70th Anniversary Conference on ‘Body weight regulation – food, gut and brain signalling’

Symposium I: Food–gut interactions

The receptive function of hypothalamic and brainstem centres to hormonal and nutrient signals affecting energy balance

Thomas Riediger
Institute of Veterinary Physiology and Centre of Integrative Human Physiology, University of Zurich, 8057 Zurich, Switzerland

The hypothalamic arcuate nucleus (ARC) and the area postrema (AP) represent targets for hormonal and metabolic signals involved in energy homeostasis, e.g. glucose, amylin, insulin, leptin, peptide YY (PYY), glucagon-like peptide 1 (GLP-1) and ghrelin. Orexigenic neuropeptide Y expressing ARC neurons are activated by food deprivation and inhibited by feeding in a nutrient-dependent manner. PYY and leptin also reverse or prevent fasting-induced activation of the ARC. Interestingly, hypothalamic responses to fasting are blunted in different models of obesity (e.g. diet-induced obesity (DIO) or late-onset obesity). The AP also responds to feeding-related signals. The pancreatic hormone amylin acts via the AP to control energy intake. Amylin-sensitive AP neurons are also glucose-responsive. Furthermore, diet-derived protein attenuates amylin responsiveness suggesting a modulation of AP sensitivity by macronutrient supply. This review gives an overview of the receptive function of the ARC and the AP to hormonal and nutritional stimuli involved in the control of energy balance and the possible implications in the context of obesity. Collectively, there is consistency between the neurophysiological actions of these stimuli and their effects on energy homeostasis under experimental conditions. However, surprisingly little progress has been made in the development of effective pharmacological approaches against obesity. A promising way to improve effectiveness involves combination treatments (e.g. amylin/leptin agonists). Hormonal alterations (e.g. GLP-1 and PYY) are also considered to mediate body weight loss observed in obese patients receiving bariatric surgery. The effects of hormonal and nutritional signals and their interactions might hold the potential to develop poly-mechanistic therapeutic strategies against obesity.

Driven by the search for therapeutic treatment strategies against obesity and associated metabolic disorders, considerable knowledge has accumulated about the control mechanisms involved in the maintenance of energy balance. The redundancy of control mechanisms, the capacity of these systems to compensate for pharmacological effects and the existence of obesity-related hormonal insensitivities make it difficult to achieve sufficient therapeutic efficiency for the reduction of body weight over longer periods of time. Evidently, at least under non-laboratory conditions, so-called non-homeostatic factors (hedonic properties and availability of food, food preferences, social factors, eating habits, etc.) and central reward mechanisms override homeostatic hormonal and metabolic signals that are considered to reflect and to control the body’s energy status.

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; AP, area postrema; APX, area postrema lesion; ARC, arcuate nucleus; CCK, cholecystokinin; DIO, diet-induced obesity; FAS, fatty acid synthase; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; mTOR, mammalian target of rapamycin; NED, non-energy diet; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; OXM, oxyntomodulin; POMC, pro-opiomelanocortin; PYY, peptide YY; STAT, signal transducer and activator of transcription.

Corresponding author: Thomas Riediger, fax +41 44 635 8932, email triedig@vetphys.uzh.ch

https://doi.org/10.1017/S0029665112000778 Published online by Cambridge University Press
In contrast to the currently available pharmacological treatment options, bariatric surgery (e.g. Roux-en-Y gastric bypass) is very effective in reducing body weight and improving glucose homeostasis in patients with type-2 diabetes mellitus\(^\text{(1,2)}\). Apparently, bariatric surgery alters gastrointestinal physiology in a way that changes nutrient-induced gastrointestinal hormone responses, which presumably contribute to these therapeutic effects. Hence, the signalling mechanisms of the gut–brain axis and the interaction between its nutritional and hormonal feedback signals are not only essential for our understanding of the body’s energy homeostasis but also for the ultimate desire to develop pharmacological approaches against the obesity epidemic. Some of the hormones (e.g. glucagon-like peptide 1 (GLP-1) and peptide YY (PYY)) discussed in this review with regard to their central actions are presumed as mediators of weight loss after bariatric surgery. Although the exact mechanisms behind bariatric weight loss surgery and their potential to be targeted pharmacologically remain to be identified, there are other promising therapeutic approaches that are based on hormonal combination treatments. Both pre-clinical studies in obese rodents and clinical trials in human subjects have established a beneficial effect of combined treatment with the anorexigenic pancreatic hormone amylin with the fat-derived hormone leptin. Amylin treatment seems to reverse leptin insensitivity in obese subjects, in which amylin and leptin exert a synergistic action to reduce body weight\(^\text{(3,4)}\).

Brain circuits involved in the control of energy balance also function as fuel sensors responding to diet-derived macronutrients. Nutrients not only alter neuronal activity in feeding regulatory brain areas, they also modulate neuronal responsiveness to hormonal stimuli. Two important brain sites that are targeted by hormonal and nutrient signals involved in the control of energy balance are the hypothalamic arcuate nucleus (ARC) and area postrema (AP) of the brainstem. The current review focuses on the receptive function of the ARC and the AP to feeding-related signals and alterations of neuronal responsiveness that are associated with obesity. It is beyond the scope of this review to describe similar hormone and nutrient-dependent signalling mechanisms in other brain areas that also contribute to the overall control of energy homeostasis.

