Ghrelin Signalling on Food Reward: A Salient Link Between the Gut and the Mesolimbic System

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‘Hunger is the best spice’ is an old and wise saying that acknowledges the fact that almost any food tastes better when we are hungry. The neurobiological underpinnings of this lore include activation of the brain’s reward system and the stimulation of this system by the hunger-promoting hormone ghrelin. Ghrelin is produced largely from the stomach and levels are higher preprandially. The ghrelin receptor is expressed in many brain areas important for feeding control, including not only the hypothalamic nuclei involved in energy balance regulation, but also reward-linked areas such as the ventral tegmental area. By targeting the mesoaccumbal dopamine neurones of the ventral tegmental area, ghrelin recruits pathways important for food reward-related behaviours that show overlap with but are also distinct from those important for food intake. We review a variety of studies that support the notion that ghrelin signalling at the level of the mesolimbic system is one of the key molecular substrates that provides a physiological signal connecting gut and reward pathways.

Key words: dopamine, ghrelin

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Introduction

The present review is concerned with the actions of a circulating appetite-promoting hormone ghrelin (1), produced mostly by the stomach, on the reward system of the brain. The idea that the gut utilises enteroendocrine hormones to communicate with pathways involved in rewarding aspects of food intake is a new and emerging field (2) suggesting the existence of an endocrine gut-brain reward axis that can modify reward signalling for food, a system important for survival. Ghrelin appears to have a physiological role in hunger and meal initiation (3), orchestrating a variety of behaviours that ensure animals go out into the environment to seek out and consume a variety and plentiful supply of nutrients. We focus especially on the effects of ghrelin on reward-linked behaviour for food, including the mechanisms and pathways involved.

The ghrelin/growth hormone secretagogue receptor type 1A (GHSR-1A) system

Ghrelin is a 28-amino acid octanoylated peptide hormone (1) secreted predominantly from a specific type of endocrine cell of the stomach located within the gastric oxyntic mucosa and named P/D1-type cells in humans (4) or A-like-type cells in rodents (5). Ghrelin mediates its actions via its unique specific receptor, the GHSR-1A (6), which is a G protein-coupled receptor highly expressed in the central nervous system (7,8). Ghrelin is the only known naturally-occurring peptide to be post-translationally
modified by a O-octanoylation, a reaction catalysed by the enzyme ghrelin O-acyl transferase (GOAT) (9). This modification is essential for ghrelin-induced activation of GHSR-1A. Ghrelin plays a well-defined variety of physiological roles and is recognised for being the only known circulating peptide hormone that stimulates food intake (10,11). As discussed below, ghrelin stimulates appetite via diverse mechanisms that promote food intake and that stimulate food reward-related behaviours. In plasma, a non-octanoylated form of ghrelin, named desacyl-ghrelin that represents more than 90% of total ghrelin immunoreactivity, has been shown to exist (12). The GHSR-1A-independent effects of this peptide on food intake regulation have been described, although the physiological significance of desacyl-ghrelin remains a matter of debate (13).

Plasma ghrelin levels fluctuate with meal cycle and energy status. One of the most striking features about the 24-h secretory pattern of ghrelin in man is the large rise in plasma levels that occur just before mealtimes (3). The precise mechanism governing preprandial ghrelin release remains an open question (14). One possibility is that ghrelin release is controlled locally in the gastrointestinal tract (15). Some of the metabolic and hormonal factors able to directly regulate ghrelin secretion have been identified. Using ghrelin-producing cell lines, it has been shown that low ω-glucose concentrations stimulate ghrelin release, whereas high ω-glucose and glucose metabolism block ghrelin release (16). Also, norepinephrine enhances ghrelin release by binding to β1-adrenergic receptors on ghrelin cells; indeed, this mechanism has been proposed to modulate ghrelin secretion during fasting when the sympathetic tone is increased (17). It has also been shown that natural (i.e. α-linolenic acid) or chemical agonist for the G protein-coupled receptor 120 inhibit the secretion of ghrelin, suggesting that the decrease of ghrelin release after feeding is induced partially by long-chain fatty acids acting directly on gastric cells (18). Importantly, ghrelin release is also coordinated by a descending pathway (e.g. via the vagus nerve) signalling either a food anticipatory signal (19) or a cry for nutrients by the brain. Anticipatory cues associated with the delivery of food have been shown to trigger ghrelin release in mice (20). Food anticipatory behaviour, including anticipation for palatable food, is decreased in models of suppressed ghrelin signalling, suggesting that ghrelin may, in turn, activate food anticipatory pathways (21,22).

