Neuroendocrinology of nutritional infertility

George N. Wade¹ and Juli E. Jones²
¹Center for Neuroendocrine Studies, University of Massachusetts, Amherst 01003; and ²Beth Israel Deaconess Medical Center, Division of Endocrinology, Harvard Medical School, Boston, Massachusetts, 02215

Wade, George N., and Juli E. Jones. Neuroendocrinology of nutritional infertility. Am J Physiol Regul Integr Comp Physiol 287: R1277–R1296, 2004; doi:10.1152/ajpregu.00475.2004.—Natural selection has linked the physiological controls of energy balance and fertility such that reproduction is deferred during lean times, particularly in female mammals. In this way, an energetically costly process is confined to periods when sufficient food is available to support pregnancy and lactation. Even in the face of abundance, nutritional infertility ensues if energy intake fails to keep pace with expenditure. A working hypothesis is proposed in which any activity or condition that limits the availability of oxidizable fuels (e.g., undereating, excessive energy expenditure, diabetes mellitus) can inhibit both gonadotropin-releasing hormone (GnRH)/luteinizing hormone secretion and female copulatory behaviors. Decreases in metabolic fuel availability appear to be detected by cells in the caudal hindbrain. Hindbrain neurons producing neuropeptide Y (NPY) and catecholamines (CA) then project to the forebrain where they contact GnRH neurons both directly and also indirectly via corticotropin-releasing hormone (CRH) neurons to inhibit GnRH secretion. In the case of estrous behavior, the best available evidence suggests that the inhibitory NPY/CA system acts primarily via CRH or urocortin projections to various forebrain loci that control sexual receptivity. Disruption of these signaling processes allows normal reproduction to proceed in the face of energetic deficits, indicating that the circuitry responds to energy deficits and that no signal is necessary to indicate that there is an adequate energy supply. While there is a large body of evidence to support this hypothesis, the data do not exclude nutritional inhibition of reproduction by other pathways and processes, and the full story will undoubtedly be more complex than this.

luteinizing hormone; estrous behavior; corticotropin-releasing hormone; neuropeptide Y; hindbrain

OF NECESSITY, the physiological controls of reproduction and energy balance are closely intertwined. Living creatures require a steady stream of oxidizable substrates to fuel their many energy-consuming activities, including reproduction. But in nature, energetic demands and opportunities for meal taking can be erratic and sometimes unpredictable, necessitating the existence of physiological and behavioral strategies to maximize chances of individual survival and permit propagation of the species (21, 22, 200, 208).

A need to eat constantly is obviated by the presence of internal caloric buffers that store glycogen and fatty acids and provide sustenance in between meals and during brief fasts. When food is abundant, animals adjust their intakes to meet all demands and keep their storage depots full (or overfull in an increasing number of cases). However, a more likely scenario in nature and during human evolution is that food can be scarce or have a very high acquisition cost. In that case, animals adjust their energetic priorities, i.e., how they partition and use the available metabolic fuels (Fig. 1). Individual survival seems to be paramount. Accordingly, some physiological processes (e.g., pumping blood, basic cellular maintenance) cannot be compromised and must be maintained to the end. Other processes can be compromised to varying degrees without threatening survival. For example, somatic growth can be slowed during lean times, and animals can reduce thermoregulatory costs by building more elaborate nests, huddling, daily torpor, or hibernation (21).

Finally, there are some activities that are not necessary for individual survival and would even be counterproductive during famine. Consequently, these activities receive a very low priority for energy utilization. Two obvious examples are fat storage and reproduction. Calories are mobilized from adipose tissue for oxidation when food is scarce or in high demand. At the same time animals suspend reproductive attempts, favoring individual survival and deferring procreation. Given that fat storage and reproduction have similarly low priorities for energy partitioning, it should come as no surprise that they are often highly correlated with one another. Unfortunately this correlation has led some to conclude, erroneously as it turns out, that these two consequences of negative energy balance are causally related (80, 82). Rather, the correlation between low body fat content and infertility is due to the fact that both are consequences of negative energy balance. As will become clear, fluctuations in fat storage and fertility are separate and independent adaptive responses to an ever-changing energetic milieu.
For a female mammal, a complete reproductive cycle from conception through weaning her offspring (and beyond) is likely the most energetically costly endeavor she will undertake in her lifetime. To initiate a reproductive attempt when calories are in short supply could be wasteful if the offspring fail to survive, or it could even cost the mother her life, precluding any future chance of reproduction. Animals use a number of mechanisms that help to maximize chances that there will be an adequate energy supply during pregnancy and lactation. A common strategy in temperate zone mammals is seasonal breeding, using environmental cues such as photoperiod to time conception so that the young are born during times of abundance (21, 250). Note that different species may breed at different times of year (i.e., in different photoperiods) depending on the duration of their pregnancies in order to time parturition appropriately. Regardless of whether species use photoperiod as a long-term predictor of energy availability, they also respond to short-term metabolic cues (21, 68, 208). When the food supply is insufficient to meet metabolic demands, reproduction is suppressed, even if other environmental cues are favorable for reproduction. For example, sheep and hamsters remain infertile in otherwise stimulatory photoperiods if they are food deprived (64, 68, 248, 250). They require both a favorable photoperiod and adequate nutrition.

Nutritional infertility manifests itself in a variety of ways. Most typically, conception is prevented by delaying puberty in juveniles or by suppressing ovulatory cycles and copulatory behaviors in adults. In this way, significant waste of energy is prevented. However, it is still possible to interrupt reproductive attempts once conception has occurred. Implantation of the blastocyst may be prevented or deferred; fetuses may be resorbed; parental care may be modified; or mothers may engage in infanticide (205, 210).

Naturally, the availability of metabolic fuels affects reproduction in human beings. In subsistence societies, ovulation is suppressed, and the interval between pregnancies is extended significantly (55, 244, 257, 258). For this reason population control measures must be adopted concurrently with improvements in nutrition in order to prevent population explosions which nullify any gains. Nutritional infertility is also seen in societies where food is abundant but women fail to eat enough to match expenditure. An obvious example is anorexia nervosa (84, 122). Indeed, one of the diagnostic criteria for this eating disorder is sustained amenorrhea. Menstrual cycles resume when anorectics return to positive energy balance. Another example is women athletes, particularly those where participants are expected to maintain a slender body type (e.g., gymnasts, figure skaters, ballet dancers). In these cases, women may remain amenorrheic until their training is interrupted (i.e., by an injury) or they increase their food intake for some reason (141, 142, 144, 165). Recently, increasing numbers of women have entered the military, and the rigor of basic training often result in amenorrhea. It has been suggested that athletic amenorrhea could be due to some kind of “stress” (143, 190, 221), but recent work indicates that exercise-induced amenorrhea is more likely due to low energy availability, rather than the stress of exercise (145). Work with food-deprived monkeys comes to the same conclusion (28). The occurrence of nutritional amenorrhea, per se, is not necessarily cause for concern, as long as women are not attempting to become pregnant. However, the associated reduction in ovarian estrogen production can cause osteoporosis, which can have far-reaching consequences. In the short-term, stress fractures can end athletic careers prematurely or interrupt basic training in the military. Osteoporosis persisting into old age can seriously diminish the quality of life (165, 202).