The hypothalamic arcuate nucleus

The ARC is considered as one of the most important hypothalamic target structures for blood-borne hormonal and metabolic factors involved in the regulation of food intake and energy homeostasis. The best-characterised hormone supposed to act via the ARC is the lipostatic and anorectic hormone leptin, a 16 kDa protein that is secreted from white adipose tissue. Genetic deficiency of leptin or its receptor in rodents causes excessive food intake, body weight gain and an obese phenotype\(^\text{(5,6)}\). Leptin acts via the ARC, which contains two functionally antagonistic populations of neurons with respect to the control of food intake. One population contains the orexigenic neuropeptide Y (NPY) and agouti gene-related peptide\(^\text{(7)}\). The other distinct population synthesises the anorectic pro-opiomelanocortin (POMC) gene product \(\alpha\)-melanocyte stimulating hormone\(^\text{(8)}\). Leptin up-regulates POMC neurons while it inhibits NPY/agouti gene-related peptide neurons in the ARC\(^\text{(9,10)}\). Similar to leptin, insulin fulfils the criteria of an adiposity signal because circulating insulin levels increase in relation to body adiposity. Furthermore, insulin has access to the brain and it influences food intake and body weight in a similar way as leptin via the ARC\(^\text{(11)}\).

Catalysed by the high importance of the ARC for body weight control and the failure of leptin therapy as an effective anti-obesity treatment because of leptin resistance\(^\text{(12)}\), the scientific interest in other factors acting via the ARC and its downstream signalling pathways began to increase. The well-established concept that the ARC is targeted by the adiposity signals leptin and insulin has been extended by numerous studies demonstrating ARC-dependent actions of hormones, neuropeptides and metabolites that change dynamically according to the status of energy intake. Notably, leptin itself shows dynamic short-term changes as a function of food intake\(^\text{(13,16)}\).

Ghrelin

One of the hormones targeting the ARC is the orexigenic gastrointestinal hormone ghrelin\(^\text{(15)}\). Plasma levels of ghrelin are modulated by the nutritional state. During fasting and shortly before meals circulating ghrelin concentrations are elevated, while feeding or nutrient intake reduce blood-ghrelin levels\(^\text{(16–18)}\). Various electro-physiological and immunohistological studies characterised ghrelin’s effects on neuronal activity of ARC neurons that have been suggested to represent target cells for ghrelin in the brain\(^\text{(19–22)}\). Ghrelin binds to the growth hormone secretagogue receptor that is highly abundant in the ARC\(^\text{(23)}\). The growth hormone secretagogue receptor is predominantly expressed in NPY neurons (94% co-localisation), while it is found in only 8% of POMC neurons\(^\text{(24)}\). Ghrelin exerts opposite effects to leptin on the activity of ARC neurons. Ghrelin or growth hormone secretagogue receptor agonists excite leptin-inhibited NPY neurons via a direct postsynaptic effect\(^\text{(19,25–27)}\). In addition to this action, ghrelin indirectly inhibits neurons of the lateral ARC, where POMC neurons are located\(^\text{(20,28)}\). The excitatory effect of ghrelin on ARC neurons and the NPYergic phenotype of these cells have been confirmed in vivo by immunohistochemical studies using c-Fos as a marker for neuronal activation\(^\text{(22,29)}\).

Whether ghrelin can be considered a physiological signal in the control of energy homeostasis has not yet been confirmed based on the general criteria that have been suggested to define a physiological relevance\(^\text{(30)}\). Owing to the weak consequences of ghrelin antagonism for feeding behaviour or body weight phenotype and because of the ineffectiveness of physiological ghrelin doses to produce feeding or body weight effects, the physiological relevance of ghrelin has not yet been fully established\(^\text{(31)}\). Based on the leptin counter-regulatory action of ghrelin, antagonism of ghrelin signalling has been proposed as a therapeutic approach against obesity. However, the role of

https://doi.org/10.1017/S0029665112000778 Published online by Cambridge University Press
Ghrelin in the pathogenesis of obesity is not clear. Ghrelin levels in obese human subjects are lower than under normal weight conditions limiting the usefulness and success of ghrelin antagonism as an anti-obesity strategy. Interestingly, weight loss in obese subjects seems to be associated with an increase in ghrelin levels suggesting that a blockade of ghrelin action might facilitate the body weight reducing effect of other therapeutic interventions. Notably, there is cumulating evidence for a role of ghrelin in reward mechanisms that involve extra-hypothalamic pathways originating in the ventral tegmental area. The potential of pharmacological ghrelin-dependet approaches with respect to this mechanism has not yet been sufficiently explored in clinical studies.

Peptide YY/glucagon-like peptide 1

The two gut hormones PYY and GLP-1 act on ARC neurons. They have received special attention in the context of energy homeostasis and as potential targets for the treatment of overweight. Both hormones might contribute to weight loss after gastric bypass surgery because Roux-en-Y gastric bypass patients have increased GLP-1 and PYY levels and blockade of gastrointestinal hormone release (including GLP-1 and PYY) by the somatostatin analogue octreotide led to increased appetite. A specific role for PYY is suggested by the absence of weight loss in PYY knockout mice subjected to gastrointestinal bypass surgery.

