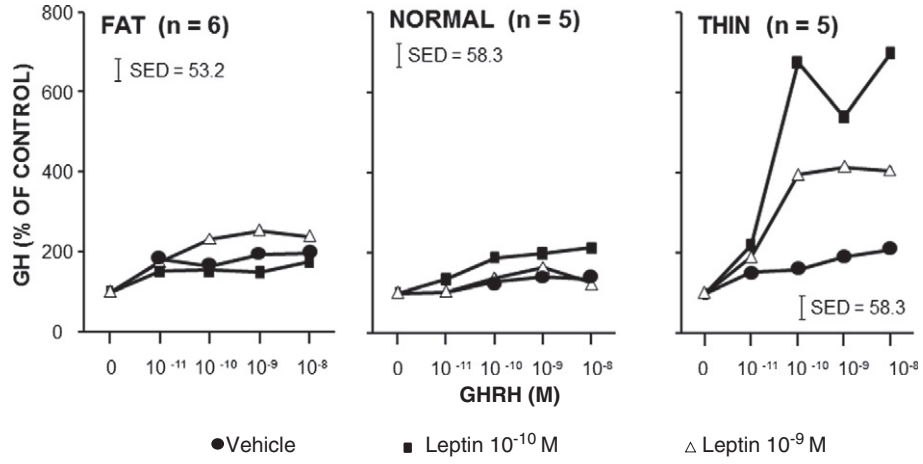

Fig. 7. Effect of body weight on the way that leptin affects the response of pituitary somatotropes to GH releasing hormone (GHRH). Pituitaries were harvested from lean, normal or fat ovariectomised ewes and plated at 250,000 cells per well. After 3 days, the cells were challenged with GHRH alone (closed squares) or with GHRH and leptin (10^{-10} M, open triangles; 10^{-9} M, closed circles) treatment. Note that the response to GHRH is much greater in pituitary cells from lean animals and the effect of leptin on response to GHRH is unmasked in these cells. Errors are shown as standard error of difference for each data set and the effect of leptin on GHRH response in lean animals was statistically significant ($P < 0.01$) by analysis of variance. *Adapted from S.-G. Roh, C. Chen and I.J. Clarke (unpublished data).*

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of the way that these hormones regulate metabolic function. Direct actions of GH, growth factors and binding proteins on the reproductive neuroendocrine system are somewhat unappreciated. Wathes (2012) have presented a case for the GH axis impacting upon the reproductive axis in cows, arguing that lowered insulin-like growth factor 1 (IGF-1) levels in early lactation could predispose to infertility. This is a complex matter involving various growth factors and binding proteins, but the effect of these factors could be via the neurons upstream of GnRH cells in the hypothalamus. The levels of insulin like growth factor (IGF)-1 fall and those of IGF binding protein 2 rise after calving.

The thyroid axis in relation to metabolic state and reproduction

Original work in starlings indicated that thyroid function was required for seasonal transition from breeding to non-breeding state (Goldsmith and Nicholls, 1984; Wieselthier and van Tienhoven, 1972). In sheep, there is a fundamental role of the thyroid in the control of seasonal breeding. In particular, thyroid hormones play a role in the suppression of luteinising hormone by oestrogen in the non-breeding season. Thyroidectomy prevents this suppressive effect of estradiol in ovariectomised ewes (Moenter et al., 1991). Thyroid hormone replacement restores the negative feedback effect of estradiol to suppress luteinising hormone in thyroidectomised, ovariectomised ewes at the time of the normal non-breeding season (Webster et al., 1991).

The control of seasonal reproduction involves an interesting mechanism whereby melatonin regulates TSH production in the pars tuberalis, which feeds back to the hypothalamus to induce de-iodinase 2 (Hanon et al., 2008). This enzyme converts thyroxine (T4) to the active form, triiodothyronine (T3) and the action of thyroid hormone regulates the function of GnRH neurons in a range of species (Yoshimura, 2013) including sheep (Saenz de Miera et al., 2013). Both TSH in the pars tuberalis and DIO-2 in the hypothalamus are upregulated in sheep on long-day photoperiod, which is the time of reproductive senescence (Hanon et al., 2008). This creates a precedent in regard to the way that the thyroid axis is intimately involved in the regulation of reproduction. Classical studies in birds showed that GnRH terminals are ensheathed in glia during the non-breeding season, but not in the breeding season (Yamamura et al., 2004). The function of the neuroglial endfeet is modified by thyroid hormone action (Nakane and Yoshimura, 2010). Thus, the function of the thyroid hormone axis is intimately involved in the regulation of reproduction. The role of thyroid hormone action in the basal hypothalamus in relation to the seasonality of metabolic state and reproductive function is reviewed in another chapter in this volume (Ebling FJP 2014, *On the value of seasonal mammals for identifying mechanisms underlying the control of food intake and body weight*).

Thyroid hormone is a well-recognised regulator of metabolic function, acting in various regions of the body. Hypothyroidism reduces food intake and body weight and central replacement of thyroid hormone reverses this in rats (Alva-Sanchez et al., 2012). In lean hypogonadotropic ovariectomised ewes, levels of T3 and T4 are lower in lean animals compared to fat animals (Henry et al., 2000), so there could be remodelling within the median eminence that affects GnRH secretion. The reasoning for this is that thyroid hormones act via tanycytes in the basal hypothalamus in response to altered TSH levels (Hanon et al., 2008) and tanycytes have an intimate association with the GnRH neuronal terminals in the median eminence (Langlet et al., 2013b). The relationship between the tanycytes and the GnRH terminals can change with reproductive state (Prevot et al., 2010). Mice with targeted deletion of DIO-2 are susceptible to diet-induced obesity (Marsili et al., 2011) and may well have remodelled median eminence. Whether there is remodelling in lean, hypogonadotropic animals is yet to be determined. Certainly there is remodelling of the mediobasal hypothalamus in obese rodents and humans, characterised by inflammation and gliosis (Thaler et al., 2012). There is also a suggestion that tanycyte arrangement in the basal hypothalamus is altered by lowered blood glucose levels in fasted animals or those injected with 2-deoxyglucose, associated with upregulation of vascular endothelial growth factor-A (VEGF-A) in tanycytes (Langlet et al., 2013a).

Photoperiodic effects on metabolic status

As indicated earlier, there is a circannual cycle of metabolic function in sheep. This has been reviewed in detail elsewhere (Clarke, 2008; Rhind et al., 2002). The circannual cycle of food intake and change in body weight is driven by changes, within the brain, in the expression of NPY and POMC gene expression (Anukulkitch et al., 2009; Clarke et al., 2000, 2003; Lincoln et al., 2001). There are also changes in the expression of pre-pro-ORX and MCH, but how this relates to food intake and energy expenditure is not well understood (Anukulkitch et al., 2009). As mentioned earlier, sheep are short-day breeders and they also have lowered appetite drive under short day photoperiod. Interestingly, although Siberian hamsters are long-day breeders, they display reduced appetite drive under short-day photoperiod as well, so the association between photoperiod, breeding period and appetite drive is not consistent across species.

When gonad-intact and gonadectomised Soay rams were transferred from long-day photoperiod (16 h light:8 h dark) to short-day photoperiod (8 h light:16 h dark), the responses of the two groups of rams were different. In essence, the food intake of both groups fell, but with continued short-day photoperiod, the gonad-intact animals became photorefractory and their food intake increased after 16 weeks; this did not happen in the gonadectomised animals (Anukulkitch et al., 2007). Whilst at minimal food intake, induced by short-day photoperiod, the gonadectomised animals gained adipose mass. In other words, these animals gained weight whilst eating less food, which can only be explained by an alteration in energy expenditure. Unfortunately, there is little information on the seasonal patterns of energy expenditure in sheep, particularly in the female.

Ghrelin and leptin are perhaps the most well recognised regulators of food intake and energy expenditure and reproduction that signal to the brain from the periphery. Since there are marked effects of photoperiod on metabolic function in the sheep as in other photoperiodic species, the question then arises as to whether this is due, at least in part, to ghrelin and leptin signalling.