One of the key physiological targets of the ghrelin/GHSR-1A system is the brain. The dedicated ghrelin receptor, GHSR-1A, was identified in 1996 and was found to be expressed in many brain areas linked to feeding control, including hypothalamic, brainstem and mesolimbic pathways (6–8). Ghrelin is able to drive a feeding response when microinjected into many of these sites, including the arcuate nucleus of the hypothalamus (ARC), ventromedial nucleus (23), lateral hypothalamic area (LHA) (24), caudal brainstem (25), ventral tegmental area (VTA), nucleus accumbens (NAC) (26,27), lateral amygdala (28) and ventral hippocampus (29). The orexigenic effects of ghrelin appear to be exclusively signalled through GHSR-1A (6) because ghrelin fails to elicit a feeding response in mice lacking this receptor (30), as well as in rats pretreated with central injection with a GHSR-1A antagonist (31). Food intake appears to be a common goal of the ghrelin-appetitory networks, an endpoint achieved through integration with brain pathways involved in many diverse constructs and behaviours. Indeed, ghrelin signalling pathways are involved in hunger sensation (32), food anticipatory behaviour (21,33), food reward (34), motivated (reward-linked) behaviour for food (35,36), memory (37), novelty-seeking behaviour (38) and anxiety/stress-like behaviour (28,39,40). In the present review, we focus especially on recent advances implicating the central ghrelin signalling system in food reward.

The first indication that central GHSR-1A signalling could be implicated in food intake regulation was noted in 1993, when it was found that the growth hormone-releasing peptide 6 (a growth hormone secretagogue that is now recognised to be a ghrelin mimetic) activates cells in the ARC, as reflected by an increase in the number of cells detected that express Fos protein (41). It emerged that approximately half of the ARC cells activated by this ghrelin mimetic expressed neuropeptide Y (NPY) mRNA, a potent orexigenic signal (42). These ghrelin-sensitive NPY neurones, which co-express another orexigenic peptide, agouti-related protein (AgRP), are considered as a major node in the appetite-regulatory circuitry. In line with this hypothesis, it was later found that NPY/AgRP neurones of the ARC express high levels of GHSR-1A (43,44). Moreover, ghrelin fails to increase food intake in mice lacking NPY and AgRP (45,46), suggesting that these peptides play a pivotal role in the effects of ghrelin on food intake. Selective re-expression of GHSR-1A in AgRP neurones partially restored this phenotype (47).

Ghrelin signalling engages a complex network of neuronal circuits to modulate feeding-linked behaviour. For example, ghrelin-induced food intake also appears to depend on orexin neurones of the LHA, where GHSR-1A is expressed (24,48) and the action of ghrelin on food reward requires intact orexin signalling (35). GHSR-1A is expressed in vagal afferent neurones of the nodose ganglia and in the dorsal vagal complex (8,49), providing an alternative route through which peripheral ghrelin could signal to appetitive neurocircuitry. Indeed, it has been suggested that vagus nerve integrity is required for ghrelin-induced food intake (50,51), although a subsequent study did not find this to be the case (52). The presence of GHSR-1A in dopaminergic VTA neurones (8,53,54), as well as other cell types in this region, supports the possibility that ghrelin can regulate rewarding aspects of eating. Ghrelin may also regulate mesolimbic circuits indirectly via the cholinergic neurones of the laterodorsal tegmental area (LDTg), which also express GHSR-1A (55,56). The action of ghrelin at the level of the hippocampus, another brain area where GHSR-1A is present in abundance (7,8), also appears to be important for both motivational and learned aspects of feeding behaviour (29). Thus, the neuroanatomical distribution of the ghrelin receptor supports a role for the ghrelin/GHSR-1A system in the regulation of both homeostatic and rewarding aspects of feeding.