For these reasons, nutritional infertility is commonly thought of as a pathology, but taken in a larger context this is certainly not the case. Rather, inhibition of reproduction by undernutrition appears to be a perfectly appropriate adaptive response to the prevailing environmental conditions. Two features of the phenomenon are pertinent here. First, nutritional infertility is reversible. Reproduction is deferred in favor of individual survival until the prevailing conditions improve and reproduction resumes. There are no lasting effects on the reproductive system, as far as we can tell. Second, animals exhibiting nutritional infertility are not ill. Reproduction, like fat storage, is inhibited well before there is any deterioration in the animals’ general health.

SCOPE

This paper reviews what is and is not known about the physiological bases of nutritional infertility in female mammals. We consider nutritional effects on both female reproductive behaviors and reproductive physiology, because they change in parallel. Although these are separate and independent responses, their physiological bases can overlap considerably (Fig. 2). Undernutrition halts ovulatory cycles by inhibiting hypothalamic secretion of gonadotropin-releasing hormone (GnRH) (12, 21, 66, 111, 200). This, in turn, slows the pulsatile release of luteinizing hormone (LH) and dampens the preovulatory LH surge (21, 107). Undernutrition also inhibits female copulatory behavior (48, 86, 117). This may be due, in part, to a disruption in the pattern of the release of ovarian steroids. But this effect is also independent of the changes in ovarian hormone secretion, because it is seen in animals that have been ovariectomized (OVX) and brought into heat by treatment with ovarian steroids (116). Indeed, food deprivation actually increases circulating levels of estradiol in OVX hamsters treated with estradiol benzoate, indicating a decreased neural responsiveness to the steroid (116). This decreased responsiveness is associated with changes in estrogen receptor-α immunoreactivity (ERIR) content in several neural loci that play a prominent role in the control of female sexual behavior (Fig. 3) (52, 97, 117, 139, 166, 167, 191).
Much of the recent animal work in this field (Fig. 2) has been directed at determining 1) the nature of the primary metabolic cues that affect fertility; 2) the loci and nature of the detectors for these signals; 3) the mechanisms by which this information is conveyed from the detectors to the neural circuits controlling GnRH secretion and female sexual behavior; and 4) the nature of the changes in these neural circuits, which result in changes in 5) estrous behavior and 6) pulsatile LH secretion. Each of these issues is considered in turn.

Recent progress in the study of nutritional infertility owes an enormous debt to the literature on the physiological controls of food intake. The reasoning has been that hunger is one of the most obvious responses to negative energy balance, and a great deal is known about the physiology of hunger. Perhaps the two systems overlap to some extent. Over the past fifteen years or so, pirating concepts and techniques from the study of eating behavior has proven to be a productive strategy with which to study nutritional infertility. As it turns out, hunger, anovulation, and reduced sexual receptivity tend to be temporally related and obey many of the same rules. Despite this association, these three responses to undernutrition are independent of one another and can be dissociated by a number of manipulations.

CALORIES MUST BE AVAILABLE FOR OXIDATION

Although reproduction can be inhibited by chronic under- or excessive caloric expenditure, neither the absolute level of food intake nor the amount of energy expended, per se, is of primary importance. Rather it is balance between intake and expenditure that matters. For example, small rodents kept at low temperatures become infertile (52, 148, 150, 179, 206), but if they have access to an unlimited food supply they can continue to reproduce at very low temperatures (21, 206). Given the enormous thermoregulatory costs of small mammals, this is quite striking. However, it is not enough for intake to meet or exceed expenditure; the calories must be available for oxidation. For example, diabetic animals are hyperphagic and hyperglycemic, yet they are almost always infertile. Both ovulation and female sexual behavior are inhibited in untreated diabetics (121, 139, 223). Although there are plenty of calories in circulation, glucose is not available for oxidation. If diabetic rats are fed diets rich in fatty acids, an oxidizable fuel for diabetics, their hyperphagia subsides (32, 72, 76), estrous behavior is increased (4), and some measure of fertility may be restored (133).

Infertility is not uncommon in obese individuals (37, 87), and some types of obesity may result in nutritional infertility. Stored calories are useful in supporting reproduction only to the extent that they can be mobilized and oxidized. Hyperphagia can be secondary to metabolic disorders that cause the sequestration of calories in adipose tissue and starve other physiological processes. That is, some animals may be hyperphagic because they are obese, and not vice versa (78, 249). Fat storage may sequester metabolic fuels such that they are unavailable for other processes. Similar processes may affect reproduction. For example, if hamsters are treated with pharmacological amounts of insulin, they fatten. If allowed to eat ad libitum, they continue to ovulate and exhibit normal levels of estrous behavior. However, if they are not permitted to overeat, insulin-treated hamsters still fatten, but they stop cycling, and estrous behavior is suppressed (167, 249).

A final example may be drawn from studies using pharmacological inhibitors of metabolic fuel oxidation, discussed in detail below. These compounds limit the organism’s ability to oxidize fatty acids and glucose, and they suppress LH release and ovulatory cyclicity and also inhibit sexual behavior (24, 48, 109, 110, 139, 156, 209). However, these animals are not

Fig. 3. Food deprivation or restriction reduces the number of detectable estrogen receptor-α immunoreactive (ERIR) cells in the ventromedial hypothalamus (VMH) of Syrian hamsters (139), mice (191), sheep (97), and rats (117). The VMH plays a crucial role in the control of estrous behavior. * P < 0.05 vs. ad libitum fed.
in negative energy balance, and circulating glucose levels are quite high (183). Again, neither positive energy balance, ample energy stores, nor high levels of circulating metabolic fuels ensures fertility. The calories must be available for oxidation.

**PRIMARY METABOLIC CUES**

How do the neural circuits controlling reproductive physiology and behaviors “know” whether sufficient calories are available to support reproduction? What is (are) the primary metabolic cue(s)? Before addressing this question, it is important to articulate the difference between the primary metabolic event(s) (1 in Fig. 2) and the secondary or tertiary neural and/or humoral responses (3 in Fig. 2) that they elicit. In discussions of nutritional infertility, authors often speculate about the function of circulating hormones such as insulin or leptin or changes in the production and release of neurotransmitters/neuropeptides. Whether or not these factors play a role in nutritional control of reproductive physiology and behavior, they cannot serve as the primary metabolic cues. These factors do not change spontaneously of their own accord; rather, they are responses to some prior metabolic perturbation(s)—the primary metabolic cue(s). It is essential to keep this distinction between primary metabolic events and secondary responses in mind while attempting to decipher the physiology of nutritional infertility.

Fifteen years ago, this was a question that many thought to have been resolved. Based on epidemiological data, Frisch and colleagues (80, 82) noted that in women and girls, body fat content and ovulatory cyclicity were positively associated. In adolescents, the onset of menses tended to occur once they had attained a certain body fat level (81, 82). Similarly, very lean adults exhibited a high incidence of amenorrhea. Frisch then posited a causal relation between these two variables and argued that there was a critical body fat content necessary to support ovulatory cycles. When women fell below this critical body fat content, they became amenorrheic. When a minimum body fat level was restored, menstrual cycles resumed. Note that this hypothesis springs from the ingestive behavior literature. It is an adaptation of Kennedy’s (126) lipostatic hypothesis of food intake in which he suggested that animals monitor some correlate of body fat content and adjust food intake such that a relatively constant level of adiposity is maintained. [Note that Kennedy’s lipostatic hypothesis is not without its shortcomings, foremost of which is that it is inherently unfalsifiable (90, 115, 259).] Kennedy also noted that puberty was more closely associated with the attainment of a certain body weight than with chronological age in female rats (127).