PYY and PYY(3–36) are two biologically active peptides arising from endocrine L-cells of the ileum and colon in response to food intake. Although PYY binds to several subtypes of Y receptors, PYY(3–36) exhibits relative specificity for the Y2 receptor. Both PYY isoforms inhibit food intake after peripheral administration; but PYY(3–36) appears to be more potent possibly due to its specificity to the Y2 receptor that is decisive for the anorexigenic response. However, the minimal doses that were required to suppress food intake in these studies were supraphysiological when compared with meal-induced changes of total PYY blood levels. Therefore, the question whether PYY(3–36) acts as a physiological satiating signal is controversial. Direct site-specific injections of PYY(3–36) identified the ARC as possible central target structure. With respect to the electrophysiological effects of PYY(3–36) in the ARC, conflicting data exist in the literature. In mice, PYY(3–36) has been demonstrated to indirectly (presynaptically) excite phenotypically identified POMC neurons, while another electrophysiological study demonstrated direct postsynaptic inhibitions in POMC neurons of mice. Among others, the reasons for these seemingly contradictory findings have not yet been identified, but might be related to experimental and technical differences concerning the slice preparation and selection of cells.

In rats, full-length PYY exerts direct inhibitory effects on ghrelin-exited ARC neurons (Fig. 1) suggesting that the NPYergic system is also affected by PYY signalling. This is consistent with the demonstration that PYY(3–36) inhibits NPY release from ARC explants in addition to stimulating a melanocytic stimulating hormone response.

Basal and postprandial PYY(3–36) levels are decreased in obese human subjects and rodents. The absence of PYY(3–36) resistance in obese subjects is in favour of using PYY agonists as anti-obesity drugs. Chronic peripheral administration of PYY(3–36) decreases body weight in different species including rhesus macaques. Whether PYY agonists can be therapeutically used in human subjects to reduce body weight awaits further evaluation. Interestingly, PYY seems to synergise with other hormones or hormone agonists affecting the control of energy balance and glucose homeostasis, including the GLP-1 agonist exendin-4 and the pancreatic hormone amylin. The neuronal correlates of these synergies are unknown.

Similar to PYY, GLP-1 is released in response to food intake from gastrointestinal L-cells. GLP-1 plays an important role in glucose homeostasis (incretin) and gastrointestinal function. In addition to its peripheral expression, GLP-1 is a neuropeptide expressed in a discrete population of enteroeceptive neurons located in the brainstem. It binds to the GLP-1 receptor (GLP-1R) that is also activated by the agonist exendin-4, a peptide that has been isolated from the saliva of the lizard Heloderma suspectum. The truncated form of exendin (9–39) (also termed exendin-9) acts as a competitive antagonist at the GLP-1R. The ARC, and other hypothalamic and brainstem structures, including the paraventricular nucleus and the AP, show high GLP-1R expression. Central and peripheral injection GLP-1R agonists decrease food intake in rodents. Furthermore, numerous studies have demonstrated a GLP-1-mediated reduction in energy intake in human subjects. Similar to ghrelin and PYY, it has not yet been confirmed that endogenous GLP-1 physiologically controls food intake.

The neuronal mechanisms mediating the action of GLP-1 on food intake have not yet been clearly identified, which might be due to the fact that different pathways seem to be activated depending on the experimental conditions, particularly the route of administration and the potency of the stimulus. While subdiaphragmatic deafferentation
blocked the anorexigenic effect of hepatic portal vein GLP-1 infusion, subdiaphragmatic deafferentation did not blunt the feeding suppressive effect of GLP-1 infused into the vena cava(61). In contrast, the effect of the long-acting GLP-1 agonist exendin-4 was not blocked in rats with subdiaphragmatic deafferentation(62). There is some evidence that the ARC represents a target site not only for GLP-1 but also for the structurally related hormone oxyntomodulin (OXM), which is co-secreted with GLP-1 and exerts an anorexigenic effect via the GLP-1R after central injection(63,64). GLP-1R activation by intra-arcuate injection of OXM decreased food intake and the OXM-mediated suppression of intraperitoneal OXM administration was blocked by injection of the GLP-1R antagonist exendin(9–39)(58). Another study did not observe a feeding suppressive effect after intra-arcuate GLP-1 injection, but hepatic glucose production was reduced under these conditions(65). However, GLP-1 consistently reduced feeding after site-specific injection into the paraventricular nucleus(65,66).

The effects of GLP-1 and OXM on neuronal activity in the ARC are similar. In rats, both hormones induced excitatory responses in ghrelin-inhibited cells(67) (see example in Fig. 2) suggesting a stimulation of POMC signalling, which is consistent with the high expression of the GLP-1R in POMC neurons(65). GLP-1 and OXM induced heterogeneous excitatory and inhibitory effects in ghrelin-excited ARC neurons, but the functional and neurochemical phenotype of these cells have not yet been identified. The absence of GLP-1R expression in NPY neurons seems to exclude the NPY system as a primary target for GLP-1 in the ARC. Despite the existing in vivo and in vitro evidence for a potential role of ARC-dependent GLP-1 signalling in energy and glucose homeostasis further support for a physiological role of endogenous GLP-1R ligands acting via hypothalamic circuits needs to be established. Although beneficial effects on body weight have been reported for anti-diabetic GLP-1 agonists(68–70), moderate effectiveness and undesired side effects limit the potential of these drugs at least as a mono-therapy against obesity.