In humans, the response of the brain’s reward system to food cues, as measured using functional resonance imaging, is enhanced by fasting (57). The relevance of the ghrelin signalling in this observation was suggested by a functional magnetic resonance imaging study showing that ghrelin can mimic the effects of fasting on the reward networks (58) and was further confirmed by another imaging study in humans showing that ghrelin increases the neural
response in brain centres implicated in rewarding aspects of feeding (59). In particular, ghrelin administration to human subjects increases the activation of some reward-related brain centres, including the substance nigra and the VTA, in response to tempting food pictures (59). A more recent imaging study has not only confirmed previous observations, but also shown that subjects with polymorphisms in the fat mass and obesity-associated gene genotype exhibited divergent neural responsiveness to peripheral ghrelin within brain regions that regulate rewarding aspects of appetite (60). Thus, several functional resonance imaging studies in healthy subjects strongly support a role for ghrelin in rewarding aspects of eating in human subjects.

Food reward: motivational and hedonic aspects of eating

All organisms have a capacity to seek out and consume food. In mammals, the neurobiological mechanisms sensing energy need, food availability and coordinating appetitive and food-seeking behaviours are complex. Feeding control involves an integrated regulatory system of homeostatic brain circuits that drive food intake depending on energy store levels: processes for which the hypothalamus and brainstem have a primary role. Food intake is also regulated by reward pathways that process information about the pleasurable (hedonic) and incentive (motivational) aspects of food intake. Homeostatic and reward circuits mediating food intake are inter-related: ‘Hunger is the best spice’, is a Swedish saying acknowledging that all foods have a rewarding value that is influenced by hunger and food availability. From an evolutionary perspective, food reward has a dual role: it not only promotes food seeking and eating when food is scarce, but also promotes overeating when food becomes available, aiming to establish sufficient energy stores for a future famine. If food is found rewarding, this will motivate animals to go out into the environment to seek it out, ensuring not only an adequate supply of calories, but also that consumed foods are of diverse nutritional composition. In the current obesogenic environment, however, food reward is no longer an evolutionary advantage for modern human beings. Instead, it contributes to the maladaptation to that environment, encouraging people to overeat calorie-dense foods to a level far beyond metabolic need.

Neuronal circuits involved in food reward

A key feature of reward processing is the activation of a major dopaminergic cell group located in the VTA of the midbrain. These dopaminergic neurones project to the NAc and the prefrontal cortex, as well as to several other brain areas, including the hippocampus and the hypothalamus (61). The VTA receives projections from many brain nuclei, including the aforementioned areas that receive projections from the VTA, cholinergic neurones of the LDtg (55), as well as taste information via afferent sensory fibres (62,63). Activation of dopaminergic VTA neurones occurs in response to both natural rewards (such as food or sex) and artificial rewards (such as alcohol or other addictive drugs of abuse). As is the case for addictive drugs, the consumption of a food reward has been found to trigger dopamine overflow/release in the NAc, as measured by microdialysis (64), and to increase phasic dopamine release in the striatum, as measured by fast-scan cyclic voltammetry (65). Accumbal dopamine overflow is coupled to VTA dopamine neurone activity (66) and so it may be inferred that foods, especially palatable foods, have the capacity to activate the VTA-NAc dopamine projection. Indeed, acute consumption of a high-fat diet activates the mesolimbic circuit by neuronal pathways that require orosensory stimulation (67). Dopamine release in the NAc potently augments the drive to obtain food rewards (68). The shell part of the NAc is particularly important for eating behaviours engaging projections to the LHA neurones that control food intake. Orexigenic LHA neurones appear to be under a tonic inhibition that can be relieved by activation of reward pathways (69,70). In addition, LHA orexin neurones send projections to the VTA, where they activate dopaminergic neurones (71,72). Thus, LHA orexin neurones have been proposed as a potential link between homeostatic and reward circuits regulating food intake (73).