In oestrogen treated, castrated male sheep that had been on long or short day photoperiods for 16 weeks, icv injection of ghrelin significantly stimulated food intake under long day photoperiod but not short day photoperiod (Harrison et al., 2008). On the other hand, the same treatment suppressed LH secretion under short day photoperiod but not long day photoperiod. It will be recalled that food intake falls under short days, so appetite drive is reduced; this may be due in part to lack of sensitivity to ghrelin, but the photoperiodic regulation of ghrelin receptors remains to be determined. Regarding the effect of ghrelin to reduce LH secretion, the significance of the effect under short day photoperiod is not apparent. Ghrelin levels in plasma were not affected by photoperiod in this model (Harrison et al., 2008).

Although changes in body weight and adiposity are seen in sheep in relation to photoperiod, whether in natural conditions or under controlled lighting, many studies indicate that there is no change in circulating leptin levels (Anukulkitch et al., 2007, 2009; Clarke et al., 2003). There are, however, reports to the contrary, reporting higher leptin levels during long day photoperiod (Marie et al., 2001) (reviewed in Rhind et al., 2002). The response, in terms of food intake and LH levels, to icv leptin treatment of oestrogen-treated gonadectomised rams was found to be dependent upon season (Miller et al., 2002). In gonadectomised sheep of both sexes, we found that the effect of leptin was greater in spring than in autumn and greater in females than in males (Clarke et al., 2001) (Fig. 13). The lack of effect on food intake could be due to a signalling defect within the ARP cells of the hypothalamus in autumn, but the effect of leptin on reproductive function may persist at this time, according to the results obtained in oestrogen-treated gonadectomised male sheep (Harrison et al., 2008). Under normal conditions, lack of effect of leptin on food intake

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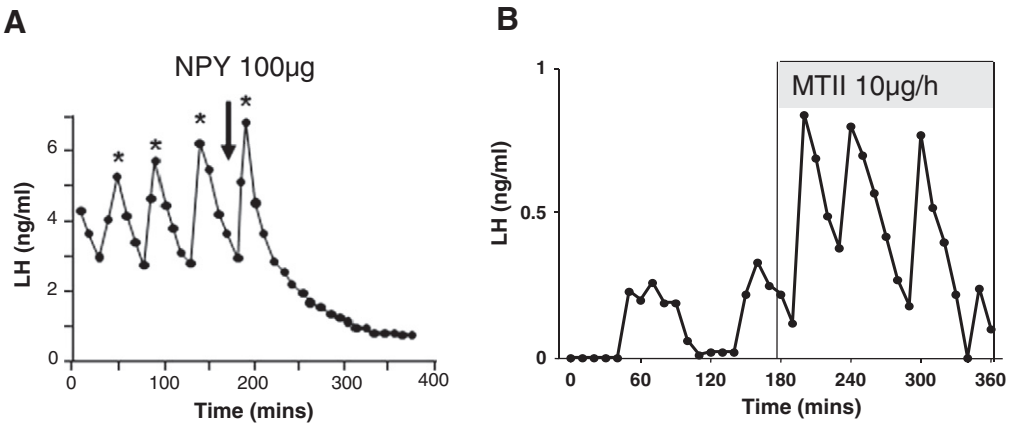

Fig. 8. Effect of NPY or a melanocortin agonist (MTII) on plasma LH levels in ewes. Panel A shows the effect of a single intracerebroventricular (icv) injection in an ovariectomised ewe. Panel B shows the effect of an icv infusion of MTII in a ewe during the luteal phase of the estrous cycle.
Panel A is adapted from Barker-Gibb et al. (1995). Panel B is adapted from Backholer et al. (2010a).

or reproductive function in the autumn or under short day photoperiod could be due to the reduced leptin transport into the brain (Fig. 14) (Adam et al., 2006). This would also limit any effect on reproductive function under such photoperiod. Transport of sex steroids into the brain of the sheep is also modulated by photoperiod (Thiery and Malpaux, 2003; Thiery et al., 2003). How photoperiod alters the access of hormones to the brain is an issue that requires further investigation.

Effects of ARP on the reproductive axis

As indicated above, ghrelin is a potent stimulator of food intake and a suppressor of reproductive function. In relation to ARP that are found in the brain, the same general rule applies, with orexigens inhibiting reproduction and anorexigens having a stimulatory effect.

Thus, NPY suppresses reproduction (Barker-Gibb et al., 1995) (Fig. 8A), whilst melanocortins are stimulatory (Backholer et al., 2010a) (Fig. 8B). NPY does not only block pulsatile secretion of GnRH/LH in the ovariectomised ewe, with free-running pulse generation, but it also blocks the oestrogen-induced GnRH/LH surge (Clarke et al., 2005); this is due to action via the Y2 receptor, whereas the orexigenic effect of the peptide in sheep is via the Y1 receptor. By contrast, the Y1 receptor mediates effects on the reproductive axis in rodents (Jain et al., 1999; Raposinho et al., 2000; Xu et al., 2000). The Y1, Y2 and Y5 receptor subtypes all participate in the regulation of feeding in rodents (Corp et al., 2001; Gerald et al., 1996; Kanatani et al., 2000; Zhang et al., 2011), and the Y1 receptor appears to be involved in the regulation of energy expenditure in mice (Shi et al., 2013). Y2 agonists act to suppress food intake predominantly at locations in the brain where there is access to neurons by circulating factors, such as the arcuate nucleus (Zhang et al., 2011). Interestingly, however, high protein diets that stimulate the production of PYY (which activates Y2 receptors) in humans do not lead to a suppression of food intake (van der Klaauw et al., 2013), consistent with the findings in sheep (see above) showing no effect of central administration of Y2 agonists on food intake.

Melanocortins are anorexigenic and also stimulate LH secretion when infused into the brain, so the effect is most likely due to action to increase GnRH secretion. As indicated above, melanocortins are produced as post-translational products of the POMC gene. The opioid peptide β-end, encoded by the same POMC gene, is thought to exert inhibitory tone on the GnRH system (Horton et al., 1987). The POMC cells of the arcuate nucleus are very interesting cells because they are receptive to a range of circulating factors by virtue of expression of the relevant receptors (Fig. 9A). These cells, however, express only a low level of ER-α (Table 1).

Most importantly, there are changes in the post-translational processing of the POMC products, which are produced by a range of enzymes (Fig. 9A). This allows regulation of the production of POMC products at 4 levels, within the cells of the arcuate nucleus (Fig. 9). We have examined the expression of these enzymes in the arcuate nucleus of the ovariectomised ewe brain, by polymerase chain reaction (I.J. Clarke, unpublished data). The expression of the gene for proconvertase 1 (PC1) is increased in the lean condition and expression of carboxypeptidase E (CPE) is reduced; proconvertase 2 (PC2) expression is similar in normal, lean and fat animals (Fig. 10). PC1 processes the POMC transcript to ACTH 1-39 and corticotropin-like intermediate lobe peptide (CLIP), so its activity affects the production of both melanocortins and β-end. PC2 processes ACTH 1-39 to ACTH 1-17 and CPE processes ACTH 1-17 to Des-acetyl-α-MSH (Mountjoy, 2010). All of these enzymes are involved in processing a variety of peptides, so these data are not specific to the POMC cell. Nevertheless, there is a strong indication that the enzymes are differentially regulated when animals become lean and it would be instructive to obtain more definitive data with respect to the various cell types of the arcuate nucleus. It would also be interesting to determine whether the changes in expression of the enzymes lead to altered balance between β-end and α-MSH production. Acetylation is mandatory for function of the melanocortins and nullifies the activity of the endorphins. Presumably the activity of an un-identified acetylase is regulated by body weight in sheep because of the changes seen in the acetylated peptide seen in the arcuate nucleus of lean animals (Backholer et al., 2010a) (see below).