**Feeding and nutrient-related effects on neuronal function**

Numerous studies have confirmed the reciprocal regulation of NPY and POMC gene expression in response to alterations in energy intake(71). Fasting not only leads to alterations in neuropeptide expression but also to alterations in neuronal activity. Food deprivation for 24 h elicits an activation of ARC neurons in mice as reflected by an increase in c-Fos expression(72–74). Refeeding of 12 h food-deprived mice completely reverses the fasting-induced ARC activation (Fig. 3) indicating that feeding-related signals exert a strong inhibitory effect on ARC neurons that are activated under negative energy balance(44,75). Hence, ARC neurons appear to respond to negative and positive changes in energy intake. As demonstrated by phenotyping studies, fasting-induced ARC activation specifically occurs in NPY neurons, but not in POMC neurons under these conditions(75,76). While this observation suggests increased hypothalamic NPY signalling as a result of fasting, it does not exclude a parallel inhibition of POMC neurons in the ARC. The induction of a fasting-induced ARC activation has also been demonstrated in rats, although considerably longer periods of food deprivation (3 d) are typically required for an immunohistochemically detectable response in this species, at least when c-Fos is used as a marker. Interestingly, there seems to be a shift of neuronal activation from the NPY system in the fasted state to the melanocortin system following refeeding(77,78). This response is consistent with the function of the NPY system to promote energy intake under negative energy balance and with the counter-regulatory function of the POMC system to limit energy intake.

Feeding of palatable non-energy diet (NED; vanilla-flavoured cellulose) with or without selective nutrient supplementation has proven to be a useful approach to explore the role of diet-derived nutrients in feeding-related modulation of neuronal ARC activity. Mice receiving NED for 12 h instead of standard chow exhibit a strong c-Fos expression in the ARC that is only slightly lower than in 12 h fasted animals(75). The same is true for fasted mice that were refed with NED. Therefore, nutrient-independent stimuli associated with feeding activity (e.g. gustatory, oropharyngeal cues and gastric distension) appear to exert a minor, but significant influence on the ARC because they only weakly counteract the stimulation of ARC activation triggered by short-term energy deprivation. When refeeding with NED that was selectively supplemented with each of the different macronutrients (carbohydrates, protein or fat), a stronger reversal of fasting-induced ARC activation was observed than that following refeeding with non-supplemented NED(75). When the intake of each nutrient was matched to specific nutrient intake of chow-fed animals, the carbohydrates were more effective than protein and fat. However, increasing the protein content in the diet or refeeding with pure fat (lard) resulted in a similar reversal of fasting-induced c-Fos expression as observed for refeeding with carbohydrate. Therefore, the relative contribution of each macronutrient to the inhibition of ARC activity in this experimental setting appeared to depend on the amount of intake. Whether isoenergetic intakes of the different macronutrients are equipotent to reverse fasting-induced ARC activation has not yet been investigated.

The mechanism underlying the fasting and feeding-related effects on neuronal activity in the ARC are likely to
ARC neurons have been shown to respond to glucose in indirect actions mediated by gastrointestinal hormones. These involve not only direct effects of metabolites but also indirect actions mediated by gastrointestinal hormones. ARC neurons have been shown to respond to glucose in excitatory and inhibitory ways. Phenotypically identified NPY neurons of mice and rats decrease their firing rate with increasing glucose concentrations (glucose-inhibited cells). Conversely, POMC neurons have been demonstrated to be glucose-excited because they decrease their discharge rate when the extracellular glucose concentration is decreased. The intracellular mechanisms transducing changes in the glucose concentration into altered neuronal activity are well understood for glucose-excited neurons, while the molecular correlates of inhibitory glucose signalling are less clear. Glucose sensitivity in glucose-excited neurons has obvious parallels to glucose signalling in other tissues, including POMC neurons of the ARC. Not only glucose but also hypothalamic fatty acid signalling contributes to the control of energy balance and glucose homeostasis because intracerebroventricular infusion of oleic acid inhibits food intake and hepatic glucose production. Besides other hypothalamic structures, the ARC has been identified as a fatty acid responsive area. Lipid sensitivity has been demonstrated for POMC neurons that were inhibited by oleic acid. In analogy to the importance of intracellular glucose metabolism, intracellular lipid metabolism has been linked to altered neuronal function and to changes in food intake and body weight. Pharmacological blockade fatty acid synthesis (FAS) pathway by central or peripheral injection of the FAS inhibitor C75 inhibited feeding and reduced body weight in mice. Among other hypothalamic sites, FAS is expressed in NPY neurons of the ARC. Peripheral applied C75 prevented the fasting induced c-Fos expression in the medial ARC and in hypothalamic downstream targets of the ARC supporting a role of lipid signalling in fasting-induced ARC activation. Different intracellular mechanisms have been proposed to mediate the effects of FAS inhibition on energy homeostasis. These mechanisms include an increase in the FAS substrate malonyl CoA, which represents an intracellular metabolic signal inhibiting food intake and body weight. This view is consistent with the development of obesity and increased food intake when malonyl CoA degradation in the mediobasal hypothalamus is enhanced by overexpression of the enzyme malonyl CoA decarboxylase. A regulatory function for cellular lipid sensing has also been postulated for the enzyme carnitine palmitoyltransferase-I that controls long-chain fatty acid transport into the mitochondria. Pharmacological or genetic inhibition of hypothalamic food intake.
carnitine palmitoyltransferase-1 reduced food intake and glucose production\textsuperscript{(97)}. Despite the cumulating evidence for a role of hypothalamic lipid sensing further confirmation is required for the physiological relevance of these processes in the context of feeding-related or metabolically induced changes in circulating mediators involved in lipid signalling.