One of the primary roles attributed to the VTA-NAc dopamine pathway is ‘wanting’ or motivational component of reward that is important for craving behaviour and that is linked to but distinct from the ‘liking’ or hedonic component of reward (74). As reviewed elsewhere (75), the mechanisms linking dopamine to rewarding aspects of eating are rather sophisticated. Novelty of the reward appears to be critical for achieving a maximal dopamine signal (76). With repeated exposure to that same reward (conditioning), the NAc dopamine response lessens (habituates) and transfers instead onto a predictive cue associated with its delivery (77–79). Thus, dopamine signalling is crucially important for the formation of associations between rewards and anticipatory cues (78,80,81). As a consequence of conditioning, the dopamine signal takes on a new role: as a predictor of reward; motivational behaviours are recruited as part of this mechanism ensuring that the expected reward is consumed. One hypothesis for the role of the dopamine signal in reward is that it serves as a ‘reward prediction error’ (82). It follows that NAc dopamine release could be important for assigning increasing reward value to food cues (83).

Multifaceted actions of ghrelin signalling on food reward

In 2006, it became apparent that ghrelin activates the dopamine system, triggering NAc dopamine release (84), dopamine turnover and VTA dopamine neurone activity (54). Consistent with this, a subpopulation of VTA dopamine neurones was found to express GHSR-1A, although the receptor was also found to be located on other cell types within the VTA (54). The idea that ghrelin may provide a physiological signal connecting gut and reward pathways paved the way to studies exploring the effects of ghrelin on behaviours linked to dopamine signalling, including hedonic and motivational aspects of eating.

In rodents, reward can be assessed in the condition place preference (CPP) test in which the animals learn to associate the experience of reward with a particular environment/chamber. They will return to that chamber, spending more time there, even when the
reward is no longer available. In satiated rats, the peripheral delivery of a GHSR-1A antagonist has been shown to abolish CPP for chocolate (85). Similarly, in mice, the delivery of a GHSR-1A antagonist suppresses/abolishes CPP for a high-fat diet (35), and even for alcohol (86) or psychostimulant drugs such as cocaine or amphetamine (87). These data using GHSR-1A antagonists, together with data from ghrelin receptor knockout mice (35,85,86), indicate that central ghrelin signalling is required for the rodents to experience reward from alcohol (an artificial reward) or food (a natural reward). Consistent with this, ghrelin appears to enhance CPP for a high-fat diet (35) and psychostimulant drugs such as cocaine (88).

Craving-like behaviour (i.e. ‘wanting’, motivated, goal-directed behaviour) for food or other reward reinforcers can be explored in rodents using the ‘operant conditioning’ paradigm in which the animals have to progressively work harder (e.g. by pressing a lever) to obtain a reward. The animals are first trained on a fixed ratio schedule to associate the pressing of the lever a fixed number of times with the delivery of a reward. Subsequently, they are tested using a progressive ratio schedule in which the animal has to work increasingly hard for each reward obtained. The number of lever presses or the number of reward earned can be used to estimate motivation or goal-directed behaviour. Motivated behaviour for sugar treats has been shown to be increased by ghrelin (administered peripherally or centrally to satiated rats) and decreased by a GHSR-1A antagonist administered to fasted rats (also delivered both peripherally and centrally) (36). Central ghrelin administration increases operant lever-pressing for sucrose in rats (36,89). Similarly, operant behaviour for a high-fat diet in mice, measured by nose pokes instead of lever presses, was increased by ghrelin (35). Also, GOAT-deficient mice display an attenuated motivation for a high-fat diet in an operant responding model and a decreased reward-linked feeding response examined in a ‘dessert effect’ protocol, in which the intake of a palatable high-fat diet pellet ‘dessert’ is assessed in calorically-satiated mice (90).