NPY levels increase and melanocortin levels fall in the lean sheep, which is consistent with the low levels of GnRH/LH secretion that prevail in the catabolic state. Definitive studies to counteract these changes and determine the effect on GnRH/gonadotropin secretion are yet to be done, but some mechanistic studies on the melanocortin system have been undertaken in sheep. In particular, we sought to determine the means by which leptin restores gonadotropic function in lean hypogonadotropic animals and tested whether this was due to upregulation of central acetylated melanocortin production. Third-ventricular infusion of leptin for 3 days restored pulsatile LH secretion and this was associated with a marked upregulation of POMC gene expression (Backholer et al., 2010a) (Fig. 11), without associated changes in NPY or AgRP gene expression in the arcuate nucleus. Des-acetylated α-MSH was the predominant form that was present in the arcuate nucleus, with similar levels in lean and normal body weight animals. Importantly, however, the levels of acetylated α-MSH were much lower in the lean animals. It remains to be determined as to whether physiological doses of leptin actively increase the acetylation of POMC derived peptides in this model, but given the differences between animals that are lean and those of normal body weight, it would seem most likely that leptin does act directly to regulate the acetylase; this has been shown in mice (Guo et al., 2004). Levels of β-end were lower in lean ovariectomised ewes, consistent with the level of POMC gene

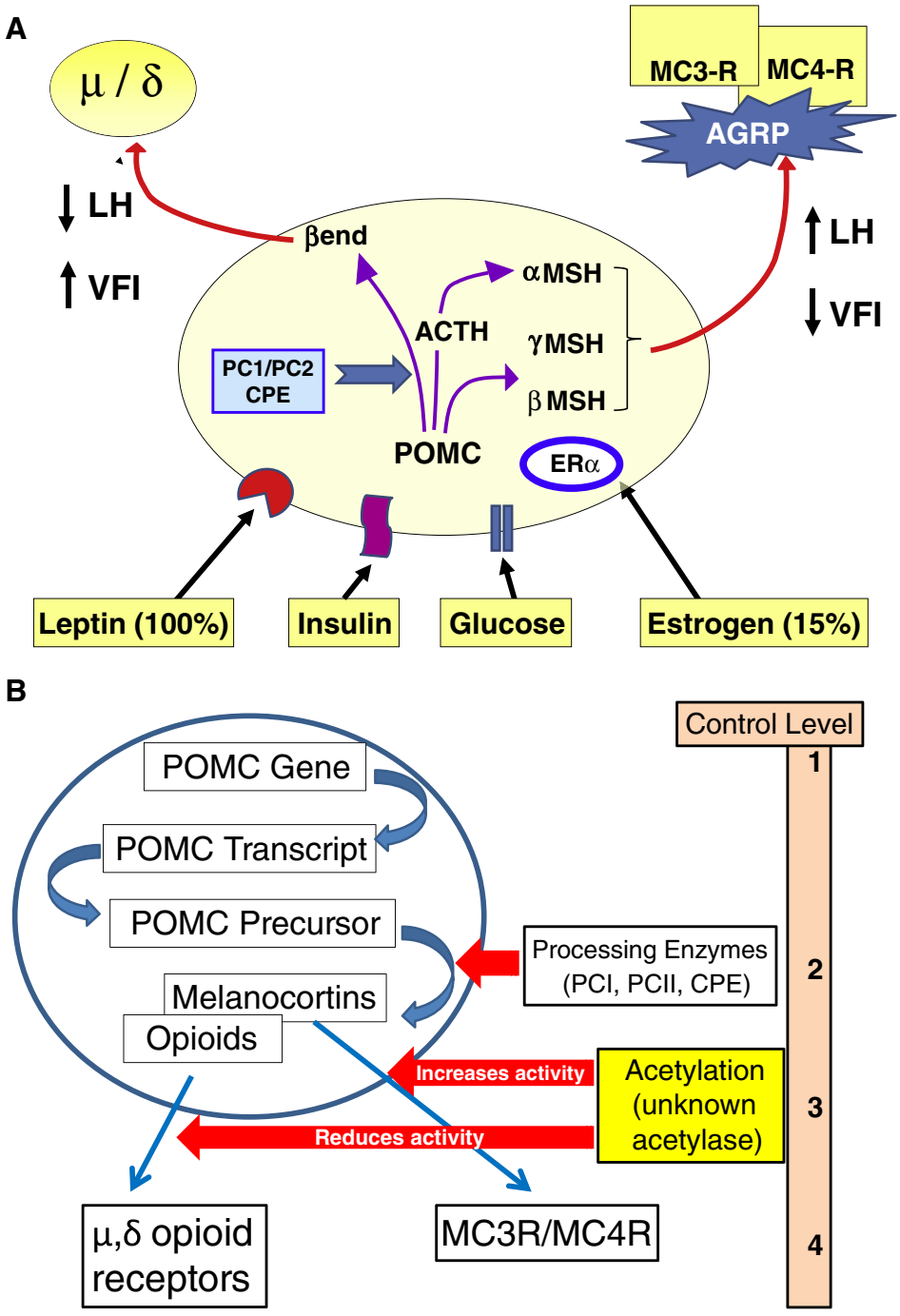

Fig. 9. POMC cells. Panel A: The POMC gene encodes a protein precursor that is post-translationally processed to produce β-end and the melanocortins (α-, β-, and γ-MSH). Sequential processing is by action of proconvertase 1 (PC1), proconvertase 2 (PC2) and carboxypeptidase E (CPE) (see text for details). The peptides are acetylated by an unknown enzyme; acetylation increases the biological activity of melanocortins and reduces the activity of β-end. The melanocortins act on MC3R and MC4R in the brain to reduce food intake, increase energy expenditure and increase GnRH/LH secretion. β-End acts through μ and δ predominantly to reduce or suppress reproductive function and stimulate food intake. The cells are regulated by metabolic factors from the periphery, such as leptin, insulin and glucose and only 15% of POMC cells express oestrogen receptor-α (ER-α). AgRP, which is produced in NPY/AgRP cells of the arcuate nucleus block the action of melanocortins at the level of their cognate receptors. Panel B: Regulation of the production of melanocortins and β-end is at 4 levels.

expression, but leptin was not able to rectify this (Backholer et al., 2010a). Perhaps longer treatment would increase melanocortin and β-end peptide levels, in line with the change in POMC gene expression. These studies highlight the fact that post-translational processing of the products of the POMC gene is a major determinant of the function of the peptides encoded by this gene.

Another potential mechanism for the regulation of the melanocortin system is at the level of the melanocortin receptors in the hypothalamus, but long-term alterations in body weight do not affect expression of MC3R or MC4R in ovariectomised ewes (Iqbal et al., 2001a). The melanocortin cells may regulate ORX cells in the dorsomedial nucleus of the sheep brain (Backholer et al., 2010b) and since the ORX cells project to GnRH cells, this is a potential means by which the melanocortins can increase reproductive function. This was tested by central infusion of MTII, a melanocortin receptor agonist, and there was specific upregulation of ORX expression in this region (Backholer et al., 2010b).

Changes in the levels of gene expression for CART, AgRP and MCH (Henry et al., 2000, 2001b) may also impact on reproductive function.