Similar to lipid signalling a role of hypothalamic amino acid signalling in the control of food intake and body weight has emerged. The serine-threonine kinase mammalian target of rapamycin (mTOR) functions as nutrient sensor implicated in cell growth and proliferation\textsuperscript{(98)}. Particularly branched amino acids such as leucine activate the mTOR pathway. Components of the mTOR pathway have been detected in 90 and 45% of ARC NPY and POMC neurons, respectively\textsuperscript{(99)}. In analogy to the changes of neuronal activity in the ARC as a result of fasting and refeeding, activity of the mTOR pathway in the ARC is down-regulated in the fasted state, while it increases in response to refeeding. Both intracerebroventricular and site-specific injection of leucine into the mediobasal hypothalamus inhibited food intake\textsuperscript{(99,100)}. POMC neurons of the ARC increase their electrical activity in response to stimulation with leucine, which is consistent with \textit{in vivo} studies demonstrating a melanocortin receptor involvement in the anorexigenic effect of mediobasal hypothalamic leucin injection\textsuperscript{(100)}.

Fasting and the ingestion of each of the single macro-nutrients lead to hormonal responses adjusting energy homoeostasis and gastrointestinal function to the metabolic and digestive requirements of the body. Some attempts have been made to explore the involvement of hormones in the feeding-related changes in ARC activity. Based on the increase in ghrelin levels under fasting conditions, ghrelin might contribute to the fasting-induced ARC activation in mice. In rats, neutralisation of ghrelin by a site-specific injection of anti-ghrelin Ig into the ARC attenuated food intake\textsuperscript{(101)}. An alternative approach to neutralise ghrelin is the use of an L-RNA-based hormone antagonist, a so-called Spiegelmer, that specifically binds and inactivates the bioactive form of ghrelin\textsuperscript{(102,103)}. Owing to its long-lasting action a single intravenous infusion of the anti-ghrelin Spiegelmer NOX-B11-3 blocked the c-Fos response in the ARC of \textit{ad libitum} fed mice induced by exogenous ghrelin injected peripherally 12 h after administration of the antagonist\textsuperscript{(21)}. In the same study, ghrelin antagonism did not significantly attenuate the fasting-induced c-Fos response in the ARC suggesting that endogenous ghrelin is not necessary for the activation of the ARC under these experimental conditions.

Among the feeding-related hormones that are likely to act via the ARC, ghrelin is the only hormone that increases during fasting. Not only increases but also decreases in hormone levels are likely to reflect alterations in energy homoeostasis and metabolism. One experimental approach to investigate this consists in the compensation of decreased hormone levels by exogenous administration of the respective hormone. Superimposed to the well-described differences in leptin levels related to body adiposity, leptin plasma levels decrease considerably (5-fold) in fasted \textit{v. ad libitum} fed lean mice\textsuperscript{(75)}. Preventing the fasting-induced decrease in leptin by repeated low-dose leptin injections during the fasting period markedly attenuated the c-Fos expression of in the ARC of food-deprived mice (Fig. 4)\textsuperscript{(104)}. Despite the limitation of this approach to precisely mimic physiological leptin levels by exogenous leptin administration, this finding supports the contribution of decreased leptin signalling to fasting-induced ARC activation. Studies in leptin deficient \textit{ob/ob} mice seem to complement these findings because fasting-induced ARC activation is exaggerated in \textit{ob/ob} mice compared with wild-type littermates\textsuperscript{(104)}. Together these studies underscore that leptin signalling has an important impact on the ARC in the context of feeding-related modulation of ARC neurons.

Using the fasting/refeeding paradigm the possible involvement of other hormones in feeding-related changes of ARC activity has been studied. Similar to the ineffectiveness of an acute leptin treatment to reverse fasting-induced ARC activation, insulin did not attenuate c-Fos expression in fasted animals regardless of whether a glucose-lowering dose of insulin was used or a dose that did not produce a reduction in blood glucose\textsuperscript{(75)}. Besides insulin the anorexigenic hormones PYY, amylin and cho-lecystokinin (CCK) were tested for their ability to reverse fasting-induced c-Fos expression in the ARC. Only PYY, but not amylin or CCK attenuated the stimulation of ARC neurons in food-deprived mice\textsuperscript{(75,105)}. The lack of effectiveness was probably not due to ineffective doses, because in the same animals both amylin and CCK elicited c-Fos expression in the AP and the nucleus of the solitary tract (NTS), respectively. As amylin and CCK primarily act via the brainstem (see later) and because the ARC is considered as a target site for PYY, these findings are consistent with the presumed central signalling mechanisms for these hormones. Despite the failure of CCK and amylin to reverse fasting-induced ARC activation, at least CCK partially prevented ghrelin-induced c-Fos expression in the ARC in \textit{ad libitum} fed mice\textsuperscript{(75)} indicating some CCK-dependent inhibitory input to the ARC. Collectively, these findings suggest diverse roles for the investigated hormones in feeding-related changes of neuronal function in the ARC. However, the use of knockout models or specific hormone antagonists is required to confirm the relevance of endogenous hormone actions.