It is clear that ghrelin enhances preference for pleasurable, sweet and fatty foods. In particular, ghrelin administration shifts food preference towards a high-fat diet (91). Ghrelin administration also increases intake of palatable saccharin solution and preference for saccharin-flavored foods in mice (92). Similarly, rats treated with a GHSR-1A antagonist consume less peanut butter and the liquid nutritional supplement Ensure® (Abbott Laboratories, Chicago, IL, USA) but fail to change intake of regular chow in a free choice protocol (85). On the other hand, ghrelin also increases food anticipatory activity, which is characterised by increased arousal, increased locomotor activity and an elevated body temperature in anticipation of a predicted meal (21,22). Ghrelin secreted in anticipation of a meal correlates with anticipatory locomotor activity and the administration of ghrelin increases locomotor activity and foraging-like activities in rodents (93–95). Moreover, GHSR-1A antagonists decrease anticipatory behaviour for a palatable meal (22). Interestingly, i.c.v. ghrelin fails to alter the avidity of licking, when lick motor patterns were recorded using lickometry, suggesting that ghrelin does not affect the hedonic valuation (i.e. ‘liking’) in rats (89). Odour also plays a role in conferring information about food availability, and ghrelin may have a beneficial role in to help

animals seek out because it has been shown to stimulate sniffing and to increase olfactory sensitivity in mice (96). Overall, there is a great deal of evidence supporting a role for ghrelin in a variety of food reward-related eating behaviours. It is perhaps not surprising therefore that ghrelin-sensitive brain networks overlap considerably with those important for feeding control (97).

Neuronal circuits mediating the effects of ghrelin on food reward

The neurobiological substrates and circuits underpinning the effects of ghrelin on food-motivated behaviour show some overlap with (but also divergence from) those involved in food intake (2,97). Although food intake can be driven by ghrelin delivery to both the VTA (26,54,85) and the NAc (26,85), food-motivated behaviour occurs only after VTA (but not Nac) microinjection of ghrelin (27). VTA-lesioned rats spend less time than control rats exploring tubes containing peanut butter in response to centrally-administered ghrelin (85). Similar effects are observed in food-restricted rats in which chronic intra-VTA administration of ghrelin enhances, whereas chronic intra-VTA delivery of a GHSR-1A antagonist blunts, operant responding for chocolate-flavored pellets (98). Collectively, these findings identify the VTA as a key target brain area for the effects of ghrelin on food-motivated behaviour and suggest that direct action of ghrelin at the level of the VTA is sufficient to drive food-motivated behaviour. Moreover, the uncoupling between food intake and food motivated behaviour elicited by intra-VTA ghrelin resonates with the collective findings that NAc dopamine signalling is not coupled to normal feeding (99,100) but rather in motivated behaviour towards palatable foods, independent of the calories consumed (101,102). Importantly, only the effect of intra-VTA ghrelin on food-motivated behaviour was found to require NAc dopamine receptor 1 and 2 signalling, whereas food intake driven by VTA ghrelin was dopamine-independent in this paradigm (103). Pretreatment of rats with a dopamine receptor 1 antagonist eliminates ghrelin-induced increases in bar pressing, without compromising generalised licking motor control, supporting a role for dopamine receptor 1 signalling mainly in the motivational feeding effects of ghrelin (89). It has been shown that mice expressing GHSR-1A selectively in tyrosine hydroxylase-containing cells, including a subset of VTA dopaminergic neurons, display a significant, albeit reduced, response to the orexigenic effects of ghrelin and a full CPP for a high-fat diet when treated with exogenous ghrelin or exposed to a chronic social defeat stress (CSDS) protocol. In the study, a nontraditional mouse model was used in which GHSR-1A gene expression is disrupted by a transcriptional blocking cassette flanked by loxP sites that enable Cre recombinase-mediated GHSR-1A gene re-expression (53). Thus, a variety of studies in rodents suggest that the action of ghrelin at the level of the dopaminergic neurons of the VTA is essential for the actions of ghrelin on both food intake and food reward.