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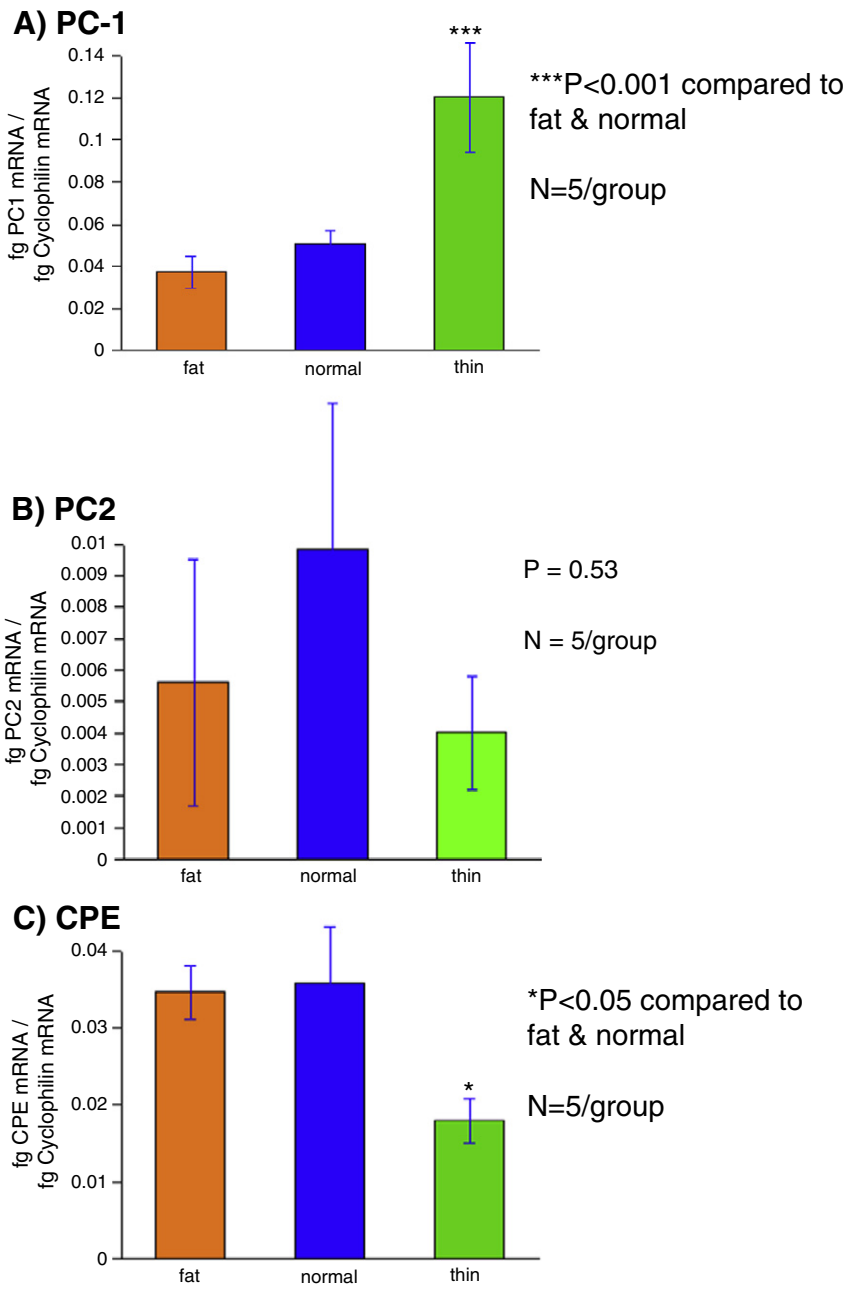

Fig. 10. Effect of altered body weight on the expression of post-translational processing enzymes in the arcuate nucleus of ovariectomised ewes. Dissected mid-arcuate nucleus sections were used for polymerase chain reaction quantification of PC-1 (Panel A), PC-2 (Panel B) and CPE (Panel C) from lean, normal or fat ovariectomised ewes. It is important to note that these data show expression for the entire nucleus and not a particular cell type, so it would be instructive to examine expression that is cell-specific.
Adapted from I.J Clarke (unpublished data).

Studies in rodent species show that AgRP, an orexigen, inhibits reproduction (Wu et al., 2012), as does ORX (Furuta et al., 2002; Tamura et al., 1999). There are, however, indications of the opposite effect of ORX (Small et al., 2003), so this is a confusing issue. ORX cells project directly to GnRH cells in the sheep brain (Iqbal et al., 2001c). Melanin concentrating hormone, which is considered to be orexigenic, appears to stimulate reproductive function (Murray et al., 2006), so it is an exception to the general rule of opposite effect on reproduction and regulation of metabolic function.

Special case of kisspeptin in relation to the lean condition

Kisspeptin cells in the ovine brain express leptin receptors and leptin treatment of lean ovariectomised ewes increases kisspeptin gene expression (Backholer et al., 2010b). It seems reasonable, therefore, to postulate that the restoration of reproductive function in lean animals, by leptin, is through this mechanism. Whereas the leptin effect is on both populations of kisspeptin cells, those of the arcuate nucleus do not project directly to the GnRH cell bodies of the preoptic area; those of the preoptic area do so (Backholer et al., 2010b; Pompolo et al., 2001). Restoration of kisspeptin stimulation of GnRH secretion could also be through the projections of the arcuate population of cells into the median eminence (Smith et al., 2011), so investigation of these projections in the lean hypogonadal ewe would be useful.

The kisspeptin cells do not express ghrelin receptors, in the mouse at least (Smith et al., 2013), so changes in the levels of circulating ghrelin with altered body weight are unlikely to regulate reproductive function at this level. It is possible that the negative effect of ghrelin is mediated