\textbf{Altered neuronal arcuate nucleus responses in obese rodent models}

Obesity is associated with hormonal and metabolic perturbations, including hyperleptinaemia, hyperinsulinaemia, leptin and insulin resistance, dyslipidaemia and hyperglycaemia and others\textsuperscript{(12,106,107)}. In rodent models of diet-induced obesity (DIO) neuronal to responsiveness of ARC neurons to leptin is disrupted. DIO mice show a reduced phosphorylation of the signal transducer and activator of transcription 3 (STAT3), a marker for leptin receptor activation\textsuperscript{(108,109)}. Furthermore, the leptin-dependent release of NPY and \textit{α}-melanocyte stimulating hormone\textsuperscript{(110)} is decreased. As recently demonstrated, obesity also seems to be associated with ghrelin resistance in the ARC because peripheral or central ghrelin injection did not induce food
intake and ARC activation in DIO mice. Moreover, ghrelin failed to induce the release of NPY and agouti gene-related peptide in hypothalamic explants from DIO mice in this study. Not only hormonal resistance but also impaired responsiveness of the ARC to metabolic stimuli has been described.

It has been demonstrated in different obesity models that obese mice show blunted neuronal responses of the ARC to alterations in energy intake. Age-related obese (late-onset obesity) and DIO mice show completely absent or a strongly attenuated fasting-induced activation of NPY neurons in the ARC depending on the duration of food deprivation (12 h or 24 h, respectively). The blunted responses in these animals were not due to age per se or due to high-fat diet feeding because age-matched controls and diet-resistant lean controls showed intense fasting-induced c-Fos responses in the ARC. The absence of fasting-induced ARC activation was associated with the absence of refeeding hyperphagia that only occurred in lean mice after fasting but not in late-onset obesity mice. The blunted ARC activation in these hyperleptinaemic obesity models contrasts with the exaggerated fasting response in the ARC of obese leptin-deficient ob/ob mice. Measurements of metabolic (blood glucose and lipids) and hormonal parameters (insulin, ghrelin and leptin) were conducted in fasted and ad libitum fed obese mice and in respective lean controls. Among these parameters, the only factor that consistently predicted the altered neuronal responses across the different obesity models was leptin because neuronal ARC activation is enhanced under leptin deficiency, prevented by exogenous leptin and blunted under hyperleptinaemia. However, the idea that obesity-related hyperleptinaemia prevents the response of the ARC to fasting is challenged by well-described leptin resistance in hyperleptinaemic obesity. Factors that limit central effectiveness of circulating leptin involve reduced transport across the blood–brain barrier, increased intracellular inhibition of leptin-signalling and reduced leptin receptor expression. Each of these factors are affected by feeding in a way that promotes leptin responsiveness under fasting conditions. Fasting increases the rate of leptin transport into the brain, reduces intracellular leptin-antagonistic signalling and increases leptin receptor expression and leptin binding to the receptor. Hence, leptin responsiveness is dynamic and linked to the feeding status. In line with this assumption, fasting not only increases the leptin-induced STAT3 phosphorylation in lean mice but also in late-onset obesity mice that did not show a significant STAT3 response in the ad libitum fed state. Consequently, the combination of increased leptin levels and increased leptin responsiveness might prevent the activation of ARC.
neurons under negative energy balance. Together, these findings suggest that dynamic changes in leptin sensitivity under hyperleptinaemia might interfere with the receptive function of the ARC for stimuli reflecting short-term excursions in energy intake.

The area postrema

The AP is a sensory circumventricular organ located in the dorsal vagal complex of the brainstem. Owing to the lack of a functional blood–brain barrier AP neurons can be reached by humoral factors including peptide hormones that do not passively cross the blood–brain barrier in other areas of the brain. The AP is neuronally interconnected with structures of the visceral enteroreceptive neuroaxis including the NTS and the lateral parabrachial nucleus. Among other neuronal efferents the AP also projects to the dorsal motor nucleus of the vagus that controls visceral motor functions. The AP plays an important protective role as a so-called chemoreceptor trigger zone that mediates aversive and emetic responses induced by noxious or toxic stimuli. This function is for example reflected by the absence of lithium chloride induced taste aversion after ablation of the AP-lesioned (APX) in rats.

Observations from APX studies suggested an AP involvement in the control of food intake and body weight. APX rats are hypophagic and show lower body weight compared with sham-lesions animals, although they over-consume highly palatable food. Furthermore, APX leads to loss of hypoglycaemic or glucoprivic feeding responses induced by insulin, 2-deoxy-glucose or 5-thio-glucose, suggesting a role of the AP in central nervous glucose sensing.

Area postrema-mediated effects of amylin on food intake

The AP has been identified as a target structure for the anorexigenic hormone amylin, which is widely accepted to contribute to the physiological control of food intake. Amylin is co-secreted with insulin from pancreatic β-cells in response to meal-related stimuli. Amylin reduces food intake at near physiological doses via a reduction of meal size. Amylin antagonism not only blocks the anorexigenic effect of exogenous amylin but also increases food intake, supporting a physiological role of endogenous amylin in energy intake. Numerous studies provided evidence that the AP mediates amylin’s suppressive effect on food intake. While vagal afferents are not required for amylin’s anorexigenic action, APX blocks feeding inhibitory action of amylin.