This “critical body fat hypothesis” was restated in many venues and became widely considered to be fact. To this day, it is accepted by many nutritionists and physicians as dogma and appears in numerous medical textbooks. Indeed, a critical body fat hypothesis is simple, elegant, and intuitive. Unfortunately, it has a significant shortcoming: a large and growing body of empirical data that disconfirms it. From the outset it was criticized on methodological and theoretical grounds, and there were reports that the association between fatness and fertility was not universal (1, 253). A large body of data from both humans and animals now shows that it is relatively simple to dissociate fatness and reproductive function (141, 200, 248, 250), indicating that while they tend to be correlated with one another, body fat content and fertility cannot be causally related.

If not body fat content, then what? Returning to the literature on the physiology of eating behavior, another possibility arises. This “metabolic fuels hypothesis” suggests that reproductive function, like food intake, is responsive to short-term (minute-to-minute or hour-to-hour) changes in metabolic fuel oxidation. Using pharmacological inhibitors of glucose or fatty acid utilization, several laboratories have shown that rapid changes in metabolic fuel oxidation produce rapid changes in food intake (Table 1) (79, 152, 183, 187, 188). For example, limiting glucose oxidation (glucoprivation) using 2-deoxy-D-glucose (2DG) or long-chain fatty oxidation (lipoprivation) with methyl palmitoxirate (MP) or mercaptoacetate (MA) rapidly elicits eating in sated rats (79, 188). A combination of 2DG + MP or 2DG + MA is particularly effective at increasing food intake (79, 188). The metabolic changes and the resulting increases in eating are much too rapid to be accounted for by changes in body fat. Thus it appears that short-term fluctuations in metabolic fuel oxidation play an important role in the control of food intake.

The same appears to be the case with reproductive function. Treatment of gonadally intact Syrian (golden) hamsters with high doses of 2DG alone mimics the effects of food deprivation and interrupts ovulatory cycles, and concurrent treatment with MP greatly potentiates the effects of otherwise subthreshold doses of 2DG (Table 1) (207). Subsequent work has shown that 2DG (glucoprivation) alone, given systemically or directly into the cerebral ventricles, rapidly suppresses luteinizing hormone (LH) pulses in sheep and rats (Fig. 4) (156, 157, 163). Thus LH (and by inference, GnRH) secretion responds very rapidly to changes in metabolic fuel oxidation. The rapidity with which metabolic inhibitors suppress LH pulses is consistent with a metabolic fuels hypothesis but not with a critical body fat explanation.

The role of fatty acid oxidation in the control of LH secretion is unsettled at this time. A common view is that LH secretion is controlled solely by glucose availability (29, 65, 140, 163). However, in hamsters, the effects of glucoprivation (2DG) on ovulatory cycles are greatly magnified by concurrent lipoprivation (MP), but high doses of 2DG alone have the same effect (207). Investigation of glucoprivation-lipoprivation interactions in the control of LH pulsatility in other species would seem to be warranted before concluding that pulsatile LH release is sensitive only to glucose availability.

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Rat Food Intake</th>
<th>Hamster Food Intake</th>
<th>Hamster Estrous Behavior</th>
<th>Hamster Estrous Cyclicity</th>
<th>LH Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2DG (low dose)</td>
<td>↑</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>?</td>
</tr>
<tr>
<td>2DG (high dose)</td>
<td>↑</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>↓</td>
</tr>
<tr>
<td>MP (low dose)</td>
<td>↑</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>↑</td>
</tr>
<tr>
<td>2DG (low dose)</td>
<td>↑</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>↑</td>
</tr>
</tbody>
</table>

↑, Increase; ↓, decrease; —, no change; ?, not known; 2DG, 2-deoxy-D-glucose; MP, methyl palmitoxirate. LH, luteinizing hormone.
Treatment with 2DG + MP also mimics the effects of food deprivation and inhibits estrous behavior in OVX hamsters treated with ovarian steroids (Table 1) (48, 139), but there are some differences when compared with the effects on ovulation and LH pulses. First, neither 2DG nor MP, given alone, has any effect whatsoever on estrous behavior, even when given in “heroic” doses (Table 1) (48). Second, unlike the rapid effects on LH pulses (156, 157, 163), prolonged treatment (>36 h) with 2DG + MP is necessary to suppress estrous behavior (113). One implication of these findings is that, while they normally occur concurrently, metabolic suppression of LH secretion and estrous behavior are actually separate processes. Thus a substantial body of evidence is now consistent with a metabolic fuels hypothesis—that the primary metabolic event in nutritional inhibition of reproductive physiology and behavior is related to short-term changes in cellular oxidation of metabolic substrates.

DETECTION OF METABOLIC CUES

Where are the metabolic fuel detectors (2 in Fig. 2) that transduce information about metabolic fuel oxidation and inform the brain about the availability of energy? Given that every cell in the body requires a continuous supply of oxidizable fuels to function, there is no shortage of candidates, but some make more sense than others (Table 2).

The liver has been suggested as a site of metabolic fuel detectors (74, 77), and it communicates with the brain via autonomic and somatic afferents (75). Indeed, selectively feeding or starving the liver can produce striking effects on food intake (238, 239).

Adipose tissue is another possibility. Adipocyte function is highly dependent on metabolic fuel oxidation, and this information can be transmitted to the brain in at least two ways. The brain could monitor adipocyte function via sensory innervation of adipose tissue (63). Adipose tissue can also communicate with the brain through changes in circulating leptin levels because adipocyte leptin production responds to acute changes in substrate oxidation (134, 135, 252).

Pancreatic beta cells represent another possible fuel detector; beta cell insulin output is highly responsive to the metabolic fuel supply and tends to parallel leptin levels. Indeed, it has been suggested that the hypothalamus monitors circulating insulin levels and adjusts food intake accordingly. That is to say, high insulin levels could indicate the presence of abundant calories, whereas lower insulin levels could signal a paucity of metabolic fuels (220, 262).

Ghrelin, a peptide hormone released by the stomach, has been posited to be an orexigenic hormone (104, 266). Circulating ghrelin levels are elevated after fasting, and systemic or intracerebroventricular (ICV) infusion of the peptide stimulates...

Table 2. Putative loci of metabolic fuel detectors in the body and how they convey information to the neural circuits controlling LH secretion and estrous behavior

<table>
<thead>
<tr>
<th>Putative Detector Site</th>
<th>Method of Transmission</th>
<th>Secondary Signal</th>
<th>Target Sites and Pathways</th>
<th>Likely Importance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver/gut</td>
<td>neural</td>
<td>vagal activity</td>
<td>hindbrain → hypothalamus</td>
<td>—</td>
<td>139, 163, 204</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>humoral</td>
<td>leptin</td>
<td>hindbrain/hypothalamus</td>
<td>+/—</td>
<td>35, 39, 212</td>
</tr>
<tr>
<td>Pancreas</td>
<td>humoral</td>
<td>insulin</td>
<td>hindbrain/hypothalamus</td>
<td>—</td>
<td>41, 99, 256</td>
</tr>
<tr>
<td>Stomach</td>
<td>humoral</td>
<td>ghrelin</td>
<td>hindbrain/hypothalamus</td>
<td>?</td>
<td>220, 262</td>
</tr>
<tr>
<td>Duodenum</td>
<td>humoral</td>
<td>CCK</td>
<td>vaginal nerve endings →</td>
<td>—</td>
<td>102, 112</td>
</tr>
<tr>
<td>Hindbrain</td>
<td>neural</td>
<td>NPY/CA</td>
<td>hypothalamus</td>
<td>+</td>
<td>110, 139, 156</td>
</tr>
</tbody>
</table>

+, Likely to be important; —, unlikely to be important; ?, not known. NPY, neuropeptide Y; CA, catecholamine; CCK, cholecystokinin.
food intake. In addition, stretch receptors in the stomach communicate with the brain via the vagus nerves (172). Thus the stomach could be yet another site of metabolic fuel detectors that modulate reproduction.