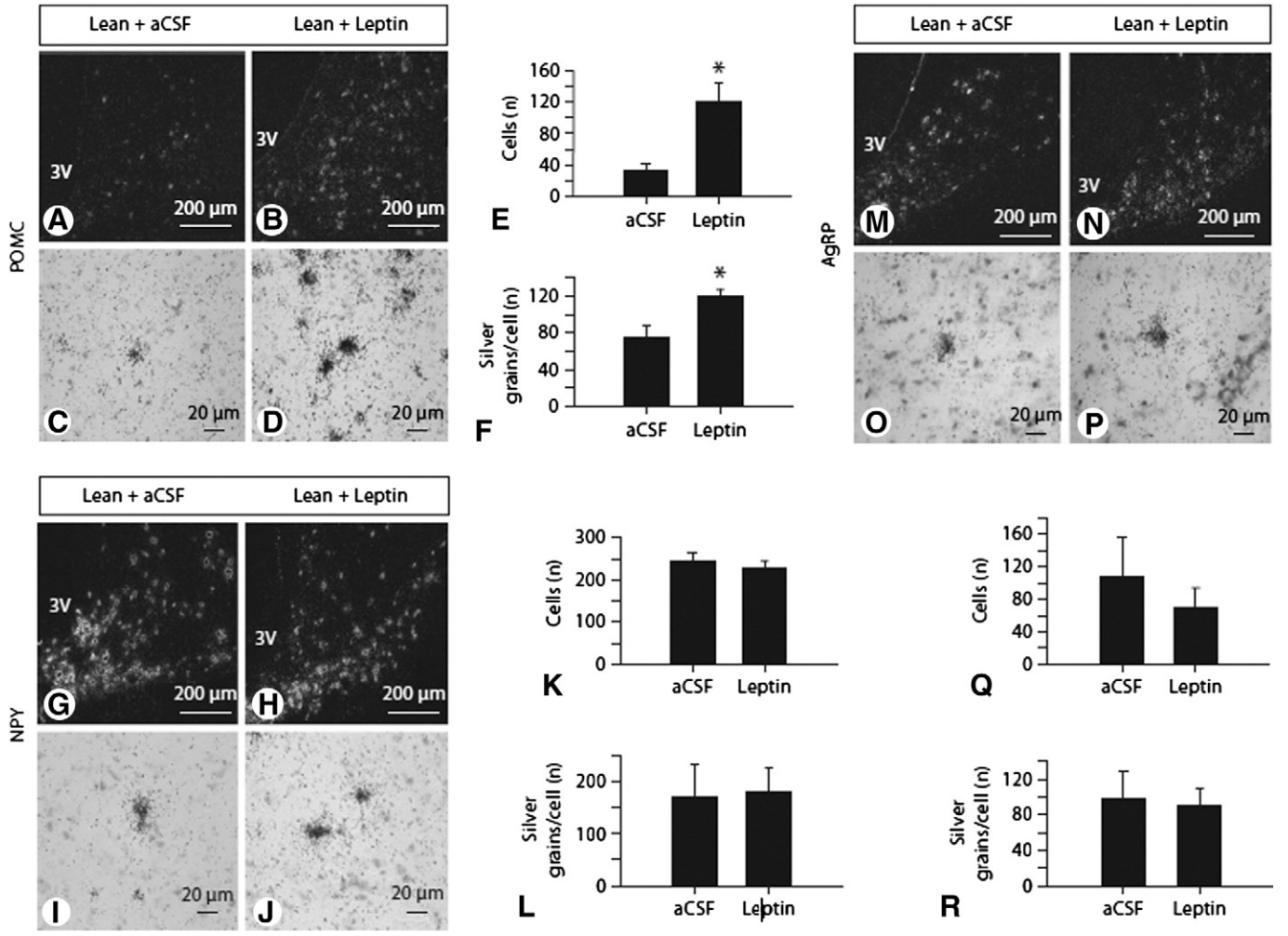

Fig. 11. Effect of reduced body weight and infusion of leptin on the expression of appetite regulating peptides in the arcuate nucleus. Leptin or aCSF was infused into the third cerebroventricle for 3 days and the brains were collected for in situ hybridisation analysis of expression of NPY, POMC and AgRP in the arcuate nucleus of the hypothalamus of lean hypogonadotropic ovariectomised ewes. Panels A–F: POMC gene expression in lean control (aCSF) and lean leptin-treated hypogonadotropic ewes. Panels G–L: NPY gene expression in lean control (aCSF) and lean leptin-treated hypogonadotropic ewes. Panels M–R: AgRP gene expression in lean control (aCSF) and lean leptin-treated hypogonadotropic ewes. Darkfield and brightfield photomicrographs are taken at 10× and 40× magnification, respectively. Data are means ± SEM. *$P < 0.05$ compared to control.
Taken from Backholer et al. (2010a).

via NPY cells that do express the relevant receptor and are stimulated by ghrelin (Grove and Cowley, 2005).

The NPY, POMC and kisspeptin cells are seen to form a network within the arcuate nucleus of the ovine brain (Fig. 12), based on confocal microscopic observations (Backholer et al., 2010b). Up to half of the kisspeptin cells in the arcuate nucleus receive input from melanocortin-staining fibres, so the stimulatory effect of melanocortins on GnRH/LH secretion could be via the kisspeptin cells. Thus, kisspeptin administration increased the expression of POMC and reduced the expression of NPY in lean ovariectomised ewes (Backholer et al., 2010b).

GH and kisspeptin in relation to reduced body weight

In cows, kisspeptin appears to stimulate the secretion of GH as well as gonadotropins (Kadokawa et al., 2008). In contrast to the situation in the sheep, where kisspeptin regulates GnRH secretion (Smith et al., 2008b), it appears to act on the pituitary gonadotropes in the cow (Ezzat et al., 2010). In sheep, kisspeptin gene expression is reduced in animals that are hypogonadotropic because of lean condition and this is partly corrected by leptin administration (Backholer et al., 2010b), as mentioned above. In sheep, kisspeptin stimulated GH axis when administered by central infusion, suggesting some mechanism for a brain-mediated effect (intravenous infusion was not effective) (Whitlock et al., 2010). Nevertheless, in the lean condition, kisspeptin expression in the hypothalamus is lowered, in sheep at least, whereas GH levels are substantially increased, so it is unlikely that the former affects the latter in this state.

GnIH as an integrator of reproduction and metabolic function

GnIH is a potent inhibitor of reproductive function in the sheep and also stimulates food intake (Clarke et al., 2012a). GnIH cells project to GnRH cells as well as to the ARP cells throughout the ovine hypothalamus (Qi et al., 2009), providing a neuronal substrate for dual effect on reproduction and regulation of food intake (Table 3).

GnIH also stimulates food intake in the rat (Johnson et al., 2007) and the non-human primate (Clarke et al., 2012a). An indication that there is an inverse relationship between reproductive function and appetite that is driven by GnIH is found in the observation that GnIH expression and input to GnRH cells are lower during the breeding season in ewes (during declining day length), when appetite drive is highest (Smith et al., 2008a). The effect of altered body weight on GnIH cell activity in sheep is, however, not known. In Siberian and Syrian hamsters, GnIH expression is paradoxically increased during long days, when the animals are breeding (Paul et al., 2009). Comparison of Syrian and Siberian hamsters is complicated by the fact that both breed during long day

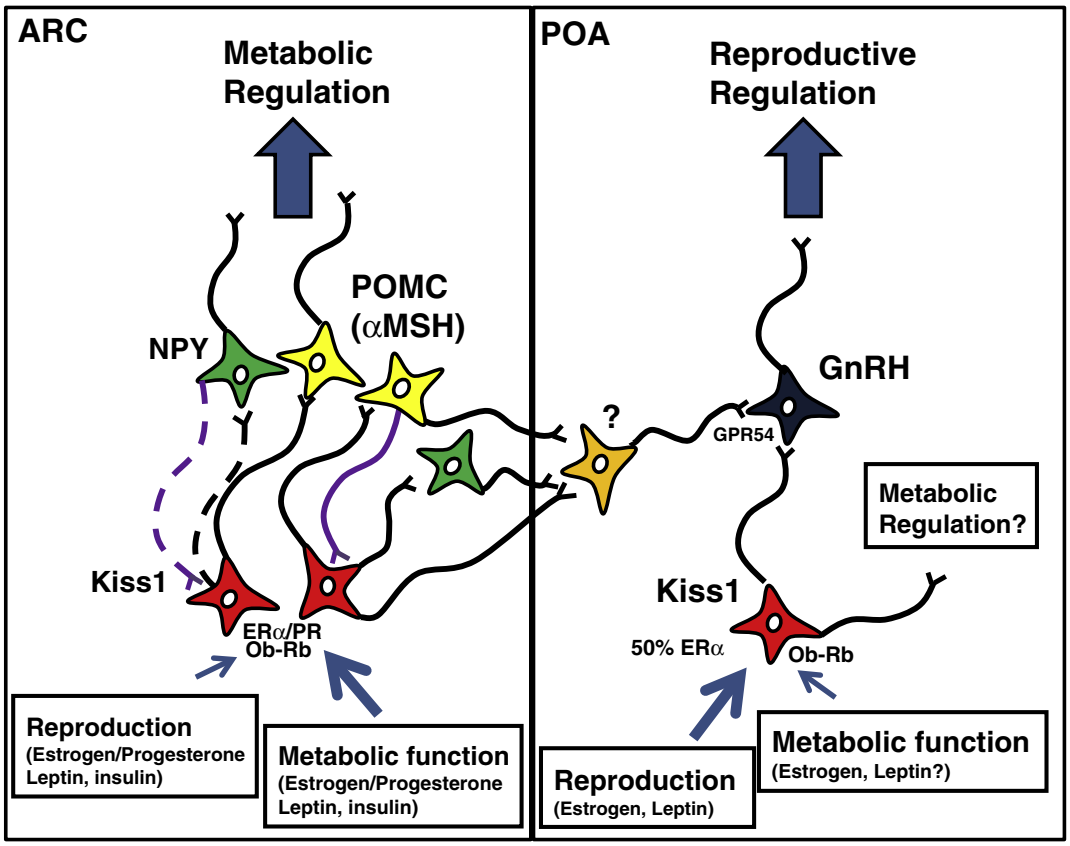

Fig. 12. Networking in the arcuate nucleus. A circuit diagram was composed, based on a series of studies incorporating confocal microscopy and effects of infusion of kisspeptin. Reciprocal connections between POMC and kisspeptin cells as well as NPY and kisspeptin cells allow integration of signalling from peripheral factors that regulate both reproductive function and appetite/energy expenditure. Importantly, the 'porous' nature of at least a part of the arcuate nucleus allows access by protein/peptide hormones as well as oestrogen. The high level of expression of ER-α in kisspeptin cells allows this steroid, and other sex steroids, to transmit information to the NPY/POMC cells by way of the kisspeptin cells. There is no direct communication of arcuate cells to the GnRH cell bodies in the preoptic area, so an unknown interneuron may be involved in the regulation of the GnRH cells by elements in the arcuate nucleus. On the other hand, direct input to GnRH neurons from preoptic kisspeptin cells is seen in the ewe brain.
Adapted from Backholer et al. (2010b).