The effects of amylin on neuronal function in the AP and the transmission of amylin signalling to other brain structures involved in the control of energy homeostasis are well established. The amylin receptor consists of a functional complex of the calcitonin receptor and so-called receptor activity modifying proteins (receptor activity modifying protein 1 and receptor activity modifying protein 3). The calcitonin receptor is densely expressed in the AP, where receptor activity modifying protein 3 gene expression has also been described. In electrophysiological studies, amylin exerted strong excitatory effects in 44% of the recorded spontaneously active AP neurons. These effects were blocked by the amylin receptor antagonist AC187 and appeared to be mediated by the intracellular second messenger cyclic GMP. The excitatory action of amylin on AP neurons is in line with studies demonstrating an amylin-induced c-Fos expression in the AP. Not only exogenous but also feeding-related endogenous amylin activates AP neurons because refeeding of fasted rats triggered a c-Fos response in the AP that was attenuated by amylin antagonism. About 50% of neurons showing amylin-induced c-Fos are noradrenergic and specific neurotoxic lesion of these cells blunted amylin’s feeding inhibitory action.

Interaction of amylin and nutrient signalling

Glucose. In support of the afore-mentioned evidence for a role of the AP as central glucose sensor, electrophysiological studies identified glucose-responsive neurons in the AP. These studies were extended by the demonstration that amylin-sensitive neurons in the AP are specifically excited by glucose, whereas amylin-insensitive neurons are unresponsive to changes in the ambient glucose concentration. Based on the importance of amylin-sensitive AP neurons for the physiological control of feeding behaviour, these findings further support a role of the AP in the metabolic control of food intake. The AP is not the only glucose sensitive structure in the brainstem, but it is located in a brainstem area where local injection of 5-thio-glucose induced the strongest glucoprivic feeding responses.

The glucose sensitivity of the AP has also been postulated as a protective mechanism against excursions in blood glucose values. Hypoglycaemia induces a counter-regulatory increase in the rate of gastric emptying that compensates the decreased blood glucose via increased gastrointestinal nutrient absorption. This mechanism interferes with hormonal and pharmacological effects that delay gastric emptying because glucose appears to be a permissive factor. Amylin potently inhibits gastric emptying via an AP-dependent action, which is overridden by hypoglycaemia. Our group recently demonstrated a similar permissive effect of glucose for amylin’s feeding suppressive effect because amylin failed to significantly inhibit feeding under hypoglycaemic conditions (C Neuner-Boyle, unpublished results). The inhibitory effect of the amylin agonist pramlintide on gastric emptying is an important therapeutic mechanism to lower postprandial glucose levels in diabetic patients. As treatment-induced hypoglycaemia is a severe risk in diabetic patients, the permissive effect of glucose on amylin’s feeding suppressive effect because amylin failed to significantly inhibit feeding under hypoglycaemic conditions (see for review).

Protein. Amylin’s satiating and effect and amylin-induced AP activation are modulated by diet-derived macronutrients, in particular by protein. An influence of diet-derived nutrients on amylin signalling was inferred from the observation that amylin-induced activation of the AP is stronger in fasted vs. ad libitum fed rats and also in
rats that received nutrient-deficient NED\(^{148}\). Selective supplementation of NED with protein but not with glucose or fat strongly attenuated the amylin-induced AP activation suggesting that protein counteracts anorexigenic amylin signalling. In light of the well-established satiating action of protein this assumption appeared unexpected, but it was confirmed by feeding studies in which amylin failed to inhibit protein supplemented NED (Fig. 6)\(^{148}\). The inhibitory effect of diet-derived protein on amylin action has been corroborated by immunohistological and feeding studies using isoenergetic diets of different protein content. Amylin more potently decreased intake of 1 and 8% protein chow than standard chow containing 18% protein. Similarly, amylin-induced c-Fos expression was higher in rats receiving less protein\(^{149}\). These effects were not secondary to altered energy intake that was similar across the experimental groups. While blood glucose levels were also similar, blood amino acid levels decreased in rats fed low-protein diet suggesting that circulating amino acids might contribute the protein-dependent attenuation of amylin-induced AP activation. Pre-treatment of rats with an intraperitoneal administration of a mixed amino acid solution also attenuated amylin’s c-Fos response in the AP, which supports this hypothesis\(^{149}\). However, the exact neuronal mechanism underlying the protein-dependent modulation of amylin signalling remains to be identified. The relationship between reduced amylin-responsiveness and protein intake might bear both physiological and therapeutic implications. Amylin’s function in the control of nutrient and energy intake might be adapted to the nutrient composition of the diet. At least under undisturbed energy homoeostasis protein is metabolically less important than carbohydrate or fat, which might suggest that amylin’s contribution to the overall control of energy intake is stronger when the intake of protein relative to glucose or fat is low. Another mutually not exclusive implication might consist of an AP-sensitising effect produced by short-term reduction of food intake or food availability in a protein-dependent manner, pre-adapting AP responsiveness to a higher level in the pre-prandial state before meal onset. In addition to the possible physiological relevance, the effectiveness of pharmacological
approaches based on amylin agonism might be influenced in a nutrient-dependent way.

_Fat._ There is not much experimental evidence for a role of the AP in lipid sensing. Although lipoprivic feeding induced by the fatty acid oxidation inhibitor merceptoacetate is blunted in rats after AP/NTS lesion, this effect has been attributed to the destruction of vagal terminal fields in the NTS (150). Accordingly, lipoprivic feeding is also blocked in vagotomised rats, confirming a mediation of vagal afferent signals (150). Similarly, c-Fos expression in the AP/NTS region after intraduodenal lipid infusion is attenuated by capsaicin-induced destruction of vagal afferents and by CCK receptor antagonism. The latter finding indicates an indirect effect of intestinal lipid sensing via CCK-mediated activation the vagal afferents signalling (151). It is reasonable to assume that amylin signalling and vagally transmitted lipid/CCK dependent signals converge at the level of the AP/NTS region. Furthermore, the existence of a satiogenic synergy between amylin and CCK (152) might imply that lipid-dependent signals indirectly enhance amylin’s feeding suppressive effect via a CCK-dependent action. Unlike the development of hormonal resistance to leptin and insulin as a consequence of prolonged high-fat diet feeding, neither short-term nor long-term high-fat diet feeding leads to amylin resistance at least when amylin’s inhibitory action on food intake is used as an index of amylin responsiveness (153).