The duodenum secretes cholecystokinin (CCK) in response to the entry of nutrients into the gut. This CCK acts on vagal nerve endings in the gut in a paracrine fashion, and the resulting vagal activation induces meal termination and reduces food intake (85, 260). It is conceivable that increased CCK secretion could stimulate reproductive function by informing the nervous system that additional calories are available.

Although it is possible to make a case for the existence of metabolic fuel detectors in adipose tissue, the pancreas, the liver, the stomach, and small intestine, much recent work has concentrated on the caudal hindbrain, particularly the area postrema (AP), as a site of primary importance. The AP is a chemosensory structure located in the floor of the hindbrain fourth ventricle, and it has a permeable blood-brain barrier. Thus it is in an ideal position to monitor the chemical composition of the blood and cerebrospinal fluid. Lesions of the AP block the appetite-stimulating actions of 2DG and related glucoprivic drugs (189).

The AP also seems to play a role in nutritional control of LH secretion and ovulatory cycles. AP lesions prevent the suppression of LH pulses by treatment with high doses of insulin (glucoprivation + lipoprivation) in rats (29). Similarly, AP-lesioned Syrian hamsters continue to cycle after treatment with 2DG (glucoprivation), unlike neurologically intact controls (213). The fact that application of glucoprivic drugs to the fourth ventricle is sufficient to inhibit pulsatile LH release in rats (156) and sheep (163) further supports the notion of critical circumventricular fuel detectors in the hindbrain (Fig. 4). On the other hand, lesions of the AP do not block the effects of food deprivation on hamster estrous cycles (166), suggesting that food deprivation provides a wider variety of metabolic signals than just glucoprivation and that not all of them inhibit LH secretion via the AP.

The situation with female sexual behavior is more straightforward. Hamsters with AP lesions do not exhibit the normal decreases in sexual receptivity in response to any metabolic challenges, including treatment with metabolic inhibitors (139), increased energy expenditure (52), sequestration of metabolic fuels in adipose tissue (167), and food deprivation (Table 3) (166). AP lesions also prevent the associated decreases in ventromedial hypothalamus (VMH) ERIR. These findings indicate that the metabolic fuel detectors that affect female sexual behavior cannot lie in the forebrain; the forebrain circuits themselves do not contain the critical fuel detectors. The fact that animals with hindbrain lesions do not respond to metabolic perturbations, despite having intact forebrain circuitry (29, 52, 139, 166, 167), would seem to preclude the existence of critical forebrain fuel detectors.

As is the case with physiological controls of food intake, there are three possible ways for fuel detectors to communicate with effector circuitry in the forebrain: 1) peripheral detectors with neural projections to the brain; 2) peripheral detectors with humoral signaling of the brain; and 3) central nervous system (CNS) detectors with neural (synaptic) transmission to the effector circuitry. We consider each of these in turn.

### Table 3. Effects of various manipulations of metabolic fuel availability on estrous behavior and on neural estrogen receptor in Syrian hamsters

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Response</th>
<th>Prevented by APX?</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2DG + MP</td>
<td>↓ estrous behavior</td>
<td>/</td>
<td>139</td>
</tr>
<tr>
<td>(inhibition of fuel oxidation at cellular level)</td>
<td>↓ VMH ERIR</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Cold exposure</td>
<td>↓ estrous behavior</td>
<td>/</td>
<td>52</td>
</tr>
<tr>
<td>(excessive expenditure)</td>
<td>↓ VMH ERIR</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Insulin treatment</td>
<td>↓ estrous behavior</td>
<td>/</td>
<td>167</td>
</tr>
<tr>
<td>(sequestration in adipose tissue)</td>
<td>↓ VMH ERIR</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Untreated diabetes</td>
<td>↓ estrous behavior</td>
<td>?</td>
<td>139</td>
</tr>
<tr>
<td>VMH ERIR</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food deprivation</td>
<td>↓ estrous behavior</td>
<td>?</td>
<td>166</td>
</tr>
<tr>
<td>VMH ERIR</td>
<td>?</td>
<td></td>
<td></td>
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</tbody>
</table>

↓, Decrease; /, prevented by lesions of the area postrema (APX); ?, not known; ERIR, detectable estrogen receptor-α immunoreactivity; VMH, ventromedial hypothalamus.

**SECONDARY SIGNALS**

Once metabolic fuel status has been detected, it must be conveyed to the forebrain circuits that control GnRH secretion and copulatory behavior (3 in Fig. 2). This requires the generation of secondary signals, neural, humoral, or both, to communicate this information. Of course, this way of thinking assumes that the forebrain circuits themselves do not contain the critical fuel detectors. The fact that animals with hindbrain lesions do not respond to metabolic perturbations, despite having intact forebrain circuitry (29, 52, 139, 166, 167), would seem to preclude the existence of critical forebrain fuel detectors.

**Peripheral Detectors with Neural Projections to the CNS**

A large body of evidence implicates neural (primarily vagal) transmission of peripheral signals to the brain in the control of food intake (218). For example, cutting the vagus nerves, which eliminates the parasympathetic sensory input from the liver and other visera, prevents the changes in food intake that normally follow manipulations of hepatic energy status (132, 184, 188, 230, 264) or gut fill (172). There is also evidence for sensory innervation of adipose tissue (63), but whether it participates in the control of energy balance has not been determined.

In contrast to the evidence for vagal participation in metabolic controls of energy balance, it is not at all clear that the vagus plays a role in nutritional infertility. One paper (26)
Peripheral Detectors with Humoral Transmission to the CNS

Based on work on the physiology of energy balance, four peripheral tissues that could communicate energetic status to the brain by secreting hormones have been identified: adipose tissue (leptin), β cells of the pancreas (insulin), the stomach (ghrelin), and the small intestine (CCK).

Leptin. There is a significant body of evidence showing that leptin, secreted by adipocytes, plays a role in the nutritional control of reproduction (2, 7, 39, 69, 125). In the absence of functional leptin signaling, animals are infertile. This includes strains that do not produce leptin as well as those with mutated leptin receptors (ObR) (7, 10, 33, 155, 164, 267). Conversely, treatment with exogenous leptin can restore some measure of fertility to animals lacking leptin (33), but not those with dysfunctional ObR (70). However, introduction of ObR into the brains of ObR-deficient Zucker rats with a viral vector restores estrous cyclicity (123). At the very least, these findings indicate that some level of leptin signaling is required for fertility.

Leptin production and release from adipocytes are directly related to body fat content, and food deprivation decreases body fat content and circulating hormone levels (6, 7, 149). Consequently, a cynic might argue that this as a more up-to-date incarnation of Frisch’s flawed critical body fat hypothesis. However, leptin secretion can change quite rapidly in response to acute changes in fuel availability (134, 135, 159), indicating that circulating leptin levels reflect more than just body fat stores. Neuronal regions implicated in the control of reproduction and energy balance contain abundant levels of the signaling form of ObR (56, 266), including neurons that express ERIR (47) and perhaps GnRH-producing neurons (234).