Apart from the VTA-NAc dopamine system, the rest of the neuronal circuitry engaged by ghrelin to modulate food reward-related behaviours is quite unclear. For example, it has been suggested that ghrelin can regulate mesolimbic circuits indirectly via the

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cholinergic neurones of the LDTg, which express GHSR-1A (55,56). Also, the action of ghrelin on food reward appears to require intact orexin signalling, as indicated by the finding that the effects of ghrelin with respect to conditioning food CPP or operant conditioning for a food reward were blocked in orexin-deficient mice and wild-type mice given an orexin 1 receptor antagonist (35). A potential pathway would involve direct binding of ghrelin to GHSR-1A present on orexin neurones of the LHA. This is supported by the finding that GHSR-1A is expressed within the LHA of the rat (8), as well as by studies showing that ghrelin can induce action potentials and depolarisation in isolated orexin neurones (104). The ghrelin-engaged orexin neurones would then project to the VTA, where orexin signalling is critical in the activation of mesolimbic dopaminergic circuit and reward-seeking behaviours (105,106). A recent study using fast-scan cyclic voltammetry in awake rats to record dopamine spikes in the NAc core showed that the central infusion of ghrelin increases, whereas a GHSR-1A antagonist suppresses, the magnitude of dopamine spikes evoked by food, respectively. In addition, potentiation of food-evoked dopamine spikes was increased by intra-LHA ghrelin and intra-VTA blockade of orexin receptors attenuated food intake induced by central ghrelin (107). Thus, orexin signalling also appears as a key mediator of the actions of ghrelin on food reward.

Additionally, ghrelin might indirectly regulate food reward-related behaviours by engaging central NPY/AgRP neurones of the ARC. In this regard, studies using DREADD technology (designer receptors exclusively activated by designer drugs) have revealed that selective activation of AgRP neurones is sufficient to drive food motivated behaviour in mice (108). NPY has been shown to be able to induce CPP when administered to the NAc (109). NPY also induces sucrose-motivated behaviour when administered to the VTA or Nac, although it only increased sucrose consumption when administered to the NAc (110). Cross-talk between these NPY- and ghrelin-sensitive networks at the level of the VTA for food intake has been suggested by studies showing that the feeding effects, but not food-motivated effects, of ghrelin were abolished by VTA pretreatment with a NPY receptor 1 antagonist. Interestingly, the converse was true for the VTA delivery of a mu-prefering opioid receptor antagonist, which suppressed the effects of VTA ghrelin on food motivation but not food intake (111). Kappa opioid receptor pathways at the hypothalamic level also appear to be a component of the ghrelin sensitive circuitry that is important for feeding control (112). Collectively, these studies outline the divergence of behaviours (food motivation and food intake), involving overlapping but also distinct neurochemical pathways.

**Physiological role of the ghrelin signalling system on food reward**

Fifteen years after its discovery, the physiological relevance of the ghrelin signalling on food intake regulation, in general, and food reward pathways, in particular, is still a matter of discussion. Notably, some evidence strongly supports a role for ghrelin in short-term food intake regulation. Ghrelin administration triggers eating in both human beings and rodents (10,11). As described above, human plasma ghrelin levels decrease rapidly in response to nutrient ingestion, and 24-h plasma profiles display marked preprandial increases and postprandial decreases associated with every meal (3). In rodents, ghrelin levels are suppressed within minutes by re-feeding or enteral infusions of nutrients (113). Interestingly, blockade of ghrelin signalling in adult animals using anti-ghrelin antibodies, GHSR-1A antagonists or anti-sense oligonucleotides report decreases in spontaneous food intake and body weight (114–116). By contrast, genetically modified mouse models, including mice overexpressing ghrelin or mice with genetic deletion of ghrelin, GHSR-1A or GOAT, are almost indistinguishable from wild-type mice in terms of ad lib. standard rodent chow diet feeding, food intake and body weights (117). The argument invariably put forward for the lack of a robust phenotype in these mutant mice is that compensatory physiological mechanisms arise during development. However, a recent study has reported that genetic ablation of ghrelin-producing cells in adult mice fails to lead to a loss of appetite or body weight, or resistance to a high-fat diet (118). Even a physiological role for plasma ghrelin concentrations in the regulation of basal food intake was questioned because the study found that only doses of ghrelin resulting in supraphysiological plasma levels of the hormone are able to increase food intake. In line with this possibility, no increase in appetite was observed in normal-weight volunteers when plasma ghrelin is raised four-fold above basal levels (119). By contrast to these data, ghrelin has been shown to increase hunger scores and food intake in a buffet test meal in both normal weight and obese subjects (120,121). Thus, further studies are required to better clarify the role of basal ghrelin signalling on food intake and food reward-linked behaviours.