photoperiod but they show opposite body weight changes. Syrian hamsters gain weight, but Siberian hamsters lose weight under short photoperiod exposure (Bartness and Wade, 1984, 1985; Wade and Bartness, 1984). Accordingly, a role for GnIH in the photoperiodic changes in body weight is not clearly apparent. Notably, these aforementioned studies showed that the changes seen in hamsters on different photoperiods were not due to differences in food intake, but to changes in food efficiency because changes in body weight preceded any changes in food intake (Bartness and Wade, 1984; Wade and Bartness, 1984). Other studies of Siberian hamsters (Atcha et al., 2000; Knopper and Bioly, 2000) show that short day photoperiod does reduce food intake, but the magnitude of this effect does not account for the body weight change that is observed. Clearly, the photoperiodic regulation of metabolic function in Siberian hamsters involves a variety of mechanisms (Warner et al., 2010).

GnIH does not appear to regulate energy expenditure, in terms of thermogenesis, at least in the sheep and the rat (Clarke et al., 2012a).

Energy expenditure and the effects of sex steroids

Ruminants are continuous grazers but can be entrained in various ways with programmed feeding. In particular, when sheep are fed during a certain window every day, a post-prandial thermogenic response is seen in fat and muscle (Henry et al., 2008). This enables a detailed study of mechanisms that regulate thermogenesis, perhaps the best known being the central effect of leptin to enhance the post-prandial thermogenic response — this was clearly seen in skeletal muscle (Henry et al., 2008), although the exact mechanisms underlying this are not yet known. The same model can be used to examine sex steroid effects. Peripheral treatment with estradiol-17β implants in ovariectomised ewes did not show any effect, whereas pulsatile i.v. administration increased thermogenic output (Clarke et al., 2013). Testosterone treatment of ovariectomised ewes reduced the resting tissue temperature, but did not alter the post-prandial response (Clarke et al., 2012b). It is most likely that at least part of the sex steroid effect on thermogenic output involves the regulatory centres of the hypothalamus, but studies on this in ruminant species are yet to be undertaken. In addition, it has now become apparent that the sternal fat of sheep is a very dense bed of brown adipose tissue (Symonds et al., 2012) and studies on the sex steroid modulation of thermogenesis at this level should prove very interesting. The nexus between day-length, sex steroids and energy expenditure is worthy of investigation.

Genetic models of obesity and reproduction

A number of rodent models of obesity are well known, but these will not be discussed here. One model of genetic 'obesity' in sheep is that originally described by Morris et al. (1997). Here, animals were selected for backfat thickness and then back-crossed. The main difference in composition of these animals is in the amount of retroperitoneal and omental fat (Francis et al., 2000). Whilst there are no reports in the literature regarding the fertility of these animals, the shepherds for the flock anecdotally report that fertility is lower in the lean line. A complicated interaction may exist between cortisol levels, relative adiposity and fertility/litter size in different breeds of pigs (Foury et al., 2007).

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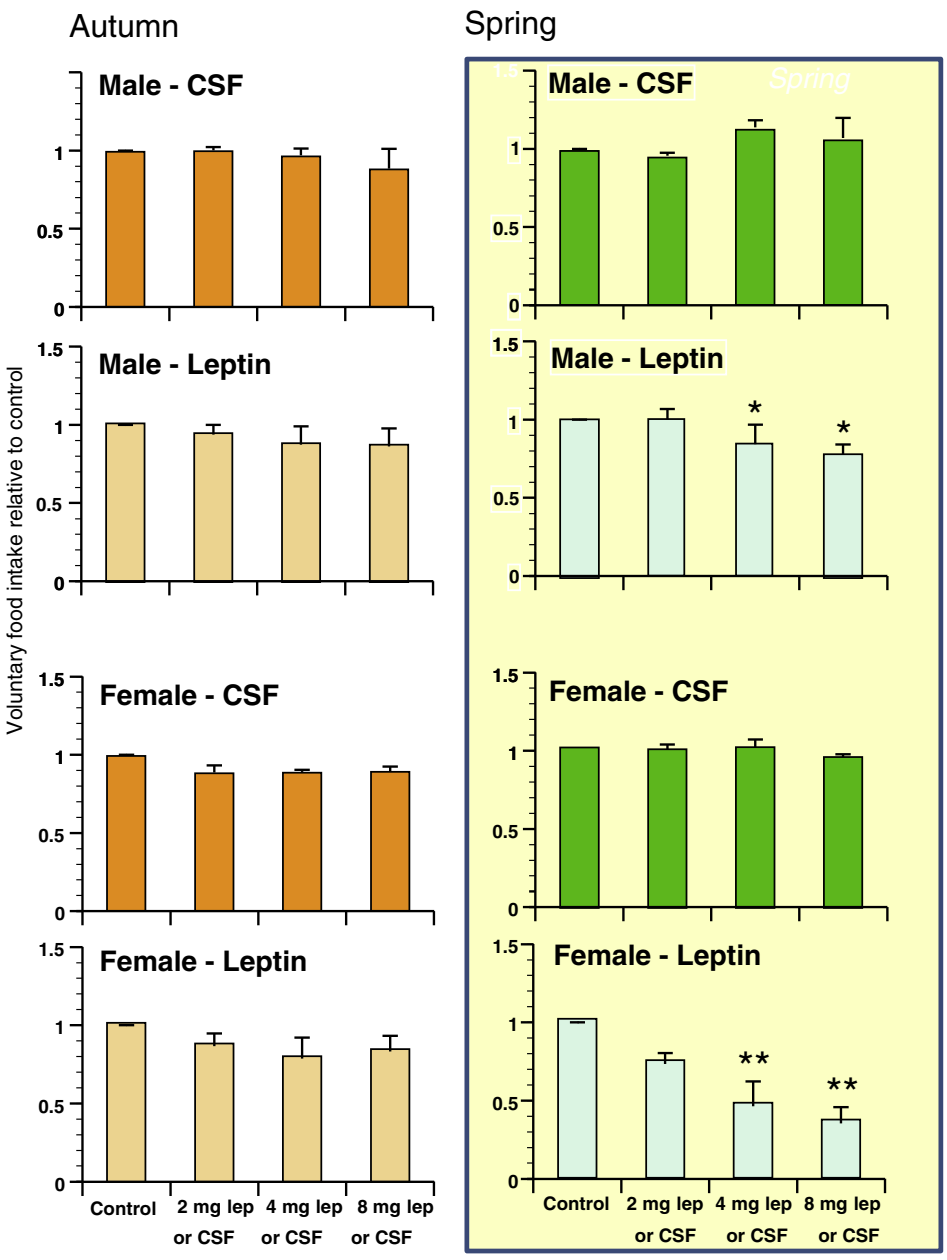

Fig. 13. Effect of sex and season on the effect of leptin to suppress food intake in sheep. The effect of sex was determined in animals that were gonadectomised and the effect of season was determined by administering increasing doses of leptin in either the spring or autumn. Note the lack of effect in autumn and the greater effect in females than in males in spring (shaded box). *P < 0.05; **P < 0.01, compared to control.
Taken from Clarke et al. (2001).