Treatment with exogenous leptin can enhance reproductive function. Treating food-deprived adults of a number of species with leptin restores normal pulsatile secretion of LH [(61, 96, 158, 161) but cf. (15, 154)]. In addition, progression through puberty requires that animals have ample metabolic fuels available (20, 127), and leptin treatments can advance puberty in both ad libitum-fed (5, 34) and food-restricted animals (5, 89). These findings are consistent with the notion that undernutrition-induced decreases in circulating leptin levels lead to an inhibition of reproduction. However, it should be noted that treatment with leptin does not facilitate estrous behavior in food-deprived hamsters (247), nor does ICV administration of leptin affect estrous behavior in fatty Zucker rats, even when given in doses sufficient to decrease food intake and body weight gain (70).

Although some leptin signaling is required for normal reproduction, and leptin treatment can affect LH secretion, it is not at all clear how important fluctuations in leptin levels within the normal physiological range are for nutritional control of reproduction (35, 200, 212). It is relatively simple to dissociate circulating leptin levels and reproductive function (174, 201). For example, when food-restricted animals are refed, normal levels of estrous behavior (113) and LH pulsatility (200, 235) are restored long before leptin levels begin to rise. Another example is the fact that up to 72 h of food deprivation suppresses circulating LH levels in female mice without decreasing circulating leptin levels (98). Even the positive effects of leptin treatment on reproductive function are difficult to interpret, because leptin can act both centrally and peripherally to increase the availability of glucose and fatty acids for oxidation (95, 105, 106, 120, 200, 265). Thus leptin treatment could stimulate reproductive function by mobilizing metabolic fuels for oxidation rather than directly signaling the brain. Furthermore, although leptin treatment can reverse the effects of food deprivation on LH secretion and ovulatory cycles (61, 96, 158, 161), it does not reverse the effects of metabolic inhibitors (159, 203, 211) that would act downstream of fuel mobilization.

In summary, circulating leptin levels, like body fat content, are usually positively correlated with several indexes of reproductive function. However, relatively simple manipulations can break this correlation down, casting doubt on the notion that this is a causal relationship. Furthermore, while leptin treatment usually, but not always (154, 247), reverses reproductive effects of food deprivation, this could be due to mobilization of fuel supplies rather than to direct signaling of the brain. All that is clear at this time is that leptin has a permissive effect on reproduction; in the complete absence of leptin signaling, animals are infertile. But given that the absence of leptin or functional ObR is extraordinarily rare in the real world, the significance of leptin for nutritional control of reproduction may be questionable. In and of itself, leptin signaling does not appear to play a primary role in nutritional control of reproduction, but this does not rule out the possibility that fluctuations within the physiological range could interact with other secondary signals.

Insulin. Insulin, like leptin, is required for reproduction; in the absence of insulin, animals are infertile (88, 233). Obviously, insulin affects reproductive physiology and behavior by altering the availability of fuels for oxidation. But the question here is whether circulating insulin levels serve a signaling function independent of their effects on fuel metabolism. The parallel with a possible role of leptin in fertility is obvious. As usual, there is a basis for this possibility in the literature on the physiology of energy balance. Basal insulin levels are directly proportional to the body's fat stores, and ICV infusion of insulin decreases food intake and body weight in baboons and male rats (31, 261), leading to the conclusion that the basal hypothalamus monitors insulin levels and adjusts food intake and energy expenditure such that energy stores are maintained within a particular range (219, 262).

On the other hand, there is scant evidence that insulin plays a signaling function in regard to reproduction. First, the theoretical underpinnings of this concept are undercut by the recent demonstration that, while ICV insulin infusion decreases food intake in male rats, it has no effect at all in females (41), the sex in which reproductive function is most sensitive to fuel availability (21). Second, while fertility and circulating insulin levels are generally correlated with one another (as are body fat content and leptin levels), it is relatively simple to dissociate circulating insulin levels and reproductive function. For example, restoration of normal levels of estrous behavior or LH...
pulsatility in animals refeed after a fast does not require an increase in insulin levels (113, 256). Third, it is not clear whether ICV insulin infusion can reverse the effects of food deprivation on LH secretion. One paper found a stimulatory effect (151), but another did not (99). Fourth, artificially high insulin levels can actually inhibit estrous behavior and LH secretion (25, 29, 40, 140, 167, 249), inconsistent with a signaling action of insulin. However, these latter results are almost certainly due to insulin-induced decreases in metabolic fuel availability, because providing the animals with an adequate energy supply prevents the inhibition of LH secretion and estrous behavior (40, 167, 249). Finally, it has been shown that ICV infusion of insulin 1 facilitates estrous behavior (129) in diabetic rats and 2 fos expression in GnRH neurons in diabetic rats (130) and 3 restores pulsatile LH release in diabetic sheep (237). However, these findings are just as consistent with improvements in fuel oxidation as they are with a signaling action of insulin, so they really do not cast any light on the issue.

As with leptin, insulin signaling does not seem to play a major role in nutritional control of fertility. Instead, its positive effects are due to an enhancement of metabolic fuel availability. Again, as with leptin, this does not rule out the possibility that fluctuations within the physiological range could interact with other secondary signals.

**Ghrelin.** Endocrine cells in the stomach secrete ghrelin during negative energy balance (94, 266), and it can act in either the hypothalamus (263) or hindbrain (60) to stimulate food intake. Thus it is conceivable that high circulating ghrelin levels could act on the neural circuitry controlling reproduction to inhibit LH secretion and copulatory behavior. As yet, there is virtually no work on the effects of ghrelin on fertility. One paper reports that ghrelin inhibits pulsatile LH release in rats (83), whereas another reports no effect in humans (236). Our own work in progress paradoxically suggests that ICV ghrelin infusion actually facilitates receptivity in hamsters (M. Furman, S. L. Dettloff, and G. N. Wade, unpublished results). Clearly, the jury is still out on possible reproductive effects of ghrelin.

**CCK.** The rapid restoration of pulsatile LH secretion in refeed animals (18, 20, 23, 67, 168, 214, 217, 254) could be due to the prandial release of CCK from the duodenum. It has been reported that CCK treatment stimulates LH secretion whether given directly into the brain (91, 128) or systemically (169, 215), but the significance of these findings for nutritional infertility is unclear. Refeeding food-restricted lambs did not affect hypothalamic levels of CCK peptide or mRNA (102). Furthermore, administration of a CCK-A or CCK-B antagonist did not affect LH secretion in refeed Rhesus monkeys (215). What about CCK effects on estrous behavior? Central infusions of CCK can either facilitate or inhibit estrous behavior in rats, depending on the animals’ hormonal status and the site of infusion (8, 17, 50). Presumably these effects are related to centrally produced and released CCK acting as a neurotransmitter. However, CCK released from the gut acts peripherally on vagal endings, not centrally, and this information is conveyed to the brain by the vagus nerve (181, 260). The single paper to have looked at the effects of systemically administered CCK on estrous behavior found no effect in either ad libitum-fed or food-deprived animals (112). Thus there seems to be virtually no support that peripherally released CCK plays any signaling role for nutritional control of LH secretion or estrous behavior.

Taken together, these results do not provide strong support for the possibility that peripheral fuel detectors affect fertility via hormonal inputs to the brain. However, it would be foolish to rule out this possibility, given that there is still much to be done and the fact that other humoral signals to the brain almost certainly remain to be discovered.