The significance of ghrelin signalling likely becomes more evident in situations in which ghrelin signalling is physiologically more relevant, such as fasting, caloric restriction or stress (122). In this regard, GHSR-1A deficient mice show important eating behaviour alterations under specific experimental conditions. For example, wild-type mice subjected to prolonged caloric restriction show enhanced CPP for a high-fat diet, whereas GHSR-1A deficient mice lack such response (35,92). Moreover, GHSR-1A deficient mice in response to scheduled meals have both attenuated anticipatory hyper-locomotion and reduced expression of the marker of cellular activation c-Fos in the mesolimbic pathway (93,123). Similarly, GHSR-1A deficient mice do not anticipate food when exposed to an activity-based anorexia model, in which mice are given free access to a running wheel and fed once per day for 2 h (21). Most humans change in their eating habits in response to stress, and an emerging literature has started to support the existence of a strong association between the ghrelin/GHSR-1A system and stress. For example, the CSDS procedure, which subjects mice to daily bouts of social defeat by aggressive male mice, has been used to study the physiological effect of ghrelin on feeding behaviours (39,53,124). Wild-type mice exposed to CSDS increase their plasma ghrelin levels and regular chow intake during and for at least 1 month after the defeat period. By contrast, GHSR-1A deficient mice fail to show CSDS-induced hyperphagia (39,124). In wild-type mice, CSDS also increases CPP for a high-fat diet, whereas such a stress-induced food reward response is not observed in CSDS-exposed GHSR-1A deficient mice (53). Thus, ghrelin signalling appears to be required
for stress-induced change in rewarding aspects of eating in mice, under these particular conditions. By contrast to these findings, wild-type mice exposed to a chronic unpredictable stress procedure, which also elevates plasma ghrelin levels, decrease food intake and body weight gain, whereas similarly-treated GHSR-1A deficient mice lack these changes (124). Notably, elevations of plasma ghrelin are observed in several stress models and ghrelin administration to either humans or rodents has been shown to induce a strong activation of the hypothalamus-pituitary-adrenal neuroendocrine axis (125,126). The physiological implications of ghrelin-induced activation of stress responses or, conversely, the impact of stress on ghrelin responsiveness are currently unknown. Thus, further work is needed to clarify the inter-relationship between of ghrelin, stress and food intake.

The ability of ghrelin to act in the brain to regulate food intake depends on the accessibility of circulating ghrelin to the above mentioned brain areas. Circulating ghrelin cannot freely cross the blood–brain barrier and it is currently unclear how this hormone enters the brain [127]. In mice, ghrelin can be transported from the brain to the circulation via a saturable transport system; however, no such system has been identified for blood to brain transport (128). A recent study using ghrelin-fluorescent tracer has shown that peripheral increases of plasma ghrelin mainly access to the ARC (127,129). The ARC is a hypothalamic nucleus located in close apposition to the median eminence, a circumventricular organ with fenestrated capillaries that allows plasma ghrelin to diffuse to the brain parenchyma (130). The area postrema is another circumventricular organ also known to participate in food intake regulation and to express GHSR-1A (8,127). Early studies using GHSR-1A agonists showed that these compounds activate cells in the area postrema and in some closely-adjacent structures (131). In the study using the fluorescent ghrelin tracer, it was also found that high levels of circulating ghrelin can directly act on area postrema neurones, which then innervate several hypothalamic and brainstem feeding centres (127,129). In line with this possibility, there are indications that long-term effects of ghrelin on feeding depend on intact signalling at the area postrema (132,133). The study using the fluorescent ghrelin tracer could not provide direct evidence for increases of plasma ghrelin reaching centres of the mesolimbic pathway, at least in an acute fashion. However, an acute effect of peripheral ghrelin on the mesolimbic pathways is supported by a study showing that intra-VTA delivery of a selective GHSR-1A antagonist blocked the orexigenic effect of peripherally-administrated ghrelin (54). Also, it has been shown that peripheral administration of ghrelin in mice increases the extracellular concentration of dopamine in the NAc measured by in vivo microdialysis (134). However, the extent to which implantation of permanent cannulas in the brain affects the blood–brain