Lactation

Lactation can be regarded as a state of negative energy balance and the function of ARP at this time has been well studied in the rat (Smith and Grove, 2002). The profound drain of energy that is brought about by lactation in rats is thought to cause the cessation of reproduction (Brogan et al., 1999; Tsukamura and Maeda, 2001). The effect in rats may be due to suckling, since pup-removal can restore LH levels (Smith and Grove, 2002; Tsukamura and Maeda, 2001). Energy expenditure during lactation exceeds energy intake, so the body stores accumulated during pregnancy are lost (Naismith et al., 1982). On the other hand, changes in circulating hormones, such as prolactin, and alteration in the levels of circulating metabolites could be factors relating to the lowered gonadotropin secretion in suckling animals. As reviewed (Smith and Grove, 2002), expression of genes for orexigenic peptides is upregulated and that for anorectic peptides is downregulated during lactation, consistent with the state of negative energy balance increasing appetite drive. Thus, in rats, the expression of the gene for the orexigenic peptide NPY is increased and POMC gene expression is decreased (Smith, 1993); levels of leptin receptor expression are also reduced in the hypothalamus (Brogan et al., 2000). Whether the situation in lactating rats pertains to the lactating ruminant is debatable. An early study in sheep (Broad et al., 1993) showed a slight elevation in the level of POMC gene expression in the hypothalamus during lactation (compared with late pregnancy). Later work indicated that hypothalamic expression of POMC is lowered and NPY and AgRP expression is increased in lactating sheep (compared to non-lactating animals) (Sorensen et al., 2002). Leptin receptor expression also increases in the lactating ewe, in association with lowered plasma leptin levels (Sorensen et al., 2002). This is contrary to the results seen in the lactating rat, but is consistent with a state of negative energy balance, driving

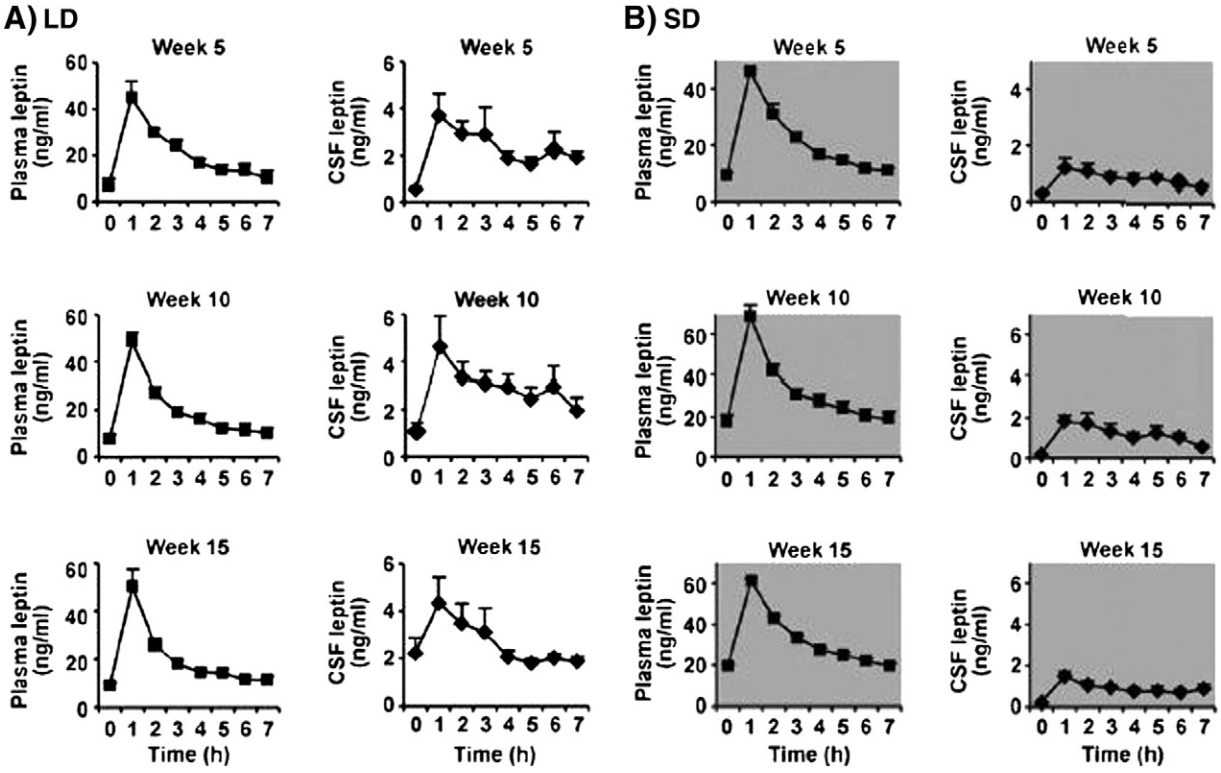

Fig. 14. Effect of photoperiod on the transport of leptin into the sheep brain. Sheep were challenged with an i.v. leptin injection at different times after the imposition of either short day photoperiod (SD — 8 h light:16 h dark) or long day photoperiod (LD — 16 h light:8 h dark). Note the reduction in transport into the brain under SD as indicated by the levels in cerebrospinal fluid.
Taken from Adam et al. (2006).

increased appetite. The difference in the leptin receptor status of lactating rats and sheep indicates an important species difference.

Milk production by dairy cows has increased substantially in the last 50 years. Associated with this, there has been a decline in the fertility of high-producing cows (Hansen, 2000), which could be due to a number of environmental, physiological and genetic factors. In addition, the reduced fertility may be due to factors that involve the brain, pituitary gland, ovaries and uterus. This can lead to poor quality oocytes and unfavourable uterine environment (Santos et al., 2010). These latter factors have been documented extensively, but there is relatively little information on the hypothalamo-pituitary factors that contribute to anovulation in post-partum dairy cows. Studies comparing fertility of Holstein–Friesians in different regions of the world show inherent strain differences on the timing of resumption of reproductive function and fertility after calving, suggesting the involvement of factors other than body condition score or energy balance (Piccand et al., 2011). There is, however, minimal information on central regulation of appetite/energy expenditure and only cursory knowledge of the integration of the energy and reproductive systems in the dairy cow. It is clear that extended post-partum anestrus in the high producing dairy cow (Peter et al., 2009) is, at least in part, associated with perturbation within the neuroendocrine hypothalamus. This may be inferred because dairy cows can be successfully bred with GnRH treatment (Ayres et al., 2013; Kile et al., 1991), pointing to a fundamental deficit at the level of the hypothalamus to perturb the ovulatory mechanism in the early post-partum period. The combined use of prostaglandin and gonadotropin releasing hormone (GnRH) to induce ovulations and procure pregnancies in high-producing dairy cows was developed in 1995 (Pursley et al., 1995) and refined until the present day (Gumen et al., 2012; Yilmazbas-Mecitoglu et al., 2012).

Studies in sheep showed that there is an increased negative feedback of oestrogen on LH secretion in the early post-partum period (Wright et al., 1981) and failure, in more than 50% of ewes up to 28 days post-partum, of the positive feedback effect of oestrogen that causes the pre-ovulatory LH surge (Wright et al., 1980). On the other hand, with respect to the associated negative energy balance in relation to reproduction, it is interesting that no effect of lactation was seen on the pulsatile secretion of LH in dairy cows (Canfield and Butler, 1991). Thus, it seems most likely that the defect in the high-producing dairy cow is in the ovulatory mechanism, rather than in basal gonadotropin secretion.

Physiological changes that occur in early lactation in animals such as dairy cows reflect negative energy balance verging on starvation. Leptin levels are lowered in lactation in various species (Smith and Grove, 2002), including sheep (Sorensen et al., 2002) and cows (Laeger et al., 2013; Wathes et al., 2007a). Plasma insulin and glucose levels are lower in early lactation than in late lactation in dairy cows and the post-prandial insulin response, which is marked in late lactation, is virtually non-existent in early lactation (Bradford and Allen, 2008; Laeger et al., 2013). Early lactating cows have markedly higher non-esterified fatty acid concentrations in plasma, with a further increase upon withdrawal from feeding, indicating mobilisation of body fat (Bradford and

Table 3
Projections of GnIH neurons to ARP and GnRH cells in the ovine hypothalamus. CRH — corticotropin releasing hormone.
Taken from Qi et al. (2009).