**Neural Detectors with Synaptic Projections to the Forebrain**

The work demonstrating that AP lesions abolish behavioral responses to fuel deprivation (Table 3) (52, 139, 166, 167) and the inhibition of LH release by insulin (29) as well as the fact that fourth ventricular infusion of 2DG inhibits pulsatile LH secretion (Fig. 4) (156, 163) provide very strong evidence that hindbrain fuel detectors play a significant role in the physiology of nutritional infertility. Of course there is abundant evidence for hindbrain fuel detectors in the literature on ingestive behaviors, too (182, 185, 186). How, then, is this information relayed to GnRH neurons and the forebrain circuit controlling estrous behavior?

Metabolic/visceral information is transmitted from the hindbrain to the forebrain via projections containing norepinephrine (NE) (46, 197), epinephrine (Epi) (242), or a combination of NE or Epi plus NPY (36, 198). Selective destruction of these projections, including those containing both NPY and a catecholamine (CA), by infusions of saporin conjugated with an antibody to dopamine-β-hydroxylase (DSAP) into the terminal fields in the paraventricular nucleus of the hypothalamus (PVN) or the medial-basal hypothalamus abolishes the increase in food intake normally induced by treatment with 2DG (71, 137, 183). These immunotoxic lesions deplete dopamine-β-hydroxylase (DBH) and NPY immunostaining neurons in the A1 and C1–C3 catecholaminergic areas of the hindbrain without damaging cells in the terminal fields (71, 137, 183). It is important to note that these hindbrain neurons send collaterals to multiple forebrain sites and that infusion of DSAP into any one of the terminal fields destroys the projections to all of them (71, 183).

The fact that DSAP lesions destroy both NPY and CA projections does not permit any conclusions about the relative importance of the two types of neurotransmitters. However, recent work with knockout (KO) mice is instructive. Although NPY KO mice appear to display nearly normal regulation of energy balance (58), they do not increase their food intake when treated with insulin or 2DG, indicating that NPY is required for normal glucoprivic feeding (Fig. 5) (225). On the other hand, DBH KO mice, which can produce neither Epi nor NE, exhibit perfectly normal glucoprivic feeding (232). Of course, these findings do not rule out a role for CA for glucoprivation-induced increases in food intake; they merely indicate that neither Epi nor NE is required, unlike NPY.

It is likely that these projections also play a significant role in nutritional infertility. Infusion of DSAP into the PVN does not affect estrous cyclicity in fed rats, but it prevents the delay of the next expected estrus caused by 2DG injections (Fig. 6) (109, 110). Thus, as with estrous behavior (52, 139, 166, 167), GnRH neurons behave as though ample calories are available for oxidation, unless they receive a signal to the contrary. Furthermore, NPY null-mutant female mice do not exhibit a
suppression of circulating LH levels after a 48-h fast, unlike wild-type controls (100) [although males do (57)]. One might argue that this failure to respond to food deprivation could be due to the absence of forebrain NPY neurons [e.g., in the arcuate nucleus of the hypothalamus (ARC)]. However, recall that an intact forebrain NPY system is insufficient to support an inhibition of cyclicity in DSAP-treated rats (110) or of estrous behavior in AP-lesioned hamsters (52, 139, 166, 167). Thus the most parsimonious interpretation is that hindbrain NPY/CA projections to the forebrain play a critical role in conveying nutritional information to GnRH neurons and the circuits controlling estrous behavior.

FOREBRAIN CIRCUITRY

Investigators studying nutritional control of LH secretion have a distinct advantage over those studying estrous behavior. There is a clear final common pathway to pituitary gonadotropes, namely GnRH neurons. On the other hand, while we know a great deal about the neural circuitry controlling estrous behavior (16, 171), we cannot pinpoint a single structure or type of neuron comparable to GnRH neurons if one exists. Although there are a number of similarities in the nutritional control of LH release and estrous behavior (4 in Fig. 2), we discuss them separately for the sake of simplicity.

Control of LH Secretion

There is ample evidence for both direct and indirect NPY/CA control of GnRH neurons (119, 224). 1) Afferents expressing both NPY and DBH (therefore originating in the hindbrain) synapse on GnRH neurons in the medial preoptic area (mPOA) in mice (243). There are also inputs expressing NPY and agouti-related gene product, which arise in the arcuate nucleus of the hypothalamus (243), but NPY projections arising in the forebrain may not be involved in nutritional infertility (52, 110, 139, 166, 167). 2) Energetic challenges such as food deprivation and diabetes increase release of NE and NPY in the forebrain (3, 118, 153, 160, 195, 231). 3) Forebrain infusions of either NPY (30, 177) or NE (27, 241) inhibit LH secretion (Figs. 7 and 8), and studies using selective

![Fig. 5](image) Left: treatment with insulin increases food intake in wild-type (+/+) mice, but the effect is absent or attenuated in neuropeptide Y (NPY) knockout (KO) (−/−) mice. Adapted from Ref. 225. Right: treatment with insulin increases food intake in both wild-type and DBH KO mice. Adapted from Ref. 232. Bars with different letters are significantly different from one another.

![Fig. 6](image) Treatment with 2DG lengthens the estrous cycle in control rats (SAP) but not in rats which had previously been given immunotoxins lesions of the NPY/CA ascending pathways by giving intracerebral infusions of saporin linked to an antibody to dopamine-β-hydroxylase (DSAP). Adapted from Ref. 110.

![Fig. 7](image) Intracerebroventricular (ICV) infusion of NPY reduces circulating LH levels, and this inhibition is prevented by prior infusion of the NPY Y5 antagonist, trans-naphthalene-1-sulfonic acid-[4-[(3-dimethylamino-propylamino)-quinazolin-2-ylamino]-methyl]-cyclohexylmethyl]-amide. Bars with different letters are significantly different from one another. Adapted from Ref. 177.

![Fig. 8](image) Infusion of norepinephrine (NE) into the PVN of ovariectomized (OVX), estradiol-treated rats inhibits pulsatile LH release. Prior infusion of α-helical corticotropin-releasing hormone (CRH) into the third ventricle prevented this effect. Open circles indicate LH peaks. Adapted from Ref. 241.
agonists and antagonists suggest that these effects are mediated by NPY Y5 and α-adrenergic receptors, respectively (27, 177).

Although a case can be made for the involvement of both NPY and CA projections from the hindbrain in the control of LH secretion, the relative importance of the two neurotransmitter systems cannot be ascertained. Recall that NPY, but not CA, is required for the expression of glucoprivic eating (Fig. 5) (225, 232). Similarly, female NPY KO mice do not exhibit a suppression of LH levels after a fast (100), but we are not aware of any comparable data with DBH KO mice. Although these findings seem to indicate an essential role for NPY, it has also been reported that ICV administration of α-adrenergic antagonists is sufficient to prevent fasting-induced decreases in LH levels (27), consistent with crucial participation of α-adrenergic transmission. Perhaps both NPY and CA signaling are required.

In addition to (or perhaps instead of) direct NPY/CA inputs to GnRH neurons, these neurotransmitters could act via intermediates, namely CRH and the urocortins. [Although there is some literature on the recently discovered urocortins (136, 180, 246) and reproductive function (103, 114, 222, 255), we will focus on CRH, knowing full well that some of the effects could actually be mediated by one of the urocortins.] CRH neurons in the forebrain receive direct inputs from hindbrain NPY/CA neurons (194, 198), and intracerebral infusion of either NPY or CA increases CRH release (173, 251). In addition, CRH terminals are found on GnRH neurons (146), and CRH is a potent inhibitor of GnRH release (170).