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**Fig. 1.** Endocrine gut–brain reward axis: a model of the effects of ghrelin on eating behaviour. Some specific conditions are known to influence eating regulation by affecting homeostatic brain circuits, which drive food intake depending on energy store levels, and/or reward brain circuits, which drive consumption based on the rewarding properties of foods. Several lines of evidence suggest a key role for the ghrelin/growth hormone secretagogue receptor type 1A system in mediating these eating behaviours. These specific conditions increase ghrelin, which in turn reaches the brain where, upon interaction with its receptor on dopaminergic neurones in the ventral tegmental area and likely in other brain nuclei, mediates an integrated and complex eating behavioural response.

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barrier integrity in these studies is unclear. Interestingly, mice expressing GHSR-1A selectively in tyrosine hydroxylase-containing cells partially respond to ghrelin-induced food intake and fully develop CPP for a high-fat diet in response to either peripheral ghrelin administration during the conditioning sessions or after CSDS (53). Thus, future studies are required to clarify the physiological relevance of the action of peripheral ghrelin on the mesolimbic pathway.

The relevance of the expression of GHSR-1A in brain areas without obvious access to circulating ghrelin is, in general, unclear. Although earlier studies suggested that ghrelin could be produced in the brain, more recent studies have clearly shown that ghrelin is not synthesised in the central nervous system (135–137). GHSR-1A mainly signals through Goq/11, phospholipase C, inositol phosphate and calcium mobilisation from intracellular stores; although it also activates other signalling pathways (138). An interesting feature of GHSR-1A is its strong constitutive activity that makes it capable to signal in a ghrelin-independent manner (139,140). Thus, the increase of GHSR-1A expression would accordingly increase activation of the downstream signalling pathways affecting, as a consequence, food intake and body weight regulation (124). Additionally, it has been proposed that an alternative mechanism by which GHSR-1A regulates food intake involves its dimerisation with other G protein-coupled receptors. GHSR-1A has been shown to heterodimerise with the melanocortin 3 receptor, the serotonin 2C receptor and the dopamine receptors, which are all involved in food intake and body weight regulation. Heterodimerisation could serve to modulate specific functions of GHSR-1A, such as signalling pathways, or to act as an allosteric mechanism to regulate signalling pathways of the other receptors, independently of ghrelin binding (141–144).

Concluding remarks

The evidence reviewed here suggests that ghrelin/GHSR-1A system is strongly linked to food reward-related pathways in addition to and partially separate from those which drive food intake. Notably, the mechanisms by which ghrelin/GHSR-1A system promotes food intake are multifaceted and are summarised in Fig. 1. The mesoaccumbal dopamine pathway appears to be a key target for ghrelin/GHSR-1A system, opening the possibility that a primary role for ghrelin is to regulate rewarding aspects of eating. The ghrelin/GHSR-1A system is not only up-regulated by hunger and in anticipation to food, orchestrating a feeding response, but also by negative energy balance conditions, or psychological stress when the activation of the mesoaccumbal dopamine pathway helps animals cope with these detrimental conditions. Thus, the action of the ghrelin/GHSR-1A system on the mesolimbic pathway is very advantageous for the survival of the animal in times of food scarcity. The constant abundance of palatable foods together to the excessive stress levels that we suffer in modern societies places the ghrelin/GHSR-1A system in a new role in which it likely cause adverse consequences, including overeating beyond metabolic need and body weight gain. Therefore, the action of ghrelin on the mesolimbic system may have been a ‘great spice’ from an evolutionary perspective, although it no longer represents an advantage for modern human beings. Our knowledge of the neuronal circuits and molecular mechanisms mediating the actions of the ghrelin/GHSR-1A system on the mesolimbic pathways has progressed considerably in recent years, yet still many novel and exciting aspects of this endocrine gut–brain reward axis likely remain to be discovered and will deserve intense research in the near future.

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Ghrelin signalling and food reward

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