_Hormonal interactions involving area postrema and brainstem mechanisms._

The dorsal vagal complex is a site of convergence for vagal and blood-borne signals controlling food intake. Brainstem structures including amylin-sensitive sites are reciprocally interconnected with hypothalamic and extra-hypothalamic areas involved in energy homeostasis (154). For some hormones known to act via the brainstem synergistic effects have been described, although the existence of a synergy is not always based on equal criteria in the literature. Synergy is often inferred from the observation that the effect of two combined stimuli is higher than the sum of the effects when each stimulus is applied alone. Sophisticated statistical models (e.g. isobolographic analysis) have been used to detect and describe synergistic actions. Several synergistic effects have been demonstrated for amylin in combination with other hormones (155). In addition to the synergy between amylin and CCK mentioned earlier, there is a synergistic effect of amylin and leptin to reduce body weight in obese and lean subjects (3,4). Several neuronal mechanisms have been associated with the synergy between amylin and leptin. Amylin seems to enhance neuronal leptin responsiveness as reflected by the reversal of the blunted STAT3 response to leptin in the ventromedial hypothalamus. Amylin also affects leptin signalling in the brainstem because amylin knockout mice tend to show reduced leptin-induced STAT3 phosphorylation in the NTS, and pre-treating leptin-resistant DIO rats with amylin increased leptin-dependent STAT3 signalling in the AP (3,4).

Anorexigenic synergy has also been reported for amylin and PYY (3–36), whereas body weight reduction was additive (49). The neuronal correlate of this anorexigenic synergy has not yet been identified. While the AP was not required for PYY (3–36)-induced suppression of feeding in one study (156,157), another study suggested an involvement of the AP in the PYY-dependent effects on gastric acid secretion (158). Binding sites for PYY of the Y receptor family are abundant in the AP (159–161). Interestingly, a dose of full-length PYY that did not trigger a c-Fos response in the AP alone, increased amylin-induced c-Fos expression in the AP (165) suggesting a possible interaction between amylin and PYY in the AP.

A recent study conducted in non-human primates demonstrated anorexigenic synergy between the potent amylin receptor agonist salmon calcitonin and the GLP-1R agonist exendin-4 (162). The mechanism underlying this synergy also remains to be identified. There is evidence that GLP-1 inhibits feeding via an AP-dependent effect after hepatic portal vein infusion (163). However, the anorexigenic effect of exendin-4 is not blocked in APX rats (164). Although amylin and GLP-1 activate AP neurons, these hormones seem to have different target cells in the AP. Similar to the aversive stimulus lithium chloride, GLP-1 and exendin-4 almost exclusively activate AP neurons that do not express the calcitonin receptor, which is considered as a marker for the primary target cells for amylin in the AP (149). The functional implication of this dissociation has not yet been specifically investigated. GLP-1R-mediated activation of the AP has been linked to the induction of aversion (165). Therefore, it is particularly important to exclude aversive mechanisms in approaches based on GLP-1R activation.

**Conclusions**

Extensive evidence has accumulated demonstrating that the ARC and the AP are responsive to hormonal and nutrient-related signals involved in the homeostatic feedback control of energy homeostasis. Overall, the neurophysiological effects that have been described by a multitude of different _in vivo_ and _in vitro_ approaches are consistent with the effects of these signals on food intake, body weight or metabolism. However, for many of the discussed mechanisms, the physiological relevance remains to be confirmed. Even when physiological relevance is established (e.g. amylin) or postulated (ghrelin, PYY and GLP-1) the predictive value of these physiological mechanisms for the effectiveness of monotherapeutic anti-obesity approaches targeting these mechanisms appears to be weak. Nevertheless, the impressive effectiveness of bariatric surgery suggests the existence of signalling mechanisms that potently reduce body weight and improve metabolic disorders without being compensated by the body. Obviously, the interaction between the different feedback signals has been underestimated, which is reflected by the promising improvements in the therapeutic outcome of combination treatments. The concept of the homeostatic feedback control of energy homeostasis needs to be

https://doi.org/10.1017/S0029665112000778 Published online by Cambridge University Press
integrated into the increasing knowledge about the non-homoeostatic controls affecting energy balance.

Acknowledgements
The author thanks T.A. Lutz (Institute of Veterinary Physiology, University of Zurich) for the critical reading of the manuscript. The research presented in this work has been continuously supported by the Swiss National Science Foundation, the University of Zurich and the Centre of Integrative Human Physiology, University of Zurich. The author declares no conflicts of interest.

References


70. Vilsbøll T, Christensen M, Junker AE, et al. (2012) Effects of glucagon-like peptide-1 receptor agonists on...
weight loss: systematic review and meta-analyses of randomised controlled trials. BMJ 344, d7771.


in the area postrema and on food intake. Neuroendocrinology 86, 124–135.