There is ample evidence to support the hypothesis that undernutrition-induced NPY and NE release inhibits pulsatile LH secretion by stimulating CRH input to GnRH neurons. Namely, ICV infusion of CRH antagonists prevents or reverses the inhibition of pulsatile LH release by PYN NE infusion (Fig. 8) (241), by food deprivation (Fig. 9) (147), and by treatment with 2DG (Fig. 10) (240). We are not aware of anyone’s having attempted to reverse NPY-induced inhibition of LH release with a CRH antagonist.

These results, particularly those in food-deprived rats (Fig. 9), indicate that transmission via CRH receptors, whether by CRH or one of the urocortins, is essential for nutritional suppression of LH release. One possible interpretation could be that NE and NPY act solely by increasing CRH activity, and that direct NPY/CA contacts on GnRH neurons are not necessary, making CRH the final common link between energy availability and GnRH secretion. Other interpretations might be that both direct and indirect inputs are required or that the indirect (via CRH) pathway is primary with direct NPY/CA inputs having a modulatory role. Whatever the mechanism(s), the findings with CRH antagonists do not preclude a role for direct hindbrain inputs to GnRH neurons.

**Control of Estrous Behavior**

Similar mechanisms seem to be operating with respect to the control of estrous behavior. Infusion of NPY or one of its agonists into the cerebral ventricles or various parenchymal sites rapidly (<30 min) inhibits estrous behavior in rats and hamsters (Fig. 11) (38, 43, 124). However, we are not aware of any work on the effects of chronic NE infusion on estrous behavior, although there is a large literature showing that acute CA administration can facilitate sexual receptivity depending on the timing and hormonal condition of the animal (16, 59). Thus, at this time there is no way of knowing whether CA might participate in undernutrition-induced inhibition of estrous behavior.

Injection of PYY3–36, an NPY agonist, into the PVN-anterior hypothalamus-mPOA region rapidly (<30 min) suppresses lordosis in estrous hamsters. On the other hand, placements in the VMH, ARC, and fourth ventricle were uniformly negative (124). The fact that the VMH was not responsive is important, given its crucial role in the control of estrous behavior. This finding is consistent with the sparse NPY innervation of the VMH (193). Lateral ventricular infusion of PYY3–36 increases expression of fos immunoreactivity (fos-
infusion of CRH rapidly (<30 min) and transiently (<4 h) inhibits estrous behavior in rats and hamsters (114, 222, 226–228). More importantly, ICV infusion of the CRH receptor 1 (CRHR1)/CRHR2 antagonist astressin prevents the inhibition of estrous behavior by CRH and by NPY and reverses the inhibition by food deprivation (Fig. 13) (114, 222). Work such as this with antagonists is particularly informative, because it implicates endogenous CRH activity and demonstrates that the effects of treatment with agonists are physiologically relevant, not simply pharmacological artifacts. Thus CRH receptor signaling plays a pivotal role in undernutrition-induced inhibition of female copulatory behavior, as may be the case for LH secretion. There is a solid neuroanatomic basis for this hypothesis. Neurons containing CRH or urocortins send projections to forebrain sites including the ventromedial hypothalamus, medial preoptic area, and lateral septum (14, 138, 245), all of which contain CRH receptors (245) and are involved in the control of estrous behavior (16, 171).

Whether these actions are due to CRH or one of the urocortins is unclear. ICV infusion of urocortins 1, 2, and 3 inhibit estrous behavior in hamsters, just as CRH does (114, 222). Urocortins 2 and 3 are selective for the CRHR2 (136, 180), indicating that activation of this receptor alone is sufficient to inhibit receptivity. Furthermore, administration of astressin 2B, a selective antagonist at CRHR2, blocks the effects of urocortin 2 on estrous behavior (222). However, astressin 2B does not block the effects of CRH, which activates both types of receptors, consistent with a role for CRHR1. The upshot of all this is that, in all likelihood, activation of either receptor type can inhibit female copulatory behavior, but we do not know which receptor type(s) is (are) physiologically relevant. This stands in contrast to the inhibition of food intake, which is mediated via CRHR2, and activation of the hypothalamic-pituitary-adrenal (HPA) axis, which is mediated via CRHR1 (9, 45, 175).

Recall that nutritional manipulations that inhibit estrous behavior also decrease ERIR in the VMH (Fig. 3) (52, 97, 117, 139, 166, 167, 191), which may contribute to the reduced behavioral responsiveness to estradiol (116). Does increased release of NPY, CRH, or the urocortins inhibit behavior by
invoking this decrease in ERIR? No one has done the experiments yet. However, regardless of any possible effects on ERIR, these neurotransmitters almost certainly affect behavior by other mechanisms, too. Estrous behavior responds slowly to decreased fuel oxidation, taking at least 36 h to develop (113), but NPY, CRH, and the urocortins take <30 min to work (43, 114, 222). Furthermore, astressin rapidly (<30 min) reverses the established inhibition of sexual receptivity by food deprivation (Fig. 13) (114). Thus the peptides seem to act far too quickly to depend on changes in steroid receptor levels. Perhaps they act concurrently to inhibit behavior via slowly developing steroid-receptor-dependent actions and rapidly through steroid-receptor-independent mechanisms. It would be interesting to look at the effects of prolonged ICV infusion of NPY and CRH on neural ERIR.

Taken together, this body of work provides strong support for the hypothesis that metabolic cues, detected in the hindbrain, are transmitted to the forebrain by NPY/CA projections that inhibit female copulatory behavior via activation of CRHR1/R2 pathways.

CAVEATS: WHAT'S WRONG WITH THIS PICTURE?

Given the available data, one can build a credible model such as ours. But of course that doesn’t mean that it is correct. Indeed, there remain conflicting perceptions and obvious lacunae in our understanding. It would be useful to note some of them.

An Objective Measure of Metabolic Fuel Availability?

While the concept of oxidizable metabolic fuels is reasonable and conceptually useful, it remains vague and poorly defined. An objective measure of fuel availability is essential in order to apply strong inference to this hypothesis. Perhaps the work on nutrient sensing (e.g., 42, 73, 162, 192) will prove to be useful in this regard.

What About Stress?

Given the pivotal role of CRH in nutritional infertility, the issue of stress arises. Indeed, there is a significant literature indicating that various stressors can inhibit LH secretion (49, 190). The experimental literature on stress, nutrition, and reproduction can be rather confusing, because the contemporary definition of a stressor, i.e., anything that disturbs physiological balance (196), is unfortunately vague. This vagueness has resulted in experimental glucoprivation/lipoprivation caused by 2DG or insulin treatment having been lumped with immune/inflammatory responses, pain, and psychological stressors such as restraint or being driven around in a truck. The problem here is that, while glucoprivation/lipoprivation may indeed be a stressor in the same sense that restraint or injury is, it also deprives animals of calories. As it turns out, it is relatively easy to dissociate LH secretion from psychological stress or activation of the HPA axis (40, 93, 108, 145, 214, 216) in a variety of species.

Despite the prevailing conventional wisdom and the attention it receives in the popular press, there is virtually no literature on the effects of stress on female copulatory behavior. We found that ICV treatment with doses of CRH or urocortin 1 that inhibited sexual receptivity in hamsters also activated the HPA axis. However, restraint stress, which also activated the HPA axis, had no effect at all on hamster estrous behavior (114).