Cell type	Region	% contacts
POMC	Arcuate nucleus	60
NPY	Arcuate nucleus	44
Orexin	LHA	21
Orexin	DMH	61
MCH	LHA	22
CRH	PVN	33
Oxytocin	PVN	29
GnRH	Preoptic area	63

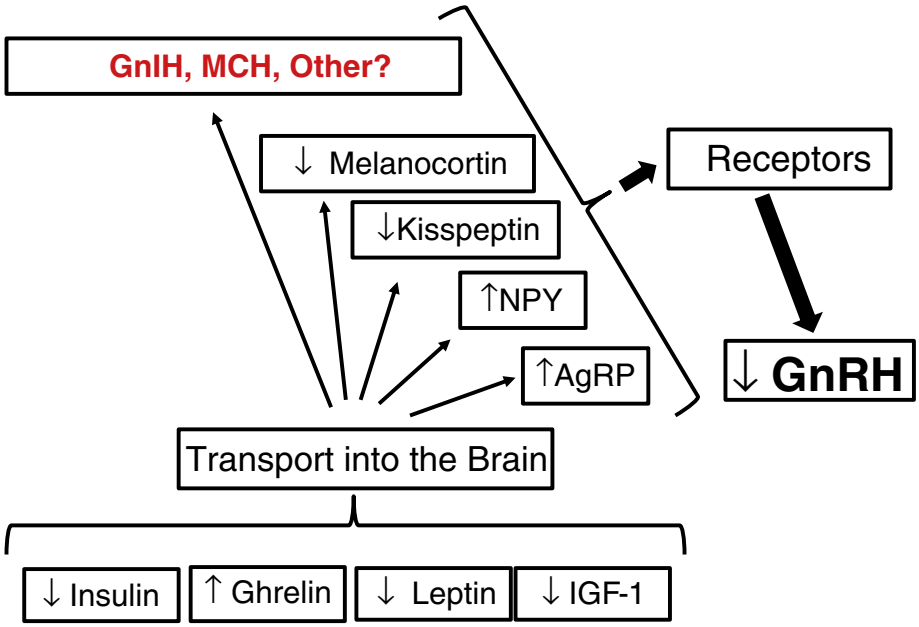

Fig. 15. The relationship between metabolic hormones and hypothalamic peptides and the regulation of reproduction — impact of negative energy balance. Changes in circulating levels of insulin, ghrelin, leptin and IGF-1 alter levels of melanocortins, kisspeptin, NPY and AgRP in the hypothalamus. There may also be changes in the expression of the receptors for the metabolic signalling factors. There are no changes in dynorphin or ORX with altered body weight in sheep. Levels of expression of receptors in the hypothalamus for the neuropeptides require investigation in most cases. The coordinated changes at each of these levels in lean animals negatively impacts on the function of GnRH neurons, leading to reduced reproductive performance.

Allen, 2008; Laeger et al., 2013). Cortisol levels rose in response to a ghrelin injection in lactating cows, but not in non-lactating cows (Itoh et al., 2006) — the significance of this finding is not apparent.

The gut-derived metabolic regulators, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1-(7-36) amide (GLP-1) and cholecystokinin (CCK) were measured in the plasma of dairy cows from 11 days before calving until 19 days afterwards (Relling and Reynolds, 2007). It was found that the generalised rise in levels of all these peptides was associated with lower levels of insulin and glucose in early lactation. The consequence of this increase in secretion of gut peptides is certainly not to reduce food intake (Relling and Reynolds, 2007). The situation regarding ghrelin status is nebulous. In one study, plasma ghrelin levels were seen to be markedly increased during early lactation in the dairy cow (Bradford and Allen, 2008), but not in another (Laeger et al., 2013). The secretion of ghrelin in dairy cows is suppressed by glucose challenge (Roche et al., 2008), but the significance of this is not yet clear.

The recent study of Laeger et al. (2013) has provided some information on the transport of metabolic substrates and circulating regulatory factors into the central nervous system of cows. Cerebrospinal fluid (CSF) and plasma samples were analysed. The CSF was taken by sampling at the level of the 6th lumbar vertebra and the sacrum by needle biopsy, so the extent to which this represents levels in the brain is open to some question. If one accepts that levels of such factors are uniform throughout the CSF, then the data are important in terms of feedback signalling to the brain. CSF glucose levels fell, in parallel with the fall in plasma levels during early lactation and this may cause greater hunger drive. CSF concentrations of fatty acids were much lower than those in plasma and the changes in plasma were not mirrored in CSF, casting doubt on whether feedback is exerted on ARP neurons by these metabolic factors. Complex changes in amino acid levels were also reported, but the relevance of these to regulation of energy balance is not clear at this stage. Resistin, which is produced by fat, may also regulate metabolic function by central action, but levels did not change in early lactation (Laeger et al., 2013). Apart from these relatively sparse data on the metabolic indicators in dairy cows, there is no information on other regulators of metabolic function, appetite and energy expenditure, such as adiponectin or interleukins.

Conclusions

It is clear that reproduction is dependent upon metabolic state and this can be seen at all levels of the HPG axis. Circulating metabolic factors and indicators of body stores (such as leptin) act on the brain to alter the levels of peptides that regulate appetite/energy expenditure as well as GnRH secretion and this has been the major focus of this review. The particular bias has been to examine these issues in ruminants, particularly the sheep and the cow. These species provide useful perspective because, as ruminants, their metabolic function differs from that of monogastrics. In spite of this, there is relatively high concordance between the two types of animal in terms of the way the metabolic control systems of the hypothalamus interface with reproductive function. Given the recently appreciated fact that GnRH secretion is most likely regulated at the level of the terminals of GnRH neurons in the median eminence, the way that ARP operates at this level would be an interesting area of investigation.

Many factors are involved in the dynamics of metabolism and reproduction. Drawing on available information, one can develop a simple working hypothesis (summarised in Fig. 15). Apart from the sex steroid feedback regulation of GnRH and gonadotropin secretion (Fig. 1), the parallel feedback actions of factors such as leptin and ghrelin affect the level of function of ARP and these, in turn, have influence on the neuroendocrine control of reproduction. An important level of control, especially in the sheep, is the transport of metabolic factors into the brain and the way this is affected by season; the response of ARP neurons to the metabolic factors is also influenced by season, as demonstrated by the photoperiodic effect on the response in terms of metabolic function and reproduction when factors such as leptin or ghrelin are administered centrally. The influence of altered metabolic state on the level of activity of GnIH, MCH and orexin neurons requires investigation and the question of how energy deficit enhances oestrogen-negative feedback through genomic or non-genomic mechanisms is one that

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deserves attention. In particular, the means by which fuel availability affects kisspeptin and GnIH function requires elucidation. In sheep, the change in oestrogen-negative feedback with photoperiod provides a model in which this issue could be addressed.

Whereas a significant body of knowledge exists to explain seasonality of reproduction, there is a paucity of information on how photoperiod and energy balance interact to determine onset and termination of the breeding season in sheep. The interaction between systems that regulate energy balance (which change with season) and those that regulate seasonality of reproduction is not delineated in large animal models. Examination of parallel changes in energy balance and reproduction across the seasons could be examined in the sheep. This review has also indicated the additional influence on reproduction and metabolic neuroendocrine systems of the GH axis and the thyroid hormones. The additional layers of influence indicate that we should view peripheral-to-central feedback modulation in the widest possible context.

In all species, lactation is a major influence on the neuroendocrine systems that regulate metabolic and reproductive function and in species such as sheep, season is an additional factor. Relatively sparse information is available for the special physiological state that exists in highly selected dairy cows and better understanding is required of the levels of function of the hypothalamic cells within the bovine brain that have dual function in the regulation of energy balance and reproduction.

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