Taken together, these findings indicate that whatever the effects of stress on reproduction, metabolic fuel deprivation can inhibit both LH secretion and estrous behavior without activating the HPA axis. There is always the possibility that CRH effects on reproduction could be mediated by CRHR2, like food intake, rather than CRHR1, which mediates the effects of stress on ACTH secretion (9, 45, 175). The fact that urocortins 2 and 3, selective for CRHR2, inhibit estrous behavior is consistent with this prospect (222). We are not aware of any published work on urocortin 2 or 3 and LH secretion.

CRH Inhibits Both Food Intake and Reproduction

Increased NPY release concurrently stimulates food intake and inhibits estrous behavior and LH secretion, which makes sense given that all these are responses to caloric deficits. But administration of CRH or one of the urocortins inhibits food intake and reproductive function, which appears to be counterintuitive if one assumes that hunger and reproduction are reciprocally regulated. However, these seemingly contradictory actions may simply be a pharmacological anomaly, due to the fact that ICV administration of CRH or urocortin activates CRH-sensitive systems throughout the brain, and not reflect what is happening in vivo. Central CRH/urocortin systems are enormously complicated (13, 138, 180, 245), and there is no reason to expect that all are activated concurrently. In the absence of data to constrain us, it is simple to posit the existence of multiple CRH/urocortin circuits controlling food intake, estrous behavior, LH secretion, energy expenditure, and

![Fig. 13. Lateral ventricular infusion of CRH (A) or NPY (B) or 48 h of food deprivation (C) inhibit estrous behavior in OVX, estradiol + progesterone-primed Syrian hamsters. ICV infusion of the CRHR1/R2 antagonist astressin reverses (C) or prevents (A and B) these effects. Adapted from Refs. 114 and 222. *P < 0.05 vs. other treatment groups.](http://ajpregu.physiology.org/doi/abs/10.1083/journal.issue.287/h11028)
so forth, with some responding to energy excess and others responding to energy deficits.

**What About Other Neurotransmitter/Neuropeptide Systems?**

We have emphasized the roles of CA, NPY, and the CRH family in nutritional infertility because the case is strongest for these neurotransmitter systems. It is almost certain that other neuropeptides will be shown to participate, too, including peptides yet to be discovered. For example, orexin, galanin, and galanin-like peptide all increase food intake and LH secretion (62, 119, 131, 176, 229), but a connection with nutritional infertility is yet to be determined. It has also been reported that melanocortins can increase circulating LH release (199) and sexual receptivity (44), but a role in nutritional infertility is doubtful (11, 101, 178). Endogenous opioid peptides (EOP) have been proposed as mediators of the effects of fuel availability on reproduction (119). EOP treatment increases food intake and inhibits LH secretion. More to the point, administration of EOP antagonists prevents the suppression of LH levels by food deprivation (51) or 2DG treatment (19) in rats and by insulin treatment in sheep (40), but not in Rhesus monkeys (92). However, as Clarke et al. (40) point out, the positive data must be interpreted with great caution. EOP antagonists increase LH secretion in otherwise untreated animals (e.g., 40, 54), so the effects may simply be a general stimulation of LH, rather than specifically reversing the effects of nutritional challenges. The list of candidates could go on and on in the absence of conclusive data.

**Food Deprivation vis-à-vis Metabolic Inhibitors**

Although many of us use metabolic inhibitors in an attempt to decipher the mechanisms by which food deprivation inhibits reproduction, they are different from one another. There are two issues here. First, do metabolic inhibitors affect reproduction by producing some of the physiological cues that food deprivation does, or do they work by producing a general malaise? The latter possibility is difficult to rule out, but as we noted above, AP lesions prevent the inhibition of estrous behavior caused by treatment with 2DG + MP (139), just as they do with glucoprivic eating (189), which would be difficult to explain by positing a general malaise. In addition, the fact that 2DG treatment concurrently increases hunger and inhibits LH secretion is also inconsistent with this kind of interpretation.

The second issue is the fact that food deprivation provides a wider spectrum of physiological cues than treatment with metabolic inhibitors, e.g., oropharyngeal sensations and gut fill. Can food deprivation inhibit reproduction via processes in addition to those activated by metabolic inhibitors? For example, AP/NTS lesions prevent glucoprivic eating (189), but not deprivation-induced increases in food intake (53), consistent with the conclusion that food deprivation affects eating by actions in addition to decreases in fuel oxidation. Similarly, AP lesions prevent the interruption of estrous cyclicity by 2DG treatments in hamsters (166), but they do not block the effects of food deprivation (213). On the other hand, AP lesions do prevent the inhibition of sexual receptivity by both food deprivation and 2DG + MP treatment (139, 166), which probably means that deprivation does not provide additional salient cues in the case of estrous behavior. Whatever the end point one is measuring, common sense dictates that we keep in mind that metabolic inhibitors are useful experimental tools, but it is important to use a variety of metabolic challenges (e.g., food deprivation, increased energy expenditure, sequestration of fuels in storage depots, diabetes mellitus).

**The Role of Leptin?**

When we reviewed this field nearly 10 years ago (250), we noted the recent discovery of leptin and, unencumbered by data, speculated that it could play some role in nutritional infertility. Hundreds of papers later, we know that some level of leptin signaling is required for normal reproduction, but it is difficult to say more than that with any degree of certainty. Highly respected scientists come down on either side of the question as to whether fluctuations in leptin levels within the physiological range provide salient information to the neuroendocrine circuits controlling reproduction. On one hand, leptin treatment can reverse the effects of food deprivation (but not metabolic inhibitors) on LH secretion. On the other hand, it is relatively simple to dissociate leptin levels and reproduction with simple metabolic manipulations. Clearly, the last has not been written on this issue.

**SUMMARY**

Over the last 15 years, neuroendocrinologists have managed to fill in many of the questions outlined in Fig. 2, and a working hypothesis to integrate this information can be concocted (Fig. 14). It is now widely accepted that the primary metabolic cue

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**Fig. 14.** Working model of the putative pathways underlying nutritional inhibition of estrous behavior and LH secretion. Decreases in the availability of oxidizable metabolic fuels (1) are detected in the hindbrain (2). This information is transmitted to the forebrain (3) via NPY/CA projections to GnRH neurons, CRH/urocortin neurons, and NPY neurons (4). The CRH/urocortin neurons then project to several forebrain sites involved in the control of estrous behavior (5) and to GnRH neurons (6). The role of the forebrain NPY neurons in nutritional infertility is unclear at this time, nor is it known whether the hindbrain NPY/CA neurons are the same cells as the fuel detectors.
that inhibits reproduction during lean times is a reduction in the short-term availability of oxidizable fuels (1 in Fig. 14) and not some aspect of body size or composition. The critical detectors for metabolic fuel availability (2 in Fig. 14) appear to lie in the hindbrain and not in the forebrain. Once this information is detected, it is transmitted to the forebrain by NPY/CA projections (3 in Fig. 14). These hindbrain projections could inhibit LH release by direct synapses on GnRH neurons or indirectly via CRH/urocortin projections (4 in Fig. 14). Inhibition of estrous behavior appears to be accomplished through CRH/urocortin intermediaries. Of course, the output pathway for LH secretion (6 in Fig. 14) and the neural loci mediating estrous behavior (5 in Fig. 14) have been known for quite some time. While there is a large body of evidence to support this hypothesis, there are substantial gaps to be filled in. There are almost certainly important pathways and processes that are not part of this simple (simplistic?) model. Fortunately, this working model is explicit enough to be falsifiable.

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